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REVIEW

539 **Health insurance status and outcomes in children, adolescents, and young adults: a systematic review and meta-analysis**
Congyang Huang, Hanshan Liu, Honglian Hu, Li Jia, Suyun Hu

ORIGINAL ARTICLES

554 **IgE mediated food allergy in Turkey: different spectrum, similar outcome**
Ayşegül Akarsu, Melike Ocak, Gizem Köken, Ümit Murat Şahiner, Özge Soyer, Bülent Enis Şekerel

564 **Enablers and barriers for enteral feeding with mother's own milk in preterm very low birth weight infants in a tertiary care neonatal intensive care unit**
Dattatray Kulkarni, Srinivas Murki, Dinesh Pawale, Soumya Jena, Deepak Sharma, Venkateshwarlu Vardhelli, Venkat Kallem, Tanveer Bashir, Vidhyadhara Naik, Sai Kiran

575 **The performance of shear wave elastography on evaluating liver changes in obese and overweight children**
Neslihan Özkul Sağlam, Sema Aksoy, Selcen Yaroğlu Kazancı, Figen Palabıyık, Sadık Sami Hatipoğlu, Ercan İnci

584 **Developmental parameters and physical fitness in preschool children with Minor Neurological Dysfunction**
Halil Alkan, Akmer Mutlu, Göknur Haliloğlu

594 **Expression of MicroRNA 146a, 155, 181 and 223 in febrile seizure**
Kürşat Bora Çarman, Yasemin Karal, Gül Gülen Mert, Arzu Ekici, Peren Perk, Didem Arslantaş, Coskun Yarar, Ener Çağrı Dinleyici

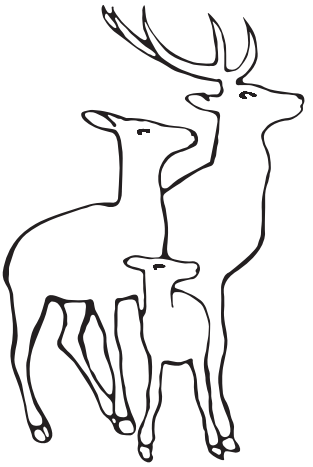
602 **Developmental evaluation in children experiencing febrile convulsions**
Rojan İpek, Khatuna Makharoblidze, Burçin Gönüllü Polat, Meltem Çobanoğulları Direk, Didem Derici Yıldırım, Mustafa Kömür, Çetin Okuyaz

612 **Clinical profile and long-term outcome of the first seizures in children**
Pınar Arıcan, Hakan Salman, Nihal Olgaç Dündar

618 **The causes of parental vaccine refusal: results of a survey from Giresun, Turkey**
Özlem Terzi, Elif Nur Gülen, Cihad Dündar

626 **Determining the effect of time dependent and time independent factors on pneumonia of children under five in North west Ethiopia**
Lijalem Melie Tesfaw, Muluwerk Ayele Derebe, Haile Mekonnen Fenta

volume 63
number 4
July-August 2021



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CONTENTS

VOLUME: 63 NUMBER: 4 JULY-AUGUST 2021

REVIEW

Health insurance status and outcomes in children, adolescents, and young adults: a systematic review and meta-analysis 539
Congyang Huang, Hanshan Liu, Honglian Hu, Li Jia, Suyun Hu

ORIGINAL ARTICLES

IgE mediated food allergy in Turkey: different spectrum, similar outcome 554
Ayşegül Akarsu, Melike Ocak, Gizem Köken, Ümit Murat Şahiner, Özge Soyer, Bülent Enis Şekerel

Enablers and barriers for enteral feeding with mother's own milk in preterm very low birth weight infants in a tertiary care neonatal intensive care unit 564
Dattatray Kulkarni, Srinivas Murki, Dinesh Pawale, Soumya Jena, Deepak Sharma, Venkateshwarlu Vardhelli, Venkat Kallem, Tanveer Bashir, Vidhyadhara Naik, Sai Kiran

The performance of shear wave elastography on evaluating liver changes in obese and overweight children 575
Neslihan Özkul Sağlam, Sema Aksoy, Selcen Yaroğlu Kazancı, Figen Palabıyık, Sadık Sami Hatipoğlu, Ercan İnci

Developmental parameters and physical fitness in preschool children with Minor Neurological Dysfunction 584
Halil Alkan, Akmer Mutlu, Göknur Haliloğlu

Expression of MicroRNA 146a, 155, 181 and 223 in febrile seizure 594
Kürşat Bora Çarman, Yasemin Karal, Gül Gülen Mert, Arzu Ekici, Peren Perk, Didem Arslantaş, Coskun Yarar, Ener Çağrı Dinleyici

Developmental evaluation in children experiencing febrile convulsions 602
Rojan İpek, Khatuna Makharoblidze, Burçin Gönüllü Polat, Meltem Çobanoğulları Direk, Didem Derici Yıldırım, Mustafa Kömür, Çetin Okuyaz

Clinical profile and long-term outcome of the first seizures in children 612
Pınar Arıcan, Hakan Salman, Nihal Olgaç Dündar

The causes of parental vaccine refusal: results of a survey from Giresun, Turkey 618
Özlem Terzi, Elif Nur Gülen, Cihad Dündar

Determining the effect of time dependent and time independent factors on pneumonia of children under five in North west Ethiopia 626
Lijalem Melie Tesfaw, Muluwerk Ayele Derebe, Haile Mekonnen Fenta

Treatment results of modified BFM protocol in pediatric high-risk Burkitt lymphoma 639
Sema Vural, Dildar Bahar Genç, Rejin Kebudi, Öner Doğan, Serap Karaman

Effects of lockdown during corona pandemic on children with neurodevelopmental disorders-A questionnaire-based survey 648
Jyotindra Narayan Goswami, Vishal Sondhi, Ashish Kumar Simalti, Manu Bamal, Shuvendu Roy

CONTENTS

VOLUME: 63 NUMBER: 4 JULY-AUGUST 2021

Asthma and risk factors in the first 6 years of life in a population-based cohort 660
Ayşe Şenay Şaşihüseyinoğlu, Dilek Özcan, Derya Ufuk Altıntaş

Assessment of sleep in children with periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome 666
Ayşin Nalbantoğlu, Burçin Nalbantoğlu

Cost of illness of RSV infection in a middle-income tropical country 673
Jefferson Antonio Buendía, Diana Guerrero Patino, Denis Sinisterra

Timeliness of postnatal surgery in newborns with open neural tube defects: a single center experience 683
Ayhan Pektaş, Mehmet Gazi Boyacı, Hilal Koyuncu, Mine Kanat Pektaş, Ahmet Afşin Kundak

CASE REPORTS

Clinical and molecular characteristics of carnitine-acylcarnitine translocase deficiency with c.270delC and a novel c.408C>A variant 691
Berrak Bilginer Gürbüz, Didem Yücel Yılmaz, Rıza Köksal Özgül, Can Koşukcu, Ali Dursun, Hatice Serap Sivri, Turgay Coşkun, Ayşegül Tokatlı

Outbreak of late-onset Group B Streptococcal disease with serotype Ib in a Neonatal Intensive Care Unit 697
Hiroko Horie-Ota, Kazushige Ikeda

Whole exome sequencing identifies a novel variant in ABCA3 in an individual with fatal congenital surfactant protein deficiency 703
Hayrunnisa Bekis Bozkurt, Yavuz Şahin

A very rare cause of protein losing enteropathy: Gaucher disease 708
Mehmet Akif Göktaş, Ersin Gümüş, Hülya Demir, Hayriye Hızarcıoğlu Gülşen, İnci Nur Saltık Temizel, Hasan Özen, Şafak Güçer, Aysel Yüce

Severe isolated sulfide oxidase deficiency with a novel mutation 716
Meriç Ergene, Nuriye Yarar, Elif Perihan Öncel, Taner Sezer, Büşranur Çavdarlı, İsmail Zafer Ecevit, Halil İbrahim Aydın

Massive true thymic hyperplasia in a 3-month-old infant: case report and literature review ... 721
Min Yang, Lin Zeng, Yi Ji, Bo Xiang, Zhi-cheng Xu

Facial colliculus syndrome due to a Herpes simplex virus infection following Herpes labialis 727
Muhittin Bodur, Rabia Tütüncü Toker, Abdullah Hakan Özmen, Mehmet Sait Okan

Intranasal supernumerary tooth in a child: a case report 731
Yoon Seok Choi, Yong-Dae Kim, Chang Hoon Bae, Hyung Gyun Na

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Review

Health insurance status and outcomes in children, adolescents, and young adults: a systematic review and meta-analysis

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ABSTRACT

Background. The impacts of health insurance status on survival outcomes in children, adolescents, and young adults (aged 0-39 years) with malignant tumors have not been addressed in depth. The present study aimed to identify significant relationships of health insurance condition with overall survival or all-cause mortality among children (age 0–14 years) and adolescents and young adults (AYAs, age 15–39 years) with malignant tumors.

Methods. PubMed, Wiley Cochrane Central Register of Controlled Trials, Econlit, CINAHL, Web of Knowledge, PsychInfo, Business Source Premier, ProQuest Dissertation & Theses Database, and SCOPUS were systematically searched from inception to February 29, 2020 with no language restriction. All related articles comparing the effect of health insurance status on the risk of overall survival and the risk of all-cause mortality in malignant conditions affecting children and AYAs were identified. Pooled risk ratios (RRs) and 95% confidence intervals (CIs) were computed using a random- or fixed-effect model as per the heterogeneity evaluated using Cochran's Q and I^2 statistics.

Results. Fourteen studies including 149,680 individuals were selected for this meta-analysis. The pooled RR for all-cause mortality with insurance versus without insurance was 0.78 (95%CI, 0.71–0.86; I^2=33.7%). Among the insurance types, patients with private insurance presented with a lower all-cause mortality (RR 0.70, 95% CI 0.60–0.82), with considerable heterogeneity (I^2=83.3%).

Conclusions. The findings of this review suggest that a lack of or insufficient insurance is related to all-cause mortality of AYAs with malignant cancers. Strategies aimed at identifying causality and reducing disparities are warranted.

Key words: children, adolescents and young adults, malignancy, health insurance, survival analysis.

The survival of malignancy-affected children[1] and adolescents and young adults (AYAs, aged 15–39 years)[2] has largely improved in the past 50 decades due to the remarkable progress in medicine, including diagnostics, pharmacology, combined treatments and techniques. Leading causes of death among all mortality reasons in this age group vary as a function of age, sex, and Socio-demographic Index (SDI) status, a composite indicator of development status generated for the Global Burden of Diseases, Injuries, and Risk Factors (GBD).[3] A data from the 2004 GBD[4] found that traffic accidents were the largest cause in both sexes, and maternal conditions were a leading cause of female deaths. Nevertheless, in data from the 2013 GBD with age range from 1 to 24 years, it was found that neoplasms had become the major cause of death in 1-9 year olds of both sexes, and in 10- to 24-year-old females in

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Malaysia[5], although the major cause of death in 10- to 24-year-old males remained road traffic injuries. Meanwhile, malignancy remains the dominant cause of disease-linked death among these age groups in most studies.[6-8] These populations do not benefit equally from these advances, owing to discrepancies in the age of clinical occurrence, malignancy type, disease stage (in solid tumor), anatomical position, geological region, or variations within regions. Discrepancies are particularly evident in resource-restricted settings, but also apply to socioeconomic groups in developed countries, although to a lesser but detectable degree.[9] In undeveloped countries, survival rates of cancer-bearing AYAs and children are dismal and lower than 10%.[10] In high-income countries, the survival rates of AYAs with cancer have improved, while the survival of younger pediatric patients and older adult patients have deteriorated because of inaccessibility to medical care and health insurance, as well as disparities in race/ethnicity and neighborhood socioeconomic status (SES).[11] American AYAs are the least likely to be covered by health insurance, and they tend to choose no insurance or public insurance.[12] Recent research implies that insurance deficiency is positively related to delayed diagnosis and later stage (and less treatable) disease in children and AYAs with cancer.[13] Insured young adults tend to undergo definitive cancer therapy and are therefore, less likely to die.[13] Additionally, lower neighborhood SES, minority race/ethnicity, public or deficient insurance, and other sociodemographic factors are all related to a greater risk of death[14] among AYAs, in addition to delayed diagnoses and undertreatment.[13]

However, the effect of health insurance on the risk of mortality in cancer-bearing AYAs (age 15–39 years) and children (age 0–14 years) has not been clearly described because of inconsistent findings.[15,16] In particular, some undetected confounders may have been neglected by model adjustment and contributed to bias. Given these concerns, we aimed to verify these findings through a systematic review.

Material and Methods

Search scheme and inclusion criteria

This study adheres to the PRISMA statement standards of quality for reporting systematic reviews. Two independent investigators searched PubMed, Wiley Cochrane Central Register of Controlled Trials, Econlit, CINAHL, Web of Knowledge, PsychInfo, Business Source Premier, ProQuest Dissertation & Theses Database, and SCOPUS with starting and ending date from inception to February 29, 2020 and with no language restriction. The combinations of terms searched included: 'insurance', 'Medicaid', 'Medicare', or 'cooperative medical scheme' and 'infant', 'child*', 'adolescent', 'youth*', 'puberty', 'prepuberty*', 'pediatric*', or 'paediatric*' and 'cancer', 'oncolog*', 'neoplas*', 'carcinom*', 'tumor*', 'malignan*', 'tumour*', 'leukemi*', 'lymphom*', 'sarcom*', 'osteosarcoma', 'nephroblastom*', 'neuroblastoma', 'rhabdomyosarcoma', 'teratom*', 'hepatom*', 'hepatoblastom*', 'medulloblastom*', 'retinoblastom*', 'meningiom*', or 'gliom*' and 'mortality', 'mortalit*', or 'survival'. The titles and abstracts of papers as-searched were reviewed. The search scheme was elaborated in Supplementary Information. The references of these papers were hand-searched using the snow-ball technique to ensure no potential articles were missed. Disagreements were resolved through mutual consensus. When detailed information needed for the analysis was unavailable, the original authors were contacted through e-mail to obtain the missing information. All authors agreed upon the final selection of included studies.

The inclusion criteria were as follows: (i) observational studies (cohort or registry), (ii) provision of endpoint for overall survival or all-cause mortality in malignancy patients aged 0–39 years with different health insurance status, and (iii) report of effect estimates: hazard ratio (HR), relative risk (RR), or Odds ratios (OR) and available relevant raw data for re-calculation.

The exclusion criteria were: (i) case report, comment, editorial, letter, quasi-experiment (non-random subject assignment), or unpublished study and (ii) abstract or conference proceeding. Of two or more articles from the same team or organization, only the latest publication or the report with the largest sample size was selected.

Data isolation and quality assessment

Two investigators independently extracted all information of interest in a standardized form, including the study design, name of first author, title, country, publication year, follow-up duration, endpoints, sample size, adjustment level, mean age, gender, analysis strategy (statistical models and adjustment factors), and effect magnitude, including HRs, RRs, or ORs, as well as relevant raw data for re-calculation.

Study quality was evaluated by two independent investigators using the *Effective Public Health Practice Project Quality Assessment Tool for Quantitative Studies*.[17] Any inconsistencies were addressed via discussions. This quality assessment tool rates the study procedures as 'strong', 'moderate', or 'weak' using eight scales (selection bias, study design, confounders, blinding, data collection methods, withdrawal/dropouts, intervention integrity, and analyses). We scored a paper as overall 'strong' or 'high quality' if no 'weak' item score existed and if at least four of the eight items were 'strong'. A paper of overall 'moderate quality' was one with only one 'weak' item score, with otherwise only 'strong' and 'moderate' item scores. The remaining studies were rated as 'weak' or 'low quality' overall.

Statistical analyses

The primary outcome measure was overall survival (freedom from all-cause mortality)[18] or all-cause mortality. Dichotomous outcomes were synthesized using HRs, RRs, or ORs, with 95% confidence interval (CIs). The percent of between-study variability due to between-study heterogeneity was estimated using I^2 statistic[19] and was classified as high, modest, and low with I^2 ≥50%, <50%, and <25%, respectively. A CI for I^2 was identified using the iterative non-central chi-square method.[20] RRs were converted to natural logarithms, and logRRs and standard errors were pooled by DerSimonian and Laird's approach in a random- or fixed-effect model as per the heterogeneity evaluated by Cochran's Q and I^2 statistics.

One-study-removed analysis was also performed to test sensitivity. Regarding the a-priori discrepancy of all-cause mortality, we conducted subgroup analyses by age (0–21 years, 15–39 years, and 0–39 years) according to the patient population of the enrolled studies. Publication bias was quantified by Egger's test (regression asymmetry) and Begg's test (rank correlation). All analyses were conducted using Stata statistical 15.0 (Stata Corp LP), at the α level of 0.05. P was set as two-sided. The 95% CI with null '1' indicated no clinical significance even if P<0.05.

Results

Study selection, characteristics, and quality assessment

The initial search found 3867 potentially feasible articles, and after title and abstract screening, 52 articles were retrieved for full-text assessment. Finally, 14 studies[11,14-16,21-30] were included (Fig. 1). Of the 14 studies, the dates of publication were between 2009 and 2019, the sample size varied between 19 and 80,855 patients, and there were four resources. Four studies were conducted among children, six among AYAs and four included both groups. Patient demographics, tumor characteristics, and treatments are shown in Table I.

Eleven of the 14 identified programs were assessed as moderate via the global rating, three as weak, and none as strong (Table II). Study design and confounders were the main weaknesses.

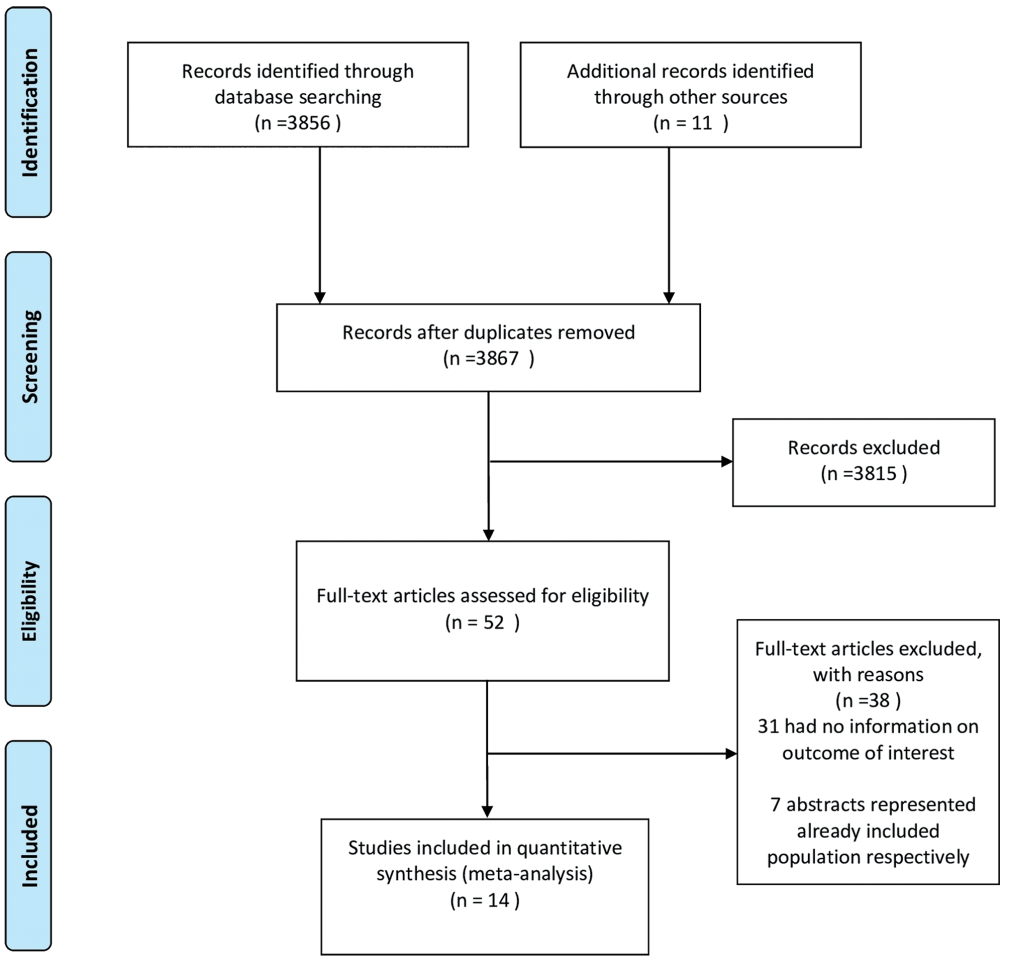

Fig. 1. Flow-diagram of study selection for the systematic literature review.

Effect of health insurance status on all-cause mortality in malignancy in patients aged 0–39 years

In 10 studies which examined the relationship between insured and uninsured patients, the pooled RR of all-cause mortality for insured versus uninsured was 0.78 (95% CI, 0.71-0.86; p <0.001) in a fixed-effects model without heterogeneity (I^2=33.7%, p=0.138; Fig. 2). A stratified analysis by contingency revealed the presence of positive outcome regarding all-cause mortality in studies among children and AYAs (HR 0.76, 95% CI 0.68-0.84; p <0.001; Fig. 3). Since Egger's test showed evidence of publication bias (p=0.012, Fig. 4), rather than replacing potential missing data, we performed a trim-and-fill sensitivity analysis and found basically similar results.

In 10 studies reporting the relationship between private and nonprivate insurance, the pooled RR of all-cause mortality for private insurance versus nonprivate insurance was 0.70 (95% CI 0.60 to 0.82; p <0.001) in a random-effect model with severe heterogeneity (I^2=83.3%, p <0.001; Fig. 5). Heterogeneity was analyzed via the sensitivity test. However, heterogeneity remained after the exclusion of single studies. The funnel plots showed evidence of systematic bias in the analysis of all-cause mortality (Begg test, p=0.21; Egger's test, p=0.005; Fig. 6). In the exploration of possible publication bias via the trim-and-fill approach, we did not substitute the probable missing data and found generally identical results.

Table I. Detailed demographic characteristics of studies included in the meta-analysis.

Study	Region	Study design	Baseline years	Sample size	Age (years)	Sex (% female)	Malignancy	Malignancy Stage	Data Resource
Kent et al (2009)	American	Retrospective Cohort	1996–2005	7,688	~39	42.1	Leukemia	NR	CCR
Fintel et al (2015)	American	Retrospective Cohort	1973-2010	574	18~30	36.0	ALL	NR	SEER
Abrahäo et al (2016)	American	Retrospective Cohort	1988-2011	3,935	~39	46.5	AML	NR	CCR
Akhavan et al (2015)	American	Retrospective Cohort	1998-2011	3,658	~30	51.4	Renal cell carcinoma	NR	NCDB
Keegan et al (2015)	American	Retrospective Cohort	1988-2010	16,827	15~39	82.8	thyroid cancer	NR	CCR
Keegan et al (2016)	American	Retrospective Cohort	1988-2011	9,353	15~39	48.8	Hodgkin lymphoma	NR	CCR
Lee et al (2017)	American	Retrospective Cohort	1998–2012	3,295	15~39	42.5	Rectal Cancer	NR	NCDB
DeRouen et al (2017)	American	Retrospective Cohort	2001–2011	80,855	15~39	59.8	Cancer[a]	NR	CCR
Martijn et al (2017)	Kenya	Retrospective Cohort	2010-2012	63	~16	29.0	non-Hodgkin's lymphoma	NR	MTRH
Garner et al (2017)	American	Retrospective Cohort	1998-2012	9,585	~21	82.6	Thyroid Cancer	None insurance: stage I57.3%; stage II42.7% Government insurance: stage I59.3%; stage II40.7% Private insurance: stage I69.1%; stage II30.9%	NCDB
Njuguna et al (2017)	Kenya	Retrospective Cohort	2010-2012	39	~16	51	Wilms Tumor	NR	MTRH

HR: adjusted hazard ratio, CI: confidence interval, OR: odds ratio, NR: Not Reported, OS: overall survival, ASM: all-cause mortality, NHIF: National Hospital Insurance Fund, ALL: Acute Lymphoblastic Leukemia, AML: acute myeloid leukaemia, CCR: California Cancer Registry, NCDB: National Cancer Database, MTRH: Moi Teaching and Referral Hospital, NOS: not otherwise specified, CNS: central nervous system, ICCC: International Classification of Childhood Cancer

[a] including breast cancer, thyroid cancer, melanoma, testicular cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, leukemia, cervical cancer, colorectal cancer, sarcoma, central nervous system cancers and ovarian cancer;

[b] [1]denoted as COX HR, reported in paper; [2]denoted as extracting HR from Kaplan–Meier curves

Table I. Continued.

Study	Region	Study design	Baseline years	Sample size	Age (years)	Sex (% female)	Malignancy	Malignancy Stage	Data Resource
Bownes et al (2018)	American	Retrospective Cohort	1998-2012	3,125	15~39	100	ovarian germ cell tumors	None insurance: stage I56.1%; stage II10.0%; stage III25.8%; stage IV8.1%	NCDB
								Government insurance: stage I59.2%; stage II5.6%; stage III27.4%; stage IV7.8%	
								Private insurance: stage I63.8%; stage II8.4%; stage III24.4% ; stage IV3.5%	
Penumarthy et al (2020)	American	Retrospective Cohort	2000-2015	1,106	~39	41.7	bone and soft tissue sarcomas	Low-income public insurance: local 36.0%; regional 26.9%; metastatic 21.3%; unknown 15.9%	CCR
								Private insurance: local 45.4%; regional 24.2%; metastatic 12.5%; unknown 17.8%	
Mitchell et al (2020)	American	Retrospective Cohort	2000-2015	9,577	~19	45.7	central nervous system tumours	NR	SEER

HR: adjusted hazard ratio, CI: confidence interval, OR: odds ratio, NR: Not Reported, OS: overall survival, ASM: all-cause mortality, NHIF: National Hospital Insurance Fund, ALL: Acute Lymphoblastic Leukemia, AML: acute myeloid leukaemia, CCR: California Cancer Registry, NCDB: National Cancer Database, MTRH: Moi Teaching and Referral Hospital, NOS: not otherwise specified, CNS: central nervous system, ICCC: International Classification of Childhood Cancer

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[b] [1]denoted as COX HR, reported in paper; [2]denoted as extracting HR from Kaplan–Meier curves

Table I. Continued.

Study	Observation group vs. control group	Survival Rates(%)	Outcome	Effect estimates[b]
Kent et al (2009)	None/unknown insurance vs. Any insurance	NR	OS	OS:HR[1](95%CI): 1.31 (1.16–1.47)
Fintel et al (2015)	Insured vs. Uninsured	Insured: 61 Uninsured:50	OS	OR(95%CI):1.60(0.98-2.63)
Abrahäo et al (2016)	Unknown/NOS vs. Private; Uninsured vs. Private; Public vs. Private	None insurance:37.9 Public insurance:43.8 Private insurance:46.5 Unknown/NOS:37.1	OS	Unknown/NOS vs. Private, HR[1](95%CI):1.27(1.07-1.51); Uninsured vs. Private, HR[1](95%CI):1.34(1.01-1.78); Public vs. Private, HR(95%CI):1.05(0.93-1.19)
Akhavan et al (2015)	Government vs. Private Insurance; Uninsured vs. Private Insurance;	NR	ASM	Government vs. Private Insurance, HR[1](95%CI):2.64(1.34-5.20); Uninsured vs. Private, Insurance, HR[1](95%CI):2.77(0.62-12.50)
Keegan et al (2015)	Public insurance/no insurance/unknown vs. Private/ military insurance	Private/military insurance:99.4 Public insurance/no insurance/ unknown:98.3	OS	OS:HR[1](95%CI): 2.56 (1.39–4.71)
Keegan et al (2016)	Public insurance/no Insurance vs. Private/military insurance; unknown vs. Private/military insurance	Private/military insurance:94.9 Public insurance/no insurance:88.4 unknown:93.9	OS	Public insurance/no Insurance vs. Private/military insurance:HR[1](95%CI):2.05 (1.58–2.66); Public insurance/no unknown vs. Private/military insurance, OS:HR[1](95%CI): 1.25 (0.70–2.24)
Lee et al (2017)	No insurance vs Private; Medicaid/Medicare/ Government vs. Private	NR	OS	No insurance vs. Private, HR[1](95%CI): 1.71 (1.08–2.70); Medicaid/Medicare/Government vs. Private, HR[1](95%CI):1.86 (1.33–2.59)

HR: adjusted hazard ratio, CI: confidence interval, OR: odds ratio, NR: Not Reported, OS: overall survival, ASM: all-cause mortality, NHIF: National Hospital Insurance Fund, ALL: Acute Lymphoblastic Leukemia, AML: acute myeloid leukaemia, CCR: California Cancer Registry, NCDB: National Cancer Database, MTRH: Moi Teaching and Referral Hospital, NOS: not otherwise specified, CNS: central nervous system, ICCC: International Classification of Childhood Cancer

[a] including breast cancer, thyroid cancer, melanoma, testicular cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, leukemia, cervical cancer, colorectal cancer, sarcoma, central nervous system cancers and ovarian cancer;

[b] [1]denoted as COX HR, reported in paper; [2]denoted as extracting HR from Kaplan–Meier curves

Table I. Continued.

Study	Observation group vs. control group	Survival Rates(%)	Outcome	Effect estimates[b]
DeRouen et al (2017)	Public/uninsured vs. Private/military; Unknown vs Private/military	15-24 yeas: Private/military 97.7; Public/none 94.1 24-34 years: Private/military 94.5; Public/none 88.5 35-39 years: Private/military 93.4; Public/none 88.8	OS	Public/uninsured vs.Private/military,HR[1](95%CI): 1.57 (1.36–1.80) Unknown vs Private/military,:HR[1](95%CI): 1.22(1.11–1.34)
Martijn et al (2017)	NHIF vs. No NHIF	NR	ASM	OR(95%CI):0.29(0.03-2.51)
Garner et al (2017)	None vs. Priviate Government vs. Priviate	NR	OS	None vs. Priviate, HR[2](95%CI):2.05(0.12-34.98) Government vs. Priviate, HR[2](95%CI):2.32(0.14-37.63)
Njuguna et al (2017)	NHIF vs. No NHIF	NR	ASM	OR(95%CI):1.2(0.27-5.4)
Bownes et al (2018)	Government vs No insurance; Priviate vs No insurance	NR	OS	Government vs. No insurance, HR[1](95%CI):. 0.82 (0.41–1.01); Priviate vs No insurance, HR[1](95%CI): 0.70 (0.33–1.18)
Penumarthy et al (2020)	Low-income public Insurance vs. Private insurance	Low-income public insurance: 49 Private insurance:63	OS	HR[1](95%CI): 1.27 (1.02–1.57)
Mitchell et al (2020)	Insured (Medicaid) vs. Insured (Private); Insured (unknown type) vs. Insured (Private); No insurance vs. Insured (Private); Unknown vs. Insured (Private)	Insured (private): 76.1 Insured (Medicaid):70.3 Insured (unknown type):80.9 No insurance:76.0	OS	Insured(Medicaid) vs. Insured (Private), HR[1](95%CI): 1.01(0.87–1.16); Insured (unknown type) vs Insured (Private), HR[1](95%CI): 0.82(0.66–1.02); No insurance vs Insured (Private);HR[1](95%CI): 0.97(0.61–1.53); Unknown vs Insured (Private),HR[1](95%CI): 1.36(0.94–1.96)

HR: adjusted hazard ratio, CI: confidence interval, OR: odds ratio, NR: Not Reported, OS: overall survival, ASM: all-cause mortality, NHIF: National Hospital Insurance Fund, ALL: Acute Lymphoblastic Leukemia, AML: acute myeloid leukaemia, CCR: California Cancer Registry, NCDB: National Cancer Database, MTRH: Moi Teaching and Referral Hospital, NOS: not otherwise specified, CNS: central nervous system, ICCC: International Classification of Childhood Cancer

[a] including breast cancer, thyroid cancer, melanoma, testicular cancer, non-Hodgkin lymphoma, Hodgkin lymphoma, leukemia, cervical cancer, colorectal cancer, sarcoma, central nervous system cancers and ovarian cancer;

[b] [1]denoted as COX HR, reported in paper; [2]denoted as extracting HR from Kaplan–Meier curves

Table II. Ratings of methodological quality by effective public health practice project quality assessment tool.

Study	Selection bias	Design	Confounders	Blinding	Data collection methods	Withdrawals and drop-outs	Intervention integrity	Analyses	Global rating*
Kent et al. (2009)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
Fintel et al. (2015)	Strong	Weak	Weak	Moderate	Strong	Strong	Strong	Strong	Moderate
Abrahäo et al. (2015)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
Akhavan et al. (2015)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
Keegan et al. (2015)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
Keegan et al. (2016)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
Lee et al. (2017)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
DeRouen et al. (2017)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
Martijn et al. (2017)	Weak	Weak	Weak	Moderate	Strong	Strong	Strong	Strong	Weak
Garner et al. (2017)	Strong	Weak	Weak	Moderate	Strong	Strong	Strong	Strong	Weak
Njuguna et al. (2017)	Weak	Weak	Weak	Moderate	Strong	Strong	Strong	Strong	Weak
Bownes et al. (2018)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
Penumarthy et al. (2020)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate
Mitchell et al. (2020)	Strong	Weak	Strong	Moderate	Strong	Strong	Strong	Strong	Moderate

*: strong: no weak ratings, moderate: one weak rating, weak: two or more weak ratings.

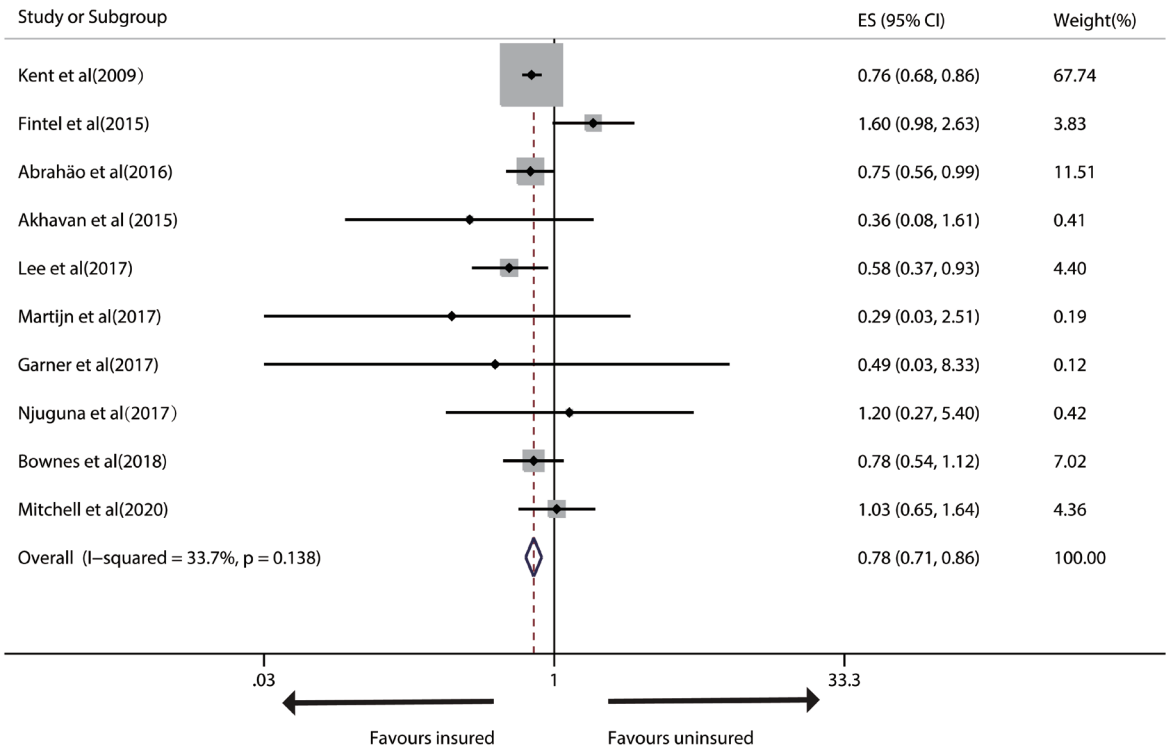

Fig. 2. Forest plot of the risk of all-cause mortality between insured and uninsured cancer patients aged 0-39 years.

Discussion

This systematic review found that uninsured malignancy patients, aged 0–39 years, relative to private insurance, had a higher risk of all-cause mortality. Many factors (such as disease stage and delayed diagnosis) related to healthcare availability may have modulated the association between deficient or public insurance and survival,[31] which in turn resulted in a later stage diagnosis (and resulting in the cancer being untreatable), delays, lower therapy reception, termination,[13] Medicaid service inhibitions, or limited accessibility of information and support services among patients and survivors.[32] The AYA Health Outcomes and Patient Experience research found that insurance shortage was related to a lower quality of life among new patients.[33] Similarly, contacts with health care services may promote adherence to therapy and follow-up instructions, which are factors that were reportedly connected with poor outcomes among AYAs.

However, the race and SES differences in survival identified among privately insured AYAs imply that broadening insurance coverage alone may not eliminate all outcome differences. Insurance coverage has often been shown to be a key modulator of race and SES differences in survival. This is supported by recent studies, including ours, on relationships between no or public insurance and cancer outcomes among AYAs after race and SES adjustments.[34] Our results verified the significance of insurance coverage, and suggested that considerable race and SES disparities still exist even among those with private insurance. Our results agree with recent findings that severe financial stress is related to a cancer diagnosis for those who are relatively young in age, lower SES, or non-white race/ethnicity, irrespective of insurance conditions.[35] For those with private insurance, financial stress can originate from out-of-pocket costs related to co-payments, co-insurance, deductibles, and out-of-network costs, that may dictate treatment plans and termination, and

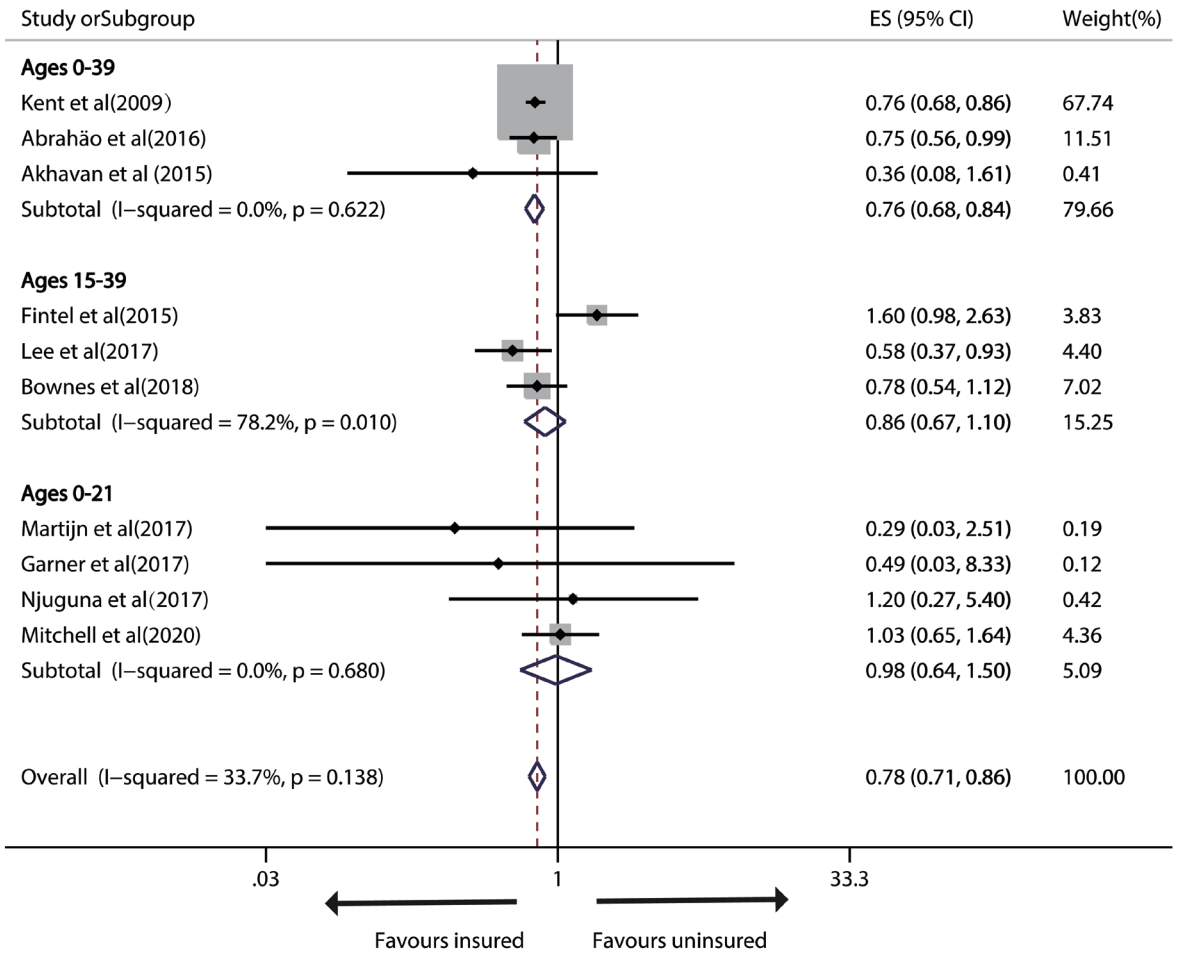

Fig. 3. Forest plot of the risk of all-cause mortality between insured and uninsured cancer patients aged 0-39 years, according to study population.

general well-being.[36] For instance, for all age groups, African Americans and low-SES citizens are less likely to undergo standard medical therapy than their Caucasian counterparts, even when they have the same insurance coverage. This disparity has been noted by other studies on specific cancer care.[37]

Race bias may affect care use (regardless of insurance condition), and chronic burden due to bias affects health outcomes.[38] Severe racial/ethnic differences for cancers resulting in some groups being more prone to avoidance of therapy indicate that disparities in socioeconomic resources (social capital) may contribute to and intensify the residual racial/ethnic disparities in cancer survival.[39] Financial concerns among minorities and low-SES groups, even with private insurance, may critically contribute to racial/ethnic and SES differences in therapy and therefore, survival. Moreover, biological divergences in cancer subtypes are probably related to some cancers. For example, African-American AYAs are more susceptible to breast cancer of specific molecular subtypes, and these are related to more adverse prognoses.[40] In addition, when considering molecular subtypes and insurance types in survival models, these decrease the relationship of a certain race/ethnicity with a severe risk of death.[41]

Our analyses have some notable limitations. First, we did not examine the roles of specific sociodemographic characteristics (e.g., income, education, and access to medical care). There may also have been some undetected

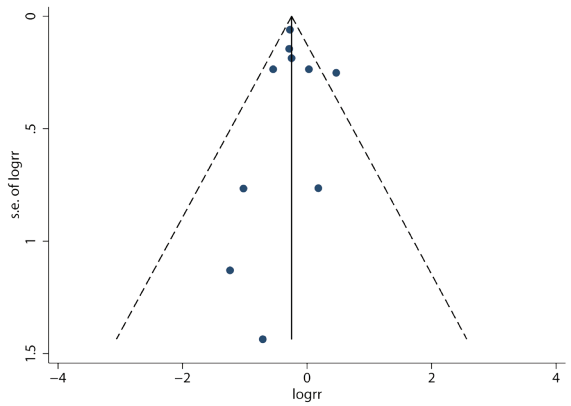

Fig. 4. Funnel plot of the risk of all-cause mortality between insured and uninsured cancer patients aged 0-39 years.

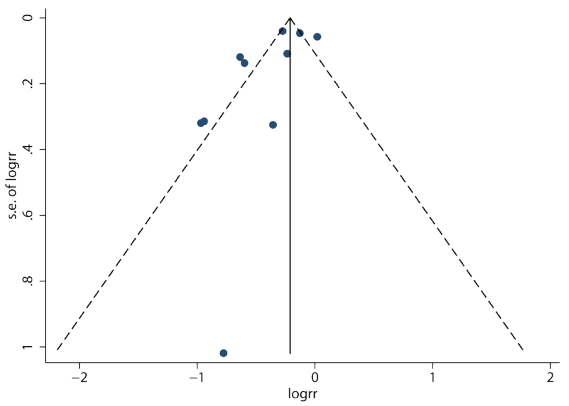

Fig. 6. Funnel plot of the risk of all-cause mortality between private and nonprivate insurance cancer patients aged 0-39 years.

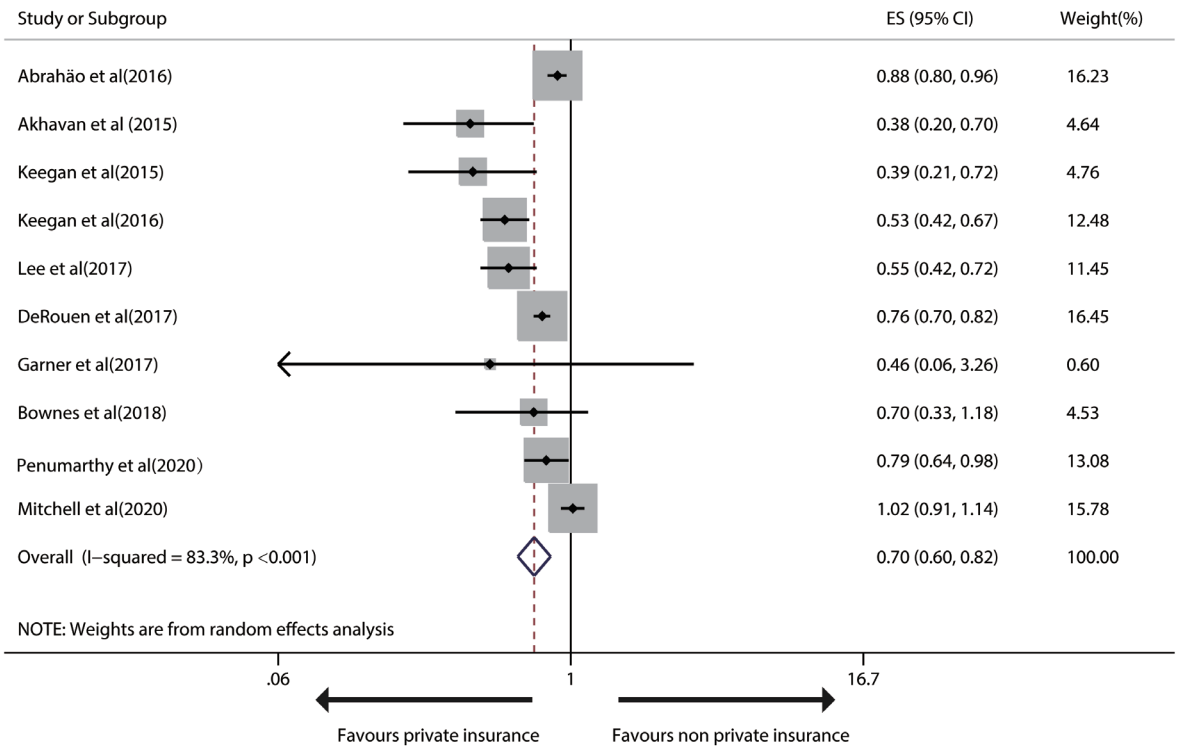

Fig. 5. Forest plot of the risk of all-cause mortality between private and nonprivate insurance cancer patients aged 0-39 years.

confounding factors that we did not account for in our findings. Second, we ignored therapeutic indices, which were often inconsistent and/or incomplete. This omission is likely to weaken our findings because we cannot clarify the extent to which the divergences in survival may be induced by differences in treatment (which are also probably related to insurance coverage). Third, we were unable to detect causality in a cross-sectional analysis and there were uncertainties regarding whether deficient or private insurance may lead to delayed diagnoses. Fourth, since insurance status was reported at diagnosis, we cannot explain the

changes in insurance status over the follow-up period. As a result, uninsured patients may be misclassified as Medicaid patients at baseline, which may lead to a lower observed survival rate among Medicaid patients.[42] We are not inferring that Medicaid is less 'protective' than private insurance. Rather, our concern is that any shortage of insurance upon diagnosis may be related to a higher risk and that patients coded as 'Medicaid-insured' may actually be uninsured before diagnosis. Fifth, any survival superiority for privately insured patients may be ascribed to lead-time bias, which was suggested as a reason for survival differences by insurance conditions among adults.[42] For instance, the lead-time of later diagnoses may give a false impression of longer survivals among privately insured patients than among uninsured or Medicaid patients. In addition, as anticipated in any systematic review, the cohort studies demonstrated remarkable heterogeneity for all-cause mortality between uninsured and privately insured patients. Moreover, Egger's test uncovered a potential publication bias, which was difficult to identify. These results suggest that we may have exaggerated the exact effect if some studies, such as abstracts or conference proceedings being a potential resource for grey literatures were excluded.

In conclusion, results from our systematic review suggest that limited or deficient insurance is heavily related to all-cause mortality in AYAs with malignancy. Strategies aimed at identifying causality and reducing disparities are warranted.

Acknowledgements

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SH, CH; data collection: HH; analysis and interpretation of results: CH, LJ; draft manuscript preparation: CH, HL. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no competing interest.

Supplementary information is available at:

http://www.turkishjournalpediatrics.org/uploads/turkjped.2021.04.001.S1.pdf

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Original Article

IgE mediated food allergy in Turkey: different spectrum, similar outcome

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ABSTRACT

Background. Food allergies (FAs) potentially differ across cultures.

Methods. All medical records of 534 children and adolescents with IgE-mediated FA over a 5-year period were reviewed to document the regional characteristics with regard to spectrum and outcome.

Results. According to their last visit, the most common FAs were tree nuts (TNs) (52.4%), cow's milk (27.3%), seeds (24.7%), egg white (23.2%) and peanuts (14.9%). Hazelnut and Anacardia nuts were the most common etiologies for TN allergies, whereas lentils and chickpeas for legumes and sesame and pumpkin seed for seeds were most common, respectively. TN allergy was in first place in school-age children (55.3%) and adolescents (57.1%) while in the second place in preschoolers (57.7%) after egg white (60%). Of these 534 children, 59.2% had at least one resolved FA (mainly egg white, cow's milk) and 21% had no residual FA during the study period. Emerging FAs (fish, shellfish, fruit, TN and seed) after the age of 3 years was reported in 94 children. The prevalence of current asthma (22.3%, 38.2%, 40%) and allergic rhinitis (11.6%, 45.2%, 60%) increased, while current atopic dermatitis (17.5%, 8.6%, 8,6%) decreased in preschoolers, school age children and adolescents, respectively.

Conclusions. The FA spectrum of Turkish children and adolescents differs from many regions of the world with high rates of TN (hazelnut, Anacardia nuts), seed (sesame, pumpkin seed) and lentils, and low rates of soy, peanut and seafood allergies. However, resolution, emergence and persistence of allergies and comorbidities are similar, which points to the limited role of the environment in the outcome.

Key words: allergic rhinitis, atopic dermatitis, asthma, food allergy, prevalence.

Although the prevalence of food allergy (FA) peaks in early childhood, there has been a shift in understanding from thinking of FA as a disease that passes over time to understanding that FA can have a heterogeneous natural history.[1] The natural history can range from early/late recovery to permanent disease, and is accompanied by the emergence of new allergies and atopic comorbidities. The focus on FA in early life is largely due to the early onset of the disorder and the disappearance of many FA in early childhood.[2] Persistent FA beyond infancy and toddler is common and clinical appearances may vary between age groups.[3]

The primary objective of this study is to reveal the spectrum of IgE-mediated FA in preschooler, school age children and adolescents in Turkey. Secondary objectives are to examine the relationship between FA and comorbid atopic diseases and the short-term prognosis of FA.

Material and Methods

Study population

This study is a retrospective analysis of the characteristics of IgE-mediated FAs in children aged 3-18 years at Hacettepe University Pediatric

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Allergy Department, a tertiary reference center for the entire country of Turkey. The children over 24-months old with IgE-mediated FA who had at least one visit between 1 January 2015 and 1 January 2020 were enrolled in the study. Approval was obtained from non-interventional clinical researches ethics committee

(Hacettepe University Non-interventional Clinical Researches Ethics Board GO20/398, 2020/09-43).

The diagnosis of FA was based on the following two criteria:

1. Positive skin prick test (SPT ≥ 3mm than negative control) and/or positive specific IgE (sIgE ≥ 0.35 kU/L) PLUS positive oral food challenge (OFC) or a consistent and clear-cut history of food related IgE-mediated symptoms within 2 hours after the ingestion of the culprit food in last 12-months.
2. SPT wheal diameters or sIgE levels of the culprit food suggesting clinical reactivity with > 95% positive predictive value (PPV) in last 12-months (Table I).[1,4,5] If 95% PPV is not clearly defined, the following criteria were used: SPT ≥ 8 mm and/or sIgE ≥ 15kU/L.[1,4,6]

In order to evaluate age group characteristics, groups were formed as preschoolers (3-5 years old), school-age children (6-12 years old) and adolescents (13-18 years old). The patients had several outpatient visits during the five-year period of the study. While examining the characteristics of the age groups to analyze prevalence of asthma, atopic dermatitis (AD), allergic rhinitis (AR), and food allergen groups, one patient was allowed to take part in two groups (Fig. 1). The analyses except the ones which depend on the age group distribution were made according to "current" (the food allergies and atopic diseases at last visit as "current FA", "current asthma" and "current AD") or "ever" status (the patients' resolved and/or current IgE-mediated food allergies and atopic diseases as "FA ever", "asthma ever", and "AD ever").[7]

In the study, tree nuts (TN) were defined as almond, hazelnut, walnut and Anacardia nuts (cashew and pistachio), legumes included lentils, peas, chickpeas and soy, seeds consisted of sesame, poppy seed, sunflower seed and pumpkin seed. Peanuts were individually analyzed, not included to the TN or legumes groups.

Resolved Food Allergies

The tolerance to the culprit food allergens was collected from the patients' full medical records and histories. The tolerance was determined with negative OFC test result according to PRACTALL.[8]

Comorbidities and Aeroallergen sensitization

The diagnosis of AD, AR and asthma/recurrent wheezing was made according to the international guidelines.[9-11] The age of asthma diagnosis was determined as the start of asthma

Table I. > 95% positive predictive values according to food allergen.[1,4,5]

Food	> 95% Positive predictive value	
	SPT (mm)	sIgE (kU/L)
Egg white	≥ 7	≥ 7
Cow's milk	≥ 8	≥ 15
Tree nut	≥ 8	≥ 15
Peanuts	≥ 8	≥ 15
Sesame seeds	≥ 14	
Fish	≥ 20	

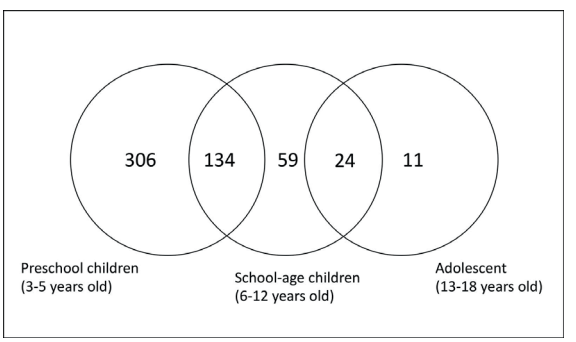

Fig. 1. Shema of the study.

controller therapy. The age of onset of AR was considered to be the age at which the patient showed signs of rhinitis when exposed to the aero-allergen that she/he was sensitive to.

Skin Prick Test, total and specific IgE measurements

SPTs were performed with culprit foods and aeroallergens from the patient's clinical history and its cross-reactive allergens as panels of aeroallergens (pollens, molds, house dust, cat and dog dander, cockroach), legumes (lentils, peas, chickpeas, peanuts), tree nuts (almonds, hazelnuts, walnuts, cashews, pistachios, sesame, peanuts) and seeds (sesame, poppy seed, sunflower seed, pumpkin seed). SPTs were applied on the volar face of forearms or upper backs of the patients and wheal size was measured after 15 minutes.[12] Total IgE and sIgE for food allergens were measured in the sera using Immuno-CAP method (Thermo Fisher Scientific, Uppsala, Sweden).

Statistical Analysis

SPSS version 22.0 statistical software package (IBM Corp., Armonk, NY, USA) was used for statistical analyses. The data including age, total IgE, absolute eosinophil count variables were not normally distributed; therefore the results were described as medians and interquartile ranges (IQR). The prevalence of all variables, including each allergen, asthma, AD, AR according to the age groups, gender, predominant initial symptoms and family history of atopy were performed using frequencies and percentages. $P < .05$ was considered significant for all analyses.

Results

A total of 534 patients (M/F: 359/175) enrolled in the study. The median ages at the diagnosis of FA and last visit were 6 months (IQR: 5 - 7.63 months) and 4.3 years (IQR: 3 - 6.9 years), respectively. The majority of the patients had an 'ever' diagnosis of AD (n=311, 58.2%) and multiple FA (≥ 2 food category) (n=364, 68.2%) (Table II).

Table II. Demographic and clinical features of patients.

Total number of patients		534
Gender (male)		359 (67.2)
Age at diagnosis of FA , months, median (IQR)		6 (5-7.63)
Cow's milk		6 (4.5-6)
Egg white		6 (5-6)
Tree nuts		12 (9-18)
Peanuts		18 (11-30)
Legumes		9 (7-13.25)
Seeds		15 (9-25)
Wheat		6 (5-8)
Fish		21 (13-28)
Shellfish		85.5 (49--)
Kiwi		25 (16.5-39.5)
Banana		41 (7.75-167.25)
Age at last visit, years, median (IQR)		4.3 (3- 6.9)
Predominant initial symptoms*		
Atopic dermatitis		216 (40.4)
Urticaria		153 (28.7)
Angioedema		45 (8.4)
Anaphylaxis		87 (16.3)
Nausea, vomiting		14 (2.6)
Diarrhea		2 (0.4)
Cough		1(0.2)
Other		16 (3.0)
Number of allergies according food groups	Current	Ever
Total tolerance	112 (21)	-
1	230 (43.1)	170 (31.8)
2	101 (18.9)	145 (27.2)
3	50 (9.4)	102 (19.1)
≥4	41 (7.7)	117 (21.9)
Atopic dermatitis	99 (18.5)	311 (58.2)
Asthma/Recurrent wheezing	154 (28.8)	196 (36.7)
Allergic rhinitis	127 (23.8)	127 (23.8)
Anaphylactic reactions after ingestion of allergic food		271 (50.7)
Family history of atopy		184 (34.5)
Total IgE, median (IQR)		181 (65.6-640)
AEC, mm^3, median (IQR)		300 (200-600)
Eosinophils, %, median (IQR)		3.9 (2.3-6.8)

Values are n (%) unless otherwise indicated
*All patients met the inclusion criteria as mentioned in Methods section.

Food Allergies at Last Visit (Current FA)

The most common FA at the last visit of patients was tree nut allergy (n=280, 52.4%) followed by cow's milk (n=146, 27.3%), seeds (n=132, 24.7%), egg white (n=124, 23.2%) and peanuts (n=80, 15%) allergies (Figs 2-4). Many patients with TN allergy suffered from multiple TN (≥ 2 TN) allergies (n=202, 72.1%). Among tree nuts, legumes and seed groups, the most common food allergens were hazelnuts, lentils and sesame, respectively (Fig. 3A, 3B, 3C). None of the patients had soy allergy.

Food Allergies and Comorbid Atopic Diseases According to Age Groups

Preschool children (3 - 5 years old)

Most of the patients had egg white allergy (n=264, 60%) followed by TN (n=254, 57.7%) and cow's milk (n=216, 49.1%) (Fig. 2A, 2B). The detection of FA in > 90% of preschool children with any kind of FA necessitated testing with 3 foods: egg white (60%) plus hazelnuts (sum = 83.5%) plus cow's milk (sum = 96.4%).

Twenty- five percent of the patients (n=113) had recurrent wheezing or diagnosis of asthma. Ninety-eight out of 113 (86.7%) patients were receiving asthma controller-therapy at their last visits. Fifty-one children (11.6%) had AR and the most common aeroallergen was pollen (n=26, 51%) followed by dust mite (n=25, 49%) (Fig. 3D). Though 63.6% (n=280) of the patients had an 'ever' AD history, 17.5% (n=77) of the patients had 'current' AD at last visit (Fig. 2C).

School-age children (6-12 years old)

Tree nut allergy (n=120, 55.3%) was the most frequent FA followed by cow's milk (n=65, 30%) and egg white (n=60, 27.6%) allergies (Fig. 2A, 2B). Among the individual food groups, the most frequent allergens were hazelnuts (n=99, 45.6%), Anacardia nuts (pistachios [n=76, 35%] and cashews [n=69, 31.8%]) and walnuts (n=69, 31.8%). The detection of FA in > 90% of school children with any kind of FA necessitated testing with the same 3 foods with preschool children: hazelnuts (55.3%) plus cow's milk (sum= 79.3%) plus egg white (sum= 91%).

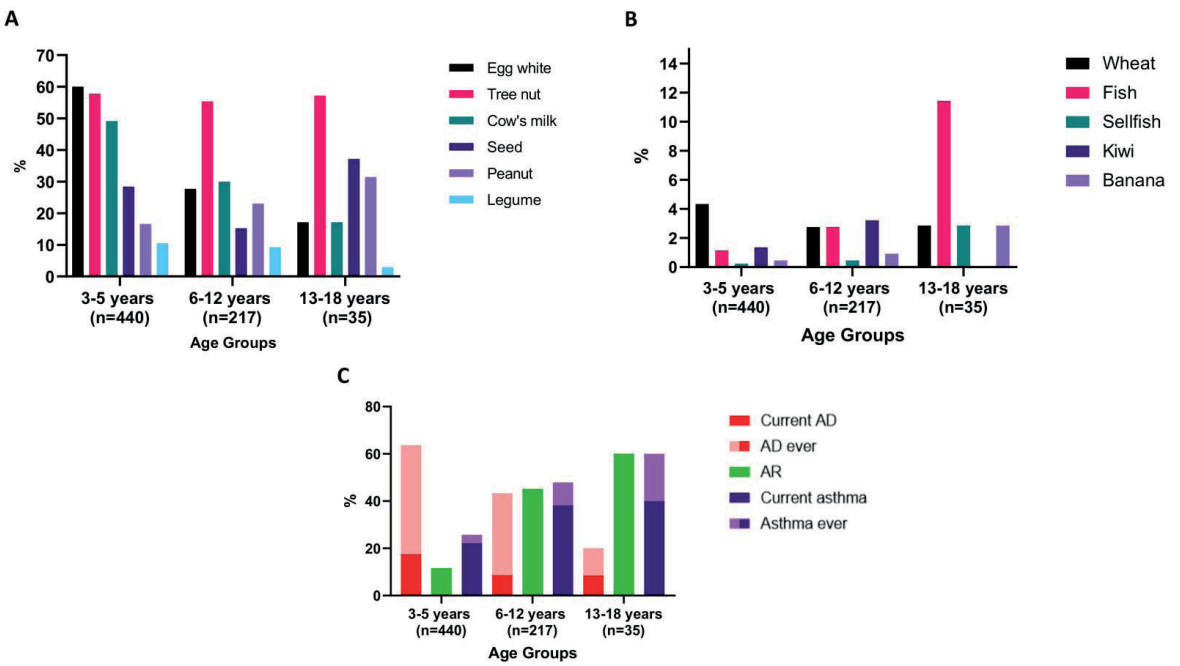

Fig. 2. Distribution of (A) most common and (B) less common food allergies, (C) current and ever atopic diseases according to age groups. AD: atopic dermatitis, AR: allergic rhinitis.

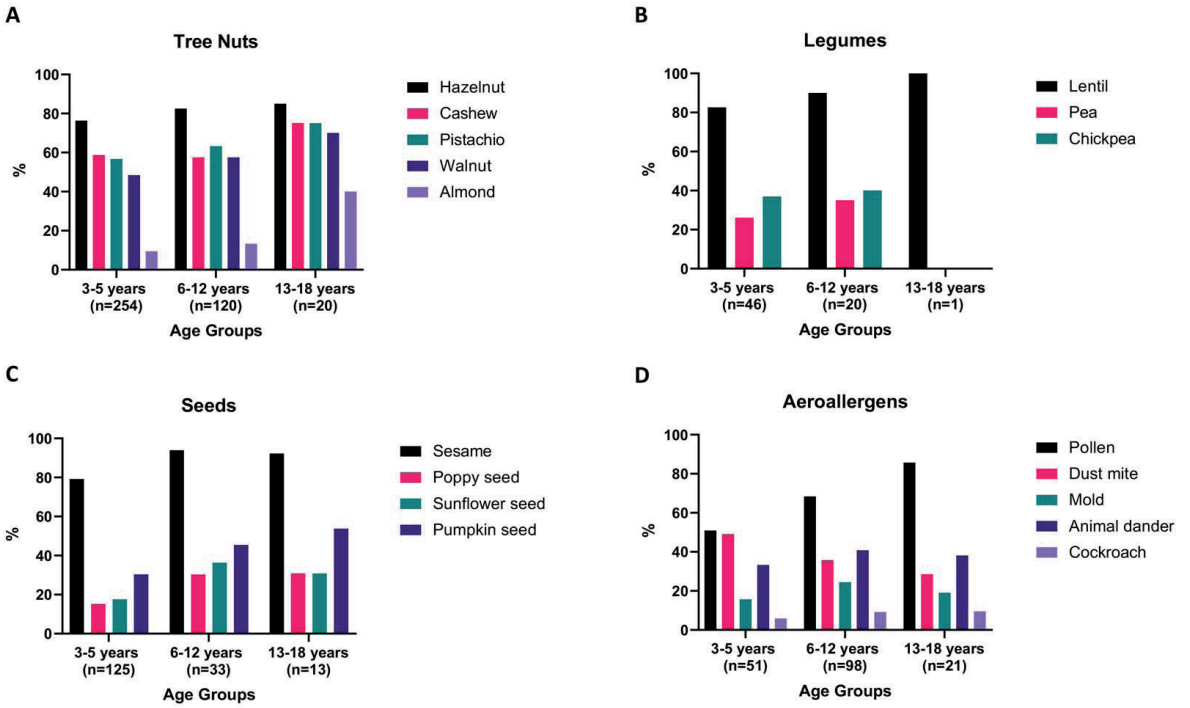

Fig. 3. Distribution of allergens by age (A) in tree nuts (B) in legumes (C) in seeds and (D) in aeroallergen sensitization.

Approximately half of the patients had asthma diagnosis (n=104, 47.9%) and AR (n=98, 45.2%) while 79.8% of the asthmatic patients (n=83) were using asthma controller therapy at their last visits. The most prevalent aeroallergens were pollens (n=67, 68.4%) and animal dander (n=40, 40.8%) (Fig. 3D). Though 43.3% (n=94) of the patients had 'ever' AD, 8.6% (n=19) of the school children had 'current' AD (Fig. 2C).

Adolescents (13-18 years old)

Tree nuts (n=20, 57.1%) were the most common food allergen followed by seeds (n=13, 37.1%) and peanuts (n=11, 31.4%) allergies (Fig. 2A, 2B). Individually, hazelnuts (n=17, 48.6%), Anacardia nuts (cashews [n=15, 42.9%], and pistachios [n=15, 42.9%]) allergies were the most frequent FAs. The detection of FA in > 90% of adolescents with any kind of FA necessitated testing with 5 foods: hazelnuts (48.6%), plus cow's milk (sum= 71.4%) plus sesame (sum=82.8%) plus egg white (sum= 88.6%) and walnuts (sum= 91.4%).

The majority of the adolescents had asthma (n=21, 60%) and AR (n=21, 60%). Most of the adolescents with asthma (n=14, 66.6%) were using any asthma-controller therapy at their last visits. Only 8.6% of the adolescents (n=3) had AD at their last visits, while 20% (n=7) of adolescents had 'ever' AD diagnosis (Fig. 2C).

Comparisons of food allergies and comorbidities by age groups

Egg white allergy frequency significantly decreased (p=0.002) in contrast to peanut allergy which increased (p=0.001) with respect to age. Current asthma and AR prevalence were higher in older children while current AD prevalence was decreasing with respect to age (Fig. 2C).

Resolved food allergies

The median follow-up period of the patients was 30.9 months (IQR:16.6-48.3 months) in the 5 year- study period. At their last visits, 316 patients (59.2%) had tolerance to at least one group of the allergens. In addition, 112 patients (21%) had tolerance to all of the foods which they previously had allergies (Table II).

According to the histories and medical records of the patients egg white (64.7%), wheat (52.6%), cow's milk (51.3%), lentils (34.6%) and fish (27.7%) allergies had higher resolution rate than those of other food allergies. Almonds (22.2%) and cashews (7.8%) had the highest and the lowest tolerance rate among TNs, respectively. In the seed group, they were sesame (21%) and sunflower seeds (3.3%), respectively. None of the patients with shellfish and kiwi allergy had tolerance to culprit foods at the last visit (Fig. 4).

Emerging food allergies

Although allergies with cow's milk, egg white and wheat have been diagnosed before the age of 3 years, some patients with seeds (n=40, 25.5%), TN (n=37, 12%), legumes (n=5, 10%), kiwi (n=5, 50%), banana (n=2, 50%), fish (n=3, 27.3%) and shellfish (n=2, 100%) allergies were diagnosed after the age of 3.

Discussion

In this study, we showed that the spectrum of food allergies differs based on age group. Egg white was the most common allergen in preschool children and the rate decreased with age whereas the majority of school age children and adolescents had TN allergy. The comparison of comorbidities based on age groups showed that the prevalence of current asthma and AR increased with age in contrast to AD.

The prevalence of individual food allergies differed significantly based on the geographic regions, the associated dietary habits and the methods of the studies. In the US, a survey analysis showed that the most frequent FA was peanuts in all age groups followed by cow's milk and TN in preschool children, shellfish and cow's milk in school-age children and adolescents.[3] A randomized telephone survey from 10 European nations indicated cow's milk allergy (38.5%) was the most frequent reported FA, followed by fruits (29.5%) and eggs (19%).[13] The meta-analysis done by EAACI Food allergy and Anaphylaxis Guidelines Group revealed that cow's milk, TN and soy allergies were the most frequent food-challenge-defined FAs in Europe.[14] In the SchoolNuts study, peanut, TN and egg were the most frequent FAs in Australian early adolescents.[15] Another study in Hong Kong Chinese preschoolers reported that the most frequent adverse food reactions were caused by shellfish, egg and peanuts.[16] In contrast to those studies, we found that most of the 3-18 years old children had TN allergy (52.4%) at last visits followed by cow's milk (27.3%) and seeds (24.7%) allergies. In addition, the frequency of peanut allergy was not as high as reported in the studies of other countries and only 2 patients (0.4%) had shellfish allergy while none of the patients had soy allergy. The differences in the distribution and prevalence

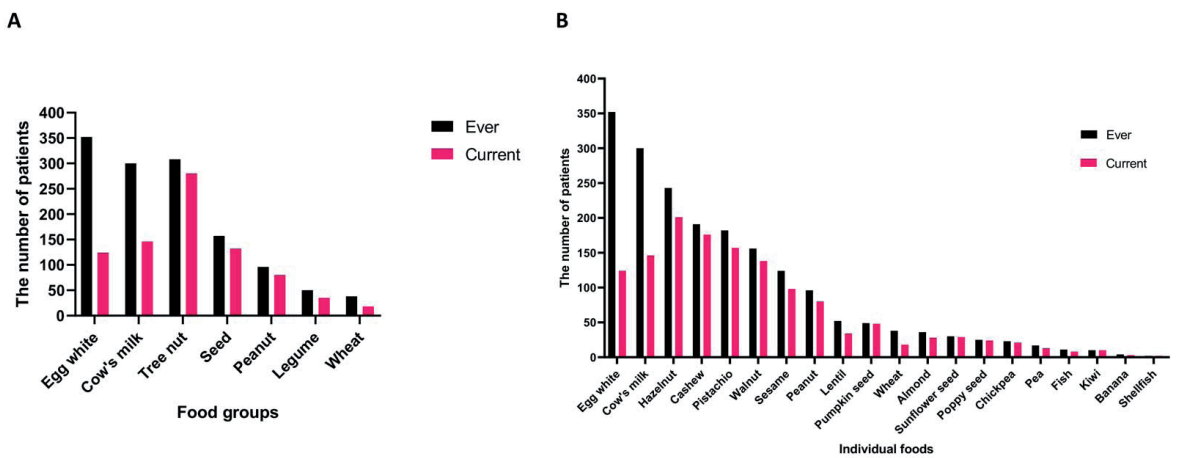

Fig. 4. Distribution of current and ever food allergies.

of FAs may arise from the culinary variations of countries and methods of the studies as self-reported or physician diagnosed. Turkey holds an important place in the production of TN in worldwide.[17] The higher rate of TN allergy in Turkey could be the result of high consumption of TN. On the other hand, peanut consumption in Turkey was not as high as in other countries that could explain the lower rate of peanut allergy in our study compared to the rates reported in the literature.[17] The recent studies reported that the prevalence of sesame allergy has been increasing in last years.[18-20] Similar to those studies, we found that sesame was one of the most important FA in adolescents. The explanation for the increase of sesame allergy may be an increased awareness of these "rare" FAs, as well as changing dietary habits. Similar to EAACI report, we found that egg white allergy was more prevalent among younger children (p=0.002), while the frequency of peanut allergy was higher among older children (p=0.001).[14] In addition, TN allergy was more common among school-age and adolescent groups. Although, seafood allergies were common in Middle East and Mediterranean areas, only 13 patients (2.4%) had seafood allergy in our cohort.[21,22]

In the previous report from Turkey which enrolled the children aged 0.1-19 years, egg white, cow's milk and hazelnuts were the most frequent FAs.[23] Orhan et al.[24], reported that beef, cow's milk and cocoa were the most frequent FA in 6-9-year-old urban schoolchildren in the eastern Black Sea region of Turkey. Moreover, the recent study from Turkey has revealed that 0-2 years age children had egg white, cow's milk and TN allergies more frequently compared to other food allergens.[20] In our cohort, egg white allergy was more common among preschool children similar to the results of previous studies, while TN allergy was the most common FA among school-age children and adolescents and none of the patients had beef or cocoa allergy. The differences in the distribution of FA from previous studies may arise from the resolution of egg white and cow's milk allergies among younger age.[25,26] Similar to our report, a food-challenged based study form Turkey showed that TN and peanuts were the most frequent FAs among adolescents.[27] In addition, we found that seed allergy, especially sesame (34.2%), had similar prevalence with peanut (31.4%) in adolescents.

Tree nut allergy spectrum also varied among studies. In the HealthNuts and SchoolNuts studies, cashews were defined as the most frequent TN allergy in the first 6 years of life and early adolescence followed by hazelnuts and pistachios, respectively.[15,28] In USA, walnuts and cashews were reported as the most common TN allergies, recently another parent-reported study showed that almonds and cashews were more common. While hazelnuts were the most frequent TN allergy in Europe, Brazil nuts, almonds and walnuts were the most common ones in the UK.[29] In our study, hazelnuts were the most frequent TN allergy followed by Anacardia nuts in all age-groups in the line of previous studies from Turkey.[30,31] Turkey supplies approximately 75% of worldwide hazelnut production and it is estimated that Turkish people rank third among nations in the consumption of hazelnuts.[17] Similarly, Turkey had an important role in the production of pistachios (in third place) and was reported as the country having the highest rate of pistachio consumption.[17] The possible reason for the high rate of hazelnut and pistachio in our study can be explained by the high consumption of these nuts. Although cashews are not frequently consumed in Turkey, because of significant co-sensitization and co-allergy with pistachios, we found that the rate of cashew allergy was as high as pistachio.[32]

In previous studies, the co-occurrence of other atopic diseases with FA has been well defined.[33] Adding to this, we reported the similar prevalence of AD, asthma and AR in children with FA. In addition, the incidence of asthma and AR was increased age although lower age for AD, in the line of previous knowledge.[34,35] These findings revealed that the frequency and natural history of comorbid allergic diseases was similar to Western countries in spite of the different spectrum of FAs.

This short-term longitudinal study documents the spectrum of food allergies while providing data on their prognosis as a secondary outcome. As the diagnosis of food allergy is not entirely based on OFC, the results differ in both OFC-based and single food-focused studies, however they are valuable to reflect daily practice. Although, it can be assumed that some allergies are only sensitization, the fact that cow's milk and hen's egg allergies have a higher tolerance rate and early age for the diagnosis compared to other allergies is compatible with the literature.[2] Similar to previous reports, we found that egg white (64.7%), wheat (52.6%), cow's milk (51.3%) allergies had higher resolution ratio than that of TN allergy (7.8% to 22.2%). We also found that the resolution rate of seed allergy (3.3% to 21%) was similar to that of TN allergy which was consistent with the previous study that reported 80% persistence of sesame allergy.[36]

In our study, the diagnosis of cow's milk, egg white and wheat allergy was made in the first 2 years of life in all patients, while some seeds, TN, legumes, fruit and seafood allergies were diagnosed after the age of 3. An older age during diagnosis can be explained either by the fact that delayed development of these allergies occurs or these patients have not encountered these foods before that age. Although the exact explanation goes beyond the scope of this study, the late onset age of these foods is compatible with the literature.[29]

The limitation of this study is that the diagnosis of FA was not totally based on OFC results. Therefore, the patients who had SPT or IgE values with the culprit food suggesting clinical reactivity with > 95% PPV were enrolled in the study to minimize the inaccuracy of the diagnosis of FA. The other limitation is that the number of patients in the adolescent group is too low to make strong conclusions. The strength of our study is that it is the first study which focuses on the FA spectrum, comorbid atopic diseases and natural prognosis in children aged 3-18 years from the Eastern Mediterranean region. The other strengths of our study are the inclusion of a large patient group, the documentation of allergy frequencies within food groups and real-life data.

In conclusion, FA spectrum of Turkish children is different from many cultures, but the outcome of allergies and comorbidities are similar pointing out to the role of the environment in the development but not in the natural history.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BEŞ; data collection: AA, MÖ, GK; analysis and interpretation of results: AA, ÜMŞ, ÖS, BEŞ; draft manuscript preparation: AA, BEŞ. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Approval was obtained from non-interventional clinical researches ethics committee

(Hacettepe University Non-interventional Clinical Researches Ethics Board GO20/398, 2020/09-43).

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Conflict of interest

The authors declare that they have no conflict of interest.

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Original Article

Enablers and barriers for enteral feeding with mother's own milk in preterm very low birth weight infants in a tertiary care neonatal intensive care unit

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ABSTRACT

Background. The management of lactation in preterm mothers is a real challenge for Neonatal Intensive Care Unit (NICU) care, providers. The study aimed to evaluate the enablers and barriers for enteral feeding with mothers' own milk (MOM) in preterm very low birth weight (VLBW) infants in a tertiary care neonatal unit.

Methods. This prospective observational study took place at a tertiary level NICU of a high-risk obstetric unit in a private hospital. All VLBW infants and mothers were incorporated into the study. Data on enablers and barriers were gathered from mother-baby dyads at the time of birth, at the end of the 7th day, and then weekly till the discharge of the baby from the unit.

Results. We studied 87 mother-baby dyads. Mean (SD) maternal age, gestation age and birth weight were 29.3 (4.7) years, 30.8 (2.0) weeks, and 1196 (196) grams respectively. We categorized our data into 2 groups based on outcome estimates done during the entire hospital stay or pre-discharge (48 hours before the discharge). On comparison of perinatal and post-natal factors, the enablers were maternal dwelling from the rural locality, number of milk expression son day 1 after the birth, number of night expressions in the first week postnatally, and MOM volume till day 3, day 7, and 2 weeks postnatally. The enablers of MOM in the pre-discharge group were the number of expressions in the first 3 days, the number of night expressions in week 1, mother's visit, and the number of maternal visits on day 1 to NICU and MOM volume expressed from day 1 until the second week after birth. The main barriers for MOM (48 hours pre-discharge) were extremely low birth weight (ELBW) and intrauterine growth-restricted infants (IUGR).

Conclusions. ELBW infants and IUGR infants are susceptible to low MOM feeding. The total of milk expressions in the first 3 days, number of night expressions in the first week, maternal visits on day 1 and the average MOM amount in the first 2 weeks are enablers for MOM feeding.

Key words: mother own milk, ELBW, IUGR, milk expression.

There are about 15 million preterm births per year worldwide. Preterm childbirth incidence has increased in the last 20 years in 62 of 65 countries with available data trends. More than 1 million children succumb to preterm birth-related complexities yearly. Preterm childbirths are the leading cause of neonatal death and the second most common cause of mortality after pneumonia for less than 5 years of age mortality rate.[1] Breastfeeding and donor human milk benefits for giving ideal nutrition and immune protection are well known.[2] It reduces the risk of respiratory infection, necrotizing enterocolitis, gastrointestinal infection, and improves neurodevelopmental outcomes. Mothers' own milk (MOM) incorporates hormones,

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growth factors, immunological factors, so it is an exhaustive nutrient source for preterm newborns. Human milk is nutritionally rich and immunologically superior so very crucial for preterm survival and has immense value in a resource-limited environment.[2,3] MOM provides optimum nutrition for both term and preterm infants. The use of human milk for preterm infants is not only nutritional but it sustains host defense, helps in the maturation of the gastrointestinal system and sensory neural development.[3,4] Mothers of preterm infants are at greater risk of low initiation of breastfeeding in contrast to term infants. The breastfeeding of term infants has been thoroughly studied and data on facilitators and obstacles are available, but preterm breastfeeding appears more complicated because of its rate and the fact that factors increasing and decreasing preterm breastfeeding have not been studied effectively when compared to term infants.[5] Breastfeeding in the term, as well as preterm, is affected by various factors like maternal, infant, social, and cultural factors. Maternal factors are sociodemographic factors, physiological aspects, earlier breastfeeding experience and support from family members whereas infant factors are birth gestation age, neonatal morbidity, and social factors are perspective of the society, culture, maternity benefits, and health care factors are staff attitude, staffing figure, advice and arrangement of the neonatal unit, which are all of importance.[6] However, very few studies are available on human milk and the factors affecting it. Recognizing the low breastfeeding rate in preterm mothers will help us understand the complicated nature of preterm breastfeeding and various factors promoting and prohibiting its uses and its outcome on preterm infants.[5]

The objective of our study was to assess enablers and barriers of favorable MOM use among preterm very low birth weight infants.

Material and Methods

A prospective observational study was carried on the preterm infants admitted to the Neonatal Intensive Care Unit (NICU) during the study period of 6 months from August 2017 to January 2018. The infant was included if they were born ≤ 34 weeks of gestation age and weighing <1500 grams at birth. The infant was excluded if they had any of the following:

- Mother admitted to ICU due to critical illness
- HIV (human immunodeficiency virus) positive mother who is reluctant to give MOM
- Infants having major congenital anomalies
- Infants depending upon surgical intervention where we must manage them nil per oral.
- Infants who died or were discharged or transferred to another hospital

The study was approved by the ethical committee of the Fernandez Hospital (reference number 24_2017). All the appropriate VLBW infants and their mothers were enrolled in the study after obtaining their informed consent.

Sample size:

A sample was obtained from the baseline phase of a QI (quality initiative) study run at Fernandez Hospital. No prior sample was estimated.

Study technique:

The data gathered consisted of the perinatal variables, milk expressions, daily proportion of enteral feeds (MOM and donor human milk (DHM)), management of breast pump and type of breast pump utilized, hours of skin to skin interaction, non-nutritive sucking, and care hours (time devoted to infant care activities) by mothers. The numbers of maternal visits, kangaroo mother care hours were also compiled prospectively. The data on enablers and barriers were gathered from the mother and family members at the time of birth, at the end of the 7th day, and weekly until the discharge of infants from the neonatal unit. We assessed 87 VLBW infants to identify the enablers and

barriers for human milk uses. The enablers and barriers assessed in this study are summarized in Table I. We recorded the volume of the milk-fed to the infants daily in the first week after birth than on the weekly basis for the total NICU stay and at the time of discharge (milk intake by the infants 48 hours before discharge from NICU) prospectively for estimation of following outcomes:

- Amount of MOM intake
- Amount of DHM
- Amount of total milk intake {amount of MOM and amount of DHM) and formula milk (FM)}

Every day from the time enteral feeding started, the amount of milk fed to the infant was prospectively recorded. For the recognition of enablers or barriers of use of MOM, very low birth weight infants with MOM intake of more than 60% of total enteral intake were regarded as infants with MOM success. We divided our infants into 2 groups as the MOM success group and the MOM failure group for assessing enablers and barriers for MOM . We counted the proportion of infants with MOM success for both during the NICU stay and at the time of discharge (48 hours before discharge from the NICU).

Outcome Measures:

Successful MOM uses for each group at the time of discharge and during the hospital stay was represented as an enteral intake of more than 60% of MOM out of the total volume of milk intake by infants.

Table I. Enablers and barriers evaluated in the study.

Maternal factors	Infant factors	Social factors
• Age	• Birth weight	• Locality of residence of mother
• Parity	• Sex	• Breast feeding awareness
• Gestation age	• Extreme low birth weight	
• Structured antenatal counselling about breast feeding	• Intrauterine growth restriction	
	• APGAR at 5 minute	
• Structured post natal counselling about breast feeding	• Respiratory distress syndrome	
	• Hemodynamically significant patent ductus arteriosus	
• Pregnancy induced hypertension	• Non nutritive sucking	
• Diabetes mellitus	• Day of initiation of NNS	
• Caesarean section	• Duration of NNS weekly in minutes	
• Milk expression		
• Time of first expression after birth		
• Mode of expression		
• number of expression in first 3 days		
• number of expression in first week		
• number of night expression in first week		
• number of weekly expression until discharge		
• Maternal visits		
• Kangaroo mother care		

NNS: Non nutritive sucking

Statistical analysis:

All the data during the study which were registered were filled in into the Microsoft XL sheet. The data was solved statistically by using statistical software SPSS version 20. All data were revealed as mean (standard deviation) and proportions where ever as relevant. The Chi-square test and student t-test were administered for all qualitative data and quantitative data respectively. A "p-value" of <0.05 was taken as significant.

Result

During the study span, a total of 93 mother-infant dyads were enrolled and 6 dyads were omitted of which five infants had died, and one was discharged against medical advice. The study outcomes were evaluated in the remaining 87-eligible mother-infant dyads that were discharged from the hospital. Of these mother-infant dyads, 32 (36.7%) had successful MOM use, pre-discharge (48 hours before discharge) and 43 mother-infant dyads (49.4%) had successful MOM use during the total hospital stay.

In the MOM (48 hours prior discharge) group and FM or DHM group, mean maternal age, mean gestation age, education level, and mother having medical disorder (pregnancy-induced hypertension and gestational diabetes mellitus), were identical. In the MOM (48 hours before discharge) group, 50% were primigravida, which were statistically not significant. The proportion of women with c-section was greater in the formula or DHM group (87 vs 72%) but not statistically significant. The proportion of mothers residing from the rural locality was greater in infants with MOM success. None of the mothers in either group had structured antenatal counseling about breastfeeding and thus the breastfeeding awareness in both groups was at 19% and 18% in the MOM group and FM or DHM group respectively. Also, none had first-day and first-week postnatal counseling on breastfeeding practice. The proportion of infants with birth weight less than 1000 grams and the incidence of intrauterine growth restriction (IUGR) were significantly larger in the FM or DHM group. The incidence of respiratory distress syndrome (RDS), hemodynamically significant patent ductus arteriosus (HSPDA), and requirement of invasive ventilation or noninvasive ventilation (continuous positive airway pressure (CPAP) heated high flow nasal cannula (HHFNC)) in MOM (48 hours prior discharge) and FM or DHM group were comparable. APGAR score at five minutes in both groups was identical, mean of 8/10 (Table II).

The evaluation of the barriers and enablers of MOM (48 hours before discharge) use and the enablers of MOM (48hours prior discharge) are given below (Table III)

- Total night feeding (expression) in the first week
- Total number of milk expression until the first 3 days after birth
- Maternal visit to the NICU on the first day
- Total number of maternal visits on the first day to the NICU
- The average volume of MOM on the first day, second day to the third day, from day four until day seven, and in the second week after birth.

The major barriers for MOM (48 hours before discharge)

- Extremely low birth weight (ELBW) (Birth weight less than 1000 grams) infants
- IUGR infants

All other factors detailed below neither increased the MOM (pre-discharge usage) or lessened its use

- Total number of milk expressions on the first day
- Time of first milk expression after the birth
- Total number of milk expressions on the first day, until day seven and during a hospital stay

Table II. Comparison of Pre-discharge (Before 48 hrs) MOM success vs. MOM Failure Group for baseline variable.

Baseline variable	MOM Success (n=32)	MOM Failure (n=55)	P value
Primigravida mother	16 (50%)	29 (53%)	0.78
Cesarean section	23 (72%)	48 (87%)	0.07
Maternal age (years)	29.09(SD±4.4)	29.45(SD±4.9)	0.73
Maternal PIH	18 (56%)	24 (44%)	0.25
Maternal diabetes	4(12.5%)	10 (18%)	0.48
Education			
Matriculation	2 (6%)	2 (4%)	0.57
Intermediate	11 (34%)	13 (24)	0.27
Graduate	11 (34%)	32 (58%)	0.03
Post graduate	8 (25%)	8 (15%)	0.22
Residence			
Rural	4 (13%)	1 (1.8%)	0.045
Semi urban	3 (9%)	13 (24%)	0.29
Urban	25 (78%)	41 (74.2%)	0.84
Breastfeeding awareness	6 (19%)	10 (18%)	0.94
Gestation (week)	30.6 (SD±1.6)	30.8 (SD±2.2)	0.64
Birth weight (gram)	1244 (SD±165)	1168 (SD±208)	0.08
Male sex	22 (69%)	30 (55%)	0.19
ELBW	2 (6.3%)	14 (25.5%)	0.02
IUGR	6 (19%)	24 (44%)	0.02
APGAR 5min	8 (IQR 8-8)	8 (IQR 8-8)	1.00
RDS	30 (94%)	47 (85%)	0.24
HSPDA	12 (38%)	27 (49%)	0.29
Ventilation	2 (6.3%)	7 (12.7%)	0.34
HFNC/CPAP	19 (59%)	38 (69%)	0.35

MOM: mother own milk, PIH: pregnancy induced hypertension, ELBW: extremely low birth weight (birth weight <1000 grams), IUGR: intra-uterine growth restriction, RDS: respiratory distress syndrome, HSPDA: hemodynamically significant patent ductus arteriosus CPAP: continuous positive pressure support HFNC: high flow nasal canula, SD: standard deviation, IQR: interquartile range

- Mode of the milk expression by electronic pump or by manual hand
- Maternal visit from day two to 3rd day, 4th to 7th day and until the end of the first week
- Mean minutes of visits to the NICU on the first day, second post-natal day to third post-natal day and day four to 7th day
- Time of the first contact of mothers with their infants
- Mean of the time of the first non nutritive sucking (NNS) and mean duration of NNS
- Mean of the time of first skin to skin contact and mean duration of skin to skin contact

On testing, of perinatal and post-natal factors during the total hospital stay for infants with MOM success with the control mother-infant dyad; baseline variables were similar between the 2 groups. The ELBW infants and IUGR infants were similar throughout the entire hospital stay which was conflicting with pre-discharge state. Maternal visits were also not significantly different between the groups. Even

Table III. Comparison of Pre-discharge (Before 48 hrs) MOM success vs. MOM Failure Group for enables and barrier.

Factors	MOM Success (n= 32)	MOM Failure (n= 55)	P value
Antenatal counseling	0	0	0
Breastfeeding awareness	6 (19%)	10 (18%)	0.94
Day 1 counseling	0	0	0
Proportion of the mothers who Expressed the milk on Day1 after birth	25 (78%)	39 (71%)	0.615
Time of first expression after birth (hours)	12 (IQR 7-24)	18 (IQR 14-27)	0.14
Median number of expressions Day1 after birth	2 (IQR 0-2)	1 (IQR 0-1)	0.057
Median number of expressions in first 3 days after birth	6 (IQR 5-8)	5 (IQR 4-6)	0.049
Median number of expressions in first week after birth	17 (IQR 14-20)	16 (IQR 13-18)	0.114
Median number of night expressions in first week	5 (IQR 4-6)	4 (IQR 2-4)	0.001
Median number of total Expressions in NICU stay	78 (IQR 61-103)	52 (IQR 30-102)	0.10
Mode of expressions			
Only pump	24 (75%)	32 (58%)	0.11
Both (manual and pump)	8 (25%)	23 (42%)	
Mother having Any Visit on Day 1	9 (28%)	3 (5.5%)	0.003
Maternal visit on day 1 (median numbers)	0 (IQR 0-1)	0 (IQR 0-0)	0.008
Maternal visit on day 2-3 (median numbers)	2 (IQR 1-4)	2 (IQR 1-3)	0.776
Maternal visit on day 4-7(median numbers)	3 (IQR 1-5)	3 (IQR 1-4)	0.308
Maternal visits in the first week (median numbers)	16 (IQR 7-29)	13 (IQR 7-30)	0.36
Minutes of visit on day 1 (median)	0 (IOR 0-5)	0 (IQR 0-0)	0.25
Minutes of visit on day 2- 3 (median)	15 (IQR 10-30)	10 (IQR 4-20)	0.92
Minutes of visit on day 4- 7(median)	40 (IQR 20-150)	32 (IQR 13-121)	0.36
Time of first contact (in hrs) after birth with infant	30 (IQR 23-50)	38 (IQR 29-72)	0.88
Time of first Non-Nutritive sucking done for infant (day of life after birth)	7 (IQR 5-9)	8 (IQR 7-13)	0.525
Non -Nutritive sucking (in Min) done for infant in the first week	5 (IQR 0-10)	0 (IQR 0-5)	0.234
Day of initiation of Skin to skin care for infant after birth	7 (IOR 5-10)	8 (IQR 6-10)	0.542
Median duration of Skin to skin (hrs) done in NICU stay	21 (IQR 11-32)	26 (IQR 5.7-43.4)	0.796
Median duration of KMC in first week	0 (IQR 0-3)	0 (IQR 0-2)	0.92
Median MOM volume on day 1	1 (IQR 0-4)	0 (IQR 0-1)	0.014
Median MOM volume on day 2-3	20 (IQR 8-44)	3.5 (IQR 0-18)	0.001
Median MOM volume on day 4-7	227 (IQR 95-512)	76.5 (IQR 17.7-246)	0.011
Median MOM volume in 2nd week	1263 (IQR 640-1588)	222 (IQR 31-606)	<0.000

MOM: mother own milk, NICU: neonatal intensive care unit, KMC: kangaroo mother care, IQR: interquartile range

though the number of mothers was less from the rural locality in the total number of studied mother-infant dyads it still preserved its effect. The numbers of expressions on day 1, number of night expressions during the first week, MOM volume from day 3 till 2 weeks postnatally were enablers for MOM feeding (Table IV and Table V). Neither mode of expression (electronic versus manual) nor infant's stay in the hospital was a factor for any of the groups.

Table IV. Comparison of baseline variables: MOM (Hospital Stay) group vs. formula or donor milk group.

Baseline variable	MOM success (n= 43)	MOM failure (n= 44)	P value
Primigravida mother	22 (50%)	23 (53.5%)	0.59
Cesarean section	34 (77.7%)	37 (86%)	0.74
Maternal age (years)	29(SD±4.3)	29.5(SD±5.1)	0.64
Maternal PIH	22 (50%)	23 (53.5%)	0.83
Maternal diabetes	9 (20.5%)	5 (11.6%)	0.38
Education			
Matriculation	18 (40.9%)	25 (58.1%)	0.10
Intermediate	14 (31.8%)	10 (23.3%)	0.30
Graduate	2 (4.5%)	2 (4.7%)	0.98
Post graduate	10 (22.7%)	6 (14%)	0.38
Residence			
Rural	5 (11.4%)	0	0.045
Semi urban	6 (13.6%)	10 (23.3%)	0.29
Urban	33 (75%)	33 (76.7%)	0.84
Gestation (week)	30.6 (SD±1.6)	30.8 (SD±2.2)	0.64
Birth weight (gram)	1213 (SD±182)	1179 (SD±208)	0.57
Male sex	22 (69%)	30 (55%)	0.19
ELBW	7 (15.9%)	9 (20.9%)	0.82
IUGR	13 (29.5%)	17 (39.5%)	0.59
APGAR 5min	8 (IQR 8-8)	8 (IQR 8-8)	1.00
RDS	41(93.1%)	36 (83.7%)	0.97
HSPDA	17 (41.46%)	19 (44.18%)	0.19
Ventilation	1 (2.2%)	6 (13.9%)	1.00
HFNC/CPAP	27 (61.36%)	30 (69.76%)	0.08

MOM: mother own milk, PIH: pregnancy induced hypertension, ELBW: extremely low birth weight (birth weight <1000 grams), IUGR: intra-uterine growth restriction, RDS: respiratory distress syndrome, HSPDA: hemodynamically significant patent ductus arteriosus CPAP: continuous positive pressure support, HFNC: high flow nasal canula, IQR: interquartile range, SD: standard deviation

Discussion

Mothers of preterm infants must express their breast milk by hand or use a pump and then the milk is given to the infant by an orogastric tube or gavage as per appropriate feeding mode for the infant in the initial phase after birth, with the aim of transitioning to direct breastfeeding. While some mothers find this transition to be easy some struggle. If the direct breastfeeding attempt is successful then this can be a sense of security and pride for mothers and if not, then they may experience shame, insufficiency, frustration, rejection, and setback which then may affect mother-infant bonding.[6] Preterm infant's mother's journey from starting milk expression to attaining a full volume of milk can be challenging, among which stress and deficient lactation support are primary causative factors. Other factors may also delay lactogenesis stage two, leading to a less number of milk expressions and less breast milk volume.[7] Other barriers for human milk feeding are the ambiguity of the premature condition of the infant, physical partition from the mother, suspicion of discharge, and maternal anxiety.[8] Hobbs et al.[9] observed that mothers who had received a C-section experienced more breastfeeding obstacles and lesser breastfeeding duration when compared to the

Table V. Comparison MOM (Hospital Stay) group vs. formula or donor milk group.

Factors	MOM success (n= 43)	MOM failure (n= 44)	P value
Antenatal counseling	0	0	0
Breastfeeding awareness	5 (12%)	11 (25%)	0.18
Day 1 counseling	0	0	0
Proportion of the mothers who Expressed the milk on Day1 after birth	32 (74%)	32 (73%)	0.94
Time of first expression after birth (hours)	16 (IQR 8-24)	18 (IQR 13-26)	0.23
Median number of expressions Day 1 after birth	2 (IQR 0-2)	1 (IQR 0-1)	0.006
Median number of expressions in first 3 days after birth	6 (IQR 5-7)	5 (IQR 4-6)	0.16
Median number of expressions in first week after birth	17 (IQR 15-20)	14 (IQR 12-18)	0.24
Median number of night expressions in first week	4 (IQR 4-5)	3 (IQR 2-4)	0.002
Median number of total Expressions in NICU stay	78 (IQR 59-102)	50 (IQR 23-108)	0.069
Mode of expressions			
Only pump	30 (70%)	26(59%)	0.29
Both (manual and pump)	13 (30%)	18 (41%)	
Mother having Any Visit on Day 1	9 (28%)	3 (5.5%)	0.11
Maternal visit on day 1 (median numbers)	0 (IQR 0-0)	0 (IQR 0-0)	0.11
Maternal visit on day 2-3 (median numbers)	2 (IQR 1-3.5)	1 (IQR 0-3)	0.33
Maternal visit on day 4-7(median numbers)	4 (IQR 3-7)	4 (IQR 2-6)	0.23
Maternal visits in the first week (median numbers)	8 (IQR 5-9.5)	6 (IQR 3-7)	0.24
Minutes of visit on day 1 (median)	0 (IOR 0-2.5)	0 (IQR 0-1)	0.11
Minutes of visit on day 2- 3 (median)	16 (IQR 7-34)	3 (IQR 0-18)	0.24
Minutes of visit on day 4- 7(median)	200 (IQR 48-521)	76 (IQR 12-227)	0.90
Time of first contact (in hrs) after birth with infant	30 (IQR 25-49)	40 (IQR 30-75)	0.74
Time of first Non-Nutritive sucking done for infant (day of life after birth)	8 (IQR 6-9.5)	7.5 (IQR 6-11)	0.69
Non -Nutritive sucking (in Min) done for infant in the first week	0 (IQR 0-10)	0 (IQR 0-5)	0.59
Day of initiation of Skin to skin care for infant after birth	8 (IOR 6-10)	7.5 (IQR 6-11)	0.88
Median duration of Skin to skin (hrs) done in NICU stay	22 (IQR 11-42)	18 (IQR 4-35)	0.91
Median duration of KMC in first week	0 (IQR 0-3)	0 (IQR 0-2)	0.90
Median MOM volume on day 1	1 (IQR 0-3)	0 (IQR 0-1)	0.064
Median MOM volume on day 2-3	16 (IQR 7-34)	3 (IQR 0-18)	0.02
Median MOM volume on day 4-7	200 (IQR 48-521)	76 (IQR 12-227)	0.024
Median MOM volume in 2nd week	1177 (IQR 660-1535)	110 (IQR 0-410)	<0.000

MOM: mother own milk, NICU: neonatal intensive care unit, KMC: kangaroo mother care, IQR: interquartile range

mothers who had delivered vaginally. Evan et al.[10] also showed that breastfeeding among the mothers who had received C-sections was significantly lesser in the first five days after the delivery when compared to mothers that had delivered vaginally. Similarly, Giannì et. al.[5] and Scott et al.[11] found that mothers who had received C-sections had significantly delayed lactation when compared to those who delivered vaginally. The mothers of preterm infants due to their early delivery had lowered milk production as there is deficient mammary gland development.[12] Also, maybe due to the delayed lactogenesis two (initiation of full milk

generation following the evacuation of the placenta after delivery), following the cesarean section which may cause a significant or lengthy maternal stress response and due to medical complications, which is especially common among the VLBW mothers.[9,13] We did not find that a C-section was a barrier to successful MOM use, contrary to the findings of many studies. C-section rates were very high in our unit and similar between the 2 groups (>70%).

Infant factors like EUGR and IUGR were shown to be significant barriers for MOM in the pre-discharge 48-hour group. To date, there are no studies concentrating on small for gestation age (SGA) newborns and the best mode of feeding. The limited data concerning premature infants less than 1500 grams shows that they are not capable of coordinated sucking, swallowing, and breathing, with little recognition of infants.[11,14] Our findings are coherent with the findings of Meier et al.[15] and Radtke et al.[16], which mentions factors related to the infants, such as the fact that the varying degree of infant developmental immaturity can complicate the feeding situation. Preterm infants present with latching difficulty, lethargy, limited sucking, and metabolic disturbances that could further incline them to poor breastfeeding outcomes. Similarly, Jones et al.[17] assessed preterm breastfeeding practices and suggested that difficulties with proper latching, positioning, and coordination are commonly experienced.

Sisk et al.[18] and Hurst et al.[19] recognized that lack of motivation and support impairs the ability to frequently express breast milk. The unavailability of lactation professionals in the NICU and scarce supply of hospital grade pumps can also lead to less human milk utilization in VLBW infants. Flacking et al.[20] and Swanson et al.[21] showed that the expression of milk required resilience, motivation, and persistence. The education of staff and family regarding the priority of human milk for preterm infants, helping mothers express their own milk either manually or pumping, and assessing the latch of the newborn has been demonstrated to improve milk generation in the mothers of VLBW.[22,23]

Giannì et al.[5] suggested positive impact factors such as receiving support from a consultant or health care professional, prenatal counseling, mother and baby rooming for a prolonged time, early expression of breast milk, and expressing milk at a frequency of at least 6.25 times per day. The participant mothers in both groups from our study had not obtained structured counseling neither during the antenatal period nor the postnatal period by health care professionals specializing in breastfeeding practices, however in our study milk expression on the first day until the third day and night expressions in the first week postnatally were significantly higher in infants with MOM success group (pre discharge) .The maternal role in terms of a visit on the first day after delivery to her infant as well as provision of MOM on the first day were also the predictors of successful MOM use in the hospital pre-discharge (48 hours prior) MOM group.

Early commencement of manual hand expression of breast milk resulted in a greater generation of human milk.[23] Parker et al.[24] detected a greater milk volume of human milk in mothers who initiated expression of milk in less than 1 hour after birth compared to those who expressed between one to six hours. In our study the first expression after the birth was more than 12 hours in both groups. Furthermore, repeated frequent expression of milk by the pump or by manual hand expression ended in a higher volume of human milk over time.[25] The mode of expression of milk did not affect the MOM use in our study.

Flacking et al.[20] indicated that maternal separation from their infants caused a loss of natural connection between the mother-infant dyad and the tie-in that starts when a term infant is breastfed right after birth was not experienced by the preterm infants and mothers after discharge. In our study, we noticed that a maternal visit on the first day and the number

of maternal visits on the first day to NICU has a decisive impact on MOM use in preterm infants. Similarly, Ikonen et al.[25] found that mothers who were separated from their infants had struggles in expressing their milk and breastfeeding, as well as feeling detachment.

The main limitations of our study was that it was a single-center study and most of the mothers were from high educational environments and that data on the psychological and emotional aspects of mothers were not incorporated in the study; hence the results may not relate to dissimilar settings.

In our study close to 50% of the mothers were predominantly feeding their infants with MOM during the entire hospital stay and nearly one third successfully preserved a full human milk supply to their infants at the time of hospital discharge. The ELBW infants and IUGR infants are at a greater risk of poor MOM use. The numbers of milk expressions until the third day, maternal visit on the first day, and average milk volume in the first 2 weeks are the crucial enablers of MOM use.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DK, SM; data collection: DP, VN, DS, VV, TB, DK, SJ, VK; analysis and interpretation of results: DK, VN, SK, DP, DS, VV, TB; draft manuscript preparation: SM, DK, SK. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the ethical committee of the Fernandez Hospital (reference number 24-2017).

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Conflict of interest

None.

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Original Article

The performance of shear wave elastography on evaluating liver changes in obese and overweight children

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ABSTRACT

Background. Real time shear-wave elastography (SWE) is a non-invasive imaging method which can quantitatively assess liver stiffness. Obesity and its complication are increasing with improving lifestyles in our century. We evaluated the performance of SWE for detecting liver changes (fatty liver, steatohepatitis) in obese and overweight children, in addition to this, we determined the diagnostic accuracy and clinical usefulness of SWE in non-alcoholic fatty liver disease (NAFLD).

Methods. Obese and overweight 41 children within the age range of 6–15 years were included in this single-center prospective study. Biochemical evaluation for aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride (TG), total cholesterol (TC) levels, as well as conventional ultrasound and SWE of the liver were performed in the patient group. These values were compared with values of 25 normal weight and healthy children in the age range of 6–16 years.

Results. The mean SWE values was 13.7 ± 5.5 kiloPascal (kPa) and 2.03 ± 0.35 meter/second (m/s) in patient group and 7.99 ± 2.81 kPa and 1.62 ± 0.21 m/s in control group ($p<0.01$). The receiver-operating characteristics (ROC) analysis was performed to determine the optimum cut-off value for elastography values (kPa) to evaluate liver changes; area under the curve was 87.5% (95% CI 79.3-95.8). When the cutoff value was set as 10.45 kPa, the sensitivity and the specificity was 69.2% and 100%, respectively. We could not observe a statistically significant difference when we compared the elastography values (kPa and m/s) according to presence of hepatosteatosis ($p=0.581$ and 0.172). There were no significant correlations between SWE and AST, ALT values.

Conclusions. SWE may be a useful and accurate imaging method to evaluate liver changes and monitor NAFLD in obese and overweight children.

Key words: child, obesity, liver, real time shear-wave elastography.

The worldwide prevalence of childhood obesity has increased constantly during the past three decades according to a report from the World Health Organization in 2011.[1,2] The population of overweight children and adolescents under 18 years of age is 17 million with an annual increase of 0.5-1 percent.[3] However, this rise has caused an increased incidence of obesity comorbidities, including hyperlipidemia and non-alcoholic fatty liver disease (NAFLD).[4] NAFLD is the most common pediatric liver disease and its prevalence has more than doubled in the last decades[5]; 3-11% of the pediatric population present NAFLD[6,7] with highest prevalence (46%) reached among overweight and obese children and adolescents.[8]

NAFLD includes conditions changing from fatty liver or steatohepatitis to cirrhosis and its complications (e.g. hepatocellular carcinoma and portal hypertension).[9] Although there are some noninvasive diagnostic tools for NAFLD, liver biopsy is the gold standard for diagnosis. It gives to the clinicians the possibility to both

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diagnose the NAFLD and assess its progression to fibrosis or cirrhosis with greater certainty than other techniques. However, the use of liver biopsy in routine clinical practice has some drawbacks. Most cases with NAFLD have good prognosis, for this reason, the risks of a liver biopsy appear to overweigh the clinical benefits. It represents an impractical screening procedure as it is invasive, expensive and has complications such as pain or hemorrhage. Furthermore, inaccurate sampling of liver biopsy can cause very serious misdiagnosis as well as staging inaccuracies, because histological lesions of non-alcoholic steatohepatitis (NASH) have an uneven distribution in liver parenchyma. As the liver biopsy is limited, clinicians have to use noninvasive and practical methods to diagnose and stage NAFLD.[9]

Recently, some noninvasive methods for measuring liver stiffness (LS), including transient elastography (TE), acoustic radiation force impulse imaging (ARFI), and magnetic resonance elastography have been developed.[10] More recently, real-time shear wave elastography (SWE), another method for measuring LS, has been developed.[11]

SWE is a novel noninvasive method that involves application of local mechanical compression on soft tissue using focused ultrasound scanning (US) and acquiring strain images that show tissue responses.[12,13] Several studies report positive results in their ability to predict the degree of hepatic fibrosis.[14-16] Unlike TE, SWE measures tissue elasticity simultaneously during B-mode ultrasound examination, and elasticity values can be measured on the basis of anatomical information. In addition, SWE provides elastography color, quantitative maps according to the degree of stiffness, allowing an assessment of homogeneity.[10] The spatial heterogeneity of liver stiffness can be visualized, and the size of the region used for a measurement can be selectively placed or adjusted. SWE is a relatively new method, therefore studies in children are limited.[17]

The aim of this study was to evaluate the performance of real time SWE for detecting liver changes (fatty liver or steatohepatitis) in obese and owerweight children, in addition to this, to determine the diagnostic accuracy and clinical usefulness of SWE in NAFLD in the absence of liver biopsy.

Material and Methods

Study population

This was a single center, cross-sectional and prospective study. The patient group included 41 children in the age range of 6–15 years who were admitted to University of Health Sciences Bakırkoy Dr. Sadi Konuk Training and Research Hospital, general pediatrics outpatient clinic between June 2015 and January 2016.

The study protocol was approved by the University of Health Sciences Bakırkoy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (07.12.2015, report number: 2015/19/01). All subjects gave signed informed consent for the research. Inclusion criteria were the presence of obesity or being overweight. Patients who had a body mass index (BMI) at or above the 95th percentile (obesity) and at or above the 85th percentile and below the 95th percentile (overweight) were included in this study. Children with known chronic disease and liver disease were excluded.

Demographic data were noted. The body weight and height were measured for all patients and BMI was calculated. To define obesity, the percentile distributions relative to gender and age which were constituted by Neyzi et al. (2015 Growth Charts) were used.[18] All of the patients underwent a physical examination. We evaluated laboratory tests, which included alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG) and total cholesterol (TC). The values below 35 U/L for both AST and ALT was considered as normal. The normal cutoff value for TG level was 150 mg/dl and for TC level was 200 mg/dl. Conventional ultrasound and real time SWE for each patient were performed. All ultrasounds

were performed by a single observer (SA). All clinical details, examinations, laboratory and radiological findings were recorded.

The control group included 25 healthy children that were admitted to our outpatient clinic, within the age range of 6-16 years, whose BMI were between the 5th percentile and 85th percentile. These children showed a normal physical examination, normal levels of transaminase and lipids. Their liver ultrasound revealed no change by conventional methods. SWE was also performed on the children in the control group.

Real time shear-wave elastography

Measurements were performed using convex probe on an Applio 500 Platinum device (Toshiba, America, medical Systems) by the same radiologist. The patients were examined lying in dorsal decubitus position with the right arm at maximal abduction in a breath suspension mid-respiratory phase.

The region of interest (ROI) (10x16 mm) was put in the right lobe of the liver. Measurements were performed 1.5 to 2.0 cm below the liver capsule through intercostal spaces in the right lobe. The area was far from the blood vessels that could be caused by artifacts. The values were reported as shear wave speed (m/s) and converted to kPa (kiloPascal; elastic modulus).

Statistical analysis

Statistical analysis was performed by using IBM SPSS (Statistics Package for Social Sciences for Windows, Version 21.0, Armonk, NY, IBM Corp.) package program. The chi-square test was used to determine the differences between categorical variables. To show the behavioral differences of group averages; analysis of variance (ANOVA) T-test was used when normality and uniformity variables were met, and the statistical analyses for continuous variables were performed using the Mann-Whitney U test. In addition, while comparing the value of any parameter between the groups, analysis of covariance (ANCOVA) analysis was performed to determine a statistically significant difference, taking into account the factors affecting this change. In calculating the correlation between any two numerical variables, the nonparametric Spearman's rank correlation test was used because the data did not have a normal distribution. The receiver-operating characteristics (ROC) analysis was performed to test optimum cutoff value for elastography values (kPa) to evaluate liver changes (fatty liver or steatohepatitis). Statistical significance for all cases was determined as $p < 0.05$.

Results

Demographics, clinical features and laboratory findings of the patient and control groups are summarized in Table I. Seven patients (17.1%) had hepatosteatosis (mild) on conventional US. Out of 41 obese and overweight children, AST was found to be raised in 1 child (2.4%) and ALT was raised in 3 children (7.3%). Only nine (21.9%) patients had TG levels of >150 mg/dl. None of the healthy children had elevated levels of TG. Only two (4.8%) patients had TC levels of >200 mg/dL. One of the healthy children had elevated level of TC.

SWE values were higher in patients, compared to controls ($p < 0.001$, Table II).

The receiver-operating characteristics (ROC) analysis to determine the optimum cutoff value for elastography values (kPa) to evaluate liver changes revealed an area under the curve of 87.5% (95% CI 79.3 - 95.8). When the cutoff value was set as 10.45 kPa, the sensitivity and the specificity was 69.2% and 100%, respectively (Fig 1).

The elastography values (kPa and m/s) were positively correlated with weight, BMI, BMI-SDS, TG values and presence of acanthosis; there was no correlation between elastography values and age, height, ALT, AST, TC (Table III).

When the patient and control group were compared in terms of elastography values with

Table I. Demographics, clinical features and laboratory findings of the patient and control groups.

Characteristics	Patient Group (N= 41)	Control Group (N= 25)	p-value
Age (year)	11.39 (9.6, 13.53)	11.7 (9, 13.2)	0.905*
Sex (female/male), n/n	19/22	13/12	0.847 **
BMI (kg/m^2)	27.87 (25.08, 31.5)	18.66 (17.2, 20.7)	<0.001*
BMI-SDS	2.31 (2, 2.79)	0.36 (-0.17, 0.86)	<0.001*
Acanthosis, n (%)	19 (46.3)	0	<0.001**
ALT (IU/L)	18 (15, 22)	13 (10.07, 16.25)	0.001*
AST (IU/L)	21 (19, 23)	23 (17.75, 25.25)	0.569*
Total cholesterol (mg/dl)	156 (142, 172.75)	137.5 (128, 149.75)	0.074*
Triglyceride (mg/dl)	107.5 (87, 135.5)	68 (59.25, 98.25)	0.014*
Hepatosteatosis, n (%)	7 (17.1)	0	0.039 **
Elastography (kPa)	13 (8.9, 16.9)	7.4 (6.8, 8.5)	<0.001*
Elastography (m/s)	2.07 (1.8, 2.21)	1.59 (1.52, 1.73)	<0.001*

Data are presented as median (Q1, Q3) or n (%).
*: Mann-Whitney U test; **: Chi-square test
ALT: alanine transaminase, AST: aspartate transaminase, BMI: body mass index, BMI-SDS: body mass index-standard deviation score.

Table II. The mean SWE values of patients and controls.

SWE values	Groups		p-value
	Patients (N= 41)	Controls (N= 25)	
Elastography (kPa)	13 (8.9, 16.9)	7.4 (6.8, 8.5)	<0.001*
Elastography (m/s)	2.07 (1.8, 2.21)	1.59 (1.52, 1.73)	<0.001*

Data are presented as median (Q1, Q3).
*: Mann-Whitney U test

Table III. Correlation analysis between elastography and certain clinical and laboratory parameters.

Parameters	Elastography (kPA)		Elastography (m/s)	
	r*	p	r*	p
Age	0.209	0.092	0.202	0.135
Height	0.21	0.091	0.167	0.219
Weight	0.559	<0.001	0.537	<0.001
ALT	0.255	0.058	0.283	0.051
AST	-0.091	0.488	-0.069	0.624
BMI	0.705	<0.001	0.688	<0.001
BMI-SDS	0.665	<0.001	0.641	<0.001
Total cholesterol	0.239	0.127	0.305	0.066
Triglyceride	0.443	0.002	0.444	0.005
Acantosis	0.439	<0.001	0.411	0.002

*: Spearman's correlation test
ALT: alanine transaminase, AST: aspartate transaminase, BMI: body mass index, BMI-SDS: body mass index-standard deviation score.

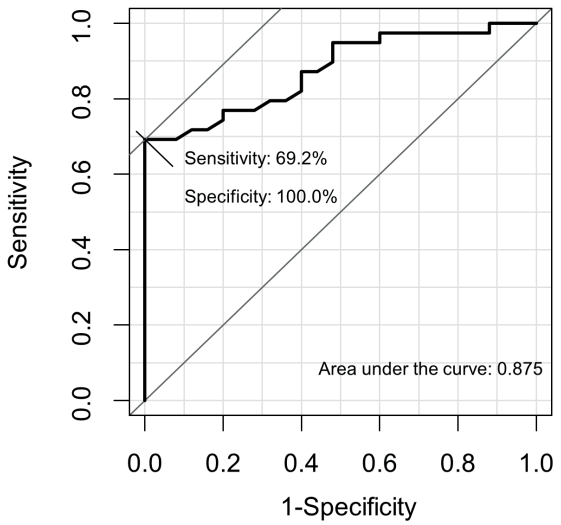

Fig. 1. The receiver-operating characteristics (ROC) analysis was performed to determine the optimum cutoff value for elastography values (kPa) to detect liver changes in obese and overweight children; area under the curve was 87.5% (95% CI 79.3 - 95.8). When the cutoff value was set as 10.45 kPa, the sensitivity and the specificity was 69.2% and 100%, respectively.

ANCOVA, we found that AST, ALT, TC did not affect these results (p values 0.373, 0.882, 0.143, respectively). We determined the effect of BMI, BMI-SDS and TG on elastography values (all p values <0.001). SWE values were higher in the presence of acanthosis (13.15±1.505 vs. 8.89±1.99 kPa; p=0.002). SWE values were comparable between groups with and without hepatosteatosis (Table IV).

Discussion

The initial step to determine the potential risk for obesity and NAFLD requires that the primary care pediatricians calculate and record the BMI for each child at each visit. NAFLD is usually "a silent liver disease" because it occurs without any warning signs. Some symptoms of chronic liver disease will occur when NASH develops with more severe liver damage. In clinical practice, NAFLD is generally suspected based on the findings of hypertransaminasemia and/or an ultrasonographic bright liver in an otherwise healthy child who is overweight or obese.[19]

Although there are some diagnostic techniques to evaluate NAFLD, liver biopsy is the gold standard for diagnosis. As liver biopsy is an invasive method and most patients with NAFLD have a good prognosis, this method causes controversy and this cannot be performed routinely in the pediatric population.[9]

Conventional US is often the first imaging modality used to evaluate fatty liver clinically, especially for screening of suspected NAFLD, due to its lack of invasiveness, wide availability, and relatively low cost.[20,21] However, in two studies that have compared liver conventional US and liver histology for measuring the degree of hepatic steatosis, they concluded that conventional US is an insensitive technique to identify mild steatosis.[22,23] Steatosis is reported to be detectable by US when more than 20% of hepatocytes contain histologically visible fat droplets, with a reported sensitivity of 79.7% and specificity of 86.2%.[24]

There are several limitations of conventional US for NAFLD evaluation: 1) It is qualitative and therefore subjective; the value of conventional US to evaluate NAFLD is limited by the subjective nature of the criteria used to differentiate fatty from normal liver and a lack of sonographic criteria for different degrees of

Table IV. The elastography values according to the condition of hepatosteatosis.

SWE values	Groups		p-value
	With hepatosteatosis (N= 7)	Without hepatosteatosis (N= 59)	
Elastography (kPa)	9.2 (7.7, 17.3)	9.1 (7.52, 14.35)	0.581*
Elastography (m/s)	2.17 (1.7, 2.34)	1.74 (1.56, 2.07)	0.172*

Data are presented as median (Q1, Q3).
*: Mann-Whitney U test

steatosis. 2) Sensitivity is limited when there are few steatotic hepatocytes.[24] 3) The sensitivity and specificity of B mode sonography decreases as BMI increases, varying between 49%-100% and 75%-95%.[25] Conventional US cannot differentiate steatosis and steatohepatitis or stage fibrosis.[26]

SWE quantitatively evaluates liver stiffness to make noninvasive evaluation of liver fibrosis and NASH clinically possible. Estimated tissue stiffness therefore provides information on the presence and degree of fibrosis.[27]

Our study was an effort to diagnose liver changes at its earliest stage in overweight and obese children with fatty liver disease using the new US technique of SWE. It was aimed to determine the accuracy of this technique in the follow-up of NAFLD findings in obese and overweight children.

Different ultrasound-based elastography techniques have been developed to noninvasively evaluate tissue stiffness like that of the liver. The radiologic imaging methods can estimate liver stiffness as a surrogate for liver fibrosis, including SWE. There are many reports related to these methods in adults and children. Guzmán-Aroca et al.[28] assessed the liver findings in 23 morbid obese adults with acustic radiation force impulse elastography (ARFI) and conventional ultrasound. They showed that the findings of the liver biopsy were correlated with ARFI measurements and considered the ARFI technique as a useful diagnostic tool for differentiating NAFLD from NASH in asymptomatic patients with morbid obesity.

Real-time SWE is a newer technic and there are limited studies in children with SWE. Tutar et al.[17] evaluated the liver stiffness with SWE in pediatric chronic liver disease. Consequently, they determined correlation with SWE measurements and fibrosis in liver. Kim et al.[29] evaluated the diagnostic performance of SWE for determining the severity of liver fibrosis in children and adolescents. They concluded that SWE is an excellent method for the assessment of the severity of liver fibrosis in children and adolescents.

In our study we compared the liver SWE measurements in obese and overweight children with a healthy control group. The values of the study group were significantly higher than the values of the control group ($p < 0.001$ for kPa). This result has shown that real-time SWE may be used for monitoring liver changes in obese and overweight children.

There are few studies with ultrasound-based elastography that evaluate the liver changes in obese and overweight children. Kamble et al.[30] have analyzed the diagnostic effectiveness of ARFI elastography with biochemical markers for evaluating hepatic changes in overweight and obese children. In this study ARFI elastography has shown excellent correlation with AST/ALT ratios in obese children. They have concluded that ARFI elastography can be used as a noninvasive method to detect NAFLD and associated hepatic changes in pediatric patients. Marginean et al.[31] determined that in the group with hepatic steatosis, the SWE values were statistically higher compared to those in healthy controls. They established positive statistical correlations between AST and SWE in the group of children with NAFLD.

As far as we know, there is only one report which evaluated the alterations in liver with SWE in overweight and obese children. Bailey at al.[32] evaluated US-SWE velocities of the liver in normal-weight and obese children, to correlate US-SWE findings with age and BMI, and to compare US-SWE values with qualitative assessment of the liver by conventional US. It was concluded that US-SWE provides a useful quantitative imaging biomarker for evaluating liver stiffness in children.

Our results showed that the liver SWE values of obese and overweight children were significantly higher than the values of the healthy control group. The calculated area under the ROC curve values indicated a good

performance of SWE for evaluating the liver changes (fatty liver) in obese and overweight children.

NAFLD is the most common cause of hypertransaminasemia in children and adolescents.[33] However elevated ALT is not a sensitive marker of disease existence and/or severity at ordinarily used thresholds.[34] According to the Screening ALT for Elevation in Today's Youth Study, normal values of transaminases for teenagers and children are presently set too high to detect liver steatosis.[35] In our study ALT levels of the patient group were significantly higher than the control group. Nevertheless, ALT, AST levels and elastography values were not significantly correlated. Therefore, we think that the serum aminotransferase levels are not reliable biomarkers to detect the degree of fatty liver in obese and overweight children.

Imaging methods, such as conventional US have been increasingly approved as noninvasive alternative methods to diagnose and monitor NAFLD/NASH. US is safe, but it is limited by the inability to detect fatty liver (liver brightness vs. kidney parenchymal echogenicity) when steatosis involves <30% of hepatocytes.[19] Wong et al.[36] reported that hepatic steatosis necroinflammation, and obesity did not affect the liver stiffness measurement. In our study when the elastography values (kPa) of the group who had hepatosteatosis were compared with those who had no hepatosteatosis, no significant difference was observed. However, the number of patients with hepatosteatosis was very low and all of them were mild hepatosteatosis. It is possible that mild hepatosteatosis may not change the value of SWE. It was considered that further studies are needed on a large number of pediatric populations to evaluate this condition.

Kamble et al.[30] reported an association between liver changes and TG levels in children. In our study, we found a significant correlation between elastography (kPa, m/s) and TG values ($p < 0.001$). We think that SWE and TG level may be useful indicators in the follow-up of NAFLD due to obesity.

In several studies, normal mean liver elasticity values in children were reported. On transient elastographic (Fibro Scan) studies, normal mean liver elasticity values were reported to range between 4.4 and 5.6 kPa.[37] In the study by Hanquinet et al.[38] normal mean liver elasticity was reported to be 1.12 m/s in healthy children. Tutar et al.[17] they found the mean elasticity of 7.41 kPa and 1.56 m/s in the liver of normal healthy children. In our study, we determined that the mean elasticity of the liver was 7.99±2.81 kPa and 1.62±0.21 m/s in healthy children.

There were some limitations in our study. We could not compare SWE measurements of the liver with the histological results of liver biopsy. This was not possible because liver biopsy is an invasive method. Secondly, measurement of the liver stiffness with SWE were made by the same operator at one time and only in one period; it is difficult to perform this method in children and families did not want to get another imaging.

In conclusion, SWE may a useful and accurate imaging method to evaluate and monitor NAFLD-related liver changes in obese children. However further longitudinal studies are required on a larger pediatric population.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NÖS, SA, FBP, SSH, Eİ; data collection: NÖS; analysis and interpretation of results: NÖS, SYK; draft manuscript preparation: NÖS. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study protocol was approved by the University of Health Sciences Bakırkoy Dr. Sadi Konuk Training and Research Hospital Ethics Committee (07.12.2015, report number: 2015/19/01).

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Conflict of interest

The authors have indicated they have no potential conflicts of interest to disclose.

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Original Article

Developmental parameters and physical fitness in preschool children with Minor Neurological Dysfunction

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ABSTRACT

Background. The preschool years constitute a critical period during which significant changes are experienced in the acquisition of locomotor skills due to maturation of the nervous system. Our aim was to investigate the developmental parameters and physical fitness in preschool children with Minor Neurological Dysfunction (MND).

Methods. The study was carried out in 212 preschool children without any known health problems. Sociodemographic characteristics of children were recorded. Denver Developmental Screening Test (DDST) II, Touwen Neurological Examination, and Preschool Physical Fitness (PREFIT) test battery were used to assess developmental parameters, neurological status, and physical fitness, respectively.

Results. There was a statistical difference in the physical fitness and developmental parameters in preschool children with MND compared with healthy peers ($p<0.05$). There was also a relationship between physical fitness and developmental parameters ($p<0.05$).

Conclusions. Early identification of problems in developmental parameters and physical fitness in preschool children with MND might help to implement early supportive physiotherapy and rehabilitation.

Key words: minor neurological dysfunction, physical fitness, preschool.

The preschool years are a critical period in which significant changes are experienced in the acquisition of locomotor skills due to maturation of the nervous system in children.[1] In this period, children gain significant progress in terms of developmental parameters in gross-motor, fine-motor, language and social-emotional domains.[2] This process, which is also affected by the environment, may show a non-optimal pattern, although it appears normal. The exact mechanism of 'misadaptation of behavior' due to the failure of the brain to develop within normal limits is not known, and this 'non-optimal developmental process' in the brain is attributed to the altered function of neurotransmitters.[3] As a result of this condition, the neurodevelopmental level of the child is reflected on problems of coordination which are uncertain movements in daily life activity, such as, impaired posture and muscle tone, insufficient gross and fine motor movements. In other words, this condition, which is defined as Minor Neurological Dysfunction (MND), may arise even as a complex MND with the increased load of involved developmental domains which further presents in the form of cerebral palsy spectrum disorder.[4] Although there is no appareant symptom in the first years of life, there is the possibility to determine definitive neurological problems at preschool or school age.[5] In this respect, it is important to assess the neurological status of preschool children at the earliest possible time, to detect possible dysfunction and to direct them to the right discipline in terms of both prognosis and rehabilitation.[6]

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Physical activity in childhood is not only important for bone, muscle, cardiovascular and brain development, but also for healthy growth and obesity prevention.[7,8] Physical fitness level is a strong indicator of health at early ages and physical activity is the main determinant of physical fitness.[9] While health-related physical fitness includes muscular strength and endurance, cardiovascular endurance, flexibility and body composition; skill-related physical fitness includes coordination, speed, agility, power, balance, and reaction time.[10] Depending on any reason, impairment of any physical fitness parameter affects physical fitness.[8]

To the best of our knowledge, there are no studies investigating the developmental parameters and physical fitness in preschool children with MND. Therefore, our aim was to investigate developmental parameters and physical fitness in preschool children, and compare the developmental profile of children with MND and healthy peers.

Material and Methods

Design and Participants

The study was carried out in 212 preschool children (107 girls and 105 boys, aged 4-6 years) without any known health problems in schools of Muş Province, Ministry of National Education between February and June 2018. Ethical permission was obtained on 29/11/2017 from Muş Alparslan University Publication Ethics Board, with the decision number E13931, and decision number 2. At the same time, with the decision dated 29/12/2017 and numbered 8822, official permission was obtained from Muş Provincial Directorate of National Education. The study was conducted in schools determined by simple random sampling method according to the guidelines given in the Declaration of Helsinki. Informed consent, witnessed and formally recorded, was obtained from all children and parents.

Inclusion criteria for the study were children aged 4-6 years currently attending kindergarten, without any known health problems, who could follow the directions of the physiotherapist. For statistical power analysis, Hacettepe University Faculty of Medicine Biostatistics Department was consulted. Using PASS 11.0 (Power Analysis and Sample Size) software, the number of children was determined with 90% power and 5% error rate. Of 250 children, 212 children who were able to complete the assessments were included in the statistical analysis.

Measurements

Demographic characteristics of the children in the study were recorded. Each child was evaluated respectively within a period of approximately 90 minutes. All measurements were performed in one season for each child and carried out by the Expert Physiotherapist, who has five years of experience in pediatric rehabilitation and has Denver Developmental Screening Test (DDST) II certificate. All measurements were carried out in the morning, at the playground or garden of the schools. The instructions in all measurement tests used were reviewed one by one and were implemented by ensuring standardization in all settings.

Touwen Neurological Examination

The Touwen examination is a method which comprehensively evaluates neural functions developed by Touwen and Prechtl in 1970, in eight areas including posture and muscle tonus, reflexes, involuntary movements, coordination and balance, fine-motor movements, associated movements, sensory functions and cranial nerve function.[11] In these eight areas, children with no dysfunction in any area are defined as "normal"; children with dysfunction in one or two areas are defined as "Simple MND"; children with dysfunction in three or more areas are defined as "Complex MND".[4] It is a standardized, detailed and age-specific assessment method that measures the quality of motor behavior in a strong and precise way, and includes

97 items. Different positions such as sitting, standing, walking and lying are used during assessments.[11] Before using this measurement method, a pilot study was carried out on 5 children by reading the Touwen Neurological Examination manual book and watching the sample case videos one by one. All the items of the Touwen neurological examination were performed in accordance with the specified guidelines.

Denver Developmental Screening Test II

Denver Developmental Screening Test (DDST) II, standardized in many countries around the world, is a screening test designed to detect developmental problems in personal-social, language, fine-motor and gross-motor areas in children aged 0-6 years.[12] This test, which is valuable in terms of defining developmental problems, verifying suspicious cases with an objective criterion and following children at risk and directing them to the relevant professionals, consists of a total of 134 items that can be assisted by asking the parents and/or caregivers as well as performance tests.[6] The norm values of this test have been conducted in Turkish children.[13,14]

Children performing the age-appropriate items or taking only one warning item for performance are determined as "normal" in terms of development. Children who are not able to perform one item, have two or more warning items are determined as "suspicious" in terms of development. Children who can not perform two or more items as are determined as "abnormal" in terms of development.[6,12] DDST II was performed according to defined, standard equipments.

Physical Fitness

To assess physical fitness, PREschool Physical FITness (PREFIT) Test Battery developed in Europe for 4-6 age range was used. This battery used in preschool children is an easy and reliable method including weight, height and waist circumference to assess anthropometry, 20 m shuttle run test (SRT) to assess cardiorespiratory fitness, handgrip strength and standing long jump (SLJ) tests to assess muscular strength, 4 × 10 m SRT to assess speed-agility, and one-leg stance test to assess balance.[15,16]

Body Composition

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, with wall stadiometer and an electronic scale, respectively. During the measurement, children were asked to be barefoot and minimally dressed. Body Mass Index (BMI) is calculated as body weight (kg)/height (m). All measurements are repeated twice and averaged for statistical analyses.[15,16]

Six-Minute Walk Test

Six-Minute Walk Test (MWT) was used to assess cardiorespiratory fitness in preschool children. Six-MWT is similar to the shuttle run test assessing cardiorespiratory fitness in preschool children. It has the advantage of being more common, and it can be conducted with a single researcher, without using a video signal and tape.[17]

Six-MWT is a very appropriate method used in a cheap, equipment-free, practical way assessing functional exercise capacity at a submaximal level with one's own walking speed. Validity studies of Six-MWT have been conducted in healthy children and adolescents, and its standard value has been specified. According to this test, children were asked to walk as fast as possible on a flat, hard surface for 6 minutes and were encouraged verbally for test. The test distance in the pediatric population is recommended as 15-20 m, and is determined as 20 m.[18] The distance measured was marked with band and the cones were placed at both ends. The total distance walked by children was recorded (m).[17]

Handgrip Strength Test

Hand dynamometer (TKK 5001, gripA, Takei, Tokyo; range 0–100 kg; accuracy 0.5 kg) was

used to assess the muscular strength of the upper body and upper extremity with a grip span of 4 (cm). Hand grip strength test assesses isometric muscle strength of the upper body and upper extremity. During the measurement, the children standing in a bipedal position were asked to squeeze the dynamometer continuously for at least 2 or 3 seconds without bending the elbow and contacting the body. The best value of two trials for each hand was chosen, and the average of both hands was registered (kg).[15,16]

SLJ Test

The SLJ test was used to assess the muscular strength of the lower body and lower extremities. This test consists of jumping as far as possible behind the starting line with feet together (separate from each other approximately at the shoulder's width on a hard, non-slippery surface,) and remaining upright. Children performed three jumps and the best of these attempts was recorded (cm).[15,16] The starting line was marked, and the jump distance was glued to the band and scaled (cm).[15]

SRT

The SRT was used to assess speed and agility. The test consists of running on a non-slippery surface between the two parallel lines (10 m apart) drawn on the floor, covering a distance of 40 m and running back and forth as fast as they can. In each round, the children had to cross the finish line with both feet and touch the hand of the researcher. Children tried the test for a second time after a short rest. The best result (minimum score) of two attempts was recorded (sec).[15,16] While the tester was on one side of the parallel lines during the test, one of the parents was placed to the other side of the parallel lines.[15]

One-leg Stance Test

One-leg stance test was used to assess the balance. According to this test, which assesses the static balance, the children maintained their balance in this position as much as they could stand on one leg while the other leg was bended from the knee. The chronometer was activated immediately after the children had lifted the free leg from the floor. Children were allowed to use their arms if it was necessary to maintain the balance position as long as possible. The test ended when the children couldn't maintain the required position, i.e. holded something, moved the supporting foot, heel or toe of the original position. The test was performed once with each leg and the mean of two attempts was registered (sec).[15,16]

Statistical analysis

Statistical analyses were performed using IBM Statistical Package for the Social Science software version 23. Visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov / Shapiro Wilk's test) were used to determine whether or not the numerical variables were normally distributed. While investigating the associations between non-normally distributed and/or ordinal variables, the correlation coefficients and statistical significance were calculated using the Spearman test. The descriptive analyses were presented using means and standard deviations for the numerical variables which show normal distribution in the comparisons, medians and interquartile range (IQR) for the numerical variables showing non-normal distribution, number and percentage values for categorical variables. The Independent Sample t test was used for the comparison of two groups with normal distribution, while the Mann-Whitney U test was used to compare two groups with at least one non-normal distribution. When comparing categorical independent two groups, Chi-square or Fisher's exact (when chi-square test assumptions do not hold due to the low expected cell counts) test was used. The total type-1 error level was accepted as 5% and the p-value as smaller than 0.05 for statistical significance.

Results

The study population included 212 preschool children without any known health problems, 107 girls and 105 boys, between the ages of 4-6 years. Out of 212 children, 19 (8.9%; 3 girls and 16 boys) who had MND were compared with the rest of the study population (Table I).

Physical fitness and developmental parameters of children who were detected to have MND according to the Touwen examination and their healthy peers were compared (Table II). There was a significant difference in terms of physical fitness (one-leg stance) and DDST II sub-parameters (personal-social, fine motor, gross motor) ($p < 0.05$). There was no significant difference for the other physical fitness parameters and language parameter ($p > 0.05$). Children with MND performed lower scores in physical fitness (one-leg stance) and DDST II sub-parameters (personal-social, fine motor, gross motor) compared to their healthy peers. Although not statistically significant, children with MND performed lower scores clinically

Table I. Descriptive characteristics of the study population.

			Mean±SD (n=212)	Min-Max
Age (months)			62±7	44-75
Weight (kg)			19.3±3	13.2-32.5
Height (cm)			109±6	92-123
BMI (kg/m^2)			16.2±1.7	12.7-22.5
	Physical fitness	Handgrip (kg)	7±1.5	5-14.2
		One-leg stance (s)	18.6±14.3	3.5-94
		SLJ (cm)	82±17	37-120
		4x10 m SRT (s)	17.55±2	14-25
		Six-MWT (m)	396±52	290-560
			n (%)	
Gender		Girls	107 (50.5)	
		Boys	105 (49.5)	
DDST II		Normal	129 (60.8)	
		Suspicious	73 (34.4)	
		Abnormal	10 (4.7)	
	DDST II sub-parameters: Personal-social	Abnormal	21 (9.9)	
		Normal	191 (90.1)	
	DDST II sub-parameters: Fine-motor	Abnormal	25 (11.8)	
		Normal	187 (88.2)	
	DDST II sub-parameters: Language	Abnormal	55 (25.9)	
		Normal	157 (74.1)	
	DDST II sub-parameters: Gross-motor	Abnormal	68 (32.1)	
		Normal	144 (67.9)	
MND		Normal	193 (91.0)	
		Simple MND	18 (8.5)	
		Complex MND	1 (0.5)	
Neurological status		MND	19 (8.9)	
		Normal	193 (91.1)	

BMI: body mass index, DDST: denver developmental screening test, MND: minor neurological dysfunction, MWT: minute walk test, SD: standard deviation, SLJ: standing long jump, SRT: shuttle run test.

Table II. Comparison of physical fitness and developmental parameters according to neurological status.

			Neurological status		p
			Normal	MND	
			n=193	n=19	
			Mean±SD	Mean±SD	
Physical fitness	SLJ (cm)		82±16	79±21	0.459
			Median (IQR)	Median (IQR)	
	Handgrip (kg)		6.7 (1.7)	6.8 (2.7)	0.299
	One-leg stance (s)		15 (16)	9 (7)	0.001*
	4x10 m SRT (s)		17 (2.1)	17 (4)	0.452
	Six-MWT (m)		390 (70)	375 (84)	0.262
			n (%)	n (%)	
DDST II sub-parameters	Gender	Girls	104 (53.8)	3 (15.8)	
		Boys	89 (46.2)	16 (84.2)	
	DDST II	Abnormal	2 (1.0)	8 (42.1)	
		Suspicious	64 (33.2)	9 (47.4)	0.000*
		Normal	127 (65.8)	2 (10.5)	
	Personal-social	Abnormal	13 (6.7)	8 (42.1)	0.000*
		Normal	180 (93.3)	11 (57.9)	
	Fine motor	Abnormal	16 (8.3)	9 (47.4)	0.000*
		Normal	177 (91.7)	10 (52.6)	
	Language	Abnormal	46 (23.8)	9 (47.4)	0.050
		Normal	147 (76.2)	10 (52.6)	
	Gross motor	Abnormal	54 (28.0)	14 (73.7)	0.000*
		Normal	139 (72.0)	5 (26.3)	

DDST: Denver developmental screening test, MND: minor neurological dysfunction, MWT: minute walk test, SD: standard deviation, SLJ: standing long jump, SRT: shuttle run test, IQR: interquartile range, *, $p < 0.01$,

than their healthy peers in terms of other physical fitness parameters (SLJ, handgrip, one-leg stance, 4x10 m SRT, Six-MWT) and DDST II sub-parameter (language). Children with MND performed worse than their peers in physical fitness and developmental parameters.

The relationship between the physical fitness and developmental parameters was also analyzed (Table III). When positive, weak, statistically significant correlation was found between DDST II and physical fitness (one-leg stance) ($p<0.05$); there was no significant relationship between other physical fitness parameters ($p>0.05$). As the developmental parameters of preschool children get closer to normal, there was an improvement in balance.

Table III. The relationship between DDST II and physical fitness in preschool children.

		DDST II	
		r	p
Physical fitness	Handgrip (kg)	-0.032	0.648
	One-leg stance (s)	0.231	0.001*
	SLJ (cm)	0.095	0.170
	4x10 m SRT (s)	-0.098	0.155
	Six-MWT (m)	0.077	0.267

*, $p < 0.01$

DDST: Denver developmental screening test, MWT: minute walk test, SLJ: standing long jump, SRT: shuttle run test.

Discussion

Developmental parameters and physical fitness of children in preschool period with a special focus on MND was evaluated in this study. Preschool children with MND had low developmental parameters and physical fitness compared to their healthy peers.

The incidence of MND in children varies according to gender, age, and prematurity.[4,19] To the best of our knowledge, our study is the first to evaluate incidence of MND in preschool children without any known health problems in our country, and was determined to be 8.9% which was in line with the literature.[20]

In the field of neurodevelopmental disorders, MND is studied under the umrella of Developmetal Coordination Disorder (DCD) as many children with DCD show MND.[21,22] MND, which is thought to stem from minor aberrations of brain function undetectable on conventional neurological examination methods, is described on the problems of coordination which are uncertain movements in daily life activity, such as, impaired posture and muscle tone, insufficient gross and fine motor movements.[23] Studies have also indicated that MND can be associated with learning and cognitive problems in children other than motor impairments.[24,25] As for DCD, it is defined by the following four criteria: (1) acquiring and execution of coordinated motor skills is far below expected level for age, given opportunity for skill learning; (2) motor skill difficulties significantly interfere with daily life activities and impact academic/school productivity, prevocational and vocational activities, leisure and play; (3) onset is in the early developmental period; (4) motor skill difficulties are not better explained by intellectual delay, visual impairment, or other neurological conditions that affect movement.[25] In other words, DCD has impairments the levels of the International Classification of Functioning, Disability and Health (ICF) such as body structure and functions (motor, sensory, cognitive, emotional), daily life activities (basic and instrumental skills), participation (at home, school and community), personal and environmental factors.[25] To our knowledge, the physical fitness of preschool children with MND has not been investigated in the literature. For this reason, the physical fitness part of our study was discussed in line with DCD contaning the MND.[21,22]

The assessment of physical fitness in children is carried out in order to determine the physical fitness level, as well as to plan exercise programs and to follow-up progression.[26] A systematic review revealed that physical fitness in children with DCD were significantly lower than their healthy peers.[27] Tsiotra et al.[28] in a study comparing children's physical fitness, clinically observed that, children with DCD showed lower performance than their healthy peers in terms of all physical fitness tests, but the statistical differences were shown in the handgrip strength, vertical jump and 40 m speed tests. Similarly, children with DCD had a statistically lower performance than their healthy peers in physical fitness tests (Six-MWT, 20 m running, jumping and ball throwing tests).[29] Ferguson et al.[30] observed that children with DCD had significantly lower handgrip strength, SLJ, 20 m SRT compared to their healthy peers. Focusing on MND population, as in DCD, we observed that children with MND showed statistically lower performance in terms of physical fitness (one-leg stance test) compared to their healthy peers. The relationship between the neurological status and physical fitness in our preschool children population also supported this result. In other words, physical fitness of children increases with being close to normal neurological status.

Minor Neurological Dysfunction is associated with impaired motor performance,[31] difficulties in learning, spelling, literacy and arithmetic skills;[32] as well as behavioral problems such as attention-deficit and social problems in school-age children.[31] In many studies involving preterm infants who were followed up until school age, it was reported that children detected with MND at school age performed lower scores in areas such as gross-motor, fine-

motor, cognitive, daily life activities compared to their healthy peers.[24,33]

Similar to the studies mentioned above, we observed that preschool children with MND had lower performance scores in DDST sub-parameters, thus, developmental parameters are more behind compared to their healthy peers. Although language domain was also impaired, there was no statistically significant difference, which can be attributed to small sample size for this parameter.

The activities of a child affected by a developmental disorder are restricted in daily life, and the child has premature fatigue, as well as decreased exercise performance and physical fitness.[34] Physical fitness levels of children with neuromotor developmental delay are lower than healthy children. The low physical fitness and long reaction times in these children are an important problem that prevents these children to perform activities independently. There are also problems in appropriate stimulus selection, adaptive response, and recording of perceived information.[35] In a systematic review evaluating the studies conducted on school-age and adolescent children with neuromotor developmental delay, physical fitness parameters of children were significantly lower compared to their healthy peers.[27] In another study, it was observed that children with neuromotor developmental delay showed significantly lower performance in terms of physical fitness (Six-MWT, muscle strength and endurance, speed tests, 5x10 m agility test and balance test) compared to their healthy peers.[34]

There are limitations of this study such as; evaluating only children from a certain geographic location, including children only attending to kindergarten which may have an effect on developmental parameters, a limited number of children with developmental delay assessed with DDST II, and PREFIT Test Battery lacking an adaptation to Turkish children.

Beyond these limitations, to our knowledge, this is the first study to investigate the relationship between physical fitness and developmental parameters in preschool children, and positive, significant correlation was found between the DDST II and the physical fitness (one-leg stance test) in this age group. In other words, as the developmental parameters of children get closer to normal, their balance increases. Thus, the relationship between these children's developmental parameters and their physical fitness can be explained. Assessment of physical fitness or developmental parameters is important for this integrity.

In conclusion, in our study population, 8.9% of preschool children without a known health problem had MND which affects physical fitness and developmental parameters. The relationship between developmental domains and physical fitness in preschool children shows that evaluation should cover all parameters. Identifying children who have problems at an early stage will help to timely implement supportive measures in terms of physiotherapy and rehabilitation.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AM, HA, GH; data collection: HA; analysis and interpretation of results: HA, AM, GH; draft manuscript preparation: HA, AM, GH. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Ethical approval was obtained on 29/11/2017 from Muş Alparslan University Publication Ethics Board, with the decision number E13931, and decision number 2.

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Conflicts of interest

The authors declare no conflicts of interest.

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Original Article

Expression of MicroRNA 146a, 155, 181 and 223 in febrile seizure

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ABSTRACT

Background. We studied microRNAs (miRNAs) -146a, -155, -181 and -223 expressions and proinflammatory cytokine levels in children with Febrile seizure (FS) and compared to febrile controls.

Methods. This prospective multicenter study examined representative populations in eight different cities in Turkey between June 30, 2018 and July 1, 2019. Blood samples were taken from all children at presentation. The real time (RT) polymerase chain reaction (PCR) were used to measure the expressions of microRNAs and tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6) levels were studied by enzyme-linked immuno-sorbent assay.

Results. The study was conducted with 60 children; 30 children with FS and 30 children in the febrile control group. The seizure was classified as simple FS in 73.3 % and half of the children were experiencing their first FS episode.

Although the expression levels of miRNAs-146a, -181a and -155 were higher in febrile seizure patients, only miRNAs 146a level was significantly higher in FS patients. Serum TNF-α, IL-1β, IL-6 levels were higher in the FS group than the controls. The results of statistical analysis showed that there were correlations within miRNA expressions in children with FS. No differences were found considering miRNA expression between FS type, number of FS experienced.

Conclusions. miRNAs-146a, -181a, -155 and -223 may be involved in FS pathogenesis. Altered miRNA expression levels might be an adaptive response to inflammation. New therapeutic approaches might be developed based on miRNA expressions in children with FS.

Key words: febrile seizure, microRNA, proinflammatory cytokine.

The pathophysiology of febrile seizure (FS) is not fully understood, and research results have revealed a complex interaction of inflammation, genetic tendency and cytokines. Tumor necrosis factor alpha (TNF- α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6) are the main cytokines involved in the pathophysiology of FS.[1,2]

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MicroRNAs (miRNAs) are small non-coding RNAs, and their main target is messenger RNAs (mRNAs). More than two thousand miRNAs have been identified thus far, and more than half of these are expressed in the human brain.[3] Alteration in the expression levels of specific miRNAs has been suggested as a possible cause in the pathophysiology of different diseases, such as cancer, Parkinson disease and epilepsy.[3,4]

miRNAs have emerged as potent regulators of inflammation.[4,5] For example, miRNA-146a is the first inflammation-associated miRNA.

It has been shown to regulate the expression of toll-like receptors and cytokine pathways. Both miRNA-146a and IL-1β were upregulated in astrocytes in epilepsy models. The purpose of miRNA-146a expression in astrocytes may be to modulate the inflammatory response stimulated by pro-inflammatory cytokine IL-1β.[5,6] miRNA-146a is significantly upregulated in tissues obtained from patients with mesial temporal lobe epilepsy (MTLE), more than half of whom had a history of FS in childhood.[5-7] In addition, increased expression of miRNA-155 has been observed in the hippocampal tissue of children with MTLE, and this increase was correlated with an increase in TNF-α in nervous tissue.[8]

Respiratory viral infections are the most common cause of fever in children with FS.[9] The miRNA system modulates viral replication and pathogenesis.[10] Studies have shown that many miRNAs have high expression, whereas thirty-five miRNAs have low expression following adenovirus infection, which commonly affects children with FS. Downregulation of miRNA-146a and miRNA-155 in patients with influenza A virus has also been reported.[11] The role of miRNAs in the pathophysiology of FS has rarely been investigated.[12,13] Thus, in the present study, we aimed to investigate miRNAs 146a, 155, 181 and 223 expressions in children with FS and compare these to levels in febrile controls.

Material and Methods

This prospective multicenter study was conducted in eight different cities between June 30, 2018 and July 1, 2019. The study was approved by the Local Ethical Committee of Eskisehir Osmangazi University (IRB Number 2020/55, Date: 02.03.2020) and supported by a university research grant. Written informed consents were obtained from the parents of all the participating children.

At each site, all children with seizures were evaluated for a one-year period, and children with FS were enrolled in the study. FS in each child was classified.[14] The childrens' previous medical history and demographical features were recorded. Detailed physical examinations were performed, including neurological examinations. The control group included age-matched children who were diagnosed with febrile disease but who did not have seizures and had no known history of previous FS. The febrile diseases include respiratory tract infections, gastrointestinal infections and other viral infections.

Blood samples (3 cc) were obtained within one hour after the seizure in a serum separation tube (SST). The serum samples were immediately separated by centrifugation and stored at -80 °C in a freezer.

Cytokine analysis: All those stored were analyzed using commercially available assay kits according to the manufacturer's instructions. Serum IL-6 values were determined using ELISA technique (Bioassay Technology Laboratory, Shanghai, China). The minimum detectable IL-6 concentration was 0.092 pg/ml. Serum TNF- α values were also measured using an ELISA technique (Bioassay Technology Laboratory, Shanghai, China). The minimum detectable TNF-α concentration was 1.52 pg/ml. Serum IL-1β values were determined using ELISA technique (Bioassay Technology Laboratory, Shanghai, China). The minimum detectable IL-1β concentration was 0.02 pg/mL. The intraassay coefficient of variations for all three cytokines were below 8%, and inter-assay coefficient of variations were below 10%.

Total RNA Extraction: Total RNA extraction was done by using a TRIzol™ Reagent kit according to the manufacturer's protocol for serum/plasma samples. In short, 200 µl human serum was added into 1000 µl TRIzol™ reagent and after vortexing incubated at room temperature for 2 min. Following the phase separation by adding chloroform with centrifugation, upper aqueous phase (containing RNA) was mixed with 500 µl of 2-Propanol. The supernatant was discarded after centrifugation. The pellet was resuspended with 75% ethanol, centrifuged

and air dry. Thirty µl of nuclease-free elution buffer was carefully added to resuspend the pellet. Then the amount of nucleic acid in the total RNA samples by fluorescence spectrophotometry (Colibri Microvolume Spectrometer, Titertek-Berthold, Germany) was measured. RNA concentrations ranged from 100 to 400 ng/µl, and the total RNA purity was verified using of A260/A280 ratios (range 1.81–1.97). The integrity of the serum RNA could not be assessed by gel electrophoresis because the levels were very low amounts in the serum and plasma. DNase I enzyme (Arcticzymes, Norway) was used to remove DNA from serum RNA by fixing 100 ng/µl.

Poly(A) Polymerisation and Reverse Transcription: In this step, poly(A) tails were added to the 3′ end of miRNAs with Poly(A) Polymerase, Yeast (ABM) kit. The reaction mixture consisted of 5 µl of 5X buffer, 11.25 µl of nuclease-free water, 1 µl Poly(A) Polymerase (1 U/µl), 1.25 µl of ATP (10mM), 2.5 µl of MnCl2 (25 mM), 5 µl (500 ng) of RNA for a 25 µl reaction and incubate at 37 °C for 20 min and 65 °C for 20 min in in Veriti™ 384-Well Thermal Cycler (Life Technologies, Carlsbad, CA, USA). These modified miRNAs were then reverse transcribed using a stem-loop poly(A) tailed RT-PCR. The reaction mixture consisted of 2 µl of M-MuLV reaction buffer (2x), 10.5 µl of nuclease-free water, 1 µl of M-MuLV RT enzyme (200U/µl), 1.25 µl of dNTP (10mM), 1 µl of miRNA specific RT primer (10mM), 0.25 µl of RNase Inhibitor (40U/µl) and 5 µl of poly(A) tailed RNA for a 20 µl reaction. Reverse transcription was performed for OneScript® Plus cDNA Synthesis (ABM) in in Veriti™ 384-Well Thermal Cycler (Life Technologies, Carlsbad, California, United States) at 42 °C for 60 min, heat inactivate the reverse transcriptase at 85 °C for 10 min.Then the product was immediately cooled to 4 °C and stored at -20 °C.

Detection of miRNAs by qPCR: The RT-qPCR reactions were performed and monitored using a real-time ABI Prism 7500 FAST qPCR System from Applied BioSystems (Life Technologies, Carlsbad, California, United States). Real time PCR was performed in duplicate for each miRNA and included non-template control. Human small non-coding RNAs miR181a, miR155, mir146a and miR223 designs (Diagen, Ankara, Turkey) were used for study and endogenous control small ncRNA SNORD47 was chosen as the internal control for normalization. The PCR reaction consisted of, 10 µl of Diagen 2X PCR Master Mix solution (2x), 3.5 µl of nuclease-free water, 3.5 µl of miRNA specific Primer Mix and 3 µl of poly(A) tailed miRNA for 20 µl reaction. Amplification was performed and monitored using a real-time instrument ABI Prism 7500 FAST qPCR System from Applied BioSystems (Life Technologies, Carlsbad, California, United States) at 95 °C for 10 min, followed by 40 cycles at 95 °C for 10 sec, 55 °C for 30 sec, 72 °C for 5 sec. Real time PCR raw fluorescence data were analyzed using 7500 Fast Software v2.0.6 with automatic baseline and threshold setting for quantification cycle (Ct) determination. PCR amplification efficiencies test were calculated to verify the primer specificity and sensitivity by use of the formula E = (10-1/slope - 1) 9 100 and to control the PCR yield was demonstrated by the synthetic miRNA sequence of each miRNA. The qPCR Ct values of the samples included in the target and control groups were calculated as fold changes in the gene expression analysis. Fold Change value is qPCR gene expression ratio of each sample. In this study, fold change values were calculated of the miRNAs miR181a, miR155, mir146a and miR223 with the comparative $2(-\Delta\Delta Ct)$ relative expression method to reference gene SNORD47. The gene expression fold change values for each gene in each sample were transformed to log2.The fact that the fold change data give extremely low or high expression gene expression coefficients affects the data distribution in statistical analyses. The purpose of logarithmic transformation is to make gene expression level more specific and to show normal distributions.[15,16] In this way, the expression load is equalized in cases of over-expression and low expression of genes, and the effect of extreme values is also eliminated.

Statistical analysis: The expression levels of miRNAs and serum cytokine levels were

presented as median, minimum and maximum values. The non-parametric tests were performed because of small sample size and the data that were not normally distributed were analyzed with Shapiro-Wilk's test. The Mann-Whitney U test was used to compare miRNA and cytokine levels between the two groups. To further potential any interrelationship, Spearman rank correlation test was performed. A p value <0.05 was deemed statistically significant. The analyses were performed with SPSS for Windows 15.0 (Chicago, IL, United States)

Results

The study was conducted with 60 children: 30 children with FS and 30 children in the febrile control group. Half of the children (15 out of 30; 50%) were experiencing their first FS episode. In 73.3% of the children (22/30), the seizures were classified as simple FS. A total of 56.6% (17/30) of the children had a family history of FS. The children's demographic and clinical features are summarized in Table I. Serum IL-6, TNF-α, and IL-1β were higher in febrile seizure group than in the controls ($p<0.01$, $p<0.01$ and $p<0.05$, respectively) (Table I).

miRNA expression analysis revealed an alteration in children with FS compared to the controls. Serum median miRNA-181, miRNA-155, miRNA-146a, and miRNA-223 levels were higher in febrile seizure group than in the control group, however, the expression levels of miRNAs 146a were significantly increased in FS patients ($p<0.05$) (Table II). No differences were found in miRNA expression between FS type and the number of seizures experienced ($p>0.05$). There were correlations between levels of TNF-α, IL-1β and IL-6 ($p<0.001$). However, no relation was found between miRNA expression and TNF-α, IL-1β and IL-6 (Table III).

Table I. Demographic, clinical findings and serum TNF-α, IL-1β, and IL-6 levels of the study group.

	Febrile seizures n (%)	Control group n (%)	p-value
Age (months) (mean ± SD; min-max)	25.17±13.23 (10–60)	40.16±18.23 (6–60)	p>0.05
Gender (boys/girls)	15/15	20/10	p>0.05
Source of fever			
Upper respiratory tract infections	23 (79.3)	25 (83.3)	p>0.05
Other infections	7 (10.7)	5 (16.7)	p>0.05
TNF-α (pg/ml)	515.58	212.58	p<0.01
Median (min-max)	(31.96–1127.71)	(39.27- 423.42)	
IL-1β (pg/ml)	3923.54	1747.13	p<0.05
Median (min-max)	(280.83–8220.80)	(221.48–7817.39)	
IL-6 (pg/ml)	326.79	109.59	p<0.01
Median (min-max)	(13.64–728.53)	(21.42–610.72)	

Table II. MicroRNA 146a, 155, 181 and 223 expression.

MicroRNA	Ct Value	Febrile Seizure Group Fold Change Median $2^{-\Delta\Delta Ct}$ (min–max)	Control Group Fold Change Median $2^{-\Delta\Delta Ct}$ (min–max)	p-value
MicroRNA-181	20.4 – 28.1	-0.4240 (-1.50–1.57)	-0.1490 (-1.77–2.17)	p>0.05
MicroRNA-155	26.1 – 32.2	-0.6200 (-1.92–0.88)	-0.0150 (-2.69–1.57)	p>0.05
MicroRNA-146a	15.2 - 27.4	-1.0600 (-2.39–1.60)	-0.0980(-1.66–2.68)	p<0.05
MicroRNA-223	17.7 - 23.7	-0.4010 (-1.69–2.15)	-0.0820 (-2.04–2.39)	p>0.05

Table III. The correlations between MicroRNAs; MicroRNAs and cytokines.

	MicroRNA-181	MicroRNA-155	MicroRNA-146a	MicroRNA-223	TNF-α	IL-1β	IL-6
MicroRNA-181	-	0.663	0.365	0.206	-0.92	0.037	-0.77
		<0.001	0.047	0.274	0.629	0.845	0.685
MicroRNA-155			0.469	0.377	0.218	0.288	0.199
			0.009	0.040	0.247	0.123	0.291
MicroRNA-146a				0.299	0.66	0.130	0.36
				0.109	0.728	0.495	0.850
MicroRNA-223					0.095	0.157	-0.039
					0.617	0.406	0.837
TNF-α						0.940	0.951
						<0.001	<0.001
IL-1β							0.871
							<0.001
IL-6							-

The level of miRNA values in predicting patient and control were analyzed using ROC (Receiver Operating Characteristics) curve analysis. When a significant cut-off model was observed, it was presented as p value, area under curve (AUC) with 95% confidence interval and cut-off value which has maximum value calculated by dividing sensitivity/specificity. According to these; the cut-off value for miR155 was 0.834 (AUC:0.69, 95%CI: 0.55-0.82, p=0.013). The cut-off value for miR146 was 1.443 (AUC:0.67, 95%CI: 0.53-0.81, p=0.028).

Discussion

This prospective multicenter study found that the expression levels of miRNA 146a were significantly increased in FS patients. Although previous reports support the hypothesis that miRNAs may contribute to the pathogenesis of epilepsy, studies on the role of microRNAs in the pathophysiology of FS are limited.[5,6,12,13,17-22]

Several pro-inflammatory cytokines increase during FS, and, as a consequence, their related miRNAs are also affected.[4,6] Omran et al.[7] revealed a negative correlation between IL-1β and miRNA146a expression. This significant increase in miRNA146a expression and its association with the low level of IL-1β might suggest that the purpose of miRNA146a expression is to modulate the inflammation stimulated by IL-1β. In contrast, the results of the present study showed a positive correlation between miRNA146a and IL-1β level. The genetic polymorphisms might play a role in this correlation. A study conducted by Issac et al.[12] revealed that rs2910164 polymorphism in the pre-microRNA-146a gene might be accompanied by an upregulation of proinflammatory cytokines.

The present study found increased miRNA-223 expression in FS patients than controls, without statistical significance. Wang et al.[13] used the hot water-bath box method with the FS model and examined the role of miRNA-223 in pathogenesis of FS. They found that, compared to normal controls, the expression of miRNA-223 in hippocampal tissues of rats in the FS group was significantly decreased, suggesting low expression levels of miRNA-223. The seizure latency was markedly prolonged while the seizure duration was significantly shortened for rats injected with miRNA-223. The results demonstrate that upregulating the expression of miRNA-223 can improve seizures in FS rats.

Lumbar puncture and cerebrospinal fluid (CSF) analysis are not recommended routinely

to evaluate FS patients so was not conducted in the present study. In a recent study, Kim et al.[23] searched the miRNA profile of CSF in FS patients. They reported 95 miRNAs were significantly higher in patients than in controls and the top 5 highly expressed miRNAs were miRNA-4486, miRNA-6850-5p, miRNA-642-3p, miRNA-7107-5p and miRNA-4281. None of these miRNAs were searched in the present study.

FS is associated with a higher risk of epilepsy in general and especially in temporal lobe epilepsy (TLE).[24] Ren et al.[25] found miRNA-181a to be increased in the temporal lobe tissues of children with intractable epilepsy. In addition, miRNA-146a and miRNA-155 were also significantly upregulated in tissues obtained from patients with MTLE.[5-8] Another study found that increased miRNA-155 expression was correlated with increased TNF-α in nervous tissue.[6] However, our results revealed no relationship between miRNAs and proinflammatory cytokines (TNF-α, IL-1β, IL-6).

It has been speculated that modulation of miRNA expression might be a therapeutic model for seizures. In a pilocarpine-induced TLE mouse model, intranasal delivery of miRNA146a antagonist reduced the percentage of animals with seizure onset to 6.7% and also resulted in an increase in seizure latency.[26] This may have been the result of a decrease in inflammatory modulators and cytokines, such as nuclear factor kB (NF-kB), TNF-α, IL-1β and IL-6. An intraventricular injection of miRNA-181a-mimic produced neuronal death in rats, whereas antagomirs against miRNA-181a reduced neuronal death after status epilepticus.[12,27]

Viral respiratory infections are commonly associated with FS. Our previous study showed that the most frequently detected viruses were adenovirus, influenza A and influenza B.[16] Human miRNAs have been suggested to have their evolutionary origin as an innate immune defense mechanism against viral infection. The miRNA system modulates viral replication and pathogenesis in several ways: a) respiratory cell miRNAs can affect viral replication; b) some viruses also encode miRNAs, and viral-encoded miRNAs target cellular genes involved in cell proliferation and anti-viral response; and c) viral-encoded miRNAs may regulate viral gene expression.[11,28]

A study conducted by Qi et al.[29] revealed that the expression levels of many miRNAs increase while the levels of more than thirty miRNAs decrease after adenovirus infection, which is one of the common viruses that affect FS patients. A recent study showed a unique expression pattern of microRNAs in influenza infection and decreased expression of miRNA-155, miRNA-146b, miRNA-29, miR-150, miRNA-299-5p and miRNA-335.[30] Huang et al.[31] showed an accumulation of miRNA-146a, miRNA-7, miRNA-132, miRNA-187, miRNA-200c and miRNA-1275 in lung tissue infected with the influenza A virus. In the present study, we did not search for respiratory viruses; therefore, we cannot analyze the correlation between viruses and miRNA.

The present study has some limitations. miRNA expression was not searched in healthy children. Since most of miRNAs analyzed in the present study were inflammation-related, febrile children were preferred as controls.

In conclusion, miRNAs -146a, -181a, -155 and -223, especially miRNA-146a might be involved in FS pathogenesis. Altered miRNA expression levels might be an adaptive response to inflammation. New therapeutic approaches based on miRNA expression could provide new perspectives for FS treatment.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: KBÇ; data collection: YK, GGM, AE, PP, ÇY; analysis and interpretation of results: KBÇ, DA, EÇD; draft manuscript preparation: KBÇ, EÇD. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Local Ethical Committee of Eskisehir Osmangazi University (IRB Number 2020/55, Date: 02.03.2020) and supported by a university research grant.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Original Article

Developmental evaluation in children experiencing febrile convulsions

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ABSTRACT

Background. The objective of this study was to determine the effect of febrile convulsion (FC) on neuromotor development.

Methods. Data of 325 patients, who were followed up at our outpatient clinic and diagnosed with FC between January 2012 and December 2018, were retrospectively evaluated. Of these patients, 203 underwent the Denver Developmental Screening Test II (DDST II) and were included in the study as the patient group and 100 healthy children as the control group.

Results. Of the study group, 84 (41.4%) were girls and 119 (58.6%) were boys (B/G: 1.4). Of all patients, 163 (80.3%) were diagnosed with simple FC, 22 (10.8%) with complicated FC, and 18 (8.9%) with FC+. There was no significant relationship found between FC subtypes and gender, family history of FC, family history of epilepsy, iron (Fe) deficiency, and Fe deficiency anemia. DDST II subtest points were significantly lower in all developmental areas in the patient group when compared to the controls ($p<0.001$), while suspected and abnormal test results were higher in all developmental areas in the patient group compared to the controls ($p=0.01$). It was also determined that the language points were lower as the age of first seizure increased ($r=-0.319$, $p<0.01$).

Conclusions. Although FC is known to usually having a good prognosis, the low DDST II test results measured in this study indicated that the FC may pose a developmental risk and patients with FC should be followed up in terms of developmental features. Because of the retrospective nature of the study, there was no "pre-convulsion" developmental evaluation. This is a major limitation of our study.

Key words: DDST II, febrile convulsion, neuromotor development, prognosis.

Febrile convulsions (FCs) are the most commonly seen age-related seizures in childhood usually with a good prognosis. These seizures have been defined by the International League Against Epilepsy (ILAE) as convulsions observed in the febrile conditions in children who had not experienced an afebrile convulsion previously, without having a cause such as a central nervous system infection, electrolyte imbalance, metabolic disorder, trauma, and intoxication or any other cause.[1] The age range of FCs has been reported between 1 month and 8 years though the highest incidence occurs between 18-22 months.[2,3] FCs have been reported higher in boys than in girls (B/G: 1:1-1.7:1).[4] Although its pathogenesis is not fully understood, the association of the FC with numerous factors has been reported, including infections related to inflammatory mediators, cytokines, iron (Fe) deficiency anemia, mineral deficiencies, high fever, and genetic predisposition.[5,6]

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FCs are known to have a good prognosis. However, there are limited studies reporting the language, motor, behavior, attention, cognitive functions, and developmental disorders in patients with the FC.[7-9] Denver Developmental Screening Test (DDST II) is a test that is used to detect the developmental status of healthy children in the personal-social, language, fine and gross motor areas by comparing them with their healthy peers of the same age.

In this study, we aimed to determine the neurodevelopmental status of children diagnosed with FC by comparing them with healthy controls using the DDST II in order to determine the effect of the FC on neuromotor development.

Material and Methods

In this study, the patients, who were diagnosed with FC at Mersin University Medical Faculty, Pediatric Neurology Outpatient Clinic and underwent the DDST II between January 2012 and December 2018, were evaluated retrospectively. The study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee of Mersin University (2020:2020/221). The inclusion criteria for the patient group were being between the ages of one month and six years, being monolingual, observing an increase in the body temperature above 38°C, not having a central nervous system infection, ruling out the other factors causing convulsions, not having any other chronic disease, and complying with the DDST II test application and determined according to the ILAE criteria for the FC diagnosis.[1] The exclusion criteria were included being out of the specified age range, being bilingual, fever and/or central nervous system infection during the seizure, the presence of other secondary causes that may affect neurodevelopment and/or lead to convulsions, those diagnosed with epilepsy later, patients with missing data and incompliance to the test application. According to the inclusion and exclusion criteria, a study flow diagram has been given in Figure 1. Of the participants, 122 patients were excluded from the study group due to incompliance with the DDST II test. Incompliance to the DDST II was described as a refusal of the test at least two times. An additional 14 (11.5%) patients were excluded from the study since they had afebrile seizures and 46 (37.7%) patients were excluded due to missing data. The number of patients who did not complete the DDST II test was 42 (34.4%) whereas the numbers of patients diagnosed with epilepsy later and having other secondary causes that may affect neurodevelopment and/or lead to convulsions were 13 (10.7%) and 7 (5.7%), respectively. As a result, 122 patients who met the aforementioned exclusion criteria were excluded from the study. The control group was comprised of 100 healthy children, whose age and gender-matched to children with FC and who had no systemic or neurological problems that could affect their neurodevelopment, and showed compliance to the test application. These participants were selected among the children regularly followed at the healthy children outpatient

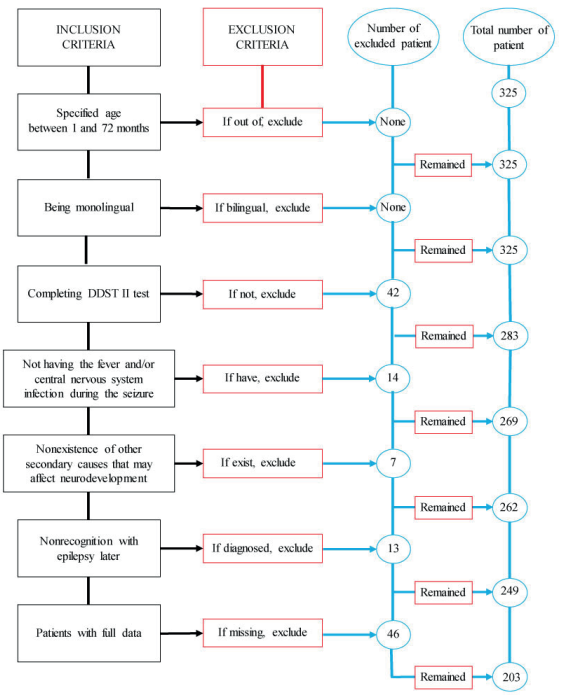

Fig. 1. Demonstrating the inclusion and exclusion criteria considered in this study.

clinic. The study and control groups were selected according to inclusion and exclusion criteria. The children included in the control group were similar to the study group in terms of socioeconomic and demographical features. Additionally, there were no bilingual children in this study.

The files of all patients were retrospectively evaluated from the time of the first seizure and recorded for the following: age, simple or complicated FC diagnosis, the number of convulsions experienced till the admission time, prenatal, natal and postnatal histories, FC and epilepsy histories in family, Fe deficiency and Fe deficiency anemia, and other factors that can influence the neuromotor development. The patients were classified according to the ILAE as simple and complicated FC and in addition to the ILAE criteria as the FC+ group. The patients having a simple FC repeating more than 3 times and a febrile status epilepticus (FSE) history and those having a complicated FC repeating more than 3 at different times were included in the FC+ group. The FC+ group was created in order to determine whether the specified factors caused a prognostic difference or not.

Denver Developmental Screening Test II (DDST II)

DDST II is a neurodevelopmental screening test developed to be carried out on healthy-appearing children between 0-6 years. The test compares the skills of children in subareas with their peers. DDST II has a significant role in screening possible developmental problems. The test can detect developmental deviations in the early screening of babies with suspected developmental delay, especially monitoring babies at risk. The test is easy to apply and interpret and only takes about 15 minutes to complete. Its other advantages are that it is easy to learn how to do and can be applied by non-physician professionals who receive training. However, it is not a test of intelligence, it cannot be employed to predict mental and adaptive ability in the future nor can it be utilized to identify learning difficulties, emotional problems, and special education requirements. The patient may require further examinations since it is a screening test. The social and cultural differences leading to errors in the evaluation can be counted among its disadvantages.

The test compares the skills of the children in the following 4 subareas with their peers: personal-social (PS), fine motor skills (FMS), language, and gross motor skills (GMS). The test form involves 134 items. The validity and reliability of the Turkish version of the test used in this study were performed by Yalaz et al.[10]

DDST II tests of all children included in the study were performed by the same person, who was trained for the test and had 10-year experience in this area. In accordance with the test application rules, it was performed on all children by allocating at least 15 minutes after they had had enough sleep and on a full stomach. Moreover, the DSST II tests were conducted at least four weeks after experiencing the convulsion to exclude the possible unpleasant experience of the patient at the health center after the FC. Both the sub developmental areas and the overall score of the DDST II was evaluated. The test result was interpreted as normal in the case of no delay and at most one caution, abnormal in the case of two or more delays, and suspected in the case of one delay and/or two or more cautions. In order to state the scores in the DDST II test, firstly, the age scale is located and each mark on the scale form represents one month till the first 24 months. After the first 24 months, each mark means a 3-month interval. Then, the age of the child is calculated. The number of items to be tested is specified after determining the age of the child. The number of items depends on the age and ability of the child. Scoring in terms of pass-child (P), fail-child (F), no opportunity (NO), and for refusal (R) is carried out after specifying the number of items. Afterward, the advanced, normal, caution, delayed, and no opportunity items are determined in regard to the testing guidelines. According to the number of cautions and delayed items, the score is designated as normal, suspected, and abnormal. Each subtest

score of the patient were separately evaluated. In each subtest, firstly, in accordance with the age of the child, the number of items in which a 90% success should be achieved by the child was determined. Afterward, the percentile score was obtained by the following formula: Subtest score = 100 x the number of items/the number of items to succeed with respect to 90%).[11-13] The 90%-reliability rate among the implementers and more than 85%-rate of yielding similar result for the repetitive measurements have been reported by Yalaz et al.[10]

Statistical Analysis

The normality of the values was analyzed using the Shapiro–Wilk test. Independent samples t-test or Mann-Whitney U test was used according to the results of Shapiro–Wilk. Spearman's correlation coefficient was calculated to determine the relationship between the continuous variables. The Chi-square test was conducted to examine the relationship among the categorical variables, and the exact test results were handled when the expected frequency percentage was lower than 25%. The comparison of the two ratios was done with the Z test when a meaningful relationship was established. Both mean±SD (standard deviation) and median (min-max) values for the continuous variables were presented whereas the categorical variables were summarized in terms of the number and percentage.

Differences were considered significant at $p<0.05$. All statistical tests were performed using software named Statistica 13.3.1.

Results

The study group consisted of 203 patients, of which 84 (41.4%) were girls and 119 (58.6%) were boys and the boys/girl ratio was 1.4. The mean age of the first seizure was 19.5±11.5 months. Of all patients, 163 (80.3%) were diagnosed with the simple FC, 22 (10.8%) with complicated FC, and 18 (8.9%) with FC+ (7 of them had the simple FC that repeated more than 3 times, 6 of them had the FSE, and 5 of them had complicated FC that repeated more than 3 at different times). The demographic features of the patient group are presented in Table I.

No significant statistical relationship was determined between the FC type and the patient's gender ($p=0.133$), the family history of FC ($p=0.558$) and epilepsy ($p=0.708$) and Fe deficiency and Fe deficiency anemia ($p=0.237$).

In the patient group Fe deficiency was seen in 50/177 (28.2%) and Fe deficiency anemia was seen in 16/177 (9%) participants. But since the control group consisted of healthy children, blood samples were not obtained from these children due to ethical reasons, so the patient and control groups could not be compared in terms of Fe deficiency and Fe deficiency anemia.

The DDST II test, which was performed at least 4 weeks after the FC, was normal in 103 (50.7%) patients, whereas suspected in 87 (42.9%) and abnormal in 13 (6.4%) patients. There was a statistically significant difference between the patient and control groups in terms of DDST II test scores ($p<0.001$).

When the patient and control groups were compared in regard to the DDST II subtest taking into consideration the normal, suspected, and abnormal scores; no significant difference was found between the two groups in terms of personal-social subtest scores ($p=0.100$). The rate of participants with suspected ($p<0.001$) and abnormal ($p=0.024$) test scores in the language area were significantly higher in the patient group compared to the control group. In addition, the numbers of suspected patients both in fine and gross motor skills were significantly higher in the patient group compared to the control group ($p=0.001$, $p<0.001$; respectively) (as indicated in Table II). There was a statistically significant negative correlation between the age of first seizure and language subtest scores in the patient group, and lower language scores were obtained as the age of the patient increased ($r=-0.319$, $p<0.001$). No statistically significant difference was found between FC subtypes in terms of DDST II test

Table I. Demographic features of the patient and control groups.

First FC age (month)		min-max 3-65 n	Mean±SD 19.5±11.5 %
Gender	Girl	84	41.4
	Boy	119	58.6
FC subtype	Simple FC	163	80.3
	Complicated FC	22	10.8
	FC+	18	8.9
FC family history	Yes	69	34.0
	No	134	66.0
Epilepsy family history	Yes	38	18.7
	No	165	81.3
Treatment	Attack treatment	115	56.7
	Intermittent prophylaxis	26	12.8
	Continuous prophylaxis	61	30.0
	No treatment	1	0.5
Iron (Fe)	Deficiency	50	28.2
	Deficiency anemia	16	9.0
	Normal	111	62.7
Control group		min-max 4-74 n	Mean±SD 27.8±15.1 %
Gender	Girl	43	43
	Boy	57	57

FC: Febrile convulsion

Table II. The relationship between the patient and control groups in accordance with subtests; personal-social, fine motor skills, language, and gross motor skills scores.

	Group		p-value
	Patient (n=203) Mean ± SD Median [min - max]	Control (n=100) Mean ± SD Median [min - max]	
Personal-social	89.2±4.3 90.0 [60.0 - 90.0]	90.0±0.0 90.0 [90.0 - 90.0]	0.045
Fine motor skills	88.2±6.2 90.0 [51.0 - 90.0]	90.0±0.0 90.0 [90.0 - 90.0]	0.002
Language	85.6±8.5 90.0 [51.0 - 90.0]	90.0±0.0 90.0 [90.0 - 90.0]	<0.001
Gross motor skills	87.3±6.8 90.0 [64.0 - 90.0]	90.0±0.0 90.0 [90.0 - 90.0]	<0.001

results.

There were statistically significant differences between the patient and control groups in terms of all subtest scores and the results are shown in Table II. In the patient group, it was determined that only attack treatment was recommended to 115 (56.7%) children, intermittent prophylaxis to 26 (12.8%) children, and continuous prophylaxis to 61 (30.0%) children and no treatment was given to 1 (0.5%) child.

As there is a possibility that it can have an effect on developmental delay those patients with Fe deficiency and Fe deficiency anemia who were on continues Fe prophylaxis were removed from the group step by step and the statistical analysis was repeated. When the patients, who received continuous antiepileptic drug prophylaxis, were excluded from the patient group, this group still showed a statistically significant difference in the field of fine motor skills (p=0.001), gross motor skills (p<0.001), personal-social (p=0.038) and language (p<0.01) when compared with the control group (Table III). When the patients with Fe deficiency and Fe deficiency anemia were excluded from the patient group, the results were similar (fine motor skills (p=0.009), gross motor skills (p<0.001), personal-social (p<0.086), and language (p<0.001) (Table IV). When both groups were excluded from the study group, the test results of the fine motor skills (p=0.003), gross motor skills (p<0.001), personal-social (p=0.148), and language (p<0.001) fields were still statistically significant compared to the control group (Table V).

Discussion

FCs are the most commonly observed seizures in children and seen more frequently seen in boys.4 In our study, the DDST II test and subtest results of 203 FC patients, of which 84 (41.4%) were girls and 119 (58.6%) were boys, were compared with the control group. The boy/girl ratio was reported as 1.[4] by Knudsen[14], and 1.3 in a study by Okumura et al.[15] with 203 patients. In this study, boy/girl ratio was found as 1.4, which is consistent with previous

Table III. The relationship between the patient and control groups in accordance with subtests; personal-social, fine motor skills, language, and gross motor skills scores: excluding the continuous prophylaxis case.

	Group		p-value
	Patient (n=142) Mean ± SD (Median)	Control (n=100) Mean ± SD (Median)	
Personal-social	89.1±4.2 (90.0)	90.0±0.0 (90.0)	0.038
Fine motor skills	87.9±6.6 (90.0)	90.0±0.0 (90.0)	0.001
Language	85.1±9.1 (90.0)	90.0±0.0 (90.0)	<0.001
Gross motor skills	87.2±7.1 (90.0)	90.0 0.0 (90.0)	<0.001

Table IV. The relationship between the patient and control groups in accordance with subtests; personal-social, fine motor skills, language, and gross motor skills scores: excluding the Fe deficiency and Fe deficiency anemia case.

	Group		p-value
	Patient (n=137) Mean ± SD (Median)	Control (n=100) Mean ± SD (Median)	
Personal-social	89.4±3.6 (90.0)	90.0±0.0 (90.0)	0.086
Fine motor skills	88.6±5.6 (90.0)	90.0±0.0 (90.0)	0.009
Language	85.7±8.5 (90.0)	90.0±0.0 (90.0)	<0.001
Gross motor skills	87.7±6.3 (90.0)	90.0±0.0 (90.0)	<0.001

Table V. The relationship between the patient and control groups in accordance with subtests; personal-social, fine motor skills, language, and gross motor skills scores: excluding both the continuous prophylaxis and Fe deficiency and Fe deficiency anemia case.

	Group		p-value
	Patient (n=96) Mean ± SD (Median)	Control (n=100) Mean ± SD (Median)	
Personal-social	89.7±2.4 (90.0)	90.0±0.0 (90.0)	0.148
Fine motor skills	88.3±6.0 (90.0)	90.0±0.0 (90.0)	0.003
Language	85.4±8.8 (90.0)	90.0±0.0 (90.0)	<0.001
Gross motor skills	87.5±6.7 (90.0)	90.0±0.0 (90.0)	<0.001

studies.[4] Although it was reported that the male gender may be a negative factor in the prognosis of FCs[16], in the current study, no relationship could be found between gender and FC subtypes (p=0.133). The age range of FC has been reported differently in studies, the highest incidence occurring between 18-22 months.[2,3] In the study of Okumura et al.[15], the average age of the FC patients was 28 months (range, 6 to 71 months), and the youngest patient of this study was a 6-month-old.

In the current study, 163 (80.3%) of the 203 patients were found to have the simple FC, 22 (10.8%) had complicated FC, and 18 (8.9%) had FC+. The rate of complicated FCs was found as 23.8% by Şen et al.[17], 35% by Shinnar et al.[18], and 27.2% by Verrotti et al.[19] In this study group, the rate of complicated FCs was slightly lower than those reported studies. The reason for the lower rate of complicated FCs rate determined in this study was attributed to the inclusion of some of the complicated patients to the FC+ group. In the literature, FC history in the family was reported between 25% and 40%.[20] The FC history in the family in the first-degree relatives was reported as 17, 34, and 26.6% by Wallace[4], Özaydın et al.[21], and Ling[22], respectively. In the current study, the FC history in the family was found as 34% and epilepsy history in the family as 18.7% of the total patients. In this study, no significant relationship was determined between the FC subtypes and the history of FC and epilepsy in the family (p=0.558 and 0.708, respectively).

The prognosis of infants and children with FC is usually good, and they are mostly neurologically and mentally normal. In a study with 398 FC children, Verity et al.[23] found that the academic performance, mental and behavioral differences of children with FC were similar to healthy children.[23] In the study of Leaffer et al.[24], it was stated that 159 patients who had a first FC did not show any difference in cognitive, motor, and adaptive behaviors compared to the healthy control group one month and one year later. On the other hand, neurologic sequelae such as cerebellar ataxia, dyspraxia, pyramidal findings, and late speech were observed during the following-up of a very low proportion of children with FC.[25,26] Learning difficulty, reading difficulty, attention deficit, and behavioral problems were more frequently accoutered in contrast to other children.[25,26] Bertelsen et al.[27] showed that the frequency of ADHD increased in children diagnosed with FC compared to the healthy control group. Weiss et al.[28] showed a decrease in receptive language and motor skill abnormalities in children with FSE compared to children with simple FC. In the present study, the number of patients having suspected and abnormal test results was statistically significantly higher than the control group (p=0.01). The rate of persons with suspected (p<0.001) and abnormal (p =0.024) test scores in the language area were significantly higher in the patient group compared to the control group.

Similarly, the patient group showed a developmental delay in the language (p<0.001),

FMS (p=0.002) and GMS (p<0.001) subtests compared to the control group, while no significant difference was found between the patient and control groups in the personal-social subtest (see Table II).

It was reported in the literature that FCs at an early age increase the risk of cognitive impairment.[25,26] In the current study, we found a statistically significant and negative relationship between the mean age of the first seizure and the DDST II language subtest score, and the severity of neuromotor delay increased as the seizure age increased (r=-0.319, p<0.001). Since the results from the current study indicated a direct relationship between the seizure age and neuromotor delay and it is known that the neuromotor delay can be one of the most significant signs of epileptic seizures, it can be stated that the FCs starting at older ages may be associated with epileptic (unprovoked) seizures triggered by the fever.[29] The oldest patient in the study herein was 65 months old. Epilepsy is a disease that can be seen at any age during childhood and in the patient group of this study there may be patients that have not developed epilepsy yet. Hermann et al.[30] reported cognitive and language anomalies even before the onset of seizures in patients with idiopathic generalized epilepsy.

In the study of Kolfen et al.[31], it was observed that a significant decrement was observed in the non-verbal intelligence of patients with prolonged FC compared to the patients with simple FCs and the control group. In patients with recurrent FC, poor performance was observed in all neuropsychological tests. In the study of Tsai et al.[32] in patients with complicated FC, it was emphasized that these children had lower intelligence scores than the healthy control group. In our study, no significant neurodevelopmental difference was detected between the simple FC, complicated FC, and FC+ groups. However, DDST II is a neurodevelopmental screening test and in this study, no comparison was made with the control group with other neuropsychological tests.

It is suggested that low serum iron decreases the convulsion threshold, however, fever further increases this adverse condition and facilitates the occurrence of convulsion. Daoud et al.[5] emphasized that the first FC was associated with low iron levels. In our study, we also found 28.2% Fe deficiency and 9% Fe deficiency anemia in the patient group. But the investigation and statistical evaluation related to the Fe deficiency and Fe deficiency anemia of the control group could not be performed due to ethical reasons. On the other hand, in the patient group included in our study, approximately 40% of Fe deficiency or Fe deficiency anemia indicates a possible relationship between Fe deficiency and FC. However, no statistically significant relation was found between Fe deficiency or Fe deficiency anemia and FC subtypes (p=0.237). Fe deficiency and Fe deficiency anemia are reported to affect neuromotor development negatively.[33,34] Therefore, patients with Fe deficiency and Fe deficiency anemia were excluded from the study group and the statistical analysis was performed again. It was observed that there was a statistically significant difference between the groups in the areas of FM (p=0.009), GM (p<0.001), Language (p<0.001), and PS (p=0.086).

The use of continuous prophylaxis was determined as 30% in the patients of the current study group. In order to exclude the possibility of antiepileptic drug use affecting neurodevelopmental test results, patients who received continuous prophylaxis were excluded and statistical analysis was re-performed It was determined that the patient group was lower in the FM (p = 0.001), GM (p<0.001), PS (p=0.018) and Language (p<0.001) subtypes than the control group.

Due to the fact that our study was retrospective, the patients included in the study were selected among the patients who applied to the hospital, and the lack of neuropsychological tests that could make more detailed comparisons reduced the strength of the study. Because our study subgroups had a smaller number of patients, the case selection bias risk cannot

be determined totally. On the other hand, in order to eliminate case selection bias, statistical analysis was performed again after excluding patients who received both Fe deficiency, Fe deficiency anemia, and continuous prophylaxis that could affect DDST II test results. According to the results obtained, it was revealed that the difference in PS (p=0.148) subtype scores disappeared. In addition, it was determined that p values of FM (p=0.003), Language (p<0.001) and GM (p<0.001) scores increased but statistically significant difference continued. Therefore, it was concluded that neuromotor development delay was due to FC rather than iron deficiency, iron deficiency anemia, or antiepileptic use.

The weaknesses of our study were 1) the fact that the DDST II is a developmental screening test, not a neurodevelopmental evaluation test, 2) lack of detailed neuropsychological test battery for neurodevelopmental evaluation, 3) retrospective and hospital-based study design, and 4) case selection bias risk due to a smaller number of patients in study subgroups. The strengths of our study were 1) although DDST II has normative data to interpret the patient's neurodevelopmental status we had a large control group for statistical analysis, 2) the total number of patients included in the study and 3) in order to eliminate the case selection bias risk, we used large exclusion criteria and detailed statistical analysis including exact test.

In conclusion, we found that patients with FC scored significantly lower in all subtest scores than the control group. In addition, we found that patients with FC had more suspicious and abnormal test results than the control group. Although FCs are generally known to have good prognosis, our study shows that they may pose a developmental risk, and children having FCs require the necessity for close clinical and developmental follow up. On the other hand, our study had a major limitation primarily being the retrospective nature of the study, and that there was no "pre-convulsion" developmental evaluation. To make a more definite decision about the developmental risk of FC, further prospective long-term follow-up studies are needed with a detailed neuropsychological test battery.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Rİ, MK, ÇO; data collection: Rİ, BGP, MÇD; analysis and interpretation of results: Rİ, KM, DDY, ÇO; draft manuscript preparation: Rİ, MK, ÇO. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Clinical Research Ethics Committee of Mersin University (2020:2020/221).

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Original Article

Clinical profile and long-term outcome of the first seizures in children

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ABSTRACT

Background. Seizures are one of the most common causes of pediatric admissions to hospitals in children. This study aims to identify the clinical profile and outcome of first seizures in children.

Methods. Children who presented to the pediatric neurology outpatient clinic and pediatric emergency service with a first-time seizure and aged one month through 18 years old were enrolled to the study. At the time of the study, enrolled children were categorized into three study groups according to seizure characteristics: febrile seizure, nonfebrile-provoked seizure and, unprovoked seizure.

Results. The study group consisted of 138 children. Of the 138 patients, 60 (43%) had febrile first seizures, 23 (17%) had nonfebrile-provoked first seizures, and 55 (40%) had unprovoked first seizures. The patients did not experience the recurrence of a seizure by the treatment of underlying cause at the eighteenth month and the eighth year follow-up in the nonfebrile-provoked seizure group. Among the children admitted for unprovoked first seizures, 33 (60%) patients had seizure recurrence during 18 month follow-up and 36 (82%) patients had seizure recurrence during eight year follow-up. Seizure recurrence rate was statistically higher in patients with abnormal EEG and cranial MRI findings in the unprovoked seizure group ($p < .05$).

Conclusions. The patients with provoked first seizure did not develop epilepsy during eight year follow-up. However, 36 patients with unprovoked seizures were diagnosed with epilepsy during eight year follow-up. It is essential to determine the causes of the seizures and treat the condition.

Key words: children, epilepsy, outcome, risk factors, seizures.

A seizure is a common neurological symptom and one of the most frequent reasons for hospital admissions in children.[1] It is suggested that approximately five percent of children will experience at least one episode of seizure in the first 16 years of life. The risk of seizures is higher in children less than three years of age and decreases with age.[2,3]

Seizures can be caused by an isolated event related to an acute situation, such as central nervous system infection, trauma, metabolic abnormality, toxic exposure, fever, or can occur unprovoked in the absence of precipitating factors.[4,5] Febrile seizures are the most common type of seizure seen in the pediatric population, especially in children younger than five years of age.[6,7]

While a seizure is defined as the occurrence of signs or symptoms due to abnormal or synchronous electrical activity in the brain, there is a paucity of data regarding acute seizures in children. In this study, we aimed to identify the clinical profile and outcome of first seizures in children.

Material and Methods

This prospective cross-sectional study was performed on children who were admitted with a history of seizure, to the pediatric neurology

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outpatient clinic and pediatric emergency service from January 1, 2010 to December 31, 2010. Demographic data, clinical reports, laboratory data, cranial magnetic resonance imaging (MRI), and electroencephalography (EEG) results of the included patients were recorded and reviewed. The study protocol was approved by the Clinical Research Ethics Committee of Süleyman Demirel University (number: 2010/19-7). Informed consent was obtained from the parents.

Children who presented to the pediatric neurology outpatient clinic and pediatric emergency service with a first-time seizure and aged one month through 18 years old were enrolled to study. Patients were excluded in the study if they had received any antiepileptic drug or had been diagnosed with epilepsy. In each patient the following laboratory tests were obtained: white blood count, C-reactive protein, serum electrolytes, and blood sugar. Electroencephalography was performed in all patients and cranial MRI was performed wherever indicated.

Patients were followed up in our pediatric neurology outpatient clinic. At the time of the study enrollment, children were categorized into three study groups according to seizure characteristics: febrile seizure, nonfebrile-provoked seizure, and unprovoked seizure. The data on seizure types were collected by definitions of parents. The seizure types were classified according to the International League Against Epilepsy classification of seizure types and the epilepsies.[8] A febrile seizure was classified as complex febrile seizure if it is focal, prolonged (with a duration of >15 minutes), or recurrent within 24 hours.[7] During 18 months follow-up, the patients were clinically examined every three months. For the long-term follow-up, data on recurrent seizures were collected by interviews of guardians by telephone in the eighth year.

Statistical analysis was performed using Statistical Package for the Social Sciences software program version 21.0. Categorical variables were summarized using percentages. Continuous variables were summarized using means. The Chi-square test and Fisher's exact test were used for comparison between independent groups of categorical data. For all statistical tests, values of $p < 0.05$ (two-tailed) were considered statistically significant.

Results

A total of 138 children were enrolled and evaluated of whom 55 (40%) were females and 83 (60%) males, the mean age was 42 months. In total, 126 (91%) patients had generalized seizures and 12 (9%) patients had focal seizures. Cranial MRI was obtained in 110 patients and was normal in 88 (80%) patients. Electroencephalography was obtained in 138 patients and was normal in 105 (76%) patients. Of the 138 patients, 60 (43%) had febrile first seizures, 23 (17%) had nonfebrile-provoked first seizures, and 55 (40%) had unprovoked first seizures (Table I). Out of a total of 108 patients, 78 (72%) patients had seizure recurrence during eight year follow-up.

Table I. Demographic characteristics and clinical features of patients presenting with first seizure.

Gender	
Female	55 (40%)
Male	83 (60%)
Age (months)(means ±SD)	42 months
Seizure type	
Generalized	126 (91%)
Focal	12 (9%)
Cranial MRI	
Normal	88 (80%)
Abnormal	22 (20%)
EEG findings, n (%)	
Normal	105 (76%)
Abnormal	33 (24%)
Seizure characteristics	
Febrile seizure	60 (43%)
Nonfebrile-provoked seizure	23 (17%)
Unprovoked seizure	55 (40%)

MRI: magnetic resonance imaging, EEG: electroencephalogram.

Febrile first seizures

Among the children admitted for febrile first seizures during the study period, 13 patients had complex febrile seizures and 47 patients had simple febrile seizures. The mean age was 24 months. During 18 month follow-up, 28 (47%) patients with febrile first seizures had seizure recurrence. Of 28 patients, 19 had complex febrile seizures and 9 had simple febrile seizures. At eight year follow-up, among all 55 patients, 22 (40%) patients had no seizure recurrence. A total of 33 (60%) patients had febrile seizure recurrence at eight year follow-up (Table II). Of 33 patients, 21 had complex febrile seizures and 11 had simple febrile seizures.

Nonfebrile-provoked first seizures

Among the children admitted for provoked first seizures during the study period, 12 patients had central nervous system infection, six patients had head trauma, three patients had hyponatremia, one patient had carbon monoxide poisoning, one patient had hypocalcemia and hypomagnesemia. The patient who had both hypocalcemia and hypomagnesemia was found to carry a homozygous mutation in the *TRPM6* gene. During 18 month follow-up, patients with nonfebrile-provoked first seizures had no seizure recurrence. At the eight year follow-up, nine patients still had no seizure recurrence.

Unprovoked first seizures

Among the children admitted for unprovoked first seizures during the study period, 22 (40%) patients had only one seizure and 33 (60%) patients had seizure recurrence during 18 month follow-up. At eight year follow-up, in a total of 44 patients, eight patients (18%) still had no seizure recurrence and three patients with a single seizure had seizure recurrence. Thirty-six (82%) patients had seizure recurrence at eight year follow-up. Twenty-one (78%) patients with abnormal EEG findings had seizure recurrence at 18 month follow-up. Thirteen (81%) patients with abnormal cranial MRI findings such as ischemic gliosis and mesial temporal sclerosis had seizure recurrence during 18 month follow-up. Seizure recurrence rates were statistically higher in patients with abnormal EEG and cranial MRI findings ($p < .05$), however, no statistically significant differences were observed according to the seizure types (Table III). There were no significant differences in febrile and unprovoked first seizures for seizure recurrence rate at 18 month follow-up ($p > .05$), however, the seizure recurrence rate was statistically higher in patients with unprovoked first seizures at eight year follow-up ($p < .05$) (Table II).

Discussion

In the literature, clinical and outcome profiles of childhood seizures can be different in limited reported data.[9,10] In this study, we explored the clinical spectrum and long-term outcome of children who were admitted to the hospital with a history of first seizure.

First-line investigations should include tests of blood glucose level and serum electrolytes. In patients with the suspected toxic or drug-induced etiology, if possible blood levels of the suspected drug must be measured.[11] In our study, the following laboratory tests were

Table II. Seizure recurrence rate of the first seizures in children.

	Febrile seizure group	Unprovoked seizure group	p value
Seizure recurrence at 18 month follow up			
Yes	28 (47%)	33 (60%)	0.191
No	32 (53%)	22 (40%)	
Seizure recurrence at 8 year follow up			
Yes	33 (60%)	36 (82%)	0.027
No	22 (40%)	8 (18%)	

Table III. Comparison between seizure free and seizure recurrence groups in patients with unprovoked first seizures.

	Seizure recurrence		p value
	Yes	No	
Gender			
Female	20 (64%)	11 (36%)	0.437
Male	13 (54%)	11 (46%)	
Family history of epilepsy			
Yes	5 (62%)	3 (38%)	0.876
No	28 (60%)	19 (40%)	
Seizure type			
Generalized	29 (57%)	22 (43%)	0.141
Focal	4 (100%)	0 (0%)	
Cranial MRI			
Normal	20 (51%)	19 (49%)	0.039
Abnormal	13 (81%)	3 (19%)	
EEG findings, n (%)			
Normal	11 (40%)	17 (60%)	0.004
Abnormal	21 (78%)	6 (22%)	

EEG: Electroencephalogram

obtained for each patient: white blood count, C-reactive protein, serum electrolytes, and blood sugar. We found that 23 (17%) patients who had nonfebrile-provoked first seizures did not experience recurrence of a seizure by the treatment of underlying cause at eighteen month and eight year follow-up. It is essential to determine the causes of the seizures, and treat the condition.

Electroencephalography and cranial MRI are useful tools in the diagnosis of epilepsy, identification of a specific syndrome, and prediction of long-term outcome.[12,13] The rates of epileptiform discharges varied from 44 to 80% in children who were admitted with seizures in reported studies.[14-16] In our study, EEG was abnormal in 24% (33) patients. The seizure recurrence rate was statistically higher in patients with abnormal EEG and cranial MRI findings. It is estimated that recurrence risk was significantly less among the patients with normal EEG and cranial MRI when compared to those with abnormal EEG and cranial MRI.

Population-based studies indicate that acute symptomatic seizures represent 40–50% of all cases of seizures.[12,17] Sartori et al.[18] reported that 32.5% of patients with acute symptomatic seizures experienced seizure recurrence at 4-year follow-up. In our study, 83 (60%) patients presented with acute symptomatic seizures. Seizures associated with reversible metabolic or toxic disturbances are associated with a minor risk of subsequent epilepsy.[5,19] In our study, none of the patients with metabolic or toxic disturbances developed epilepsy at eight year follow-up. Approximately one-third of children with a first febrile seizure will experience a recurrence, and 10% will have three or more febrile seizures.[20,21] Our study showed that 60% of patients who were admitted for febrile first seizures had febrile seizure recurrence at eight year follow-up.

The first unprovoked seizure has a recurrence risk of 30-50%, however, the second unprovoked seizure has a recurrence risk of 70-80%, justifying the diagnosis of epilepsy.[5] In a systematic review and meta-analysis by Garcia Pierce et al.[22], it estimated a recurrence rate within 3 years of 45% after a first unprovoked seizure. Sartori et al.[18] reported that 60% of

patients with unprovoked seizures experienced seizure recurrence over the four-year follow-up. The strongest risk factors for recurrence of unprovoked seizures are EEG abnormalities, family history of epilepsy, and pre-existing static brain abnormalities.[23,24] In our study, the febrile seizures were the most common type of first seizures, respectively followed by unprovoked seizures and nonfebrile-provoked seizures. The patients with provoked first seizure did not develop epilepsy at eight year follow-up. However, 36 patients with unprovoked seizures were diagnosed with epilepsy at eight year follow-up.

Our study has some limitations. The data on seizure types were collected by definitions of parents. Also, we could not make detailed investigations about possible underlying etiologies of first unprovoked seizures due to the limited resources at that period in 2010. Prospective studies are needed to better define the clinical profile and outcome of first seizures in children.

Ideally, the goals in the management of acute seizures are to stabilize the patient, identify any electrolyte imbalance and hypoglycemia, terminate the ongoing seizure activity as soon as possible, determine the underlying etiology, and decide on the need of long-term antiepileptic drugs. Misdiagnosis carries the potential risk of legal problems, can cause family anxiety and lead to an excessive hospital stay. The most important factor in diagnosing seizures is to rule out the possibility of a nonepileptic event. It can be useful to record the seizure-like events with a digital or video camera by the patient's parents. Investigations should include prompt EEG. It is important to delineate a detailed description of the clinical seizure for the correct diagnosis, treatment, and prognosis.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NOD; data collection: HS; analysis and interpretation of results: NOD, PA, HS; draft manuscript preparation: NOD, PA. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study protocol was approved by the Clinical Research Ethics Committee of Süleyman Demirel University (number: 2010/19-7).

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Conflicts of interest

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Original Article

The causes of parental vaccine refusal: results of a survey from Giresun, Turkey

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ABSTRACT

Background. While efforts have raised immunization levels in developing countries, high rates of vaccine refusal in both developing and developed countries are causing concern worldwide. We aimed to determine the causes of vaccine refusal among parents refusing or postponing the vaccination of their children.

Methods. This descriptive, cross-sectional study was performed in the Giresun province of Turkey. The study population included families who were unwilling or refused to vaccinate at least one of their children under the scope of the Turkey Enhanced Program of Immunization. Data were obtained during the year 2018 by face-to-face interviews with each vaccine hesitant parent (VHP).

Results. Vaccine refusal incidence was 1.2% in the year 2018. In 8 (14.8%) of the children, the vaccination was recorded to be postponed due to health problems, while 46 (85.2%) children were not vaccinated due to parental vaccine refusal. Two-thirds of unvaccinated children were living outside the province center of Giresun. The parents consisted of young adults whose average age was 30.6±6.6 years. VHPs were mostly university graduates (61.1% of mothers-70.3% of fathers). While at least one of the VHPs was a religious official in 24.1%, at least one of the either VHP was a teacher in 20.3%. The most common reasons for vaccine refusal were "fear of vaccine side-effects" (55.6%), and "problems in previous vaccinations" (33.3%). In 44.4 % of refusal cases, no specific reason was stated.

Conclusions. Primary healthcare providers, who are in close contact with parents, have an important role to provide the right health information. Various in-service training can be provided to improve the communication skills of healthcare providers. In these training sessions, besides comprehensive information about vaccines, it should be aimed to provide parents with the ability to understand their concerns, to approach them sensitively and to present the information they need effectively.

Key words: childhood vaccination, immunization, vaccine hesitant parents, vaccine refusal.

Immunization is one of the most effective public health tools for preventing contagious diseases, reducing mortality and disease rates, ensuring improved health, and eliminating such diseases as smallpox. According to the World Health Organization (WHO), thanks to vaccination procedures, more than 100 million children are inoculated before the age of one year, and 2.5 million child deaths are avoided every year.[1,2]

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While efforts are being made to raise immunization levels in developing countries and in communities with low socioeconomic levels[3,4], increasing vaccine refusal not only in developing but also in developed countries which have high immunization levels is causing worldwide concern.[5-7] The latest data from the United States of America (USA) have revealed an increase in alternative vaccination programs in addition to vaccination for preschool children.[3,4] Vaccine hesitancy is not a new occurrence, its negative impact on preventive services is gradually increasing. It has been shown that rejection or hesitation of vaccinations are associated with higher socioeconomic status,

education level of the mother, and shared cultural beliefs which tend to be clustered geographically.[8,9] Another important reason for this phenomenon is that many vaccine-preventable diseases are not perceived as a serious risk. Since most parents are not familiar with these diseases today, their threats are less tangible and concerns about these diseases have been replaced by the concern of vaccine safety.[8] In addition to these concerns, the increment of the number of routinely applied vaccines in time became another fear factor for parents who believe that vaccines could affect their children's immune system.[10]

The Turkish Ministry of Health provides all childhood vaccines free of charge within the scope of its Expanded Program on Immunization (EPI). In accordance with the vaccination schedule in the EPI circular issued by the Ministry of Health, family physicians monitor the vaccination times of all children via an electronic record system.[11] Additionally they remind families not attending the vaccination appointment on time, and provide vaccinations by inviting families to the family health center by telephone or by visiting them at home.[12] When family health center staff came across a vaccine hesitant parent (VHP), they inform the hesitant parent face to face or by phone and try to convince them to complete the refused or postponed vaccine. VHPs who do not accept the invitation are reported to the District Health Directorate. The vaccination teams of the district health directorate inform VHP's by phone and invite them to the directorate. Those who accept the invitation are interviewed face to face and tried to be persuaded for vaccination. This pursuance is repeated in every vaccination period for VHP's. Although vaccination rates are rising continuously in Turkey, debates concerning immunization are also becoming more frequent, as in many countries with high immunization rates. While the number of families who refused vaccines in Turkey was 183 in 2011, 913 in 2013, and 5091 in 2015, it has risen to over 10,000 in 2016.[13] Vaccine refusals, which were rare in our country, grew like an avalanche in 2015 after a prosecutor won the health precaution lawsuit filed in order not to vaccinate his twins.[13] According to the statement made by the Ministry of Health, the number of families who refused vaccination reached 23,000 in 2018.[14] The purpose of this study was to determine the causes of vaccine refusal among parents refusing or postponing the vaccination of their children in Giresun, Turkey.

Material and Methods

This cross-sectional study was performed in the province of Giresun with a population of 454 thousand between 1st January and 31st December of the year 2018. Giresun is a coastal town located in the Black Sea Region of Turkey with a mid-size population and surface area. The health-care services provided in this city are similar to the ones provided in the other cities of Turkey. The number of children scheduled to receive a vaccination was 4428 by the record of the Giresun Provincial Health Directorate in the year 2018. The number of children who were reported as not having been vaccinated due to vaccine refusal or postponement was 55 (1.2%) until the end of the year. The study group consisted of parents who refused or postponed one or more childhood vaccines of their children. According to the Health Ministry's ordinance, family physicians report all parental vaccine refusal cases to the district health directorate at the end of each month. When a new vaccine refusal case is reported by the family physician, the VHP was invited to the district health directorate. Those who refused the invitation were contacted by telephone, both aiming to persuade them for vaccination and also to collect relevant data.

One of the parents refused the phone interview, and the research was carried out with 54 parents. We obtained verbal and written informed consent from all participants. The study received ethical approval from the Ondokuz Mayis University Clinical Research Ethics Committee (KAEK: 1558) on 13 April 2018.

A questionnaire form developed by a literature review was used as a data collection tool. It was tested on a group that consisted of 25 participants who refused a vaccination in the previous year. After modifying some questions accordingly, the questionnaire was reorganized. This form consisted of 22 open-ended and multiple-choice questions evaluating demographic characteristics and reasons for vaccine refusal. After obtaining verbal and written informed consent, patients were questioned face-to-face or on the telephone. The questionnaire was filled separately for both the participants and their wives or husbands.

Parents who postponed scheduled vaccination or who totally refused one or more childhood vaccines were considered as VHPs.

Statistical analysis

The continuous data were expressed as mean ± standard deviation (SD) values. We presented the categorical variables as frequencies with percentages and compared them according to the independent variables using the chi-square test. The p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using the SPSS version 22.0 (IBM Corporation, Armonk, NY, USA) package program.

Results

Fifty-four of 4428 (1.2%) scheduled children were identified as not having been vaccinated due to vaccine refusal or postponement. In 8/54 (14.8%) of these cases, the vaccination was postponed due to health problems, while in 46 children (85.2%) the procedure was refused by parents. Health problems reported as cause of vaccination postponement were chickenpox (n=3), allergy (n=2), epileptic seizure (n=1), constipation (n=1), and low birth weight (n=1). The mean age of the children with VHPs was 20.3±8.5 months, and 29 of 54 cases (53.7%) were girls. In addition, 67.3% of unvaccinated children were living outside of the province center (in villages within the borders of the province Giresun).

The number of children who did not receive any vaccine was nine (16.7%). Among the rejected vaccines, pentavalent vaccine (acellular pertussis and tetanus toxoid + inactive polio vaccine + Haemophilus influenza pediatric dose vaccine) (61.8%), Conjugated Pneumococcal vaccine (52.7%) and Oral Polio Vaccine (50.9%) took the first three places (Fig. 1).

Most of the participants who were interviewed were mothers (n=43, 79.6%). Fathers (16.7%) or both parents (3.7%) were interviewed in the remaining cases. The decision of vaccination refusal of 19 (35.2%) children was made by mothers and in 35 (64.8%) cases by both parents. All VHPs were married, certain sociodemographic characteristics are presented in Table I.

The most common reasons reported by the VHPs were "fear of vaccine side-effects" (55.6%), "no reason" (44.4%), and "some problems experienced by their child after previous vaccinations" (33.3%) (Table II). Five (9.1%) of the parents stated that they refused the vaccination due to the mercury content of vaccine preparations that is believed to cause autism. Experienced problems after vaccinations were swelling and redness (12%), or pain at the injection site (16%), fever (30%), headache (24%), muscle pain (13%) and loss of appetite (23%).

At least one of either parent of 13 (24.1%) children with VHP was a religious official. The rate of refusal of vaccination due to religious beliefs in this group was 30.8%, while the most common reason (61.5%) given for refusal was "No reason, I simply do not want my child to be vaccinated".

While the most common refusal reason was "fear of side-effects of all vaccines" (62.5%) in parents who were health workers, it was "No reason, I simply do not want vaccination" (43.8%) in parents who were teachers. Parents living in rural areas (n= 36, 54.1%) stated that they refused to vaccinate because of fear of the side effects of all vaccines.

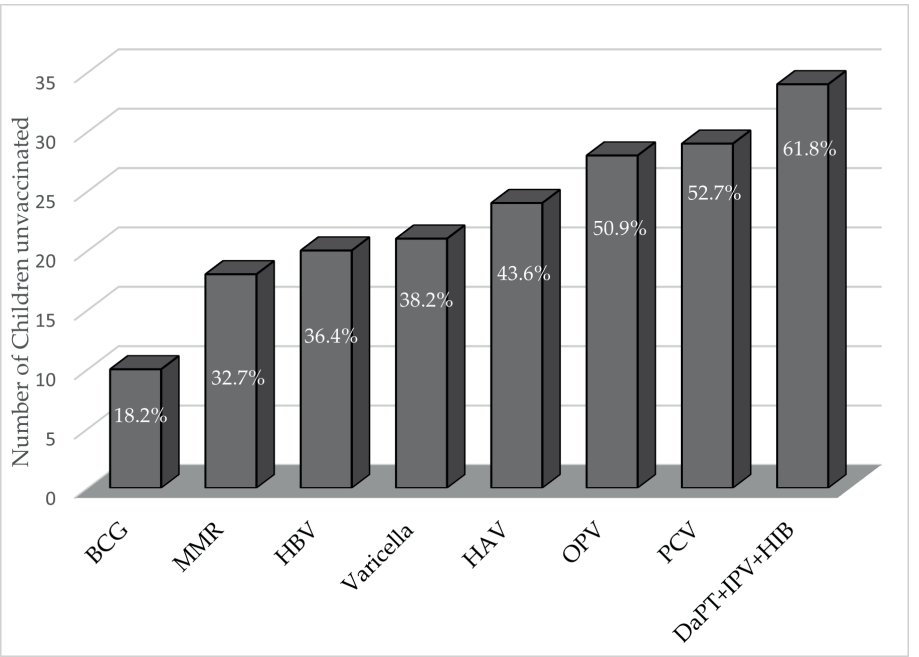

Fig. 1. Vaccines refused or delayed.

BCG: Bacille Calmette-Guérin vaccine, MMR: Measles. Mumps. Rubella vaccine, HBV: Hepatitis B pediatric dose vaccine, Varicella: Varicella vaccine, HAV: Hepatitis A pediatric dose vaccine, OPV: Oral Polio Vaccine, PCV: Pneumococcal conjugate vaccine, DaPT+IPV+HIB: Pentavalent Diphtheria and tetanus toxoid with acellular pertussis + Inactivated polio vaccine +Haemophilus influenza pediatric dose vaccine

Table I. Sociodemographic characteristics of vaccine-hesitant parents.

Variables		Mother (n: 54)	Father (n: 54)
Age (year)		28±7	31±8
Graduation n (%)	Elementary	4 (7.4)	1 (1.8)
	Primary school	7 (13.0)	1 (1.8)
	High school	10 (18.5)	14 (25.9)
	Collage-University	33 (61.1)	38 (70.3)
Occupation n (%)	None /Unemployed	32 (59.4)	1 (1.9)
	Health worker	6 (11.1)	2 (3.8)
	Religious official	4 (7.4)	11 (20.4)
	Teacher	8 (14.8)	14 (25.9)
	Public sector employee	3 (5.6)	6 (11.1)
	Self-employee	1 (1.9)	20 (36.9)

Discussion

To the best of our knowledge, this is the first epidemiological study conducted by a one-to-one interview with VHPs in North Anatolian region of Turkey. We found that the VHP rate in our research increased by a third (1.2%) compared to the previous year (0.9%). In fact, when we review the research carried out on VHPs, we were expecting a higher incidence. Because survey studies in various countries have reported varying rates of vaccine refusal, from 2% to 12%.[5,6,15,16] One of the reasons for the low incidence in our study may be that it involved parents who were definitely confirmed as VHP. In many studies with high vaccine rejection rates, the data were obtained

Table II. Vaccine-hesitant parents' reasons for vaccine refusal (n: 54)

Reasons	n* (%)
I fear the side-effects of all vaccines	30 (55.6)
No reason, I simply do not want vaccination	24 (44.4)
My child experienced some problems after his/her previous vaccinations	18 (33.3)
My child will not catch the disease requiring this vaccine	12 (22.2)
I think that the vaccine may be harmful	9 (16.7)
Due to my child's health problems	8 (14.8)
I experienced some problems after vaccination	8 (14.8)
I am opposed to vaccination because of my beliefs	8 (14.8)
I do not believe that vaccination is beneficial	7 (13.0)
Leaders/elders/my family/friends are unwilling	7 (13.0)
My child has already had this disease*	3 (5.6)
I have needle phobia	2 (3.7)

*Multiple options are marked. †for chickenpox vaccination only

through self-reports about the participants' attitude and vaccine refusal was not confirmed by medical records.[5,17,18] Compared to other regions/countries, the other reasons for the lower refusal rate may be that vaccination services in Turkey are provided by the nearest family health center, vaccination is entirely free of charge, and family physicians and nurse practitioners are responsible for monitoring childhood vaccination schedules.

Concern about side effects was the most common reason for vaccine refusal and postponement in our study. In particular, although the perceived link between the vaccine and autism is not supported by reliable scientific evidence, nevertheless, it is a significant factor increasing anxiety over vaccine safety.[19,20] As shown in our study, there was also a belief in Turkey that vaccines can lead to autism.[21] Different studies have also shown that parents regard vaccines as having low efficacy and reliability.[17,22] Giving information on potentially confusing issues such as the efficacy and safety of vaccines by different categories of healthcare professionals can provide a positive impact on parents' decision-making process.[6,23] It can be considered as a good opportunity for missed vaccines when a child visits a clinic for any reason. After this child's physical examination, the allocation of time to inform the parents about vaccinations will be of great benefit in eliminating vaccine hesitations.

We found that approximately a quarter of parents in our study did not fully perceive the importance of vaccine-preventable diseases. Topçu et al.[21] reported that 36% of parents believed that their children would not contract a vaccine-specific disease in Turkey. In a systematic review of factors affecting vaccine uptake in young children, it was reported that many VHPs thought that their children had low susceptibility to such diseases, or that such diseases were of low severity.[24] Yet two cases of polio, previously thought to have been eradicated, were observed in Europe in 2015, and one technical report described this as a 'warning sign' resulting from vaccine refusal.[22] Thanks to the high immunization rates among children under five, the majority of vaccine-preventable diseases have fallen to historically low levels particularly in developed countries. Consequently, new generation young parents are thought to be unaware of health problems and threats faced by earlier generations deriving from once widely prevalent contagious infections. The fact that the VHPs constituting the research group consisted of young adults with an average age of 30 years supports this claim.

Another important reason for vaccine refusal was some health problems experienced after immunization by VHPs' children or relatives. We believe that if the family physicians and the nurse practitioners provide information about the problems that will be experienced after a vaccination and solutions through effective face-to-face communication, then vaccine hesitations will be reduced. For example, the parents of babies crying due to injections may have additional concerns that the baby will experience a negative effect after the injection. The training of a correct procedure for the reduction of pain due to injections, the use of psychological expedients like simply distracting the child at the time of injection, can make a difference and be beneficial.[25]

Another noteworthy finding in the present study were that two-thirds of the VHPs was university graduates, and more than one-fourth of them were teachers. Several studies have also observed greater vaccine refusal in families with higher education levels.[6,17,18] However, there are some studies that have reported that education level was not significantly linked with vaccine hesitancy.[26,27] Highly educated individuals are also expected to have a high level of health literacy. Meppelink et al.[28] showed that people with high health literacy and negative beliefs about childhood vaccination perceive negative information as more persuasive than positive information. That's because anti-vaccination websites often promote alternative medicine, while promoting parents' autonomy and responsibility. We think that the incidence of VHPs can be reduced by ensuring that parents have access to information from reliable sources, even if their health literacy is otherwise high.

The incidence of VHPs due to faith-related objections in the present study was 14.8%. On the other hand, at least one parent of every four unvaccinated children was a religious official. We think that, in an almost entirely Muslim country such as Turkey, vaccine refusal by parents who are also religious officials may have an adverse influence on other members of society. Studies from Africa and Asia suggest that the lower immunization rates in Muslim communities, in particular, may be due to parents avoiding immunization for religious reasons.[29,30] The most common theological reason for vaccine refusal in Muslim communities has been shown to be using pork containing ingredients in vaccines.[31] However, a systematic review study showed that participation by religious and traditional leaders has a positive impact in central African countries and places such as Afghanistan, India and Europe.[32] That study also showed greater increases (> %20) in knowledge, awareness or attitudes with programs aimed at overcoming the particular concerns of particular groups compared to other activities. In order to relieve the anxiety of religious parents, in addition to the cooperation of religious leaders with health authorities at the national level, in order to reach the local people, especially in rural areas, primary healthcare providers can visit the places of worship and provide parents with face-to-face information or joint meetings with religious officials.

Our main limitation in this study was VHP's were not open to communication. Therefore, the interview may have failed to show the exact underlying cause of vaccination rejection. Additionally, the health problems reported as a reason for postponing the vaccination could not be verified through the health records. Our study group is not representative of the general Turkish population; therefore, future studies should be extended to broader geographic areas and socioeconomic populations.

In conclusion, a proactive strategy should be adopted to protect children from vaccine-preventable diseases. Local health workers, parents, teachers, volunteers, and peer role models are needed to protect every child in the community. If necessary, initiatives such as door-to-door interviews where easing fears that parents may have and reaching every single child should be encouraged and supported. Efforts such as media campaigns, vaccine pro blogs and initiatives focused on

the most vulnerable population can turn this negative trend into an effective positive era. As a result, we think that understanding the social determinants of vaccine rejection and trying to eliminate vaccine hesitations in our society is essential for improving vaccination rates. Multilevel collaboration can increase vaccination rates, strengthen herd immunity, and prevent vaccine refusal.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ÖT, ENG; data collection: ENG; analysis and interpretation of results: ÖT, ENG, CD; draft manuscript preparation: ÖT, CD; review & editing, supervision: CD. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study received ethical approval from the Ondokuz Mayıs University Clinical Research Ethics Committee (KAEK: 1558) on 13 April 2018.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Original Article

Determining the effect of time dependent and time independent factors on pneumonia of children under five in North west Ethiopia

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ABSTRACT

Background. Lower Respiratory Tract Infections including pneumonia are the fourth cause of death globally. In Ethiopia, pneumonia is the leading cause of death for children under five. However, so far, only a few studies that used longitudinal design and time dependent covariates determined the significant factors of pneumonia. This study sought to determine whether respiratory rate changes differed for under five-year-old pneumonia patients who had been receiving different treatments over time and whether the change was effected by time dependent and independent covariates.

Methods. A longitudinal study design involving marginal Poisson regression models and conditional Poisson regression models was used. After comparing the two, the final interpretation was made using a conditional Poisson regression model owing to its relative powerfulness.

Results. Four hundred and fifty-three under five pneumonia patients were included, of which 44.37% were female. It is found that, compared to rural children, urban children had an estimated mean respiratory rate decrease of 3%. It is also found that, compared to children whose mother practiced only exclusive breastfeeding in the first six months, children whose mother practiced both breastfed and complementary were more likely to be exposed to pneumonia. The estimated mean respiratory rate of children having asthma was 1.073 times that of children who had diarrhea.

Conclusions. In northwest Ethiopia, weight, residence, previous disease history, breastfeeding and temperature are significant factors of pneumonia among children under five. The effectiveness of treatments was dependent on the number of times children visited the hospital. A significant variation of baseline pneumonia status among under five pneumonia patient children was noted in the hospital.

Key words: pneumonia, respiratory rate, GEE, GLIMM, under five children.

Acute respiratory infection is an infection of the upper and lower respiratory system. Lower respiratory tract infections including pneumonia, affect the airways below the epiglottis specifically the lungs and are the cause of a high proportion of acute respiratory infection burden and are the fourth cause of death globally.[1] Pneumonia is an acute illness of the lung alveolar air spaces that is diagnosed using respiratory rate (RR). Among patients hospitalized for respiratory system infections, children with pneumonia have a high proportion. According to 2019 UNICEF reports, compared to other infectious diseases, pneumonia which claims 2200 lives per day, nearly 800,000 per year, is the leading cause of mortality among children under five.[1,2]

Pneumonia, birth asphyxia, preterm birth complications and diarrheal diseases are the four major causes of mortality for under five children globally. Of these, Pneumonia ranked first cause of mortality and roughly accounts for 18% of the deaths among children under five.

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Malnourishment, AIDS, measles, malaria and environmental factors, such as crowded living conditions and exposure to indoor air pollution are more likely to contribute and an increased susceptibility to pneumonia in children.[3] While possible causes of pneumonia are many, bacteria and viruses are among the more common. Though it is commonly diagnosed for the elderly (age ≥ 65 years) and the very young (age <5 years), it is not potentially life threatening for adults.[4]

In developing countries in particular, of all deaths caused by acute respiratory infection among children aged under five years, pneumonia accounted for about 75% of the cases.[2] A study conducted in East Africa reported that history of acute respiratory tract infection, wood as a fuel source, cooking food in the living room, being unvaccinated, exclusive breastfeeding, and prenatal smoking are independent important factors of pneumonia.[5,6] Nevertheless, although several precautions have been taken against pneumonia among under five children in Ethiopia, it remains the leading cause of death.[7] A hospital-based prospective study found that the cut-off for breaths per minute was 50, 40 and 30 for infants (aged less than 12 months), children aged 12-35 months and children aged 36-60 months, respectively. The study concluded the presence of high RR or fast breathing as an important indicator of pneumonia in all three age groups.[2] Moreover, pneumonia of children under five was associated with low paternal education, the number of persons in the household and young maternal age.[8]

Compared to children who did not have a history of diarrhea, the odds of pneumonia were higher among children under five who had a history of diarrhea during the past fifteen days.[9] In children with non-severe pneumonia in Pakistan, a placebo-controlled trial of amoxicillin showed insignificance benefit of amoxicillin over placebo. The study also noted a variation between children of Africa and Asia concerning etiology and the clinical course of pneumonia. This study further indicated that the body temperature of children at baseline was an important factor affecting their RR.[10]

UNICEF Ethiopia promoted a better and higher-quality diagnostic aid for pneumonia among young children through a public and private partnership.[11] While immunization and breastfeeding are among the preventive, administration of amoxicillin tablets and other antibiotics were employed as curative methods.[7] The study also indicated that female rather than male children were more likely to acquire pneumonia, indicating that the sex of children was an important factor.[12] However, in a study of children 2 to 59 months old in the Arsi zone, Ethiopia, male children were more likely to develop pneumonia as compared to female children.[13] Another study[4], which diagnosed asthma and pulmonary TB indicated that, among adults, these did not have consistent significance on the community's acquisition rate of pneumonia. Compared to fully vaccinated children, unvaccinated children were more likely to develop pneumonia, and children whose parents practiced both breastfed and complementary fed during the previous six months were more likely to develop pneumonia as compared to children whose parents practiced exclusive breastfeeding.[14,15]

Several studies have demonstrated different factors that are significantly associated with pneumonia in children.[7,10,12,13] However, to the best of our knowledge, none of the studies considered time dependent factors but only time independent factors. Thus, this research paper aimed to build new insight on how to estimate the effect of time varying covariate including non-time varying covariates using an appropriate statistical model. Therefore, the objective of this study was to determine whether RR changes differently for patients receiving different treatment over time and whether the change depends on time dependent and time independent patient characteristics.

Material and Methods

Four hundred and fifty-three under five pneumonia patients were selected randomly from the Felege Hiwot Referral Hospital (FHRH) located in Bahir Dar, Amhara Region, Ethiopia, using stratified random sampling by considering their residence as strata. The data was collected from September 09 to 15, 2019. In FHRH as well as in Ethiopia, when a patient with pneumonia is hospitalized, registering the RR on the patients' registry card is mandatory. RR was measured by counting the respiratory chest movements, via inspection, and sometimes auscultation. So as to have reliable measured value of RR, a period of at least 30 seconds was allotted to account for the variability in measures of the breathing rate. In cases where the RR was difficult to detect within 30 seconds, data of RR over a period of 60 seconds or in two blocks of 30 seconds was used.[2]

The sample size was determined using single proportion formula with the following assumptions: estimated proportion of pneumonia patients 60% (= 0.60), 95% CI: ($Z_{\alpha/2}$ = 1.96), and a 5% margin of error (d= 0.05).[10,14] Hence, the final sample involved 453 pneumonia patients. Based on the recommendation of the physician in the hospital, patients under five with pneumonia were included. was determined as greater than 50 breaths per minute for those < 2 months , 40 breaths per minute for those between 2-11 months, and greater than 30 breaths per minute for those between 1-5 years of age.[2] The investigation didn't include those patients whose ages were greater than five years and children whose ages were under five years but had less than six visits in the study period.

Variable description

The outcome variable for this study was number of RR per minute in pneumonia patients under five years of age. The explanatory variables were categorized into two as: (i) time dependent covariates (TDC) such as treatment type (amoxicillin, ampicillin, ceftriaxone, gentamicin, penicillin), vomiting status (yes, no), temperature and time; (ii) time independent covariates (TIDC) such as sex (male, female), weight (in kg), previous disease history (asthma, tuberculosis, malaria, diarrhea), immunization status (yes, no), weather (autumn, spring, summer, winter), breastfeeding (exclusive (B), complementary (C), and both breastfed and complementary fed and residence (urban, rural).

Poisson Regression model (GEE approach)

The generalized estimating equations (GEE) approach is a widely used estimation method for longitudinal marginal models.[16,17] The within-subject correlations among the repeated measures are taken into account by using a working correlation structure and employing that structure for the parameter estimations. The statistical Poisson regression model is given by:

$$\log(\mu ij) = \beta_0 + \beta_1 X_{ij1} + \beta_2 X_{ij2} \ldots + \beta p X_{ijp} \quad (1)$$

where, μ_{ij} is the expected RR of the i^{th} patient at the j^{th} visit; i=1, 2, ..., 453 and j=1, 2, ..., 6; β_0 is the intercept while $\beta_1, \beta_2, \ldots, \beta_p$ are the coefficients of independent variables $X_1, X_2, \ldots, X_p$ respectively.

Conditional Poisson Regression model

GEE approach doesn't yield reliable results for a longitudinal model including random effects

and time-dependent covariates. Thus, generalized linear mixed model (GLMM)[17,18] was applied to taking into under-consideration the random effects (random intercept and slops) and time dependent covariates, as given below by:

$$log(\mu ij) = \beta_0 + \beta_1 X_{1ij} + \beta_2 X_{2ij} + \ldots + \beta_p X_{pij} + \beta_1(t_{ij}) X_{1ij} + \beta_2(t_{ij}) X_{2ij} + \ldots + \beta_p(t_{ij}) X_{pij} + \alpha_i + b_i t_{ij} \quad (2)$$

where $\beta_1, \beta_2, \ldots, \beta_p$ are the only unknown coefficients of time independent covariates (TIDC) assumed to be constant while $\beta_1(t_{ij})$, $\beta_2(t_{ij})$, ..., $\beta_p(t_{ij})$ are unknown coefficient function of time that indicate coefficients of TDC change over time besides the corresponding constant

coefficients β_1, β_2, ..., β_p. Whereas, α_i and b_i are random intercept and random slope of the i^{th} child assumed to be independent and normally distributed with mean zero and constant variance.

Permission to undertake the study was obtained from both the ethical committees of Bahir Dar University and Felege Hiwot Referral Hospital (IRB number: 01-018/02.0). The researchers committed to the protection of the privacy of patients/participants medical information. Accordingly, identification of patients/participants was done only through numerical codes and collection of medical information was made based on Ethiopian Ministry of health legislation to which FHRH is also committed. The children's parent gave written informed consent after having been introduced to the procedures, benefits and possible risks of participation in the study.

Results

Exploratory data analysis

Of the 453 pneumonia patients in the study, 44.3% were female, 77.26% were immunized/vaccinated, and 50.55% were from a rural area (Table I). The majority of children (70.2%) had no previous disease history while 13.91%, 9.27%, 4.42% and 2.21% of the children had diarrhea, asthma, tuberculosis and malaria respectively. Most of the children (31.57%) were born in the summer season. The mothers of 270 (59.6%) children practiced both breastfed and complementary fedthe first six months of life. The proportion of children who received ceftriaxone, penicillin, gentamicin, amoxicillin and ampicillin treatment were 38.41%, 36.87%, 10.15%, 7.51% and 7.06% respectively on their first visit (day 1). Similarly, 297 (65.56%) children had persistent vomiting on their first visit (day 1).

For time dependent numerical variables, RR and temperature summary statistics were done for the first visit (day 1). On average, the temperature of under five pneumonia patients during their first visit was 37.8 °C (Table II).

The individual profile plot in Figure 1 shows the evolution of RR of each child over time (six consecutive days), each line representing a child. The profile plot suggests that in nearly all of the children the RR declined with an increase in the time (in days) of a child's stay in the hospital. It also suggests the need for random intercepts to correct for the fact that the children started with different RR. Whereas, the slope for each profile plot of a child looks similar. The intermediate red line across the profile plots indicates the average estimated RR of a child which declined over time. We also indicated in Table III the average RR at day 1 was 58.67 and declined to 40.87 on day 6.

Table I. Frequency distribution of under-five children within levels of categorical covariates.

Variable	Level	Frequency(%)
Sex	Female	201 (44.37)
	Male	252(55.63)
Weather	Autumn	143 (31.57)
	Summer	119 (26.27)
	Spring	80 (17.66)
	Winter	111 (24.5)
Immunized	No	103 (22.74)
	Yes	350 (77.26)
Residence	Rural	229 (50.55)
	Urban	224 (49.45)
	Asthma	42 (9.27)
	Diarrhea	63 (13.91)
Previous disease	Malaria	10 (2.21)
	No	318 (70.2)
	Tuberculosis	20 (4.42)
	Exclusive (E)	159(35.1)
Breast feeding	Complementary(C)	24 (5.3)
	Both breastfed and complementary fed	270 (59.6)
Vomiting status	No	156 (34.44)
	Yes	297 (65.56)
	Amoxicilline	34 (7.51)
	Ampicillin	32 (7.06)
Treatment	Ceftriaxone	174 (38.41)
	Gentamicin	46 (10.15)
	Penicillin	167 (36.87)

Table II. Summary statistics of numerical covariates of children under-five who visit FHRH.

Variable	N	Mean	St. Dev	Minimum	Maximum
Respiratory rate	453	58.67	9.24	32.00	84.00
Temperature	453	37.80	1.02	32.50	40.10
Weight	453	7.83	2.91	2.00	18.00

Table III. Average and standard deviation (St.dev) of respiratory rate per day.

Statistic	Time (in days)					
	Day1	Day2	Day3	Day4	Day5	Day6
Average	58.67	54.72	50.67	47.19	43.97	40.87
St.dev	9.24	8.97	8.30	7.97	7.39	6.87

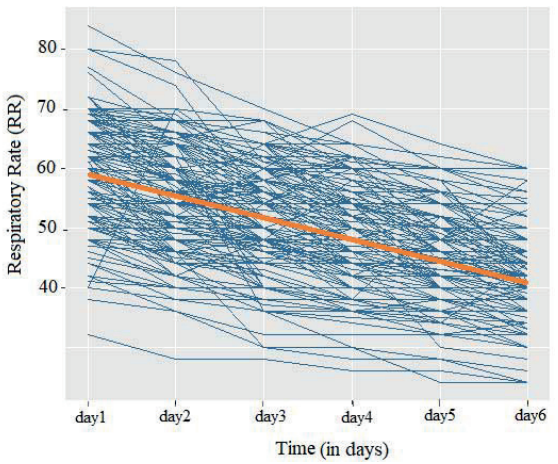

Fig. 1. Individual profile plot of respiratory rate of children over time (in days).

In addition to trends of average RR, the variability of RR declined over time (9.24 on day 1 to 6.87 on day 6) indicating that the RR of children in the hospital becomes more consistent as the number of days of staying in the hospital increased.

Figure 2 revealed the progress of the average RR of children over six consecutive days per treatment type. The average RR of children per each treatment type declined (supported from Fig. 1) and became close to similar. The average RR of children treated with Amoxicillin was the lowest throughout all of the days.

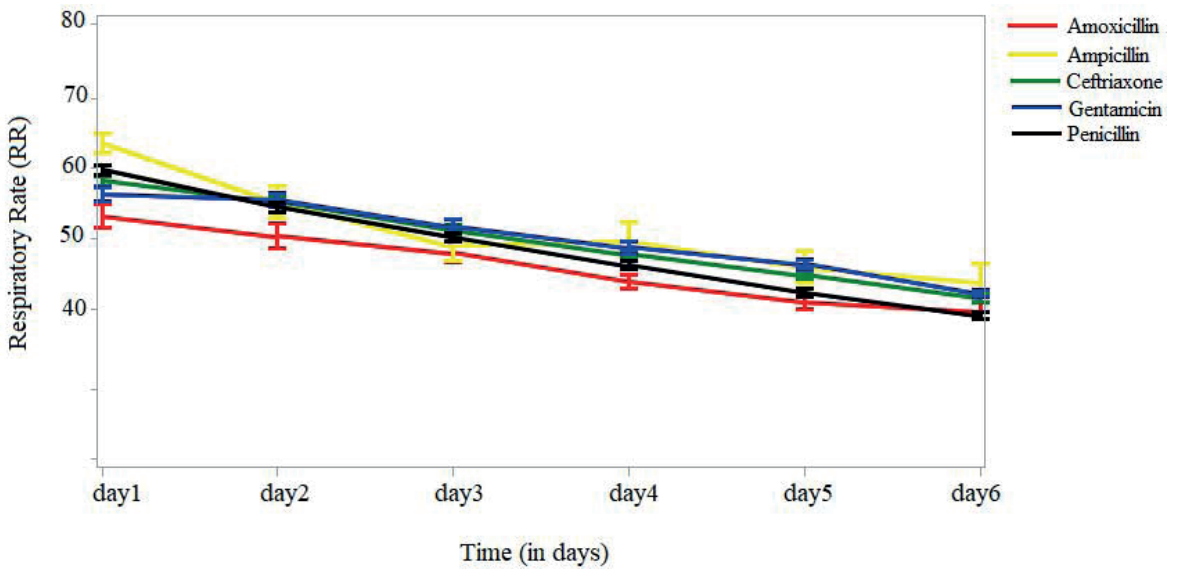

Fig. 2. Average respiratory rate of children per each treatment type represented as Amoxicillin, Ampicillin, Ceftriaxone, Gentamicin and Penicillin with red, yellow, green, blue and black color line respectively.

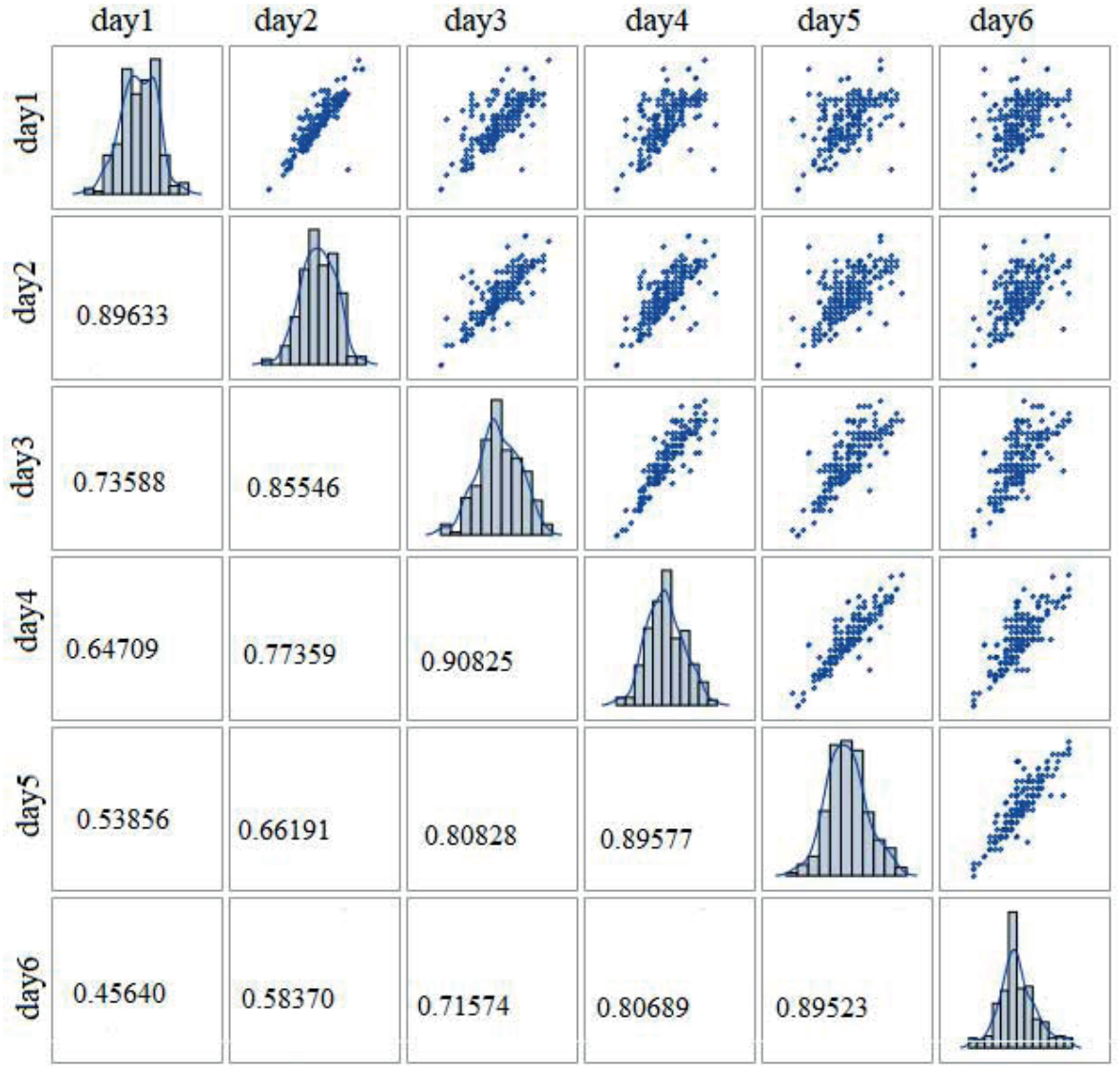

Fig. 3. Scatter correlation matrix.

The scatter matrix plot in Figure 3 indicates a positive correlation between RR on different days as there is a positive slope. The correlation was presented in form of a scatter plot (see the diagonal matrix in Fig. 3) and corresponding numerical expression (see the diagonal matrix in Fig. 3). The correlation between RR decreased with an increase in time (in days). The decrease in correlation overtime has an implication of autoregressive working correlation[19], decline in the correlations with increasing separation in time.

Parameter estimates

The parameter estimates for the covariates in the study are presented in Table IV and V. Table IV presents the Poisson regression model obtained using GEE which was compared according to different working correlations such as independent GEE (IGEE), compound symmetry GEE (CSGEE) and autoregressive GEE (ARGEE). The comparison was made using QIC, and the minimum the QIC, the better the model.[16] In this regard, a Poisson regression model using ARGEE is the best model, with QIC=440. In the standard Poisson regression model that

Table IV. Parameter estimates using Poisson regression model (standard and GEE approach).

Par.		Standard		IGEE		CSGEE		ARGEE	
		Estimate (std.error)	P-value	Estimate	P-value	Estimate	P-value	Estimate	P-value
Intercept		3.175(0.128)		3.091(0.280)		3.383(0.166)		3.647(0.129)	
Sex	Male	0.018(0.006)**	0.003	0.018(0.012)	0.156	0.0284(0.0132)*	0.0324	0.0206(0.0121)	0.0896
	Female	0.000		0.000		0.000		0.000	
weight		0.0064(0.0012)***	<.0001	0.0064(0.0032)*	0.0396	0.0089(0.0034)**	0.0072	0.0076(0.0030)*	0.0098
Weather	Summer	0.0224(0.0077)**	0.0015	0.0224(0.0161)	0.3704	0.0318(0.0172)	0.0308	0.0276(0.0150)	0.0609
	Spring	0.0043(0.0084)		0.0043(0.0160)		0.0213(0.0168)*		0.0188(0.0157)	
	Winter	0.0191(0.0078)*		0.0191(0.0200)		0.0213(0.0213)		0.0221(0.0195)	
	Autumn	0.000		0.000		0.000		0.000	
Immunize	Yes	0.0265(0.0083)**	0.0013	0.0265(0.0188)	0.1592	0.0225(0.0187)	0.2306	0.0344(0.0168)*	0.0438
	No	0.000		0.000		0.000		0.000	
Residence	Urban	0.0277(0.0059)***	<.0001	0.0277(0.0134)*	0.0395	0.0299(0.0141)*	0.0341	0.0271(0.0129)*	0.0371
	Rural	0.000		0.000		0.000		0.000	
Previous disease	Diarrhea	0.0843(0.0125)***	<.0001	0.0843(0.0264)**	0.0012	0.0514(0.0280)	0.0314	0.0718(0.0248)**	0.001
	Malaria	0.1015(0.0217)***		0.0171(0.0206)		0.0162(0.0237)		0.0182(0.0195)	
	No	0.0686(0.0102)***		0.0157(0.0204)		0.0106(0.0210)		0.0196(0.0191)	
	Tuberculosis	0.2160(0.0170)***		0.1317(0.0443)**		0.1226(0.0514)*		0.1184(0.0474)*	
	Asthma	0.000		0.000		0.000		0.000	
Time		0.0631(0.0019)***	<.0001	0.0631(0.0026)***	<0.0001	0.066(0.0021)***	<.0001	0.0676(0.002)***	<.0001
Breast Feeding	Both breastfed and complementary fed	0.0564(0.0071)***	<.0001	0.0564(0.0162)***	0.0005	0.0373(0.0166)*	0.0051	0.0414(0.0153)**	0.0024
	Complementary (C)	0.0152(0.0133)		0.0152(0.0268)		0.0636(0.0297)*		0.0431(0.0261)	
	Exclusive Breast (EB)	0.000		0.000		0.000		0.000	
Temperature		0.0268(0.0034)***	<.0001	0.0268(0.0075)***	0.0001	0.0213(0.0044)***	<.0001	0.0137(0.0034)***	<.0001
Treatment	Ampicillin	0.0675(0.0181)***	<.0001	0.0675(0.0323)*	0.0098	0.0113(0.0197)	0.1710	0.0054(0.0132)	0.1886
	Ceftriaxone	0.0856(0.0111)***		0.0856(0.0214)***		0.0169(0.0141)		0.0144(0.0102)	
	Gentamicin	0.0843(0.0139)***		0.0843(0.0261)**		0.007(0.0174)		0.0188(0.0117)	
	Penicillin	0.0633(0.0113)***		0.0633(0.0198)**		0.0232(0.0140)		0.0117(0.0104)	
	Amoxicilline	0.000		0.000		0.000		0.000	
Vomiting	Yes	0.0378(0.0058)***	<.0001	0.0378(0.0119)**	0.0016	0.0157(0.1235)	0.1311	0.0205(0.0072)**	0.0063
	No	0.000		0.000		0.000		0.000	
QIC		/		836		721		440	

Key: *** =p-value ≤ 0.001, ***=p-value ≤ 0.01, *=p-value ≤ 0.05

Table V. Parameter estimates using conditional Poisson regression model.

Effect		Between				Within			
		RI		RS		RI		RS	
		Estimate (std.error)	P-value	Estimate (std. error)	P-value	Estimate (std. error)	P-value	Estimate (std. error)	P-value
Intercept		3.296(0.165)		3.297(0.165)		3.082(0.257)		3.203(0.257)***	
Sex	Male	0.0204(0.0136)	0.134	0.021(0.014)	0.129	0.021(0.014)	0.118	0.021(0.014)	0.118
	Female	0.000		0.000		0.000		0.000	
Weight		0.007(0.003)**	0.008	0.007(0.003)**	0.008	0.007(0.003)**	0.009	0.007(0.003)**	0.009
Weather	Summer	0.021(0.018)	0.284	0.022(0.018)	0.262	0.019(0.018)	0.320	0.020(0.018)	0.320
	Spring	0.007(0.019)		0.0079(0.019)		0.008(0.019)		0.008(0.019)	
	Winter	0.024(0.018)		0.025(0.018)		0.023(0.018)		0.023(0.018)	
	Autumn	0.000		0.000		0.000		0.000	
Immunize	Yes	0.022(0.019)	235	0.023(0.019)	0.219	0.022(0.019)	0.248	0.022(0.019)	0.248
	No	0.000		0.000		0.000		0.000	
Residence	Urban	0.031(0.014)*	0.025	0.031(0.014)*	0.026	0.031(0.014)*	0.025	0.031(0.014)*	0.025
	Rural	0.000		0.000		0.000		0.000	
Previous disease	Asthma	0.072(0.029)*	<0.0001	0.071(0.029)*	<.0001	0.071(0.029)*	<.0001	0.07132(0.029)*	<.0001
	Malaria	0.010(0.048)		0.010(0.048)		0.010(0.048)		0.010(0.048)	
	No	0.011(0.020)		0.011(0.020)		0.012(0.021)		0.012(0.021)	
	Tuberculosis	0.146(0.036)***		0.145(0.036)***		0.150(0.036)		0.150(0.036)***	
	Diarrhea	0.000		0.000		0.000		0.000	
Time		0.065(0.002)***	<.0001	0.065(0.002)***	<.0001	0.031(0.071)	0.750	0.004(0.071)	0.750
Breast Feeding	Both breastfed and complementary fed	0.056(0.016)***	0.0003	0.055(0.016)***	0.0004	0.053(0.017)**	0.0006	0.052(0.017)**	0.0006
	Complementary (C)	0.027(0.031)		0.029(0.031)		0.030(0.031)		0.030(0.031)	
	Exclusive Breast (EB)	0.000		0.000		0.000		0.000	
Temperature		0.023(0.004)***	<.0001	0.023(0.004)***	<.0001	0.027(0.007)***	<.0001	0.027(0.007)***	<.0001

Key: *** =p-value ≤ 0.001, ***=p-value ≤ 0.01, *=p-value ≤ 0.05 , RI= Random intercept, RS=Random slope

Table V. Continued.

Effect		Between				Within			
		RI		RS		RI		RS	
		Estimate (std.error)	P-value	Estimate (std. error)	P-value	Estimate (std. error)	P-value	Estimate (std. error)	P-value
Treatment	Ampicillin	0.022(0.027)	0.154	0.023(0.027)	0.155	0.048(0.039)	0.0001	0.120(0.029)**	0.0001
	Ceftriaxone	0.045(0.013)*		0.045(0.018)*		0.077(0.029)**		0.073(0.031)*	
	Gentamicin	0.034(0.023)		0.034(0.023)		0.048(0.035)		0.043(0.016)**	
	Penicillin	0.038(0.018)*		0.038(0.018)*		0.1204(0.029)***		0.072(0.027)**	
	Amoxicilline	0.000		0.000		0.000		0.000	
Vomiting	Yes	0.027(0.009)**	0.004	0.027(0.009)**	0.0048	0.023(0.016)	0.145	0.023(0.016)	0.145
	No	0.000		0.000		0.000		0.000	
Time* Temperature						0.002(0.002)	0.239	0.002(0.002)	0.239
Time*Treatment	Ampicillin					0.004(0.011)	<.0001	0.026(0.007)***	<.0001
	Ceftriaxone					0.009(0.006)		0.022(0.010)*	
	Gentamicin					0.005(0.008)		0.017(0.004)***	
	Penicillin					0.026(0.007)***		0.022(0.005)	
	Amoxicilline								
Time* Vomiting	Yes					0.002(0.004)	0.603	0.002(0.004)	0.603
Intercept (σ^2_I)				0.015(0.001)**		0.015 (0.001)**		0.001	
Slope (σ^2_S)				$2.8*10^{-5}(5.310^{-5})$				0.000	
Generalized χ^2/DF		1.61		0.98		0.90		0.92	

Key: *** =p-value ≤ 0.001, ***=p-value ≤ 0.01, *=p-value ≤ 0.05 , RI= Random intercept, RS=Random slope

assumes each observation is independent, all the variables included in the model had a significant association (p-value<0.05) with RR. However, when the correlation between measurements is considered, the number of variables having a significant association with RR decreased. This indicates that considering the correlation measurement within each child is necessary. However, as considering the correlation only may not be effective, identifying the working correlation is also important.

As we observed from the scatter matrix plot in Figure 3 and checked with the minimum QIC, the best working correlation of the marginal model is ARGEE. Characteristics of children such as weight, immunization, residence, previous disease history, number of hospital visits, breastfeeding, body temperature and vomiting status have a significant effect (p-value<0.05) on RR. The estimated mean RR count decreased by 1% ($e^{-0.0076}$ = 0.99) for a unit weight increase by keeping other characteristics constant. The estimated mean RR count of children who were living in an urban area decreased by 3% ($e^{-0.0271}$=0.97) meaning that children who were born in an urban area were less likely to have pneumonia. When the number of visits of a child to the hospital increased by one, the estimate RR decreased by 7% ($e^{-0.0676}$ = 0.93).

Moreover, compared to children whose mothers practiced both breastfed and complementary fed, children whose mothers practiced exclusive breastfeeding had greater decrement RR ($e^{0.0414}$=1.042). The estimated mean RR increased by 1.4% ($e^{0.0137}$=1.014) for a unit increase in temperature. The estimated mean RR of children who were currently vomiting was 1.021 ($e^{0.0205}$) times of the children who weren't vomiting. This indicates that the RR of vomiting children were greater by 2.1%.

The model includes determining the random effects (random intercept and random slope) within decomposition (within and between) of the time-dependent covariates (Table V). The conditional Poisson regression model was fitted by including a decomposition into "within" and "between" components of time-dependent covariates in Table V. In each of "within" and "between" time dependent covariates decomposition of the random intercept (RI) and random slope (RS) was presented. Once the parameters are estimated, the conditional Poisson regression model comparison was made using generalized χ^2/DF as a fit statistic that incorporate over dispersion. The model with lower generalized χ^2/DF and parsimony is the better[15] and hence, a random intercept Poisson regression model (χ^2/DF=0.90) that considers the dynamic relationship of time dependent covariates such as treatment, temperature and vomiting status is preferred. The model illustrates that the baseline RR of children differ (random intercept, σ^2 = 0.015) while the progress of RR (random slope) of children over time didn't, which matches with the profile plot in Figure 1.

Demographic characteristics of the children such as weight, residence, previous disease history, breastfeeding and temperature have significant effects on the RR. Similarly, the dynamic effect of treatment over time (treatment*time) also has a significant effect (p-value<0.05). The estimated mean RR of pneumonia patients decreased by 1% ($e^{-0.007}$=0.99) per a unit increase of weight (in Kg). Besides, compared to the estimated mean RR of children from rural areas, urban children have an estimated mean RR decreased by 3% ($e^{-0.031}$ = 0.97). This indicates that children from urban areas were more likely to have a lower RR, and were less vulnerable to pneumonia.

Compared to the estimated mean RR of both breastfed and complementary fed children, the estimated mean RR of children receiving exclusive breastfeeding, was higher by 5% ($e^{0.053}$ = 1.054). This indicating that compared to mothers who practice both breastfed and complementary fed during the first six months, mothers who practiced only exclusive breastfeeding reduced the susceptibility of their child from pneumonia. On the other hand, for a unit increase of temperature, the estimated mean RR increased by 2.7% ($e^{0.027}$ = 1.027). There was also a significant estimated

mean RR difference between children with and without previous diseases history of malaria, tuberculosis, asthma, and diarrhea. For instance, the estimated mean RR of children with asthma was 1.073 ($e^{0.071}$) times that of the estimated mean RR of children with diarrhea.

Discussion

The main aim of this study was to describe the evolution of RR of pneumonia in children under five and to determine the effect of treatment, and time dependent and independent characteristics on RR. Two statistical model approaches were used: marginal Poisson regression model (standard and GEE approach) and conditional Poisson regression model (random intercept and slope with the decomposition of time dependent covariates). Model comparison was carried out and parameters estimated using the better of the two models. Both approaches ended up with similar results except that the conditional Poisson regression model analysis added up another information about the baseline RR difference and dynamic relationship of time dependent covariates. This indicates that parameter estimates that used the conditional Poisson regression model was more powerful to meet our objectives.

In general, for the six visits (day 1 to day 6) a decrease of RR counts was observed, this indicates that an increase in the number of days visiting the hospital from (day1 to day 6) correlated with a decrease in the RR. The baseline RR had significant variability which is consistent with a previous study[10], in which both infants and older children showed a random variation in RR over relatively short periods of time or inaccuracies in measurement. The decrease in the RR of each child was nearly similar. The average RR of children in each treatment type declined and was close to each other for each treatment. Ceftriaxone was the most commonly prescribed treatment (38.41%) while Ampicillin treatment was the least prescribed (7.06%). The distribution of urban and rural patients was nearly equal (50.55%, and 49.45% respectively). It was noted that most children (13.91%) had a previous disease history of diarrhea. Of the participants 65.56% had vomiting.

The place of residence of the children (rural or urban) and previous disease history had a significant effect on their RR. These findings are in line with a study that reported that prior disease history such as malaria and environmental factors, such as crowded living conditions and exposure to indoor air pollution may contribute to increase susceptibility to pneumonia.[3] A study on children between 2 to 59 months in Arsi zone, Ethiopia, reported that among factors associated with pneumonia, compared to female children, male children were more likely to develop pneumonia.[13] This study, however, didn't find a significant RR difference in male and female children. Compared to children who had no previous disease history, children with tuberculosis and diarrhea had an increased susceptibility to pneumonia. However, in another study it was reported that among adults with community acquired pneumonia the diagnosis of asthma and pulmonary TB didn't have a consistent significance.[4] Compared to children whose mother practiced only exclusive breastfeeding, children whose mother practiced both breastfed and complementary fed were more likely to catch pneumonia especially in children who are in the first six months of age.[14] Baseline body temperature of children during diagnosis had a significant effect on the RR of under five children, that comply with the finding of a study by Muro et al[10], which reported the higher the temperature of the children, the higher their RR. The effect of treatment on RR varied within time in the sense that treatment effectiveness differed according to the number of times the children visited the hospital. Finally, as the weight of the children decreased, they were more likely to have a higher RR indicating that the weight of under five children has a significant effect on their RR. Therefore, this study concludes that weight, residence, previous disease history, breastfeeding and temperature were found

to be significant factors of pneumonia among children under five in northwest Ethiopia. The effectiveness of treatments co-varied with the number of times the children visited the hospital. A significant variation of baseline pneumonia status was also noted among the children.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HMF, MAD, LMT; analysis and interpretation of results: LMT; draft manuscript preparation: LMT. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Permission to undertake the study was obtained from both the ethical committees of Bahir Dar University and Felege Hiwot Referral Hospital (IRB number: 01-018/02.0).

Availability of data and material

The datasets for the generated analyses during the study is freely available in FHRH, Bahir Dar, Ethiopia by offering formal request of the research proposal.

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Conflicts of interests

The authors declare that they have no competing interests.

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Original Article

Treatment results of modified BFM protocol in pediatric high-risk Burkitt lymphoma

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ABSTRACT

Background. Chemotherapy with high dose methotrexate is the mainstay of treatment for Burkitt lymphoma (BL), especially to manage central nervous system (CNS) disease. However, methotrexate administration requires close drug level monitoring for appropriate folinic acid rescue, which might not be readily available in all centers. In this study, we assessed the long-term treatment outcomes of a modified Non-Hodgkin lymphoma (NHL)-Berlin-Frankfurt-Munster (BFM) 90 regimen in pediatric high-risk BL without CNS involvement.

Methods. Between 1999 and 2011, 42 patients (median age: 7 years) with advanced-stage BL were treated with modified NHL-BFM 90 regimen (methotrexate at a dose of 1 g/m^2). Demographic data, stage, lactate dehydrogenase (LDH) and treatment results were retrospectively evaluated. The patients were assessed for toxicity, survival and CNS recurrence.

Results. Thirty-six patients had Stage III and six had Stage IV disease, respectively. The median LDH level was 1,432 IU/L. Four patients died of infectious and metabolic complications. One patient had local recurrence at the 48th month of the follow-up and he is in the second remission for 72 months. In Kaplan-Meier analysis, the overall survival and event-free survival rates at 10 years were found as 90 % and 88 %, respectively. None of our patients died of treatment failure.

Conclusions. The administration of the reduced dose of methotrexate seems to not compromise treatment success nor increase the risk of CNS recurrence in high-risk BL without CNS involvement. The limitation of the study is that it is not randomized. Our treatment scheme might be considered for centers without methotrexate measurement facility.

Key words: Burkitt lymphoma, BFM, methotrexate.

Lymphomas constitute the third most frequent cancer of children in developed countries.[1] However, in developing countries including Turkey, lymphomas (with a percentage of 18.1%) are the second most common malignancy following leukemia.[2] Burkitt lymphoma (BL) cases benefit from intensive short pulse chemotherapy[3] and the treatment intensity is tailored according to previously defined risk factors. Depending on the protocol, risk group (RG) definitions may vary.[1] Generally, the patients with advanced stage disease, elevated lactate dehydrogenase (LDH) levels and/or central nervous system (CNS)/bone marrow (BM) involvement are assigned to high risk (HR) groups (Table I).

For HR patients enrolled in NHL (Non-Hodgkin lymphoma)-BFM (Berlin-Frankfurt-Munster) and other collaborative groups' protocols, HD-Methotrexate (MTX) (3-8 g/m^2) administration has led to remarkable survival advantage possibly by increasing drug penetrance into

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Table I. Risk stratification according to NHL-BFM 90 and NHL-BFM 95 studies.

NHL BFM 90[4]	R3	*Stage III + LDH≥500 U/L, *Bone marrow involvement *CNS involvement *Multifocal bone lesions
NHL BFM 95[25]	R3	*Stage III and LDH >500, <1000 U/L *Stage IV+B-AL and LDH<1000 U/L and CNS involvement (-)
	R4	*Stage III and IV and B-AL and LDH>1000 U/L *CNS involvement

CNS: Central nervous system

sanctuary sites like CNS.[4,5] However, serum drug level monitoring as well as status of methylene tetrahydrofolate reductase (MTHFR) gene polymorphisms is crucial for safe and effective HD-MTX administration to install a proper folinic acid (FA) rescue schedule.[6] Overdosed FA rescue might abolish the anti-proliferative activity of MTX whereas inadequate doses of FA might lead to increased toxicity.[7-9] In one of the studies reported from our country, NHL high-risk patients were treated with 5 g/m^2 MTX schedule (NHL-BFM 90 protocol) and FA was empirically administered due to lack of MTX measurement facility. The treatment-related toxicity was high and significant treatment delays were observed between the chemotherapy blocks. Increased mortality due to toxicity and recurrent disease led to lower survival rates in comparison to results of the original BFM studies.[10] Therefore, the dose of MTX was reduced to 1g/m^2 in high-risk BL patients without CNS involvement. This study aims to assess the results of the modified NHL-BFM 90 protocol by decreasing the MTX dose from 5 g/m^2 to 1 g/m^2 in HR BL patients without CNS involvement in a developing country.

Material and Methods

Patient eligibility: All HR BL patients without CNS involvement aged younger than 18 years and treated in our center, between 1999 and 2011 were eligible. Disease stage and risk group (RG) stratification were determined according to criteria described in NHL-BFM 90 protocol. Risk group 3 (advanced stage) included the patients with Stage III (St. Jude) and LDH≥500 U/L or/and patients with bone marrow involvement or/and central nervous system disease or/and multifocal bone lesions.[4] Patients with CNS involvement, patients treated with higher doses of MTX (i.e. 5 g/m^2) and patients in the medium risk group were excluded.

Data collection: Patients' files were retrospectively reviewed. Age, gender, stage, site of primary involvement, diagnostic method, LDH, RG, treatment response, toxicity and disease status were recorded. There was no missing data, and no bias in patient selection. Consent was obtained from the parents.

Pretreatment work-up: A histopathological diagnosis was obtained in all patients. BL was diagnosed by the integration of morphological, immune-phenotypic and genetic studies. Routine chemistry profile, chest x-ray, abdominal ultrasound, BM examination, spinal fluid analyses, computed tomography (CT) scan or magnetic resonance imaging (MRI) of the primary site and metastatic sites were performed.

Treatment protocol: Patients received six cycles of chemotherapy based on the NHL- BFM 90 protocol. The details of the original treatment scheme have been reported previously.[4] MTX was administered as 1 g/m^2/36 hours in our study instead of 5 g/m^2/24 hours, which is the dose described in the original protocol. Ten percent and the rest of the dose were infused within 30 minutes and 35.5 hours, respectively. Racemic FA (15 mg/m^2) was empirically administered at

48 and 54 hours after the beginning of the MTX infusion. In the course CC, vincristine (1.5 mg/m^2) was administered instead of vindesine due to its unavailability in Turkey. Requirements for the start of chemotherapy (except for the first course) were as follows: Neutrophil counts higher than 500/mm^3 and platelet counts higher than 50.000/mm^3 after the nadir of post-chemotherapeutic cytopenia has resolved. The minimal and maximal interval between successive chemotherapy blocks were two and three weeks, respectively.

Toxicity and response assessment: Toxicity was evaluated in accordance with World Health Organization (WHO) Criteria.[11] Tumor response to therapy was evaluated after each course of therapy by physical examination, biochemistry, and abdominal ultrasound. In patients with BM involvement, control BM examination was performed until the BM was cleared from blasts. After two blocks of chemotherapy, abdominal CT or MRI was also done.

Complete remission (CR) was defined as the clinical disappearance of the disease. Partial remission (PR) was accepted as more than 50% of tumor regression. Progressive disease (PD) was defined by the appearance of new disease during treatment or as incomplete regression of local tumor followed by progression during the treatment protocol. Relapse was defined as evidence of disease after at least 1 month in CR.

Ethics Approval

The study is approved by our Hospital Ethics Committee (University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital Ethics Committee-Number 3003/2020).

Statistical Analysis:

Patient characteristics were summarized using descriptive statistics. Last evaluations were conducted in December 2017. Event- free survival (EFS) was calculated from the day of diagnosis to the date of last observation without events (relapse, secondary malignancy, and death from any cause). Overall survival (OS) was the time interval between the date of diagnosis and the date of death from any cause or the date of the last follow-up on which the patient was known to be alive. The survival estimates were assessed by the Kaplan-Meier method. Survival was censored at the date of the last follow-up if death was not observed. A p value < 0.05 was statistically significant.

Results

Patient's characteristics: During the study period, 55 children with BL were treated. Two patients with CNS involvement, 11 patients in the medium risk groups were not eligible. Forty-two patients were included in the study. Seventy percent of the patients were referred from cities other than Istanbul. Thirty-four boys and eight girls had a median age of 7 years (range 3-14 years). The abdomen was the most common site of primary involvement and laparotomy was the most frequent method for diagnostic tissue sampling. Thirty-six patients had Stage III and six had Stage IV disease, respectively, with a median LDH value of 1432 U/L (550 - 9080) (Table II). All the patients came from families of lower socioeconomic status (had income equal to or below the minimum wage).

Outcome: We did not observe treatment failure-related mortality during the study period. Two patients died during prophase due to sepsis and one patient died after the first AA block due to sepsis and tumor lysis syndrome. The remaining 39 patients were evaluated for treatment response after 2 courses (AA+BB) of chemotherapy. Twenty-eight (72%) patients achieved CR and 11 patients had a PR (28%). Two patients had radiological evidence of residual lesions after completing the treatment. The pathological examination of resected residual masses revealed complete necrosis in all cases. One patient in remission died of neutropenic fever and sepsis after the last course of treatment. We observed one case of local (abdominal) recurrence at the 48th month of the follow-up and the patient was salvaged by second-line chemotherapy. He has been in

remission for 72 months since the recurrence date. All the remaining 38 patients are alive with no evidence of disease. In Kaplan-Meier analysis, the OS and EFS rates at 10 years were found as 90 % and 88 %, respectively (median follow-up of 121 months (one week-210 months) (Fig. 1). The most common treatment-related adverse effect was grade III and grade IV hematological toxicity (79%). Febrile neutropenia was observed after 70% of chemotherapy blocks. Severe mucositis was seen in 42% of courses.

Table II. Clinical and demographic data of patients.

Factor	Results
Age-Median (range)	7 years (3-14 years)
Sex	
Female	8
Male	34
Primary tumor site	
Abdomen only	36
Abdomen and cervical lymph node	2
Abdomen and orbita	1
Abdomen and jaw	1
Abdomen and tonsil	1
Abdomen and paravertebral area	1
Stage	
III	36
IV	6
LDH -Median (range)	1432 (550-9080) IU/L
Diagnostic procedure	
Laparotomy	16
Tru-cut biopsy	13
Excisional/incisional biopsy	4
Fine needle biopsy	1
Peritoneal fluid cytology	7
Bone marrow aspiration	1

Discussion

Patients with mature B cell lymphomas have been treated with chemotherapy protocols of varying intensity and duration according to the assigned risk groups.[4,5] A comparison in terms of treatment success across the study groups is quite difficult since the risk stratification and chemotherapy protocols are not uniform. In the present study, 80% of our BL patients were in the RG3 group according to NHL-BFM 90 risk criteria. The percentage of RG3 patients was quite high in our study, in comparison to that of the original BFM 90 series (43%). Even if we were to stratify our patients according to NHL-BFM 95 protocol, the percentage of our RG3 and RG4 patients would have still been higher than that of the original BFM NHL 95 protocol (18 % and 64% vs. 16% and 28%, respectively).[4,12] Our RG distribution appears to be similar to that of other developing countries.[13-15] The number of

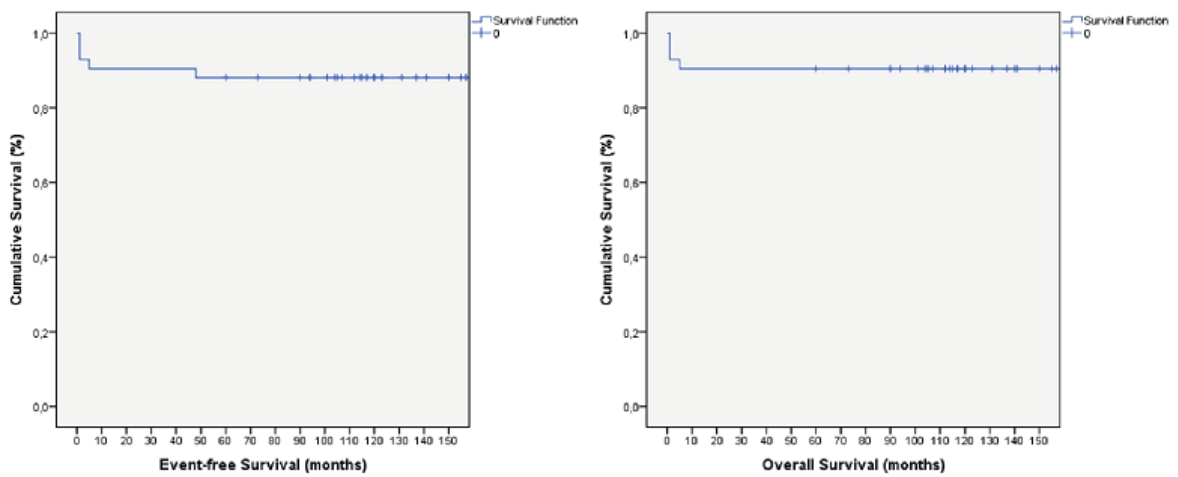

Fig. 1. Survival curves of high-risk CNS-negative patients treated according to modified BFM approach.

HR patients in our country differs according to the city and to the center of the study.[16,17] During the period of the study, our center admitted patients of low socioeconomic status, and most of the patients were referred from medically underserved rural areas of Turkey. Most of the severely ill patients were transferred under inappropriate conditions and most patients underwent unnecessary laparotomy leading to delayed referral. The above circumstances might have contributed to the higher number of advanced stage patients.

The MTX dose and the duration of administration are not standard among different BL protocols, and the optimal dose and the duration of MTX infusion are not clear.[4,5] This issue raises the question of whether the higher doses of MTX (e.g. 5-8 g/m^2/cycle) are essential in the treatment of BL. High-dose MTX chemotherapy necessitates stringent supportive care and appropriate folinic acid administration adjusted according to serum levels of MTX. Lack of these measures might increase toxicity and lead to prolonged intervals between the cycles with inferior treatment outcomes. The lower doses of MTX administration have been especially preferred in centers without MTX level measurement facilities.[17-20]

Due to methodological differences between the studies with modified protocols, it is not possible to make a reliable comparison among these studies as well as with the original BFM study. Although some study groups did not present subgroup (i.e. RG) analysis data in their reports, the EFS or OS rates were reported to range between 50-82% in modified BFM protocols with 1-2 g/m^2 MTX, with or without Rituximab (Table III). In our study, the EFS rate was 89 % with 1 g/m^2 MTX without rituximab in all our RG3 patients without CNS involvement. The survival rate of our patients seems comparable to the results reported from BFM groups (which used MTX 5 g/m^2 (NHL-BFM 90 R3 Stage III 81%, and NHL-BFM 95 R3 85±4%, R4 81±4%))[4,12] and seems superior than the survival rates of several studies with 5 g/m^2 MTX from developing countries and Turkey (Table III).[13,14,16,17,20-23]

Table III. Doses of methotrexate and treatment outcomes of previous BFM-based studies with high-risk Burkitt lymphoma.

Authors	Country	Protocol	MTX (g/m^2/course)	EFS	OS
Reiter A et al.[4]	International	BFM 90	5	78	
Woessmann et al.[25]	International	BFM 95	5	81	
Kavan et al.[26]	Czech Republic	BFM 90	5	57*	
Müller et al.[27]	Hungary	BFM 90/95	5	81	83
Pillon et al.[28]	Italy	AIEOP LNH92 (BFM 90)	5	73	
Celkan et al.[16]	Turkey	BFM 90	5	71	
Karadeniz et al.[17]	Turkey	Modified BFM 90	1	80*	81*
		Modified BFM 90	3	92*	92*
		Modified BFM 95	5		
Kebudi et al.[18]	Turkey	BFM 90	5	44*	44
		Modified BFM 90/95	1	69*	81*
Sun al.[29]	China	Modified BFM-90	5	72	
Cervio et al.[30]	Argentina	Modified BFM 90/95	2	70	
Chantada et al.[31]	Argentina	Modified BFM-90	2	82-50 (RG3-4)	81*
Klumb et al.[20]	Brazil	Modified BFM 90	2	74	
Márky al.[32]	Nordic countries	NOPHO 95, BFM 90-95	5	91*	
Dokmanovic et al.[33]	Serbia	BFM 95	5	95*	92*
Samochatova et al.[34]	Russia	Modified BFM 90 with Rituximab	1 (first two blocks)		82

*Results of all risk groups, no documented analysis for subgroups, namely RG3/RG4

CNS involvement is the most important prognostic factor in various studies. Our study represents the treatment outcome of an exclusive group of patients without CNS disease. The comparison of our results with those of the treatment groups with CNS involvement as well as the lack of a control group might be considered as limitations of our study. However, Salzburg et al.[24] reported the outcome of patients with CNS involvement in three consecutive BFM studies and the probability of 5-year EFS was found as 81% in CNS negative stage IV Burkitt's lymphoma/ B cell ALL patients. This might indicate non-inferiority of our modified BFM approach.

There are no randomized clinical trials comparing the efficacy and the toxicity of 1 g/m^2 MTX against 5 g/m^2 MTX, with similar infusion schedules in high-risk lymphoma treatment. As most of the studies submit cumulative therapy outcomes as well as toxicities of all groups together, it is difficult to sort and compare the toxicity rates by each risk group among different studies. In the original NHL-BFM 90 protocol results, the incidence of grade III and IV mucositis was found to be approximately 48% after the blocks containing 5 g/m^2 MTX.[4] We observed grade III and IV mucositis in 40% of our patients after chemotherapy blocks. Celkan et al.[16], from our country, reported mucositis as a common side effect of 5 g/m^2 MTX administration, but the incidence was not reported. Grade III and IV hematologic toxicity was seen in all their HR patients but the incidence of febrile neutropenia was lower (40%) in comparison to our study. Karadeniz et al.[17], again from Turkey, did not reveal a difference in toxicity rates between treatment schedules administering 1 g/m^2, 3 g/m^2, and 5 g/m^2 MTX. The mucositis rate was similar to our results. Grade III-IV hematologic toxicity rates and the incidence of febrile neutropenia episodes were 80% and 70%, respectively, similar to the rates observed in our study. The hematologic toxicity profile was not presented in the original NHL BFM 90 protocol but the febrile neutropenia episodes were observed after 37% and 31% of AA and BB courses, respectively.[4] The toxic death rate for B cell lymphomas ranges between 3% and 20% depending on the development status of the country.[4,5,14,21] Treatment-related mortality rate was found to be 9% in our study. Three of our patients died due to infection, one patient died due to sepsis and tumor lysis syndrome. Deceased patients were transferred from underprivileged regions of our country under inappropriate conditions. Their general status was already poor upon arrival (one with severe tumor lysis syndrome, two with infection in the operative bed, one with spinal cord compression with neurogenic bladder, and a urinary catheter). Our cohort consisted of patients treated between 1999 and 2011. Deaths (including the patient who died while in remission immediately after last chemotherapy cycle) were observed early in the study period when our patients were treated in common crowded wards. Besides, inadequate access to both pediatric intensive care units and to appropriate supportive care were major problems associated with increased mortality at that time. In the study of Celkan et al.[16], treatment-related mortality was higher in the 5 gr/m^2 MTX arm whereas another study from our country did not report any toxic death with 1 gr/m^2, 3 gr/m^2 or 5 gr/m^2 MTX.[17] The difference in treatment-related mortality rates between studies might also be attributed to the infrastructure of the medical center, study era, and the patient-related factors.

We submit the treatment results of the HR BL patients without CNS involvement treated with the modified NHL BFM 90 protocol (i.e. 1 gr/m^2 MTX.) Despite the low dose of MTX, objective response was achieved in all our patients. We did not observe CNS disease in long-term follow-up. None of the patients died due to treatment failure. The survival rate achieved in our study seems comparable to that of original BFM-90 protocol. Although serial MTX measurements and MTHFR gene polymorphism status remain essential for safe MTX administration, the encouraging results of our study raise the question of whether

administration of 1 g/m^2 MTX would be adequate for high-risk BL patients without CNS involvement in developing countries without necessary facilities.

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Author contributions

The authors confirm contribution to the paper as follows: study conception and design: SV, DBG; data collection: SV, İÖD; analysis and interpretation of results: DBG, SV, SK, RK; draft manuscript preparation: SV, DBG. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study is approved by our Hospital Ethics Committee (University of Health Sciences, Şisli Hamidiye Etfal Training and Research Hospital Ethics Committee-Number 3003/2020).

Source of funding

No funding has been secured for the study.

Conflicts of interest

The authors of this report certify that they don't have any relevant financial, personal or professional relationships with other people or organizations that pose a conflict of interest or that could potentially influence or bias the results of the study described in the manuscript.

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Original Article

Effects of lockdown during corona pandemic on children with neurodevelopmental disorders-A questionnaire-based survey

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ABSTRACT

Background. Lockdown due to Corona pandemic is an unprecedented event, which has had a profound impact on the lives of children across all ages. Its effects on children with Neurodevelopmental Disorders (NDD) has not been adequately studied. This study was performed in order to explore the effects of lockdown during the Corona pandemic on children with NDD and their parents.

Methods. The survey was conducted in three Indian tertiary-care hospitals wherein parents of children with NDD were requested to respond to an online questionnaire. The questions attempted to elicit various aspects of the children's therapies and behavioural profiles as well as their parents' experiences during the pandemic related lockdown.

Results. 135/188 (71.8%) parents of children with Autism Spectrum Disorder (ASD)(n=104), Attention Deficit Hyperactivity Disorder (ADHD) (n=26) and Learning Disability (LD)(n=5) responded. Pre-lockdown, 133 (99%) children were receiving regular institution-based therapy, which ceased intra-lockdown. Mean cumulative home-based therapy duration significantly increased during lockdown (p=0.03). Parents reported significantly increased temper tantrums in children (p=0.02). They perceived that during lockdown, their children were bored and their interactions and speech worsened. Majority of parents reported worsening of own qualities of life, but felt confident of taking care of their children during lockdown.

Conclusions. To conclude, children with NDD and their parents were significantly affected by Corona pandemic-related lockdown. Institutional therapy discontinuation, behavioural deterioration (especially among ASD and ADHD) and parental stress were prominent challenges whereas parental motivation and reliance on home-based therapy were the positive highlights. The survey points to the role of regular parent-administered home-based therapy in children with NDD, especially to tide over similar unexpected adverse scenarios.

Key words: corona pandemic, COVID-19, lockdown, neurodevelopmental disorders.

Coronavirus-2019 Disease (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was declared as a global epidemic by World Health Organization (WHO) on 11 March 2020.[1] Lockdown was an unprecedented, emergency measure, which was adopted across several countries to prevent the exponential spread of the disease. India was placed under lockdown from 24 March 2020, which was extended till 31 May 2020 with subsequent gradual relaxation of restrictions. At the time of submission of this article (16 August 2020), a partial lockdown was in vogue in India. Lockdown is having profound repercussions on day-to-day functioning of people from all walks of life, including children. Children with neurodevelopmental disorders (NDD) constitute a sizeable segment

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of pediatric population with high healthcare service requirements compared to the general population.[2] Discontinuation of almost all services except skeletal emergency services, socioeconomic implications, compounded with the disruption of daily routine and a novel, uncertain environment are likely to have varied effects on children with NDD depending on their underlying neurological diagnoses and disease severity. Parents/primary caregivers are also likely to be affected. These effects are hitherto unknown. Hence, our study was formulated in this backdrop to explore the salient effects of lockdown during the COVID-19 pandemic on children with NDD.

Material and Methods

Study design and population

This online cross-sectional survey was conducted simultaneously at three tertiary care referral hospitals in India having pediatric neurology services. The Institutional Ethical Committee approved the study and it was registered with the Clinical Trials Registry- India (CTRI) (No: CTRI/2020/06/025795). All parents/primary caregivers of children diagnosed with either Autism Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD) or Learning Disorder (LD) who were on follow-up at any of the three centers were approached telephonically for participation. The children were excluded if they had any coexisting neurodegenerative or neuromuscular disorder.

Procedure

A pragmatic anonymized survey was developed and piloted by authors (JNG, SR). The questions were thereafter hosted online (Google Form ™) between 15 April 2020 to 15 June 2020. Parental consent was obtained. A participant information page was included at the beginning of the survey. The survey included 21 questions (Table I) and a 5-point Likert Scale having 10 questions (Table II). If the parents/primary caregivers agreed to participate in the survey, the online Google Form link was shared with them. If they did not respond within the subsequent 72 hours, then two reminder telephone calls 72 hours apart were made. If they still did not fill the online form after a total of three telephone calls, no further attempts were made to contact them. For parents/primary caregivers who were uncomfortable with online filling of the form, telephonic assistance for filling the form was provided.

Outcome measures

The primary outcome of the study was to assess the effect of COVID-19 related lockdown on children with ASD, ADHD, and LD. The secondary objective of the study was to assess the effects of the lockdown on the parents/primary caregivers of these children.

Statistical analysis

Continuous data were presented as mean ± Standard Deviation (SD) and categorical data were expressed as number (%). Group differences were tested using Chi-square test and Wilcoxon rank sign test. All statistical analyses were performed using SPSS software (version 20.0, IBM), p values < 0.05 were considered statistically significant.

Results

Among 188 families that were contacted, 135 (71.8%) responded. These included 104 children with ASD (82 boys), 26 with ADHD (20 boys) and 5 with LD (3 boys). Table III outlines the baseline characteristics of participating children. Respondents included 83 (51%) fathers and 52 (49%) mothers (statistically similar; p= 0.41).

Therapies

Prior to implementation of lockdown, some form of institution-based therapy was being regularly administered to 133 children (99%) (Fig. 1). These included occupational therapy [ASD =101(97%), ADHD = 3(12%), LD = 0], speech therapy [ASD = 82(79%), ADHD =5

Table I. Parental questionnaire.

1.	My child is a (boy/girl).
2.	His/her is.....years.....months old.
3.	He/she is under follow-up of pediatric neurology OPD since.....
4.	My child is a patient of .....
5.	His/her total average daytime sleep duration (in hours) was..... and total average night-time sleep duration (in hours) was ..... prior to lockdown.
6.	His/her total average daytime sleep duration (in hours) is..... and total average night-time sleep duration (in hours) was ..... during lockdown.
7.	He /she had sleep problems prior to lockdown. (Yes/No)
8.	He/she is having sleep problems during lockdown. (Yes/No)
9.	He/she had feeding issues prior to lockdown. (Yes/No)
10.	He/she is having feeding issues during lockdown. (Yes/No)
11.	He she had significant temper tantrums prior to lockdown. (Yes/No)
12.	He/she is having significant temper tantrums during lockdown. (Yes/No)
13.	He/she used to receive following institutional therapy/therapies regularly before lockdown .....
14.	Among therapies mentioned in Serial No 13, following are not being administered during lockdown .....
15.	Total average weekly institutional therapy/therapies administered to the child prior to lockdown was.....hours/week.
16.	Total average weekly institutional therapy/therapies administered to the child during lockdown is.....hours/week.
17.	He/she used to receive following therapy/therapies regularly at home before lockdown .....
18.	Among therapies mentioned in Serial No 17, following are not being administered during lockdown .....
19.	Total average weekly home-based therapy/therapies administered to the child prior to lockdown was.....hours/week.
20.	Total average weekly home-based therapy/therapies administered to the child during lockdown is.....hours/week.
21.	Child was getting .....number of drugs daily before lockdown. During lockdown .....drugs were not given

OPD: outpatient department

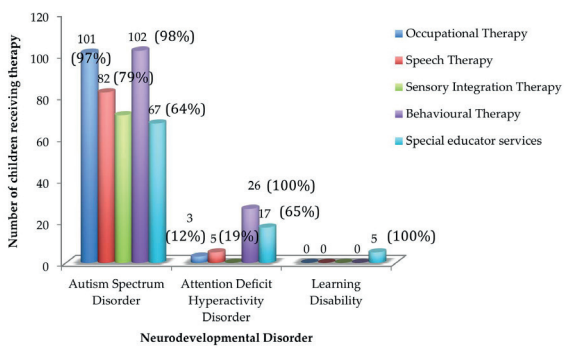

Fig. 1. Clustered bar diagram displaying institution-based therapies administered to children with NDD prior to lockdown.

(19%), LD = 0], sensory integration therapy [ASD =71(68%), ADHD =0, LD =0), behavioral services [ASD = 102 (98%), ADHD = 26 (100%), LD =0] and special educator services [ASD =67(64%), ADHD =17(65%), LD =5(100%]. During lockdown, no child could avail of institution-based interventions as these facilities were temporarily suspended. Hence, difference between the pre-lockdown and intra-lockdown mean cumulative institution-based therapies administered to the children was significant (p=0.02, Table IV).

Table II. Parental Likert scale.

Part A						
Serial number	Question	Choose one of the following responses				
		(A) Totally agree	(B) Somewhat agree	(C) No comment	(D) Partly disagree	(E) Totally disagree
1	I feel as confident of taking care of my child during lockdown as before					
2	My child appears to be bored during major part of the day during lockdown					
3	Home-based therapy given to my child during lockdown is inadequate for him/her					
4	Lockdown has adversely affected my child's therapy					
Part B						
Serial number	Question	Choose one of the following responses				
		(A) Significantly worse	(B) Somewhat worse	(C) Neutral	(D) Somewhat better	(E) Significantly better
1	My overall quality of life during lockdown compared to what it was prior to lockdown					
2	Child's speech during lockdown					
3	Child's sleep-related problems during lockdown					
4	Child's temper tantrums during lockdown					
5	Child's interaction with parents and sibling(s) during lockdown					
6	My child's behavioural problems during lockdown compared to those before lockdown					

Though parents of all children with NDD were advised home-based therapy as a part of institutional protocol, the number of children actually being administered some form of home-based therapy was variable. Details of pre-lockdown and intra-lockdown home-based therapy are illustrated in Fig. 2. The mean cumulative duration of home-based therapies administered to the entire cohort of children with NDD during lockdown was significantly greater than that being administered prior to lockdown (p=0.03, Table IV).

The number of children with ASD who received home-based therapy during pre-lockdown and intra-lockdown periods was comparable. These therapies included home-based occupational therapy (pre-lockdown: 62, intra-lockdown: 70, p=0.25), home-based sensory integration therapy (pre-lockdown: 10, intra-lockdown: 12, p=0.65), home-based behavior therapy (pre-lockdown: 50, intra-lockdown: 55, p=0.49) and home-based special educator services (pre-lockdown: 2, intra-lockdown: 2, p=1). Similarly, pre-lockdown and intra-lockdown variations in the numbers of children with ADHD receiving

Table III. Demographic profile of children with neurodevelopmental disorders enrolled in the study.

Gender	Boys: 105, Girls: 30	
Neurological Diagnosis	Autism Spectrum Disorder (ASD)	104 (boys: 82, girls: 22)
	Attention Deficit Hyperactivity Disorder (ADHD)	26 (boys: 20, girls: 6)
	Learning Disability (LD)	5 (boys: 3, girls: 2)
Age bands	1-3 years	ASD: 9 ADHD: 3 LD: 0
	4-6 years	ASD: 31 ADHD: 6 LD: 0
	7-9 years	ASD: 49 ADHD: 13 LD: 1
	10-12 years	ASD: 15 ADHD: 4 LD: 4
Mean duration of prior follow-up in OPD	ASD	23 (QI: 7, Q3: 54)
	ADHD	19 (Q1: 3, Q3: 26)
(Median, 1st quartile,3rd quartile in months)	LD	47 (Q1: 32, Q3: 63)
Children receiving pharmacotherapy pre-lockdown	ASD	78 (1 drug: 45, 2 drugs: 33)
	ADHD	26 (1 drug: 3, 2 drugs: 14, 3 drugs: 9)
	LD	0
Children who had discontinuation of pharmacotherapy during lockdown	ASD	2 (all on mono- drug therapy)
	ADHD	0
	LD	0

OPD: outpatient department

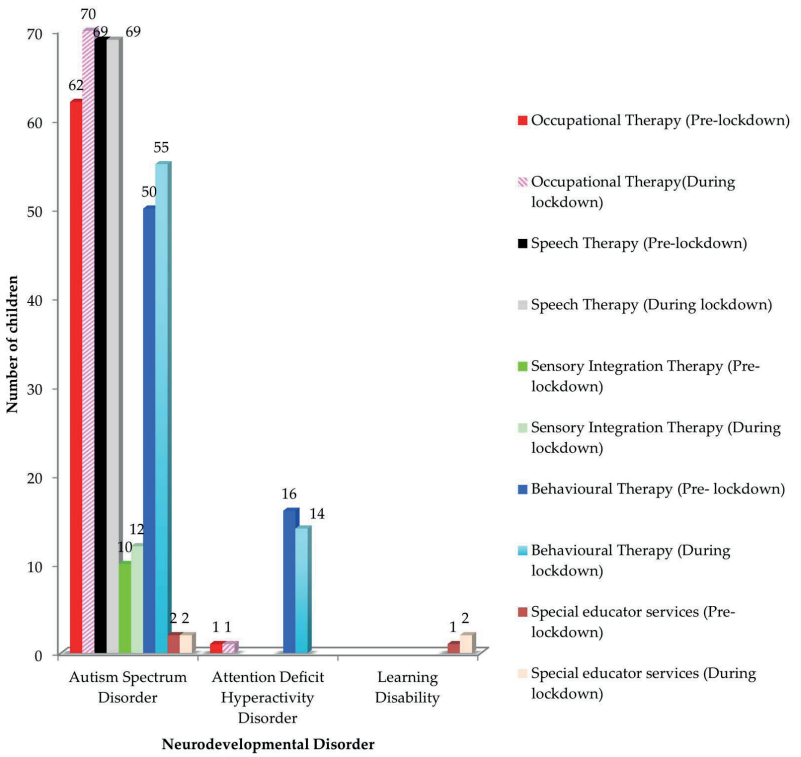

Fig. 2. Clustered bar diagram representing home-based interventions administered to children with NDD prior to implementation of lockdown and during lockdown period.

Table IV. Tabular representation of key target behaviours of enrolled children and the various therapies offered to them prior to and during lockdown period (based on parental response).

Variable	Pre-lockdown	During lockdown	p value
Daily mean cumulative sleep duration (night) (hours)	8.8 (Q1:7.5, Q3: 9; SD: 2.4)	7.3 (Q1:6, Q3:9; SD: 3)	0.7
Daily mean cumulative sleep duration (daytime) (hours)	3.2 (Q1:1.4, Q3:4; SD: 2.1)	2.7 (Q1:1.9, Q3:3; SD: 1.8)	0.86
Number of children with parentally reported sleep problems	96	82	0.72
Number of children with parentally reported feeding issues	82	94	0.13
Number of children with parentally reported significant temper tantrums	108	122	0.02
Mean duration of consolidated institutional occupational therapy (occupation + speech + behavioral therapy) (hours/week)	5.4 (Q1: 2.5, Q3:7; SD: 3.7)	0	0.02
Mean duration of Home –based intervention (hours/week)	11.2 (Q1: 7.8, Q3: 15.2; SD: 5.4)	15 (Q1: 8.1, Q3: 18.3; SD: 4.6)	0.3

Q1: first quartile, Q3: third quartile, SD: standard deviation

home-based therapy [occupational therapy (pre-lockdown: 1, intra-lockdown: 1, p=1), behaviour therapy (pre-lockdown: 16, intra-lockdown: 14, p=0.32)] and those with LD receiving home-based special education (pre-lockdown: 1, intra- lockdown: 2,p=0.48) were statistically insignificant.

Behavioural changes

Parents reported a significant increase in their children's temper tantrums during lockdown (p=0.02, Table IV). No significant biorhythm alteration was noted in the children. Pre-lockdown and intra-lockdown sleep patterns were similar with comparable daytime and nocturnal sleep durations and sleep related problems. Though greater number of children were reported to have feeding issues during lockdown period compared to that during pre-lockdown period, the difference was statistically insignificant (p=0.3, Table IV).

Parental Perceptions

Parental responses to the Likert Scale questionnaire have been represented through Fig. 3, Fig. 4 while a diagnosis-based stratification of the core responses are represented through Table V. The percentages of parents of children with ASD/ADHD/LD who responded to the various questions as 'agreed' were similar (N = 30; p = 0.20). Percentages of parents who responded as 'disagreed' were also similar (N = 30; p=0.39). When comparing across the groups, the results are significant [H statistic is 15.8393 (4, N = 50), p: 0 .00324]. Significantly larger number of parents/primary caregivers felt that lockdown affected their children's therapy adversely (Table V). Subclass analysis revealed that this difference was not significant in the responses of parents/primary caregivers of children with LD (p=0.22). Significant proportion of parents/primary caregivers responded that their children were bored during lockdown and that their behavioral problems, interactions with parents and peers, temper tantrums and sleep related issues had worsened during lockdown. The reported worsening in speech problems during lockdown was statistically insignificant (Table V). Parents/primary caregivers felt that the quantity of home-based therapy administered

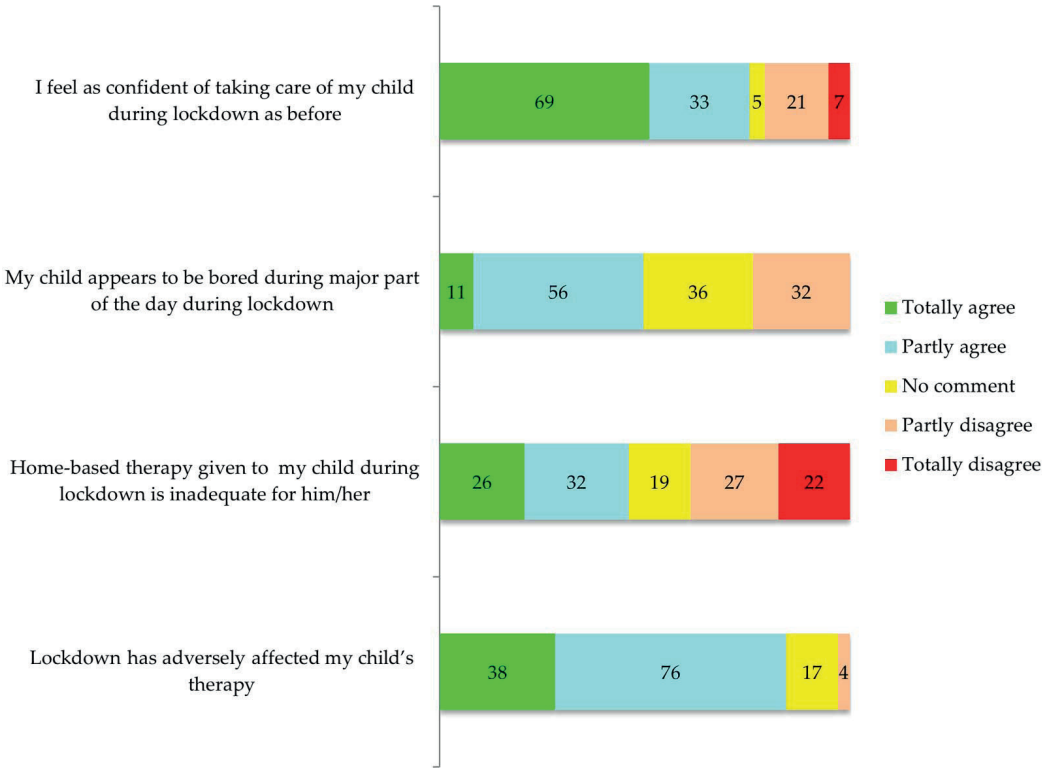

Fig. 3. Graphical representation of parental responses to 'Likert Scale Questionnaire: Part A'.

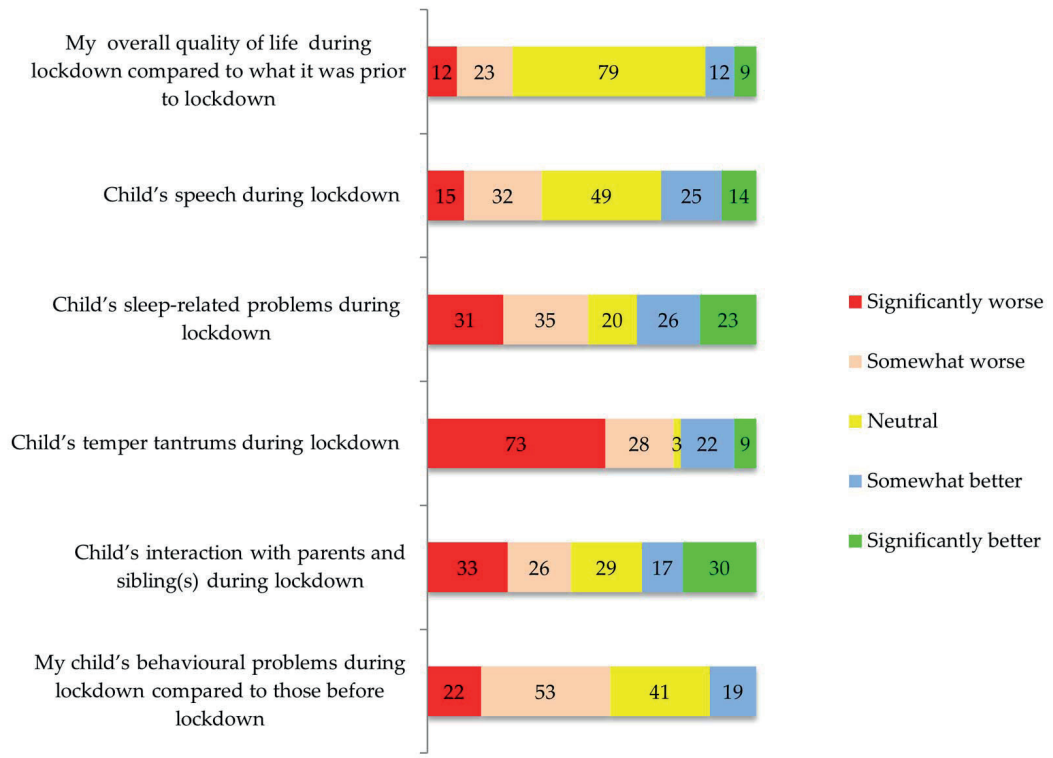

Fig. 4. Graphical representation of parental responses to 'Likert Scale Questionnaire: Part B'.

Table V. Tabular representation of parental responses to Likert Questionnaire along with statistical interpretation.

Questions	Responses						p value (comparison of 'Agree (totally/partially)' versus 'Disagree (Totally/partially)'
	AGREE (Totally/Partly)			DISAGREE (Totally/Partly)			
	Parent of child with ASD	Parent of child with ADHD	Parent of child with LD	Parent of child with ASD	Parent of child with ADHD	Parent of child with LD	
Has lockdown adversely affected your child's therapy ?	93 (89%)	18 (69%)	3 (60%)	3 (3%)	0	1 (20%)	<0.0001
Is amount of home-based therapy given to your child during lockdown inadequate for him/her?	47 (45%)	10 (38%)	1 (20%)	39 (38%)	8 (31%)	2 (40%)	0.26
Does your child appear to be bored during major part of the day during lockdown?	45 (43%)	17 (65%)	5 (100%)	30 (29%)	2 (8%)	0	<0.0001
Do you feel as confident of taking care of your child during lockdown as before?	86 (83%)	13 (50%)	3 (60%)	10 (10%)	14 (54%)	4 (80%)	<0.0001
Have your child's behavioural problems worsened during lockdown?	51 (49%)	23 88%)	1 (20%)	5 (5%)	11 (42%)	3 (60%)	<0.0001
Has your child's interaction with parents and sibling(s) worsened during lockdown?	45 (43%)	11 (42%)	3 (60%)	39 (38%)	7 (27%)	1 (20%)	0.007
Has your child's temper tantrums worsened during lockdown?	79 (76%)	21 (81%)	1 (20%)	24 (23%)	3 (12%)	4 (80%)	<0.0001
Has your child's sleep-related problems worsened during lockdown?	54 (52%)	12 (46%)	0	36 (35%)	8 (31%)	5 (100%)	0.037
Has your child's speech worsened during lockdown ?	40 (38%)	7 (27%)	0	23 (22%)	15 (58%)	1 (20%)	0.3
Has your overall quality of life worsened during lockdown compared to what it was immediately prior to it?	27 (26%)	7 (27%)	1 (20%)	14 (13%)	6 (23%)	1 (20%)	0.037

during lockdown was adequate. (Table V). Significantly larger number of parents/primary caregivers opined that their quality of lives had worsened during lockdown period compared to pre-lockdown period (Table V). However, the parents/primary caregivers of children with LD did not report significant worsening of their quality of lives.

Discussion

Lockdown due to the corona pandemic is a novel situation with possible impact in multiple sectors of societal functioning including routine healthcare delivery and rehabilitation services. In addition to the closure of various day-to-day social and professional activities except essential services, the lockdown also has socioeconomic and psychological dimensions. Children, more so those with special needs, stand to be affected due to the limited health care access during this period.[3] Children with NDD form a significant proportion of clientele enrolled in regular pediatric neurology follow-up programmes.[4] Hence, children with NDD form the target cohort in the current study.

ASD comprises of a large segment of NDD with varying prevalence ranging between 1-2%.[5,6] ADHD has been reported to have higher prevalence rates ranging upto 1.9%.[7] In the index study, number of children with ASD significantly outnumbered those with ADHD and LD, probably reflecting differential prevalence as well as bias since the study was a hospital-based one. Moreover, regular institutional therapy was practiced for most ASD and some ADHD patients. Learning Disability management, on the other hand, hinges on a multimodal approach with remedial education measures being the pivot.[8]

The study centres practiced institution-based interventions for NDD in consonance with current global practice where protocolized care is provided under closed supervision of trained personnel. The lockdown scenario drastically hampered institutional rehabilitation as depicted through the survey. Long-term effects of therapy discontinuation are yet to be evaluated. Though advocated in all, there are variable rates of compliance to home-based therapy as elucidated through the index survey. Adherence to a home-based rehabilitation programme depends on multiple factors such as motivation, social support and previous adherence to therapies.[9] The lockdown scenario motivated parents/primary caregivers who were already administering home-based therapy to their children, to increase the number of activity-hours and also led few to start administering these modalities afresh. This parental behaviour pattern is indicative of the fact that home-based therapy may be a feasible modality for ensuring continuity of rehabilitation of children with NDD in unexpected lockdown-like scenarios. In the absence of robust head-to-head trials between institutional versus home-based therapy, a hybrid approach may be advocated as an effective model.[10] Such a model would be effective at times when institutional therapy is disrupted as in the case of the current lockdown.

COVID-19 pandemic is akin to a disaster-like situation which is likely to trigger immense psychological problems in general pediatric clientele' ranging from anxiety, behavioural issues, sleep disorders and anorexia.[11] The psychological effects of this lockdown, specifically on children with NDD have not been published till date. Index survey hints at these behavioural issues by revealing significant parental concerns about increase in behavioural problems, temper tantrums, interaction and sleep issues though the perceived increase is not statistically significant. There is evidence to support the fact that disruptive behaviours of children with ADHD, rather than the disorder itself, significantly affects parental psychopathology and leads to parental stress.[12] The survey is based on parental perceptions, which have a strong subjective element. Parental perceptions are crucial as parents form the core of the NDD management team. The successful implementation of any therapeutic programme in children with NDD is dependent on perspectives of parents/primary-caregivers.[13]

Hence, even though statistically insignificant, concerns about increased behavioural problems as in the index survey, need to be addressed. The perception of increased behavioural problems in children with NDD may be hypothesized to be secondary to the interplay of multiple factors such as therapy discontinuation, daily-routine alteration, monotony, excessive parental involvement, reflection of parental stress and undue expectations which all need to be addressed on an individualized fashion post lockdown relaxation. Children with milder variants of NDD may be additionally affected by their perceptions about the uncertain environment and fear psychosis as it has been adequately reported that exposures during natural disasters constitute a risk factor for poor psychological health in children and adolescents.[14] In similar lines, 83% among 2111 individuals up to the age of twenty-five years who had history of mental illness, remarked in a survey conducted in the United Kingdom that their conditions had deteriorated due to COVID-19 pandemic.[15] These aspects were not considered in the current survey.

The strengths of the study are the simplistic design, relevant questions and emphasis on parental perceptions as highlighted above.

The most salient limitation of the study is its non-generalizability in the community setup as the study is a hospital-based one and the cohort is skewed towards ASD. Behavioural issues are more common in children with ASD and ADHD than with LD. Interventions to improve social skills is a major constituent of ASD rehabilitation programmes.[16] It is intuitive to hypothesize that the disruption in these activities due to lockdown might have led to a surge in behavioural problems leading to the results of the survey. This may not be the issue with children with LD. Few entities that may be classified under the umbrella of NDD such as children with refractory epilepsy and Down syndrome were not included in the study.

Even two-year old children adversely affected by the disruption of their daily routines have been noted to become well adapted after adequate communication.[17] Parental communication with children diagnosed with NDD is complex and depends on factors such as the nature and severity of the underlying disorder and parental motivation. This aspect was not factored in the current study design.

The earthquake in Italy had displayed that children with ASD who have been exposed to disasters, might have chronic psychological effects, which may be mitigated by early resumption of routine and targeted rehabilitation.[18] In-depth psychological review, which could give an early indication of the impending effects, has not been performed in the current survey. Factors such as age, socioeconomic status, educational qualifications and psychological status of the parents/primary caregivers are likely to affect their responses. These were not taken into account in the study.

To conclude, lockdown during the Corona pandemic is a unique situation, whose multidimensional effects on children with NDD and their parents/primary caregivers were explored through an online, multi-centric questionnaire-based survey. Children with ASD, ADHD and LD between one to twelve years of age were enrolled. Discontinuation of multimodal institutional therapies was noted to be a significant effect of the lockdown. Home-based therapies were continued mostly by those, who were already receiving this modality earlier. There was no significant discontinuation of pharmacotherapy during lockdown. Parents/primary caregivers perceived that their children had increase in behavioural issues and that their quality of lives deteriorated during lockdown. Among the subsets of children, those with ASD and ADHD were maximally affected. However, most parents felt confident that they would be able to continue providing adequate care of their children. The survey points to the role of regular home-based therapy in children with

NDD, especially to tide over similar unexpected scenarios. An open feedback loop between healthcare providers and parents based on a web-based platform may be hypothesized to be useful and needs to be studied. Re-evaluating the children after the pandemic using assessment tools and combining the results would make the study more valuable.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: JNG, SR Data collection: SR,VS,AKS,MB,JNG Analysis and interpretation of results:VS,SR,JNG Draft manuscript preparation: JNG,MB All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The Institutional Ethical Committee approved the study and it was registered with the Clinical Trials Registry-India (CTRI) (No: CTRI/2020/06/025795).

Source of funding

Nil.

Conflicts of interest

Nil.

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Original Article

Asthma and risk factors in the first 6 years of life in a population-based cohort

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ABSTRACT

Background. The frequency of asthma increases in childhood and asthma is associated with risk factors varying across age groups. The aim of our study was to assess the prevalence of asthma and its associated risk factors in the first six years of life.

Methods. Within the scope of the Adana Pediatric Allergy and Risk Factor (ADAPAR) birth cohort study, 203 infants that had experienced at least one wheezing attack during the first year of life were followed for asthma development until the age of six years. Additionally, 223 infants that were followed within the scope of the same study and had no wheezing attacks in the first year of life were assigned to the control group.

Results. At the end of the sixth year, 46 (22.7%) infants were diagnosed with asthma and the use of antibiotics of the mother during pregnancy (OR: 2.98), the presence of allergic diseases in the mother (OR: 4.70) and sibling (OR:2.11), the presence of atopy (OR:4.76), and recurrence of wheezing in the first age (OR:17.35) were identified as risk factors for asthma.

Conclusions. The prevalence of asthma at six years of age was higher than that of other studies. Prevention of infections at an early age and during pregnancy can reduce the prevalence of asthma.

Key words: asthma, wheezing, childhood, risk factor.

Asthma is a common chronic respiratory disease in children.[1] The disease can be caused by numerous environmental and genetic factors and is associated with risk factors varying across age groups. Meaningfully, knowledge of these risk factors will help to prevent asthma development. Birth cohort studies can contribute substantially to the understanding of health and diseases. The aim of this study was to assess the incidence of asthma and associated risk factors in the first six years of life as a part of the Adana Pediatric Allergy and Risk Factor (ADAPAR) birth cohort study.

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Material and Methods

Study design

The prospective study was performed as a part of the ADAPAR birth cohort study. The study protocol was approved by Çukurova University local ethics committee (02.03.2018/75-46) and was performed in accordance with the Helsinki Declaration. The details of this birth cohort study has been described in detail elsewhere.[2] Within the scope of ADAPAR study, 203 infants who had at least one wheezing attack in the first year of life were included in this study. Additionally, 223 infants that were followed within the scope of ADAPAR birth cohort study and had no wheezing attacks in the first year of life were included into the control group. An informed parental consent was obtained from all the participants before inclusion.

Follow-up assessment

All the infants were followed at six-month intervals for a total period of six years. Cord blood samples were taken and a physical examination was performed after the birth for each participant. A baseline questionnaire that probed questions on pregnancy conditions, environmental exposures, and family history of allergic disorders was administered to the mothers after birth. Additionally, follow-up questionnaires were administered following physical examination at each visit to assess patients' allergic symptoms and environmental exposure. Each year, a skin prick test was performed using food allergens (egg, wheat, milk, peanut), and major inhalant allergens (Allergopharma, Germany) including dust mites (dermatophagoides pteronyssinus and D. Farinae), tree pollens (hazel, elm poplar, alder, and sallow), grass pollens (timothy, oat, barley, wheat grass, rye, orchard, meadow fescue, blue grass and velvet), and molds (Alternaria alternata, Fusarium moniliforme and Cladosporium herbarum). A wheezing attack was defined as an acute wheezing episode reported by parents . At the end of the sixth year, participants were evaluated for asthma development. Asthma was defined as presence of asthma symptoms within the last 12 months in the absence of a cold (respiratory distress, wheezing, wheezing or shortness of breath after exercise), 12% increase in forced expiratory volume in 1 second (FEV1) after bronchodilator, or receiving regular medication for asthma within the last one year.

Statistical analysis

Data were analyzed using SPSS for Windows version 22.0 (IBM Corp. Released 2013, Armonk, NY). Categorical data were compared using Chi-square test or Fisher's exact test. Continuous data were expressed as mean and 95% confidence interval (CI) or median and interquartile range (IQR) as appropriate. Skewed data were compared using Mann-Whitney U test. Unadjusted and adjusted odds ratios (OR) with 95% CI were determined. A p value of < 0.05 was considered significant.

Results

A total of 1,475 infants were born in the period between February 2010 and February 2011 at Çukurova University Medical Hospital. Of these, 98 infants were excluded due to insufficient parental knowledge of ADAPAR. Of the remaining 1,377 infants, 220 (15.9%) of them who had experienced at least one wheezing attack during the period between birth and the end of the first year of age were planned to be included in this study. However, 17 out of 220 infants were excluded since their family refused to enroll in the study and thus the remaining 203 children were included. Table I presents the evaluation of risk factors for wheezing at the end of first year of life.

At the end of the sixth year, 46 (22.7%) infants were diagnosed with asthma (Table II) and the risk factors for asthma were revealed as maternal use of antibiotics during pregnancy, presence of allergic diseases in the mother and sibling, presence of atopy, and recurrence of wheezing attacks in the first year of age (Table III).

Discussion

Wheezing is a common symptom in infants and it is difficult to predict whether wheezing symptoms will develop into asthma in later periods. Asthma prevalence in childhood has been reported to be 8-22%.[3-6] Children experiencing a wheezing attack during the first year of age have a higher risk of asthma development.[7]

Risk factors for the development of wheezing symptoms during the first year of age have been investigated in numerous studies.[8-11] In cohort studies, the incidence of wheezing during the first year of life has been reported to be 18-28%.[12-14] In our study, it was found to be 15.9% and it was revealed that 2.7% of the infants had two or more wheezing attacks. Accordingly, the wheezing prevalence in our study was lower than that of other studies. A previous study evaluated the wheezing symptoms described

Table I. Evaluation of risk factors for wheezing at the end of the first year.

Risk factors	N (%)	Infants who had a wheezing attack at least once in the first year		
		n (%)	OR (% 95 CI)	p
Gestational age (weeks)				
<37	219 (15.9)	46 (20.9)	1	0.002
≥37	1158 (84.1)	174 (79.1)	0.25 (0.10-0.59)	
Domestic pet at home				
No	1271 (92.3)	209 (95)	1	0.046
Yes	106 (7.7)	11 (5)	0.32 (0.10-0.97)	
Upper respiratory infection				
0	243 (17.6)	8 (3.6)	1	
1	648 (47)	90 (40.9)	4.74 (1.69-13.31)	0.003
2-3	440 (32)	103 (46.8)	9.47 (3.34-26.89)	0.001
≥ 4	46 (3.4)	19 (8.6)	24.91 (5.89-105.24)	0.001
Lower respiratory infection				
0	1280 (93)	151 (68.6)	1	
1	89 (6.4)	63 (28.6)	16.83 (7.31-38.75)	0.001
2-3	8 (0.6)	6 (2.8)	5.92 (0.59-58.93)	0.129
Food allergy				
No	1344 (97.6)	211 (96)	1	0.716
Yes	33 (2.4)	9 (4)	1.34 (0.26-6.75)	
Cord blood Ig-E			1.05 (0.43- 2.53)	0.910

by parents as in our study and reported that the risk factors for wheezing attacks within the first year included eczema in the child, damp housing and asthma in the mother or sibling, and family history of allergic disease. In our study upper and lower respiratory tract infections, presence of a pet at home, and birth before 37 weeks of pregnancy were established as risk factor for wheezing within the first year of age. However, although recurrent wheezing is presumed to be an atopic condition, no correlation was found between food allergy and this condition.

The investigators of RESPIR (Registro y Análisis Epi-demiológico de las Sibilancias y el Asma en una PoblaciónInfantil en La Ribera),7 ISAAC (International Study of Asthma and Allergies inChildhood),[15] and TCRS (Tucson Children's Respiratory Study)[16] reported the prevalence of asthma at six years of age as 12.8%, 9.6% and 9.8%, respectively. In our study, this prevalence was found to be 22.7%.

Alfonso et al.[7] determined the risk factors for school asthma as atopic dermatitis, at least one attack of wheezing within the first year of age, prematurity, and a family history of asthma. The authors also reported that experiencing one wheezing attack during the first age increased the risk of asthma at six years of age. In our study, however, more than one wheezing attack was found to increase the risk of asthma.

In the BAMSE (Barn/Children, Allergy and Milieu in Stockholm, an Epidemiological) study that evaluated children that experienced wheezing at the age of 2-8 years, Neuman et al.[17] defined wheezing and asthma in a similar way to our study and found the prevalence of asthma as 14% and also reported that increased frequency of wheezing was a risk factor for childhood asthma

Whether exposure to a pet at home is a protector or a risk factor for asthma remains controversial.[18] In our study, presence of a pet

Table II. Features of participants and relationship between asthma development and risk factors.

Variable	Asthma (+) n (%)	Asthma (-) n (%)	p
Gender			
Female	22 (11.5)	169 (88.5)	0.164
Male	39 (16.6)	196 (83.4)	
PRM at pregnancy			
No	59 (14.8)	341 (85.2)	0.556
Yes	2 (8)	24 (92)	
Use of antibiotics of during pregnancy			
No	45 (12.5)	314 (87.5)	0.022
Yes	16 (23.9)	51 (76.1)	
Tobacco smoke exposure during pregnancy			
No	50 (14.1)	304 (85.9)	0.618
Yes	11 (16.4)	61 (83.6)	
Tobacco smoke exposure at home			
No	45 (14.8)	260 (85.2)	0.406
Yes	16 (13.2)	105 (86.8)	
Allergic diseases of sibling			
No	38 (10.6)	322 (89.4)	0.001
Yes	23 (34.8)	43 (65.2)	
Allergic diseases of mother			
No	35 (9.9)	319 (90.1)	0.001
Yes	26 (36.1)	46 (63.9)	
Allergic diseases of father			
No	59 (14.3)	353 (85.7)	1.000
Yes	2 (14.3)	12 (85.7)	
Domestic pet at home			
No	52 (13.4)	336 (86.6)	0.091
Yes	9 (23.7)	29 (76.3)	
Dampness at home			
No	47 (12.9)	317 (87.1)	0.045
Yes	14 (22.6)	48 (77.4)	
Environment			
Countryside	8 (28.6)	20 (71.4)	0.045
Urban	53 (13.4)	345 (86.6)	
Day care attendance			
No	12 (8.7)	126 (91.3)	0.026
Yes	49 (17.1)	239 (82.9)	
Upper respiratory infection in first year			
No	7 (16.3)	36 (83.7)	0.650
Yes	54 (14.1)	329 (85.9)	
Use of antibiotics in first year			
No	5 (10.6)	42 (89.4)	0.658
Yes	56 (14.8)	323 (85.2)	
Wheezing attack in first year			
No	15 (6.7)	208 (93.3)	0.001
Yes	46 (22.7)	157 (77.3)	
Atopy			
No	31 (15.7)	166 (84.3)	0.001
Yes	16 (47.1)	18 (52.9)	

PRM: Premature rupture of membranes

Table III. Risk factors for asthma at the sixth year.

Risk factors	OR (95 % CI)	p
Use of antibiotics during pregnancy	2.98 (1.35-6.55)	0.007
Allergic diseases in sibling	2.11 (0.98-4.56)	0.057
Allergic diseases in mother	4.70 (2.29-9.64)	0.001
Presence of atopy	4.76 (2.19-10.33)	0.001
Wheezing attack at first age		
1	1	
2	15.53 (5.47-44.12)	0.001
≥ 3	17.35 (5.28-59.99)	0.001

in the house was found to be a risk factor for wheezing although no correlation was found between exposure to pet at home and asthma at the age of six. In contrast, a few studies indicated that exposure to a pet at home, particularly a cat, was a risk for asthma.[18-20]

A recent study conducted with children aged 9-12 years indicated that the use of antibiotics during the first six months of life is an independent risk factor for asthma, as shown in our study.[18] Another study showed that the use of antibiotics during the first weeks of life is a risk factor for asthma in the following years.[21] In our study, it was also revealed that maternal use of antibiotics during pregnancy is a risk factor for asthma. Stokholm et al.[22] reported that maternal use of antibiotics in pregnancy was associated with an increased risk of childhood asthma like in our study. On the other hand, it has been hypothesized that both the risk of allergic diseases and the methylation levels of imprinted genes increase with intrauterine antibiotic exposure.[23,24] In a similar way, a recent retrospective cohort study by Yoshida et al.[25] found a significant relationship between antibiotic exposure during fetal period and early development of asthma.

Our study was limited since no respiratory test was performed, which was due to the fact that young children are not suitable for the equipment used for lung function tests.

In conclusion, risk factors for asthma at six years of age were found to include maternal use of antibiotics during pregnancy, presence of allergic diseases in the mother and sibling, presence of atopy, and recurrence of wheezing and upper respiratory tract infection in the first year of life.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DÖ, DUA; data collection: DÖ; analysis and interpretation of results: AŞŞ, DÖ; draft manuscript preparation: AŞŞ.

Ethical approval

The study protocol was approved by Çukurova University local ethics committee (02.03.2018/75-46) and was performed in accordance with the Helsinki Declaration.

Conflicts of interest

There is no conflict of interest among the authors of this article.

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Original Article

Assessment of sleep in children with periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome

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ABSTRACT

Background. Periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome is the most common cause of periodic fever in childhood. This study aimed to investigate sleep patterns and possible factors that are associated with sleep disturbances among children with PFAPA syndrome.

Methods. Sixty-two patients with PFAPA and 68 age and sex matched healthy controls were enrolled in the study. Patients who had an attack during the former 2 weeks were not included. Demographic and anthropometric data, duration of fever episodes, laboratory results, and clinical manifestations of patients were recorded. The Children's Sleep Habits Questionnaire was administered.

Results. The total sleep scores of patients with PFAPA were significantly higher than the control group (49.6 ± 10.7 vs. 38.3 ± 7.5, p = 0.002). Children with PFAPA had significantly higher scores regarding sleep-onset delay, sleep anxiety and night wakening (p=0.003, p=0.007, and p=0.014, respectively). Total sleep durations were similar between children with PFAPA and the control group. There was a significant positive correlation between the total sleep score and disease duration (r=0.425, p=0.002). Also there was a significant positive correlation between disease duration and sleep onset delay (r=0.561, p<0.001) and night wakening (r=0.327, p=0.003).

Conclusion. This study showed for the first time that patients with PFAPA have significantly disturbed sleep when compared to otherwise normal children. This study emphasized the need to assess sleep problems in children with PFAPA

Key words: PFAPA, children, sleep, periodic fever.

Periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome is the most common cause of periodic fever in childhood and generally seen among young children.[1] Fever episodes occur in regular three to eight week cycles. However, the general condition of the patient is good, despite the high fever and other disease features. Between these episodes, patients are asymptomatic. Most patients have resolution of episodes until adolescence.[2]

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The exact mechanism and etiology of PFAPA syndrome remains unclear. A dysregulation of the immune system or abnormal response to an unknown infection trigger is suspected. Recent studies support the hypothesis that PFAPA syndrome involves inflammasome-driven proinflammatory interleukin (IL)-1β production during febrile episodes.[3] Acute phase parameters such as leukocyte counts, the erythrocyte sedimentation rate, C-reactive protein, and the excretion of proinflammatory cytokines increase during the fever episodes.[4] Also hypovitaminosis D can be a significant risk factor for PFAPA recurrence.[5] This strengthens the presence of underlying immune dysregulation.

Sleep problems are much more prevalent among medically ill children when compared with the general population and the presence of co-morbidity may adversely affect medical outcomes and the quality of life of these patients.[6] Sleep problems are important to assess due to both the potential for short and long term negative outcomes such as daytime behavioral problems, inattention, reduced academic performance and reduced health related quality of life. In previous studies, sleep problems have been described in several chronic rheumatological conditions such as juvenile dermatomyositis, familial Mediterranean fever (FMF), juvenile idiopathic arthritis (JIA), and systemic lupus erythematosus (SLE).[6-9] PFAPA shares many similar clinical features with monogenic auto-inflammatory recurrent fever syndromes, even though no genetic causes of PFAPA have been documented. PFAPA is generally considered to be a benign disease, however fever attacks have been shown to have a substantial impact on daily activities, school and family functioning.[10] There is only one study in the literature demonstrating that the well-being of children with PFAPA is poor, with a major impact on psychosocial functioning and increased fatigue.[11] Previous studies show that sleep disturbances may be associated with inflammation and inflammatory mediators effect the regulation of sleep in the central nervous system.[6-9] Therefore as an inflammatory disease, sleep is a prominent issue, even though this has not yet been studied in PFAPA patients. The aim of this study is to assess the sleep habits of children and possible factors that are associated with sleep disturbances using an objective and reliable tool among children with PFAPA syndrome.

Material and Methods

This study was conducted on patients recruited from the out-patient clinic of our institute, between 1 February 2020 and 1 May 2020. We consecutively evaluated 62 patients aged between 4-8 years, after having excluded other causes of recurrent fever. All patients were enrolled in the study during an attack free period. Hence patients who had an attack during the former 2 weeks were not included. The diagnosis of PFAPA was made according to international criteria suggested by Thomas et al.[12] We recorded demographic and anthropometric data, duration of fever episodes, laboratory results, and clinical manifestations of patients. Exclusion criteria from the study were; patients who had infections, any chronic or psychiatric diseases other than PFAPA, and any chronic drug use. None of the patients had enlarged tonsils, adenoid problem or morbid obesity, which may cause obstructive apnea.

The control group included 68 patients aged between 4-8 years without PFAPA history and were recruited from the children who visited the well child outpatient clinic of our department. Demographic data of the control group was also collected. The control group were matched for age, sex, economic status and parental education with the patient group. The control group had no history of any sleep disorders or used any medication that could cause sleep problems.

Tekirdağ Namık Kemal University Ethics Committee approved the study protocol (25/02/2020-2020.35.02.09). All legal guardians of patients were informed about the scale and written informed consent was received.

Children's Sleep Habits Questionnaire

The Children's Sleep Habits Questionnaire (CSHQ) is a parent-report instrument that was used to measure the external validity of the family inventory of sleep habits and assess the typical sleep patterns of preschool and school-age children.[13,14] The CSQH is a retrospective, 33-item parent questionnaire that has been used in a number of studies to examine sleep behaviors in children, and appears to have adequate validity and reliability. The validity and the reliability of the Turkish version of the CSHQ has been previously shown.[15] Parents are asked to recall sleep behaviors occurring over a typical recent week. Items are rated on a 3-point

scale for frequency of the sleep behavior: usually, 5-7 times/week; sometimes, 2-4 times/week; and rarely, 0-1 times/week. Scores are adjusted so that a higher score was indicative of more disturbed sleep. In addition to a total score, 33 items of the CSHQ were grouped into the following 8 subscales: (1) bedtime resistance, (2) sleep onset delay, (3) sleep duration, (4) sleep anxiety, (5) sleep-disordered breathing, (6) night wakening, (7) parasomnias, and (8) morning wakening/daytime sleepiness.[15] If the patients have an unusual event in the week prior to the questionnaire, parents were asked to fill it according to the nearest typical week. A total CSQH score of higher than 41 has been reported to be a sensitive clinical cut off for screening purposes and identification of probable sleep problems.[14]

Statistical analysis

Data were analyzed using SPSS 21 (IBM SPSS, Chicago, IL, USA). Kolmogorov-Smirnov test was used to check for whether the data was normally or non-normally distributed. Non-normally distributed data were expressed as median and minimum-maximum values; normally distributed data were expressed as the mean ± standard deviation. Student's t-test and Mann-Whitney U test were used as appropriate. The Chi-square test was used to test differences in categorical variables between the two groups. Intercorrelations between parameters were computed through Pearson's correlation analysis. P values <0.5 was accepted as significant. Internal consistency of the scale was determined by calculating Cronbach's coefficient alpha. Scales with reliabilities of 0.70 or greater are recommended for comparing groups.

Results

Table I shows the demographic data of the groups. The total sleep scores of the patients with PFAPA were significantly higher than the control group (49.6 ± 10.7 vs. 38.3 ± 7.5, p = 0.002). In patient group 41 (66.1%) patients had CSQH scores higher than 41 (clinically significant sleep disturbance), whereas in the control group 23 (%33.8) had CSQH scores higher than 41 (p=0.001). Children with PFAPA had significantly higher scores regarding sleep-onset delay, sleep anxiety and night wakening (p=0.003, p=0.007, and p=0.014, respectively) (Table II). Total sleep durations were comparable between children with PFAPA and the control group (8.7 ± 1.7 vs. 9.0 ± 1.2 hours, p=0.36). The total sleep scores of the male PFAPA patients were significantly higher than the female PFAPA patients (51.1 ± 9.9 vs. 46.4 ± 9.6; p=0.025).

There was no significant correlation between the total sleep score and number of attacks in the last year (r=0.006, p=0.54). There was a significant positive correlation between the total sleep score and disease duration (r=0.425, p=0.002). Also there was a significant positive correlation between disease duration and sleep onset delay (r=0.561, p<0.001), and night wakening (r=0.327, p=0.003).

Internal consistency reliability alpha coefficient for CSHQ in the patient group and in the control group were 0.79 and 0.72, respectively.

Table I. Demographic data of patient and control groups.

Characteristics	PFAPA group (N=62)	Control group (N=68)	P value
Sex (male), n (%)	35 (56.5%)	35 (51.5%)	0.694
Age (years)	5.8 ± 2.1	6.1 ± 2.2	0.752
Duration of fever (days)	5 (0.7 – 11)		
Time between the attacks (week)	7 (1.3 – 10)		
Disease duration (years)	4.8 (1.1 – 6.8)		

Continuous variables are presented as mean ± standard deviation or median (minimum - maximum), as appropriate.

Table II. Sleep parameters of patient and control groups.

Parameters	PFAPA group (N=62)	Control group (N=68)	P value
Total sleep duration (hours)	8.7 ± 1.7	9.0 ± 1.2	0.360
Total sleep score	49.6 ± 10.7	38.3 ± 7.5	0.002
Sleep score >41	41 (66.1%)	23 (33.8%)	0.001
Subscales			
Bedtime resistance	7.7 ± 3.9	8.1 ± 3.2	0.067
Sleep-onset delay	2.7 ± 1.3	1.6 ± 0.9	0.001
Sleep duration	4.1 ± 1.7	4.2 ± 1.9	0.765
Sleep anxiety	6.5 ± 2.5	4.9 ± 1.9	0.001
Night wakening	4.8 ± 1.2	3.7 ± 1.4	0.002
Parasomnias	8.3 ± 2.3	8.8 ± 2.4	0.923
Sleep-disordered breathing	3.8 ± 1.5	3.6 ± 0.9	0.628
Day-time sleepiness	14.8 ± 4.8	14.1 ± 3.8	0.743

Data are presented as mean ± standard deviation or n (%).

Discussion

The results of this study indicated that patients with PFAPA had more sleep problems than their healthy peers. The patients with PFAPA reported higher scores of sleep-onset delay, sleep anxiety and night wakening, compared to healthy controls. Clinically significant sleep disturbance rate was higher in PFAPA syndrome (66.1% vs 33.8%; p=0.001). Also, there was a significant positive correlation between the total sleep score and disease duration. To the best of our knowledge, this is the first study investigating sleep problems among children with PFAPA.

Inflammatory cytokines can affect the regulation of sleep in the central nervous system and sleep may play a vital role in immune regulation.[15] Many inflammatory cytokines such as IL-1β and IL-6 were elevated either during or between attacks in PFAPA patients.[16] IL-6, a proinflammatory cytokine, elevation was shown to affect the organism, with a focus on sleep-related symptoms and fatigue.[17] As a result of this data, we are of the opinion that inflammation can contribute to sleep disturbance of PFAPA patients.

Makay et al.[6] conducted a study assessing sleep problems in children with FMF using CSHQ. They found significantly higher scores regarding sleep-onset delay, sleep anxiety, night wakening and sleep-disordered breathing in patients with FMF when compared to healthy controls. The findings of our study is similar to their results, but we found no relationship between number of attacks per year and sleep scores unlike their study. Also, in our study, there was a significant positive correlation between the total sleep score and disease duration. We found no correlation between sleep disordered breathing and PFAPA. Despite the diversity of clinical presentation, there are some common features between PFAPA and FMF.[1] In some of the PFAPA patients *MEFV* gene mutation may be present similar to FMF.[20] Potential prophylactic benefits of colchicine reported in the literature supports a similar immunological basis for PFAPA and FMF.[21] Also, sleep problems have been described in several chronic rheumatological conditions such as juvenile dermatomyositis, FMF, JIA, and SLE.[6-9] In a study conducted by Bloom et al.[16], patients with JIA had higher total score on CSHQ, as well as subscales assessing night wakening, parasomnias, sleep anxiety, sleep-disordered breathing, and morning wakening/ daytime sleepiness.

In a previous study by Grimwood et al.[11] investigating the health related quality of life (HRQOL) in children with PFAPA syndrome,

they showed that the wellbeing of PFAPA children is poor, with a major impact on psychosocial functioning and increased fatigue. They found the quality of life of PFAPA children to be even lower than that of FMF patients, for whom a lower than normal HRQOL has already been demonstrated. Although PFAPA is known as a self-limited and benign condition, it may have major impact on HRQOL and sleep patterns in both patients and their parents as suggested by our study.

Gender differences in total sleep scores were observed in our cohort, boys had higher scores than girls which is consistent with previous studies.[22,23] An explanation for the gender difference is unclear; however, it is possible that girls may require more sleep than boys, that parenting practices or social obligations may vary, or that girls may be more attentive to their sleep needs.[23]

Adequate sleep in young children is important for both healthy development and optimum daytime functioning. Sleep problems in young children may be problematic for parents, but such problems may be related to additional problems for the children both concurrently and over time.[24] Increased sleep problems have been related to increased internalizing and externalizing problems, increased injury rates and although many children experience improvements with sleep over time, for a substantial subset of children who exhibits sleep disturbances at a young age are more likely to exhibit persistent sleep problems.[23] Persistent sleep problems are more likely to have other difficulties over time such as negative behavior and poor academic skills.

Parents' education regarding what is adequate sleep hygiene may be the start for treatment in PFAPA patients. Physical activity, which, when moderate, has a beneficial effect on sleep. At least 3 hours before the child's established bedtime, he/she should be involved in relaxing activities; over stimulation should be avoided. Activities involving electronic media (TV, computer, tablet, and mobile phone) should also be restricted and avoided at least 1 hour before bedtime. The bed room environment is also a sleep hygiene factor. It should be well ventilated, quiet and dark, at an adequate temperature, and have a comfortable bed. Positive routines can also help the child learn appropriate sleep behaviors and reduce stress. In addition to defining bedtime, consistent routines (activities that help prepare for sleep) should be established and repeated every night. Brushing the teeth, performing other hygiene routines, putting on pajamas, reading a story or spending some time with parents, turning off the lights could be given as examples. It is important to make sure that the time set for the routine is sufficient, so that they can be conducted calmly, without harming total sleep time.[25,26] Despite these interventions, if the problem still continues, patient may be referred to a sleep expert.

The limitations of this study is the small sample size and the inclusion of children who were attending the same clinic, which may limit the generalizing the results of this study to all children with PFAPA. As diagnostic limitations we did not perform a polysomnography and the data for sleep disturbances was collected by a questionnaire, but owing the reason that our study is the first work on this topic, we think it is still worth sharing. The strength of this study includes the use of a pediatric sleep habit questionnaire with previously demonstrated reliability and validity in pediatric rheumatic diseases.

The present study underlines the need to assess and manage sleep problems in children with PFAPA for the first time. Our results showed that patients with PFAPA have significantly disturbed sleep when compared to otherwise normal children. Given the sleep problems in children with PFAPA, as well as the potential for negative consequences associated with sleep problems, it is important to be able to identify and intervene with sleep problems at an early

age. Poor sleep in children has been associated with family stress and health issues as well. The results of this study to assess the sleep problems of PFAPA children refutes the belief that PFAPA is a benign disease and demonstrates that the consequences of PFAPA has probably been largely underestimated. To our opinion, sleep habits of PFAPA patients must be questioned, and every effort including sleep hygiene techniques should be made to resolve sleep problems, in order to avoid long term negative consequences. However, further studies using polysomnography with larger sample sizes and from different centers are needed to confirm this hypothesis.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AN, BN; data collection: AN; analysis and interpretation of results: AN, BN; draft manuscript preparation: AN, BN. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

Tekirdağ Namık Kemal University Ethics Committee approved the study protocol (25/02/2020-2020.35.02.09).

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Conflict of interest

Author A. Nalbantoğlu and B. Nalbantoğlu declare that they have no conflict of interest.

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Original Article

Cost of illness of RSV infection in a middle-income tropical country

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ABSTRACT

Background. Despite the burden of disease of Respiratory syncytial virus (RSV) infection in children, there are important gaps in knowledge about the potential impact in terms of health as well as social and healthcare resources. The aim of this study was to describe the economic burden of RSV in the first two years of life in Colombia.

Methods. We conducted a cost-of-illness study, taking a population prevalence-based approach. A decision tree model was constructed with a time horizon of two years. We defined the following outcomes: death, RSV infection with long term complications, RSV with acute complications, RSV without complications. Inpatient and outpatient costs were collected directly from medical invoices of patients who attended a tertiary referral hospital. Results. The mean cost per patient with an RSV infection was US$ 178.35 CI 95% (30.7-541.67 US$). The total cost of RSV infection in children less than 2 years in Colombia was US $ 64 443 616 per year (CI 95% US$11 092 902 – US$195 722 867). In the probabilistic sensitivity analysis, the mean cost per patient with RSV infection was only sensitive to changes in the cost of recurrent wheezing, cost of outpatient visits and cost of hospitalizations.

Conclusion. The infection by RSV in Colombia generates a high economic burden on the health system. Generating comprehensive data on healthcare resource use and costs associated with RSV will help to provide valuable information for the development of cost-effectiveness models, and help guide prevention strategies against RSV.

Key words: cost, respiratory syncytial virus, Colombia.

Respiratory syncytial virus (RSV) is the most frequent cause of bronchiolitis worldwide.1 In 2005, 33.[1] million episodes of RSV infections worldwide resulted in about 3.2 million hospital admissions, and 59,600 in-hospital deaths in children younger than five years.[2] Worldwide, RSV infection is the second cause of postnatal infant death after malaria, causing 137,000 deaths each year (equal to 6.7% of all newborn deaths).[3]

Despite the increased risk of RSV in children and the frequency of their complications, there is an important gap in the knowledge concerning the potential impact in terms of health as well as social and healthcare resources. Most of the economic studies focused on assessing the efficiency of palivizumab for the prophylaxis of RSV infection and have not properly evaluated the impact of this infection beyond the acute episode phase.[4-7] Many of these studies have been designed from the perspective of the payer and not from a social perspective, without including indirect costs such as those associated with job loss and family expenses. Likewise, no studies have evaluated the economic impact of such an infection in developing countries; in which the problem in terms of morbidity and mortality is growing.[8] The aim of this study was to describe the economic burden of RSV infection in the first two years of life in Colombia.

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Material and Methods

Choice of model

We conducted a cost-of-illness study, taking a population prevalence-based approach. A decision tree model was constructed to estimate the cost of each episode of RSV infection (Fig. 1). The reason for using a decision tree to estimate the expected cost per patient is that this analytical approach reduces the possible selection bias in the population studied that over represents or underestimates both the frequency of the outcome and the derived costs. With this structure, the only factor that weighs the cost are the probabilities of the analytical model and not the chance that such events may or may not occur in the population secondary to selection bias.[9-12] The study protocol and their informed consent was reviewed and approved by the Institutional Review Board of Clinica Somer (No 281015) and the University of Antioquia (No 18/2015).

Target population

Children younger than two years of age admitted to the pediatric ward with a diagnosis of RSV infection were included in the study. Patients older than two years, without lower respiratory compromise, with positive bacterial cultures on admission, confirmed whooping cough or those referred from another hospital center were excluded.

Setting and location

Rionegro is a city and municipality in Antioquia Department, Colombia, located in the subregion of Eastern Antioquia, at an average elevation of 2,125 meters above sea level. The average annual precipitation varies between 1,800 and 2,500 millimeters with an average temperature of 17 °C, with a peak in the presentation of RSV between March and April.[13] The municipality of Rionegro has a total population of 101,046 inhabitants, the sixth largest populated area in Antioquia, with two tertiary referral hospital.[14] Colombia's health system is composed by a social security sector and a private sector. The basis of the system is the General Social Security Health System, which has two plans, contributory and subsidized; all with a decentralized referral mechanism. The contributory regimen covers salaried workers, pensioners, and independent workers, with the subsidized plan covering anyone who cannot pay. The National Health Authority's functions under the system include an increase in the quality of health care and supervision, surveillance, and control of health insurance. Enrollment in the General Social

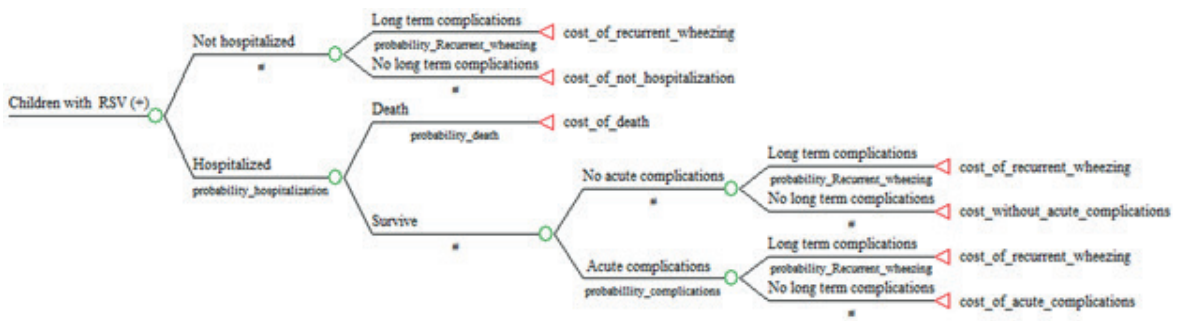

Fig. 1. Decision tree model.

cRecurrent_Wheezing: cost of RSV patient with recurrent wheezing
cNoHosp: cost of RSV patient Not hospitalized
cWithoutComp: cost of RSV patient hospitalized without complications
cComplications: cost of RSV patient hospitalized with complications
cdeath: cost of RSV patient death
pHosp: probability of hospitalization
pCompl: probability of complications
pdeath: probability of death
pRecurrent Wheezing: probability of recurrent wheezing

Security Health System is obligatory and is handled through public or private health promotion agencies. Health care is provided by institutional health service providers. Those who can afford to purchase health insurance coverage on their own and who can pay for any uncovered fees out-of-pocket use the private sector.

Study perspective and Time horizon

We conducted a cost-of-illness study, taking a population prevalence-based approach. A decision tree model was constructed with a time horizon of two years. This analysis was performed from a societal perspective including direct and indirect costs. No discount rate was applied to the cost due to the short time horizon evaluated.

Choice of health outcomes

We defined the following outcomes according to the natural history of RSV infection: death, RSV infection with long term complications (recurrent wheezing), RSV infection only with acute complications, RSV infection without complications. In this model, the development of recurrent wheezing was assumed as the only long-term complication; since it is the most frequent respiratory complication in the first two years after the first infection.[15] Among the acute complications included: pneumonia, atelectasis, sepsis, pleural effusions, and pneumothoraxes.[16]

Measurement and evaluation of probabilities of model.

The probabilities of the model were obtained from literature and are listed in Table I. The search was performed in February 2019 and was limited to primary literature published in the English or Spanish language, human subjects, and children (birth to 5 years). The following engines were searched for the periods specified: MEDLINE from 1950 on, EMBASE from 1974 on, BIREME from 1980 on. To avoid missing any articles published we performed a search using Google search engine and we reviewed the first 100 results returned. Terms for these database searches included keywords closely matching the relevant medical field headings: *respiratory syncytial virus, and respiratory syncytial pneumovirus*. The authors (JAB, DG) reviewed all potentially relevant references independently and selected relevant publications. The study inclusion criteria were studies: (1) reporting the frequency or incidence of outcomes during the episode of community acquired, medically attended, severe RSV infection in children < 2 years for data analysis. (2) reporting data on laboratory confirmed diagnosis of RSV through enzyme-linked immunosorbent assay, polymerase chain reaction (PCR; Multiplex), immunofluorescence (IF), culture, direct fluorescent antibody test (DFA), or by relevant International Classification of diseases-9 (ICD-9) diagnosis codes. Population estimates of cases with RSV infection aged <2 years, was obtained from the National Institute of Health of Colombia (1).

Estimating resources and costs

To estimate resources and cost we conducted a prospective study in infants under two years of age admitted to the tertiary centers, in Rionegro due to an RSV infection (ICD-10 code: J21.0, according to the national clinical guideline of bronchiolitis[17]) from January 2015 to December 2016. Although the costs were derived from tertiary centers of Rionegro, all values as

Table I. Probabilities used in the model.

	Probability	CI 95%	Distribution
Hospitalization	0.014[15]	0.008-0.021	
Death	0.009[15]	0.003-0.014	Beta
Acute complications	0.144[15]	0.075-0.195	
Recurrent wheezing	0.281[13]	0.206-0.357	

detailed below were subjected to sensitivity analysis with a certain range and distribution in order to increase the external validity of our results.

Inclusion criteria were defined as children younger than two years of age admitted to the pediatric ward with a diagnosis of RSV confirmed using direct immunofluorescence (Light Diagnostics TM Respiratory Panel 1 DFA, Merck-Millipore Laboratory). Patients older than two years, without lower respiratory compromise, with positive bacterial cultures on admission, confirmed whooping cough, and those referred from another hospital center were excluded. After receiving informed consent from the parent or caregiver, the patient was interviewed and their electronic medical records were reviewed. We collected the following variables: age, sex, weight, height, signs, and symptoms at admission (e.g. fever, chest indrawing, chest auscultation abnormalities like rhonchi or crepitation), history of prematurity, bronchopulmonary dysplasia confirmed by a specialist physician on discharge from the neonatology unit, comorbidities (congenital heart disease, neurological disease), results of chest X-rays or other medical test, drugs and other treatments, and complications (pneumonia, atelectasis, sepsis). The clinical and sociodemographic characteristics of the patients from whom the cost information was extracted are presented in Table II.

The cost derivation follows a bottom-up approach based on the following formula: Number of "A" unit's x costs per unit "A" = Total cost of "A" units. Inpatient and outpatient costs, data of hospitalizations, and their costs were collected directly from medical invoices and health records. The direct costs considered in the analysis include: medical consultation at the emergency room, specialist referrals, chest physiotherapy, diagnosis support (laboratory, electrocardiogram, x-ray, etc.), medication (oxygen, nebulization, antibiotics, corticosteroids, bronchodilators, etc.) , medical devices, day- bed on the intensive care unit, and day-bed on the general medical ward. We used US dollars (Currency rate: US$ 1.00 = COP$ 3,000) to express all costs in the study. For the evaluation of the indirect costs associated with the loss of productivity, the human capital method was used. In this, the cost-opportunity of the productivity loss at the workplace and the caregiver were assessed based on the minimum wage without including the transportation assistance for the year 2016 (U$ 229.81 per month). Because all patients with RSV infection included were children, we assumed that at least one family member accompanied the patient permanently during hospitalization.

Table II. Sociodemographic and clinical characteristics of patients.

Variable. N (%)	N (%)
Age (months), median(ds)	5.66(0.38)
Male %(n)	113(58.55)
Premature birth	28(14.51)
Comorbidities (CHD, neurological)	11(5.71)
Atopy	21(10.88)
SpO2.median(ds)	88(0.93)
O2 support, n (%)	178(92.33)
Clinical & laboratory parameter	
Fever	53(27.46)
Chest indrawing	102(52.85)
Tachypnea	30(15.54)
Rhonchi	78(40.41)
Crepitation	36(18.65)
Leukocytosis (> 15.000/cm)	31(16.76)
Increased C-reactive protein (> 4 mg/lit)	59(44.81)
Chest X-ray	
normal	22(12.36)
peribronchial thickening	63(35.39)
hyperinflation	33(18.54)
atelectasis	5(2.81)
bilateral interstitial infiltrates	33(18.54)
alveolar infiltrates	22(12.36)
Length of hospital stay, median (range)	5.88(0.39)
Complications	
pneumonia	23(11.92)
Sepsis	9(4.66)
Atelectasis	5(2.59)
ICU	3(1.5)

CHD: congenital heart defect, ICU: intensive care unit

For the cost associated with transportation and food (does not include a stay), care was assumed to correspond to 50% of the day's cost for productivity loss at the workplace.

Data Analysis

Using the decision tree analysis, the cost of a patient with RSV infection was estimated. The validity of the estimates was evaluated, first by a tornado graph and the analysis of the permissible limit values to determine the variables with the greatest influence on the sensitivity analysis. In addition, a probabilistic sensitivity analysis was made using the Monte Carlo technique with a simulation of a hypothetical cohort of 10 000 patients in which each parameter varied randomly according to certain distributions (beta distribution in the case of probabilities, and gamma distribution in the case of costs) according to the recommendations of Briggs; to generate 95% confidence intervals (95% CI).[18] The Tree age 3.5 statistical package was used in all analyses.

Results

Resource use and cost.

Overall, the diagnostic tests most frequently requested by the attending physicians were hemogram in 185 (95.85%) and chest radiography in 178 (92.22%) patients. The medications most often prescribed were nebulized or inhaled beta 2 agonists in 108 (55.96%), and nebulized hypertonic saline in 180 (93.26%) patients, see Table III. Overall, the major contributors to the hospitalization costs consisted of room costs (31.5%), drugs (21.8%) and indirect costs (14.9%). The medications with the highest average costs were nebulization with a hypertonic solution, systemic antibiotics, and parenteral fluids, see Table III. Diagnostic tests of both images and laboratory tests contributed to 9.32% of the costs per patient. Among these, chest radiography, blood cultures, C-reactive protein were the ones that most added to the costs per patient, see Table III.

Table III. Cost associated with RSV infection.

	Cost/patient/day	CI 95%	
Specialist referrals	10.457	10.117	10.798
Chest physiotherapy	5.049	4.805	5.293
Chest radiography	2.788	2.643	2.933
Others diagnostic imaging	0.005	0.000	0.022
Complete blood cell counts	1.190	1.134	1.247
RSV test	2.892	2.568	3.215
C-reactive protein and another test	3.988	3.914	4.063
Oxygen	1.463	1.382	1.544
Nebulization	20.558	19.665	21.451
Parenteral fluids	1.367	1.334	1.399
Systemic antibiotics	1.291	1.193	1.389
Systemic or inhaled corticosteroids	0.089	0.000	0.915
Bronchodilators	0.041	0.036	0.046
Medical devices	10.664	10.138	11.190
Hospital stay	23.925	22.745	25.106
The daily cost of the emergency ward	12.833	12.200	13.467
Indirect cost per day	17.236	16.386	18.087

Cost of an episode of RSV infection estimated by the model.

In the decision tree model, the mean cost per patient with RSV infection was US$ 178.35 CI 95% (30.7-541.67 US$). The final cost estimated by the model for each outcome can be seen in Table IV. In the probabilistic sensitivity analysis, the mean cost per patient with RSV infection was stable; being only sensitive to changes in the cost of recurrent wheezing, cost of outpatient visits and cost of hospitalizations, see Figure 2. In the other variables of costs and probabilities, there were no significant variations during this analysis.

Cost estimated by RSV infection in Colombia

Taking the total of national reports of RSV infection reported to the national surveillance system; and according to the prevalence of RSV infection found in local studies[19]; the total cost of RSV infection in children less than 2 years in Colombia were of US $ 64 443 616 per year (CI 95% US$11 092 902 – US$195 722 867).

Discussion

The results of this study show the high economic impact of RSV-related bronchiolitis in a tropical country. The total cost of RSV infection in children less than two years in Colombia were of US $ 64 443 616 per year; this is equivalent to more than 0.7% of the total health spending in Colombia for 2018.[20] Nevertheless, the proportion of cases of RSV infection in children under two years are less than 0.1% of the total of the event for any disease in Colombia.[21] There is a clear imbalance between the number of patients treated and the cost that they generate, and the burden of expenses, perhaps mostly due to the use of medications or unjustified diagnostic tests.[22,23]

The mean cost per patient with RSV infection were US$ 178.35 CI 95% (30.7-541.67 US$); this value being the most complete estimation of cost concerning this disease in any country in Latin America because we included indirect costs such as those associated with job loss

Table IV. Cost estimated by the model for each outcome.

	Cost/patient/day	SD	Distribution
Not hospitalized	171,73	228,92	
Hospitalized without long term complications	515,72	16,34	Gamma
Hospitalized with long term complications	840,52	189,79	

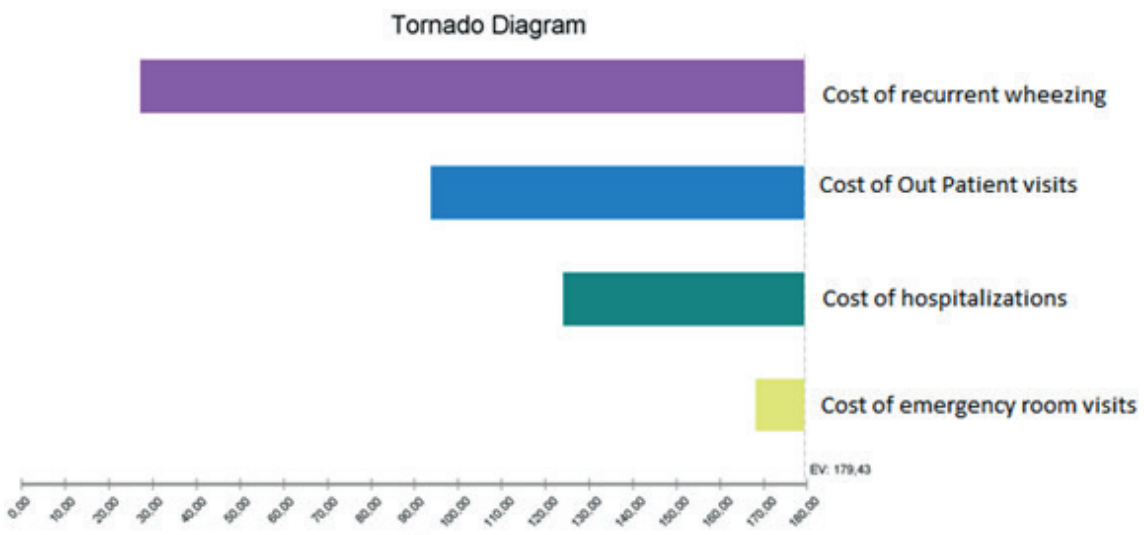

Fig. 2. Probabilistic sensitivity analysis.

and family expenses, and the cost of long term complications of RSV infection in the first two years. These values were similar to those reported from Bangladesh (US $ 62)[24], China (US$ 571)[25], and Chile (US $ 632).[4] The study from Bangladesh was made from a social perspective, and in Chile and China using a payer perspective. As is expected, due to the greater willingness to pay, our value per patient is lower than found in developed countries (For example: United States (US $ 2664-3799)[7,26], United Kingdom (US $ 3117)[27] and Finland (US $ 955 1)[28]). Not all previous studies were performed in the same population; most of them included the cost in children under five years and not only in the first two years. This may be difficult, in a practical sense, because the cases of RSV infection under two years are usually managed within clinical protocols of acute bronchiolitis in which the use of bronchodilators, corticosteroids, etc. are not recommended; while in older patients, especially if they have a history of wheezing episodes, are treated within asthma protocols which involve greater use of medications and diagnostic tests.[29,30] In those studies that included only patients under one-year-old or preterm infants[4]; patients in which the initial treatment is usually more aggressive because of the risk of bacterial infection[29], their cost per patient also will be higher. For this reason, the values obtained in the different studies are not comparable, and it is not appropriate to infer differences in the resources used for health care in the diverse populations studied.

In respect to the impact of RSV infections concerning morbidity, RSV is frequently linked to hospital admissions, which results in a large burden to the health care system. For example, in 2015, around 45% of the hospitalizations and deaths occurred in RSV- in infants < 6 months.[2] Interventions to lower the prevalence and costs are deficient. No effective RSV-specific antivirals for active infection or preventive vaccines are available. RSV immune prophylaxis with targeted monoclonal antibodies (mAbs) is convenient for a limited population of high-risk infants. The number of RSV products in development highlights the need for up-to-date information to estimate the impact of vaccines, antivirals, and mAb on disease burden, once approved.

In our study, the mean costs per patient with RSV infection were sensitive to changes in the cost of recurrent wheezing, cost of outpatient visits, and cost of hospitalizations. It is also widely documented in other populations studied, that variables such as the cost of long-term complications and wheezing are those that have the most impact on the total cost per patient.[30,31] The cost of hospitalizations may be related to the increase of inappropriate use of antibiotics for RSV infections, which directly and indirectly increases the direct costs and days of hospital stay for patients.[32] This highlights the need to mitigate, through early detection in patients at risk of developing wheezing to recurrence as a strategy to be evaluated for the cost containment in this population.

Indirect costs are the costs of those resources for which no payment is made, but for which there is an opportunity cost. Guidelines for economic evaluation studies in health care recommend the inclusion of indirect costs.[33] The addition of indirect costs usually had a substantial effect on the efficiency ratio, especially in pediatric diseases where hospitalization mobilizes the family, increasing the economic impact on society because of this disease. The non-inclusion of such costs otherwise leads to underestimating the real cost of the disease and the effect that preventive interventions may have to reduce the frequency and duration of hospitalizations. In our study, about 15% of the cost generated by the RSV infection is attributable to indirect costs. If we take into account that in Colombia there are about 361 332 annual cases of low acute respiratory infection due to RSV, according to the national epidemiological surveillance registries[34], and in our study the indirect costs are 17 dollars per day of hospitalization; this expense they represent more than 6 million dollars per day for our society. This fact highlights the impact that

preventive strategies may have on infections such as this one to reduce the opportunity cost and improve efficiency in the prioritization and allocation of health resources.

Our study has the following limitations: there may be differences in the costs used in this study when compared with the costs of other hospitals in Colombia. However, there is adequate adherence in our country to the use of health reference cost manuals, which means that there is low variability in the rates of each of the associated direct costs between each hospital.[35] All costs were subjected to probabilistic sensitivity analysis, and none of them showed a significant change in the result of the study. Likewise, the probabilities were not obtained directly from the Colombian population. However, they were extracted from the Latin American population, and were also evaluated in the probabilistic sensitivity analysis. Likewise, the time horizon of this study is 2 years, no temporary discount value was included in the costs due to the short follow-up time of the modeled cohort. Constant discount rates devalue the long-term health benefits of prevention strongly and are usually not recommended for modeling short duration acute events.

The RSV infection in Colombia generates a high economic burden on the health system. Generating comprehensive data on healthcare resource use and costs associated with RSV will help to provide valuable information for the development of cost-effectiveness models and help guide prevention strategies against RSV.

Acknowledgment

None to declare.

Ethical approval

The study protocol and their informed consent was reviewed and approved by the Institutional Review Board of Clinica Somer (No 281015) and the University of Antioquia (No 18/2015).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: JAB, DG, DS; data collection: JAB, DG, DS ; analysis and interpretation of results: JAB, DG, DS ; draft manuscript preparation: JAB, DG, Author. All authors reviewed the results and approved the final version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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Original Article

Timeliness of postnatal surgery in newborns with open neural tube defects: a single center experience

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ABSTRACT

Background. This study aims to evaluate the experience of a tertiary health center on the timeliness of postnatal management in newborns with open neural tube defects (NTDs).

Methods. This is a retrospective review of 38 neonates with NTDs who were treated surgically at a tertiary health care center between January 2009 and January 2019. Five neonates with genetic syndromes were excluded.

Results. Twenty-six neonates with NTD underwent surgery on the first postnatal day while 12 neonates with NTD had surgery after the first postnatal day. The reasons for the latency in operative treatment were the delay in the referral of the affected newborn from other health care centers (n=8) and the transient abnormalities in coagulation tests (n=4).

Rural residence was significantly more frequent, gestational age at delivery was significantly lower, preterm delivery was significantly more frequent and prenatal diagnosis was significantly less frequent in neonates that underwent surgery for NTD repair after the first postnatal day (p=0.001, p=0.048, p=0.024 and p=0.003 respectively). Postoperative motor dysfunction was significantly more severe (p=0.002), postoperative complications were significantly more frequent (p=0.008), the reoperation and postoperative mortality rates were significantly higher (p=0.009 and p=0.048 respectively) and the duration of hospital stay was significantly longer (p=0.033) for the neonates who underwent surgery after the first postnatal day.

Conclusions. Our study appears to favor the early repair of NTD's within the first 24 hours of life. Such an approach may reduce the risk of infectious and neurological complications significantly.

Key words: morbidity, mortality, neural tube defects, operative surgical procedures.

Neural tube defects (NTDs) are the second most common birth defects of the whole system and the most common congenital anomaly of the central nervous system. These defects emerge when part of the neural tube fails to close normally during the third to the fourth week after conception.[1,2]

Most of the isolated NTDs are caused by folate deficiency, likely in combination with genetic or other environmental risk factors consisting of fever/hyperthermia, obesity, pregestational diabetes, exposure to pesticides, nitrosatable drugs and clomiphene citrate.[3,4] These defects may be associated with syndromes such as Edwards', Patau, Meckel-Gruber, Roberts, Jarcho-Levin as well as triploidy, limb-body wall complex, and cloacal exstrophy.[5,6]

The improvements in diagnostic equipment and therapeutic procedures have caused a dramatic increase in survival and life expectancy rates of the individuals affected by NTDs.[7,8] Despite this fact, the risk of morbidity and mortality still remains significantly increased for newborns diagnosed with these congenital defects.[8,9] It has been hypothesized that morbidity and

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mortality associated with the management of NTDs is more pronounced in developing and underdeveloped countries with limited resources and access to health care.[9,10]

Approximately 80% of NTDs are open lesions such as myelomeningocele (spina bifida), meningocele, encephalocele, and anencephaly.[11] These lesions can be associated with cerebral ventriculomegaly.[12] The standard of treatment for open NTDs is the prenatal surgical repair or postnatal repair within the first few days of life.[13] The rationale behind the recommendation of prompt postnatal closure is to prevent infectious complications and protect the exposed nerves from possible trauma.[12,13]

This study aims to evaluate the experience of a tertiary health center on the timeliness of postnatal management in newborns with open NTDs.

Material and Methods

The present study was approved by the Institutional Review Board and Ethical Committee of Afyonkarahisar Health Sciences University (02.08.2019-261).

This was a retrospective review of 43 neonates with NTDs who were treated surgically at a tertiary health care center between January 2009 and January 2019. The parents of these neonates were counseled for the management of NTDs and they were provided with written informed consent for surgical treatment.

Data related with prenatal diagnosis, gestational age at the time of delivery, birth weight, birth length, sex, mode of delivery, and Apgar scores were obtained from the medical records.

Data concerning family history, maternal age, maternal residence, maternal smoking and use of anti-epileptic drugs were also derived from the hospital files. In addition, the type and localization of NTDs, co-existing congenital anomalies, timing and type of surgery were designated. The duration of hospital stay, findings of neurological imaging, postoperative complications, postoperative mortality and the reasons for re-operation were also specified.

Statistical analysis

Collected data were analyzed with Statistical Package for Social Sciences version 22.0 (SPSS IBM, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (range: minimum-maximum) whereas categorical variables were denoted as numbers or percentages. Mann Whitney U test was used to compare the continuous variables and chi square test was utilized to compare the categorical variables. Two-tailed p values less than 0.05 were accepted to be statistically significant.

Results

Twenty-six neonates with NTD underwent surgery on the first postnatal day (68.4%) while 10 neonates with NTD had surgery on the second postnatal day (26.3%) and 2 neonates with NTD had surgical repair on the third postnatal day (5.3%). The reasons for the latency in operative treatment were the delay in the referral of the affected newborns from primary and secondary health care centers (n=8) and the transient abnormalities in coagulation tests (n=4).

Two neonates who also had Meckel Gruber syndrome, one neonate who was also diagnosed with Edwards' syndrome, one neonate who also had Patau syndrome and one neonate who was also diagnosed with Roberts' syndrome were excluded from the study.

Table I compares the sociodemographic characteristics of the neonates with NTDs with respect to the timing of surgery. Rural residence was significantly more frequent, gestational age at delivery was significantly lower and preterm delivery was significantly more frequent in neonates that underwent surgery for NTD repair after the first postnatal day (p=0.001, p=0.048 and p=0.024 respectively). Maternal age and smoking, maternal use of anti-epileptic

Table I. Sociodemographic characteristics of the neonates with neural tube defects.

	Surgery at first postnatal day (n=26)	Surgery after first postnatal day (n=12)	p
Maternal age (years)	34.6±4.7	33.8±5.6	0.554
Maternal residence (Urban/Rural)	21 (80.8%) / 5 (19.2%)	2 (16.7%) / 10 (83.3%)	0.001*
Maternal smoking	2 (7.7%)	0 (0.0%)	0.324
Maternal use of anti-epileptic drugs	1 (3.8%)	0 (0.0%)	0.491
Positive family history	2 (7.7%)	0 (0.0%)	0.324
Gestational age at the time of delivery (weeks)	39.3±2.5	36.2±3.8	0.048*
Preterm delivery (<37 weeks)	4 (15.4%)	6 (50.0%)	0.005*
Birth weight (grams)	3012.7±154.8	2715.5±127.8	0.117
Birth length (cm)	50.4±4.1	49.3±5.4	0.514
Head circumference (cm)	42.3±2.9	39.2±3.7	0.492
Male / Female	13 (50.0%) / 13 (50.0%)	4 (33.3%) / 8 (66.7%)	0.337
Vaginal / Cesarean delivery	2 (7.7%) / 24 (92.3%)	1 (8.3%) / 11 (91.7%)	0.946
1st minute Apgar score	7.2±1.9	7.3±2.8	0.911
5th minute Apgar score	8.8±3.1	8.4±3.5	0.876

*p<0.05 was accepted to be statistically significant.

drugs, positive family history, birth weight, birth length, head circumference, sex, mode of delivery and Apgar scores were statistically similar in both groups of neonates (p>0.05 for each).

Table II compares the clinical characteristics of the affected neonates based on the timing of postnatal surgery. Prenatal diagnosis was significantly more frequent in the neonates who underwent NTD surgery on the first postnatal day (p=0.003). The neonates who had NTD repair on the first postnatal day and the neonates who had surgery after the first postnatal day were statistically similar in regards to gestational age at prenatal diagnosis, NTD type and localization, coexisting congenital anomalies and imaging findings (p>0.05 for each).

Table III compares the clinical outcomes of the neonates with NTDs depending on the timing of the surgery. Postoperative motor dysfunction was significantly more severe (p=0.002), postoperative complications were significantly more frequent (p=0.008), the reoperation and postoperative mortality rates were significantly higher (p=0.009 and p=0.048 respectively) and the duration of hospital stay was significantly longer (p=0.033) for the neonates who underwent surgery after the first postnatal day than the neonates who had surgery on the first postnatal day (Fig. 1).

Discussion

The morbidity and mortality related to NTDs in newborns vary worldwide. This variation has been attributed to age, sex, ethnicity, level and severity of the lesion, co-existence of multiple birth defects as well as availability and use of treatment modalities.[8-10] It has been reported that the survival and well-being of the neonates born with NTDs are negatively affected by low birth weight and lesions located at relatively higher levels of the spinal cord.[14,15] Another poor prognostic factor for newborns with NTDs has been addressed as concurrence of NTDs with other congenital abnormalities, chromosomal aberrations and genetic syndromes.[14,16] The major reason for this observation is that isolated cases of NTD tend to be less complicated.[16] On the other hand, it has been shown that early intervention after birth enhances the survival

Table II. Clinical characteristics of the neonates with neural tube defects.

	Surgery on the first postnatal day (n= 26)	Surgery after the first postnatal day (n= 12)	p
Prenatal diagnosis	24 (92.3%)	6 (50.0%)	0.003*
Gestational age at prenatal diagnosis	18.6±2.4	19.3±1.7	0.266
Type of neural tube defect			0.213
Myelocele	0 (0.0%)	1 (8.3%)	
Encephalocele	2 (7.7%)	0 (0.0%)	
Meningomyelocele	24 (92.3%)	11 (91.7%)	
Localization of neural tube defect			0.784
Lumbosacral	11 (42.3%)	5 (41.7%)	
Thoracolumbar	8 (30.8%)	4 (33.3%)	
Thoracolumbosacral	5 (19.2%)	3 (25.0%)	
Lumbar	2 (7.7%)	0 (0.0%)	
Co-existing congenital anomalies			0.526
Hydrocephaly	14 (53.8%)	6 (50.0%)	
Pes equinovarus	7 (27.0%)	3 (25.0%)	
Congenital cardiac disease	5 (19.2%)	2 (16.7%)	
Hydronephrosis	0 (0.0%)	1 (8.3%)	
Neurological imaging findings			0.761
Hydrocephaly	15 (57.7%)	5 (41.6%)	
Colpocephaly	5 (19.2%)	3 (25.0%)	
Arnold-Chiari malformation	4 (15.4%)	2 (16.7%)	
Agenesis of corpus callosum	2 (7.7%)	2 (16.7%)	

and well being of the neonates diagnosed with NTDs.[14,15]

The timing of surgery for postnatal treatment of NTD remains a controversial topic in the literature. It has been hypothesized that surgical repair of NTD within the first two days of life prevents further impairment in motor functions, contributes to the well being of the genitourinary system and decreases the risk of infectious complications.[17,18] A retrospective review of 401 children with myelomeningocele reported significantly higher incidences of febrile urinary tract infections, vesicoureteral reflux and hydronephrosis for the patients who underwent primary neurosurgical repair after 72 hours following delivery.[17] Pinto et al.[13] claimed that surgical repair carried out immediately after delivery (at time zero) was associated with significantly lower incidence of preoperative myelomeningocele rupture, postoperative dehiscence and neurodevelopmental delay during the first year of life. Oncel et al.[18] also supported the early surgical intervention in NTD patients as surgery within the first five days of postnatal life came up with significantly shorter hospital stay and antibiotic therapy duration as well as significantly lower complication rate. A retrospective cohort study conducted in 70 patients with meningomyelocele showed that surgical repair performed after 48 hours of life increased the risk of central nervous system infections 5.72 times.[19]

However, an analysis of a nationwide database failed to find any significant difference in rates of infection between same-day and 1-day waiting for NTD surgery. It has been found that the prolongation of two or more days in surgery time led to an increase of infection rates

Table III. Clinical outcomes with respect to timing of surgery.

	Surgery on the first postnatal day (n= 26)	Surgery after the first postnatal day (n= 12)	p
Type of surgery			0.505
NTD repair	6 (23.1%)	4 (33.3%)	
NTD repair & Ventriculoperitoneal shunt	20 (76.9%)	8 (66.7%)	
Postoperative motor dysfunction			0.002*
Complete loss above L4	6 (23.1%)	6 (50.0%)	
Partial loss above L4	2 (7.7%)	4 (33.3%)	
Partial loss at L4 and below	1 (3.8%)	2 (16.7%)	
Postoperative complications			0.008*
CSF leakage	4 (15.4%)	4 (33.3%)	
Wound infection	3 (11.5%)	3 (25.0%)	
CSF leakage & Wound infection	1 (3.8%)	3 (25.0%)	
Meningitis	0 (0.0%)	2 (16.7%)	
Re-operation			0.009*
Wound infection	5 (19.2%)	5 (41.7%)	
CSF leakage	3 (11.5%)	3 (25.0%)	
Recurring hydrocephaly	1 (3.8%)	3 (25.0%)	
Postoperative mortality	1 (3.8%)	3 (25.0%)	0.048* 0.033*
Duration of hospital stay	43.7±11.6	78.6±19.9	

NTD: Neural tube defect, CSF: Cerebrospinal fluid
*p<0.05 was accepted to be statistically significant.

by 65% and 88%, respectively. The presence of infection was associated with a 54% increase in the duration of hospital stay. Thus, the delay of NTD closure for more than 1 day after birth was found to correlate with a significant rise in infection and hospitalization rates.[20]

Radcliff et al.[21] held out a retrospective, statewide and population-based study for infants born in Florida between 1998 and 2007 and examined the neonates born with spina bifida as confirmed by the Florida Birth Defects Registry. They determined that the affected newborns that were delivered at primary and secondary health care centers were less likely to undergo surgery on the first postnatal day. It has been suggested that the lower prevalence of timely surgical repair in newborns with spina bifida might be due to the lack of prenatal diagnosis and related lack of appropriate referral to a tertiary health care center.

Complying with the literature, this study indicated a significantly less frequency of prenatal diagnosis in newborns that had surgery after the first postnatal day. Moreover, the reason for the latency in surgical repair was the delay in the referral from primary and secondary health care centers for 8 newborns. The lack of prenatal diagnosis for NTD might prevent the in utero referral of affected fetuses for delivery in tertiary health care centers and result in the birth of affected fetuses in primary and secondary health care centers. This might cause prolongation in the time needed for a transfer to a hospital with a higher level of health care.

Radcliff et al.[21] also concluded that preterm infants born with spina bifida were more likely to have delayed surgical repair than term infants. Similarly, in this study, the frequency of preterm delivery was significantly higher in newborns that had delayed surgery.

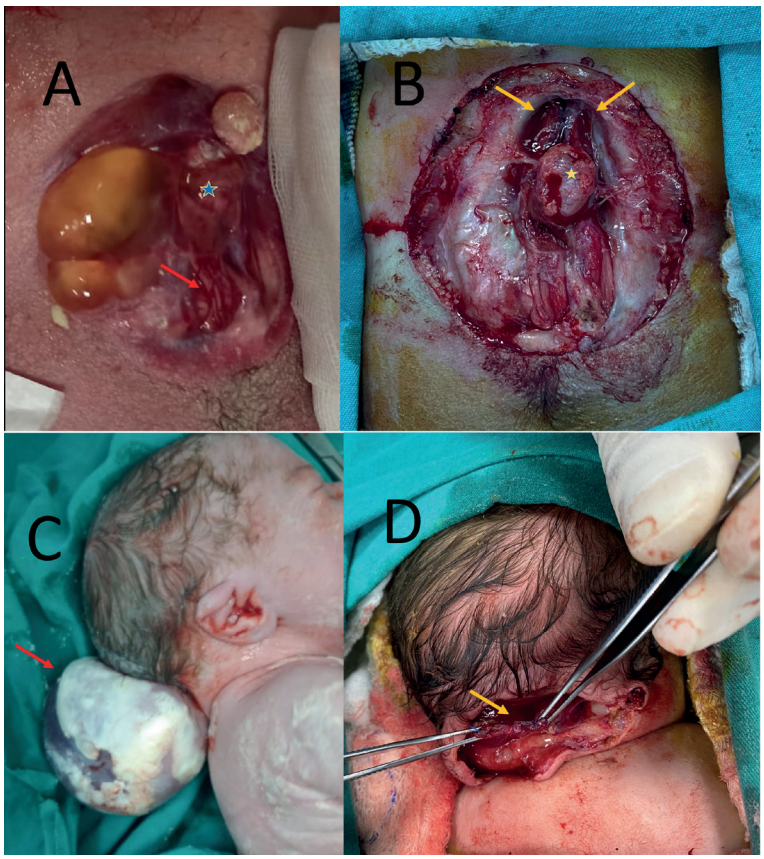

Fig. 1. (a) Meningomyelocele and diasthometamyelia can occur concurrently, (b) When the myelomeningocele sac is excised, the spinal cord split by a bone fragment becomes more obvious, (c) Encephalocele sac is marked with an arrow, (d) After the excision of encephalocele sac, the dura mater is sutured.

On the contrary, Radcliff et al.[21] failed to detect any differences in the timing of spina bifida surgery by type of the lesion. They also speculated that the simultaneous existence of other medical conditions may not be an underlying cause for the latency in the surgical treatment of spina bifida. As for the present study, the neonates who had surgery on the first postnatal day and the neonates who had surgery after the first postnatal day were statistically similar in respect to the NTD type. In order to avoid bias related to coexisting medical conditions, all newborns diagnosed with concurrent NTD and genetic syndromes were excluded from this study.

In this study, postoperative motor dysfunction was significantly more severe, postoperative complications were significantly more frequent and the duration of hospital stay was significantly longer for the neonates who underwent surgery after the first postnatal day than the neonates who had surgery on the first postnatal day. Our study appears to favor the early repair of NTD within the first 24 hours of life. Such an approach may reduce the risk of infectious and neurological complications significantly and allow the pediatric neurosurgeons to use the pregnancy as a period of time for comprehensive counseling of the parents who need to give consent for the surgical treatment of their newborns.

The findings of the present study should be interpreted carefully as its power is limited by the retrospective study design, small sample size and lack of data about ventriculoperitoneal shunt infections. Further research is warranted to clarify the long-term consequences of early postnatal management in newborns with NTD.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AP, AAK; data collection: HK, MGB; analysis and interpretation of results: AP, MKP, AAK; draft manuscript preparation: AP, MGB, MKP. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The present study was approved by the Institutional Review Board and Ethical Committee of Afyonkarahisar Health Sciences University (02.08.2019-261).

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Conflict of interest

The authors declare no conflict of interest.

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Case Report

Clinical and molecular characteristics of carnitine-acylcarnitine translocase deficiency with c.270delC and a novel c.408C>A variant

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ABSTRACT

Background. Carnitine-acylcarnitine translocase deficiency (CACTD) is a rare, autosomal recessive, and highly lethal fatty acid oxidation (FAO) disorder caused by defective acylcarnitine transport across the mitochondrial membrane. CACTD is characterized by severe episodes of hypoglycemia and hyperammonemia, seizures, cardiomyopathy, liver dysfunction, severe neurological damage, and muscle weakness. Herein, we described the clinical features, biochemical, and molecular findings of three patients with CACTD, presented with poor feeding, hypoglycemia, liver dysfunctions, and hyperammonemia, but died despite intensive treatment.

Cases. All cases had similar signs and symptoms like poor feeding and respiratory failure associated with liver dysfunction. Urinary organic acid profiles in the presence of hypoglycemia and hyperammonemia led us to the possible diagnosis of one of fatty acid β-oxidation defects. Results of the molecular analyses were compatible with CACTD. In addition to known mutation (c.270delC;p.Phe91Leufs*38) we detected a novel one (c.408C>A;p.Cys136*).

Conclusions. All three cases died despite a very intensive therapy. Based on our experience with these three cases, it can be said that CACTD has a relatively poor prognosis, molecular studies are of most importance in suspected cases for the final diagnosis and such studies might be of help while giving genetic counselling and guidance to parents for future pregnancies.

Key words: fatty acid oxidation defects, carnitine, aclycarnitines, carnitine acyltransferases, carnitine acylcarnitine translocase.

Fatty acid β-oxidation (FAO) to acetyl coenzyme A in the mitochondrial matrix requires an energy-yielding pathway controlled by specific enzymes.[1] This energy pathway is crucial for cardiac and skeletal muscles during long-term exercise and prolonged fasting.[2] The transfer of acyl-coenzyme As (acyl-CoAs) through the mitochondrial membrane needs L-carnitine, two types of carnitine palmitoyl transferases (CPT I and II), and carnitine-acylcarnitine translocase (CACT), which is a critical enzyme in the carnitine cycle.[3] Carnitine-acylcarnitine translocase deficiency (CACTD) is a rare, autosomal recessive and highly lethal FAO-disorder caused by defective acylcarnitine transport across the mitochondrial membrane, and characterized by severe episodes of hypoglycemia and hyperammonemia, seizures, cardiomyopathy, liver dysfunction, severe neurological damage, and muscle weakness.[2,4,5] Most CACTD cases have developed extreme metabolic decompensation and unexplained early death in the first year of their lives.[6]

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The gene encoding CACT (*SLC25A20*) consists of nine exons, spans about 16.5 kb, and is located on chromosome 3p21.31.[7,8] At least 42 different pathogenic/possible pathogenic mutations, including 20 missense/nonsense mutations, 10 small deletions, 2 small insertions, 1 small indel, 4 gross deletions, and 5 splicing mutations have been reported in this gene.[6] The frequently reported variation in patients with CACTD is c.199-10T>G splice site mutation in Asian population.[5,6,9] No patients with CACTD have previously been reported from Turkey.

In this study, we aimed to describe the clinical, biochemical, and molecular features of three CACTD patients.

Case Reports

Three patients followed up with a diagnosis of CACTD were reported. The patients' data were retrieved from the hospital's electronic patient registry (Nucleus Automation System) and patient files.

Genetic investigations

Genomic DNA was isolated from peripheral blood samples (10 ml) using a standard salting-out method. Whole exomes sequencing (WES) was performed on patient 1 and WES data was evaluated by bioinformatics analysis. We filtered variants according to their minor allele frequency lower than 1% within different open access population allele frequency databases (1000 Genomes Project and dbSNP138). Then, exonic and non-synonymous variants were retrieved. We removed variants that were already found in our in-house variant database. Variants present in the 'Genome Aggregation Database Browser' (gnomAD) as homozygous state were ruled out. The novel homozygous variant in the *SLC25A20* gene was visually inspected using Integrative Genomics Viewer for patient 1. This novel variant was further confirmed and familial segregation analysis was performed by Sanger sequencing. On the other hand, the gene panel including around 450 genes accounting for inborn errors of metabolism was performed in patient 2 and patient 3. Using this panel, detected nucleotide changing was also verified by Sanger sequencing in the *SLC25A20* gene for patients 2 and 3.

All three patients were girls, born to young mothers following 37-39 gestational weeks, and presented with poor feeding in the first few days of life. The most remarkable clinical findings of all patients are summarized in Table I.

Patient 1

This female neonate was the third child of consanguineous parents whose second baby died 6w postpartum. Her birth weight was 2,630 g. The patient was hospitalized because of respiratory failure and poor feeding on the second day of life. Her laboratory findings showed hypoglycemia (20 mg/dl; normal range: 70-130 mg/dl), hyperammonemia (1019 mmol/L; normal value ≤110mmol/L), elevated LDH (656 IU/L) and transaminases (ALT 168 IU/L, AST 194 IU/L). LC-MS/MS analysis of her dried blood sample showed increased levels of long-chain acylcarnitines, particularly C_{16}, $C_{16.1}$, C_{18}, $C_{18.1}$ acylcarnitines, and decreased concentrations of C_0 free carnitine.

Her diet was adjusted to include medium-chain triglycerides (MCT) and carbohydrates, limiting long-chain fatty acids. The blood carnitine levels before and after carnitine treatments are shown in Table II. Additionally, the urinary organic acid analysis demonstrated elevated lactic acid (0.5-fold), and an increased urinary excretion of 3-OH isovaleric acid (0.5-fold), adipic acid (1.3-fold), suberic acid (2.5-fold), and sebacic acid (2.5-fold). Mutation analyses in *SLC25A20* gene (NM_000387.6) revealed a novel homozygous c.408C>A(p.Cys136*) nucleotide change. This nucleotide changing c.408C>A (p.Cys136*) in exon 4 in the *SLC25A20* gene was homozygous in patient 1 (II-3) and heterozygous in the parents (I-1 and I-2) and healthy sibling (II-1) (Figs 1A and 1B). The patient was given a special diet containing 20-25% fat, and supplemented with multivitamins, and oral carnitine 30 mg/kg/day, and advised to avoid long fasting periods. The patient was discharged on day 16, but she died at 10 months of life.

Table I. Characteristics of CACTD patients.

	Patient 1	Patient 2	Patient 3
Age of mother (year)	26	32	31
Sex	Female	Female	Female
Birth weight (g)	2,630	3,480	3,540
Gestational age (week)	37	37	39
Parental consanguinity	Present	Absent	Absent
Previous deceased siblings	First child	One sister died	Two brothers died
Day of onset	2nd day of life	1st day of life	10th day of life
Signs and symptoms	Poor feeding, respiratory failure	Poor feeding, respiratory failure	Poor feeding
Age at death	10 months	12 months	52 days
Genotype	Homozygous c.408C>A (p.Cys136*)	Homozygous c.270delC (p.Phe91Leufs*38)	Homozygous c.270delC (p.Phe91Leufs*38)
ALT (IU/L)	168	59	80
AST (IU/L)	194	68	147
LDH (IU/L)	656	452	502
Ammonia (mmol/L)	1019	834	1025

ALT: alanine aminotransferase, AST: aspartate aminotransferase, LDH: lactate dehydrogenase.

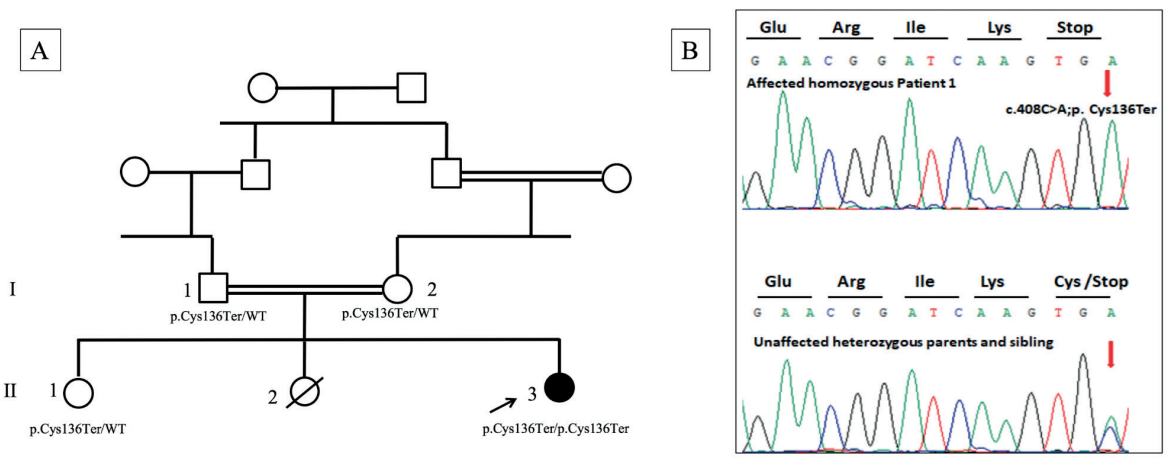

Fig. 1. Pedigree of Patient 1 (A), and Sanger sequencing of p.Cys136* (c.408C>A) variant in *SLC25A20* gene (NM_000387.6) among family members (B).

Patient 2

This baby girl was born to non-consanguineous parents after a normal gestation and delivery, weighing 3,480 g at birth. Her older sister had reportedly died two months after birth because of a suspected but remained undiagnosed metabolic disease. She presented on the first day of life with poor feeding, respiratory failure, hypotonia, hypoglycemia (20 mg/dl) and hyperammonemia (834 mmol/L). The results of LC-MS/MS analysis of a dried blood sample showed increased levels of long-chain acylcarnitines, particularly C_{16} acylcarnitine, and decreased concentrations of C_0 free carnitine (Table II).

Furthermore, the urinary organic acid analysis showed highly elevated levulinic acid, oxalic acid, fumaric acid, suberic acid, and adipic acid, whereas mildly elevated methylmalonic

Table II. Acylcarnitine concentrations in dried blood spots before and after low dose L-carnitine supplements treatment.

Acylcarnitine (mmol/L)	Patient 1		Patient 2		Patient 3		Normal range
	Before	After	Before	After	Before	After	
C_0 (free carnitine)	4.39	11.89	4.23	8.65	3.10	15.85	10-60
C_{14} (myristoil carnitine)	0.45	0.47	0.66	1.86	0.44	0.99	0-0.36
C_{16} (palmitoil carnitine)	8.04	6.26	12.87	8.94	2.62	3.94	0-1.51
$C_{16.1}$ (palmitoil carnitine)	0.52	0.61	0.95	0.86	0.33	0.60	0-0.27
C_{18} (steraoil carnitine)	1.41	1.5	1.38	1.31	0.78	0.99	0-0.61
$C_{18.1}$ (oleil carnitine)	1.92	1.78	4.01	3.27	2.44	2.72	0-1.51

acid, 3-OH sebacic acid, dodecanedioic acid, and 5-OH hexanoic acid excretions. Feeding was initiated with a diet rich in MCT and carbohydrates, limiting long-chain fatty acids. The patient received oral carnitine (30 mg/kg/day). A homozygous c.270delC deletion (p.Phe91Leufs*38) was detected in the *SLC25A20* gene (NM_000387.6). Following supportive treatment and dietary adjustment, the patient was discharged on day 42; however, she died at 12 months of life.

Patient 3

This female neonate, the third child of healthy non-consanguineous parents, was born at full term. Her birth weight was 3,540 g. Her older two brothers had died on the second and fifth days of their lives respectively due to unknown causes.

In the first ten days of life, the patient was followed up with suspected diagnosis of neonatal hepatitis. After ten days, when she stopped receiving breast feeding, and started to vomit, hepatomegaly and hyperammonemia were noted. Her serum ALT, AST, and LDH levels were 80, 147, and 502 IU/L, respectively. Blood ammonia was measured as 1025 mmol/L. LC-MS/MS analysis of her dried blood sample yielded decreased concentrations of C_0 free carnitine and increased concentrations of C_{14}, C_{16}, $C_{16.1}$, C_{18}, and $C_{18.1}$ (Table II). Additionally, the urinary organic acid analysis showed elevated adipic acid (2/3-fold), sebacic acid (1.5-fold), 3-OH sebacic acid (2/3-fold), and 3-OH dodecanedioic acid (1/6-fold) excretions. Mutation analysis of the *SLC25A20* gene revealed a homozygous c.270delC (p.Phe91Leufs*38) deletion. Despite all supportive measures and dietary adjustment (rich in MCT and carbohydrates, limiting long-chain fatty acids), the patient died at on the 52nd day of life.

Ethical approval for this study was obtained from Hacettepe University Faculty of Medicine, Clinical Research Ethics Committee (GO: 20/530-2020/11-39).

The parents of the patient were informed, and written and oral consent was obtained according to the principles of the Helsinki Declaration.

Discussion

CACTD is a rare inborn disorder of the carnitine cycle that is highly lethal before one year of age.[10] More than 60 patients have been reported worldwide during the last two decades.[5] The clinical features of this disorder are generally resulted from a combination of energy deprivation and endogenous toxicity due to accumulation of long-chain acylcarnitines.[11] Brain, liver, muscle, and heart are the primarily affected organs in this disorder which accounts for CACTD patients suffering from neurological abnormalities, muscle damage, cardiomyopathy, and liver dysfunctions.[12]

In this report, we presented three CACTD patients, all of whom had similar features, including liver dysfunctions, respiratory failure, and poor feeding. Presence of hypoglycemia and hyperammonemia along with dicarboxylic

aciduria in urinary organic acid and LC-MS/MS results led us to consider defects in fatty acid β-oxidation. As in previously reported cases, our patients had high blood levels of ammonia, ALT, AST, LDH, highly elevated C_{16}, $C_{16.1}$ carnitine esters, moderately elevated C_{18} carnitine esters, and low concentrations of C_0 free carnitine.[1-3,5,6,11] The diets given, treatment options, biochemical and clinical features were alike in all three CACTD patients. Unfortunately, their life spans were limited despite supportive care efforts.

Genetic mutation analyses of the *SLC25A20* gene showed that Patient 1, 2, and 3 have homozygous mutations: a novel c.408C>A (p.Cys136*) nonsense mutation, which has not been reported yet and a previously-reported c.270delC (p.Phe91Leufs*38) frameshift mutation.[13,14] Previously reported cases indicate that c.270delC mutation might be associated with severe phenotype in patients.[11,15] In this study, patients 2 and 3, who had c.270delC mutations showed a severe phenotype that gives further support to this assumption. These two patients died on the 12th month and 52nd day of life respectively. Carnitine-acylcarnitine translocase protein which belongs to the mitochondrial carrier family consist of 6 transmembran α-helices (two in each repetitive domain) domains. Previously reported in consanguineous Turkish family as pathogenic variant c.306delC in exon 3 frameshift mutation give rise to the stop codon at amino acid residue 127 which is predicted to cause premature protein truncation. This mutant protein that would lack the second and third transmembrane domains and would presumably result in nonfunctional translocase enzyme.[13]

Patient 1, who had a novel homozygous c.408C>A (p.Cys136*) nonsense mutation, had also severe phenotype, and she died on the 10th month of her life. This truncating mutation is predicted to occur in the second intra mitochondrial hydrophilic loop and resulting protein would lack fourth, five and sixth transmembrane domains and would therefore be completely inactive. Hence, our data further reinforce that the c.270delC (p.Phe91Leufs*38) mutation is associated with a severe phenotype of CACTD and a novelc.408C>A (p.Cys136*) variation has the potential to be associated with severe phenotype too.

In this paper, we reported three cases of CACTD. Although significant progress in early recognition and appropriate treatment is crucial and has been made according to clinical signs and symptoms developing shortly after birth, even with expanded newborn screening, including detecting urinary organic acid excretions, and C_0, C_{16}, $C_{16.1}$, C_{18}, and $C_{18:1}$ acylcarnitines in dried blood spots, this disorder has still a high mortality rate.[16,17] On the other hand, apart from the frameshift mutation c.270delC, we identified a novel c.408C>A nonsense mutation. Almost all patients with CACTD who had a genotype of those mutations present with severe clinical phenotypes. The available evidence clearly shows that CACTD is generally a highly severe and lethal metabolic disorder with clinical, biochemical, and molecular heterogeneity. Although CACTD causes early neonatal death, molecular diagnosis is quite imperative for providing better and effective reproductive guidance for future pregnancies.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BBG, AT, AD, HSS, TC; data collection: BBG, CK, DYY; analysis and interpretation of results: BBG, DYY, CK, RKÖ; draft manuscript preparation: BBG, AT, TC. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors have no conflict of interest in this study.

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Case Report

Outbreak of late-onset Group B Streptococcal disease with serotype Ib in a Neonatal Intensive Care Unit

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ABSTRACT

Background. Hospital outbreaks of invasive group B streptococcus (GBS) infection are rare. There are only a few published reports of late-onset GBS outbreaks in neonatal intensive care units (NICUs). We report here three cases of late-onset GBS in our NICU.

Case. Three preterm very low birth weight (VLBW) infants born at 24 –27 weeks gestation developed late-onset GBS sepsis within four weeks. Two asymptomatic GBS carriers were identified in the NICU prior to the outbreak. Tests of maternal rectovaginal GBS colonization were negative in all three cases; as such, vertical transmission was unlikely. All three GBS isolates were capsular serotype 1b, with comparable antibiotic susceptibility profiles.

Conclusion. Preterm delivery and VLBW are associated with an increased risk of invasive late-onset GBS infection. This report underscores the ongoing risk of nosocomial transmission of GBS in the NICU.

Key words: Group B Streptococcus, sepsis, outbreak, very low birth weight infant, NICU.

Group B streptococcus (GBS) is a leading cause of neonatal sepsis and meningitis and accounts for 50–60% of all cases in Japan. Infant invasive GBS disease is classified as early-onset disease if it occurs within the first 6 days of life; by contrast, late-onset disease (LOD) develops seven or more days after birth.[1] The frequency of early-onset GBS infection has decreased substantially since the introduction of routine screening for maternal rectovaginal GBS colonization and the use of intrapartum antibiotic prophylaxis (IAP).[2] However, late-onset GBS infection is unaffected by chemoprophylaxis and continues to cause significant morbidity and mortality in newborns. The pathogenesis of late-onset GBS infection remains controversial[3]; there are only a few published studies reporting outbreaks of late-onset GBS specifically in neonatal intensive care units (NICUs).[4] In this report, we describe a cluster of nosocomial LOD GBS infections in a regional NICU that were diagnosed over a period of four weeks involving three very low birth weight (VLBW) infants.

Patient presentations have been given in Table I.

Case 1

A male infant weighing 722 g was delivered by cesarean section at 24 6/7 weeks of gestation who required immediate resuscitation via endotracheal intubation at birth. Maternal rectovaginal GBS colonization was negative, although preterm premature rupture of the membranes (PPROM) had been recognized at 23 4/7 weeks of gestation. The patient serum IgM was detected at 33 mg/dL, suggesting intrauterine infection. Antibiotic therapy with ampicillin and gentamicin was initiated at birth (day 0) and continued through postnatal day 5; hydrocortisone was also administered (postnatal days 0 – 9) to prevent bronchopulmonary dysplasia. Mechanical ventilation was discontinued on postnatal day 40; however, on

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Table I. Characteristics of the three patients with LOD in the NICU.

	Case 1	Case 2	Case 3
Sex	Male	Male	Male
Gestational age (weeks)	24 6/7	26 1/7	27 5/7
Birth weight (grams)	722	620	1158
Delivery	Cesarean	Cesarean	Cesarean
Apgar score (1/5 min)	3/7	4/6	6/5
Onset (days after birth)	55	19	43
WBC (/μL)	4,820	10,700	10,250
C-reactive protein (mg/dL)	1.66	0.17	2.55
Blood culture	GBS +	GBS +	GBS +
CSF culture	Negative	Negative	(Not done)
Maternal rectovaginal culture	GBS -	GBS -	GBS -
Maternal milk culture	GBS +	GBS -	GBS -

WBC: white blood cell, CSF: cerebrospinal fluid, GBS: group B Streptococcus.

postnatal day 55, he again required respirator support due to frequent apnea. GBS was detected in cultures of peripheral blood on the same day; cerebrospinal fluid cultures remained negative. Of note, weekly surveillance cultures of both stool and respiratory secretions were negative for GBS before the infection. GBS was later found in maternal breast milk.

Case 2

A male infant weighing 620 g was delivered by emergency cesarean section at 26 1/7 weeks of gestation because of suspected placental abruption. Maternal rectovaginal GBS colonization was negative. He was intubated immediately after birth and provided with surfactant replacement therapy; hydrocortisone was administered for hypotension. He was extubated on postnatal day 4. On postnatal day 19, he developed lethargy and poor feeding behavior; his laboratory tests were notable for hypoglycemia and an elevated level serum C-reactive protein. He was diagnosed with late-onset GBS sepsis; GBS was detected in blood but not in cerebrospinal fluid cultures. Weekly surveillance cultures from stool were GBS positive for the first time on day 5 of the infection. GBS was not detected in maternal breastmilk.

Case 3

A male infant weighing 1,158 g was delivered by cesarean section at 27 5/7 weeks of gestation due to premature uterine contractions associated with fetal footling presentation. Maternal rectovaginal GBS colonization was negative. He was admitted to the NICU where he underwent mechanical ventilation from 0 – 2 days of age. Frequent apnea was noted on postnatal day 43 associated with an elevated serum level of C-reactive protein. GBS was detected in his blood on the same day; his overall condition precluded sampling and evaluation of his cerebrospinal fluid. His weekly surveillance cultures were negative for GBS before the diagnosis of bacterial sepsis. GBS was not found in maternal breastmilk.

Informed consent was obtained from the parents of all three patients featured in this study.

Discussion

We report here three cases of preterm VLBW infants who developed LOD GBS sepsis in the NICU within four weeks (Fig. 1). Serotype analysis of the strains isolated from the infants and from maternal breast milk revealed that all samples were capsular type Ib; interestingly, this capsule type is not typically associated with

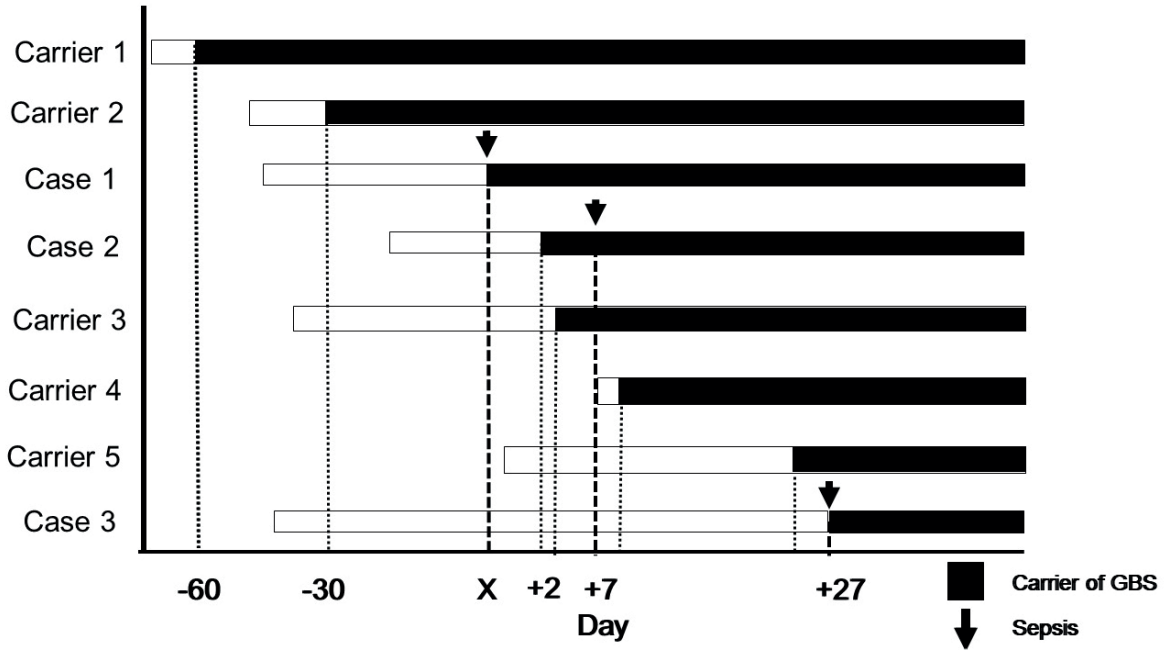

Fig. 1. Time course of the outbreak. Black arrows indicate the onset of LOD in each patient. Black bands represent the period of GBS carrier.

LOD (Table II)[5] The antibiotic susceptibility profiles of all isolates were comparable to one another.

In this report, maternal rectovaginal GBS colonization was negative throughout; as such, a mechanism involving vertical transmission of this pathogen was unlikely. Bacterial colonization surveys of all the neonates in the NICU were performed routinely on a weekly basis. Of note, two asymptomatic infants were identified in the NICU prior to the development of GBS sepsis in the index case (Fig. 1; carriers 1 and 2). Patient case 2 was identified as a GBS carrier prior to the onset of sepsis; however, two of the three patients (patient cases 1 and 3) were not GBS carriers before developing LOD. Of note, an additional three new carriers (carriers 3, 4, and 5) were identified in the NICU after the onset of disease observed in patient case 1. After the outbreak, we conducted similar prevalence surveys focused on possible sources of GBS infection, including equipment (i.e., milk warmers, feeding bottles, and shared breast pumps). No GBS was detected in any swab samples from NICU equipment. Breast milk from the mother of patient case 1 was later identified as positive for GBS. On the other hand, maternal breastmilk from patients 2 and 3 was negative. Given these findings, we hypothesize that patient case 1 may have acquired the infection from maternal milk or asymptomatic carriers; cases 2 and 3 may have developed GBS sepsis via transmission from an asymptomatic carrier on the medical staff.

NICU-associated outbreaks of invasive GBS disease are rare and the mechanisms associated with transmission and development of severe LOD remain controversial. One group has reported that more than half the mothers of infants who develop LOD are carriers of GBS.[3] Routine screening for maternal GBS carriage at 35 to 37 weeks' gestation and consequent IAP may not eradicate maternal colonization; as such, the mother may remain a source of GBS. However, horizontal transmission from nosocomial sources has also been reported.[6]

At this time, there are only a few published reports of GBS outbreaks in NICUs. Boyer reported that 34% of the nursing staff were identified as GBS carriers in association with

Table II. The susceptibility of antibiotics and capsular serotype of isolated GBS in each patient.

	Penicillin	Ampicilin	Erythromycin	Clindamycin	Levofloxacin	Capsular serotype
Case 1 (blood)	S	S	R	R	R	-
Case 1 (pharyngeal mucosa)	S	S	R	R	R	Ib
Case 1 (maternal milk)	S	S	R	R	R	Ib
Case 2 (blood)	S	S	R	R	R	-
Case 2 (stool)	S	S	R	R	R	Ib
Case 3 (blood)	S	S	R	R	R	Ib
Carrier 1 (stool)	S	S	R	R	R	Ib
Carrier 2 (stool)	S	S	R	R	R	Ib
Carrier 3 (stool)	S	S	-	-	R	Ib
Carrier 4 (stool)	S	S	R	R	R	-
Carrier 4 (pharyngeal mucosa)	S	S	R	R	-	Ib
Carrier 5 (stool)	S	S	R	R	R	Ib

S: susceptible, R: resistant.

a cluster outbreak of this infection.[7] Recently, Collin reviewed the overall incidence and prevalence of GBS clusters in healthcare settings, and identified only 12 reports focused on neonatal LOD over 50-year period.[4]

Infant-to-infant or staff member-to-infant spread may occur via physical contamination. Cross-transmission has been associated with high staff workloads, patient crowding and deficient cot-spacing and/or high patient-to-nurse ratios.[4] Likewise, poor infection control practices, including inadequate hand hygiene and care taken when preparing infant formula feedings are magnified in premature infants who are then exposed to GBS. Environmental contamination from inadequate disinfection of equipment or surfaces is also appreciated as a reservoir for invasive pathogens. Shared breast pumps, laryngoscope blades, patient monitors, and other surfaces that are handled frequently have all been implicated as sources of infection.[8]

While breast milk plays an important role in protecting infants against GBS infection, it may also represent a significant source of pathogens associated with LOD and infection.[9] It has been estimated that ~0.8 to 3.5% of mothers carry GBS in their breast milk; this pathogen is among the more common causes of mastitis. However, the role of breast milk and its role in promoting neonatal LOD remains incompletely understood.[10] In this report, breast milk from the mother of the index case (patient case 1) was GBS positive. As such, breast milk was suspected to be one of the potential sources of GBS LOD in this patient.

Several published reports have defined risk factors associated with LOD.[3,11] We note that all three cases featured here were VLBW infants born prematurely, from 24 to 27 weeks of gestation, and developed GBS sepsis at postnatal days 19–55 days after birth (Table I). Preterm delivery and low birth weight have been associated with an increased risk of invasive GBS infection and LOD. Important factors that influence progression from GBS colonization to infection include immune status and integrity of the gastrointestinal mucosa as well as the bacterial load and virulence. Compared with infants born at term, preterm infants typically have a more poorly-developed immune system as well as transient hypogammaglobulinemia. The other sources of infection associated with LOD include the use of invasive devices as well as administration of steroids[12] that may be required for life-supporting care.

Other than scrupulous attention to hygiene and infection control practices, there are no known mechanisms that might be put in place to prevent the spread of GBS. Infection control practices typically include standard precautions such as hand hygiene, careful preparation and thawing of frozen breast milk, and weekly surveillance of bacterial colonization; all of these measures are critical toward efforts to prevent GBS LOD and sepsis in the NICU. Physicians and nursing staff need to be alert to the presence of GBS in routine surveillance cultures, as well as other pathogens, including multiple drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus*. To prevent GBS outbreaks in the NICU, Jauneikaite and colleagues note that a single case of GBS LOD should be considered as a sentinel of a future outbreak[13]; all cases of GBS infection should prompt enhanced prospective and retrospective surveillance throughout the facility.

There are two limitations to our findings. First, although we provide some information about potential routes of GBS transmission, the precise mode of transmission was not fully determined because we did not perform surveillance on the healthcare personnel who were in the NICU during this period. Second, while our results may be suggestive of a single source, this could not be concluded with certainty without molecular typing information for each bacterial isolate.[14]

In summary, we report here an outbreak of GBS LOD among VLBW preterm infants in a NICU. In a recent publication, Collins reported that the risks associated with GBS outbreaks specifically in the setting of the NICU have increased over the past 9 years.[4] Indeed, some reports recommended that a single case of GBS LOD might be treated as a sentinel, and should be followed by enhanced surveillance measures.[13] This report underscores the need to be aware of the risk of nosocomial transmission of GBS infection in the NICU and the need to be alert to any indication of GBS contamination in routine surveillance cultures.

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Author contribution

The authors confirm contribution to the paper as follows: clinical data and preparation of the manuscript: HH; study conception and design: KI. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Case Report

Whole exome sequencing identifies a novel variant in ABCA3 in an individual with fatal congenital surfactant protein deficiency

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ABSTRACT

Background. Adenosine triphosphate-binding cassette subfamily A member 3 (ABCA3) gene variants, which cause severe respiratory distress syndrome (RDS) in term newborns, can cause death, especially due to the lack of congenital surfactant protein. The relationship between the types, pathophysiology and effects of ABCA3 gene variants on surfactant metabolism and the clinical phenotype have not yet been fully clarified, but the ABCA3 genotype is known to affect clinical severity.

Case. In our study, in a term newborn with a diagnosis of RDS resulting in death, we detected the c.3677 T> C (p.Leu1226Pro) variant homozygous variant in the ABCA3 gene according to the NM_001089.3 transcript, which, to our knowledge, was identified for the first time in the literature.

Conclusions. We consider that this case report contributes to the literature on RDS by showing the presence of c.3677 T> C (p.Leu1226Pro), a new homozygous variant of ABCA3 in our patient.

Key words: ATP-binding cassette transporter, subfamily A; neonatal respiratory distress syndrome, surfactant protein B deficiency, gene variant.

Adenosine triphosphate-binding cassette subfamily A member 3 (ABCA3) is an important glycoprotein expressed in the membrane of type 2 alveolar cells and plays a role in lung surfactant metabolism.[1] Type 2 alveolar cells are essential for surfactant synthesis and release. Surfactant is a phospholipid and protein complex that is vital in transferring ventilation from the placenta to the postnatal lungs in the intrauterine period and prevents lung collapse by increasing the alveolar surface tension.[2] To date, more than 200 mutations have been reported for ABCA3, which plays a critical role in lipid transport for surfactant synthesis and release.[3] Biallelic variants in ABCA3 are known to cause autosomal recessive (AR) Pulmonary Surfactant Metabolism Dysfunction type 3 (OMIM #610921).[3]

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Understanding the genetic pathophysiology of the ABCA 3 molecule will open new doors in diagnosis, treatment and management. In our study, we detected the c.3677 T> C (p.Leu1226Pro) variant homozygous variant in the ABCA3 gene according to the NM_001089.3 transcript, which, to our knowledge, has not previously been available in the literature. We aimed to discuss the conditions of our patient during the follow-up and treatment process and the gene variant we detected in order to shed light on this issue.

Case Report

This paper presents the case of a male infant born at 38 gestational weeks and 2,680 grams by C-section from a 23-year-old healthy mother. It was her second pregnancy and second birth. In his family history, his mother and father were first cousins and he had a 2.5-year-old brother followed up with the diagnosis of

non-classical congenital adrenal hyperplasia. In addition, the twin children of the patient's aunt were followed up with the diagnosis of primary ciliary dyskinesia, and his aunt also had a consanguineous marriage. The pedigree of the family is shown in Figure 1A. The infant's APGAR score was 7 at the first minute and 8 at the fifth minute. In the delivery room, due to the infant having tachypnea and expiratory grunting, he was admitted to the intensive care unit with the initial diagnosis of transient tachypnea of the newborn (TTN). When the patient was admitted to the intensive care, no pathology was detected in the electrolyte, complete blood count, C-reactive protein (CRP) and blood gas parameters but he had intercostal retractions, expiratory grunting and nasal continuous positive airway pressure (CPAP) treatment was started. Because there was no chorioamnionitis and prolonged premature rupture of membranes and the delivery was a term, the baby was considered to be at low risk for sepsis. Pneumonia was not considered in the foreground because the CRP was negative and there was no infiltration image in favor of pneumonia on chest radiography. Although the first X-ray did not have a significant aeration excess and diaphragmatic flattening; the absence of signs compatible with RDS such as lack of aeration, increased air bronchograms, prominent reticulogranular opacities and the baby's birth was term via C-section this made us think of TTN and we started respiratory support and total parenteral nutrition support first. But, with clinical need; fiO_2 was increased as the patient's saturation decreased in the following hours. However, the clinical severity gradually increased and there was no improvement, he was intubated on the postnatal 2nd day. Thereupon, we moved away from the TTN diagnosis and investigate the other potential causes of respiratory distress.

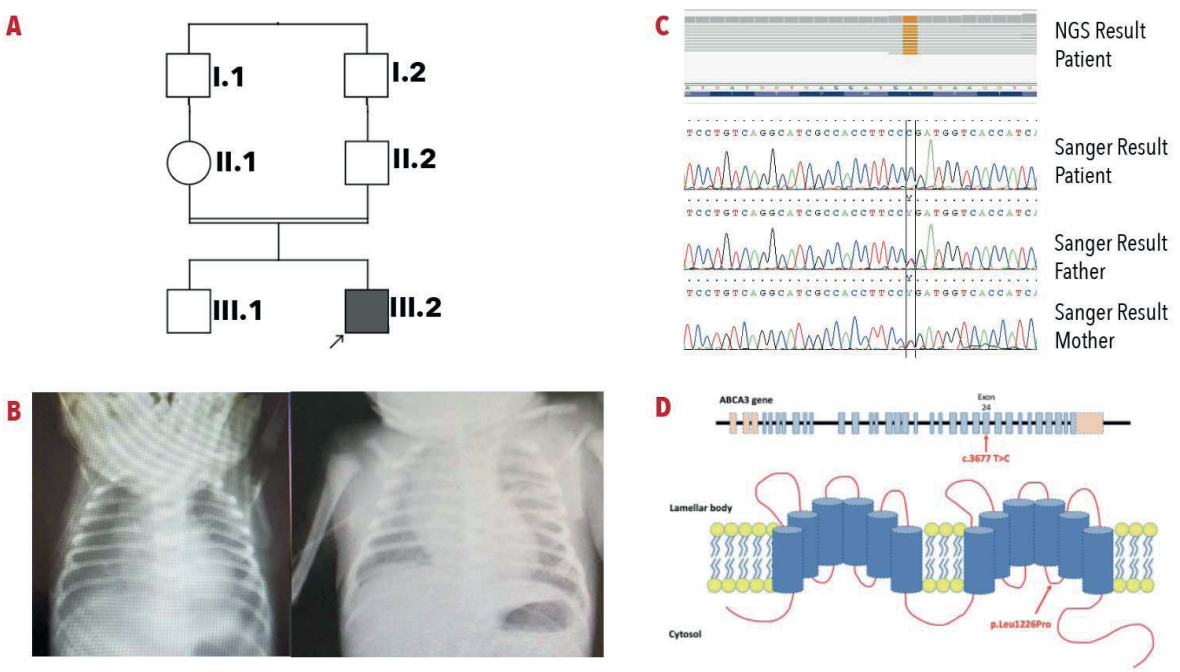

Fig. 1. A) Pedigree of the family, B) X-ray of the chest. First X-ray on the first day of hospitalization and on 3rd day before surfactant treatment, C) Display of Integrative Genomics Viewer showing novel ABCA3 c.3677 T>C missense variant in homozygous state in patient while their unaffected parents in heterozygous state. The gray letters and brown letters represent the wild type nucleotides even so the brown letters represent a single base-nucleotide alteration. Parts of the Sanger sequencing electropherograms are depicted and demonstrate homozygosity for the variant c.3677 T>C in patient, heterozygosity in father and mother, D) Structure of the ABCA3 gene, coded protein, protein domains. Red arrow marks the present variant.

In terms of persistent pulmonary hypertension, the postnatal 2nd day echocardiography was normal, and there were no findings on the X-ray that were thought to be consistent with pneumothorax or diaphragmatic hernia. For asphyxia, no pathology was found in the blood gas or neurological examination. The absence of clinical improvement on the postnatal 3rd day, the need for intubation, the onset of air bronchograms and the onset of lack of aeration on the X-ray excluded the diagnosis of TTN (Fig. 1B). From this point of view, differential diagnosis of malignant TTN, sepsis, congenital surfactant protein deficiency was considered. Blood values and CRP value (0.2 mg / dL) were normal, a blood sample was taken from the patient for whole exome sequencing analysis and sent to a genetic laboratory in Ankara, Turkey and 100 mg / kg surfactant was administered to the patient. Surfactant treatment (100 mg/ kg) was administered endotracheally three times on the 3rd, 7th and 12th days. During his follow-up, the patient was fed with total parenteral nutrition. Although there was no growth in the blood culture sent on the first day of the patient's hospitalization, on the 5th day, blood culture, urine culture and control CRP value were sent again as there was no clinical improvement. The patient was started on broad spectrum antibiotic therapy (ampicillin + gentamicin combination) with suspected sepsis pre-diagnosis because CRP was 0.7 mg / dL (> 0.5 mg / dL). Antibiotic treatment was stopped on the seventh day. Abdominal and transfontanelle ultrasonography was performed routinely on the seventh postnatal day and revealed normal findings. Upon the development of pulmonary hypertension on the 20th day, inhaled nitric oxide and sildenafil combination treatment was started. However, the patient was still dependent on intubation. Despite all the supportive treatments on the 24th day of his life, he died due to severe RDS and respiratory failure. The family was referred to genetic counseling. Written consent was obtained from the patient's family to publish this report.

Genetic analyses

Genomic DNA was extracted from peripheral blood cells according to the manufacturer's standard procedure (DNeasy Blood & Tissue Kits - QIAGEN). gDNA was broken into 150–500 bp fragments using a BGI enzymes kit (Segmentase, BGI), The fragments were collected using magnetic beads. Extracted DNA was amplified using a ligation-mediated polymerase chain reaction (LM- PCR). A mean exome coverage of more than 99% was obtained. The sequencing depth was greater than 100× for capture regions. Lastly, the qualified products were sequenced with PE100 + 100 on MGISEQ-2000 (BGI, China).

The raw data obtained was aligned with the Burrows-Wheeler Aligner (BWA) algorithm. SamTools and Picard programs were used to sort and discard polymerase chain reaction duplications. Genome analysis toolkit (GATK) program v. 3.7 was used to invoke variants. ANNOVAR, (a bioinformative tool algorithm, was used to reveal the variants, and the raw data was prepared for analysis.

Variants were filtered based on frequency, inheritance pattern, clinical phenotype and pathogenicity. After that, the c.3677 T> C (p.Leu1226Pro) variant was detected to be homozygous according to the NM_001089.3 transcript in the ABCA3 gene, which was considered to explain the patient's clinical state. Sanger sequencing was performed to verify the variant at proband and parents. There was no significant variant in primary ciliary dyskinesia genes recorded at OMIM database.

Discussion

In our study, we detected the c.3677 T> C (p.Leu1226Pro) variant as homozygous in the ABCA3 gene according to the NM_001089.3 transcript. To our knowledge, this variant is novel. The child had inherited the variant from the unaffected mother and father, both in a heterozygous state (Fig. 1C,1D). The identified genetic variation was not found in any databases,

including the1000Genome projects, esp6500, ExAC, gnomAD, and our in-house database (n=1978 Turkish individuals). At the same time, it is located in a highly conserved residue and GERP score showed the mutated region is conserved among the species. Bioinformatic Prediction analysis with in silico algorithms, such as Mutation Taster, Polyphen-2, CADD, Revel, M-CAP, and SIFT showed this alteration to be pathogenic and disease causing.[4-7]

Missense ABCA3 gene variants are thought to cause disruption in intracellular ABCA3 protein traffic and therefore cause disease.[8] This missense variant is located in the intracellular loop, which deviates in intracellular interactions that probably have a severe effect on protein function. We demonstrated the theoretical 3D structure of protein and mutant amino acid residue by using HOPE. HOPE analysis revealed the alteration can affect the protein function. (https://www3.cmbi.umcn.nl/hope/progress/5e9f34796f82a694596d3fa1/).

Mitsiakos et al.[9] reported a case with a missense variant located in exon 23 of the ABCA3 gene affecting intracellular interactions with total (surfactant) deficiency and fatal early onset. Our patients missense variant located in exon 24 resulted in the substitution of a neutral leucine residue for a non-polar hydrophobic proline residue at position 1226 (p.Leu1226Pro) which can affect intracellular interactions. The variant was submitted to the CLINVAR database (Submission ID: SUB7881781).

In a study by Wambach et al.[10], it was found that the p.R288K and p.R1474W variants caused severe neonatal RDS by reducing the ATPase activity while the c.875A>T (p.Glu292Val) variant was associated with a milder form of the disease. Another study showed that missense variants in the ABCA3 gene could be molecular methods, and the p.Q215K and p.R288K variants led to early death and severe RDS.[1,11] The survival of biallelic ABCA3 variants until adulthood has rarely been observed, and they are shown to present as interstitial lung injury. However, the variant of these patients are of partial, small frame types that do not lead to the loss of total function.[12] In a study in which 185 infants and children with ABCA3 variants were included; frameshift and nonsense variants were classified as 'null', while splice site, insertion, deletion, missense variants were classified as 'other' and all individuals with 'null/null' variants died under the age of 1. However, in this study, it was observed that only 36% of those with a 'null / other' or 'other/other' variation, survived after the age of 1.[13] We detected the missense mutation as homozygous in our patient and our patient died in the neonatal period.

In the differential diagnosis of term newborn RDS, primary pulmonary hypertension, diabetic mother, primary ciliary dyskinesia, congenital surfactant protein deficiencies or hereditary disorders causing congenital surfactant deficiency should be considered.[14] ABCA3 gene variants lead to the deficiency of surfactant proteins B and C. A recent study showed that hemagglutinin-tagged wild type ABCA3 and its variants p.Q215K, p.A1046E, p.K1388N, p.G1421R can be corrected, and all mutant proteins except M760R ABCA3 are rescued in vitro.[15] Although genetic therapies are promising for the future, lethal RDS due to congenital surfactant metabolism disorder still has no effective treatment other than lung transplantation.[14] The DNA sequence analysis of our patient was completed approximately 15 days after his death. The patient was connected to mechanical ventilation and given supportive therapy.

In conclusion, we consider that this case report contributes to the literature on RDS by showing the presence of c.3677 T> C (p.Leu1226Pro), a novel homozygous variant of ABCA3 in our patient. Further studies are needed to explain the in vitro genetic pathways of ABCA3 variants. Such studies are important for future therapeutic studies and the determination of treatment alternatives to lung transplantation.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HBB, YŞ; data collection: HBB; analysis and interpretation of results: HBB, YŞ; draft manuscript preparation: HBB, YŞ. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

None.

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Case Report

A very rare cause of protein losing enteropathy: Gaucher disease

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ABSTRACT

Background. Mesenteric lymphadenopathy is a rare manifestation of Gaucher disease (GD) in children and can be accompanied by protein losing enteropathy (PLE). PLE is a difficult-to-treat complication of GD. To date, only a few pediatric GD cases with PLE and massive mesenteric lymphadenopathies have been reported.

Case. Here, we report a girl with chronic neuronopathic GD, whose disease course was complicated by massive mesenteric lymphadenopathies with resultant protein losing enteropathy despite a regular and appropriate enzyme replacement therapy of 60 IU/kg/biweekly until the development of mesenteric lymphadenopathies and 120 IU/kg/biweekly thereafter.

Conclusions. PLE is a devastating and life threatening complication of GD developing despite long term use of high dose ERT. Clinicians should be alert for this complication particularly in GD patients presenting with progressive abdominal distension, edema, ascites and diarrhea or in patients who have already developed mesenteric lymphadenopathies. Timely diagnosis may allow early intervention with previously suggested surgical or medical treatment options. Although there is no specific and effective treatment, surgical and aggressive medical interventions in addition to ERT were reported to relieve diarrhea and halt progression of mesenteric lymphadenopathies.

Key words: enzyme replacement therapy; lymphadenopathy; lysosomal storage disorder; Gaucher disease.

Gaucher disease (GD) is a rare, autosomal recessive genetic disorder caused by deficiency of lysosomal enzyme, glucocerebrosidase, which results in the accumulation of glucocerebroside-laden macrophages (Gaucher cells) in the reticuloendothelial system.[1] Mesenteric lymphadenopathy is a rare manifestation of GD in children and can be accompanied by protein losing enteropathy (PLE). PLE is a difficult-to-treat complication of GD. To date, only a few pediatric GD cases with PLE and massive mesenteric lymphadenopathies have been reported.[1-4] Here, we report a girl with chronic neuronopathic GD whose disease course was complicated by massive mesenteric lymphadenopathies with resultant protein losing enteropathy despite a regular and appropriate enzyme replacement therapy (ERT).

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Case Report

The patient was the first child of non-consanguineous parents and the diagnosis of GD was made at the age of 18 months. At the time of diagnosis, she had severe hepatosplenomegaly

and pancytopenia (Table I). Her neurological examination was completely normal initially. Gaucher cells were seen on bone marrow aspirate and leukocyte enzyme assay showed decreased beta glucocerebrosidase activity. Diagnosis was confirmed with genetic testing showing a compound heterozygosity (L393V and L444P mutations) in the *GBA* gene. ERT with imiglucerase at an intravenous dose of 60 IU/kg/biweekly was started immediately. A splenectomy was performed because of hypersplenism and intractable pancytopenia at the age of 3.5 years (Table I). On routine follow-up visit at the age of 5 years, physical examination revealed an ill-defined abdominal mass. Ultrasound and computed tomography (CT) of abdomen demonstrated a lobulated, heterogeneous mass reaching a diameter of 8 cm and consisting of multiple conglomerated mesenteric lymphadenopathies with cystic degeneration and calcification (Fig. 1). A simultaneously performed CT of the chest also revealed accompanying axillary, supraclavicular, paratracheal, and hilar lymphadenopathies. A tru-cut biopsy from abdominal mass was performed with a high suspicion of lymphoma. Histopathology of biopsy specimens revealed a completely effaced lymph node structure by diffuse infiltration of Gaucher cells with no evidence of malignancy.[5] Treatment dose of imiglucerase was escalated to 120 IU/kg/biweekly. The patient remained clinically stable without any change in the size of lymph nodes for 7 years following dose escalation. Imiglucerase-specific antibodies were not detected at routine controls. At 10 years of age oculomotor apraxia and hearing loss developed and she was diagnosed as type 3 Gaucher disease.

The patient was hospitalized with the symptoms of chronic watery diarrhea and progressive abdominal distention at the age of 12 years. Physical examination showed ascites and generalized edema in addition to a palpable abdominal mass. Laboratory examinations revealed hypoalbuminemia and hypoproteinemia with electrolyte imbalance and alkalosis. Hepatic synthetic function tests and urine evaluation for albuminuria were normal. Acute phase reactants were negative. There was no evidence of pathogenic bacteria, viruses and parasites in stool examinations. Echocardiographic evaluation was normal. Fecal alpha-1 antitrypsin level was elevated suggesting an excessive protein loss from the intestinal system. Abdominal ultrasound confirmed the presence of moderate ascites and persisting giant conglomerated mesenteric lymphadenopathies (Table I). Gastrointestinal symptoms were relieved partially with supportive treatment including intermittent albumin infusions, fluid and electrolyte replacement, medium chain triglyceride (MCT)-enriched oil supplementation and parenteral

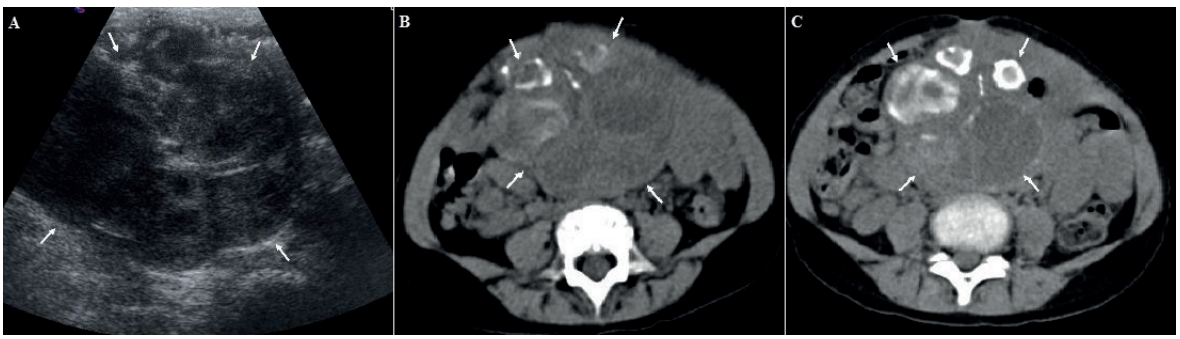

Fig. 1. Ultrasound imaging (A) and CT scan of the abdomen (B) showing a large, lobulated and heterogeneous abdominal mass consisting of conglomerated mesenteric lymphadenopathies some of which exhibiting cystic degeneration and calcification (5 years of age). Follow-up CT scan of the abdomen (C) showing similar radiological findings with areas of cystic breakdown and more extensive calcification in enlarged lymph nodes (7 years of age). No regression in radiological findings was observed despite high dose ERT (120 IU/kg/biweekly).

Table I. Laboratory and imaging parameters of the patient at critical time points.

Parameters	Time point (age of the patient)			
	Diagnosis (18 months)	Splenectomy (3.5 years)	Development of mesenteric lymphadenopathies (5 years)	Development of protein losing enteropathy (12 years)
Complete blood count				
Hemoglobin (g/dl)	5	5.3	12.9	13.3
White cell/lymphocyte count (/mm^3)	5,900/2,900	5,700/3,600	15,900/7,100	12,900/900
Platelet count (/mm^3)	17,000	23,000	487,000	442,000
Serum biochemical profile				
ALT/AST (U/L)	8/37	5/33	19/49	92/81
GGT/ALP (U/L)	17/117	27/85	21/154	35/128
Total / conjugated bilirubin (mg/dl)	0.83/0.39	0.46/0.18	0.12/0.01	0.24/0.05
Albumin / total protein (g/dl)	3.42/5.97	3.5/6.7	4.32/7.19	2.2/4.33
BUN / creatinine (mg/dl)	7.1/0.19	13/0.27	15.1/0.18	8.24/0.18
Sodium / potassium / chloride (mEq/L)	135/3.2/106	139/4.5/101	134/5/100	135/2.5/97
Triglyceride / cholesterol (mg/dl)	163/37	-	134/196	-
Further evaluation	Increased ACE level Gaucher cells in BMA Decreased glucocerebrosidase activity Compound heterozygosity of L393V and L444P in the *GBA* gene	Histopathology of the splenectomy specimen: Compatible with Gaucher disease	Slight neutrophil predominance on the peripheral smear. Normocellular BMA with no evidence of blastic infiltration Histopathology of the tru-cut biopsy specimen: Completely effaced lymph node structure by diffuse infiltration of Gaucher cells	Chemical and microbiological stool examinations: Normal Urinalysis: Normal Serum APR levels: Normal Echocardiography: Normal Increased fecal alpha-1 antitrypsin level
Abdominal imaging	Hepatomegaly, massive splenomegaly, splenic parenchymal heterogeneity, peripheral hypodense splenic lesions suggesting areas of splenic infarction		A large, lobulated and heterogeneous abdominal mass consisting of conglomerated mesenteric lymphadenopathies some of which exhibiting cystic degeneration and calcification, hepatomegaly	
	Insufficient response to ERT in terms of spleen volume		Stable findings over time	

ALT: alanine aminotransferase, ALP: alkaline phosphatase, APR: acute phase reactants, AST: aspartate aminotransferase, BMA: bone marrow aspiration, BUN: blood urea nitrogen, ERT: enzyme replacement therapy, GGT: gamma-glutamyl transpeptidase.

nutrition while continuing high dose ERT. However, the patient died of complications of PLE during outpatient follow-up.

Informed consent was received from the family.

Discussion

Including the present case, only five pediatric cases with GD whose disease course were complicated with PLE have been reported to date (Table II).[1-4] Parallel with our observations, all previously reported cases were on ERT before presenting with this life threatening complication. ERT is a safe and effective option for the treatment of visceral and hematological manifestations of GD; however, central nervous system, lymph nodes and lungs have been reported to show insufficient response to this treatment or even unresponsive to ERT compared to other tissues.[6] It is postulated that these organs are relatively inaccessible to intravenously administered enzyme. The mechanistic basis for poor permeability of enzyme to lungs and lymph nodes is still a matter of debate. Effacement of normal lymph node architecture with Gaucher cells and resultant histological changes such as fibrosis and calcification may reduce drug access to lymph nodes.

Calcified mesenteric lymphadenopathy seems to be a common finding in GD complicated by PLE. Autopsy of a boy with GD who developed progressive mesenteric lymphadenopathies and PLE showed an acellular material with areas of calcification in place of normal lymphoid tissue.[7] Lymph node calcification was also reported previously in a 16-year-old boy and in a 4.6-year-old boy both of whom had neuronopathic GD and PLE.[2,3] We also observed progressive calcification of mesenteric lymph nodes in our patient (Fig. 1).

Another contributing factor might be the development of antibodies against the enzyme. Neutralizing antibodies might possibly lead to a reduction in treatment efficacy. Lee et al.[2] reported a patient who was tested positive for antibodies to enzyme and developed progressive mesenteric lymphadenopathy with resultant PLE.

Splenectomy might also be considered as a contributing factor in the development of lymphadenopathies as it may lead to deposition of substrates in other reticulaendothelial organs.[8] It is recommended to spare splenectomy for cases of splenic rupture or cases not responding to well conducted ERT with persistent severe cytopenia related to massive splenomegaly, as was the case in the present patient. To our knowledge none of the previously reported patients had a history of splenectomy. Removal of the spleen might have contributed to the enlarged mesenteric lymph nodes in our patient and played an indirect role in the development of PLE.

Lymph node involvement in GD is not restricted to intraabdominal region. Abdelwahab et al.[4] reported eight children with GD type 1 and 3 developing mediastinal lymphadenopathy in addition to mesenteric lymph node enlargement and underscored the importance of awareness of this complication as it can easily be confused with malignancy both clinically and radiologically. Despite being less prominent, mediastinal, axillary and supraclavicular lymphadenopathies were also present in our patient and we had to perform a biopsy to rule out lymphoma.[5]

All patients in the literature including the present case were chronic neuronopathic GD patients carrying L444P mutation in *GBA* gene (Table II). However, it is impossible to make a definitive conclusion regarding phenotype-genotype relationship as there is very limited data. Although PLE has not been reported with non-neuronopathic phenotype, mesenteric lymphadenopathies were also reported to develop in children with type 1 GD.[4]

PLE in a GD patient most likely results from secondary lymphatic obstruction. Enlarged mesenteric lymph nodes block mesenteric lymphatic outflow resulting in

Table II. Clinical characteristics of the present case and previously reported pediatric patients with Gaucher disease and protein losing enteropathy.

	Case 1	Case 2	Case 3	Case 4	Present case
Age (years)	5	4.6	16	5	12
Gender	Male	Male	Male	Male	Female
Genotype	D409H/L444P/A456P/K79N	L444P/L444P	L444P/L444P	L444P/L444P	L393V/L444P
Phenotype	Chronic neuronopathic	Chronic neuronopathic	Chronic neuronopathic	Chronic neuronopathic	Chronic neuronopathic
Preceding ERT	(+)	(+)	(+)	(+)	(+)
Preceding splenectomy (indication)	(-)	(-)	(-)	(-)	(+) (Hypersplenism and intractable pancytopenia)
ERT/SRT	(+) / (-)	(+) / (-)	(+) / (-)	(+) / (-)	(+) / (-)
(Dose at last follow-up)	60 IU/kg/weekly	120 IU/kg/biweekly	100 IU/kg/biweekly / 400 mg/day	60 IU/kg/biweekly	120 IU/kg/biweekly
Accompanying manifestations	Thrombophilia, ascites, peripheral edema, mediastinal lymphadenopathy, parenchymal and interstitial lung disease, *A. israelii* and *C. difficile* infections	Retroperitoneal, inguinal, and paraesophageal lymphadenopathies, abdominal distention and respiratory difficulty	Weight loss, abdominal pain	Pelvic abdominal masses with cystic breakdown and multiple flecks of calcification, hilar and mediastinal lymphadenopathies, ascites	Axillary, supraclavicular, paratracheal and hilar lymphadenopathies, progressive abdominal distention, ascites, peripheral edema

ERT: enzyme replacement therapy, MCT: medium chain triglyceride, PLE: protein losing enteropathy, SRT: substrate reduction therapy.

Table II. Continued.

	Case 1	Case 2	Case 3	Case 4	Present case
Mesenteric lymphadenopathy	(+)	(+)	(+)	(+)	(+)
Treatment for PLE	-ERT: Increase in dosing frequency - Symptomatic management with MCT containing diet, fluid and electrolyte replacement, albumin supplementation, total parenteral nutrition was needed later in disease course	-ERT: Dose escalation -Intermittent albumin infusions, high-protein diet with supplementation of MCT	-ERT: Dose escalation -SRT: Continued as it was -Low fat diet supplemented with 30 ml of MCT oil 3 times a day, along with 9 mg daily oral budesonide and total parenteral nutrition (overnight, 6 nights a week)	-ERT: No change -Albumin infusions	-ERT: Dose escalation -Intermittent albumin infusions, fluid and electrolyte replacement and daily MCT-enriched oil supplementation
Lymphadenectomy	(-)	(+) Serial partial excisions of lymph nodes with adhesiolysis	(-)	(-)	(-)
Treatment response	No reduction in lymphadenopathies, there is progression of sequelae of PLE, succumbed to death at 12.5 years due to continued progression of his many comorbidities	Diarrhea was resolved	Decrease in number and density of lymph nodes, diarrhea and abdominal pain were resolved, patient gained weight and serum albumin normalized	Died of an unexplained cause	No reduction in lymphadenopathies, stable with supportive care but died of an unexplained cause during outpatient follow-up
Reference	Burrow et al. (2, 8)	Lee et al. (3)	Mhanni et al. (4)	Abdelwahab et al. (5)	Present report

ERT: enzyme replacement therapy, MCT: medium chain triglyceride, PLE: protein losing enteropathy, SRT: substrate reduction therapy.

increased lymphatic pressure and intestinal lymphangiectasia which eventually leads to PLE. Development of PLE seems to deteriorate disease course by causing further complications. Difficult-to-control thrombophilia with multiple venous thromboses, need for recurrent hospitalizations due to the severe diarrhea complicating with abdominal distention and respiratory difficulty, and growth retardation with significant weight loss were reported previously in these patients.[1-3] Our patient was clinically stable until development of PLE even in the time period between detection of giant lymph nodes and initiation of PLE associated diarrhea. After that, she was hospitalized several times for supportive treatment before she succumbed to death.

There is no specific and effective treatment for mesenteric lymphadenopathies and PLE in GD. Neither increasing intensity of ERT nor switching to a combination therapy with substrate reduction therapy seems to be effective.[3] Surgical excision of lymph nodes was recommended by Lee et al.[2] They reported that diarrhea was resolved with serial partial excision of lymph nodes with adhesiolysis and concluded that surgical intervention might help control the serious complication of PLE.[2] However, it should be considered if removal of enlarged lymph nodes will result in deposition of Gaucher cells in other organs as it does in splenectomy.

Another successful treatment approach was reported by Mhanni et al.[3] In addition to increasing ERT dose in combination therapy, they provided a low fat diet supplemented with MCT oil, along with oral budesonide and total parenteral nutrition. They achieved clinical, laboratory and even radiological improvement with this treatment regimen.[3] Although we followed a similar management approach except corticosteroid use, our patient failed to respond to supportive treatment and showed an intractable disease course. The patient Mhanni et al. reported might have benefited from budesonide therapy. Budesonide has been used in PLE developing after Fontan procedure as a common and devastating complication. A recent meta-analysis showed that it is effective in ameliorating serum albumin levels in this setting.[9]

Long-term complications of GD which were not observed before the availability of ERT are becoming more apparent with increased life expectancy due to ERT. All five reported GD cases with PLE belong to post-ERT era. Mesenteric lymphadenopathy is not always accompanied by PLE highlighting non-predictability of the development of this complication. As we still do not know in whom and when these complications will develop, clinicians should be alert to this life threatening complication particularly in GD patients presenting with progressive abdominal distension, edema, ascites, and diarrhea or in patients who have already developed mesenteric lymphadenopathies. Timely diagnosis may allow early intervention with previously suggested surgical or medical treatment options. Further studies are warranted to understand why lymphadenopathy and PLE develop despite ERT in some patients. Alternative treatment modalities should be investigated for treatment of poorly accessible organs including central nervous system, lungs and lymph nodes which are involved in GD.

Author contribution

The authors confirm contribution to the paper as follows: Conception and design, data collection, interpretation of data, draft manuscript preparation: MAG, EG, HHG; preparation of tables and figures: EG, HHG; drafting and revising the article: HD, HO, INST, SG, AY; critical revision: AY. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Case Report

Severe isolated sulfide oxidase deficiency with a novel mutation

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ABSTRACT

Background. Isolated sulfite oxidase deficiency (ISOD), caused by mutations in *SUOX* gene, is an autosomal recessive disease manifesting with early onset seizures, developmental delay, microcephaly, and spasticity. It mimics hypoxic-ischemic encephalopathy (HIE) in the neonatal period and is characterized by progressive severe neurological impairment due to accumulation of toxic metabolites.

Case. This report presents a late diagnosed male patient with ISOD manifesting with neonatal-onset seizures, developmental delay, microcephaly, and spastic quadriplegia. Brain magnetic resonance imaging of the patient showed bilateral subcortical multi-cystic encephalomalacia involving bilateral parieto-occipital regions. A novel homozygous c.590_595delAGCCTC in-frame deletion in *SUOX* gene was identified in the patient, while both parents were heterozygous for that mutation.

Conclusion. The mutation identified in our patient causes severe ISOD. Early diagnosis of ISOD is essential for accurate genetic counseling and achieving prenatal diagnosis. Screening for urinary sulfite in patients with neonatal or early infantile onset seizures, developmental delay, microcephaly and cystic encephalomalacia in neuroimaging mimicking HIE helps in early diagnosis.

Key words: sulfite oxidase, isolated sulfite oxidase deficiency, ISOD, SUOX, seizure.

Sulfite oxidase (SO) is a mitochondrial, molybdenum-cofactor dependent enzyme encoded by *SUOX* gene. It catalyzes the oxidation of toxic sulfite to non-toxic sulfate which is excreted into the urine. SO also participates in the electron transfer from sulfites by means of cytochrome-c in mitochondria. Isolated sulfite oxidase deficiency (ISOD; MIM #272300) is an autosomal recessive disorder characterized by severe neurological impairment caused by mutations in the *SUOX* gene.[1] Eighteen different mutations in *SUOX* gene have been described in about 50 patients to date.[1-4] Most of the patients reported had severe neurological findings, mainly related with sulfite toxicity, presenting in the neonatal period. The severity of the disease depends on the mutation and associated residual enzyme activity.[1,2] Herein, we present a patient with severe ISOD presenting with intractable neonatal-onset seizures, feeding difficulty, microcephaly, spastic quadriplegia and brain magnetic resonance imaging (MRI) findings compatible with hypoxic ischemic encephalopathy (HIE) due to a novel six-base-pair deletion mutation (c.590_595delAGCCTC) in the *SUOX* gene at the age of six.

Case Report

This study has been approved by the appropriate ethics committee and the informed consent was taken from the parents. The patient is the first born child with a birth weight of 3220 grams at term after prolonged labor by induced vaginal delivery to first-

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degree consanguineous parents. His prenatal history was unremarkable. We learned that he had generalized cyanosis resolved within minutes with oxygen of %40 at birth. He had been presented with agitation, excessive crying, poor suckling, myoclonic jerks in the arms and pedalling movements of the legs in the second day of life. Seizure control had been achieved with phenobarbital treatment in that time. Initial assessments for inborn errors of metabolism (IEM), thrombophilic conditions, and screening for intrauterine infections had been found to be normal. Brain MRI of the patient on the 3rd day of life showed hemorrhagic changes in the bilateral periventricular areas. Brain MRI at one month of age revealed features of hypoxic-ischemic brain injury and diffuse subcortical cystic encephalomalacia, especially in the parieto-occipital lobes, and corpus callosum hypoplasia. The patient was first diagnosed as HIE due to perinatal asphyxia. In the follow-up, the patient hasddeveloped intractable seizures, progressive microcephaly, axial hypotonia, severe spasticity, and recurrent respiratory infections.

At one year of age, the patient was admitted to our hospital with severe respiratory tract infection. He was taking levetiracetam, lamotrigine, phenobarbital and clonazepam for resistant epilepsy. First-degree consanguinity, prominent progressive microcephaly (43 cm; <3rd percentile), axial hypotonia, severe spastic quadriplegia, severe seizures starting in the neonatal period, inconclusive metabolic investigations including normal serum uric acid levels (3.4 mg/dl) and multicystic changes in brain MRI (Fig. 1) further raised the suspicion of ISOD. Elevated levels of sulfide [80 mg/L (normal <15)] and thiosulfate [980 micromol/gram creatinine (normal <400)] were detected in the urine. Mutation analysis of *SUOX* gene in the patient revealed a homozygous c.590_595delAGCCTC variation, while both parents were heterozygous for that mutation.

Discussion

The incidence of neonatal seizure in full-term newborns is approximately 1-3.5/1000 live births. Inborn errors of metabolism account for approximately 1-3% of neonatal seizure cases, while HIE is the most common cause of neonatal seizures accounting for about two-thirds of cases.[5,6] Among them, the inherited inability of conversion of sulfite to sulfate caused by SO deficiency has clinical presentation resembling those in neonatal HIE. SO deficiency may be caused by ISOD or defects in the biosynthesis of molybdenum cofactor which is an essential component of three molybdenum-requiring enzymes including SO.[1] ISOD and molybdenum cofactor deficiency (MoCoD) have similar clinical manifestations characterized by neonatal-onset severe and progressive neurologic deterioration and intractable seizures, progressive microcephaly and feeding difficulties. The main neuroimaging finding of ISOD and MoCoD is multicystic encephalomalacia, which resembles HIE. The progressive neurologic deterioration is mainly related with toxic sulfite accumulation in tissues, which mainly affects the central nervous system in both conditions.[7] Increased urinary sulfite excretion that can be detected by dipstick testing in a very fresh urine sample, is a practical approach in identifying patients with ISOD and MoCoD from HIE. Differential diagnosis of two conditions can be easily achieved by measuring plasma uric acid level, which is normal in ISOD. Other laboratory findings include increased thiosulfate in urine, increased S-sulfocysteine and taurine in plasma and urine; normal plasma methionine, lowered plasma cysteine and homocysteine in ISOD.[1-4] Our patient's amino acid analysis including homocysteine was found to be normal.

ISOD was suspected in our patient with findings of intractable seizures, microcephaly, axial hypotonia, spastic quadriplegia, bilateral subcortical multi-cystic encephalomalacia

on brain MRI, normal plasma uric acid level, elevated urine sulfite and thiosulfate levels. The identification of homozygous c.590_595delAGCCTC mutation in the patient, and heterozygosity of the mutation in the parents further confirmed the diagnosis of ISOD. The clinical spectrum of ISOD varies from an early-onset severe disease (classical ISOD) to a late-onset mild disease. Classical ISOD is characterized by neonatal-onset intractable seizures, feeding difficulties, rapidly progressive encephalopathy presenting as abnormal tonus followed by progressive microcephaly, profound intellectual disability, lens subluxation/dislocation and even death at an early age. ISOD was reported first in 1967 and the majority of approximately 50 patients reported to date presented with classical ISOD.[1-4] Our patient presented with intractable seizures, axial hypotonia and feeding difficulty in the neonatal period which is compatible with classical ISOD. Cerebral-cerebellar atrophy, ventricular distension, thin corpus callosum, abnormal intensities in globus pallidi, diffuse subcortical cystic encephalomalacia seen in our patient are compatible with previous imaging findings reported in ISOD patients (Fig. 1).

SUOX gene encodes SO enzyme, a 545 amino acid protein, localized to the mitochondrial intermembrane and catalyzes the oxidation of sulfur-containing amino acids.[1,3] The missense mutations in the transit peptide and cytochrome b5 heme-binding domain of the enzyme cause to milder presentations of ISOD; while all kinds of mutations of the other domains (missense, nonsense, or frameshift) are associated with

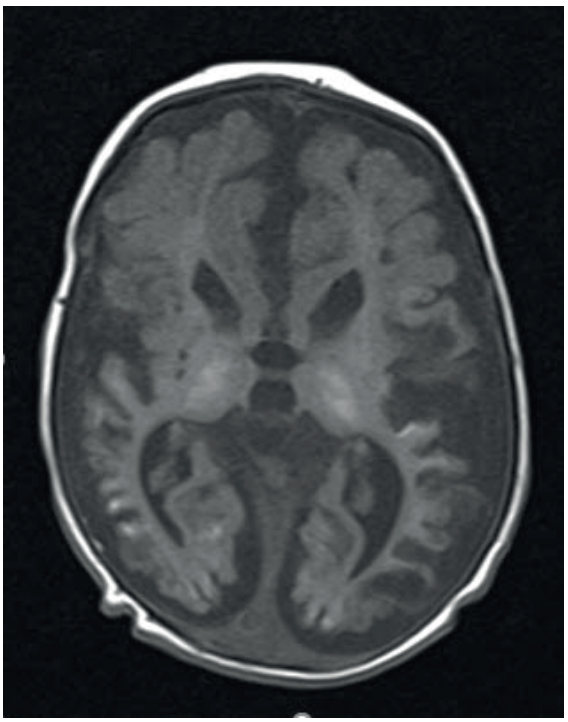

Fig. 1. Coronal T1-weighted axial magnetic resonance imaging showing bilateral subcortical multi-cystic encephalomalacia mainly in the parieto-occipital regions with bilateral hyperintensities in the corticomedullary junction and thalamus.

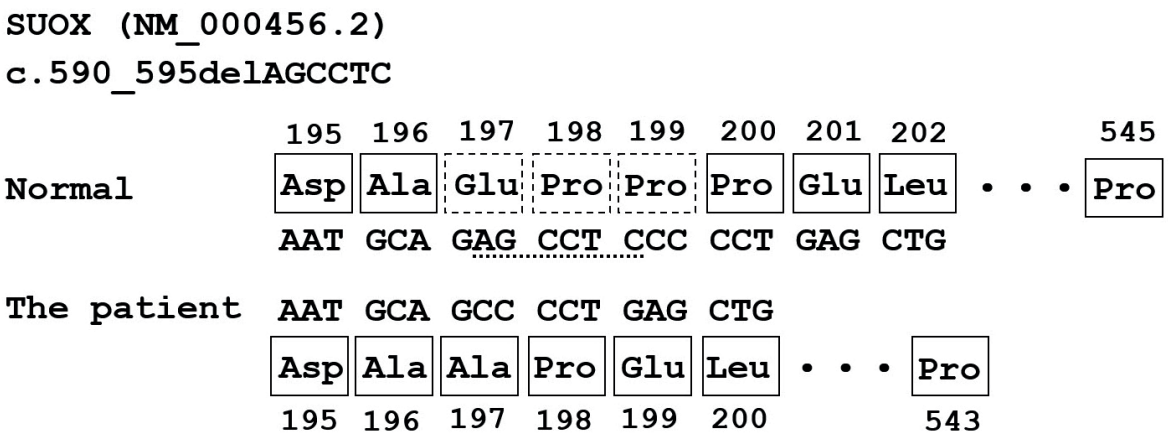

Fig. 2. *SUOX* gene encodes sulfide oxidase enzyme which is constructed by 545 amino acids. The mutation detected in our patient results in the substitution of glutamic acid with alanine at the 197 locus and the deletion of two proline amino acids.

severe clinical manifestations with onset within the first year of life.[1] The mutation identified in our patient is an in-frame deletion that does not disturb the reading frame of the sequence. However, any in-frame variant in *SUOX* gene has not been described before as a disease-causing mutation, the reported variant resulted in the substitution of glutamic acid with alanine at the 197 position and the deletion of two proline amino acids at the 198 and 199 positions (Fig. 2). The deletion described in our patient is between the hinge and molybdopterin-binding domains and close to the molybdopterin-binding domain. The c.599C>T (p.Pro200Leu) variant next to the amino acids changed in our patient has been described as likely pathogenic in a patient with delayed speech, language and global development, central hypotonia, synophry, low anterior hairline.[8] The SO enzyme has one conserved three consecutive proline (tripled PPP) sequence at the 198-200 amino acids. The c.590_595delAGCCTC mutation causes the missing of the tripled PPP sequence. The role of a single PPP motif is not known exactly, but human proteins richest in polyproline motifs are involved in DNA binding and transcription, actin cytoskeleton, RNA processing, splicing and metabolism, and signaling/ligand/receptor.[9] Mitochondrial valyl-tRNA synthetase has also one conserved tripled PPP sequence. The tripled PPP sequence of valyl-tRNA synthetase is critical for $tRNA^{Val}$ charging and editing activities and mutations within the proline triplet of valyl-tRNA synthetase reduce growth and viability of E. coli.[10] We could not perform SO enzyme analysis or functional studies on the c.590_595delAGCCTC mutation, but we suggest that the mutation described in our patient may disturb the binding of molybdenum to the enzyme and/or the missing of the tripled PPP sequence on SO may cause the loss of the enzyme activity.

The clinical and radiological similarity to HIE, the presence of a normal uric acid levels in ISOD, limited awareness of the condition, and misbelief of needing sophisticated tests to reach a presumptive diagnosis of IEM delay the diagnosis of ISOD by using routine screening tests. Early diagnosis is essential in treatable IEM to prevent permanent damage. No curative treatment exists for the underlying metabolic defect in ISOD, but early diagnosis of an untreatable inherited disease is also essential for accurate genetic counseling and achieving prenatal diagnosis.[1] Clinical and radiological findings, elevated urine sulfite and thiosulfate levels let the diagnosis at six year of age in our patient. Prenatal diagnosis was not done in the next pregnancy of the mother because of no precise diagnosis of the index patient at that time, fortunately the sibling was not affected. She is now four years old with normal growth and development, and was found heterozygous for the mutation.

We report here a late diagnosed patient with a novel mutation in *SUOX* gene causing classical ISOD. We suggest that the c.590_595delAGCCTC mutation described here may cause the loss of the SO enzyme activity by preventing the binding of molybdenum to the enzyme and/or the effects of the missed tripled PPP sequence on the enzyme. Further functional studies are needed to understand the effects of that mutation on the enzyme.

Early diagnosis of ISOD requires high index of clinical suspicion. To diagnose ISOD, sulfite levels in a very fresh urine sample should be checked carefully in every patient with neonatal or early infantile onset seizures, developmental delay, microcephaly and cystic encephalomalacia in neuroimaging mimicking HIE. Genetic counselling for future pregnancies is crucial to reduce the incidence of inherited diseases.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HİA, TS, İZE; data collection: ME, NY, EPÖ, BÇ; analysis and interpretation of results: HİA, BÇ,

TS, İZE; draft manuscript preparation: ME, NY, EPÖ, HİA. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare no competing interests.

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Case Report

Massive true thymic hyperplasia in a 3-month-old infant: case report and literature review

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ABSTRACT

Background. True thymic hyperplasia (TTH) is characterized as a distinct increase in both size and weight of thymus, which retains normal microscopic and immunohistochemical appearances. Massive true thymic hyperplasia (MTTH) is an extremely rare but significant subtype of TTH in pediatric ages due to its potentially serious consequences. It was reported that the age of cases with MTTH was predominantly between 1 and 15 years, while those before 1 year rarely occurred. By presenting the diagnosis and treatment process of our case as well as reviewing the related literature, we aimed to analyze the clinical characteristics of MTTH for patients younger than 1 year.

Case. A 3-month-old male infant was admitted to our department with a chief complaint of gradually increasing polypnea over 9 days, whose preoperative imaging examination showed a large intrathoracic soft tissue shadow predominantly on the right side. The percutaneous fine-needle biopsy guided by ultrasonography was performed to identify its diagnosis. However, proliferating lymphocytes and Hassall's corpuscles were seen microscopically in the biopsy tissues, which were immunohistochemically positive for CD3, CD19, CD20, CD99, TdT, PCK and Ki67 (>90%). Due to the aggravating symptoms, a second operation with total thymectomy was carried out successfully for this infant, which confirmed the diagnosis of TTH again by both morphological study and immunohistochemical staining from the surgical specimen.

Conclusions. By reviewing the literature, there were only 10 cases with MTTH reported between 1975 and 2020 for children aged <1 year of life, together with our present one. In MTTH patient's sex had an obviously male predominance (70%). Nine out of 10 presented initial symptoms or signs related to respiratory system and 6 patients showed respiratory distress. All patients were successfully treated by surgical thymectomy without any postoperative complications. The prognosis of MTTH was very successful.

Key words: true thymic hyperplasia, massive true thymic hyperplasia, infant, biopsy, thymectomy.

The thymus is an important immune organ for children, whose size and weight decreases with age.[1,2] True thymic hyperplasia (TTH) is characterized as a distinct increase in both size and weight of thymus, which retains normal microscopic and immunohistochemical appearances.[3-5] Massive true thymic hyperplasia (MTTH) is an extremely rare but significant subtype of TTH in pediatric ages due to its potentially serious consequences, which is rather difficult to be distinguished from anterior mediastinal tumors such as thymic lymphoma and thymoma.[6] MTTH rarely occurs in patients younger than 1 year.[1,2] Here, we report a 3-month-old infant who was clinically diagnosed as MTTH. We also reviewed the clinical features of MTTH for children aged <1 year of life in the literature.

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Case Report

A 3-month-old male infant was admitted to our department on July 20th 2019, with a chief

complaint of gradually increscent polypnea over 9 days, especially after crying or aggravating activities. Without any specific past medical history or family history, the boy weighed 7 Kg on admission, with a height of 65 cm. Physical examinations revealed absent breath sounds in the right hemithorax. Laboratory investigations demonstrated a slight leukocyte rise of 13.29 × 10^9/L with 81.3% lymphocytes, while tumor markers such as alpha fetoprotein and neuronspecific enolase were in the normal range. Chest X-ray showed a large intrathoracic soft tissue shadow predominantly on the right side, occupying most of the right hemithorax (Fig. 1a).

A percutaneous fine-needle biopsy guided by color Doppler ultrasonography (US) was performed for this infant on July 24th 2019, in which 6 pieces of tissues from different directions inside the lesion were obtained to identify diagnosis. This patient was transferred to a pediatric medical ward on July 26th 2019 and prepared to receive targeted antitumor chemotherapy based on biopsy results. Unexpectedly, proliferating lymphocytes and Hassall's corpuscles were seen microscopically in the biopsy tissues, which were immunohistochemically positive for CD3, CD19, CD20, CD99, TdT, PCK and Ki67 (>90%) (Fig. 2a-2h). The fluorescence in situ hybridization (FISH) of EBER 1/2 was also negative and gene rearrangement (PCR+GENESCAN) detected no TCRG amplification peak, which further supported the pathological diagnosis of TTH.

On August 27th 2019, this infant was referred to our department again for surgical resection by his family. Thoracic contrast-enhanced computed tomography (CT) was carried out, which showed a solid inhomogeneous predominantly hypodense hypervascularized soft tissue mass in the right thoracic cavity. This lesion was contiguous to the anterior

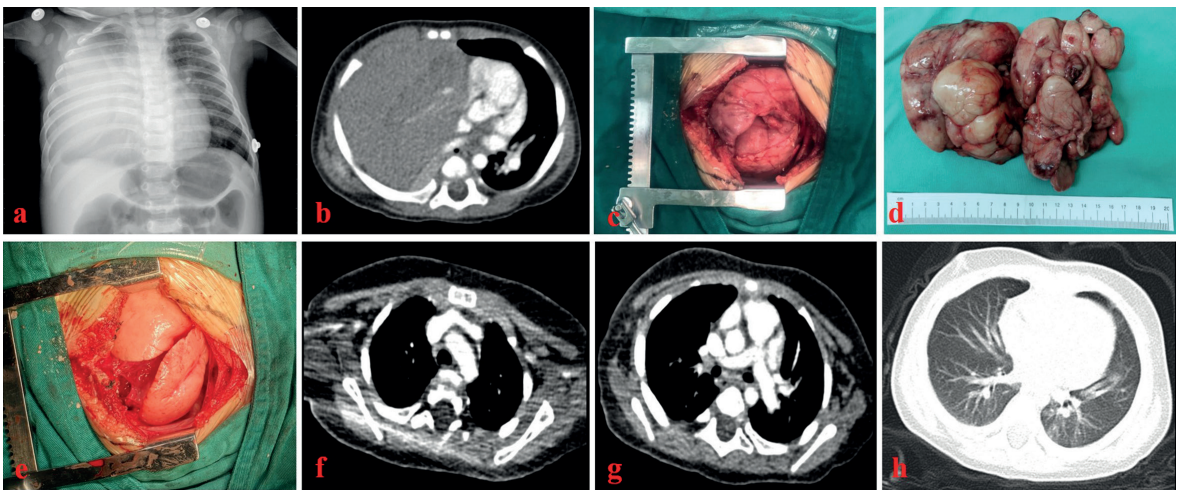

Fig. 1. a. Chest X-ray showed a large intrathoracic soft tissue shadow predominantly on the right side, involving most of the right hemithorax. **b.** Thoracic contrast-enhanced CT detected a solid predominantly hypodense, heterogeneous, hypervascularized soft tissue mass in the right thoracic cavity, significantly compressing the right lung with complete atelectasis and compressing the heart and large blood vessels with mediastinal shift towards the left. **c.** Exploratory thoracotomy found that a giant mass originating from the mediastinum filling most of the right thoracic cavity, pushing the right lung backwards and upwards. **d.** The mass was lobulated, fatty and soft in texture with clear boundary and complete capsule, measuring about 17× 11 × 4 cm and 240g in weight. **e.** After resection of the whole mass, the right lung immediately re-expanded well with normal function. **f.** Four months after second operation, thoracic contrast-enhanced CT detected no recurrence and residual of lesion in the mediastinum or thoracic cavity. **g.** Ten months after second operation, thoracic contrast-enhanced CT also detected no recurrence and residual of lesion in the mediastinum or thoracic cavity. **h.** The patient's right lung expanded well 4 months after his second operation.

mediastinum, significantly compressing the right lung with complete atelectasis and squeezing the heart and large blood vessels with mediastinal shift towards the left, while the normal thymus was not well identified (Fig. 1b).

On August 30th 2019, under general inhalation anesthesia with endotracheal intubation, we performed a right anterolateral exploratory thoracotomy for this infant, in which a giant mass originating from the mediastinum was detected (Fig. 1c). The mass occupied most of the right thoracic cavity, leading to the right lung being visibly pushed backwards and upwards. Unfolding the mediastinal pleura, the mass was lobulated, fatty and soft in texture with clear boundary and complete capsule, which was compatible with that of thymus. During the operation, the fast-frozen pathologic examination from tissues of the mass indicated thymus hyperplasia once again. Finally, thymectomy with removal of homogeneous pale tissue was performed successfully for the infant, in which the whole mass measured about 17 × 11 × 4 cm in volume and 240g in weight (Fig. 1d). After resection, his right lung immediately re-expanded with normal function (Fig. 1e).

The postoperative course of this infant was uneventful. This patient was transferred to a general ward from the intensive care unit on the fifth postoperative day with a normal respiratory pattern and discharged from our hospital on September 8th 2019. The postoperative pathological report from the surgical specimen by both morphological study and immunohistochemical staining confirmed the diagnosis of TTH as well, which was in agreement with the previous results by fine-needle biopsy (Fig. 2). Ten months after the second operation, the boy had no new symptom and recurrence was detected. The chest CT detected no recurrence and residual of lesion in the mediastinum or thoracic cavity (Fig. 1f; Fig. 1g), and his right lung expanded well (Fig. 1h).

Patient has provided informed consent for publication of the case.

Discussion

Located in the anterior mediastinum, the thymus embryologically derives from the ventral pouch of the third and fourth branchial arch. The size and weight of thymus varies with age. For a newborn, the mean weight of thymus is approximately 15 g. For infant at 1 to 3 years,

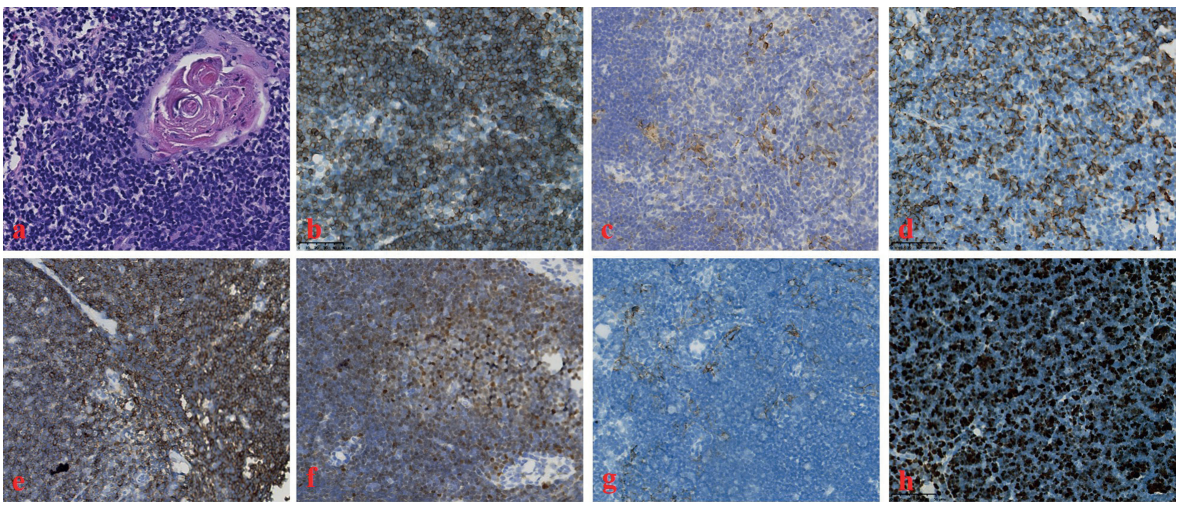

Fig. 2. Pathological analysis and immunohistochemical staining of the lesions by 400-fold optical microscope: **a.** Hematoxylin and eosin stain; **b.** Strong expression of CD3; **c.** Strong expression of CD19; **d.** Strong expression of CD20; **e.** Strong expression of CD99; **f.** Strong expression of TDT; **g.** Strong expression of PCK; **h.** Strong expression of Ki-67 (>90%).

normal thymus weight ranges between 23 and 55 g ± 13 g. Thymus gradually involutes during the first 3 to 4 years of life.[1,2] The thymus of a 3-month-old boy in our study weighed 240 g, notably beyond the average.

Thymus is the primary site of T-cell proliferation and maturation, whose hyperplasia has been usually defined by three classifications.[3,5,7] One is TTH with distinct increase in both size and weight, which retains normal microscopic and immunohistochemical appearances. The second one is lymphoid hyperplasia consisting of lymphoid cells with germinal centers predominates, regardless of the size or weight of thymus. The third one is lymphoepithelial sialadenitis like thymic hyperplasia. The second classification is often associated with myasthenia gravis, while the present case fell into the first category. As in our case (Fig. 2a-2h), TTH is histologically normal thymic tissue, which is composed of lobules with clearly demarcated cortex, medulla, and Hassall corpuscles.[5] Referring to the radiologic findings, TTH usually demonstrated a massively enlarged lesion extending from anterior mediastinum to the right upper and middle pleural cavity with underlying lung collapse without compression of the airway.[8] The present case showed similar imaging results, which was also rather difficult to be distinguished with other thymus masses preoperatively (Fig. 1a, 1b).

As a variant of TTH, MTTH is usually asymptomatic but occasionally produces symptoms related to compression of adjacent structures, such as dyspnea, dysphagia, pulmonary infections, or less commonly as an incidental finding.[6] Although there are no generally accepted definitions of MTTH, the following guidelines have been proposed in the literatures:[5-8] (i) the thymus should be greater than the heart shadow on posterior-anterior chest radiograph; (ii) it should weigh several times the expected weight for the age of the patient; (iii) it should represent more than 2% of the body mass. According to the description of clinical features above, our case met all three criteria.

The clinical manifestations and instrumental examinations of MTTH are atypical, which is often misdiagnosed as either anterior mediastinal tumor-like lesions, such as thymic lymphoma, thymoma, and germ cell tumors, or other causes of thymus enlargement, such as myasthenia gravis.[2,8] Distinguishing these entities requires pathological analysis of issues by fine-needle biopsy or surgical resection. In the present study, mediastinal malignancy was highly suspected for this infant at the beginning, while analysis by percutaneous US-guided fine-needle biopsy was compatible with TTH. This situation might be caused by the misdiagnosis from insufficient or inaccurate biopsy tissues, which was however confirmed once again by the analysis of resected specimens from his second operation. Finally, this boy was managed surgically with complete excision of thymus, as advocated by most authors.[5,8-10]

It has been reported that the age of cases with MTTH is predominantly between 1 and 15 years, while those before 1 year or after 15 years rarely occurs.[1,2,8] To the best of our knowledge, there were only 10 cases with MTTH reported in the literature between 1975 and 2020 in children aged <1 year of life, including our present case (Table I).[2,5,6,11-16] We identified 7 males and 3 females, whose mean age at diagnosis was 5.9 months, ranging from the youngest one of 1 months to the eldest of 11 months. Nine out of 10 presented initial symptoms or signs related to the respiratory system and 6 patients showed respiratory distress. Only 3 patients received glucocorticoids therapy which all failed to shrink the volume of thymus and to relieve the symptoms. Finally, all patients were successfully treated by surgical thymectomy without any postoperative complications. The mean weight and mean largest diameter of resected thymus was 296.4 g (ranging from 200 g to 550 g) and 14 cm (ranging from 8.5 cm to 18 cm), respectively. According to the available prognostic data of each case, 8 patients were thoroughly asymptomatic at follow-up (80%). One patient with Beckwith-Wiedemann Syndrome had a hepatic hemangioma and was

Table I. Clinical features of MTTH in children aged <1 year of life, including present case.

Authors	Date	Sex	Age	Symptoms	Treatments	Thymus size	Prognosis (age)
Katz et al[11]	1977	M	7 mo	Hepatomegaly	Surgical thymectomy	224 g 9 × 8 × 6 cm	Asymptomatic (4 y)
Lamesch et al[12]	1982	F	7 mo	Respiratory distress	1. Steroid (ineffective) 2. Surgical thymectomy	230 g 18 × 11 × 8.5 cm	Asymptomatic (7 y)
Linegar et al[6]	1993	F	2 mo	Respiratory distress	Surgical thymectomy	220 g	Asymptomatic (3 mo)
Lee et al[13]	1996	M	11 mo	Fever and upper respiratory symptoms	Surgical thymectomy	500 g	Asymptomatic[A]
Woywodt et al[14]	1999	M	11 mo	Pneumonia	Surgical thymectomy	550 g 17 × 5 × 3 cm	Asymptomatic (6 y)
Regal et al[2]	2007	M	5 mo	Respiratory distress	Surgical thymectomy	380 g	Asymptomatic (2 y)
Tan et al[5]	2010	F	9 mo	Fever and upper respiratory symptoms	1. Steroid (ineffective) 2. Surgical thymectomy	200 g 17.5 × 11 × 5 cm	Asymptomatic (at the discharge from hospital)
Sayed et al[15]	2016	M	3 mo	Respiratory distress, failure to thrive	Surgical thymectomy	219.7 g 14 × 12 × 5 cm	Hepatic hemangioma and managed conservatively[B]
Weis et al[16]	2017	M	1 mo	Respiratory distress	1. Steroid (ineffective) 2. Surgical thymectomy	200 g 8.5 × 7.5×3.8 cm	Recurrence and 2nd resection, asymptomatic (1 y)[C]
Present case	2020	M	3 mo	Respiratory distress	Surgical thymectomy	240 g 17 × 11 × 4 cm	Asymptomatic (1 y)

[A]: Author did not list time of follow-up.
[B]: Patient was clinically diagnosed as MTTH with Beckwith-Wiedemann syndrome. At follow-up, he presented with hepatic hemangioma which was managed conservatively.
[C]: Patient showed recurrence of MTTH soon after first surgery. A second surgical resection was performed again. Child asymptomatic at 1y follow-up.
MTTH: massive true thymic hyperplasia, M: male, F: female.

managed conservatively. One patient showed a recurrence of MTTH soon after the first surgery and a second surgical resection was performed successfully, who was asymptomatic at 1 year old.

In conclusion, we described a case of 3-month-old infant who was clinically diagnosed with MTTH. Together with our present one, there were only 10 cases with MTTH reported in the literature between 1975 and 2020 in children aged <1 year of life. Patients gender in MTTH had an obviously male predominance. Glucocorticoids might be ineffective, while surgical thymectomy could be perfectly performed for the patients. The prognosis of MTTH was very well.

Author contribution

In this paper, M. Yang and L. Zeng. Contributed equally to this work as co-first authors: ZCXu; contributed as senior author. MY, LZ; extracted

the data and wrote the manuscript together: BX; made the figures: YJi; made the references review: BX, ZCXu; had important intelligent contributions and critically revised the manuscript. All authors in read and approved the final manuscript.

Conflicts of interest

The authors declared no conflict of interest.

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Case Report

Facial colliculus syndrome due to a Herpes simplex virus infection following Herpes labialis

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ABSTRACT

Background. The facial colliculus is an elevated area that is formed by fibers from the motor nucleus of the 7'th cranial nerve as they loop over the abducens nucleus. Clinical signs and symptoms of facial colliculus lesions occur primarily due to injury to the abducens nerve nucleus, the facial nerve fibers around the abducens nucleus, paramedian pontine reticular formation, and the medial longitudinal fasciculus. The etiology of facial colliculus lesions varies by age. While tumors, demyelinating lesions, and viral infections can be involved in young individuals' etiology, vascular ischemia is a common causative factor in older people.

Case. In this paper, we present a case of facial colliculus syndrome due to its rare occurrence in a young patient; who developed the signs and symptoms after a herpes infection.

Conclusion. Facial colliculus syndrome is rare and the treatment is based on etiology.

Key words: facial colliculus syndrome, herpes infection, diplopia.

Horizontal eye movements occur as products of internuclear connections in the brain stem. A conjugated ipsilateral and a contralateral gaze is achieved via the interactions among the ipsilateral abducens nucleus, contralateral oculomotor nucleus, white matter tracts, and the extraocular muscles.[1-3] The pons is the primary center for the control of horizontal eye movements.[1] The paramedian pontine reticular formation (PPRF) is the interconnection structure for horizontal eye movements.[1,4] As a signal from the PPRF is transmitted to the abducens nucleus, it's simultaneously transmitted to the abducens nerve and the contralateral oculomotor nucleus via medial longitudinal fasciculus (MLF).[1,2] This neural pathway explains how different brainstem lesions can lead to various disorders affecting horizontal eye movements.[1] Different syndromes have been defined according to the affected brainstem region. "Eight and a half" syndrome is the rare association of "one and a half" syndrome (conjugated horizontal gaze palsy and internuclear ophthalmoplegia) with ipsilateral fascicular cranial nerve VII palsy.[5,6] First described in detail by Eggenberger in 1998, it is caused by a selective unilateral lesion of pontine tegmentum involving the 6'th cranial nerve nuclei, the internuclear fibers of the ipsilateral medial longitudinal fasciculus, and the adjacent facial colliculus.[7] The facial colliculus is an anatomical name given to the elevated area formed by the nucleus of the abducens nerve and the looping around facial nerve fibers in the intrapontine area. Clinical signs and symptoms of facial colliculus lesions ("Eight and a half" syndrome) occur mainly due to injury to the abducens nerve nucleus, the facial nerve fibers around the abducens nucleus, PPRF, and MLF.[8] In this paper, we present a case with "Eight and a half" syndrome due to a facial colliculus lesion in a young patient who developed the signs and symptoms after a herpes infection; because of its rare occurrence.

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Case Report

A 12-year-old and 9-month-old girl, known to be healthy, developed a herpetic rash on the lip about a week prior to her admission to our clinic, two days after the emergence of the herpetic rash, redness, and swelling developed around the right eye (Fig. 1). Oral acyclovir and topical acyclovir cream were prescribed to the patient at another medical center. The patient presented to our clinic with diplopia starting over the last two days in addition to her other complaints described above. The medical history of the patient was non-specific. The family history revealed consanguinity between her parents. The patient's physical and neurological examination revealed a bilateral inward gaze restriction during conjugate horizontal gaze (Fig. 1), outward gaze restriction in the right eye, nystagmus and double vision with the upward gaze, and bilateral peripheral facial paralysis being more prominently on the right. The examination of other body systems revealed normal findings.

The laboratory examinations revealed average results for the complete blood count, blood biochemistry, protein C, S, antithrombin 3, anticardiolipin antibodies, antinuclear antibodies, anti dsDNA, and homocysteine. HSV-1 IgM serology was negative, and HSV-1 IgG serology was positive in the blood samples. In the direct examination of the lumbar puncture specimen, 416 erythrocytes were counted, no leukocytes were observed. The biochemistry tests of the cerebrospinal fluid (CSF) samples were normal. CSF samples were sent for polymerase chain reaction (PCR) testing for herpes simplex virus (HSV), and the PCR result was positive for HSV-1. The cranial magnetic resonance imaging (MRI) revealed a signal increase in an area of almost 3.5 mm in diameter on the T2A and FLAIR images at the facial colliculus level in the pons posteriorly. The contrast-enhanced MRI showed mild contrast uptake in this region (Fig. 2). The orbital, spinal, and cranial magnetic resonance angiography (arteriovenous) findings were normal. The diagnosis of facial colliculus syndrome secondary to a herpes infection was considered based on the present results. Treatment with intravenous acyclovir (10 mg/kg three times per day) was initiated. Diplopia regressed on the 2nd day of the acyclovir therapy, and facial paralysis reverted on the third day of the treatment. The lateral gaze paralysis regressed on the 10th day of the treatment. After two weeks of intravenous acyclovir treatment, the patient received another week of oral acyclovir treatment. Informed consent was obtained from the family.

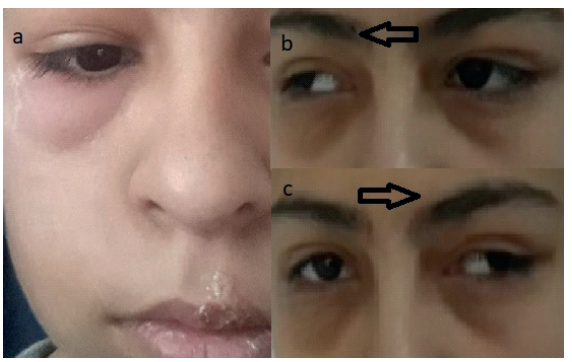

Fig. 1. a. Herpetic eruptions of the case. **b-c.** Bilateral inward gaze restriction during conjugate horizontal gaze.

Discussion

The facial colliculus is the anatomical name given to the elevated area formed by the abducens nerve's nucleus and the surrounding facial nerve fibers in the intrapontine area. Clinical signs and symptoms of facial colliculus lesions occur primarily due to injury to the abducens nerve nucleus, the facial nerve around the abducens nucleus, PPRF, and MLF.[8] "Eight and a half" syndrome is the rare association of "one and a half" syndrome (conjugated horizontal gaze palsy [the "one"] and internuclear ophthalmoplegia [the "half"]), with ipsilateral fascicular cranial nerve VII palsy.[5,6] First described in detail by Eggenberger in 1998, it is caused by a selective unilateral lesion of pontine tegmentum involving the cranial nerve VI nuclei, the internuclear fibers of the ipsilateral medial longitudinal

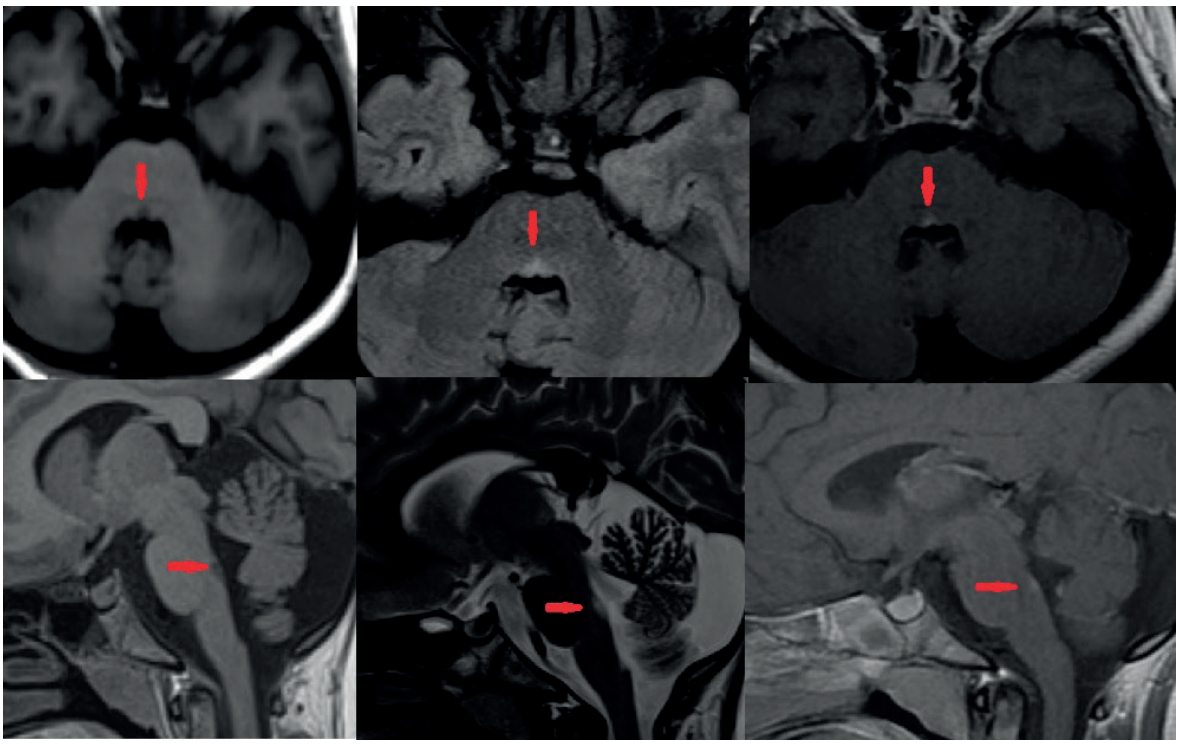

Fig. 2. Cranial MRI: The signal increase in an area of approximately 3.5 mm was observed in the T2A and FLAIR images at the colliculus facialis level at the pons posterior level.

fasciculus, and the adjacent facial colliculus.[7] Facial colliculus lesions involve the facial nerve, resulting in facial paralysis affecting both the face's upper and lower sides. This condition can often be misdiagnosed as Bell's palsy, an idiopathic cause of peripheral facial paralysis. The abducens nerve innervates the ipsilateral lateral rectus muscle and provides an outward gaze on the same side. It also connects with the contralateral oculomotor nucleus via MLF to achieve control of the contralateral eye's conjugated inward gaze. Therefore, any pathology of the abducens nucleus and PPRF impairs the ipsilateral eye's outward gaze and the conjugated inward gaze of the contralateral eye. This situation is different from abducens nerve lesions manifested by limitations in abduction only in the ipsilateral eye. The etiology of facial colliculus lesions varies by age. While tumors, demyelinating lesions, and viral infections can be involved in young individuals' etiology, vascular ischemia is a common causative factor in older people.[8,9] Facial colliculus syndrome is a clinical condition characterized by the peripheral paralysis of the ipsilateral facial nerve resulting from a facial colliculus lesion, the paralysis of the lateral rectus muscle on the same side, and commonly a conjugate gaze palsy associated with the paralysis of the contralateral medial rectus muscle.[8] A conjugate gaze palsy can result from an MLF lesion or the involvement of interneurons traveling toward MLF at the abducens nucleus level. Therefore, a combination of peripheral facial nerve palsy, lateral rectus palsy, and conjugate gaze palsy should suggest a potential insult to the facial colliculus. A cranial MRI is a diagnostic test to observe the precise location of the causative lesion.[8,9] The treatment of facial colliculus syndrome is based on etiology.[10] The presenting complaint of double vision in the patient; the neurological examination findings of bilateral inward gaze restriction during conjugated horizontal gaze; cranial MRI finding of the involvement of the posterior pons suggested the facial colliculus syndrome. The herpetic lesions of the lip starting in the previous week and the persistence of these lesions at the time

of admission to our clinic suggested that the herpes infection could explain the etiology, and this was proved by CSF analysis and serologic studies. Therefore, iv acyclovir therapy was given to the patient for 14 days and oral treatment for seven days. Improvements in the presenting complaints of the patient were observed in the follow-up visits. Facial colliculus syndrome is rare, and the treatment is based on etiology.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MB, RTK, AHÖ, MSO; data collection: MB, RTT, AHÖ, MSO; analysis and interpretation of results: MB, RTT, AHÖ, MSO; draft manuscript preparation: MB, RTT, AHÖ, MSO.

All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Case Report

Intranasal supernumerary tooth in a child: a case report

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ABSTRACT

Background. Ectopic eruption of supernumerary teeth in the nasal cavity is extremely rare, and most cases usually involve the maxillary sinus or are accompanied by dental cysts. It is usually discovered during adulthood.

Case. A 5-year-old patient presented with an intranasal mass and intermittent nasal bleeding that lasted for 1 year. He was taking medication for symptoms of allergic rhinitis, such as nasal obstruction and intermittent epistaxis, without any endoscopic evaluation for 1 year. On nasal endoscopy, a needlelike whitish mass was observed on the left nasal floor. On paranasal sinus computed tomography, it appeared as a pointed high-density mass covered by soft tissue. The intranasal mass which was a supernumerary tooth was completely removed using a pediatric endoscope.

Conclusions. Detection of supernumerary teeth in the nasal cavity of children without symptoms is difficult, and it can be delayed; although the child, in this case, had nonspecific nasal symptoms, supernumerary teeth was not considered in the diagnosis. This case report raises awareness and provides evidence for the clinical characterization and optimal treatment of supernumerary teeth in children.

Key words: supernumerary tooth, nasal cavity, child, nasal obstruction, epistaxis.

Supernumerary teeth refer to deciduous and permanent teeth that occur in addition to the normal number of teeth; the prevalence of supernumerary teeth is approximately 0.1-1% of the general population. The biological and genetic mechanisms underlying supernumerary nasal teeth remain largely unclear. Supernumerary teeth are generally characterized by a specific morphology. The most common form is the conical type, with the tuberculate or barrel-shaped and molariform types occurring less frequently.[1]

Ectopic eruption of supernumerary teeth in the nasal cavity is extremely rare, and most cases usually involve the maxillary sinus or are accompanied by dental cysts. In the oral cavity, the most common supernumerary tooth is the mesiodens, which is found between the upper central incisors. Other rare locations of supernumerary teeth include the mandibular condyle, coronoid process, orbit, and nasal cavity.[2] Although most cases are asymptomatic, supernumerary teeth in the nasal cavity have been reported to cause recurrent nasal bleeding, facial pain, headache, nasal septal abscess, and oroantral fistula.[3] It may also impact normal tooth eruption and lead to the development of malocclusion in children.

Most cases are discovered during adulthood, with most patients showing rhinologic symptoms.[4] However, we report the case of a 5-year-old child with ectopic eruption of a tooth on the nasal floor, which was characterized by nonspecific symptoms.

Case Report

A 5-year-old boy presented to the hospital with an accidentally discovered intranasal mass

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and recurrent nasal bleeding. The patient had allergic rhinitis and was under medication. Both his parents were also under medication for allergic rhinitis. On anterior rhinoscopy and nasal endoscopy, a sharp-pointed white mass enclosed in mucous membrane was discovered on the left nasal floor (Fig. 1). The mass occupied the nasal floor, and its sharp tip protruded toward the inferior turbinate. Other nasal findings were normal.

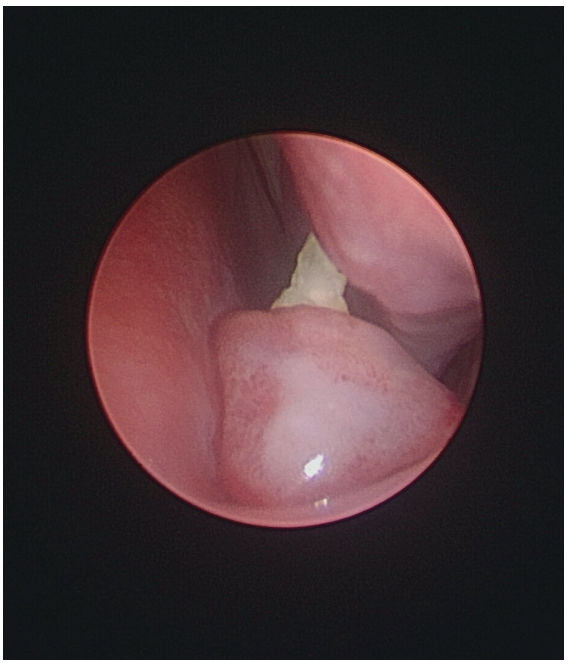

Fig. 1. Endoscopic finding, showing sharp-pointed white mass enclosed in a mucous membrane discovered on the left nasal floor.

Computed tomography (CT) of the paranasal sinus showed a pointed, high-density mass covered by soft tissue; there were no other significant findings (Fig. 2).

The mass was surgically removed under general anesthesia. It was located 3 cm posterior to the nostril and was firmly embedded in the floor of the nasal cavity. We initiated surgery by injecting 2% xylocaine and 1:100,000 adrenaline near the mass. Subsequently, we attempted to extract the mass with dental forceps, but we could not insert it due to the young age of the patient and the small size of the nostrils. Eventually, a complete resection involving the surrounding mucous membrane was performed using a Bovie needle while observing the mass with a pediatric endoscope. There was no osteoclasia of the surrounding tissues or other malignant findings. The excised mass was approximately 10 mm in length, with the crown facing the nasal cavity; the root structure was not clear (Fig. 3). The defect in the left nasal cavity resulting from the excision of the mass was not sutured. Subsequently, a nasal packing material was inserted into the defect, and pressure was applied to control bleeding. No abnormal findings were observed after surgery, and the patient was discharged on postoperative day 2. The nasal congestion and bleeding resolved after excision, and no abnormal findings were observed during the one-year follow-up assessments.

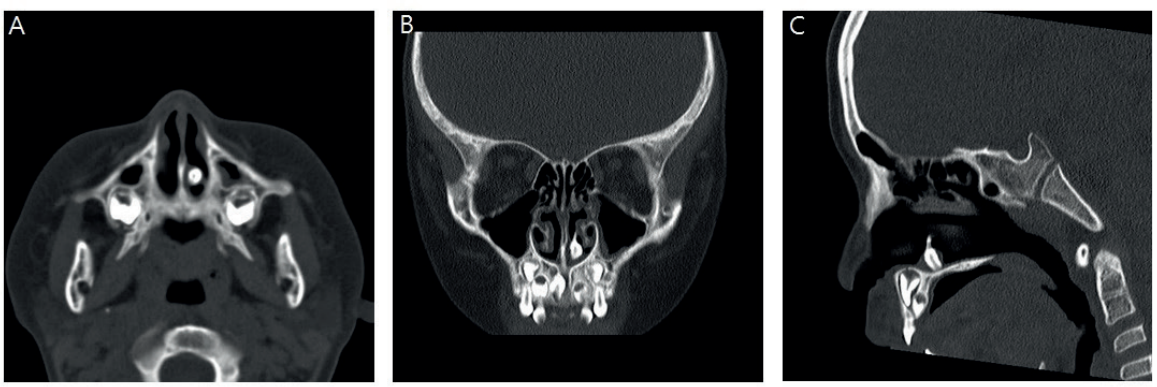

Fig. 2. Computed tomography (CT) of paranasal sinuses: Tooth observed in left nasal cavity floor. A. axial view, B. coronal view, C. sagittal view.

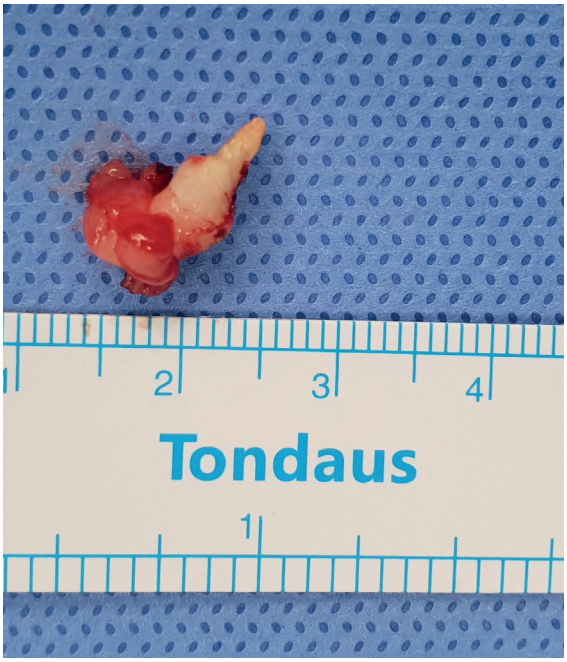

Fig. 3. Supernumerary tooth, showing supernumerary tooth was completely removed.

This study was approved by the Institutional Review Board for Human Studies of the Yeungnam University Medical Center, Daegu, Korea. Permission was obtained from the child's parents for the publication of this case, and informed consent was obtained from the family.

Discussion

The etiology of supernumerary teeth is unknown; however, possible causes include an obstruction at the time of tooth eruption due to crowded dentition, persistent deciduous teeth, and developmental disturbances such as cleft palate, infection, trauma, and cysts.[5] Ectopic teeth in the nasal cavity, as observed in this case, usually occur during tooth development, when the dental sac in the maxilla is inserted into the nasal cavity before palatal closure. Supernumerary teeth in the nasal cavity often present as a firm, fixed, and whitish mass that is often enclosed in granulation or necrotic tissue.

Radiologic testing may be useful for diagnosis, and the presence of a supernumerary tooth can be identified from the shadows observed in Waters' or Caldwell's views. However, in our patient, a complete radiographic series was not performed as the diagnosis was made on endoscopic physical examination and CT. CT may be used as an additional diagnostic tool for such cases. In most cases, the supernumerary tooth is found as a mass with a shadow that has a similar density to the teeth and bone.[6] The tooth may also show permeability, suggesting the central location of the pulp cavity within the tooth. In addition to the confirmation of ectopic tooth growth, CT imaging may be a useful diagnostic tool to determine the presence of dental cysts, examine the relationship with surrounding tissues, and detect hidden ectopic tooth growth. For pediatric patients, in particular, the paranasal sinus CT should not be used for routine diagnosis; however, it may be used in cases of suspected tumors, application from sinusitis, and before sinus surgery to confirm the surgical anatomy. As observed in this case, a supernumerary tooth in the nasal cavity should be differentiated from a foreign body, rhinolith, benign or malignant mass, tuberculosis, mycotic infection, and osteoma in the nasal cavity.

The most common cause of nasal congestion in children is inflammation associated with conditions such as the common cold, paranasal sinusitis, and allergic rhinitis[7]; hence, the diagnosis of supernumerary teeth is often delayed, and the symptoms are neglected. However, when nasal symptoms are present, misdiagnosis can be prevented by suspecting a congenital or iatrogenic mass; a systematic approach involving history taking and physical examination should also be employed. The most effective method to diagnose sinonasal problems is through direct examination with a flexible endoscope. When a patient complains of symptoms, direct examination with an endoscope should be prioritized. Prompt treatment is recommended to prevent complications and structural changes. As observed in our patient, ectopic tooth eruption in children is often accidentally discovered during

endoscopy or CT. Diagnosis is also delayed because patients are often asymptomatic. In this case, the child's family members had severe allergic rhinitis; thus, nasal congestion was attributed to this condition, and the child continuously took medications for allergic rhinitis. However, he visited our institution due to worsening nasal congestion and bleeding, and was subsequently diagnosed on visual inspection. As demonstrated in this case, visual inspection and diagnostic evaluation are important when nasal congestion is present in children.

Ectopic teeth in children are removed to alleviate symptoms and prevent complications such as interference with normal tooth eruption. However, nonerupted teeth without symptoms may be left untreated and periodically observed for progression. Owing to advances in endoscopy, these teeth can be removed with minimal injury to the surrounding tissues or mucosa. Furthermore, ectopic teeth within the maxillary sinus can be easily removed by securing a good field of view with the Caldwell-Luc procedure. In this case, the patient was symptomatic, but with episodes of intermittent bleeding as the tooth had erupted in the nasal floor. We completely removed the tooth using nasal endoscopy, ensuring minimal injury to the surrounding tissue.

As in the reported case, the differential diagnoses of ectopic teeth include foreign bodies, exostoses, and odontomes. Calcifying odontogenic cysts or malignant tumors such as chondrosarcomas and osteosarcomas are also relevant differential diagnoses as they are associated with several nonspecific nasal symptoms.[8,9] In addition, nasal endoscopic evaluation is essential for patients with rhinologic symptoms that are not sufficiently controlled by medical treatment. During physical examination, surgeons and physicians should rule out pathologies using flexible endoscopy, even in patients with a history of allergic rhinitis and chronic rhinitis.

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NHG, CYS; data collection: BCH; analysis and interpretation of results: KYD, BCH, NHG; draft manuscript preparation: NHG. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Mehmet Akif Göktaş, Ersin Gümüş, Hülya Demir, Hayriye Hızarcıoğlu Gülşen, İnci Nur Saltık Temizel, Hasan Özen, Şafak Güçer, Aysel Yüce

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Meriç Ergene, Nuriye Yarar, Elif Perihan Öncel, Taner Sezer, Büşranur Çavdarlı, İsmail Zafer Ecevit, Halil İbrahim Aydın

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