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CONTENTS

ORIGINAL ARTICLE

Prevalence of Asthma Symptoms Among Turkish Cypriot Schoolchildren 413
Ö. Kalaycı, Y. Saraçlar, B.E. Şekerel, G. Adalıoğlu
S. Kuyucu, G. Ergör, H. K. Bozer, A. Tuncer

Safety, Tolerability and Immunogenicity of a Hemophilus Influenzae Type b Vaccine Containing Aluminum Phosphate Adjuvant Administered at 2, 3 and 4 Months of Age 421
G. Kanra, S. Viviani, K. Yurdakök, E. Özmert, S. Yalçın
A. Baldini, B. Mutlu, A. Kara, M. Ceyhan, A. Podda

Neonatal Outcome Following Early Onset Preterm Premature Rupture of the Membranes-A Case Controlled Study 429
B. Acunas, A. Greenough, G. Dimitriou, H. R. Gamsu

Relationship between High Leukocyte Count and Cell Size in Childhood Acute Myeloblastic Leukemia 437
L. Olcay, U. Ertem, H. Okur, İ. Etikan, A. M. Tuncer

Serum Leptin Levels During Childhood and Adolescence: Relationship with Age, Sex, Adiposity and Puberty 447
B. Kırel, N. Doğruel, N. Akgün, F. S. Kılıç
N. Tekin, B. Uçar

Blood Levels of Leukotrienes (LCT_4, D_4, E_4, B_4) and Synthesis of Leukotriene B4 by Peripheral Leukocytes in Children with Acute A and B Hepatitis 457
E. Kasırga, I. Çoker, S. Aydoğdu, R. V. Yağcı
B. Taneli, A. Gousseinov

Autoimmune Hemolytic Anemia with Warm Antibodies in Children: Retrospective Analysis of 51 Cases 467
A. Gürgey, İ. Yenicesu, T. Kanra, Ş. Özsoylu, Ç. Altay
G. Hiçsönmez, S. Yetgin, M. Tuncer, F. Gümrük, M. Çetin

CEA, CA 125 and CA 19-9 Levels in Congenital Gastrointestinal Anomalies 473
A. Baykal-Erkılıç, M. Erkılıç, M. Melikoğlu, A. Aksu

Four Different Hernias are Encountered in the Anterior Part of the Diaphragm 483
A. B. Salman, F. C. Tanyel, M. E. Şenocak, N. Büyükpamukçu

Hemostatic System in Early Respiratory Distress Syndrome: Reduced Fibrinolytic State? 489
M. Yurdakök, Ş. Yiğit

CASE REPORTS

Congenital Microvillous Atrophy in a 4-Month-Old Girl 495
Y. Acar, D. Ertem, E. Özgüven, İ. Okar
R. Ahıskalı, E. Pehlivanoğlu

Neurological Crisis Mimicking Acute Pancreatitis in Tyrosinemia Type I 501
H.S. Kalkanoğlu, T. Coşkun

MRI of Fibromatosis Colli: Case Report 505
H. Çakmakçı, A. Kovanlıkaya

A Case with Acute Leukemia Presenting with Cardiac Tamponade 509
A. Söğüt, K. Yılmaz, N. Yalman, K. Şahin
M. Babalıoğlu, R. Eker-Ömeroğlu, T. Cantez

A Case of Hemophilia A Associated with Hodgkin's Disease 517
A. Koç, A. Varan, M. Büyükpamukçu, A. Gürgey

Cardiac Dysrhythmia that Simulates Seizure Disorder in Two Children 521
G. Turanlı, R. A. Apak

Bronchiectasis due to Ciliary Aplasia in Turner's Syndrome 525
U. Özçelik, M. Tuncel, A. Göçmen, S. Balcı
M. Erbil, L. Yel, N. Kiper

Two Female Siblings from Turkey with Langer Mesomelic Dysplasia (Homozygous Leri-Weill Dyschondrosteosis Syndrome) 531
S. Balcı, Y. Zafer, M. Ünsal

Hepatitis as the Presenting Symptom of Childhood Systemic Lupus Erythematosus 541
R. A. Apak, N. Beşbaş, S. Özdemir, H. Özen
A. Bakkaloğlu, Ü. Saatçi

Psoriasis in a Patient with Neurofibromatosis 545
S. Çelebi, S. Ş. Kılıç, M. Okan

ANNOUNCEMENTS 551
AUTHOR INDEX TO VOLUME 41 557
ARTICLE INDEX TO VOLUME 41 560
KEY WORD INDEX TO VOLUME 41 563

The Turkish Journal of Pediatrics 1999; 41: 413-420 *Original*

PREVALENCE OF ASTHMA SYMPTOMS AMONG TURKISH CYPRIOT SCHOOLCHILDREN*

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SUMMARY: Kalaycı Ö, Saraçlar Y, Şekerel BE, Adalıoğlu G, Kuyucu S, Ergör G, Bozer HK, Tuncer A. (Allergy and Asthma Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Prevalence of asthma symptoms among Turkish Cypriot schoolchildren. Turk J Pediatr 1999; 41: 413-420.

We assessed the prevalence of symptoms suggestive of asthma in Turkish Cypriot schoolchildren and the associated risk factors using a slightly modified version of the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire. The questionnaire and questions regarding risk factors were issued to the parents of 2,822 children aged six to 14 years. The response rate was 89.6 percent. The cumulative and 12-month prevalence of wheezing were 14.7 and 4.8 percent, respectively. The prevalance of physician-diagnosed asthma was 11:4 percent. Family history of atopy was the strongest risk factor for "ever wheezing" (odds ratio [OR] 1.71, 95% confidence interval [CI] 1.52-1.92) and physician-diagnosed asthma (OR 1.71, CI 1.53-1.93). This study demonstrates that symptoms suggestive of asthma are quite common and constitute a major health problem in Northern Cyprus. *Key words: bronchial asthma, ISAAC questionnaire, wheezing.*

Asthma, as the most common disease of childhood[1] poses a mojor social and economic threat, especially in developing countries where health care sources are quite limited. Therefore, as one of the initial steps for planning national health care policies, it is essential to determine the extent, i.e. the prevalence, of asthma within a given country. Although it is one of the most populous islands in the Mediterranean, a survey of the medical literature failed to reveal any data about the prevalence of asthma in Cyprus, which has a Turkish Cypriot population of 201,300 according to the 1996 census.

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Toward enabling international and temporal comparisons of childhood asthma, a standardized written questionnaire, the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire, was developed, and many studies using this protocol have already appeared in the medical literature[2-5]. The purpose of this study was to determine the prevalence of symptoms that are suggestive of asthma in a large sample (n=2,529) of Turkish Cypriot children using a modified version of the ISAAC questionnaire. We also attempted to document the relationship between asthma-like symptoms and some potential risk factors, including age, gender, passive smoking, pet ownership, family history of atopy, and socioeconomic status.

Material and Methods

Study population: The study was conducted in April 1997 in 16 schools in the Turkish Republic of Northern Cyprus. The country is divided into three administrative cities with a total student population of 26,653 attending the first eight grades. Using the stratified sampling method[6] and taking 15 percent, according to the result of a recently completed survey in Ankara[4] as the expected rate of cumulative prevalence, the number of students that would be representative of each region was calculated. Schools were selected randomly until the determined number of students was reached within each region. If, however, the result of the random selection produced a number that exceeded the originally calculated number of students, the former population was included in the survey, thus resulting in a larger sample size than calculated of 2,529 children whose parents returned the questionnaire.

Questionnaire: Turkish translation of the ISAAC protocol, similar to the one used previously in Ankara and Melbourne[4,7], was used. In this questionnaire, ISAAC questions are supplemented with six additional questions (see Appendix). As there is no equivalent for the word "wheeze" in the Turkish language, it was translated as a "whistling sound coming from the chest". This translation was previously validated in the prevalence study in Ankara[4].

On a separate page, questions regarding some potential risk factors for asthma-like symptoms, including passive smoking, pet ownership, family history of atopy, and socio-economic status, were added to the questionnaire. Family history of atopy was considered positive if at least one of the first-degree relatives had physician-diagnosed asthma, allergic rhinoconjunctivitis or atopic dermatitis. In order to determine the socioeconomic level of the family, a previously developed composite index was calculated[4]. The total score had a minimum of 1 and a maximum of 16.

Statistical analysis: Results are expressed as the percentage of positive responses to each question. Ninety-five percent confidence intervals (95% CI)

APPENDIX

Respiratory Symptoms Questionnaire

1. Has your child ever had wheezing or whistling in the chest at any time in the past?
2. Has your child ever had asthma?

If yes to question 1 or question 2, then:

3. In the last 12 months, has your child had a wheezing or asthma attack?
4. In the last 12 months, how frequent were the wheezing attacks?
5. In the last 12 months, has any wheezing attack woken your child at night?
6. In the last 12 months, has any wheezing attack been severe enough to limit speech to only one or two words at a time?

Everyone to answer the following questions:

7. In the last 12 months, has your child sounded wheezy during or after exercise?
8. In the last 12 months, has your child had a dry cough at night? (apart from a cough associated with a cold or chest infection).
9. In the last 12 months, has your child usually brought up any phlegm or mucus from the chest, first thing in the morning?
10. In the last 12 months, has your child woken with a feeling of tightness in the chest first thing in the morning?
11. In the last 12 months, has your child had tightness in the chest or become short of breath when near animals, feathers or dust?
12. In the last 12 months, has your child been treated at any time with any of the following medications? (Ventolin, Salbutol, Salbulin, Bricanyl, Pulmicort, Flixotide, Becloforte, Becotide, Intal, Kromolin, Zaditen, Kofilin, Teo kap, Theo-dur, Aminokardol)

At any time in the past:

13. Has your child ever suffered from bronchitis?
14. Has your child ever suffered from wheezing with bronchitis or with a cold?

were calculated using simple random sample methods. Prevalence of wheezing, asthma and risk factors are calculated with 95% CI. Logistic regression analysis was done using backward elimination method. All analyses were done using the SPSS 6.0 package program.

Results

Of the 2,822 questionnaires that were issued, 2,529 were returned, with a response rate of 89.6 percent. Age and gender distribution of the study population are summarized in Table I. The age range was six to 14 years. The number of boys and girls were almost equal with a girl/boy ratio of 0.97.

Table I: Age and Gender Distribution of the Study Population

Age (years)	Boys n (% of boys)	Girls n (% of girls)	Total n (% of total)
6	134 (10.4)	128 (10.3)	262 (10.4)
7	138 (10.8)	103 (8.3)	241 (9.5)
8	104 (8.1)	109 (8.7)	213 (8.4)
9	141 (11.0)	135 (10.8)	276 (10.9)
10	132 (10.3)	126 (10.1)	258 (10.2)
11	139 (10.8)	130 (10.4)	269 (10.6)
12	162 (12.6)	185 (14.8)	347 (13.7)
13	189 (14.7)	182 (14.6)	371 (14.7)
14	144 (11.2)	148 (11.9)	292 (11.6)
Total	1283 (50.7)	1246 (49.3)	2529 (100.0)

Cumulative and 12-month prevalence rates of asthma symptoms and risk factors reported by the parents are summarized in Tables II and III. Family history of atopy appears to be the strongest risk factor both for "ever wheezing" (OR=1.71, 95% CI=1.52-1.92) and physician-diagnosed asthma (OR=1.71, 95% CI=1.53-1.93). Interestingly, it is not a risk factor for "wheezing in the past 12 months" (current wheezing). Increasing age seems to be associated with a slightly increased risk for physician-diagnosed asthma (OR=1.08, 95% CI=1.05-1.14). Female gender was associated with a lower risk of "ever wheezing" and physician-diagnosed asthma (OR=0.85 and 95% CI=0.76-0.96 for both). Parental smoking, socioeconomic level and pet (cat, dog and bird) ownership did not appear to be risk factors for asthma-related symptoms for Turkish children in Northern Cyprus.

Utilization of this validated standardized questionnaire enables regional and international comparisons to be made. Two studies conducted with expanded versions of the ISAAC questionnaire in Ankara[4] and İstanbul[5], Turkey, have shown variable prevalence rates compared to those obtained in the present study. Although cumalative wheezing and current wheezing prevalences in Northern Cyprus (14.7 and 4.8%, respectively) and Ankara (14.4 and 4.7%, respectively) were similar, the values obtained in the İstanbul study (15.1 and 8.2%, respectively) were rather different. However, prevalence of physician-diagnosed asthma in Northern Cyprus (11.4%) was higher than in İstanbul (9.8%) and Ankara (8.1%). Additionally, bronchodilator use was more prevalent in Cyprus than in Ankara (11.1 vs 5.0%). These results suggest that wheezing is more readily recognized as a sign of bronchial asthma, and that bronchodilators are more

Table II: Responses of Parents Regarding Their Children's Respiratory Symptoms*

Symptoms	Boys (n=1283)	Girls (n=1249)	Total (n=2529
Ever whezed	16.5 (14.5-18.5)	12.9 (11.0-14.8)	14.7 (13.3-16.1)
Ever asthma	12.8 (11.0-14.6)	10.00 (8.3-117)	11.4 (10.2-12.6)
Symptoms in past 12 months			
Wheezing	5.5 (4.3-6.8)	4.1 (3.0-5.2)	4.8 (4.0-5.6)
Number of episodes:			
<4	4.1 (3.0-5.1)	2.6 (1.7-3.4)	3 3 (2.6-4.0)
4-12	0.5 (0.1-0.8)	0.9 (0.4-1.4)	0.7 (0.4-1.0)
>12	0.6 (0.2-1.0)	0.3 (0.0-0.6)	0.4 (0.2-0.7)
Sleep disturbance	2.8 (1.9-3.7)	2.6 (1.7-3.5)	2.7 (2.1-3.3)
Severe episode	1.8 (1.1-2.5)	1.8 (1.1-2.5)	1.8 (1.3-2.3)
Exercise-induced wheezing	6.1 (4.8-7.7)	5.6 (4.3-6.9)	5.9 (5.0-6.8)
Night cough	23.9 (21.6-26.2)	24.6 (22.2-27.0)	24.4 (22.5-25.9)
Morning tightness	2.9 (2.0-3.8)	3.0 (2.1-4.0)	3.0 (2.3-3.7)
Morning mucus	17.7 (15.6-19.8)	19.5 (17.3-21.7)	18.6 (17.1-20.1)
Wheezing with allergens	2.8 (1.9-3.7)	3.9 (2.8-5.0)	3.3 (2.6-4.0)
Use of bronchodilators	12.1 (10.3-13.9)	10.0 (8.3-11.7)	11.1 (9.9-12.3)
Bronchitis			
Ever	26.1 (23.7-28.5)	21.9 (19.6-24.2)	24.0 (22.3-25.7)
Wheezing with cold or bronchitis	17.5 (15.4-19.6)	15.2 (13.2-17.2)	16.4 (15.0-17.8)

* Values are the percent (95% confidence intervals) of boys/girls and total number of children completing a questionnaire and responding postively.

Table I: Age and Gender Distribution of the Study Population

	Girls n (% of girls)	Boys n (% of boys)	Total n (% of total)
Family atopy	430 (34.5)	411 (32.0)	841 (33.3)
Pet ownership	728 (58.4)	806 (62.8)	1534 (60.7)
Parental smoking	839 (67.3)	871 (67.9)	1710 (67.6)
Socioeconomic status			
Low (Score 1-6)	345 (27.7)	365 (28.5)	710 (28.1)
Moderate (Score 7-11)	751 (60.3)	755 (58.9)	1506 (59.6)
High (Score 12-16)	150 (12.0)	163 (12.7)	313 (12.4)

frequently administered by the physicians in Northern Cyprus. Other asthma surveys in Turkey have produced significantly different prevalance rates[8-10]. In one of these epidemiologic surveys, Kalyoncu et al.[9] found a cumulative wheezing prevalence of 23.3 and current wheezing prevalence of 11.9 percent among 6-to 12-year-old children in Ankara. However, this study was conducted including 1,226 children attending one single school and, therefore, may not be representative of the population. Due to the differences in methodology, it is quite difficult to compare these figures with those obtained by the ISAAC protocol.

We attempted with this study and the one conducted in Ankara[4] to estimate the role of allergens in asthma-related symptoms. Out of 121 children who wheezed within the last 12 months in Northern Cyprus, 83 (69% of the wheezers) were reported to have wheezed because of the allergens. In Ankara, on the other hand, only 44 percent of the current wheezing was thought by the parents to be due to allergens. This suggests that children are exposed to higher concentrations of allergens in Northern Cyprus. This would be expected since Cyprus has a significantly more humid climate. Another possible explanation would be exposure to higher levels of animal antigens. Pet ownership was more common in Northern Cyprus (60.7%) than in Ankara (21.4%). However, it did not turn out to be a risk factor for wheezing. It should be noted that the ISAAC questionnaire takes into account only animals, feathers and dust as possible allergens, excluding pollens from this list. Generally, allargens are assumed to be a more important contributing factor than the number obtained in this survey[11,12]. The results of the second phase of the ISAAC protocol[13] is expected to shed some light on this question.

The figure obtained for bronchitis in children, similar to that found in Ankara[4], was quite high (24.0%). Although no official figure is available for the prevalence of bronchitis in Cyprus, its prevalence in children is generally estimated to be low. Since there is no gold standard for the diagnosis of either asthma or bronchitis, it may be diffucult to differentiate between the two entities, simply by using responses to standard questionnaires. We think that the inability to distinguish between bronchitis and asthma is one of the main limitations of questionnaire-based methodologies.

An international comparison indicates that the cumulative and one-year prevalences of wheezing found among Turkish Cypriot children (14.7 and 4.8, respectively) are lower than those reported for Bochum (33 and 20%), West Sussex (48 and 29%), Wellington (44 and 28%), Adelaide (40 and 29%) and Sydney (45 and 30%)[2], but are close to that reported for St. Gallen[3]. In another Mediterranean island, Malta, an ISAAC study conducted among 13 to 15-year-old children showed that the prevalences of cumulative wheezing and current

wheezing were 27.9 and 16 percent, respectively[14]. These rates are much higher than the corresponding values of the present study. This is surprising, since both the Maltese Islands and Cyprus are located within the Mediterranean zone with similar climatic and ecological properties. On the other hand, resultant outdoor pollution from busy Maltese roads was determined to be a risk factor for "ever wheezing" and nocturnal cough in that study. However, outdoor pollution has not yet become a serious problem in Northern Cyprus, which may explain the observed differences between these two island. Recently, the results of the global (worldwide) ISAAC Phase One study, in which 721,601 children from 56 countries were studied, have been reported[15]. There were marked variations in the prevalence of asthma symptoms, with up to 15-fold differences between countries, the attempted explanations for which were environmental factors.

The prevalence of wheezing in the last 12 months in Northern Cyprus (4.8%) is quite low when compared to global values that ranged from 4.1 to 32.1 percent in the 6-7 years of age group and 2.1 to 32.2 percent in. 13-14 years of age group. Countries that had 12-month prevalence of wheezing under 10 percent were found mainly in Asia, Northern Africa, Eastern Europe and the Eastern Mediterranean regions, and those over 20 percent were found mainly in the United Kingdom, Australasia, North America and Latin America. These values support the hypothesis that a "western life style" is associated with a high prevalence of childhood asthma. We believe that the second phase of this study will provide clues in clarifying some of the factors that are responsible for differences in observed prevalence rates.

In our survey, family history of atopy emerged as the most significant risk factor for wheezing, lending further support to the hereditary basis of the disease. Although this study showed that passive smoking is a significant health problem (67.6%) for Turkish Cypriot children, it failed to demonstrate an association to asthma-related symptoms. Similarly, no association was found between pet ownership and socioeconomic status. However, as this is a point prevalence study, the findings regarding passive smoking, pet ownership and socioeconomic status reflect only the current time point and do not provide data about antecedent conditions. They should, therefore, be interpreted with caution.

In conclusion, this study shows that bronchial asthma is an important public health problem for Turkish Cypriot children and should be a major concern upon formulation of health care policies. Public and medical education programs should be strongly advocated in an effort to increase awareness of the disease and thus decrease the social and economic burden imposed by this common condition.

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The Turkish Journal of Pediatrics 1999; 42: 421-427 Original

SAFETY, TOLERABILITY AND IMMUNOGENICITY OF A HAEMOPHILUS INFLUENZAE TYPE b VACCINE CONTAINING ALUMINUM PHOSPHATE ADJUVANT ADMINISTERED AT 2, 3 AND 4 MONTHS OF AGE*

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SUMMARY: Kanra G, Viviani S, Yurdakök K, Özmert E, Yalçın S, Baldini A, Mutlu B, Kara A, Ceyhan M, Podda A. (Infectious Diseases and Social Pediatrics Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey and Chiron Vaccines Clinical Research Chiron SpA, Siena, Italy). Safety, tolerability and immunogenicity of a Haemophilus influenzae type b vaccine containing aluminum phosphate adjuvant administered at 2, 3 and 4 months of age. Turk J Pediatr 1999; 41: 421-427.

The primary aim of this study was to assess the tolerability and immunogenicity of a new Haemophilus influenzae type b (Hib)/$AlPO_4$ (CHIRON, SpA) vaccine, in two-month-old healthy infants. Twenty-three subjects were enrolled and administered the new Hib vaccine containing $AlPO_4$ adjuvant at two, three and four months of age concomitantly with diphtheria-pertussis-tetanus (DPT) and hepatitis B vaccines according to the local program. Children were observed for 30 minutes after each immunization for any immediate local and systemic reactions. An active surveillance for side effects was performed on the 2nd and 7th days following each immunization by telephone. Families also filled out diaries for the first seven days. From the 2nd day to the next immunization only data about adverse events necessitating a physician's visit or about serious adverse events were collected. Blood samples were obtained before the first immunization and one month after the third dose for evaluation of anti-polyribosylribitol phosphate (PRP) antibody response. Local reactions at the Hib site were mild and less frequent compared to those observed at the DPT site. Systemic reactions noted after the three immunizations were fever in 70 percent,

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irritability in 48 percent, persistent crying in 26 percent, change in eating habits in 22 percent, diarrhea in 17 percent, sleepiness in 17 percent, vomiting in 9 percent, and unusual crying in 4 percent of the cases. There was no serious adverse event. One hundred percent and 95 percent of children achieved an anti-PRP antibody response over 0.15 µg/ml and 1.0 µg/ml, respectively. The geometric mean titer was 15 µg/ml and the geometric mean ratio 84. It was concluded that the new (Hib)/$AlPO_4$ vaccine is safe and well tolerated, and induced a good PRP antibody response in healthy two-month-old infants. *Key words: aluminum phosphate adjuvant. Haemophilus influenzae type b vaccine, safety, immunogenicity.*

In those countries where haemophilus influenzae type b (Hib) conjugate vaccines have been used for routine infant vaccination, Hib invasive disease has been virtually eradicated[1-4].

The first generation of Hib vaccines consisted of plain capsular polysaccharide (PRP). These vaccines elicited a fairly good immunological response in adults and children > 18 months of age. Anti-PRP antibody level was, however, of short duration and, in addition, as a PRP a T-cell independent antigen immunological memory was not primed[5,6].

Priming of immunological memory and elicitation of anti-PRP antibody in infants are instead achieved with Hib conjugate vaccines. Plain Hib polysaccharide becomes a T-cell dependent antigen, and is thus able to prime immunological memory by conjugation to a protein[7,8].

Several Hib conjugate vaccines are used worldwide. In the PRP-T vaccine, the plain polysaccharide is conjugated to tetanus toxoid; in the PRP-OMP vaccine, conjugation is made to an outer membrane protein complex of Neisseria meningitidis; in HbOC vaccine, the protein used in CRM_{197}, a non-toxic mutant of diphtheria toxin.

Some years, ago, Chiron Vaccines in Siena, Italy developed a HbOC-like vaccine (VaxemHib®) consisting of oligomers of capsular PRP of Haemophilus influenzae type b conjugated to CRM_{197}[9,10].

The vaccine is presented in two vials for mixing before injection: one containing the Hib conjugate to CRM_{197} protein and the other aluminum hydroxide as adjuvant. In view of the inconvenience of mixing the two vials before injection, an improved formulation has ben recently developed at Chiron Vaccines in Siena. The adjuvant has been replaced by aluminum phosphate which permits formulation of the vaccine in a stable solution presented in a ready-to-use vial.

As preclinical studies on stability and immunogenicity showed promising results, an expanded pilot study has been carried out.

We present here results of a clinical pilot study to assess the safety of the new Hib formulation in two-month-old healthy infants, and to obtain preliminary data on immunogenicity.

Material and Methods

This trial was designed as an open label, controlled, single center, pilot study. The study was approved by the Ethic Board of Hacettepe University, Medical Faculty (Ankara, Turkey) and by the General Directorate of Medicine and Pharmacy, Ministry of Health, Ankara, and written informed consent was obtained from the parents.

Study subjects were recruited in July 1998 at the Department of Pediatrics Infectious Diseases Unit of Hacettepe University, among two-month-old healthy infants eligible to receive routine EPI vaccines. At the time of the study, the Hib vaccine was not part of the EPI in Turkey. Excluded from the study were infants who had previously received one dose of Hib vaccine, had presented a previous disease potentially related to Hib, had household contact and/or intimate exposure in the previous 30 days to an individual with ascertained Hib disease, had experienced fever >38 °C within the past three days, had significant acute or chronic disease or known or suspected congenital or acquired immune suppression, had received parenteral immunoglobulin, had a history of anaphylaxis or any serious vaccine reaction or had participated in another trial within 30 days.

Infants were immunized with Hib/$AlPO_4$, diphtheria-pertussis-tetanus (DPT) and oral poliovirus vaccine (OPV) vaccines according to the two, three and four months schedule. Two doses of hepatitis B vaccines were administered at the 2^{nd} and 3^{rd} months, and a third dose was administered at 9-11 months of age after the completion of the study.

The Hib/$AlPO_4$ vaccine under investigation (Chiron Vaccines SpA, Siena Italy, lot number N32P18H1) contained 10 µg CRM_{197}- Hib conjugate as oligosaccharide, 0.3 mg aluminium phosphate, 0.01% thimerosal and 0.005% polysorbate (Tween) 80 and qs 0.5 ml phosphate buffered saline. It was provided as a single dose pre-filled syringe and was administered as an intramuscular injection in the left thigh. The concomitant DPT (Pasteur Merieux Connaught) was administered as an intramuscular injection in the right thigh and hepatitis B vaccine (HBV) (Smith Kline Beecham) as an intramuscular injection in the right deltoid.

After vaccination, subjects were observed for 30 minutes for local signs and symptoms (redness, induration, pain) at the injection site and for systemic reactions (rash, drowsiness, change in eating habits, unusual cry, persistent cry, vomiting, diarrhea). Parents were instructed to measure rectal temperature and to complete a diary card to describe local reactions and systemic reactions that developed within six hours after each immunization and daily for a total of seven days. All adverse events regarding any visit or consultation during the

study period were noted. All subjects were followed up the entire study period for the use of prescribed medications. Telephone calls were made two and seven days post-immunization to obtain reaction data and to assess the subject's clinical status. Diary cards were collected at the following visits. A serious adverse event was defined as any experience that suggested a significant hazard, contraindication, side effect or precaution. It included any experience fatal or life threatening, permanently disabling, or requiring or prolonging in-patient hospitalization, or any congenital anomaly or cancer. Blood samples were obtained from subjects on the day of first immunization and four to six weeks after the third immunization. Sera samples were stored frozen at –20 °C until serological testing.

IgG antibody response to PRP was measured by ELISA technique adapted from the FDA ELISA method[11] used for measuring Hib antibodies. The FDA standard reference serum, with known antibody titers as determined by radioimmunoassay (RIA), was used as standard. Immunogenicity measures were the percentage of subjects with an anti-PRP ELISA titer ≥ 1.0 µg/ml one month after the third immunization. The proportion of subjects with an anti-PRP ELISA titer ≥ 0.15 µg/ml and geometric mean titers for PRP antibodies were also evaluated.

Local and systemic reactions were evaluated for each of the three immunizations as well as for the three immunizations combined. If a subject experienced multiple adverse events that mapped to the same assigned term, the adverse event was counted only once.

All statistical analyses were performed using SAS® version 6.12 (SAS Institute, Cary, NC). Percentages of subjects experiencing local or systemic reactions, after any immunization, were calculated.

Geometric mean titers (GMTs) of anti-PRP antibodies and 95% confidence intervals (CIs) were constructed by exponentiating (base 10) the means and the lower and upper limits of the 95% confidence intervals of the logarithmically transformed (base 10) titers. For statistical analysis, antibody levels less than minimum level of detection were set to half of that limit.

Results

Twenty-three healthy infants whose parents had signed informed consent were enrolled into the study. Their mean age was 63.1 days (range 44-83) and 52 percent were males. They all simultaneously received three doses of Hib, DPT and OPV. The second and third immunizations were given 31 (range 31-33) and 64 (range 62-76) days after the first immunization, respectively.

Local and systemic post-immunization reactions are reported in Table I. There were no deaths, serious adverse events or adverse events leading to premature

withdrawal from the study. One subject was noted to have agitation, anorexia and vomiting for eight days starting the day of the third immunization, classified as possibly related to vaccine.

Anti-PRP antibody titers before and after vaccination with Hib vaccine are shown in Table II. After three doses, 100 percent of infants (95% CI 85-100%) had a PRP antibody titer ≥ 0.15 µg/ml versus 59 percent before vaccination (95% CI 36-79%), and 95 percent had a PRP antibody titer ≥ 1.0 µg/ml (95% CI 77-100) versus 5 percent before vaccination (95% CI 0-23%). GMT of anti-PRP antibody increased from 0.18 (95% CI 0.11-0.32%) before immunization to 15 (95% CI

Table I: Percentages of Subjects Reporting Local and Systemic Reactions within Seven Days after the Three Immunizations

Reaction	n=23
Local reactions at	
Left thigh (Hib)	
Tenderness	9%
Erythema	13%
Induration	0%
Right thigh (DTwP)	
Tenderness	22%
Erythema	35%
Induration	61%
Systemic reactions	
Rash	0%
Change in eating habits	22%
Sleepiness	17%
Unusual crying	4%
Persistent crying	26%
Irritability	48%
Vomiting	9%
Diarrhea	17%
Rectal temperature ≥ 38 °C	70%

Table II: Anti-PRP Antibody Titers Before the First and after the Third Immunization

	n	Titer ≥ 0.15 µg/ml %	(95% CI)	Titer ≥ 1.0 µg/ml %	(95% CI)	GMT µg/ml	(95% CI)	GMR ratio GMT	(95% CI)
Day 0	23	59	(36-79)	5	(0-23)	0.18	(0.11-0.32	–	
Day 84	23	100	(85-100)	95	(77-100)	15	(8.21-29)	84	(36-196)

GMT: geometric mean titer GMR: geometric mean ratio CI: confidence interval

8.21-29%) after immunization, which corresponds to a geometric mean ratio (GMR) of 84.

Discussion

This was a pilot study to primarily evaluate the tolerability of a new Hib vaccine with $AlPO_4$ as adjuvant. Adjuvants have been used to augment the immune response in vaccinations for more than 60 years, but they may be responsible for some of the vaccine side effects[12,13]. The most commonly used adjuvants for human vaccines are aluminum hydroxide, aluminum phosphate and calcium phosphate[12]. In animals it has been shown that aluminum phosphate may be a more potent adjuvant than aluminum hydroxide for several antigent[14,15]. In addition, the aluminum hydroxide adjuvant may lead to catalytic depolymerization of PRP[16]. The substitution of the aluminum hydroxide adjuvant has also led to a more convenient presentation of the vaccine which can be formulated in a single container.

In this study there was no serious side effect or death reported during the study period. The local side effects at the Hib site were less frequent and mild compared to those observed at the DPT site. Systemic reactions were more frequent than local reactions, including persistent crying, irritability, sleepiness, and febrile reactions most likely attributable to the DPT vaccine[17].

The anti-PRP antibody response after three doses of vaccine was highly satisfactory. All infants achieved an anti-PRP level over 0.15 µg/ml, which is considered protective, and 95 percent achieved an anti-PRP antibody level over 1.0 µg/ml, which is indicative of long-term protection. The GMTs were comparable to those published earlier with vaccines proven to be protective[18-20] despite the presence of maternal antibodies.

The results of this study indicate that three doses of Hib/$AlPO_4$ (CHIRON SpA) vaccine are safe and well tolerated and induce a good PRP antibody response. A further large scale clinical trial to assess the safety and immunogenicity of this vaccine is in progress.

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NEONATAL OUTCOME FOLLOWING EARLY ONSET PRETERM PREMATURE RUPTURE OF THE MEMBRANES - A CASE CONTROLLED STUDY*

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SUMMARY: Acunas B, Greenough A, Dimitriou G, Gamsu H. (Children Nationwide Regional Neonatal Intensive Care Centre, King's College Hospital, London, United Kingdom). Neonatal outcome following early onset preterm premature rupture of the membranes - a case controlled study. Turk J Pediatr 1999; 41: 429-436.

A case-controlled study was performed to determine whether preterm premature rupture of the membranes (PPROM), particularly if occurring in the second trimester, increased the duration of ventilatory support or hospital admission. Infants born after membrane rupture of at least 24 hours duration and prior to 37 weeks of gestation were identified. It was possible to match for gestational age and birthweight 40 PPROM infants, 15 of whom had onset of rupture of the membranes (ROM) prior to 27 weeks of gestation, with a control (an infant whose mother had not suffered PPROM). A greater proportion of the mothers of the PPROM infants had received antenatal steroids ($p<0.01$), had an antepartum hemorrhage ($p=0.06$) or delivered vaginally ($p<0.02$). More PPROM infants had pulmonary hypoplasia ($p<0.03$) or infection ($p<0.01$). Overall, however, and if only those matched pairs where membrane rupture had occurred prior to 27 weeks of gestation were considered, there were no statistically significant differences in the duration of ventilatory support or hospital admission. Step-wise regression analysis confirmed that in the study population overall and in the matched pairs where membrane rupture had occurred at less than 27 weeks of gestation, neither the duration of ventilation nor hospital admission significantly related to PPROM. These findings have implications when counselling parents. *Key words: antenatal steroids, infection, preterm premature rupture of the membranes, pulmonary hypoplasia.*

Preterm, premature rupture of the membranes (PPROM) is a common pregnancy complication. Antenatally, parents are counselled that their infant is likely to be at increased risk of poor outcome due to the development of pulmonary hypoplasia or infection. Yet, comparison of infants whose mothers had or had not ruptured their membranes prior to 37 weeks of gestation demonstrated that infants born following PPROM did not require significantly more respiratory

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support or a longer hospital admission[1]. A possible explanation for that surprising finding was the greater use of antenatal steroids in mothers who had PPROM. Certain complications associated with PPROM, most importantly pulmonary hypoplasia, are more likely if membrane rupture occurs prior to 27 weeks of gestation[2]. Thus, it is essential that a comparison of the duration of therapy involves that high risk population. The aim of this study was, therefore, to perform a case-controlled study of infants admitted to the neonatal intensive care unit (NICU) following PPROM to determine, in particular in those whose mothers had membrane rupture prior to 27 weeks of gestation, whether their duration of ventilatory support or hospital admission differed from matched controls.

Material and Methods

Infants admitted to the NICU during a 12-month period who were born after membrane rupture of at least 24 hours duration and prior to 37 weeks of gestation were retrospectively identified. Matching for gestational age and birth weight was then attempted with an infant whose mother had not suffered PPROM (a control). The control's birth weight had to be within 95-105 percent of the corresponding PPROM infant's.

The maternal notes were then examined to obtain data regarding the timing and duration of PPROM, occurrence of antepartum hemorrhage (APH), administration of antenatal corticosteroids and mode of delivery. Membrane rupture was diagnosed from the maternal history and confirmed if fluid, seen in the posterior vaginal fornix at a sterile speculum investigation, gave a positive nitrazine test. During the study period, routine policies were followed regarding antenatal administration of corticosteroids, antibiotics or tocolytic agents. Corticosteroids were routinely administered between 26 and 32 weeks of gestation to promote lung maturation. Antibiotics were given only if there was significant maternal infection or Streptococcus agalactiae was grown from a low vaginal swab. Spontaneous labor was not inhibited at any time following membrane rupture. Delivery was prompted if there was evidence of chorioamnionitis: vaginal discharge, uterine tenderness, fever, tachycardia or labor.

The neonatal notes were reviewed. The infant's gestational age was determined from the mother's last menstrual period and an ultrasound examination prior to 21 weeks of gestation, and from the infant's physical appearance and neurological score. Throughout the study period, routine policies were followed regarding respiratory management, in particular with respect to resuscitation and criteria for intubation, mechanical ventilation and surfactant administration. The Apgar scores, maximum inspired oxygen concentration and peak inspiratory pressure for each infant were noted. The infant's respiratory diagnosis was made by the clinician in charge of the case. Respiratory distress syndrome (RDS) was

diagnosed if the infant developed tachypnea, retractions, and grunting and/or cyanosis within four hours of birth persisting for longer than 24 hours, in association with a chest radiograph appearance demonstrating symmetrically affected opaque lung fields with a ground-glass appearance[3]. Exogenous surfactant therapy was administered routinely in the latter part of the study to infants with RDS if they were ventilated and required an inspired oxygen concentration of at least 30 percent.

Pulmonary hypoplasia was diagnosed on the basis of clinical and radiological criteria[2] and in non-survivors, whenever possible, by postmortem examination. Chronic lung disease (CLD) was diagnosed if the infant had required intermittent positive pressure ventilation (IPPV) for at least three days during the first week of life and had ongoing evidence of respiratory distress (tachypnea, retraction, rales and oxygen dependence) for at least 28 days. Congenital infection was diagnosed if the infant had positive blood cultures or gastric aspirate immediately after birth. All infants of less than 33 weeks of gestational age had regular cranial ultrasound examinations in the first week of life.

Statistical analysis

Differences between the PPROM and control groups were assessed for statistical significance using either chi-square or Wilcoxon rank sum test as appropriate. To assess the significance of relationships, Spearman's correlation coefficients were calculated. Step-wise regression analysis was performed to determine the relationship of the duration of mechanical ventilation or hospital admission to PPROM, antenatal steroid usage, APH, mode of delivery, gestational age, birthweight, gender, surfactant usage, occurrence of infection and pulmonary hypoplasia.

Results

Forty of the 51 infants born following PPROM could be matched with a control infant. A greater proportion of the mothers of PPROM infants had received antenatal steroids or delivered vaginally (Table I). In addition there was a trend for more PPROM mothers to have suffered from an APH.

Significantly more PPROM infants had pulmonary hypoplasia or infection (Table II). Three PPROM infants died of pulmonary hypoplasia with membrane rupture at 23, 26 and 26 weeks of gestation respectively; a fourth died of congenital sepsis with membrane rupture at 28 weeks. One control died of congenital septicemia and another of renal failure; both were born at 23 weeks of gestation. If only infants with PPROM prior to 27 weeks of gestation and their matched controls are considered (Table III), the use of antenatal steroids and the occurrence of pulmonary hypoplasia differed significantly between the two groups. There were no significant differences, however, (Table II), nor in the

subgroup with PPROM ≤26 weeks of gestation (Table III), between the PPROM and control groups in either the duration of ventilation or hospital stay.

Table I: Maternal and Infant Demographic Data Expressed as Median (Range) or n (%)

n	PPROM 40	No PPROM 40	p
Gestational age (weeks)	29 (23-36)	29 (23-36)	ns
Birth weight (g)	1423 (490-2790)	1390 (510-2740)	ns
Male gender	31 (78%)	24 (60%)	ns
Vaginal delivery	31 (78%)	20 (50%)	<0.02
Antenatal steroids	18 (45%)	4 (10%)	<0.001
Antepartum hemorrhage	10 (25%)	3 (8%)	=0.06
PPROM duration (days)	5 (1-94)	–	–
Apgar score at 1 minute	6 (0-9)	5 (0-10)	ns
Apgar score at 5 minutes	8 (1-10)	8 (4-10)	ns

PPROM: preterm premature rupture of the membranes.

Table II: Neonatal Outcome Data Expressed as Median (Range) or n (%)

n	PPROM 40	Controls 40	p
Ventilated	27 (68%)	28 (70%)	ns
Maximum inspired oxygen concentration (FiO_2)	0.55 (0.26-1.0)	0.60 (0.25-1.0)	ns
Max PIP (cmH_2O)	28 (16-45)	19 (18-26)	ns
Duration of ventilation (days)	2 (0.5-41)	5 (0.5-38)	ns
RDS	19 (48%)	21 (53%)	ns
Surfactant administration	5 (13%)	6 (15%)	ns
Pulmonary hypoplasia	6 (15%)	0	<0.03
Infection	20 (50%)	8 (20%)	<0.01
Air leak	6 (15%)	4 (10%)	ns
CLD	6 (16%)	6 (16%)	ns
Intracranial hemorrhage	6 (15%)	7 (18%)	ns
Death	4 (10%)	2 (5%)	ns
Duration of hospital stay (days)	26 (1-125)	32 (3-127)	ns

PPROM: preterm premature rupture of the membranes.
PIP : peak inflating pressure.
RDS : respiratory distress syndrome.
CLD : chronic lung disease.

Table III: Outcome of Infants with Membrane Rupture ≤26 Weeks of Gestation

n	PPROM 15	Controls 15	p
Gestational age (weeks)	27 (23-31)	27 (23-31)	
Birth weight (g)	1082 (490-1460)	928 (510-1740)	ns
Antenatal steroids	10 (67%)	1 (9%)	<0.002
APH	7 (47%)	2 (13.3%)	<0.06
Vaginally delivered	13 (87%)	9 (40%)	ns
PPROM duration (days)	14 (2-94)	-	
Male	12 (80%)	6 (40%)	=0.06
Ventilated (n)	15 (100%)	15 (100%)	
Max FiO_2	0.88 (0.30-1.00)	0.62 (0.25-1.00)	ns
Max PIP (cmH_2O)	28 (16-45)	23 (19-26)	ns
Duration of ventilation (days)	8 (0.5-41)	7 (0.5-38)	ns
RDS	9 (60%)	12 (80%)	ns
Surfactant	5 (36%)	3 (20%)	ns
Pulmonary hypoplasia	6	0	<0.02
Infection	10 (67%)	5 (33%)	ns
Chronic lung disease	4 (27%)	6 (46%)	ns
Duration of hospital stay (days)	46 (4-125)	58 (23-127)	ns
Deaths	3 (20%)	2 (13%)	ns

PPROM: preterm premature rupture of the membranes.
APH : antepartum hemorrhage.
PIP : peak inflating pressure.
RDS : respiratory distress syndrome.

Pulmonary hypoplasia ($p<0.003$) and infection ($p<0.08$) tended to occur in the immature infants, and the duration of ventilation was inversely related to the gestational age at the onset of membrane rupture ($p<0.05$). In the study population overall, step-wise regression analysis demonstrated that prolonged ventilation related significantly only to low gestational age ($p<0.01$) and not PPROM and also that the duration of admission was significantly related to infection, low gestational age and male gender only ($p<0.01$). In the infants with early onset membrane rupture, both the duration of hospital stay and ventilatory support were significantly associated with low gestational age ($p<0.01$); the duration of hospital stay was also significantly related to lack of antenatal steroids ($p<0.01$).

Discussion

These data demonstrate that even infants born following very early onset PPROM do not require an increased duration of ventilatory support or hospital admission

compared to matched controls. Although pulmonary hypoplasia was, as expected, commoner in the PPROM infants, the number of deaths was very similar in the two groups and thus did not bias our results. The infants were matched for gestational age and as closely as possible for birth weight, but there were significant differences between them. APH was commoner in mothers who had PPROM, regardless of whether or not they delivered very immaturely. Meta-analysis has demonstrated that women with pregnancies complicated by PPROM are three times as likely to develop placental abruption[4]. The groups also differed significantly with respect to mode of delivery and antenatal steroid use, factors both known no impact on neonatal status[5-7]. We have previously demonstrated that steroid administration is more common in pregnancies complicated by PPROM than in pregnancies without that complication, but delivering at a similar early gestation[1]. Antenatal corticosteroid therapy has very few contraindications, but to be most effective should be administered for at least 24 hours prior to the delivery[6-8]. Patients with pregnancies complicated by PPROM may deliver many days after their initial presentation and thus there is sufficient time to give them antenatal steroids. This is in contrast to the control population who were selected only because of a similarly premature delivery and many of whom would have delivered with little warning. In a previous series[1] we demonstrated that, without antenatal steroid therapy, infants born following PPROM tended to require a longer duration of ventilation and hospital stay than controls. Thus, in the present population, the significantly higher steroid usage in the subjects may have improved their outcome. Indeed, regression analysis confirmed that the occurrence of PPROM was not significantly related to prolonged respiratory support or hospital admission; those adverse outcomes were explained by other factors, including lack of antenatal steroids as well as immaturity.

There were no significant differences in the incidence of RDS between our two groups, and the proportion of patients affected was similar to that quoted in previous series[9,10]. Antenatal steroid administration has not been suggested to reduce the incidence of RDS in PPROM[9], but our previous results suggest it may reduce its severity[1]. In the present series, the maximum peak inflating pressure (PIP) tended to be higher, but not significantly so, in the PPROM infants, likely due to the fact that only that group included infants with pulmonary hypoplasia. Only a small proportion of our population received surfactant. Yet, had it been routinely available throughout the study period, as a similar number of babies in each group were diagnosed as suffering from RDS and hence eligible for surfactant administration, this would not have changed the results.

The reported incidence of pulmonary hypoplasia following PPROM varies. This reflects the difficulty of diagnosing this condition accurately in survivors[11]. The importance of early gestational age at onset of membrane rupture associated

with an increased incidence of pulmonary hypoplasia has been highlighted in a number of studies[12,13]. In the present report, all six of the 40 PPROM infants in whom pulmonary hypoplasia was diagnosed had PPROM prior to 27 weeks of gestation. There remains controversy, however, whether the duration of membrane rupture is significantly associated with the development of pulmonary hypoplasia[14-16]. Not all studies reported whether the patients with PPROM had oligohydramnios, but it has been claimed[16] that only if that association occurs will pulmonary hypoplasia follow. It should be noted, however, that a later series[13] suggested that gestational age at membrane rupture and oligohydramnios were independent predictors of pulmonary hypoplasia.

Neonatal sepsis is increased following PPROM, perhaps as high as a five-fold increase in frequency compared to controls[17], but that assumption was based on a positive blood culture rate of three in 208 infants. The occurrence of infection following PPROM inversely relates to gestational age[16], as found in the present series. We also noted that the incidence of sepsis was significantly greater in the PPROM compared to the control group, but that did not significantly influence the duration of admission. It may, however, have long-term consequences. Infants born following PPROM have been reported to be at higher risk of subsequent moderate-to-severe neurological and developmental impairment[18]; 28 percent of infants in one cohort exhibited major neurological or developmental deficits when membrane rupture occurred in the mid trimester[19]. A possible mechanism is via prenatal intrauterine infection resulting in elevation of pro-inflammatory cytokines such as IL-6 and tumor necrosis factor-α[20]. In our series, nine of the PPROM infants had positive blood cultures taken immediately after birth and thus were likely to have prenatal infection.

We conclude that, even when PPROM occurs in the mid trimester, if the mother is given antenatal steroids, it is not associated with a significantly increased duration of either respiratory support or hospital admission.

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RELATIONSHIP BETWEEN HIGH LEUKOCYTE COUNT AND CELL SIZE IN CHILDHOOD ACUTE MYELOBLASTIC LEUKEMIA*

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SUMMARY: Olcay L, Ertem U, Okur H, Etikan İ, Tuncer AM. (Hematology Unit, Department of Pediatrics and Department of Biostatistics, Hacettepe University Faculty of Medicine and Division of Oncology, Dr. Sami Ulus Children's Hospital, Ankara, Turkey). Relationship between high leukocyte count and cell size in childhood acute myeloblastic leukemia. Turk J Pediatr 1999; 41: 437-445.

In order to determine the significance of cell size together with high leukocyte count (>30x10^9/L) in acute myeloblastic leukemia (AML), we evaluated the percentages of small, medium and large cells in 33 children with AML. All of the 10 patients with a high leukocyte count and 14 of the 23 patients with a low leukocyte count (<30x10^9/L) died or experienced a relapse within the first year. The mean small cell percentage of patients with high leukocyte counts was significantly lower than that of patients with low leukocyte counts ($p<0.05$). The percentages of small, medium and large cells of patients with high leukocyte counts and of patients with low leukocyte counts who died or experienced a relapse within the first year were similar. The percentage of medium cells of patients with high leukocyte counts was significantly higher than that of surviving patients with low leukocyte counts ($p<0.05$). The mean percentages of small, medium and large cells were similar in patients who died or experienced a relapse and surviving patients with low leukocyte count. We conclude that cell size has prognostic significance when the leukocyte count at admission is over 30x10^9/L, although confirmation seems necessary with a larger population of patients. *Key words: cell size, acute myeloblastic leukemia, high leukocyte count.*

The known prognostic factors for acute myeloid leukemia (AML) are not as definite as for acute lymphoblastic leukemia (ALL)[1].

High leukocyte count (total leukocyte count >100x10^9/L) at admission is known to be a poor prognostic factor for patients with AML. High leukocyte counts are generally encountered in FAB M4 and M5 subtypes, and M4 patients with high leukocyte counts are especially at high risk for extramedullary infiltration and central nervous system involvement[2].

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However, the number of studies examining the biologic and prognostic significance of myeloblast size are few in contrast to studies with ALL[3-8].

The aim of this study was to determine whether myeloblast size has prognostic significance in relationship to a high leukocyte count at admission of children with AML.

To our knowledge, this is the first such study.

Material and Methods

Patients: Children who were diagnosed as AML between November 1993 and June 1996 were included in the study (mean age: 9.80; range 1.5-16; years 16 female, 17 male). Of these, 32 were de novo AML, and one was secondary to Fanconi's aplastic anemia (diepoxybutane [DEB] positive). According to French-American-British (FAB) criteria, seven cases had M1, 14 cases M2, four cases M3, five cases M4, two cases M6, and one case M7.

In 10 patients, the leukocyte count at admission was found over $30x10^9$/L (33.8-480.0x10^9/L, mean: 119.1x10^9/L). The age range was 2-14 (mean: 7.3 years).

In 23 patients, the leukocyte counts were below $30x10^9$/L.The leukocyte count ranged between 1.4-26.4x10^9/L (mean 9.6x10^9/L); the age of the patients ranged between 18/12-16 (mean: 10.8 years).

Five of the 33 patients were diagnosed and treated in Dr. Sami Ulus Children's Hospital and 28 in Hacettepe İhsan Doğramacı Children's Hospital. Thirty-two patients received Hacettepe AML protocol[9] (11 received the 1993 and 21 the 1995 version of the same protocol) and one received Denver protocol[10].

Leukocyte counts over $30x10^9$/L were accepted as a "high leukocyte count" and below $30x10^9$/L as a "low leukocyte count", although a leukocyte count over $100x10^9$/L is generally accepted as high and a poor prognostic factor[2]. All of the patients in our study with leukocyte counts over 3010^9/L died or experienced a relapse within the first 12 months.

Cytogenetic analysis was done in 18 patients. Abnormal cytogenetic findings were as follows: In patients with leukocyte counts over 3010^9/L, trisomy 22 and trisomy 8 plus 22 were established in one patient. In patüients with leukocyte counts below $30x10^9$/L, 20 percent hypodiploidy, t(4;8)(q31,2;p23,1) dup(17)(q24); del (Y)(q11,23); t(8;21) and del 13 (q12,1) and t(X;21)(q27,3:q21,1) were established in the same patient and trisomy 22 in another one.

Flow Cytometric Analysis: Heparinized peripheral blood or aspirated bone marrow samples were obtained before the treatment. The mean percentage of leukemic blasts determined morphologically was 66.5 percent for bone marrow samples and 76.0 percent for peripheric blood samples. Bone marrow and peripheral

blood were prepared for flow cytometric analysis using the density gradient separation technique. The size of the blasts were determined by flow cytometer by Lysis II (FAC Scan, Becton Dickinson Immunology Systems, BDIS, San Jose, CA, USA). All PMT and forward scatter standardization were performed using calibration standard beads (5, 10, 15 μm latex beads, immunopreb and lymphotreb). The blast concentrations of the samples were increased by a back-gating procedure using a mixture of CD14+CD45 and CD33+CD34 prior to gating. The MoAbs used were acute leukemia phenotyping kit (Becton Dickinson Immunology Systems, San Jose, CA, USA) and fluorescein-isothiocyanate conjugated antibodies (Ecx. CD15FITC, CD14PE and CD34PE). In addition, by separating the cells by ficoll gradient[11], the concentration of the blasts increased considerably. Hence, blast concentrations on the slides of the cytospin material made up completey of mononuclear cells exceeded 90 percent.

Cells between 200-400, 400-600, and 600-800 on forward scatter were considered as "small", "medium" and "large" cells, respectively (Fig. 1). Cells were determined as percentage. The percentages of small, medium and large cells were determined for each patient.

Statistical Analysis: Mann-Whitney U test was used for comparison. For determination of differences between the percentages of small, medium and large cells in individual groups, the Wilcoxon rank sum test was used.

Results

All 10 patients with leukocyte counts over $30x10^9$/L died or experienced a relapse within the first 12 months (7^{th} day-10^{th} month) of therapy. Fourteen of 23 patients with leukocyte counts below $30x10^9$/L (60.8%) died or experienced a relapse between the 5^{th} day-12^{th} month of treatment; nine have survived for more than twelve months. The characteristics of the patients are summarized in Tables I and II.

In patients with leukocyte counts over $30x10^9$/L, the percentage of medium cells was found higher than that of large cells (45.96±4.44 vs 24.81±3.63, $p<0.05$), but the percentages of large and small cells (24.81±3.63 vs 29.65±5.11) and of small and medium cells (29.65±5.11 vs 45.96±4.44) were similar ($p>0.05$) and >0.05). In the group of patients with leukocyte counts below $30x10^9$/L, the percentage of medium cells was greater than that of large cells (36.87±2.78 vs 20.91±2.91, $p<0.01$) and the percentage of small cells was greater than that of large cells (41.28±3.60 vs 20.91±2.91, $p<0.05$), but the percentages of small and medium cells were similar (41.28±3.60 vs 36.87±2.78, $p>0.05$).

In our first classification made to evaluate together with cell size the prognostic role of leukocyte counts over $30x10^9$/L at admission, small, medium and large

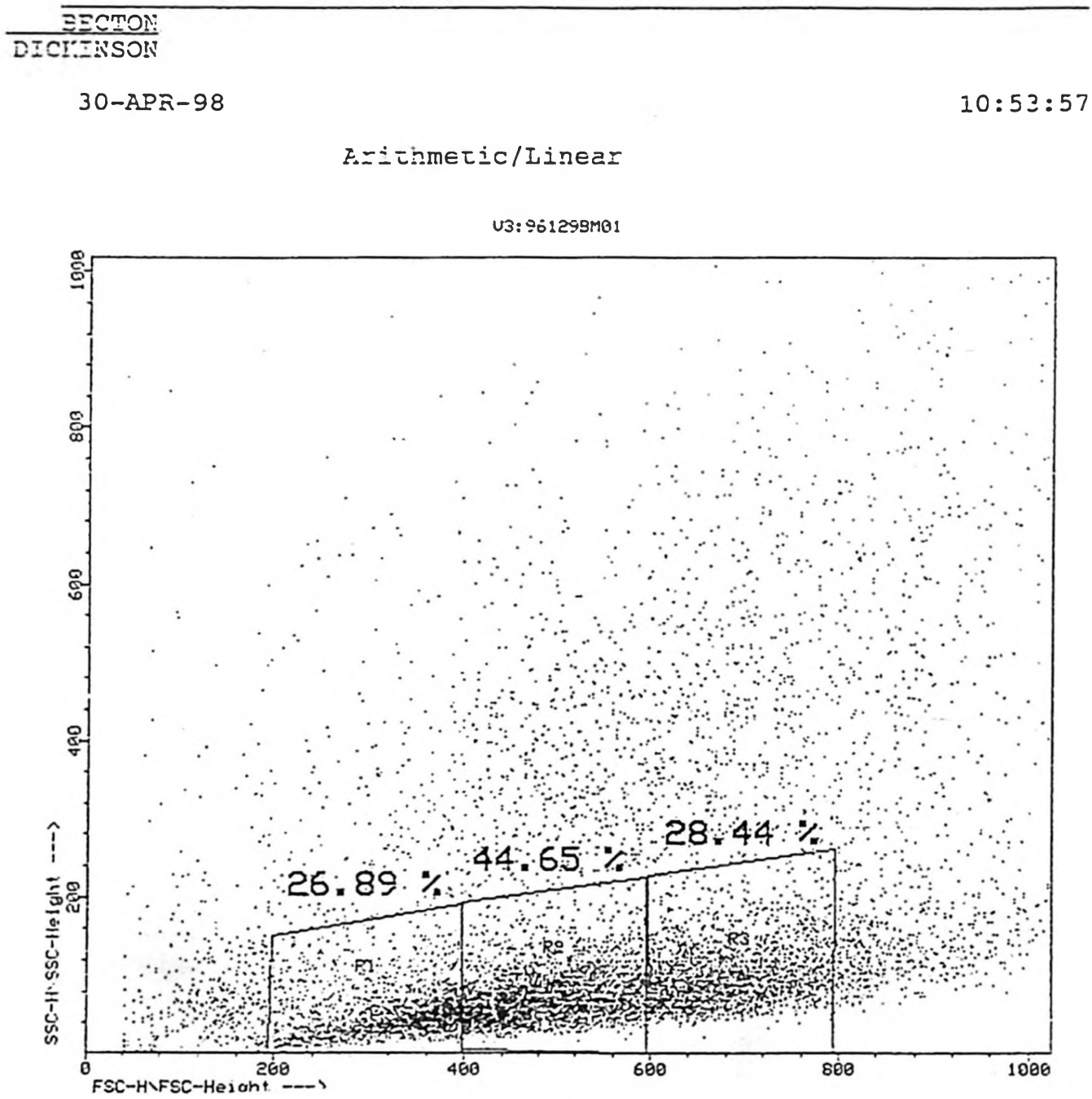

Fig. 1: The flow cytometric appearance of the cells. The cells between 200-400, 400-600 and 600-800 on forward scatter were considered as "small", "medium" and "large" cells, respectively.

cells of the patients with leukocyte counts over $30x10^9$/L were compared with those of the patients with leukocyte counts below $30x10^9$/L (Table IIIa). It was striking that the mean small cell percentage of patients with leukocyte counts over $30x10^9$/L (29.65±5.11) was significantly lower than that of patients with leukocyte counts below $30x10^9$/L (41.28±3.60) ($p<0.05$), while the mean percentages of medium and large cells of patients in both groups were similar ($p>0.05$) (Table IIIa).

Table I: Patients with Leukocyte Counts Over 30x10^9/L

Patient	Age (yrs) sex	Extramedullary involvement	Hb (g/L)	WBC (x10^9/L)	Platelet (x10^9/L)	FAB	Time of remission	Time of death or relapse
MT	3,M	right hilar LAP	80	67.2	25.0	M4	no remission	21st day, death
SÖ	5,F	pleura	122	40.2	22.0	M6	30th day	60th day, death
MA	13,F	No	95	73.2	10.0	M1	no remission	7th day, death
SK	2,F	lung	37	90.5	32.0	M1	8th day	9th month, relapse
MA	10,F	No	77	100.0	70.0	M2	4th month	10th month, relapse
Rİ	6,F	No	97	88.3	20.0	M2	36th day	4th month, relapse
AG	8,M	No	111	480.0	190.0	M2	no remission	2nd month, death
İZ	5,M	No	57	133.9	26.0	M2	no remission	2.5 month, death
RÇ	7,M	pericardium	73	84.0	32.0	M2	46th day	7th month, death
MÇ	14,F	No	67	33.8	27.0	M2	15th day	35th day, death

Hb: hemoglobin; WBC: white blood cells; FAB: French-American-British criteria; LAP: lymphadenopathy.

Table II: Patients with Leukocyte Counts Below 30x10^9/L

Patient	Age (yrs) sex	Extramedullary involvement	Hb (g/L)	WBC (x10^9/L)	Platelet (x10^9/L)	FAB	Time of remission	Time of death or relapse
MG	12,F	No	91	20.4	108.0	M4	2nd month	15th month, death
DÜ	13,F	No	79	14.2	44.0	M2	no remission	18th day, death
EG	13,M	No	55	1.4	12.0	M3	no remission	25th day, death
SÇ	13,F	No	80	10.0	32.0	M3	no remission	16th day, death
HH	4,F	No	60	11.0	25.0	M2	no remission	58th day, death
SU	15,M	No	53	9.2	20.0	M2	24th day	83rd day, death
SÖ	14,F	No	109	5.0	160.0	M2	1st month	6th month, death
İG	11,M	tonsilla, gingiva	81	28.6	23.0	M4	26th day	7th month, relapse 9th month, death
EU	10,M	No	76	8.0	22.0	M2	no remission	38th day, death
BT	11,M	No	87	1.8	15.0	M7	2nd month	5th month, death
LÇ	12,M	No	122	1.4	20.0	M3	no remission	5th day, death
MAK	2,M	gingiva, palatinum	78	6.0	30.0	M4	53rd day	12th month, death
İA	14,F	gingiva	34	12.8	25.0	M1	37th day	10th month, relapse
MEE	6,M	No	40	15.2	33.0	M2	15th day	8th month, death
NK	16,F	palatinum	74	26.4	10.0	M4	36th day	Alive and in remission for 4 years
BŞ	15,M	scapula, gingiva	89	5.5	11.0	M1		Alive and in remission for 2 years 11 months
SA	4,M	No	94	6.0	200.0	M3	30th day	Relapse at 21st month, (not remitted yet)
SMG	15,F	No	94	3.0	70.0	M1	9th day	Alive and in remission for 2 years
KP	15,M	No	70	6.5	80.0	M1	16th day	Relapse at 22nd month, (not remitted yet)
GD	16,F	No	94	2.6	27.0	M1	20th day	Relapse at 13th month, (not remitted yet)
MAT	10,M	bone	83	5.4	170.0	M6	5th day	Alive and in remission for 2 years
BY	13,F	No	41	19.1	25.0	M2	14th day	Alive and in remission for 4 years
AA	10,M	No	89	6.2	30.0	M2	8th day	2nd year 1st month, relapse 2nd year 4th month, death

Hb: hemoglobin; WBC: white blood cells; FAB: French-American-British criteria

Table IIIa: Percentage of Myeloblasts with Different Size According to Leukocyte Count

	Small		Medium		Large	
	WBC O*	WBC B**	WBC O	WBC B	WBC O	WBC B
N	10	23	10	23	10	23
Mean + SE	29.65 ± 5.11	41.28 ± 3.6	45.96 ± 4.44	36.87 ± 2.78	24.81 ± 3.63	20.91 ± 2.91
Range	10.85-64.72	17.97-86.77	17.66-64.95	5.54-53.9	7.24-41.8	1.53-53.3
P		0.04		0.06		0.31

* WBC O: Leukocyte count over $30x10^9$/L (10/10, 100% died or relapsed within the first 12 months).
** WBC B: Leukocyte count below $30x10^9$/L (14/23, 60.8%, died or relapsed within the first 12 months).

Table IIIb: Percentage of Myeloblasts with Different Size in Patients Who Died or Experienced Relapse Within the First 12 Months According to Leukocyte Counts Over and Below $30x10^9$/L

	Small		Medium		Large	
	WBC O*	WBC B**	WBC O	WBC B	WBC O	WBC B
N	10	14	10	14	10	14
Mean + SE	29.65 ± 5.11	36.79 ± 3.16	45.96 ± 4.44	40.07 ± 2.52	24.81 ± 3.63	23.11 ± 3.60
Range	10.85-64.72	17.97-66.03	17.66-64.95	27.01-53.9	7.24-41.8	1.53-46.14
R		0.14		0.21		0.76

* WBC O: Leukocyte counts over $30x10^9$/L (10/10, 100% died or relapsed within the first 12 months).
** WBC B: Leukocyte counts below $30x10^9$/L (14/14, 100%, died or relapsed within the first 12 months).

The mean percentages of small, medium and large cells of patients with leukocyte counts over $30x10^9$/L who died or experienced a relapse within the first 12 months were compared with those of the patients who died or experienced a relapse within the first 12 months and had leukocyte counts below $30x10^9$/L. The mean percentages of small, medium and large cells of these patients were similar ($p>0.05$) (Table IIIb). The mean percentage of medium cells of patients who died or experienced a relapse within the first 12 months of treatment and had leukocyte counts over $30x10^9$/L was significantly higher than that of surviving patients with leukocyte counts below $30x10^9$/L (45.96±4.44 vs 32.63±6.04) ($p<0.05$). Percentages of small and large cells of patients who died or experienced a relapse and had leukocyte counts over $30x10^9$/L were similar to those of surviving patients with leukocyte counts below $30x10^9$/L (Table IIIc). Percentages of small, medium and large cells in the group of patients who died or experienced relapse and had leukocyte counts below $30x10^9$/L were similar to those of surviving patients with leukocyte counts below $30x10^9$/L ($p>0.05$, >0.05, >0.05) (Table IIId).

Table IIIc: Percentage of Myeloblasts with Different Size in Patients Who Died or Experienced Relapse and Had Leukocyte Counts Over $30x10^9/L$ and in Surviving Patients with Leukocyte Counts Below $30x10^9/L$

	Small		Medium		Large	
	WBC O*	WBC B**	WBC O	WBC B	WBC O	WBC B
N	10	9	10	9	10	9
Mean + SE	29.65 ± 5.11	47.32 ± 8.12	45.96 ± 4.44	32.63 ± 6.04	24.81 ± 3.63	20.04 ± 5.13
Range	10.85-64.72	15.22-86.77	17.66-64.95	5.54-52.80	7.24-41.80	5.75-53.30
R		0.06		0.04		0.22

* WBC O: Leukocyte counts over $30x10^9/L$ (10/10, 100% died or relapsed within the first 12 months).
** WBC B: Leukocyte counts below $30x10^9/L$ (all 9 were alive for more than 12 months).

Table IIIc: Percentage of Myeloblasts Size of Patients with Leukocyte Counts Below $30x10^9/L$ According to Prognosis

	Small		Medium		Large	
	Deceased	Alive	Deceased	Alive	Deceased	Alive
N	14	9	14	9	14	9
Mean + SE	36.79 ± 3.16	47.32 ± 8.12	40.07 ± 2.52	32.63 ± 6.04	23.11 ± 3.6	20.04 ± 5.13
Range	17.97-66.03	15.22-86.77	27.01-53.9	5.54-52.80	1.53-46.14	5.75-53.30
R		0.28		0.61		0.41

Discussion

In ALL, blast size is known to have prognostic significance. Presence of blasts with large diameters is an independent poor prognostic factor for survival in ALL[5]. In AML, which is heterogeneous morphologically and biologically, the significance of cell size has been the subject of few studies[3,4,6-8].

Kawada et al.[3] demonstrated that myeloblast size affected the prognosis being dependent on surface markers. In a preliminary study[4], we reported that the mean percentage of large cells and the mean ratio of large cell percentage to small cell percentage was higher in deceased than in surviving patients, but in another study[7], we could not establish any relationship between cell size at admission and prognosis. Moreover, we also determined that cell size did not have prognostic value according to biphenotypy[8].

Cell proliferation and DNA synthesis rate were higher in the larger hematopoietic cells than in the smaller cells in infectious mononucleosis[12] and healthy individuals[13]. It was demonstrated that DNA synthesis increased as blast size

increased[14] and that the cell volume gradually increased during mitosis, reaching a maximum at the 18th hour and then gradually decreasing[15]. Thus, we consider the large cells of AML determined by flow cytometry as young cells with a high proliferation capacity.

The mean percentage of small cells of patients with leukocyte counts below $30x10^9$/L was significantly higher than that of patients with leukocyte counts over $30x10^9$/L, all of whom died or experienced a relapse within the first 12 months of treatment.

These data suggest that the number of cells of different size varies according to high leukocyte counts. Establishment of no difference in cell size of surviving patients with leukocyte counts below $30x10^9$/L and deceased patients with leukocyte counts below $30x10^9$/L suggests that the distribution of the blasts with different size does not differ according to prognosis when the leukocyte count declines below $30x10^9$/L.

The mean percentage of medium cells of patients with leukocyte counts over $30x10^9$/L, all of whom died or experienced a relapse within the first 12 months, was significantly higher than that of surviving patients with leukocyte counts below $30x10^9$/L.

These data suggest that the distribution of the cells according to blast size is dependent on the leukocyte count at admission being higher or lower than $30x10^9$/L and thus on prognosis. One reason for the poor prognosis in our patients with leukocyte counts over $30x10^9$/L seems to be the reduction of the number of small cells, generally more mature than the medium and/or large cells, which have less proliferating capacity than the smaller cells, and enhancement of the number of the middle cells. Therefore, cell size has prognostic significance when the leukocyte count at admission is over $30x10^9$/L.

Another significant finding, in the group of patients with leukocyte, counts below $30x10^9$/L was that large cells were the smallest in number. This supports our consideration of large blasts as young with a high proliferation capacity. Absence of significant differences between small-large or small-medium cells of the group of patients with leukocyte counts over $30x10^9$/L may be due to the limited number of patients. It is interesting that there was no significant difference between the percentages of small, medium and large cells of patients with leukocyte counts below $30x10^9$/L, whether they survived or died or experienced a relapse within the first 12 months of treatment. Thus, cell size of the blasts does not seem to have prognostic significance when the leukocyte count at admission is less than $30x10^9$/L. On the other hand, establishment of no difference in the mean percentage of small, medium and large cells of the deceased or relapsed patients with leukocyte counts higher and lower than $30x10^9$/L suggests that there are

more poor prognostic factors influencing cell size other than leukocyte counts over $30x10^9$/L.

It appears necessary to confirm these findings in patients with higher leukocyte counts (higher than $100x10^9$/L) and with a larger patient population.

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SERUM LEPTIN LEVELS DURING CHILDHOOD AND ADOLESCENCE: RELATIONSHIP WITH AGE, SEX, ADIPOSITY AND PUBERTY*

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SUMMARY: Kırel B, Doğruel N, Akgün N, Kılıç FS, Tekin N, Uçar B. (Department of Pediatrics, Osmangazi University Faculty of Medicine, Eskişehir, Turkey). Serum leptin levels during childhood and adolescence: relationship with age, sex, adiposity and puberty. Turk J Pediatr 1999; 41: 447-455.

We studied serum leptin levels in 189 healthy children to evaluate related factors during childhood and adolescence. Leptin corrrelated with body mass index (BMI), triceps skinfold thickness ($p<0.001$) and body weight ($p<0.01$). Obese children and girls had higher leptin levels than non-obese children and boys, respectively ($p<0.001$). In girls, leptin correlated positively with age, skinfold thickness and BMI ($p<0.001$). In boys, leptin correlated negatively with age ($p<0.001$) and positively with skinfold thickness ($p<0.05$). Prepubertal boys had higher leptin levels than prepubertal girls and pubertal boys ($p<0.05$). Pubertal girls had higher leptin levels than prepubertal girls and pubertal boys ($p<0.001$). Leptin levels in girls were higher at Tanner stages 4 and 5 than at stage 1 ($p<0.001$). In conclusion, serum leptin levels are related with adiposity, have obviously age-related gender differences during childhood and adolescence, and may be involved in the maturation of reproductive capacity. *Key words: childhood, leptin, obesity, puberty.*

In 1994, Zhang et al.[1] identified an obesity gene (ob) exclusively expressed in adipose tissue. Its protein product, leptin, acts as a satiety signal and regulates body-fat mass by affecting energy intake and expenditure at the hypothalamic level[2-7]. Little is known about leptin metabolism. Studies in animals and humans have demonstrated a direct relationship between leptin and adiposity. Obese humans have both higher serum leptin concentrations and higher leptin mRNA levels in adipose tissue than found in normal-weight adults and children. There is a strong positive correlation between serum leptin concentrations and percentage of body fat and body mass index (BMI)[7-13]. Females have higher serum leptin concentrations than males[11-18]. Reproduction and puberty appears to be affected by leptin[19,20]. Leptin is proposed to signal the onset of puberty. In some studies, leptin levels increased in parallel with age during prepubertal

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years and varied at different stages of puberty in both sexes, independent of adiposity, whereas in others, leptin levels did not change during the prepubertal or pubertal period[15,16,21-26]. There is limited data on leptin levels during childhood and adolescence and related factors, or on the role of leptin in growth and development. We therefore studied serum leptin levels, and also evaluated the relationship between leptin and adiposity, sex and puberty in children.

Material and Methods

This study included 189 (94 girls, 95 boys) healthy children admitted to our hospital for a hepatitis B vaccination. Ages ranged from six months to 19.5 years, with a mean of 12.56 ± 0.3 years. No children had clinical evidence of any diseases and were receiving no medication at the time of the study. Children and parents consented to participate in the study. Physical examinations were normal. Pubertal development was classified as defined by Tanner[27].

Body weight (BW), height, triceps skinfold thickness, and waist and hip circumference were measured by the same examiner. Height and BW measurements were compared with standard physical development tables for sex and age[27]. Percentage expected weight (PEW) was calculated as BW/ expected weight for height at 50th percentile x 100. Obesity was defined as weight above 120 percent. Body mass index (BMI) was calculated as BW/height^2. Triceps skinfold thickness was measured at the midarm using skinfold calipers.

Waist circumference was measured at umbilicus and hip circumference at widest horizontal distance around the buttocks. Waist/hip ratio (WHR) was calculated for estimating body fat distribution.

Fasting blood samples were obtained and serums were stored at –70°C until analysis. Serum leptin levels were determined by a commercially available radioimmunoassay (RIA) kit (Linco Research Inc.).

Relationships between leptin levels and anthropometric data were assessed by Pearson Product Moment Coefficient. One-way ANOVA and independent samples t test were used for comparing data of both sexes, of prepubertal (Tanner 1: breast development in girls, genital development in boys) and pubertal Tanner 2-5) children and of different Tanner stages. Data are presented as mean ± SEM.

Results

Clinical data of the children are presented in Table I. For the entire study group, leptin levels strongly correlated with BMI, triceps skinfold thickness and BW ($r=0.34$, $r=0.45$, $p<0.001$ and $r=0.19$, $p<0.01$, respectively), and did not correlate with age, waist and hip circumference or WHR ($p>0.05$).

Table I: Clinical Data of the Children

	Girls	Boys
Age (yr)	12.95 ± 0.43 (n=94)	12.17 ± 0.45 (n=95)
Body weight (kg)	47.8 ± 1.8 (n=90)	47.7 ± 2.0 (n=95)
Height (cm)	148 ± 2.2 (n=92)	148.5 ± 3.0 (n=95)
BMI (kg/m²)	20.85 ± 0.5 (n=89)	20.3 ± 0.44 (n=94)
Skinfold thickness (cm)	1.3 ± 0.06* (n=57)	0.92 ± 0.43 (n=50)
Waist (cm)	64 ± 1.1 (n=60)	65.96 ± 1.3 (n=62)
Hip (cm)	87.4 ± 1.86 (n=60)	83.05 ± 2.0 (n=62)
WHR	0.74 ± 0.01** (n=60)	0.84 ± 0.03 (n=62)
Leptin (ng/ml)	3.95 ± 0.3*** (n=94)	2.43 ± 0.22 (n=94)

Values are expressed as mean ± SEM.

*p<0.05, **p<0.01, ***p<0.001, girls versus boys.

BMI: body mass index; WHR: waist/hip ratio.

Leptin levels were higher in obese children than in non-obese children (4.8 ± 0.5 and 2.8 ± 0.2 ng/ml, respectively) (p<0.001).

Girls had higher leptin levels than boys (p<0.001) (Table I). There was no difference in BMI for girls and boys (p>0.05). Skinfold thickness (p<0.001) and WHR (p<0.01) were significantly different for girls and boys (Table I). In girls, leptin levels positively correlated with age (Fig. 1), skinfold thickness and BMI (r=0.45, r=0.56, r=0.5, p<0.001, respectively) and negatively correlated with WHR (r=–0.26, p<0.05). In boys, leptin negatively correlated with age (r=–0.4, p<0.001) (Fig. 1) and positively correlated with WHR and skinfold thickness (r=0.49, p<0.001 and r=0.33, p<0.05, respectively).

When the effects of puberty were investigated, leptin levels were higher in boys than in girls in prepubertal children (3.16 ± 0.42, 2.02 ± 0.31, p<0.05, respectively). In prepubertal girls, leptin did not correlate with age, BMI, or WHR (p>0.05) but did with skinfold thickness (r=0.98, p<0.001). In prepubertal boys, leptin negatively corelated with age (r=–0.38, p<0.05) but did not correlate with the other anthropometric data (p>0.05).

Pubertal girls had higher leptin levels than prepubertal girls and pubertal boys (4.5 ± 0.3, 2.02 ± 0.3, p<0.001, respectively). Pubertal boys had lower leptin levels than prepubertal boys (1.97 ± 0.24, 3.16 ± 0.42, p<0.05, respectively).

Leptin levels were significantly higher in girls than in boys in pubertal children (4.5 ± 0.3, 1.97 ± 0.24, p<0.001, respectively). When girls and boys in the pubertal group were compared, skinfold thickness (1.47 ± 0.07, 0.96 ± 0.06,

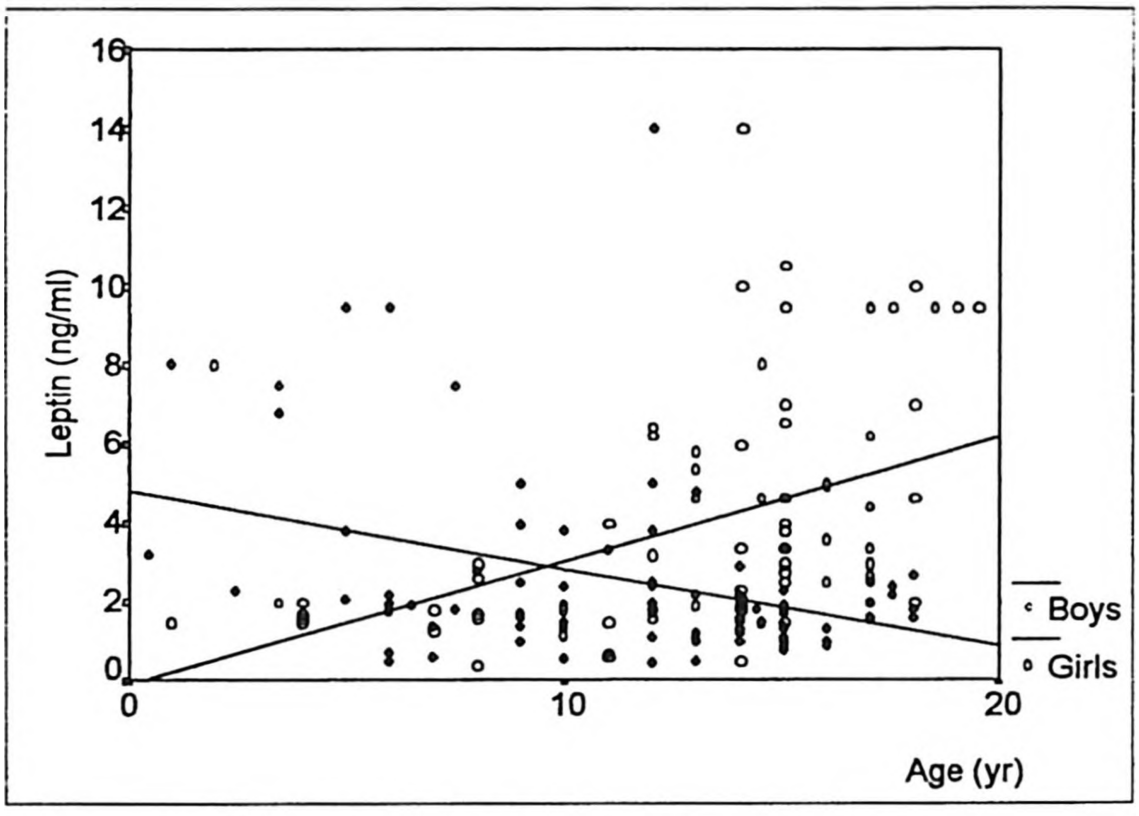

Fig. 1: Relation between age and leptin levels in both sexes.

p<0.05, respectively) and WHR (0.72 ± 0.01, 0.78 ± 0.01, p<0.001, respectively) were different. In pubertal girls, leptin correlated with age, BMI, BW (r=0.42, r=0.42, r=0.48, p<0.001, respectively) and skinfold thickness (r=0.49, p<0.01). In pubertal boys, leptin correlated with BMI (r=0.35, p<0.01), skinfold thickness (r=0.47, p<0.05) and WHR (r=0.54, p<0.001).

For the entire study group, there was no difference in at different Tanner stages (p>0.05). The findings at different Tanner stages for both sexes are shown in Table II. When an analysis was done according to sexes, BMI in girls was significantly different at Tanner stages 3, 4 and 5 than at stage 1 (p<0.0001). Both leptin and skinfold thickness were higher at stages 4 and 5 than at stage 1 (p<0.001) (Fig. 2). BMI in boys was higher at Tanner stage 5 than at stage 1 (p<0.05), but leptin levels and other measurements did not change at different Tanner stages (p>0.05). Although leptin levels at Tanner 5 in boys were slightly higher, as seen in Figure 2, they were not statistically different than in previous stages, and were still lower than at stage 5 in girls (p<0.001).

Discussion

We found serum leptin levels higher in obese children than in those normal-weighted. Leptin also strongly correlated with BMI, skinfold thickness and BW, as described previously in adults and adolescents[7-13]. We attributed the negative

Table II: Adiposity Parameters and Leptin Levels in Different Puberty Stages

Tanner Stage		BMI (kg/m²)	Skinfold thickness (cm)	WHR	Leptin (ng/ml)
1	Girls	16.6 ± 0.4 (n=20)	0.9 ± 0.0 (n=16)	0.8 ± 0.0 (n=15)	2.02 ± 0.3 (n=20)
	Boys	18.7 ± 0.7 (n=36)	0.9 ± 0.0 (n=20)	0.9 ± 0.0 (n=23)	3.2 ± 0.4 (n=36)
2	Girls	20.9 ± 2.3 (n=10)	1.1 ± 0.3 (n=4)	0.8 ± 0.0 (n=4)	2.7 ± 0.6 (n=10)
	Boys	20.3 ± 1.2 (n=15)	1.1 ± 0.6 (n=5)	0.7 ± 0.0 (n=6)	2.4 ± 0.8 (n=15)
3	Girls	20.3 ± 1** (n=14)	1.3 ± 0.3 (n=9)	0.7 ± 0.0 (n=9)	3.7 ± 0.6 (n=14)
	Boys	19.9 ± 1.4 (n=11)	0.8 ± 0.1 (n=7)	0.7 ± 0.0 (n=7)	1.7 ± 0.3 (n=19)
4	Girls	21.3 ± 0.6** (n=25)	1.5 ± 0.4† (n=18)	0.7 ± 0.3 (n=18)	4.6 ± 0.6† (n=27)
	Boys	19.1 ± 0.5 (n=13)	0.7 ± 0.1 (n=11)	0.7 ± 0.1 (n=13)	1.4 ± 0.1 (n=13)
5	Girls	24.2 ± 0.9** (n=20)	1.6 ± 0.4† (n=10)	0.7 ± 0.0 (n=14)	5.5 ± 0.6† (n=23)
	Boys	22.5 ± 0.8* (n=20)	0.9 ± 0.1 (n=7)	0.8 ± 0.0 (n=15)	2 ± 0.1 (n=20)

Values are expressed as mean ± SEM. *p<0.05, Tanner 5 versus Tanner 1 in boys, **p<0.0001, Tanner 3, 4, 5 versus Tanner 1 in girls, †p<0.001, Tanner 4-5 versus Tanner 1 in girls.
BMI: body mass index; WHR: waist/hip ratio.

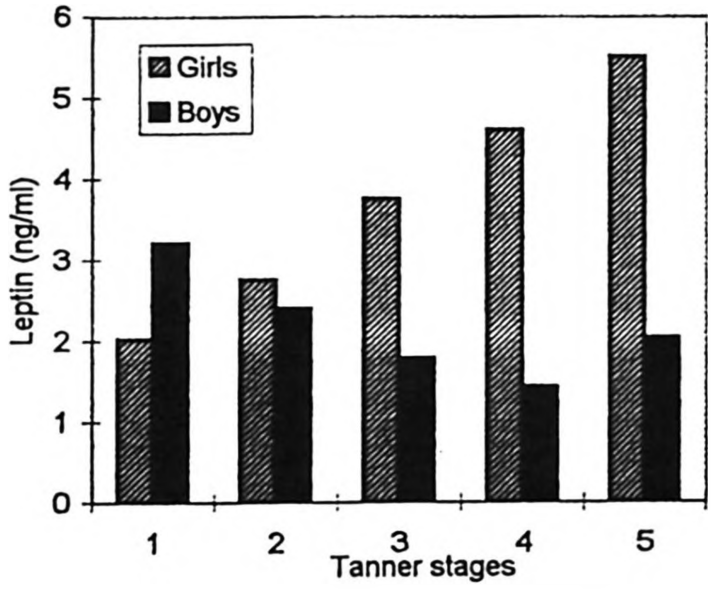

Fig. 2: Leptin levels at different puberty stages in both sexes.

correlation in girls and the positive correlation in boys between leptin and WHR to the existence of different distributions of body-fat stores. This finding also suggests a relationship between serum leptin levels and body-fat content.

The role of leptin in the pathophysiology of human obesity is not yet understood. The ob/ob mouse, which has a genetic leptin deficiency, has obesity and diabetes. Administration of exogenous leptin to these mice caused a decrease in food intake, weight loss, increased caloric expenditure and reversed insulin resistance[1-4]. In contrast, obese humans have higher leptin levels in spite of much more body-fat mass. This finding indicates that they have a decreased

sensivity or resistance to the satiety action of leptin in the brain, possibly at the hypothalamic level via appetite-stimulating hypothalamic neuropeptide Y, which is found to be inhibited by leptin in obese animals[6,8]. It was hypothesized that leptin is delivered to the brain by a saturable transport system. A decreased capacity to transport leptin to the brain via cerebrospinal fluid has already been shown in obese humans despite their having higher leptin concentrations than found in lean individuals. This is another mechanism that may provide an explanation for leptin resistance in obese individuals[28].

In our study, leptin increased with age in girls and decreased with age in boys, as reported by Garcia-Mayor et al.[15], and Blum et al.[29]. These results suggest that leptin metabolism has age-related sexual differences during childhood. Although leptin levels correlated with adiposity parameters in both sexes, we found that girls had higher body fatness in regard to skinfold thickness than boys and higher leptin levels, which may be related to their high body-fat percentage. On the other hand, leptin levels were still found to be higher in women when compared to men with similar body composition. Gender differences in leptin, independent of body fatness, suggest that females have genetic leptin resistance or that there are sex-related differences in leptin metabolism[14,18].

Studies on the reproductive system and puberty in both animals and humans have provided some explanation on gender differences in leptin levels that seem to be related with sex steroids[14,18,30]. Lower leptin levels were found in postmenopausal women than in premenopausal women, indicating that leptin metabolism is related to estrogen and/or progesterone. But postmenopausal women had higher leptin levels than males who were similar in body-fat mass to postmenopausal women[18]. It was also found that testosterone substitution normalized leptin levels in hypogonadal men whose leptin levels were found to be 3-fold higher than normal males with a similar BMI[30]. These results suggest that gender differences in leptin seem to be caused by suppressive effects of androgens on leptin metabolism[18,30].

The onset of puberty requires adequate nutrition and critical body fat stores and BW. Both pathological obesity and malnutrition are associated with delayed puberty[31]. A metabolic signal between adequate energy stores and the neuroendocrine axis is also necessary for the onset of puberty. Recently, in view of that known about the relationship between leptin and adiposity[7-18] and neuroendocrine function[32], it was hypothesized that leptin may be a metabolic signal triggering puberty. Treatment of ob/ob mice, with a genetic leptin deficiency and infertility, with leptin resulted in increased LH levels and ovarian weight in females and increased FSH levels and testicular mass in males, in spite of more weight loss than in pair fed controls. There were stimulation findings of gonadal

function on histologic examination[19]. Chehab et al.[20] gave leptin to normal prepubertal female mice and observed earlier maturation of the reproductive tract and earlier reproduction than controls, indicating that leptin regulates the neuroendocrine system in the hypothalamus, pituitary and ovary.

In prepubertal children, we found that boys had higher leptin levels than girls. Leptin was not related with age in girls. It was negatively correlated with age in boys, and it was only correlated with skinfold thickness in girls, whereas there was no correlation between leptin and anthropometric data in boys. Previously, either higher leptin levels in girls than in boys or similar leptin levels in both sexes in prepubertal children have been reported[13,15,22,29]. The etiology or importance of gender differences in leptin levels is unclear during this period when the effects of sex steroids are absent.

We found that pubertal boys had lower leptin levels than prepubertal boys. But, pubertal girls had higher leptin levels than both prepubertal girls and pubertal boys. These results confirmed the results of previous studies[15,22,29]. In the study Garcia-Mayor et al.[15], leptin levels rose in parallel with BW until 10 years of age, then a striking difference was observed in both sexes. While leptin levels decreased in boys after this age and testesterone, FSH and LH rose, leptin levels progressively rose, followed a rise in FSH and later LH and estradiol in girls during puberty. In another study, leptin levels in boys rose just before the onset of puberty and decreased to approximately baseline values and remained stable for more than two years, while testosterone progressively rose after the initiation of puberty[24]. Clayton et al.[22] reported an inverse relationship between leptin and testosterone levels and testicular volume. These clinical studies suggest that leptin plays a role in the initiation of puberty and also confirm that gender differences may be due to a negative effect of testosterone on leptin metabolism.

In this study, leptin levels in boys did not change at different Tanner stages whereas BMI increased. However, pubertal girls had higher leptin levels at stages 4 and 5 than at stage 1, as reported by Carlsson et al.[21]. The importance of the increase of leptin levels at this stage remains to be determined. Increased leptin levels at this stage may be related with the onset of the menarche, which occurs at midpuberty. During puberty, high leptin levels were found to be associated with a decline in age at menarche. An increase of 1 ng/ml in serum leptin lowered the age at menarche by one month. Similarly, an increase of body fat content wis inversely related to the age at menarche in human females[25], suggesting that leptin is a mediator between gonads and adipose tissue in women.

In conclusion, serum leptin levels are mainly related with adiposity, and there are obvious age-related gender differences during childhood and adolescence.

Leptin may be involved in the maturation of reproductive capacity. Further prospective studies are needed to understand the role of leptin in growth and development during childhood and adolescence and in the reproductive function.

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The Turkish Journal of Pediatrics 1999; 41: 457-465 Original

BLOOD LEVELS OF LEUKOTRIENES (LTC_4, D_4, E_4, B_4) AND SYNTHESIS OF LEUKOTRIENE B_4 BY PERIPHERAL LEUKOCYTES IN CHILDREN WITH ACUTE A AND B HEPATITIS*

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SUMMARY: Kasırga E, Çoker I, Aydoğdu S, Yağcı RV, Taneli B, Gousseinov A. (Division of Gastroenterology, Department of Pediatrics, Ege University Faculty of Medicine, İzmir Turkey). Blood levels of leukotrienes (LTC_4, D_4, E_4, B_4) and synthesis of leukotriene B4 by peripheral leukocytes in children with acute A and B hepatitis. Turk J Pediatr 1999; 41: 457-465.

Leukotrienes (LTs) are cell-membrane derived lipid inflammatory mediators, synthesized and eliminated by the liver. LTs have effects on liver cells in some pathological conditions. In this study, we measured plasma endogenous and liberated leukotriene (LT) concentration in peripheral blood leukocytes stimulated in vitro by the calcium ionophore (CaA23187) and platelet-activating factor (PAF). Production of LTs was measured in type A (n=37) and type B (n=10) acute hepatitis patients and control subjects (n=10). LTs levels were measured by high performance liquid chromatography (HPLC) and radioimmunoassay (RIA). The concentration of LTB_4 measured in plasma and stimulated peripheral blood leukocyte supernatants of children with hepatitis A infection was found to be statistically elevated and in positive correlation with serum alanine aminotransferase (ALT) levels. In plasma samples of hepatitis B patients, LTC_4 and LTE_4 were measured in significantly elevated concentrations. These results suggest that LTB_4 may be a critical mediator of hepatitis A virus-induced hepatocellular injury.
Key words: leukotrienes, lipid mediators, acute viral hepatitis.

Leukotrienes (LTs) are lipid inflammatory mediators that have important roles in inflammation and anaphylactic reactions[1]. These mediators are derived from arachidonic acid, released from cell membranes and during the 5-lipoxygenase pathway[2]. LTs are synthesized and released from cells such as neutrophils, monocytes, macrophages, mast cells, eosinophils, platelets, lymphocytes, Kupffer cells, renal glomerular cells, gastric epithelial cells and vascular endothelial cells[3,4]. Peptide LTs lead to edema and inflammatory cell infiltration caused by vasoconstriction and increased venopermeability at the inflammation site[1,2,5]. LTB_4, a nonpeptide leukotriene (LT), is known to be a potent chemotactic agent[1,2]. LTB_4 is responsible for activation, chemotaxis and degranulation of

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inflammatory cells[6]. At the end of these reactions, free O_2 radicals and proteases, cytokines, cationic proteins, serotonin and thromboglobulin are released from neutrophils, monocytes, lymphocytes, eosinophils and platelets[2,6]. Many laboratory studies have been performed during recent years in rats. CCl_4 or galactosamine with lipopolysaccharides and frog virus-3 were used for the induction of toxic and viral hepatitis, respectively, in rats[7-9]. It has been shown that liver destruction can be prevented using LT synthesis inhibitors or LT receptor antagonists[10,11]. LTs undergo omega and beta oxidation by N-acetylation in the liver parenchymal cells and then are excreted in bile[12]. LTs are synthesized in the liver during different pathological conditions. It is known that the liver is the target organ for many of these biologically active mediators[3].

The aim of this investigation was to study the role of LTs in the pathophysiologic mechanisms in hepatitis A and hepatitis B infections.

Material and Methods

Forty-seven patients with acute viral hepatitis (37 hepatitis A; 10 hepatitis B) seen in the outpatient Department of Pediatrics at Ege University School of Medicine were studied. The male/female ratios were 19/18 and 5/5 for the hepatitis A and B groups, respectively. The patients' mean ages (mean±SD)' were 8.67±2.40 and 11.2±3.52 years for hepatitis A and hepatitis B virus infections, respectively. All patients had acute hepatitis symptoms. There were no clinical or laboratory findings that suggested chronic liver disease in this group.

Biochemical and Serological Analysis: Liver function tests, anti-HAV IgM, anti-HAV IgG, HBsAg, anti-HBc IgM, anti-HBc total, HBeAg, and anti-HBe were assayed in all patients. Anti-HAV IgM (+), HBsAg (+) and anti-HBc IgM (+) patients were included in the study. Serological tests were performed by an ELISA method (Organon, Boxtel, Holland). Serum alanine aminotransferase (ALT) levels were measured on Hitachi 911 autoanalyzers by the Chod PAP enzymatic method.

Leukotriene Extraction from Plasma: One ml fresh plasma was separated from heparinized blood that was mixed with cold phosphate buffered saline without calcium at a 1:10 ratio and then centrifuged at 5,000 rpm for 10 min at + 4 °C. LTs were extracted from plasma using Maxi Clean C18 600 mg columns (Alltech, Co., USA) which had been conditioned and preactivated with 20 ml of methanol and then 20 ml of distilled water. The eluate was collected after applying 2 ml of acetonitrile solution and then passed through a 0.22 µ cellulose membrane filter (Alltech, Co., USA) in evaporator tubes. The acetonitrile solution was evaporated to dryness by a vacuum speed concentrator. The residue was dissolved in 20 µl of high performance liquid chromatography (HPLC) solution and stored at -70 °C until testing.

Leukocyte Isolation from Peripheral Blood: 10 ml venous blood was collected in a heparinized tube at a 0.01:10 ratio of heparin: blood and then a 10 percent gelatine solution was added to the blood at a 1:0.15 ratio of gelatin solution: blood. This mixture was incubated at 37 °C for 45 min. The cells were obtained from the lukocyte-rich plasma which settled as the upper phase by centrifugation at + 4 °C at 1,500 rpm for 7 min. The cells were then resuspended in HANKS balanced salt solution without Ca^{++} and Mg^{++} and washed twice. Finally, the cells were resuspended in 2 ml HANKS solution and a 20 µl cell suspension aliquot was taken out and diluted 3% acetic acid solution at a 1:20 ratio and counted in a Neubauer Chamber. The cell suspension was adjusted to a concentration of $1x10^7$ cells per milliliter in HANKS solution with Ca^{++} and Mg^{++}. The cell suspension was divided into two tubes for either calcium ionophore (CaA23187) or platelet-activating factor (PAF) stimulation. Leukocytes were incubated at 37 °C in an ultrasonic bath with 20 µl of 5 µm calcium ionophore for 15 min and 10 µl of 1 µm synthetic PAF C16 for 10 min. Incubations were stopped by adding 1 ml of cold deionized water and the leukocyte suspension was centrifuged immediately at + 4 °C at 6,000 rpm for 3 min. LT extraction was performed from the supernatant; the leukocyte sediment was discarded.

Leukotriene B_4 Extraction from Stimulated Leukocyte Supernatant: 0.5 ml of 2-propanol and 30 µl of 5 M formic acid were added to 1 ml of the supernatant from the leukocyte stimulation experiments and incubated for 10 min. Ether (1.5 ml) was then added dropwise and the solution mixed well and left until the appearance of two phases. The upper ether phase was removed and 22 µl of NH_3 was added to it. This mixture was poured into the microcentrifuge system (Alltech, Co., USA). A 0.22 µ diameter membrane filter was placed on the filtration part of this system. After centrifugation for 5 min, the filtrate which passed through the lower tube was dried by a vacuum speed concentrator and then stored in 20 µl HPLC solvent at -70 °C until testing.

Purification of Leukotrienes by the HPLC Method: Analysis of LTs was performed using the Waters 625 LC System. This system consisted of a four channel multidelivery pump, Waters 486 tunable absorbance UV detector, powerline system controller, Rheodyne 7012 injector with 20 µl sample loop and Baseline 810 HPLC software program. LT separation was performed on a Separon SG X C18 super analytical column (250x2.0 mm I.D) and precolumn (100x2.0 mm I.D) with a (methanol: water: acetic acid) gradient measuring at 280 nm wavelength and a 1.5 ml/min flow rate. LTs were collected from HPLC UV detector output according to their retention times and were dried again in the vacuum speed evaporator. Retention times were 7', 11', 13' and 15' for LTE_4, LTB_4, LTD_4 and LTC_4, respectively. The amounts of LTB_4, LTC_4, LTD_4 and LTE_4 were quantitatively measured by using LTB_4 (^3H), LTC_4 (^3H), LTD_4 (^3H) and LTE_4 (^3H) assay systems

(Amersham Life Science, UK). The procedure was performed according to kit instructions. LTB_4, LTC_4, LTD_4 and LTE_4 concentrations were measured by Beta-liquide scintillation counter (TRI-CARB-1600 TR, LSA-Packard, Canberra Company).

Results of LTs were expressed as ng/ml and ng/10^7 cells in plasma samples and peripheral leukocyte secretions, respectively.

Statistics: Data on LT concentrations and ALT values are expressed as mean, standard error and ranges. Differences found between the patients and controls were analyzed by Student's two-tailed *t* test. Correlation coefficients were calculated with the Spearman rank test. A p value of less than 0.05 was considered significant.

Results

Endogenous LTs concentrations were determined in the plasma samples from 23 of our 37 children with hepatitis A infection. Released LTB_4 concentration was evaluated in the supernatants of PAF-stimulated peripheral blood leukocytes from 36 of our 37 hepatitis A patients. Produced and released LTB_4 concentration was examined in the supernatants of CaA23187 stimulated leukocytes obtained from 29 of 37 hepatitis A patients. Endogenous LTs and released LTB_4 were determined in the plasma and stimulated leukocyte supernatants from all of the 10 patients with acute hepatitis B infection. In contrast to the hepatitis patients, normal subjects had almost undetectable blood levels of LTs. After stimulation with CaA23187, release of LTB_4 was observed in healthy subjects. On the other hand, in PAF-stimulated healthy subjects' leukocyte supernatants, LTB_4 was undetectable. In plasma, LTC_4 was untedetectable in 96.5 percent (28/29) of samples of children with hepatitis A and in 10 percent (1/10) of samples of patients with hepatitis B infection. Plasma samples of LTD_4 levels were undetectable in 100 percent (29/29) of patients with hepatitis A and in 70 percent (7/10) of patients with hepatitis B.

There were significant differences in the endogenous plasma LTs concentrations in the hepatitis A and B groups. LTB_4 was higher in plasma samples of patients with hepatitis A compared with those with hepatitis B ($t=2.15$, $p<0.05$). In plasma samples, LTC_4 and LTE_4 concentrations in children with type B hepatitis were found to be significantly higher when compared to patients with type A hepatitis ($t=6.55$, $p<0.001$ and $t=3.64$, $p<0.001$, respectively). Levels of LTD_4 were low and not different between type A and B hepatitis groups (Table I).

In the type A hepatitis group, it was shown that stimulation by CaA23187 and PAF yielded higher LTB4 release from peripheral leukocytes than in the hepatitis B group ($t=4.48$, $p<0.001$ and $t=2.37$, $p<0.05$, respectively) (Table II).

Table I: Concentrations of LTs in Plasma Samples (Mean±SEM)

Leukotrienes	Hepatitis A Group (n=29)	Hepatitis B Group (n=10)	p
LTB_4	1.8±0.1 (0.48-3.46)	1.1±0.6 (0.6-2.1)	<0.05
LTC_4	0.02±0.02 (0-0.64)	0.6±0.1 (0-1.93)	<0.001
LTD_4	ND	0.1±0.07 (0-0.61)	NS
LTE_4	1.5±0.1 (0.46-2.9)	2.4±0.2 (0.9-3.6)	<0.001

Leukotrienes values are expressed as ng/ml, (range).
ND: non-detectable, NS: not significant.

Table II: Concentration of LTB4 in the Supernatant of PAF and CaA23187-Stimulated Peripheral Blood Leukocytes (Mean±SEM)

LTB_4	Hepatitis A Group	Hepatitis B Group	p
	(n=36)	(n=10)	
PAF	9.7±0.8 (2.1-19.4) (n=30)	5.7±0.5 (3.9-9.2) (n=10)	<0.05
CaA23187	32.5±1.5 (18.4-49.6)	20±1.2 (14.2-26.4)	<0.001

Leukotrienes values are expressed in ng/107 cells, (range).
PAF: platelet-activating factor.

LTB_4 production was significantly higher in CaA23187 stimulated peripheral blood leukocytes from patients associated with hepatitis A and B virus infections compared with healthy control subjects ($t=11.07$, $p<0.001$ and $t=14.07$, $p<0.001$, respectively). Serum ALT levels in patients with hepatitis A (mean±SEM: 1369.7±115.1, range: 593-3312) were found to be significantly higher than in children with hepatitis B (mean±SEM: 732.6±172.9, range: 83-1811) ($t=2.65$, $p<0.05$). In the type A hepatitis group, there was a statistically significant positive correlation between serum ALT levels and the plasma concentrations of LTB_4 and LTB_4 release from peripheral leukocytes after in vitro PAF stimulation ($r=0.41$, $p<0.05$ and $r=0.44$, $p<0.01$, respectively). A significant correlation was not found between serum ALT levels and secreted LTB_4 concentrations from peripheral leukocytes after CaA23187 stimulation. On the other hand, there was no statistically significant correlation between serum ALT levels and LTs in the hepatitis B group.

Discussion

A considerable fraction of the LTs formed intrahepatically or systemically is excreted in the bile, but a portion gets into the circulation[11,13]. LTs which are in the circulation are either released from the liver or formed by further metabolization in the plasma. In the plasma, LTD_4 is formed from LTC_4, and LTE_4 is formed from LTD_4. Therefore, the fact that the level of LTE_4 determined in the plasma in both types of hepatitis was higher compared to the concentrations of LTC_4 and LTD_4 can be explained by accumulation of the end-product (Table I). By activating the peripheral blood leukocytes, the LTs synthesized in the liver and released into the circulation can initiate secretion of LTs also from cells. Accordingly, it may be wrong to say that the source of the endogenous LTs determined in the plasma is solely the liver. Thas is, these mediators are released both by the liver and the peripheral blood leukocytes. The difference between the activities of the enzymes that take part in the synthesis and metabolism of LTs and the effects of the hepatitis viruses on Kupffer cells and hepatocytes may cause the different LT concentrations in the plasma of type A and B hepatitis patients. Differences between these enzyme activities have been demonstrated in mice in the studies of Kawada and collaborators[14]. It is thought that in hepatitis A and B, the peripheral blood leukocytes participate in the inflammatory reaction by infiltrating into the liver and that they then pass into the peripheral circulation, having been sensitized. This contribution happens by a secretion of biologically active substances, especially by cells in the activated inflammation zone. During hepatitis it is shown that local mediators are secreted by activated Kupffer cells[15,16]. This secretion increases even more during viral stimulation. In 1987 Hagmann et al.[8] stimulated Kupffer cells which they had isolated with in vitro FV-3 (Frog virus 3) and they showed that both peptide and non-peptide LTs were secreted in large amounts. Additionally, they determined that the LT receptor antagonists which were used in vivo and in vitro inhibited the hepatocellular destruction that occurs as a result of the virus effect. The different amounts and spectrum of LT secretion, depending on the type of inflammation and as a result of the in vitro stimulation of leukocytes obtained from patients having different inflammatory reactions, has been shown[17-19]. PAF is a powerful otocoid mediator that plays a very important role in all stages of immune and infectious inflammation, and it is made from the cell membrane[20,21]. It is known that PAF is secreted in the liver by Kupffer cells and that at the same time it is effective on the liver[22,23]. LTB_4 and peptide LTs are formed with PAF and they potentiate the effects of each other[3]. Aiming to show that LTs are secreted as a result of the effect of PAF and to simulate the effects of these secreted LTs on the liver under in vitro circumstances, the peripheral leukocytes obtained from our patients of both hepatitis groups were stimulated by PAF. After doing so, very interesting differences are observed (Table II).

LTB_4 and PAF, very strong chemotactic factors released in the intrahepatic region, have different cells migrated into the liver. These infiltrated cells can lead to the release of a local mediator by degranulation, or pass into the peripheral circulation in an active form and then activate other intact cells by intracellular PAF or LT.

Inflammatory reactions are based on an increase of intracellular calcium and include LT synthesis by changing the composition of membrane phospholipids. The CaA23187 also caused LTB_4 secretion from peripheral leukocytes obtained from healthy children as a control group. Leukocytes from hepatitis patients in vitro secrete active LTB_4 when stimulated by CaA23187 (Table II). It can be concluded that membrane calcium channels are very active in peripheral leukocytes in patients with acute viral hepatitis.

Serum liver enzyme levels increase rapidly just after the prodromal period in viral hepatitis. Increased liver enzyme levels could be used as an indicator of cell damage[24]. Serum ALT levels, plasma LTB_4 concentrations and LTB_4, which were secreted from peripheral blood leukocytes after PAF treatment, were correlated to each other in hepatitis A and these correlations were statistically significant. However, in patients with hepatitis B there was no statistically significant correlation between serum ALT levels and LTs. It can be speculated that hepatitis B can cause slow pathogenic reactions while hepatitis A may cause rapid reactions. This can be a leading factor for progressive chronic viral hepatitis B, among the many factors responsible. Interferon (IFN) synthesis is one of the host originated and intracellular factors affected by LTB_4. Other factors like decreased MHC-II expression and IFN activity in chronic viral hepatitis B can cause defects in synthesis or secretion of LTB_4. It can be claimed that decreased amounts of MHC-II type receptors, which are necessary in the production of anti-HBs antibodies, can cause problems in amplification of immune reactions by decreased interactions between T and B cells. Anti-HBs antibody synthesis, the antibody responsible for extracellular virus clearance, can decrease at the end of these reactions[25,26].

A major difference between LTs concentrations in hepatitis A and B patients may be an important prognostic indicator. A high LTB_4 response in hepatitis A may prevent chronic hepatitis A infections. Serum ALT levels and chemotactic LTB_4 originating from PAF-stimulated leukocytes and plasma are correlated in hepatitis A and this finding indicates the above-mentioned assumption can be considered. This difference between hepatitis A and B may be related to the cytopathic effects of the hepatitis A virus.

We thus conclude that a high response rate LTB_4 secretion by peripheral leukocytes to stimulants like CaA23187 and PAF and correlation between either endogenous or PAF-stimulated LTB_4 and ALT levels suggest that LTB_4 has a very important place in A type acute viral hepatitis pathophysiology and clinical course.

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AUTOIMMUNE HEMOLYTIC ANEMIA WITH WARM ANTIBODIES IN CHILDREN*

Retrospective Analysis of 51 Cases

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SUMMARY: Gürgey A, Yenicesu İ, Kanra T, Özsoylu Ş, Altay Ç, Hiçsönmez G, Yetgin S, Tuncer M, Gümrük F, Çetin M. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Autoimmune hemolytic anemia with warm antibodies in children: retrospective analysis of 51 cases. Turk J Pediatr 1999; 41: 467-471.

In this paper, research based on 51 children with a positive antiglobulin test is presented. Eighteen of the children had acute anemia and 33 had chronic anemia. Two clinical patterns were distinguished: an acute transient type and a prolonged chronic type. Corticosteroid therapy was effective in all acute cases but its results were variable in the chronic cases. The acute form was more frequent in young children, while chronic autoimmune hemolytic anemia (AIHA) occurred mainly among children at puberty. In the chronic form of the disease, it was sometimes necessary to add immunosuppressive drugs and in two cases to perform a splenectomy. *Key words: autoimmune hemolytic anemia, Coombs' test, warm antibody.*

Autoimmune hemolytic anemia (AIHA) is a rare disorder in childhood and adolescence, and it has been shown by some of the clinical and laboratory findings to be quite different from adult AIHA[1]. Antibodies of the IgG are usually responsible for autoimmune hemolytic anemia in children. We would like to report here on the retrospective evaluation of 51 children with AIHA.

Material and Methods

This study reviews 51 children with AIHA whose ages ranged between two months and 16 years (median 6.2±5.35 years) and who were seen at Hacettepe University, Ihsan Doğramacı Children's Hospital between 1975 and 1996.

The patients were selected if they fulfilled the following criteria:

1. Clinical and laboratory onset of hemolysis prior to 16 years of age.
2. A positive direct and indirect antiglobulin test.
3. An adequate clinical and hematological follow-up.

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They were divided into two groups based on the duration of the disease, as a) acute transient cases if complete resolution of the disease was obtained within six months and b) chronic cases, of a duration longer than six months.

Routine hematological tests were carried out by standard procedures, immunohematological studies were conducted using previously described methods, and a battery of serologic tests were done for infectious diseases[2]. Immunologic investigations were done in 23 cases.

Results

Acute transient cases. Acute cases were diagnosed in 18 children (13 males and 5 females), whose ages at onset of the disease ranged from two months to 16 years (median 6.26±5.58) (Table I).

Table I: Age and Sex Distribution of 51 Patients with AIHA

	Acute Form n*=18	Chronic Form n=33	Total n=51
Male/Female	13/5	14/19	27/24
Age (years)			
<2	5 (9.8%)	5 (9.8%)	10 (19.6%)
3-5	5 (9.8%)	7 (13.7%)	12 (23.5%)
6-10	6 (11.7%)	6 (11.7%)	12 (23.5%)
11-16	2 (3.9%)	15 (29.4%)	17 (33.3%)
Total	18 (35.2%)	33 (64.7%)	51 (100%)

*n: number.
AIHA: autoimmune hemolytic anemia.

In half of the 18 patients, onset of AIHA was during the first five years of life. In five (27.7%) of the patients, hemolysis was observed after an upper respiratory tract infection (URTI). Parvovirus, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) were identified in only three patients (Table II); a causative organism could not be identified in the others. Some of the clinical and hematological data of the patients at the time of diagnosis are shown in Table III. Constant physical findings were pallor, tachycardia and jaundice. Moderate splenomegaly and hepatomegaly were noted in 11 patients. Hemoglobin levels ranged from 2.8 g/dl to 9.2 g/dl. Sixteen patients received steroid therapy (2 mg/kg prednisolone), and two patients who did not respond to a standard dose of steroid therapy were treated with intravenous immunoglobulin (1 of them was also given a high dose of methylprednisolone - 30 mg/kg/day for 7 days). One patient in each group had Evans' syndrome. Exchange transfusion was performed in two patients

(1 of them was also treated with steroid), and both died of profound hemolysis shortly after the procedure. One patient with mild hemolysis received no therapy and recovered spontaneously in a short period of time.

Table II: Associated Disorders in Patients with AIHA

	Acute	Chronic
URTI	5	2
CMV	–	1
EBV	–	2
SLE	–	3
JRA	–	3
Immune deficiency	–	2
Autoimmune disease	–	3
Chronic hepatitis	–	1

AIHA: autoimmune hemolytic anemia,
URTI: upper respiratory tract infection, CMV: cytomegalovirus, EBV: Epstein-Barr virus, SLE: systemic lupus erythematosus, JRA: juvenile rheumatoid arthritis.

Follow-up studies disclosed total permanent recovery in 16 patients. Interestingly, Hodgkin's disease developed in one patient nine years after the diagnosis of AIHA.

Chronic cases. This group included 33 children (14 males and 19 females) whose ages at onset of the disease ranged from five months to 16 years (median 6.8±5.9). Fifteen patients were above 11 years of age. None of the patients had a family history of AIHA. The clinical and hematological data of the patients at the time of presentation are shown in Table III. In two patients hemolysis occurred during the course of URTI. In 12 patients associated disorders were observed during the follow-up period. Six patients developed collagen disorders (3 systemic lupus erythematosus [SLE], 3 juvenile rheumatoid arthritis [JRA] and immunodeficiency (1 common variable immunodeficiency, 1 selective immunoglobulin M deficiency). Autoimmune diseases were associated in another five patients, and chronic persistent hepatitis was observed in one patient (Table II). Steroid was prescribed for all patients, and four were also given a high dose of methylprednisolone (HDMP). At the time of subsequent attacks, 10 patients (2 had HDMP) remained sensitive to the steroid but the effect was transient. Splenectomy was performed in two patients who were resistant to prednisolone therapy. In both patients hemolysis was completely cured following splenectomy. Immunosuppressive agents, 6-mercaptopurine and azathioprine were administered to two patients in whom hemolysis could not be controlled by standard or HDMP. Remission was obtained within one month. Unfortunately, three patients with associated disorders died. They suffered cerebral involvement of SLE, intestinal perforation (in a patient with immune deficiency), and disseminated CMV infection (in a case with immune deficiency).

Table III: Initial Clinical and Hematological Data in Acute and Chronic Cases

	Number of Patients	
	Acute (n=18)	Chronic (n=33)
Pallor	12	20
Jaundice	4	4
Splenomegaly	7	4
Hepatomegaly	7	3
Hemoglobinuria	3	2
Anemia 8-11 g/dl	3	30
<8 g/dl	15	3
Reticulocytosis <5%	10	31
>5%	7	2
Reticulocytopenia (<1%)	1	–
Direct antiglobulin test (+)	15	21
Indirect antiglobulin test (+)	3	12
Thrombocytopenia (<100,000/μl)	1	1

Discussion

Autoimmune hemolytic anemia occurs less commonly in children and adolescents than in adults[1]. Among children the peak incidence is the first five years of life[3]. Among our subjects, 41 percent of all (both acute and chronic) cases were diagnosed before the age of five years. In the present study, 64.7 percent of cases followed the chronic course (Table I). This figure has been reported to be as high as 74 percent during childhood in some previous studies[3-4]. Among the chronic cases, the majority were older than 11 at the onset of the AIHA. In the same age group, only two patients (10%) followed the acute course. It seems that the disease follows a chronic course in patients older than 11 years of age. In seven patients, hemolysis followed after upper respiratory tract infections. This finding is similar to those of the other studies[5]. CMV infection was found in only two patients with immune deficiency syndrome; it was not found to be an important etiological factor in our study group. AIHA associated with underlying chronic disorders was seen in 12 patients with chronic AIHA in our series. Multiple autoimmune disorders were observed in three patients whose cases have been reported previously[6], and Hodgkin's disease, which is well known to be associated with AIHA, developed nine years after AIHA in one patient[7]. It has been reported that thrombocytopenia occurred in about 14-32 percent of patients with autoimmune hemolytic anemia[8]. In this study thrombocytopenia was documented in two of our patients; reticulocytopenia was also observed but in only one patient. Autoantibodies directed against erythroid progenitors or precursors are thought to have been responsible for the reticulocytopenia[9].

Response to steroid therapy was observed in 16 of 18 of our patients with the acute form, and 10 patients with the chronic form showed a temporary improvement. Exchange transfusion was unsuccessful in two patients because of profound hemolysis leading to cardiac failure. It has been reported that if antibody production is an ongoing process, the effect of exchange transfusion or plasmapheresis is transient; success is limited possibly because more than half of the IgG is present in the extravascular compartment[10]. Several reports indicated that intravenous immunoglobulin (IVIG) may be useful in selected patients[10]. In our series, two patients received IVIG and steroid therapy together. A complete cure of hemolysis was observed in both patients.

Removal of the major site of red cell destruction has been shown to be an effective therapeutic strategy in IgG-induced hemolytic anemia, with a success rate of approximately 50 to 70 percent[10]. Therefore, splenectomy was performed in two of our patients who were resistant to steroid therapy, and a complete remission was obtained in both. Several chemotherapeutic agents have been used in the treatment of childhood AIHA[10]. Our two patients received 6-mercaptopurine and azathioprine and complete remission was achieved.

In conclusion, our study showed that chronic AIHA occurred mainly in older children and females. Corticosteroid was effective in the acute form of the disease but none of the chronic cases recovered fully. However, splectomy and immunosuppressive agents produced a clinical cure in patients with the chronic disease. Although the number is too small to be conclusive, it seems that splenectomy, immunosuppressive agents and IVIG should be used in the treatment of chronic cases if they are resistant to corticosteroid therapy.

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CEA, CA125 AND CA19-9 LEVELS IN CONGENITAL GASTROINTESTINAL ANOMALIES*

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SUMMARY: Baykal-Erkılıç A, Erkılıç M, Melikoğlu M, Aksu A. (Departments of Biochemistry, Nuclear Medicine and Pediatric Surgery, Akdeniz University Faculty of Medicine, Antalya, Turkey). CEA, CA125 and CA19-9 levels in congenital gastrointestinal anomalies. Turk J Pediatr 1999; 41: 473-481.

Preoperative and postoperative serum samples of 35 patients with different congenital gastrointestinal anomalies were analyzed for the markers CEA, CA 125 and 19-9 by immunoradiometric assay during a period of three years. The majority of the anomalies were aganglionic megacolon and hypertrophic pyloric stenosis. CA 125 and CA 19-9 were likely to indicate logistic model probabilities for babies with anomalies, while CEA was not ($F=35.78$, $p<0.05$ for CA CA 125 and $F=4.36$, $p<0.05$ for CA 19-9). Probability of no congenital anomaly for babies was:

$$p\ (Normal)=e^{4.41-0.13CA125-0.05CA19-9}/1+e^{4.41-0.13CA125-0.05CA19-9}$$

Using CA 125 as a marker, babies with congenital anomalies were determined with 83.3 percent probability ($F=11.33$, $p<0.05$). On the other hand, it was not possible to predict the type of anomaly with these three markers. CEA, CA 125 and CA 19-9 seem to be prognostic variables associated with congenital anomalies. These biological markers provide information that can be incorporated into the diagnosis of anomalies but without doubt results of markers should be supported by clinical findings.
Key words: tumor markers, anomalies, radioimmunoassay.

Tumor marker production is a reflection of the synthesis and secretion capability of the tumor, the rate of cellular growth, necrosis and programmed cell death[1]. Therefore, tumor markers play an important role in the assessment of patients with some types of malignant tumors[2]. These are not, however, specific markers that precisely predict the occurrence of a particular disease and furthermore, they may be increased in nonmalignant conditions as well[3].

In gastrointestinal tumors, the tumor markers CEA and CA 19-9 are accepted as markers and are usually increased. These markers are very useful in detection of postoperative recurrences and metastases. They are specific but are also detectable in different tumoral processes. Serum from patients with

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gastrointestinal carcinoma often contains increased CA 19-9 concentrations[4]. Increases in the serum concentrations of CEA have been associated with progression of cancers of the gastrointestinal tract, lung and breast. Despite its ubiquitous application as a tumor marker, CEA may be increased in patients with benign diseases of the same organs[5]. Many so-called markers currently used do not discriminate well enough between benign and malignant cells.

The value and distribution of tumor markers in congenital anomalies have not been sufficiently investigated. One can expect some changes of gastrointestinal tumor markers such as CEA and CA 19-9 in babies with congenital gastrointestinal anomalies.

On the other hand, CA 125, which is adequate as a marker for monitoring patients with known ovarian cancer and even for discriminating malignant from benign pelvic masses, has been suggested as an abnormal secretory product from the genital tract and organs[6]. Many factors like pregnancy, menstruation, benign gynecological diseases, and pleural and peritoneal inflammation can lead to increases of CA 125[7,8]. Increased concentrations of CA 125 can also be found in patients with various gastrointestinal malignancies[9].

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genital tract and organs[6]. Many factors like pregnancy, menstruation, benign gynecological diseases, and pleural and peritoneal inflammation can lead to increases of CA 125[7,8]. Increased concentrations of CA 125 can also be found in patients with various gastrointestinal malignancies[9].

We planned this study to investigate the distribution of these three gastrointestinal related tumor markers in congenital gastrointestinal anomalies.

Material and Methods

Patients

Thirty-five babies (23 males and 12 females; mean age 129±25 days) with congenital gastrointestinal anomalies were studied for a period of three years. All patients were evaluated by physical examination, ultrasonography and X-ray studies. The mean age of the mothers of babies with anomalies was 25.2 years. Sixteen males and eight females served as controls. The blood samples of these normal babies and their mothers, with a mean age of 26.3 years, were collected 123±30 days after delivery.

The study protocol conformed to local ethical standards and the Helsinki declaration of 1975, as revised in 1983.

Samples

Venous blood (3 ml) was collected from each patient pre- and postoperatively (4 weeks after the operation) for the measurement of serum tumor marker levels, into plain glass tubes. The serum sample was allowed to clot for 20 minutes then centrifuged at 1000 g for 10 minutes and stored at -20 °C until analysis. We also collected venous blood into plain glass tubes from age and sex-matched control subjects who did not have congenital anomalies. We centrifuged and stored the samples as previously described. Analysis was performed within one month after collection.

Analyses

We performed the following analyses on the samples from patients and controls: serum CEA, CA 19-9 and CA 125 levels were measured with solid phase two-sites immunoradiometric assays employing monoclonal antibodies (Cis biointernational, France).

Following the formation of the coated antibody/antigen/iodinated antigen sandwich, the unbound tracer was easily removed by a washing step. The radioactivity remaining at the tube wall was measured by gamma scintillation counter. Intraassay precisions were 4 percent, 7 percent, and 5 percent respectively.

Statistical Analysis

Student's paired t-test was used to assess the significance of any differences in serum tumor marker concentrations of the preoperative and postoperative babies with congenital gastrointestinal anomalies and of controls. Stepwise logistic regression analysis was used to assess the significance of CA 125, CEA and CA 19-9 levels in determination of congenital anomalies. Specificity of the markers for anomalies was determined with Stepwise discriminant analysis.

Results

Table I lists the characteristics of the study group of 24 normal babies and 35 babies with congenital anomalies.

Table I: Characteristics of 35 Babies with Various Congenital Gastrointestinal Anomalies

	n (Female, Male)	Age, Days±SD	Type of Anomaly
Baby with anomaly	9 (4,5)	124±23	Congenital aganglionic megacolon
	2 (1,1)	150±24	Esophageal atresia
	8 (1,7)	115±30	Congenital hypertrophic pyloric stenosis
	2 (1,1)	130±38	Gastrochisis
	5 (2,3)	138±20	Intestinal atresia
	5 (1,4)	140±26	Anal atresia
	1 (0,1)	110±21	Omphalocele
	3 (2,1)	130±17	Ectopic anus
Baby without anomaly	24 (9,15)	123±30	

When tumor marker levels in preoperative babies were compared with the concentrations in control subjects, babies with anomalies had significantly higher concentrations than the controls ($p<0.05$). Though it was not statistically significant, serum tumor marker levels tended to decrease with operation, and the concentrations of patients were not significantly higher than those of controls after the operation (Fig. 1). When tumor marker levels in mothers of babies with anomalies were compared with those of normal babies' mothers, CA 19-9 and CEA levels were increased, but there was not a statistically significant difference (Fig. 2).

With Stepwise logistic regression analysis, CA 125 and CA 19-9 were likely to indicate logistic model probabilites for babies with anomalies, while CEA was not ($F=35.78$, $p<0.05$ for CA 125 and $F=4.36$, $p<0.05$ for CA 19-9).

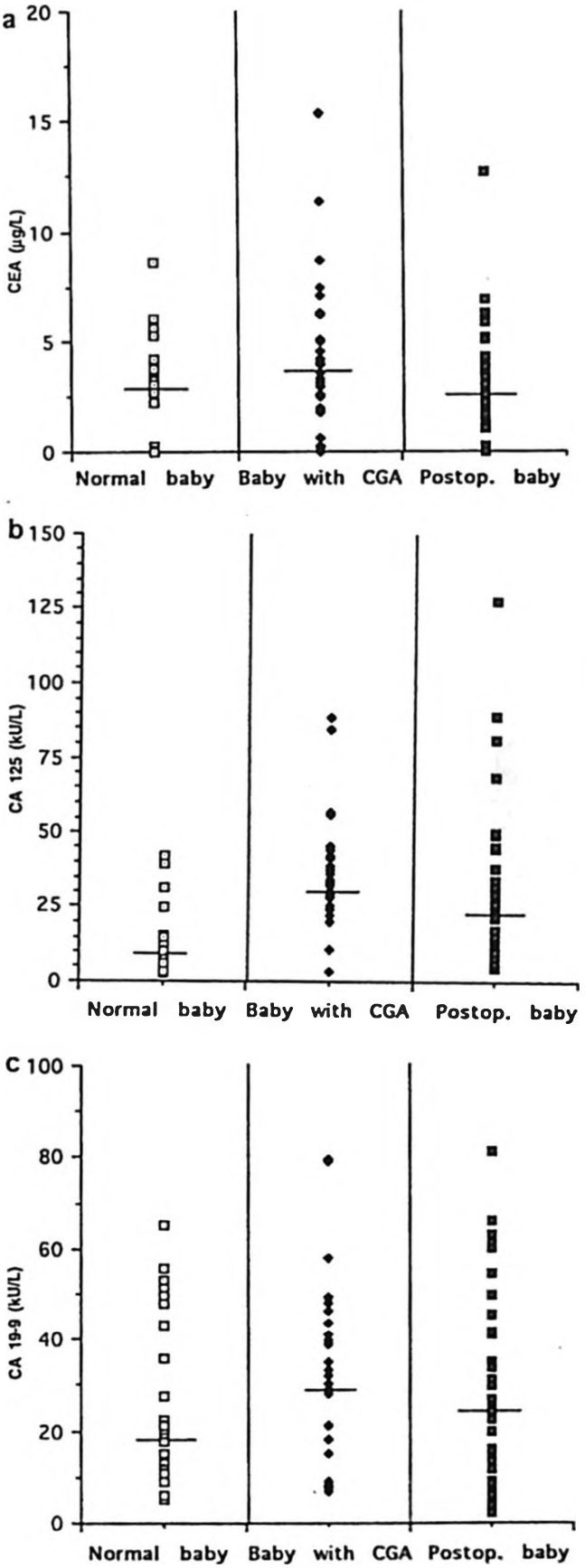

Fig. 1: Serum a) CEA, b) CA 125 and c) CA 19-9 concentrations in babies with congenital gastrointestinal anomalies preoperatively (◆) $p<0.05$, postoperatively (◘) $p>0.05$ and in age- and sex-matched controls (⊡). CA: congenital gastrointestinal anomalies. Horizontal lines indicate the median values.

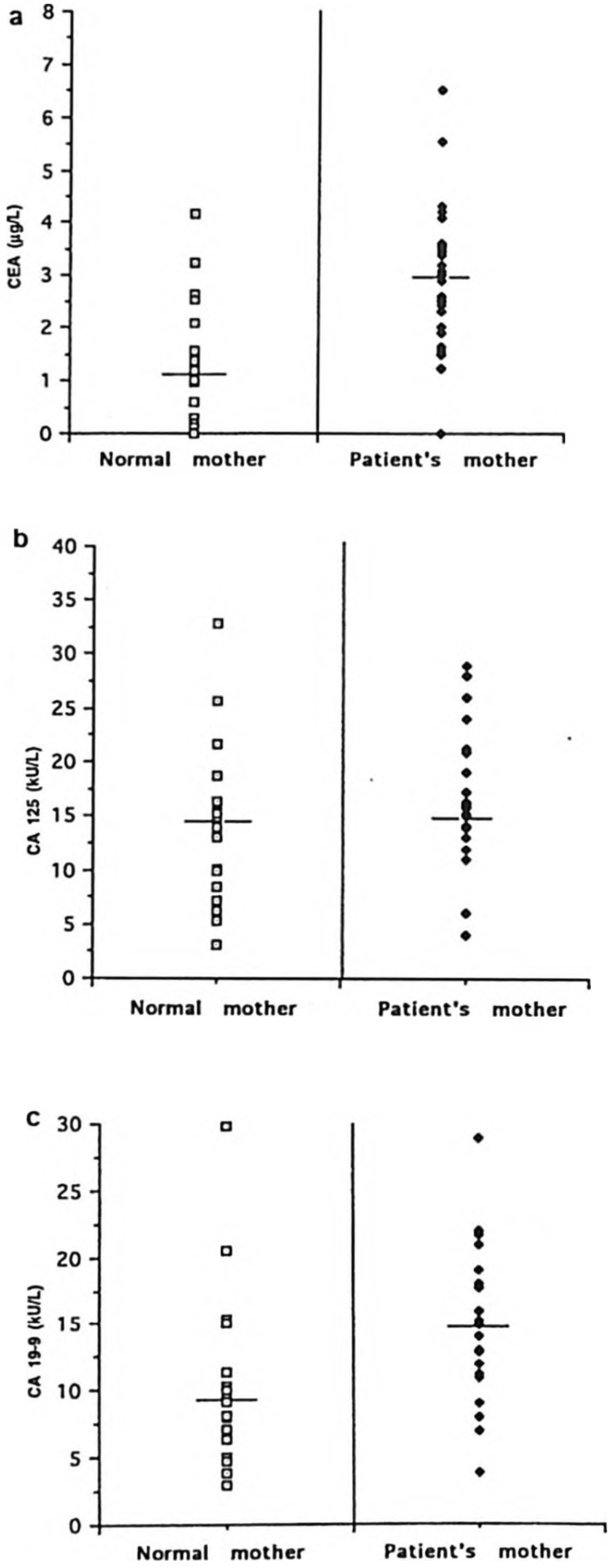

Fig. 2: Serum a) CEA, b) CA 125 and c) CA 19-9 concentrations in mothers of babies with congenital gastrointestinal anomalies (◆) p>0.05 and in mothers of control babies (⊡). Horizontal lines indicate the median values.

Probability of no congenital anomaly for babies was;

$$p\ (Normal)=e^{4.41-0.13CA125-\ 0.05CA19-9}/1+e^{4.41-0.13CA125-\ 0.05CA19-9}$$

Specificity of the markers for anomalies was determined with Stepwise discriminant analysis. This analysis was performed for babies with aganglionic megacolon and hypertrophic pyloric stenosis, as the number of babies in the other groups was not high enough for discriminant analysis. Thus, the relationship of marker level with type of anomaly was investigated in normal babies and babies with aganglionic megacolon and hypertrophic pyloric stenosis; mean marker levels in these anomalies and control babies are shown in Table II. It was found that, when using CA 125 as a marker, babies with congenital anomalies were determined with 83.3 percent probability ($F=11.33$, $p<0.05$).

Table II: Relationship Between Type of Anomaly and Serum Concentrations of CEA, CA125 and CA19-9

	Mean (SD)		
	CEA, ug/L	CA125, kU/L	CA19-9, kU/L
Congenital aganglionic megacolon (n=9)	4.57 (4.6)	42.75 (25.81)	46.43 (22.01)
Congenital hypertrophic pyloric stenosis (n=8)	3.37 (2.52)	29.29 (18.89)	37.75 (9.38)
Normal babies (n=24)	2.36 (2.39)	12.83 (10.89)	28.37 (23.07)

It was possible to classify aganglionic megacolon patients (44.4%) and hypertrophic pyloric stenosis patients (12.5%). In total, 61 percent were classified correctly.

Discussion

We observed that tumor marker levels were affected in congenital anomalies, as babies with anomalies had significantly higher concentrations of tumor markers than controls ($p<0.05$). Tumor markers lack specificity in that they are present or increased in subjects with benign disease or even in normal individuals[3,5]. These are not truly cancer-specific substances. Tumor markers are usually due to differentiation during fetal life and thus are oncofetal antigens. We suggest that the increased levels of tumor markers indicate the patient's work-up should include an assessment of non-neoplastic disease. Congenital anomalies seem to be one of the conditions in which tumor markers are increased. It is also possible that babies with anomalies are more susceptible to carcinogenesis when they grow older. According to Potter's[10] deletion hypothesis, cytoplasmic reactions may control cell division; carcinogens may cause loss of these control reactions. It is obvious that control of cell division is lost in congenital anomalies. The Vogelstein hypothesis[11] incorporates the proposal that most colorectal carcinomas arise from preexisting benign adenomas. Congenital anomalies in which control of cell division has been lost may contribute to the development

of colorectal carcinomas. Such reasoning suggests that further research on cancer incidence in babies with anomalies should be encouraged, as chromosomal changes and point mutations are crucial events in both carcinogenesis and anomalies and lie at the heart of all current genomic models.

CA 125 and CA 19-9 were increased in babies with anomalies, and these variables indicated the logistic model probabilities of having anomalies. Increases of CEA in serum were seen in benign and malignant diseases of the pancreas, colon, liver, lung and breasts[5], but it did not increase in babies with congenital anomalies. Assuming that assessment of marker in mothers could provide valuable information, measurements were also performed using sera of mothers. CEA and CA 19-9 levels were increased in mothers of ill babies when compared with those of normal babies' mothers, but there was not a statistically significant difference, suggesting that these markers cannot be used to monitor development of congenital gastrointestinal anomalies. Whether the marker levels of mothers increased during pregnancy and birth remains to be determined.

The effects on CA 125 in babies were more pronounced than effects on the other two markers. One can expect placental crossing of the CA 125 antigen, but its natural half-life in serum was estimated at 4.8 days[12]. Thus, maternal transfer due to pregnancy or to benign gynecologic problems present in the mothers of this group was ruled out. Appropriate timing of blood collections for tumor marker assays is crucial, so blood was collected from babies when they were 129±25 days old, taking into account the half-lives of markers. Again, the high tumor marker values, which could be due to their necrotic releases after operation which were erroneously reported as false-positive increases, was ruled out by collecting the samples four weeks' postoperatively. Blood samples were also taken postoperatively in the hope of using results as a marker for monitoring disease course. Venous sera of babies were collected four weeks after operation. Though serum tumor marker levels tended to decrease with operation, this was a statistically nonsignificant difference when compared with preoperative values. One reason for this may be the need of a second operation for some babies. It was reported that tumor marker levels which do not return to normal are reflective of residual disease[13,14]. It is also possible that markers will return to normal after a longer period of time.

Tumor markers were thought to be increased in malignancies due to cellular proliferation. When we classified our patients as having proliferative or atretic anomalies, there was no difference in tumor marker values of these two groups.

CEA, CA 125 and CA 19-9 seem to be prognostic variables associated with congenital anomalies. In the future, risk factors and markers will be used, to determine who is destined to develop diseases. Although this study may not yet confirm the use of markers in early detection of congenital anomalies, we

believe there is a need for studies on this subject so that use of these markers as criteria for congenital anomaly diagnosis would be justified by available information. Aside from the issue of cost, standardization of these serum assays is well advanced and interlaboratory reproducibility has been steadily improving. There are no complications in this type of diagnosis, and these biological markers provide information that can be incorporated into diagnosis of anomalies. Still, there is no doubt that marker results should be supported by clinical findings.

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The Turkish Journal of Pediatrics 1999; 41: 483-488 Original

FOUR DIFFERENT HERNIAS ARE ENCOUNTERED IN THE ANTERIOR PART OF THE DIAPHRAGM*

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*Nebil Büyükpamukçu MD****

SUMMARY: Salman AB, Tanyel FC, Şenocak ME, Büyükpamukçu N. (Departments of Pediatric Surgery, Atatürk University Faculty of Medicine, Erzurum and Hacettepe University Faculty of Medicine, Ankara, Turkey). Four different hernias are encountered in the anterior part of the diaphragm. Turk J Pediatr 1999; 41: 483-488.

A retrospective clinical study was performed to evaluate the hernias encountered in the anterior part of the diaphragm. Twenty patients (14 males, 6 females; aged 7 days-7 years) with hernias located in the anterior part of the diaphragm who were treated surgically formed the study group. The exact locations, contents and additional malformations were evaluated. The locations were parasternal in 14 and retrosternal in six. Parasternal locations were the right side in 11, left side in two and bilateral in one patient. Three patients had trisomy 21 syndrome. A sac was presented in all cases and included the colon in 12 patients. A patient with retrosternal location also had trisomy 21 syndrome. The patients with retrosternal hernias also presented with sacs, and the colon was the most commonly included viscus. In the presented series, no intrapericardial herniations or anteromedial defects were encountered. Comparison of previously reported patients and the present series suggests that the anterior part of the diaphragm hosts various hernias of congenital origin in its different locations. According to the exact location and the presence or absence of sacs, four different types of hernias occur in this area: retrosternal hernias with a sac, intrapericardial herniation, and parasternal and anteromedial hernias with either unilateral or bilateral involvements. Since four different hernias were distinguished, the term Morgagni hernia does not include or define all the hernias of the anterior part of the diaphragm. We believe they should, therefore be designated according to the location and presence or absence of a sac. *Key words: congenital diaphragmatic hernia, Morgagni hernia.*

Material and Methods

During a 15-year period from 1980-1995, 20 patients diagnosed with hernias located in the anterior part of the diaphragm in the Departments of Pediatric Surgery of Hacettepe Children's Hospital and of Atatürk University Hospital were evaluated retrospectively.

The exact localization of the defect was determined through preoperative radiological evaluations and operative findings.

The presence or absence of a sac, included organs and additional anomalies were recorded.

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Results

Twenty patients were diagnosed with hernias in the anterior part of the diaphragm. The locations were parasternal in 14 (10 males, females) six (4 males, 2 females) and retrosternal in six two (Table I). Parasternal locations were the right side in 11 (Fig. 1), left side in two (Fig. 2), and bilateral in one patient of the 14 with parasternal hernias, eight patients were younger than one year of age. Three patients, including one with ventricular septal defect (VSD) and patent ductus arteriosus (PDA), had trisomy 21 syndrome. A sac was presented in all cases and included the colon in 12 patients. Only one patient had malrotation.

Six patients with retrosternal hernias, five patients were younger than one year of age. One had trisomy 21 syndrome. The patients with retrosternal locations also had sacs and the colon was the most commonly included viscus (Fig. 3).

Midline epigastric incisions were used in 19 patients and a right thoracotomy was performed in one patient.

Postoperative courses were uneventful.

Table I: Clinical Characteristics of Patients

Patient	Sex	Age at Presentation	Site of Hernia	Organs in Hernia	Associated Anomalies
M.K.	F	7 days	Retrosternal	Left lobe of liver, small bowel	
Ö.B.	M	3 months	Right parasternal	Left lobe of liver, small bowel	
A.K.	M	4 months	Retrosternal	Colon	
K.G.	M	7 months	Right parasternal	Left lobe of liver, colon	Malrotation
S.G.	F	7 months	Right parasternal	Colon	
F.A.	F	8 months	Retrosternal	Left lobe of liver, colon	
K.S.	M	8 months	Right parasternal	Colon	Trisomy 21
T.C.	M	8 months	Right parasternal	Colon	
R.O.	M	9 months	Retrosternal	Colon, omentum	Trisomy 21
S.S.	F	9 months	Right parasternal	Colon	
H.B.	F	9 months	Left parasternal	Colon	Trisomy 21
H.A.	M	9 months	Left parasternal	Colon	
G.A.	M	11 months	Retrosternal	Colon	
Ö.Y.	M	4 years	Bilateral parasternal	Right colon, left small bowel	
S.I.	F	5 years	Right parasternal	Omentum	VSD+PDA Trisomy 21
C.F.	M	5 years	Right parasternal	Colon	
F.G.	M	5 years	Right parasternal	Colon	
M.K.	M	6 years	Right parasternal		
B.K.	M	6 years	Retrosternal	Colon	
H.B.	M	7 years	Right parasternal	Colon	

VSD: ventricular septal defect
PDA: patent ductus arteriosus.

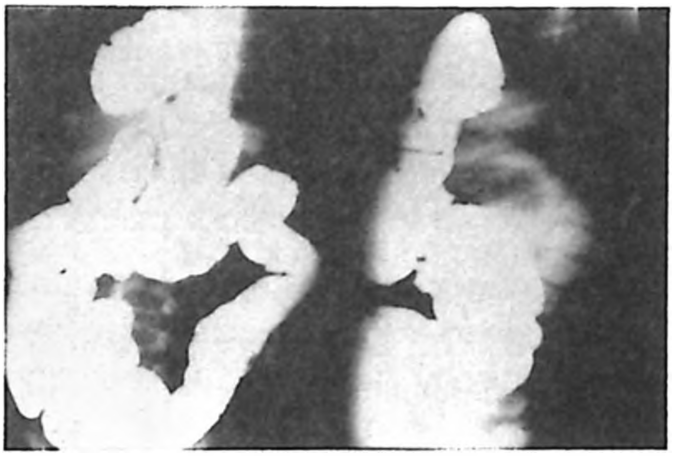

Fig. 1: Parasternal hernia involving the right side (left: posteroanterior view; right. lateral view).

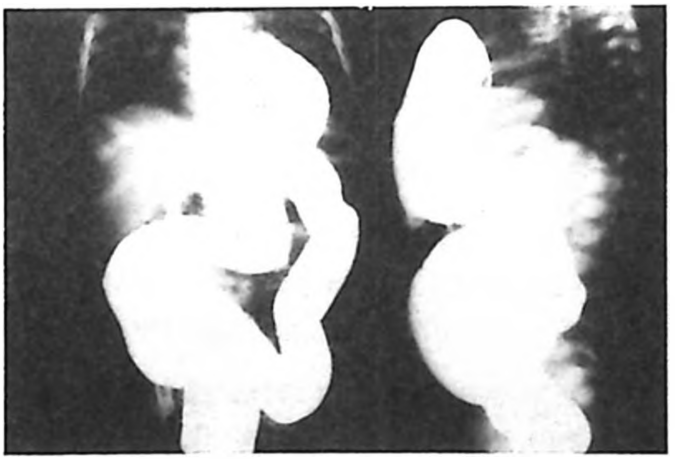

Fig. 2: Parasternal hernia involving the left side. (left: posteroanterior view; right: lateral view).

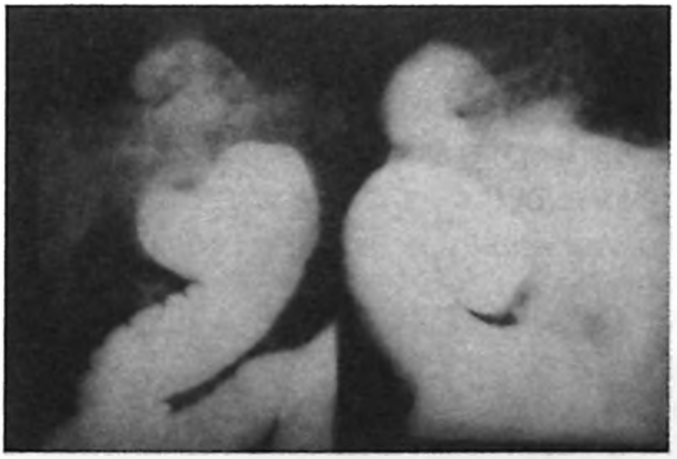

Fig. 3. Retrosternal hernia (left: posteroanterior view; right: lateral view)

Discussion

Although hernias are also encountered in the anterior part of the diaphragm, the term congenital diaphragmatic hernia describes the posterolateral form[1]. In contrast to the abundant studies and large chapters in textbooks, limited information is available for hernias encountered in the anterior part of the diaphragm.

Since the description of an anterior diaphragmatic hernia in an adult by Morgagni in 1761[14], all hernias encountered in this region are traditionally named as hernia of Morgagni. The hernia originally described by Morgagni had a parasternal location. The congenital origin has been questioned, and a direct herniation through the foramen of Larrey has also been suggested[2,15]. Therefore, the traditional nomenclature of Morgagni hernia may not satisfactorily explain the hernias seen in the anterior part of the diaphragm during the neonatal period and infancy. Additionally, these hernias have been evaluated under different titles, which suggests different anatomical locations. However, most of the cases were evaluated under the title of Morgagni hernia, and thorough evaluation of those reports fails to clarify the exact location and characteristics of the pathologies[8,16,17]. In any case, although evaluated under the title of Morgagni hernia, some reports suggest locations other than those described by Morgagni[11,12,18].

The evaluation of our clinical material revealed two different locations, parasternal and retrosternal, for hernias encountered in the anterior part of the diaphragm, with a parasternal location occurring more frequently.

The parasternal hernia was more frequent among males, and among those under one year of age in the present series. It frequently occurred in the right side. The 94 cases in the literature which were clearly defined with a parasternal location, also revealed a male predominance. Thirty were children and 64 were elderly. While the side in three patients was not clarified, right side, bilateral and left side locations were reported in 11, 9 and 5 patients, respectively[8,9,11,12,14,16,19,20]. All patients except one had sacs. One patient had an intrapericardial herniation. This suggests the case was suspect for a parasternal hernia. Although manuscripts do suggest the possibility of absence of sac, the two reports always referred to in this regard are subject to question on this point[16,17]. Hernias with parasternal location have right side predilection, but there is a tendency towards bilateral occurrence. The age distribution of those with parasternal locations resembles that of inguinal hernia, appearing more commonly among the elderly and infants.

Retrosternal hernia was more frequent among males and infants in the present series. All the patients also had sacs. Among six cases in the literature with hernias located retrosternally, four were children and two were adults and a had sacs[2,18,21,22]. A newborn patient with a retrosternal hernia also had bilateral posterolateral hernias.

Review of the literature also reveals patients with anteromedial defects and intrapericardial herniations. Anteromedial defects are also referred to as ventral diaphragmatic or septum transversum defects. They are described as semilunar defects of the ventral diaphragm extending dorsally almost to the vena caval opening and anterolaterally to the rib cage in the mid-clavicular line[8]. All 14 patients reported in the literature with anteromedial hernias were children. The frequency among males and females was equal. Three patients, including one with Cantrell's pentalogy and two others with omphalocele, had intrapericardial herniations[13,23,24].

Results of our clinical series and literature findings of location and age distribution suggest the parasternal hernia to be different from others. Retrosternal hernia, anteromedial hernia, and intrapericardial herniation might be the components of a spectrum. However, in Cantrell's pentalogy, retrosternal hernia with sac has not been reported[25]. Contrary to male predominance among patients with retrosternal hernia, equal sex distribution is encountered in anteromedial hernia, which may also occur without the other components of Cantrell's pentalogy. Isolated intrapericardial herniation however, has not been clearly documented. Therefore, it seems possible to suggest that four different hernias are encountered in the anterior part of the diaphragm: parasternal and anteromedial hernias with either unilateral or bilateral locations, and retrosternal and intrapericardial hernias. Since the normal embryologic development of the diaphragm remains controversial[26], proposing an embryologic basis for the hernias of the anterior part of the diaphragm is speculative.

The features of hernias encountered in the anterior part of the diaphragm are different from the features encountered in Bochdalek's hernia. Bochdalek's hernia is more frequent and follows a grave course. It is known that Bochdalek's hernia is usually without a sac and involves the left side, with a minimal tendency towards bilateral occurrence. Additionally, Bochdalek's hernia has an increased incidence among patients with trisomy 13 and 18[6], while the hernias of anterior diaphragmatic portion are more frequent in trisomy 21[14,12,20,27,28].

Since four different types of congenital hernias are encountered in the anterior part of the diaphragm, the traditional nomenclature of Morgagni's hernia is unsatisfactory. The anatomical location and presence or absence of a sac should be defined to clarify the exact type of the hernia in this region.

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HEMOSTATIC SYSTEM IN EARLY RESPIRATORY DISTRESS SYNDROME: REDUCED FIBRINOLYTIC STATE?*

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SUMMARY: Yurdakök M, Yiğit Ş. (Neonatology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine. Ankara, Turkey). Hemostatic system in early respiratory distress syndrome: reduced fibrinolytic state? Turk J Pediatr 1999; 41: 489-493.

Previous studies suggest that there is a systemic activation of clotting and fibrinolysis in preterm infants with advanced respiratory distress syndrome (RDS). However, there are no data on the hemostatic status in the early stages of the disease; therefore, we studied some of the hemostatic parameters in these patients and made several studies at different times in preterm infants who did or did not develop RDS, using similar protocols. We found normal plasma fibrinogen, protein C, protein S, C4b-binding protein, thrombomodulin, antithrombin III, thrombin-antithrombin III complex, prothrombin fragment 1.2, plasminogen, tissue plasminogen activator, alpha-1 antitrypsin, alpha-2-macroglobulin and protein Z. However, lower D-dimer and higher plasminogen activator inhibitor and von Willebrand factor antigen levels were found within six hours of life in infants who later developed RDS compared to the control group. These findings suggest that disseminated intravascular coagulation is not prominent in the early stages of RDS. Moreover, reduced D-dimer and increased plasminogen activator inhibitor and von Willebrand factor antigen levels are probably related to the abnormalities in the fibrinolytic mechanism due to lung damage in RDS, but further studies are needed to show their pathogenic significance in RDS. *Key words: disseminated intravascular coagulation, fibrinolysis, hemostatic system, newborn infants, respiratory distress syndrome.*

Clinical and animal studies demonstrate abnormalities in the coagulation systems in adult respiratory distress syndrome (ARDS). Common ARDS-related coagulation disorders include disseminated intravascular coagulation (DIC) and inhibition of fibrinolysis. Disruption of the alveolocapillary membrane integrity in RDS results in leakage of coagulation factors into the alveolus. The combination of high levels of alveolar-activated procoagulant factors and inhibited fibrinolysis leads to alveolar fibrin deposition and hyaline membrane formation in RDS. DIC may not only be associated with RDS, but it, may be a predisposing factor for developing RDS. Since fibrinogen and fibrin-degradation products (FDP) are known to be potent inhibitors of surfactant and thrombin, FDP and plasmin may increase pulmonary damage by inducing chemotaxis and aggregation of neutrophils. Therefore, it has been suggested that attenuation of lung injury may occur and increased survival may be achieved by interrupting the coagulation cascade early in RDS[1].

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It has been reported that there is a systemic activation of clotting, fibrinolysis, and kinin-kallikrein in infants with RDS within 12 to 24 hours of birth[2]. However, there are no data on the hemostatic status in the early stages of the disease. Therefore, we studied some of the DIC parameters in these patients. The main limitation in these studies is the ethical dilemma of attaining enough blood samples in the first few hours of life to evaluate the various parameters of the coagulation and fibrinolytic systems simultaneously. For this reason, we mad several studies in preterm infants at different times using similar protocols. All neonates included in the study received vitamin K_1 (1 mg) intramuscularly upon delivery. Infants who were in a stable clinical condition served as the control group. The study group comprised infants who developed RDS, which was considered to be present if all the following diagnostic criteria were fulfilled: symptoms of respiratory distress within one hour after birth and present for at least 24 hours, respiratory support including mechanical ventilation, and typical findings on lung x-ray and arterial blood gas analysis. None of the infants with RDS had any other disease. All blood samples for hematological testing were obtained from a peripheral vein within six hours after birth.

In developed DIC, reduced fibrinogen, protein C and antithrombin III (AT-III) levels, as well as elevated levels of FDP, including D-dimers (D-D), are found[3]. Although decreased levels of AT-III have been described in preterm infants who developed RDS[4], we found normal AT-III and protein C levels within six hours after birth in preterm infants who developed RDS[5].

Protein S, a vitamin K-dependent cofactor for activated protein C, exists in plasma in a free active form and in an inactive form bound to C4b-binding protein (C4b-BP). The increased protein S anticoagulant activities (i.e. free protein S levels) compared with low total protein S levels are probably due to low or undetectable levels of C4b-BP in newborn infants[6]. On the other hand, C4b-BP is an acute phase reactant protein and present a strong increase in inflammation. The increased levels of C4b-BP can affect the distribution of protein S in plasma, producing a shift in protein S to the complex inactive form[7]. Therefore, we studied total protein S and C4b-BP in pretem infants with or without RDS in the first few hours of life. Although the free protein S levels could not be determined, we found normal total protein S and C4b-BP levels (unpublished data).

Thrombomodulin is a high-affinity thrombin receptor present on endothelial cells. It binds and inactivates thrombin and simultaneously activates protein C. Thrombomodulin is also present in the circulation, and this soluble form of thrombomodulin appears to be active in its endothelial form[8]. Hypoxemia[9], adult RDS[10] and DIC[11] may cause an increase in plasma thrombomodulin levels; however, we found normal plasma thrombomodulin levels in preterm infants who developed RDS in the first six hours of life[12].

Fibrin-degradation products, including D-D, are the end products of fibrinolysis which result from the conversion of an inert plasma proenzyme (plasminogen) into a proteolytic enzyme (plasmin)[3]. Therefore, evaluation of plasminogen levels is important in the biological interpretation of serum FDP in cases of DIC. If the concentration of plasminogen is very deficient, a low level of FDP cannot exclude DIC. We found normal plasma plasminogen levels in preterm infants who developed RDS[13]. Therefore, the reduced D-D levels in preterm infants who developed RDS could not be explained by normal plasminogen levels. Reduced D-D levels may also be due to increased clearance of FDP or overconsumption during DIC. The finding of normal plasma fibrinogen, AT-III and protein C, but of reduced D-D levels within six hours after birth in preterm infants who developed RDS suggests that DIC is not prominent in the early stages of RDS[5].

Theoretically, plasma protein C, AT-III, and even D-D levels are not sensitive indicators of DIC. The specific detection of thrombin should be suitable for use in the active state of DIC. However, thrombin is very rapidly bound and thereby inactivated by its main physiological inhibitor, AT-III. For this reason, a more direct method to evaluate the thrombin level is to measure the thrombin-antithrombin complex (TAT) formed with AT-III[3]. Prothrombin fragment 1.2 (PF1.2), which is released from prothrombin during its activation to thrombin, is also important in the diagnosis of active DIC[3]. However, we found normal TAT and PF1.2 levels in the first few hours of life in preterm infants who developed RDS[14]. These relatively more specific parameters (i.e., TAT and PF1.2) of DIC support our hypothesis, as DIC is not a prominent event in early-stage (or developing) RDS[5].

We found normal tissue plasminogen activator (tPA) and higher plasminogen activator inhibitor (PAI) levels in preterm infants who later developed RDS compared to the control group[4]. Both tPA and PAI are isolated from many cells, including the endothelium[15]. The increased levels of PAI in developing RDS may be related to endothelial damage, because in the early stages of RDS we found increased levels of von Willebrand factor antigen (vWF-Ag)[16], which is a sensitive marker for systemic and/or lung endothelial damage[17]. However, the reason for increased PAI levels without any increase in tPA levels remains to be elucidated.

Alpha-2-macroglobulin and alpha-1-antitrypsin are produced and secreted by various cells, including endothelial cells. Therefore, increased levels of these slow thrombin inhibitors may be expected in RDS; we found normal levels in these patients in the earlier stages of the disease[18,19].

Although the physiological function of protein Z, a vitamin K-dependent protein, is still unknown, the observation that thrombin associates with phospholipid surfaces in the presence of bovine protein Z has prompted the suggestion that this phenomenon may provide a mechanism whereby thrombin is kept from

diffusing into the vascular lumen and away from the site of injury[20]. However, we found normal plasma protein Z levels in the first few hours of life in preterm infants who developed RDS[21].

Increased TAT formation, increased tPA plasma concentrations and increased plasma kallikrein activity within 12 to 24 hours of birth in infants with RDS suggested that there is a systemic activation of clotting, fibrinolysis, and kinin-kallikrein[2]. However, the abnormalities in clotting and fibrinolysis in the early stages of RDS are presumably different from that seen in the later stages of the disease. We found, for example, normal plasma fibrinogen. AT-III, protein C, thrombomodulin, protein S, C4b-BP, plasminogen and tPA, but lower D-D and higher PAI levels within six hours of life in preterm infants who later developed RDS (Table I). Therefore, activation of clotting is not prominent in the early stages of RDS and a reduced fibrinolytic system is more prominent. Further studies will clarify the changes in the hemostatic system in earlier and later stages of RDS will and show their pathogenic significance in developing RDS.

Table I: Some Hemostatic Parameters in Infants with RDS in the First Few Hours of Life (mean ± SD)

	Controls	Infants with RDS	Ref.
Fibrinogen (mg/dl)	170.3 ± 16.4	74.4 ± 20.9	5
Protein C (%)	40.3 ± 46.7	45.5 ± 56.0	5
Total protein S (%)	40 ± 8	37 ± 11	Up
C4b-binding protein (%)	123 ± 163	141 ± 193	Up
Thrombomodulin (ng/ml)	18.3 ± 22.8	8.1 ± 10.2	12
Antithrombin III (mg/dl)	18.4 ± 1.1	18.9 ± 4.4	5
Thrombin-antithrombin III complex (µg/L)	78.1 ± 72.8	64.8 ± 69.9	14
Prothrombin fragment 1.2 (nmol/L)	9.0 ± 7.4	9.9 ± 5.7	14
D-dimer (µg/ml)	4.0 ± 0.9	2.1 ± 0.8*	5
Plasminogen (%)	55.3 ± 33.1	41.4 ± 19.2	13
Tissue plasminogen activator (mg/ml)	17.1 ± 8.8	19.2 ± 11.4	5
Plasminogen activator inhibitor (IU/ml)	7.1 ± 6.0	34.8 ± 14.4*	5
von Willebrand factor antigen (%)	65.9 ± 11.6	73.3 ± 12.5**	16
Alpha-1 antitrypsin (g/dl)	1.1 ± 0.4	0.9 ± 0.4	18
Alpha-2-macroglobulin (mg/dl)	116.7 ± 30.5	104.8 ± 34.4	19
Protein Z (µg/ml)	0.28 ± 0.14	0.34 ± 0.20	21

Mann-Whitney U test: * $p < 0.001$, ** $p < 0.05$

RDS: respiratory distress syndrome; UP: unpublished.

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CONGENITAL MICROVILLUS ATROPHY IN A 4 - MONTH - OLD GIRL*

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SUMMARY: Acar Y, Ertem D, Özgüven E, Okar İ, Ahıskalı R, Pehlivanoğlu E. (Department of Pediatric Gastroenterology, Marmara University Faculty of Medicine, İstanbul, Turkey). Congenital microvillus atrophy in a 4-month-old girl. Turk J Pediatr 1999; 41: 495-500.

Congenital microvillus atrophy is a severe generalized enteropathy with ultrastructural abnormalities of the intestinal brush border. It is a rather new clinicopathological entity which needs to be differentiated from other enteropathies within the spectrum of intractable diarrhea of infancy. The presented case was a four-month-old girl with a chronic, intractable diarrhea, beginning at birth. The diagnosis was established only after the electron microscopic examination of small intestinal mucosa which revealed the characteristic features of the disease. Congenital microvillus atrophy is a rare autosomal recessively inherited disorder and bowel transplantation becomes a realistic option of treatment. Therefore, it should be specifically considered in the differential diagnosis of chronic intractable diarrhea of infancy. *Key words: congenital microvillus atrophy, intractable diarrhea, infant.*

Intractable diarrhea in infancy denotes a clinical symptom complex characterized by the onset of noninfectious diarrhea before three months of age which lasts longer than two weeks and results in severe malabsorption and malnutrition[1]. Within this group of heterogeneous patients, congenital microvillus atrophy is a specific entity with abnormal ultrastructural features of the small intestine that distinguish it from other causes of congenital diarrhea with normal ultrastructure of the enterocytes[2-6]. Clinical features of the disease are a protracted diarrhea starting at or soon after birth, which is resistant to all therapeutical interventions and results in failure to thrive[2,3,5]. The diagnosis of microvillus atrophy can be made by demonstration of abnormal periodic acid-Schiff (PAS) stained enterocytes on light microscopy, increased numbers of secretory granules in the apical cytoplasm of enterocytes, and the presence of microvillus inclusions detected on electron microscopic

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examination of the small intestinal biopsy[2-5]. We present this case because congenital microvillus atrophy is a rare but perhaps an important cause of intractable diarrhea of infancy. It could be easily overlooked if it is not specifically considered.

Case Report

A four-month-old girl was transferred to the Department of Pediatric Gastroenterology and Nutrition at Marmara University School of Medicine for the evaluation of chronic diarrhea, beginning at birth. The patient was born at term to healthy, consanguineous Turkish parents with two healthy boys, after a normal pregnancy and an uncomplicated delivery. The infant was vigorous at birth and the birth weight was 2500 g. At home, the newborn breastfed poorly, had several loose, watery stools and began to vomit after the feeds. She developed peripheral edema within the first days of life which resolved spontaneously. At the end of the first month, the edema reappeared on the face, the diarrhea persisted and the severe episodes of vomiting led to a poor weight gain. At 10 weeks of age, the patient was taken to a local hospital where she was hospitalized because of pneumonia and was given an appropriate antibiotic therapy. During the hospital stay, the initial hemoglobin level was 6.5 g/dl, and she was transfused twice. Laboratory investigation revealed hypoproteinemia (total protein 5.9 g/dl, albumin 2.7 g/dl with a normal urine analysis. Thereafter, the patient was transferred to our unit for further investigation of the chronic diarrhea and hypoproteinemia.

The physical examination showed a malnourished infant with a weight of 3530 g and a length of 53 cm. Both measurements were below the 3rd percentile and her nutritional index was 45 percent. She appeared pale with edema on cheeks, tibiae, and dorsum of feet and hands, and had hepatosplenomegaly.

Initial laboratory studies showed anemia (hemoglobin 9.3 g/dl), leukocytosis (18700/mm^3), elevated transaminases levels (AST 83 U/L, ALT 66 U/L), hypoproteinemia (protein 5.0 g/dl, albumin 2.0 g/dl), and elevated cholesterol and triglyceride levels (203 mg/dl and 319 mg/dl, respectively). Her renal functions and serum electrolyte levels were within normal ranges with a normal arterial blood gas analysis. Further investigations revealed normal glucose homeostasis, and normal serum lactate, pyruvate and ammonia levels. Her urine analysis did not reveal any reducing sugar, ketone or protein in the urine. Serum and urine amino acid profiles were normal. The circulating autoantibodies, including antinuclear, antimitochondrial, antismooth muscle and anti liver-kidney microsomal antibodies were all negative. Thyroid function tests and serum immunoglobulin levels were within normal ranges. The examination of the stool showed a normal pH and chymotryptic activity, and there was no pathological alpha1-antitrypsin excretion. The repeated stool cultures were negative for any pathogenic microorganism. The quantitative sweat chloride test was normal. Screenings for hepatitis A, B, C viruses and cytomeglovirus were also negative.

On the clinical course, the patient's diarrhea and vomiting persisted, and enteral feeding was not tolerated. Total parenteral nutrition (TPN) was initiated and the patient became TPN dependent with only minimal tolerance to enteral feeding. The upper and lower gastrointestinal endoscopies performed at this time were macroscopically unremarkable. The histopathological examination of hematoxylin and eosin (H&E) stained esophageal, gastric, duodenal and rectal biopsies were normal under light microscopy (Fig. 1). On the electron microscopy (EM), however, the ultrastructural examination of the duodenal mucosa revealed partial microvillus atrophy on the apical membrane of the enterocytes (Fig. 2). After the diagnosis of microvillus atrophy was confirmed by the EM, the duodenal specimens which were stained with periodic acid-Schiff (PAS) revealed an abnormal accumulation of PAS-positive material within the apical cytoplasm of the epithelium under light microscopic examination (Fig. 3).

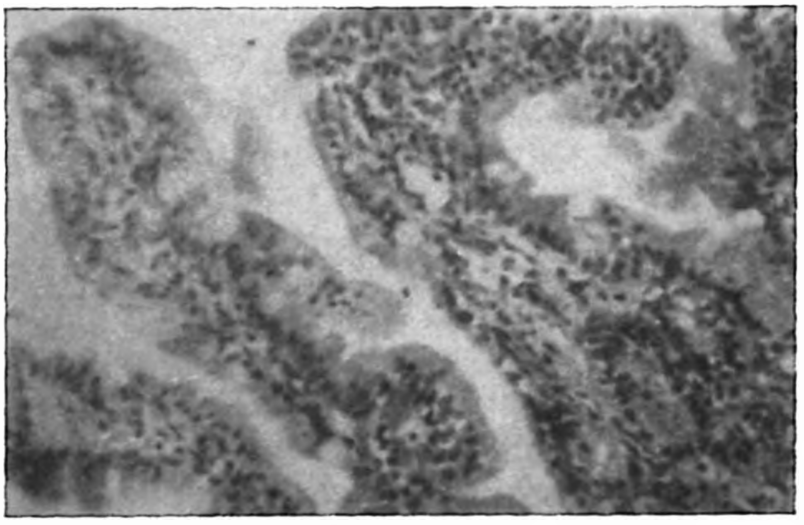

Fig. 1: The light microscopic appearance of the proximal small intestinal mucosa was normal in the patient with congenital microvillus atrophy (H&E x 40).

Fig. 2: Electron microscopy revealed a partial microvillus atrophy (arrows) on the apical membrane of the enterocyte (x 25,000).

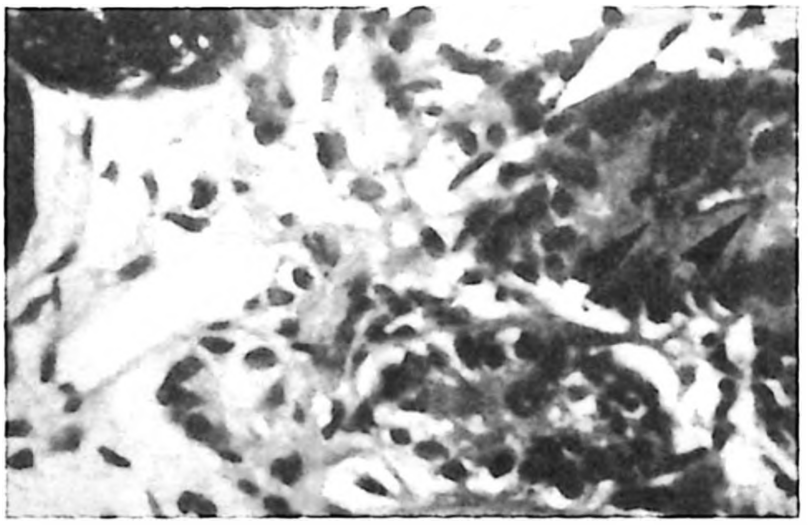

Fig. 3: Abnormal accumulation of PAS-positive material (arrows) in the apical cytoplasm of epithelial cells seen in duodenal specimen (PAS x 40).

The hepatic biopsy which was performed because of persistent hepatomegaly and increased transaminases levels showed diffuse macrosteatosis. The patient was still receiving TPN when she died due to gram-negative sepsis at eleven months of age. Unfortunately, a postmortem work-up could not be performed.

Discussion

Congenital microvillus atrophy or microvillus inclusion disease is an autosomal recessive disorder, associated with a high mortality. It may be the most common cause of severe, refractory diarrhea in the neonatal period[1,2,6,7]. Although the basic defect is not known, it has been suggested that there is a defect in the transport of substances normally destined for the brush-border, which then build up within the cells[2,3,8]. This defect may represent an inborn error of intracellular transport which leads to aberrant assembly of the components of the enterocyte surface membrane[2,8]. The characteristic features of the disease include a thin mucosa, an abnormal accumulation of PAS-positive material within the apical cytoplasm of epithelial cells, an increase in epithelial cell secretory granules, and the presence of microvillus inclusions[3-5,9]. Although intracytoplasmic inclusions are one of the diagnostic features of microvillus atrophy, they are not limited to this disease[3]. There are also published case reports showing ultrastructural abnormalities of congenital microvillus atrophy without inclusions[5]. However, the accumulation PAS-positive material within the apical cytoplasm of intestinal cells, which has only been reported in microvillus atrophy, appears to be a specific defect of this disease[3,8].

Intractable diarrhea of infancy may result from a variety of known disorders including infections, inflammatory conditions, enzymatic defects, hormonal and metabolic diseases, and anatomical abnormalities[1,6,9]. It is therefore important

to exclude these disorders through apropriate investigations. The diagnosis of congenital microvillus atrophy as a cause of intractable diarrhea of infancy has to be established on the ground of the morphological and ultrastructural features which are characteristic for the disease[3,4,9,10]. Since the H&E-stained sections did not show any characteristic features, the abnormalities which could be detected by PAS stain can be easily overlooked[3]. Ultrastructural abnormalities can involve not only the small and large intestinal epithelia but also the biliary epithelium[3,10]. At present, the only reliable way of diagnosis could be the electron microscopic examination of jejunal or rectal biopsy specimens[2,3,5].

Currently, no treatment is available, and patients with congenital microvillus atrophy are supported by total parenteral nutrition and intravenous fluids for the replacement of their massive intestinal losses[2-5]. The majority of the affected patients eventually die of septic complications or hepatic insufficiency stemming from TPN-induced cholestasis[2,3,5,6]. Recently, Oliva et al.[11] reported the first successful intestinal transplant for microvillus inclusion disease in a patient 2.5 years old. Thereafter, Herzog et al.[12] published their experience for a combined bowel-liver transplantation in a seven-month-old infant with microvillus atrophy. Advancements in immunosuppressive therapy have allowed the small bowel transplantation to become a realistic option, with a good survival of both patients and grafts[11,12].

We present this case because congenital microvillus atrophy is a rare but perhaps one of the most common causes of intractable diarrhea of infancy. It could be easily overlooked if it is not specifically considered. It is important to recognize and diagnose infants with congenital microvillus atrophy because small bowel transplantation becomes a realistic option for the future treatment of these patients.

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NEUROLOGICAL CRISIS MIMICKING ACUTE PANCREATITIS IN TYROSINEMIA TYPE I*

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SUMMARY: Kalkanoğlu HS, Coşkun T. (Nutrition and Metabolism Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Neurological crisis mimicking acute pancreatitis in tyrosinemia type I. Turk J Pediatr 1999; 41: 501-504.

Hereditary tyrosinemia results from an inborn error in the final step of tyrosine metabolism. Neurological manifestations have been reported in nearly half of patients during illness to have characteristics of altered consciousness, weakness, anorexia, vomiting, and pain in the extremities and abdomen. His physical findings and laboratory results pointed out acute pancreatitis. There have been some reports of acute and chronic pancreatitis in patients with metabolic diseases; however, this is the first case with tyrosinemia type I who exhibited clinical and biochemical findings of acute pancreatitis during neurological crisis. The presented case suggests the possibility that the pancreas is affected in neurological crisis. The determination of amylase concentration both in serum and urine samples of further cases will clarify the association between pancreatitis and neurological crisis. *Key words: tyrosinemia type I, neurological crisis, acute pancreatitis.*

Hereditary tyrosinemia (tyrosinemia type I) is an autosomal recessive disorder caused by a defect of the final enzyme in tyrosine metabolism[1-2]. Affected individuals manifest severe liver disease, a reversible renal Fanconi syndrome, hepatocellular carcinoma, porphyria-like abdominal crisis and frequent neurological manifestations. A syndrome of acute neurological crisis which has striking similarity to the acute porphyrias is seen in as many as 40 percent of the children with this disorder[3]. We describe severe neurological crises associated with extremely high serum and urine amylase concentration as in acute pancreatitis in a child with tyrosinemia type I.

Case Report

This case was first referred to Hacettepe University Children's Hospital with marked growth retardation, wasting, pathologic fractures at the lower extremities, and hypophosphatemic rickets at the age of three years. The laboratory analyses revealed the presence of renal Fanconi syndrome characterized by a generalized aminoaciduria, phosphaturia, bicarbonaturia and glucosuria. The result of urinary amino acid analysis and serum amino acid quantitation showed an increased

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level of tyrosine. Fumarylacetoacetase activity was found to be low in cultured skin fibroblasts, consistent with the diagnosis of hereditary tyrosinemia type I.

The treatment consisted of continuous supplementation of vitamin D and an oral phosphate for the rickets and of sodium-potassium citrate solution for the acidosis and hypokalemia. A low-tyrosine and phenylalanine diet was used as well.

When he was 16 years old, he was hospitalized for an illness characterized by extreme weakness, anorexia, vomiting and pain in the extremities and in the abdomen. On physical examination, the patient's height was 130 cm and weight 23 kg, which were below the fifth percentile, and his blood pressure was 150/110 mmHg. He had generalized abdominal tenderness without rebound tenderness and tenderness in the legs and arms. He was unable to walk and had generalized hyperreflexia. Neurological findings included delirium and altered consciousness such as somnolence and stupor. He also had disorientation as well as visual, auditory and olfactory hallucinations.

Laboratory data revealed normal serum electrolyte values with the exception of severe hypokalemia. Serum calcium and phosphate levels were low. Serum bicarbonate level was 12.2 mmol/L. Serum transaminases levels were slightly elevated and amylase concentration was 445 U/L and 635 U/L on two consecutive measurements (normal 50-180 U/L). Urinary amylase level of 507 U/L led to amylase clearance which was 6.6 percent. Urinary porphobilinogen was 1.3 µg/ml (normal ≤ 1), coproporphyrin 36.7 mg/ml (normal ≤ 60), uroporphyrin 7.8 µg/L (normal ≤ 10), delta aminolevulinic acid 5.2 µg/L (normal ≤ 4.5), serum coproporphyrin 48.2 µg/dl (normal ≤ 4.5), blood ammonia 125 µg/dl (normal ≤ 120), and prothrombin time was normal.

Urinary and serum amino acid chromatography revealed an increased concentration of tyrosine (serum tyrosine level was 6.8 mg/dl). Abdominal ultrasonography showed an irregular pattern of the liver parenchyma. Alpha fetoprotein was 5.3 ng/dl (normal ≤ 5.9) Electromyography and cranial magnetic resonance imaging were normal and EEG showed no epileptic focus. Plasma renin activity was 16.2 ng/ml/h (normal ≤ 5.7) and urine succinylacetone level was 14 µmol/L.

Initial treatment included intravenous fluids containing bicarbonate and potassium, and oral phosphate supplement. His blood pressure was regulated by a beta-blocker. After starting the 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione (NTBC) therapy, all his signs and symptoms resolved and he was discharged. The patient continues to use NTBC and anti-hypertensive drugs.

Discussion

Hereditary tyrosinemia type I is an autosomal recessive disorder of amino acid metabolism caused by a deficiency of fumarylacetoacetate hydrolase, the final

enzyme in the metabolic pathway of tyrosine breakdown. This disorder, with an incidence of 1 in 100,000, leads to liver failure in the first year of life or cirrhosis and hepatocellular carcinoma in the first two decades. Renal tubular dysfunction and neurological crisis may complicate the clinical course[4]. As in the acute porphyrias the episodes of acute neuropathy associated with tyrosinemia are best correlated with ALA excretion and are due to competitive inhibition of ALA dehydrase by succinylacetone, a metabolite of tyrosine degradation[5]. Neurological manifestation of this disorder may include depression, delirium, psychosis, altered consciousness (somnolence to coma), and seizures[6].

Signs and symptoms of acute intermittent porphyria, crises-related weakness, myalgia and abdominal pain and anorexia and elevated urinary excretion of ALA were detected in our patient. Delirium and paranoia manifested later, further deteriorating his neurological state. During this crisis symptoms of abdominal pain, high levels of serum and urine amylase and amylase clearance mimicked acute pancreatitis. In recent years, there have been an increasing number of reports of acute and chronic pancreatitis in patients with inborn errors of metabolism, such as maple syrup urine disease, isovaleric acidemia, methylmalonic acidemia, propionic acidemia, 3-OH-3-methyl glutaric aciduria, homocystinuria, Pearson syndrome, cytochrome c oxidase deficiency, glycogen storage disease type I, carnitine palmitoyltransferase II deficiency and glutaric acidemia types I and II[7-16]. Pancreatitis is relatively rare in childhood and difficult to diagnose. The common signs and symptoms are non-specific, with gastrointestinal manifestations such as anorexia, nausea, vomiting and abdominal pain. Laboratory data is not always useful for the diagnosis, and pathological evaluation of the pancreas may be necessary. In our case, the pathological findings of acute pancreatitis were absent. The symptoms, physical examination and laboratory results pointed out a new neurological crisis, the nature of which has not been encountered or reported before in literature. In this case, the causative factor underlying the development of pancreatitis was obscure. Speculatively, the pathogenesis depends on the direct effect of succinylacetone or the accumulation of the porphyrin metabolites. There is no documentation about any pancreatic involvement in the neurological crisis of patients with type I tyrosinemia.

In conclusion, the presented case highlights the fact that pancreatitis may be a component of neurological crisis in patients with hereditary tyrosinemia type I. Therefore, physicians treating such patients should be aware of this possibility and perform all necessary tests to confirm the diagnosis of pancreatitis. Further reports will clarify whether or not pancreatitis is a component of neurological crisis in tyrosinemia type I patients, particularly in those with severe abdominal pain.

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MRI OF FIBROMATOSIS COLLI*

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SUMMARY: Çakmakçı H, Kovanlıkaya A. (Department of Radiology, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). MRI of fibromatosis colli: case report. Turk J Pediatr 1999; 41: 505-508.

Magnetic resonance imaging (MRI) appearance of fibromatosis colli has been reported in only two cases in the literature. We herein describe the MRI findings in a case of fibromatosis colli: the signal intensity of the fusiform mass on T2 weighted images was slightly less than on T1 weighted images, consistent with the presence of some fibrous tissue within the muscle mass. *Key words: magnetic resonance imaging, fibromatosis colli.*

Fibromatosis colli is a benign fusiform mass associated with torticollis arising from the sternocleidomastoid muscle in the anterior neck. A history of birth trauma, difficult delivery, or breech delivery common[1-3]. The sonographic and computed tomography (CT) findings of fibromatosis colli have been described[1,2]. Magnetic resonance imaging (MRI) appearance has been described in very few cases in the literature[3,4]. This paper describes the MRI findings in a case of fibromatosis colli.

Case Report

A three-month-old girl, born by uncomplicated vaginal delivery, presentted to the ear, nose and throat (ENT) physician with a right neck mass. It was first noticed while the baby was crying or being fed. Initial ultrasound examination done at an outside facility revealed a right neck mass which had ill-defined margins with neighboring right thyroid and right submandibular glands (Fig. 1). The patient was referred to our MRI unit to delineate the relationship and determine the origin of the mass. The parents were also told that the infant had a potential malignancy. T1 and T2 weighted images showed a fusiform enlarged, mildly hypointense right sternocleidomastoid muscle (Fig. 2). No other neck mass was demonstrated. After MRI examination, radiological and clinical findings confirmed the diagnosis of fibromatosis colli.

Discussion

Fibromatosis colli has characteristic ultrasonographic and clinical features[1]. The diagnosis is made based on these findings. Usually a neck mass arises in a

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neonate approximately two weeks after birth and is associated with torticollis in 14-20 percent of cases. The mass may continue to increase in size for two four weeks or months. The infants are otherwise healthy. A history of birth trauma such as breech presentation, forceps, delivery, or difficult delivery is common[2-4]. Pre-existing intrauterine torticollis may contribute to a difficult delivery. An etiologic factor is traumatic compression of the neck during delivery which may

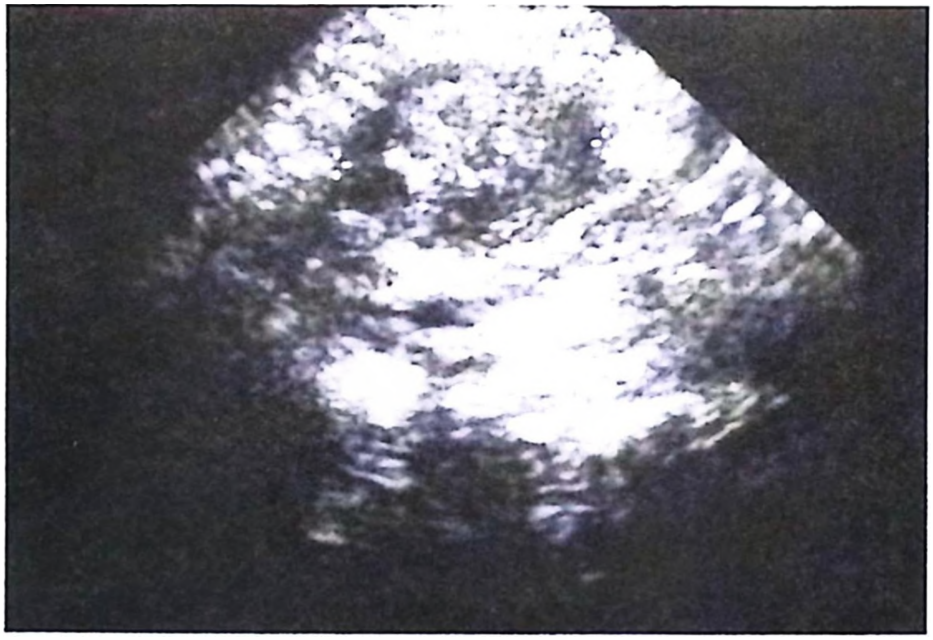

Fig. 1a

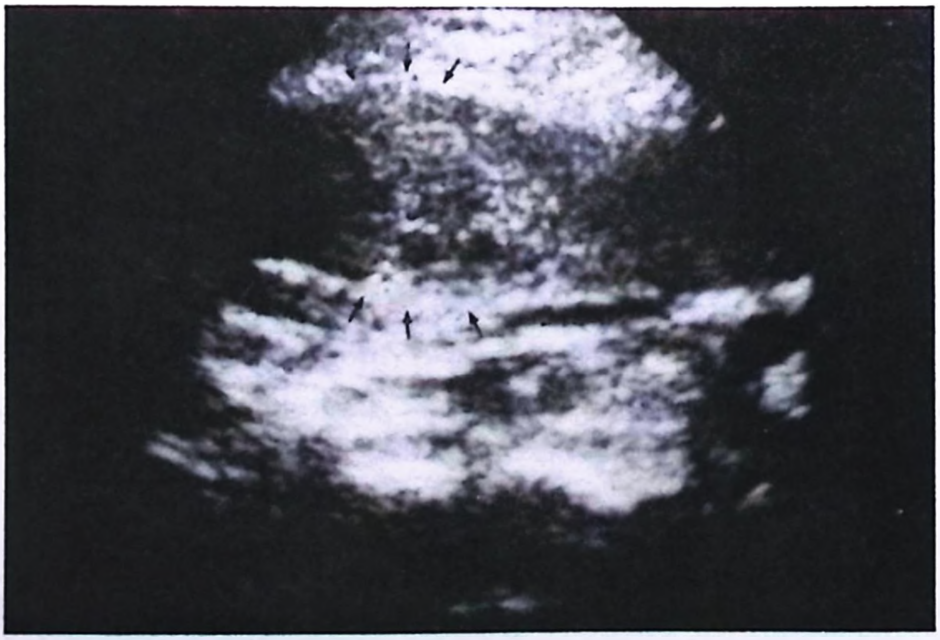

Fig. 1b

Fig. 1: Ultrasound of the right neck in transverse view (a) shows isoechoic mass-like appearance but longitudinal view (b) reveals fusiform enlargement of the right sternocleidomastoid muscle (arrows) at involved region of the neck.

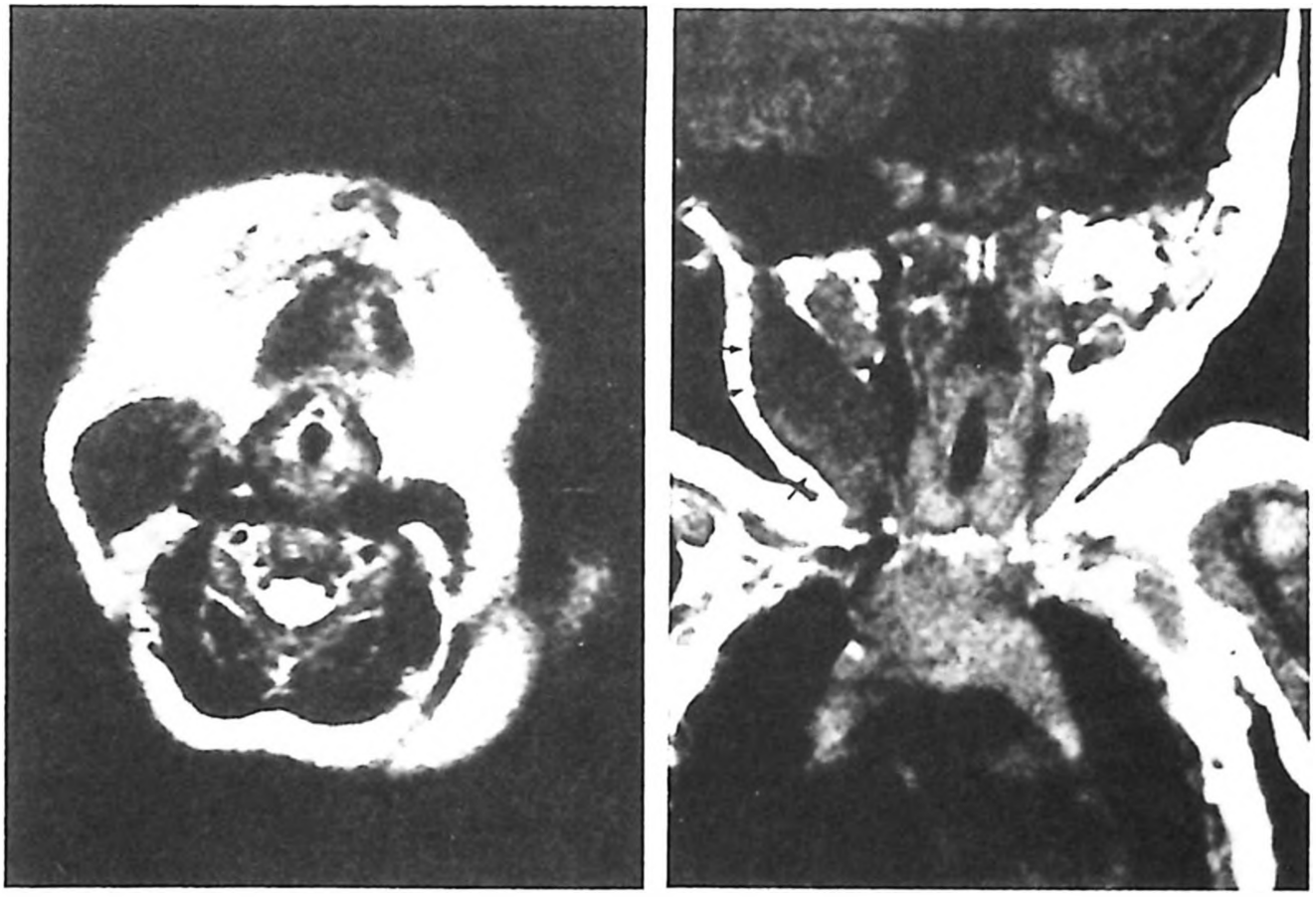

Fig. 2a

Fig. 2b

Fig. 2: Axial T2 weighted (a) and coronal T1 weighted (b) MR images confirm the fusiform enlargement of the right sternocleidomastoid muscle. On T2 weighted images, the signal intensity of the right sternocleidomastoid muscle is less than that of the normal left sternocleidomastoid muscle.

cause pressure necrosis and/or occlusion of the venous outflow of blood from the sternocleidomastoid muscle, resulting in edema in the muscle, degeneration of the muscle fibers, and then, fibrosis of the muscle[4]. Usually fibromatosis colli is unilateral and is more common in the right (73%) than the left (22%) neck. The mass usually resolves spontaneously over four to eight months with conservative management, either with stretching exercises or no treatment. The mass has been erroneously called a hematoma[1-5].

Sonography is the procedure of choice for diagnosis. On sonography focal or diffuse enlargement of the sternocleidomastoid muscle is present, usually in a fusiform configuration and in the lower two-thirds of the muscle. The mass moves synchronously with the sternocleidomastoid muscle. The echogenicity of the mass may be hyperechoic, isoechoic, or hypoechoic relative to normal muscle. Echogenic foci with acoustic shadowing due to calcifications have been reported[1].

Computerized tomography imaging of fibromatosis colli demonstrates focal or diffuse enlargement of the sternocleidomastoid muscle. Although the diagnosis of fibromatosis colli can be made by CT, ultrasound is the preferred modality because it is noninvasive, less expensive, easier to perform and does not involve radiation[2].

If an infant fails to follow the typical clinical course or develops other symptoms and/or physical findings not typical of the condition, further evaluation with CT and/or biopsy is necessary for diagnosis. MRI may be helpful for further evaluation of fibromatosis colli, by demonstrating the signal intensity of the mass and localizing the mass in the sternocleidomastoid muscle, as well as by differentiating it from neck masses arising from different anatomic structures. The solid soft tissue masses of the anterolateral neck include: lymphoma, rhabdomyosarcoma, other soft tissue sarcomas, neuroblastoma, inflammatory masses, and infectious or metastatic adenopathy. Rare benign soft tissue tumors include aggressive fibromatosis, cervicothoracic lipoblastomatosis, parathyroid adenoma, and plexiform neurofibroma. Imaging findings that are not characteristic of fibromatosis colli and may suggest the presence of another solid neck mass would include: irregular margins, mass extending beyond the confines of the sternocleidomastoid muscle, poor definition of surrounding fascial planes, and/or mass associated with adenopathy, bone involvement, intracranial or intraspinal extension, vascular encasement and airway compression[3-5]. In this case, on T2 weighted images the signal intensity of the mass was slightly less than on T1 weighted images, consistent with the presence of fibrous tissue within the muscle.

In order to avoid unnecessary invasive procedures to distinguish a benign condition from a malignant neck mass, such as fine needle aspiration biopsy, it is important to know the clinical and imaging features of fibromatosis colli, and sonography should be the initial modality of choice. If unexpected symptoms or physical findings develop, further evaluation with CT and/or MRI and/or biopsy are necessary for diagnosis.

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A CASE WITH ACUTE LEUKEMIA PRESENTING WITH CARDIAC TAMPONADE*

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*Rukiye Eker Ömeroğlu MD******, *Talat Cantez MD******

SUMMARY: Söğüt A, Yılmaz K, Yalman N, Şahin K, Babalıoğlu M, Ömeroğlu RE, Cantez T. (Department of Pediatrics, İstanbul University Faculty of Medicine, Çapa-İstanbul, Turkey). A case with acute leukemia presenting with cardiac tamponade. Turk J Pediatr 1999; 41: 509-516.

Pericardial effcisions and cardiac tamponade are rare and severe complications of leukemia. They often develop during the radiation therapy, chemotherapy, or infections in the course of leukemia. However, some cases present with pericardial effusion and tamponade. We report a three-year-old girl who was admitted with cardiac tamponade and needed urgent pericardiocentesis. Clinical evaluation and laboratory results revealed myeloid markered-T cell acute lymphoblastic leukemia (ALL) and pericardial invasion. She is the youngest patient with cardiac tamponade who was diagnosed acute lymphoblastic leukemia in the English-language literature. *Key words: pericardial effusion, acute lymphoblastic leukemia.*

A variety of cardiac and noncardiac conditions such as congestive heart failure, infections, autoimmune and renal diseases and cardiac surgery may cause pericardial effusion. Leukemia is one of the rare causes of pericardial effusion. It occurs mainly as a consequence of chemotherapy, radiotherapy, or of infections while treating patients with leukemia. However, pericardial effusion is rarely the initial presentation of acute leukemia. There are 12 cases who presented with cardiac tamponade as the initial manifestation of acute leukemia in English-language literature[4-14]. We present a three-year-old girl who was referred because of cardiac tamponade. She needed urgent pericardiocentesis and then an acute lymphoblastic leukemia (ALL) and pericardial invasion were determined.

Case Report

A three-year-old girl whose complaints were cough, fever, dyspnea and swelling of the eyelids was refferred to our hospital prediagnosed as pericarditis.

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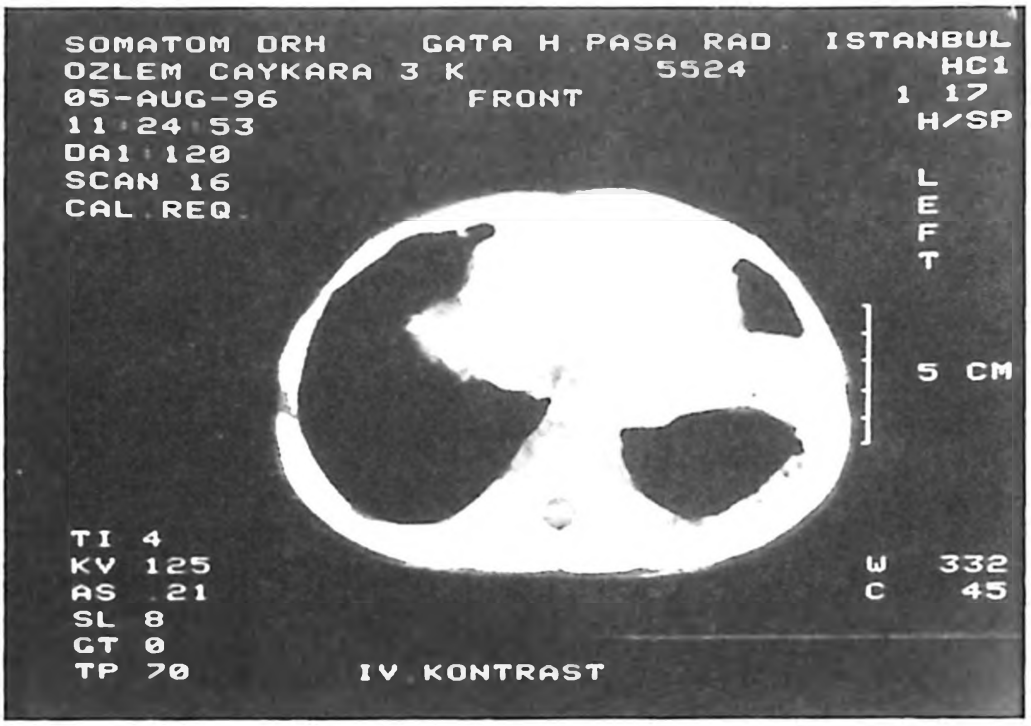

Fig. 1: Pericardial and pleural effusion in chest tomography.

The patient had been treated with iron tablets because of pica and iron deficiency for a year. She was brought to a local hospital one month previously with complaints of cough, fever, dyspnea and fatigue. Ceftriaxone was administered for the diagnosis of bacterial bronchopneumonia. However, an enlargement of the cardiac silhouette was noticed in control chest X-rays. Echocardiography, thoracal and abdominal ultrasonography and chest tomography (Fig. 1) revealed pericardial and minimal pleural effusion and mild hepatosplenomegaly, but no mediastinal or abdominal lymphadenopathy or mass. Other laboratory findings were as follows: hemoglobin: 8.1 g/dl; white blood cell count: 8000/mm^3 with 60 percent lymphocytes, 4 percent reactive lymphocytes (not blasts), 30 percent neutrophils, 4 percent band forms, 2 percent others; platelet count: 112,000/mm^3 and erythrocyte sedimentation rate: 65 mm/h. She had been treated at the local hospital for bronchopneumonia for 10 days without success and, when she deteriorated, was sent to our hospital for further evaluation.

Upon admission to our hospital, pallor, restlessnes, tachypnea, dyspnea, sweating and periorbital edema were noticed on physical examination. Her pulse rate was with 160/min and blood pressure was 90/60 mmHg, and she had marked jugular vein distension. Heart sounds were weak, but there were no murmurs or friction rubs. Crackling rales were heard over basal areas of both lungs. The liver was 4 cm and the spleen 3 cm palpable below the respective costal margins. The rest of the physical examination was normal.

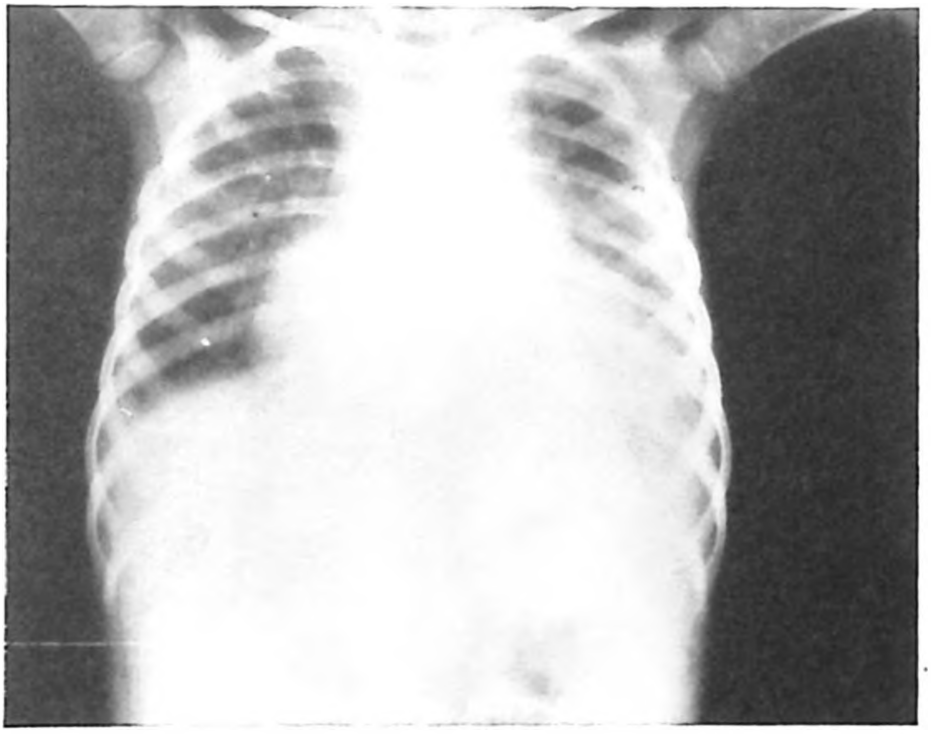

Fig. 2. Cardiomegaly in telecardiography before pericardiocentesis.

The telecardiography before pericardiocentesis showed a marked cardiac enlargement (Fig. 2). Sinus tachycardia, low QRS voltages and negative T waves on V_{1-6} were present in ECG tracings. On echocardiography, pericardial effusion, collapse of right atrial wall and anterior wall of right ventricle during diastole period was present (Fig. 3). Minimal pleural effusion, bilateral but more apparent

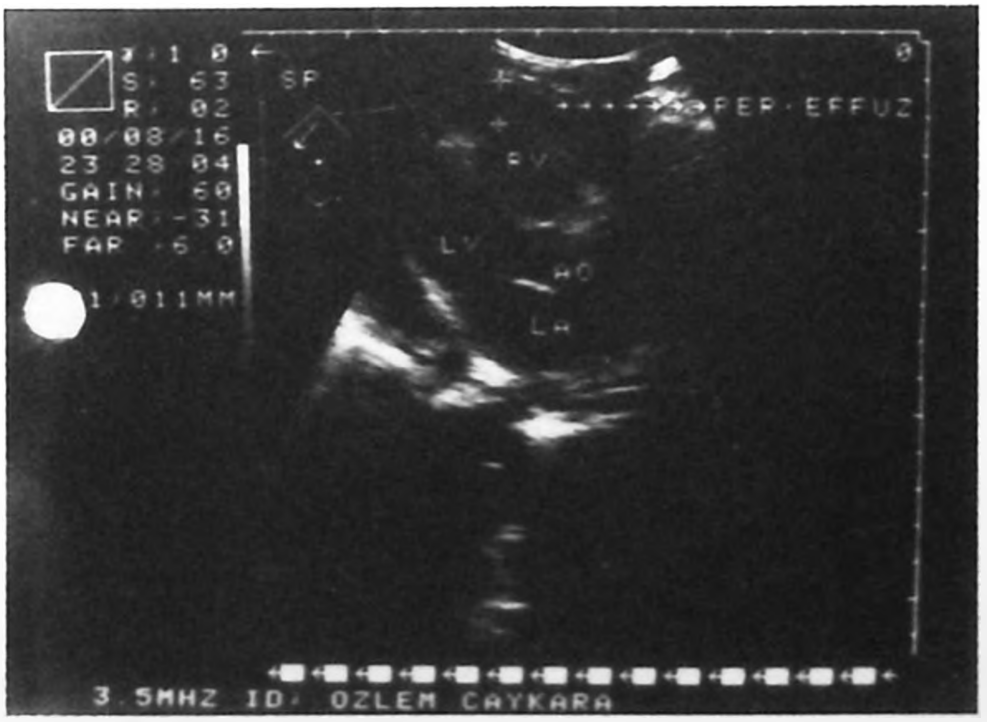

Fig. 3. Pericardial effusion on echocardiography

on left side, was also observed by thorax ultrasonography. Clinical and echocardiographical evaluation revealed a pericardial tamponade requiring an urgent pericardiocentesis. Pericardiocentesis yielded 200 ml of serohemorrhagic fluid. Fluid analysis revealed glucose 74 mg/dl, protein 2.85 g/dl, LDH 4,000 IU/L, simultaneous serum protein 6.3 g/dl and LDH level 2,872 IU/L. No

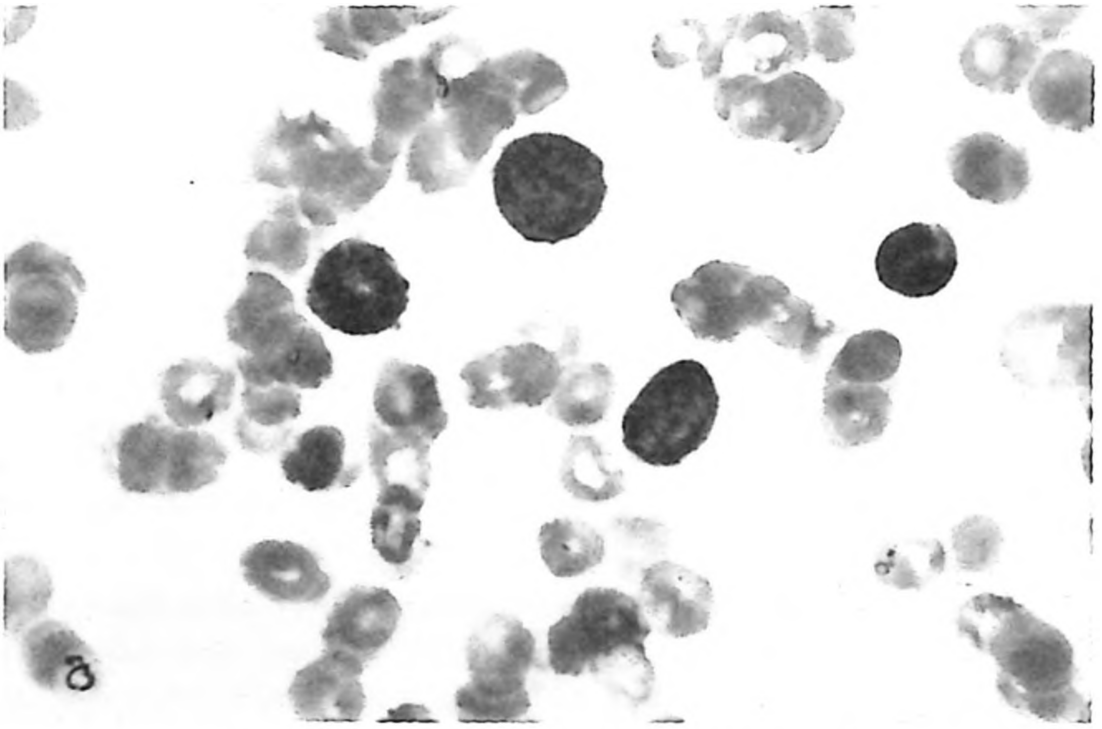

Fig. 4: Lymphoblastic cells in pericardial fluid.

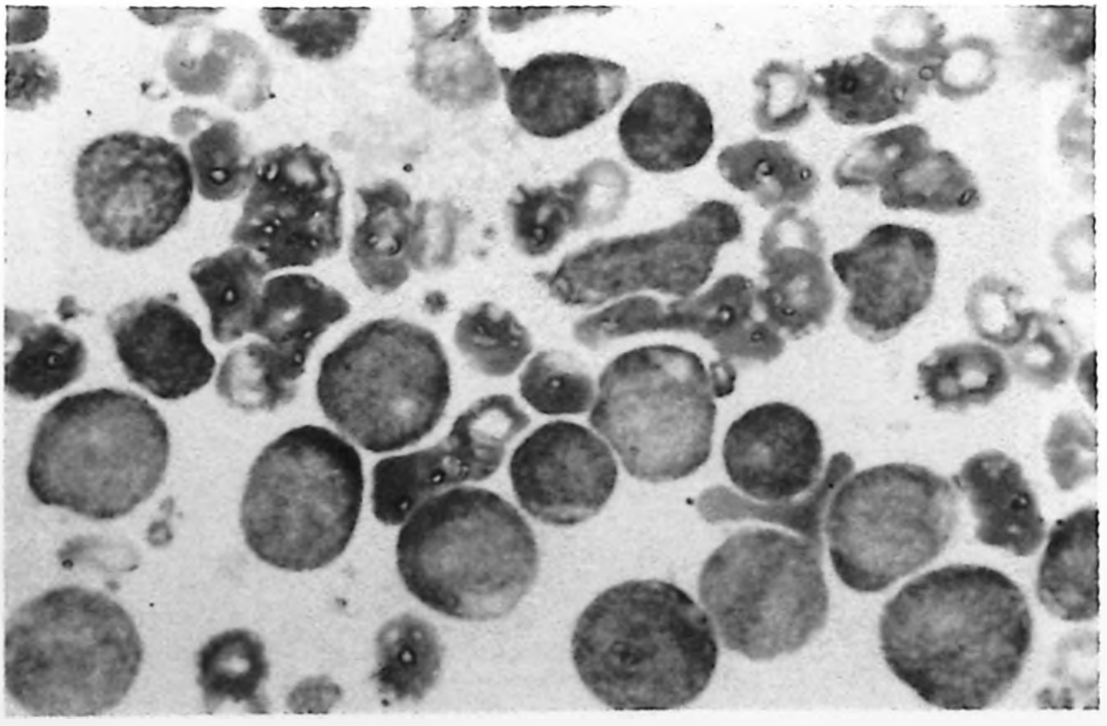

Fig. 5: L_2 type lymphoblasts on bone marrow.

microorganisms or M. tuberculosis were detected in the fluid. Among cells, 25 percent microlymhoblastoid cells were observed in the pericardial fluid (Fig. 4). Hematological values were as follows: hemoglobin 6.3 g/dl; hematocrit 19.7 percent; mean corpuscular volume (MCV) 81 fl; platelet count 180,000/mm^3; and white blood cell count 16,000/mm^3 with 20 percent neutrophils, 70 percent lymphocytes, and 10 percent lymphoblasts. Blood urea, ALT, AST and serum electrolytes and routine urine examination were all normal. Bone marrow aspiration revealed a hyperplastic marrow with L_2 type mononuclear cell infiltration (Fig. 5). CALLA, CD 19, CD 20, CD 4, CD 8, CD 13, CD 14 were negative and CD 2, CD 7, CD 34, CD 33 were positive by immunohistochemical and immunophenotypical studies in bone marrow and, following these results, myeloid markered-T cell ALL was diagnosed. Central nervous system involvement was not detected. She was administered the augmented BFM-86 CCG-1882 chemotherapy protocol. Pericardial and pleural effusion disappeared two weeks later (Fig. 6) and, after one year, her bone marrow is still in remission.

Discussion

Cardiac involvement is a well-known feature in hematological malignancies, and it has been reported at autopsy in approximately 20 percent of patients with lymphomas or leukemias[1]. Most are asymptomatic. Infiltration of the pericardium is a much more common finding in postmortem examinations of patients with acute leukemia (AL)[2]. Generally, pericardial involvements detected before death

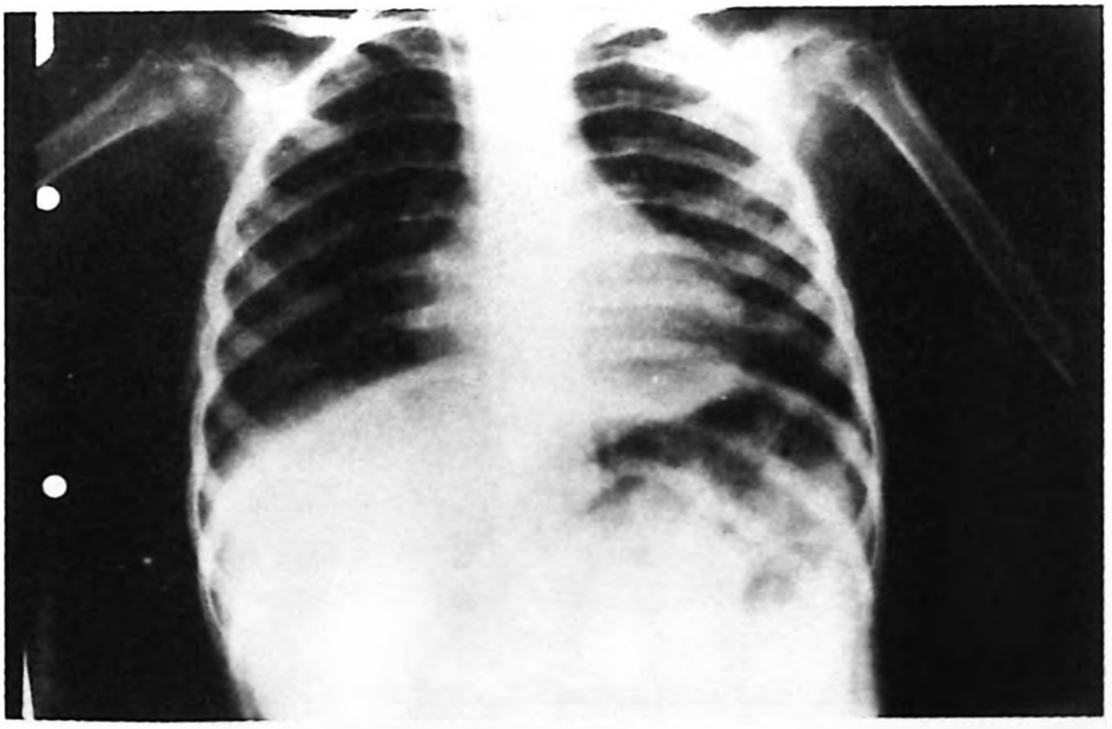

Fig. 6. Normal telecardiography after chemotherapy

have been diagnosed in the advanced stages of neoplastic blood diseases[2,3]. In contrast to the high frequency of pericardial involvement in the course of AL, pericardial effusion is rarely present at the onset of AL, and is rarely the initial manifestation of AL. The occurrence of pericarditits after bronchopneumonia without any finding suggesting acute leukemia was also noted in our case. Although our case developed cardiac tamponade, she did not demnostrate any abnormality in hematological parameters or imaging consistent with AL in the beginning. The patient was admitted to our hospital with severe cardiac tamponade. An emergency pericardiocentesis was performed and microlymphoblastoid cells were detected on the smear of the pericardial effusions. Thereupon, a peripheral blood smear and bone marrow aspiration were done, and blastic cells were seen on both.

Battle et al.[13] reported a review in 1991 containing 10 patient reports plus their own patient, and Spottswood et al.[14] also reported another case. The age range was two to 45 years. Four cases had acute myeloblastic leukemia and nine acute lymphoblastic leukemia; our case is the youngest patient with acute lymphoblastic lekemia. A summary of these cases is present in Table I.

Table I: ALL Cases with Pericardial Involvement in English-Language Literature

	No of Reference	Age (year)	Sex	Type of AL	Blasts in the Pericardial Effusion
Rab et al.[4]	4	26	M	ALL	Present
Battle et al.	5	6	F	ALL	Present
Jaffe et al.	6	15	F	ALL	Present
Jaffe et al.	6	5	M	ALL	Not Performed
Chia et al.	7	19	F	ALL	Present
Krause	8	16	M	AML	Present
Sobolewski et al.	9	12	M	ALL	Not Perfomed
Chu et al.	10	5	M	AML	Present
Mancuso et al.	11	18	F	ALL	Absent
Leung et al.	12	45	F	ALL	Not Performed
Battle M et al.	13	18	F	AML	Present
Spottswood et al.	14	2	F	AML	Present
Our case et al.		3	F	ALL	Present

Abbreviations: AL: acute leukemia; ALL: Acute lymphoblastic leukemia; AML: Acute myeloblastic leukemia; F: Female; M: Male.

Pericardiocentesis was performed on nine patients. Blastic cells were detected in eight of nine pericardial fluid samples. Pericardial effusion responded to chemotherapy and did not recur, although AL relapsed. We had to perform pericardiocentesis because of our patient's cardiac tamponade. We diagnosed the same day and started to administer chemotherapy four days later. Pericardial effusion resolved in two weeks and did not reaccumulate.

The optimal management in cardiac tamponade as a result of leukemic infiltration is still controversial. Pericardiocentesis is only to be performed to relieve the patient with cardiac tamponade and to attain hemodynamic stabilization. Systemic chemotherapy is the essential therapeutic approach. Although surgical drainage with subxiphoid pericardiotomy might be recommended to prevent the reaccumulation of fluid or to evacuate the reaccumulated fluid, perhaps the best approach is drainage followed by rapidly systemic chemotherapy. The prognosis depends on the leukemia itself, rather than on pericardial involvement.

The cases mentioned above demonstrate that, although rare, leukemia my present with cardiac tamponade. Careful cytologic examination of pericardial fluid peroxides valuable diagnotic information in such cases.

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A CASE OF HEMOPHILIA A ASSOCIATED WITH HODGKIN'S DISEASE*

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SUMMARY: Koç A, Varan A, Büyükpamukçu M, Gürgey A. (Hematology and Oncology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). A case of hemophilia A associated with Hodgkin's disease. Turk J Pediatr 1999; 41: 517-520.

Lymphoreticular malignancies are more common in patients with hemophilia, but it is usually attributed to human immunodeficiency virus (HIV) infection associated with repeated use of blood products. However, there are a couple of hemophiliac patients with malignancies but without HIV infection in the literature. We report a case of a hemophiliac patients who had Hodgkin's disease at 2.3 years old without any congenital or acquired immunodeficiency and without use of any blood products. This patient showed that malignancy can develop in hemophiliacs without HIV infection, but further studies are needed to clarify whether hemophiliacs are more susceptible to malignancies. *Key words: hemophilia, Hodgkin's disease, malignancy.*

The inherited immunodeficiency states such as ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable immunodeficiency, and some hematological disorders such as Fanconi's anemia are associated with a high incidence of malignancy[1,2]. Non-Hodgkin's lymphoma (NHL) is the most common malignant disease in patients with hemophilia, but it is usually attributed to human immunodeficiency virus (HIV) infection associated with repeated use of blood products[3,4]. Other malignancies in hemophiliacs are also reported, with or without HIV infection[5-7].

We report the case of 2.3-year-old hemophiliac child with Hodgkin's lymphoma without HIV infection.

Case Report

A 2.3-year-old by with hemophilia A was brought to İhsan Doğramacı Children's Hospital in June 1997 with left sub-auricular and upper cervical mass. When he was two years old, he was diagnosed as having hemophilia A, without any

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bleeding, because of his family history, which included three patients diagnosed with hemophilia A. The patient had no underlying predisposing factor for development of cancer. The laboratory tests revealed the following prothrombin time (PT) of 13 seconds and an activated partial thromboplastin time (aPTT) of 67 seconds. Bleeding time was normal. A thromboplastin generation test indicated that the boy had factor VIII deficiency (Factor VIII was 4% and Factor IX was 75%). He did not receive any blood product as he had no severe bleeding episodes.

Physical examination revealed a well developed but pale child. Massive cervical lymphadenopathy on the left side was noted. The liver was palpable 3 cm and the spleen 4 cm below the respective costal margins. The hemoglobin level was 7.8 g/dl, leukocyte count 8,000/mm^3 with 53 percent neutrophils, 36 percent lymphocytes, 8 percent monocytes and 2 percent eosinophils. Bone marrow aspiration was normal. In immunological and viral studies, HIV was negative, anti CMV IgG>250 IU/ml, IgM (+); anti rubella IgG (–), IgM (–); anti toxoplasma IgG (–), IgM (–); nitroblue tetrazolium test (NBT): 100%, quantitative immunoglobulins: IgG 1600 mg/dl (N 604-1941), IGM 271 mg/dl (N 71-235), IgA 369 mg/dl (N 26-296); PPD (–). Computerized tomographic (CT) examination of the cervical region showed left cervical necrotic mass through to superior mediastinum and thoracal CT showed upper mediastinal mass. Abdominal CT was normal. Mass biopsy was performed, and Hodgkin's disease (HD) with lymphocyte depletion was diagnosed.

He had been treated with adriamycin (25 mg/m^3), bleomycin (10 mg/m^3), vinblastine (6 mg/m^2), and dacarbazine (375 mg/m^2) (ABVD protocol). After initial treatment, he did not respond to his therapy during the following three months, and in October 1997 his treatment was changed to the ABVD-COPP (cyclophosphamide, oncovin, procarbazine, prednisone) alternate treatment protocol. Radiotherapy was given in December 1997. In January 1998, six months after he started chemotherapy, his lymphoma relapsed. He did not respond to therapy and died two weeks after the relapse, in February 1998, because of overwhelming infections.

Discussion

Children with inherited and acquired immunodeficiency syndromes and those with iatrogenically induced immunodeficiency have a much higher risk of developing lymphoreticular malignancies than expected[3,4,8,9]. Since HIV infection leads no progressive loss of cellular immunity, it is probable that these malignancies result from the progressive reactivation or loss of immunologic control of latent oncogenic viruses[4,10]. NHL in particular was found to be more frequent in a hemophiliac who had been infected with HIV[3,4]. The mean CD 4

at presentation of NHL was extremely low in NHL associated with HIV infection in hemophiliac patients[3]. It is possible that the CD 4 number in hemophiliac patients at NHL presentation reflects not only the result of underlying HIV infection with a quantitative decrease in CD 4, but is also related to chronic foreign protein and antigenic exposure with chronic blood transfusions[3,11-15]. There are several reports of diminished helper/suppressor T lymphocyte ratios and natural killer activity in recipients of repeated blood transfusions without HIV infection[4,11-15].

Lymphomas and other malignancies have also been reported in hemophiliac patients without HIV infection. Ragni et al.[3] reported three NHL cases in hemophiliacs without HIV infection, two of them with mild hemophilia, one treated only with cryoprecipitate and one who had never been treated with blood products. Bouhasin et al.[7] and Green et al.[6] reported acute leukemia in patients with hemophilia. Altay et al.[5] reported two acute leukemia cases with mild hemophilia from our clinics previously. One of them had acute lymphoblastic leukemia at the age of 10 years, and he had only one blood transfusion and one fresh frozen plasma before leukemia was diagnosed. The other had acute myelomonocytic leukemia at the age of 1.5 years and the had had only two plasma infusions. Levine et al.[16] reported familial nasopharyngeal carcinoma cases also with hemophilia.

Our patient had clinically mild hemophilia and had not received any blood products before HD was diagnosed. He did not have HIV infection or other congenital or acquired immunodeficiency states.

Hodgkin's disease is rarely diagnosed in children younger than five years[8]. Most cases of HD associated with HIV infection have a pathologically high stage and bone marrow involvement with the initial diagnosis, and are histologically subclassified as mixed cellularity and nodular sclerosis[9,17-19], contrary to the cases of our patients.

In conclusion, malignancy can develop in hemophiliacs without HIV infection. It is also possible that the association of Hodgkin's disease in our patient was coincidental. However, further studies are needed to clarify whether hemophiliacs are more susceptiple to malignancies.

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CARDIAC DYSRHYTHMIA THAT SIMULATES SEIZURE DISORDER IN TWO CHILDREN*

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SUMMARY: Turanlı G, Apak RA. (Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Cardiac dysrhythmia that simulates seizure disorder in two children. Turk J Pediatr 1999; 41: 521-523.

Cardiac dysrhythmia can present signs and symptoms of a seizure disorder and sometimes they are clinically indistinguishable. We present two children with a presumed seizure disorder but also with an underlying and associated cardiac problem. Therefore, we suggest that both conditions must be considered concomitantly, and that each patient with a newly diagnosed seizure disorder requires both neurological and cardiological evaluation. *Key words: seizures, dysrhythmia.*

Cardiac dysrhythmia can present signs and symptoms of a convulsive disorder and in many cases both disorders may be clinically undifferentiated[1]. There are no clues or criteria to differentiate these two conditions, and this may result in inappropriate and also ineffective treatments. Sometimes, the underlying cardiac abnormality may be fatal and may lead to sudden death of the patient with presumed epilepsy[2]. Early diagnosis and treatment of an underlying cardiac problem in these patients could, therefore alleviate symptoms and prevent sudden deaths

We report two children with an initial diagnosis of epilepsy having an underlying, unsuspected cardiac problem.

Case Reports

Case 1

A 12-year-old boy had a three-year history of a presumed seizure disorder slightly responsive to antiepileptic treatment. Initial episodes were precipitated by exercise and preceded by an aura that included nausea and vomiting. Later, he developed pallor and perioral cyanosis with tonic deviation of the eyes and tonic-clonic jerks in all extremities. He experienced six more seizures of the same type and had been prescribed phenytoin treatment after the second episode by his family physician. After his last seizure he was found to be

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bradycardiac, and was then referred to our center for cardiologic work-up. On admission, physical examination, including a detailed neurological examination, was normal except for a pulse rate of 48/min. Blood pressure measurements both at sitting and standing positions were normal. Electrocardiography (ECG) showed atrioventricular dissociation. Twenty-four hour Holter monitoring revealed ventricular extrasystoles and attacks of ventricular tachycardia. Echocardiography, routine electroencephalography (EEG), sleep-deprived EEG and EEG video monitoring testings were normal. He was diagnosed as sick sinus syndrome and started on β-blocker treatment; phenytoin was withdrawn progressively. At his one year of follow-up, he had not experienced any seizure.

Case 2

A nine-year-old girl was admitted with a history of loss of consciousness, occurring at school following sudden risings preceded by nausea and blackout, she could not be aroused for five minutes. She was otherwise healthy. She had a milder episode about one year ago and had complained of a paroxysmal, blunt, frontal headache since then. She had no family history for syncope, migraine or epilepsy. On admission, physical examination, including a detailed neurological examination, was normal. Pulse rate and blood pressure both at sitting and standing positions were normal. An EEG revealed generalized paroxysmal activity provoked by photic stimulation. Brain magnetic resonance imaging (MRI) was normal. She was diagnosed as having photosensitive epilepsy and was started on valproic acid treatment. Despite her regular antiepileptic treatment, she experienced two more syncopal attacks and needed further cardiologic evaluation that revealed a normal ECG with a normal corrected QT interval, normal Holter monitoring and echocardiography. However, tilt-table test was positive on the 7th minute. She was advised not to rise suddenly or stand for a long time. Within the following six months, her EEG returned to normal and she became seizure-free.

Discussion

Many reports in the literature have shown a well-documented relationship between seizures, dysrhythmias and syncope[3-5]. Seizures sometimes may be the leading clinical manifestation of an underlying cardiac dysrhythmia, notably in ventricular tachyarrhythmia; however, there are no specific established criteria to distinguish these conditions[6]. Among these, long QT syndrome (LQT) is one of the important disoders that causes syncope, seizure and sudden death from cardiac dysrhythmias, particularly ventricular dysrhythmias such as torsade de pointes and ventricular fibrillation[7]. LQT can be acquired or inherited, although most cases are probably caused by some combination of environmental and genetic

factors[8]. Therefore, an initial baseline ECG to establish an underlying cardiac dysrhythmia is recommended, especially in newly diagnosed seizures, but a normal ECG does not rule out an underlying disorder and further cardiac investigation may also be required. on the other hand, some reports[9] stated that seizures may lead to cardiac dysrhythmias such as asystole and supraventricular tachyarrhythmias and furthermore, that these dysrhythmias may lead to convulsive activity and a variety of EEG abnormalities. In our seires, the first patient was misdiagnosed with an epileptic disorder until a cardiac problem was suspected after which the precise diagnosis of sick sinus syndrome was made and appropriate treatment was started. This patients no longer needed further antiepileptic treatment, but his convulsive symptoms improved only with β-blocker treatment. In the second patient on the other hand, a cardiac dysrhythmia was most likely present, but EEG was significantly abnormal. These cases demonstrate that convulsive and cardiac disorders may simulate their symptoms as well as associate. To resolve this enigma, further studies to detect the incidence of cardiac dysrhythmia among newly diagnosed and refractory seizure disorders, or clinical trials designed by simultaneous ambulatory monitoring of EEG and ECG in epileptics, are needed.

In conclusion, we suggest that cardiac dysrhythmia contributes to seizure disorder in children more often than previously thought and must be considered in the early management of patients.

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BRONCHIECTASIS DUE TO CILIARY APLASIA IN TURNER'S SYNDROME*

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SUMMARY: Özçelik U, Tuncel M, Göçmen A, Balcı S, Erbil M, Yel L, Kiper N. (Chest Disease Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Bronchiectasis due to ciliary aplasia in Turner's syndrome. Turk J Pediatr 1999; 41: 525-529.

A seven-year-old girl with Turner's syndrome, who suffered from recurrent respiratory system infections since birth, was investigated to determine the etiology of bronchiectasis. Electron microscopy of recurrent nasal biopsy specimens revealed ciliary aplasia. Ciliary aplasia in Turner's syndrome, has not previously been reported.
Key words: bronchiectasis, ciliary aplasia, primary ciliary dyskinesia, Turner's syndrome.

Recurrent lower and upper respiratory system infections are not common findings in Turner's syndrome. In this report, we describe ciliary aplasia, a rare congenital disorder, in a girl with Turner's syndrome who suffered from recurrent respiratory system infections and bronchiectasis. To our knowledge no such association has been reported previously.

Case Report

A seven-year-old girl with Turner's syndrome was admitted to Hacettepe University Children's Hospital because of chronic cough, purulent sputum and chronic rhinitis since birth. There was a third-degree consanguinity in her parents; all four of her siblings were healthy. Previously, she had been followed with the diagnosis of bronchiectasis at another hospital, and a lobectomy of the right inferior lobe had been performed a year ago.

On physical examination, she had a height of 97 cm, and a weight of 15.5 kg, both of which were below the 3rd percentile. Her height and bone ages were

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3 years and 9/12 months, and 6 years, respectively. She was showing typical physical findings of Turner's syndrome[11]: short and webbed neck, low posterior hair line, cubitus valgus, short metacarpals, and widely spaced nipples. There was a purulent nasal and postnasal discharge. On chest examination, the breath sound was decreased on the right basal area, and rales were auscultated over both lung areas. The cardiovascular system examination was within normal limits except for bilaterally weak arterio-femoral pulses. Clubbing was present.

On laboratory examination, the peripheral chromosomal studies showed 45,XO Tuner's genotype. Coarctation of the aorta was diagnosed by echocardiographic and angiographic studies. Her chest radiograph showed chronic changes and bronchiectasis in both lung areas. The thoracal computerized tomography (CT) performed before the lobectomy demonstrated significant bronchiectasis of the right lower lobe. The follow-up thoracal CT, one yar after the operation, showed left lower lobe bronchiectasis. Histopathological examination of the lobectomy material showed bronchiectasis and reactive lymphoid hyperplasia. Sweat chloride and alpha-1-antitrypsin values were normal. The immunological tests which were performed were in normal limits: IgG: 1600, M: 205, A: 299 mg/dl; immunoglobulin G subclasses: G1: 1030, G2: 266, G3: 61, G4: 5.4 mg/dl; her blood group was A Rh positive; and anti B titer was 1/54. Polio antibody titers were T1: 1/4, T2: 1/8, T3: 1/8. Lymphocyte response to mitogens (phytohemagglutinin, concanavalin A) was normal; CH50:33 U/ml; T lymphocyte subsets: total T cell: 63%, B cell: 18%, helper/inducer: 34%. She did not cooperate with the nasal saccharin test. Nasal biopsies were done on three separate occasions over a six-month period, when the child was clinically well. The tissue was fixed immediately in two percent glutaraldehyde solution and processed for transmission electron microscopy. Cilia were rarely found in any of the three nasal biopsies. Whole areas of the sample were examined and a minimum of 250 cells were analyzed under the electron microscope. Epithelial cells were covered with numerous normal microvilli (Fig. 1); cilia and basal bodies were not found except in small number of cells having a few cilia (Fig. 2). These cilia had basal bodies and did not show any structural abnormalities (Fig. 3).

Discussion

Bronchiectasis is not one of the phenotypic changes in Turner's syndrome. Some immunological defects have been described in Turner's syndrome[2,7,8]. Abnormalities of the proportions of peripheral blood lymphocyte subpopulations and of immunoglobulin serum levels have been described in some patients affected by Turner's syndrome. However, we could not find such defects in our patient, and all the other immunological tests which were performed were in normal limits. All the other tests done to determine the cause of bronchiectasis,

Fig. 1: Electron microscopy of epithelial cells with surrounding microvilli and goblet cells. Cilia are not present (m: microvilli) (magnification: x3,000).

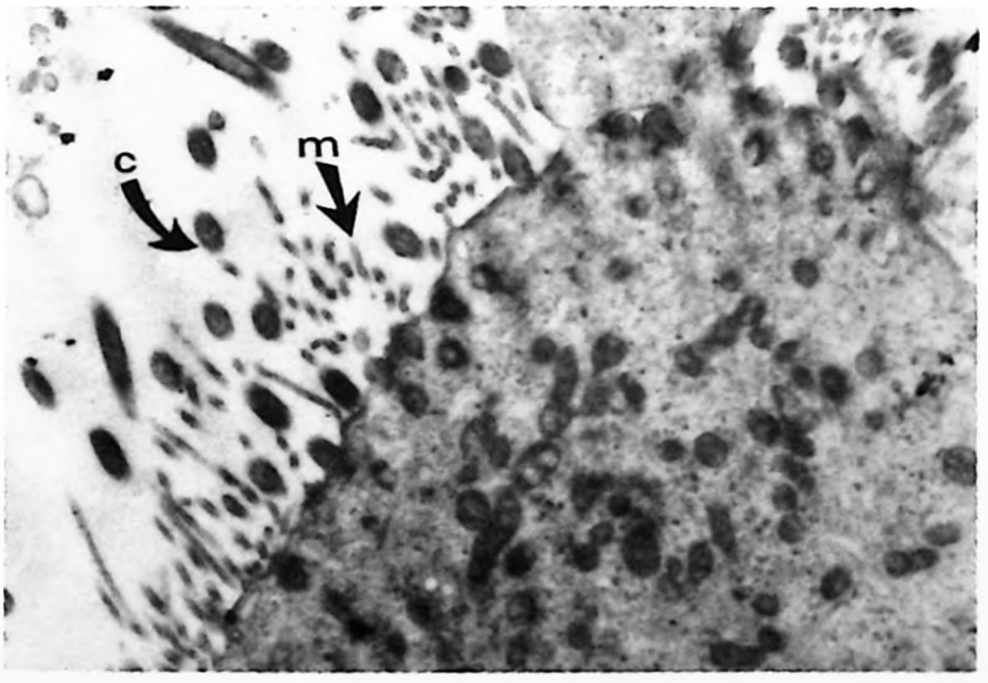

Fig. 2: Electron microscopy of epithelial cells shows absence of cilia and basal bodies in most of the cells (m: microvilli, c: cilia) (magnification: x10,000).

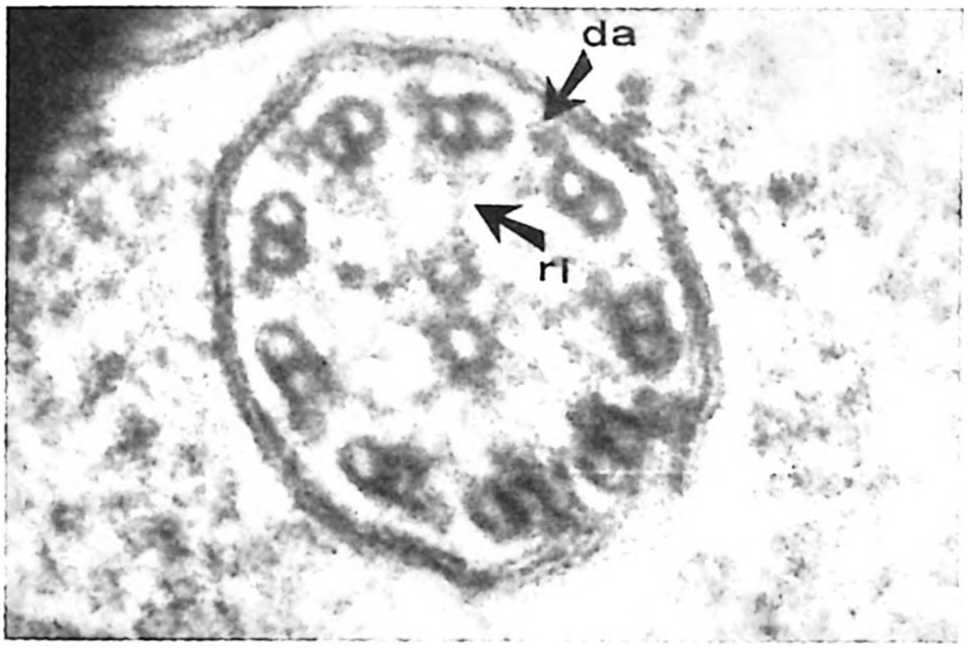

Fig. 3: Normal cross-section of cilium (d: dynein arms, r: radial spoke) (magnification: x100,000).

except for the nasal biopsies, including sweat chloride and alpha-1-antitrypsin, were in normal limits.

Repeated nasal mucosa biopsies showed lack of cilia and basal bodies in almost all cells examined. It is known that viral and bacterial infections appear to be the most frequent cause of secondary ciliary aplasia[3,10]. However, although we could not perform an in vitro cell culture for ciliogenesis, ciliary aplasia was documented three times over a six-month period, when the patient was well. In addition, ciliary basal bodies, which are expected to be present in secondary ciliary dysplasias[4], were not seen in these biopsies.

Ciliary aplasia is a very rare condition. Some authors consider ciliary aplasia as type 4 dyskinetic cilia (abnormal ciliated cells lacking axonal structure within the ciliary shafts)[1]. As is similar in other primary ciliary dyskinesias, association of ciliary aplasia with abnormalities such as dextrocardia, azoospermia, and hydrocephalus has been noted in the literature[1,4-6,9]. Bronchiectasis is a very common finding of primary ciliary dysplasia. In the series of Barlocco et al.[1], the incidence of bronchiectasis was reported to be 85 percent. Ciliary aplasia is considered an autosomal recessively inherited disease, as are the other forms of primary ciliary dyskinesia. Although the parents of our patient were consanguineous, her siblings were healthy. In this report, ciliary aplasia was described in a girl with Turner's syndrome who showed typical clinical findings of primary ciliary dyskinesia syndrome. To our knowledge, association of these two different genetic diseases has not been reported previously.

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TWO FEMALE SIBLINGS FROM TURKEY WITH LANGER MESOMELIC DYSPLASIA (HOMOZYGOUS LERI-WEILL DYSCHONDROSTEOSIS SYNDROME)*

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SUMMARY: Balcı S, Zafer Y, Ünsal M. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Two female siblings from Turkey with Langer mesomelic dysplasia (homozygous Leri-Weill dyschondrosteosis syndrome). Turk J Pediatr 1999; 41: 531-539.

Leri-Weill dyschondrosteosis is an autosomal dominant syndrome of which the characteristic features are mild-to-moderate shortness of stature and Madelung deformity of the wrist. The homozygous state of the gene for Leri-Weill syndrome causes Langer mesomelic dysplasia which is characterized mainly by shortening of the long tubular bones, more markedly in the middle than in the proximal and distal segment of the extremities. In this paper, we present two sisters with Langer mesomelic dysplasia (12 years and 6 months of age, respectively), from consanguineous parents. The mother of our cases had Madelung deformity. Father, mother and grandmother also had a slight deformity of both forearms. Unfortunately, despite the well documented case of the older sister with Langer mesomelic type dysplasia, the first and second trimester ultrasonographies of the younger sister were performed by inexperienced staff of a local urban hospital and the prenatal diagnosis of this case was not made. In this paper, we also discuss the prenatal diagnosis of Langer type mesomelic dysplasia. *Key words: Langer mesomelic dysplasia, homozygous Leri-Weill dyschondrosteosis syndrome, hypoplastic ulna-fibula, hypoplastic mandible, prenatal diagnosis.*

Leri-Weill dyschondrosteosis is an autosomal dominant syndrome of which the characteristic features are mild-to-moderate shortness of stature and Madelung deformity of the wrist. The homozygous state of the gene for Leri-Weill syndrome causes Langer mesomelic dysplasia[1]. The first Turkish case of Langer type mesomelic dysplasia was reported from Germany in 1980[2] and the second by Diren et al.[3] 1985. This report from Turkey is the third such report of Langer type mesomelic dysplasia, documented in this case in two siblings.

Case Reports

Case 1

The 12-year-old female patient was the first child born to consanguineous parents who were cousins. She was first seen at 21 months of age with chief complaints

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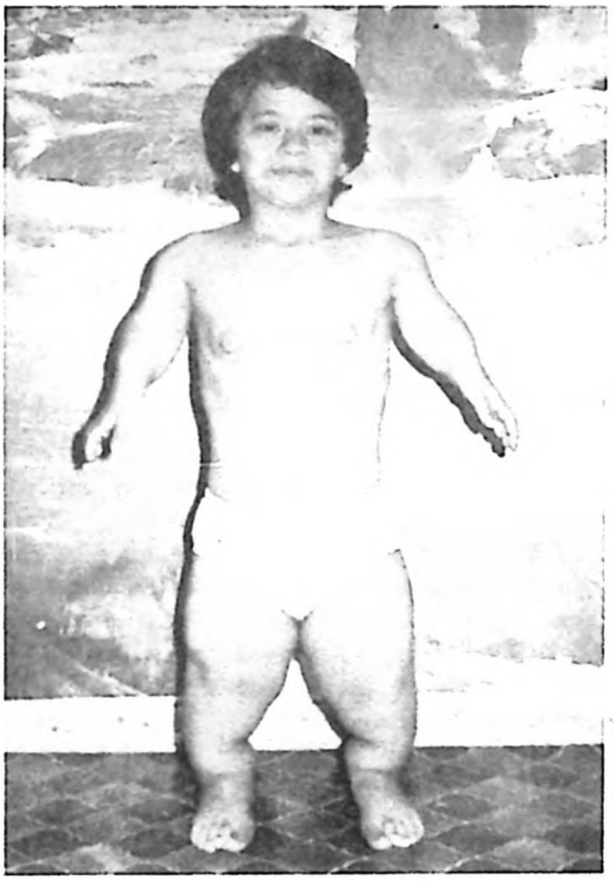

Fig. 1: The first case at the age of 12 years. Note the striking shortness of the middle segments of the extremities, ulnar deviation at the wrists, and normal appearance of hands and feet.

of short stature mainly due to short extremities. Her length was 67 cm (<3rd percentile), weight 12 kg (50th percentile), and head circumference 48 cm (75th percentile). She was seen again at 12 years of age (Fig. 1). Her psychomotor development was normal. Physical examination revealed marked bilateral shortening of the proximal and middle segments of upper and lower limbs. Her height was 106 cm (<3rd percentile) and weight 30 kg (<3rd percentile). She had hypertelorism, pectus excavatum and lumbar lordosis.

Radiological examination: Skull X-ray showed basilary impression and mildly hypoplastic mandible (Fig. 2). Symmetrical shortening of the radius and ulna was associated with bowing of the radius. Humerus was also short and thickened. Caput humeri showed varus deformity and the distal epicondyle was broad. Tuberositas deltoidea humeri was prominent. Proximal portion of the radius and distal portion of the ulna were hypoplastic (Fig. 3).

Spina bifida occulta malformation at 5th lumbar vertebra was observed. The femur and the tibia had a broad shaft and were shortened. Diaphysis of the femur was also short and trochanter major, trochanter minor and condyles were broad (Fig. 4).

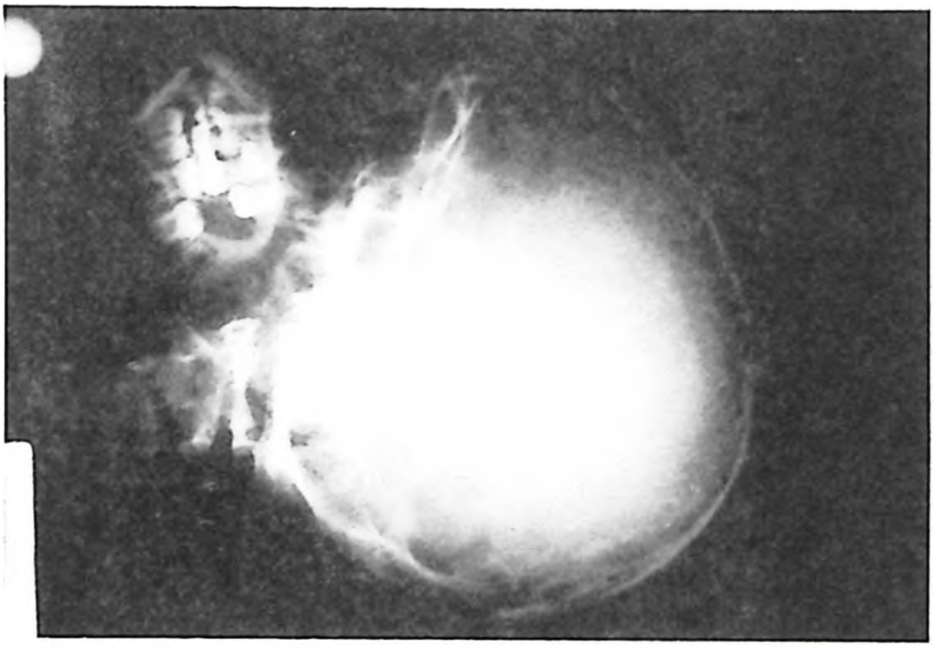

Fig. 2: Skull X-ray: basilary impression and mildly hypoplastic mandible.

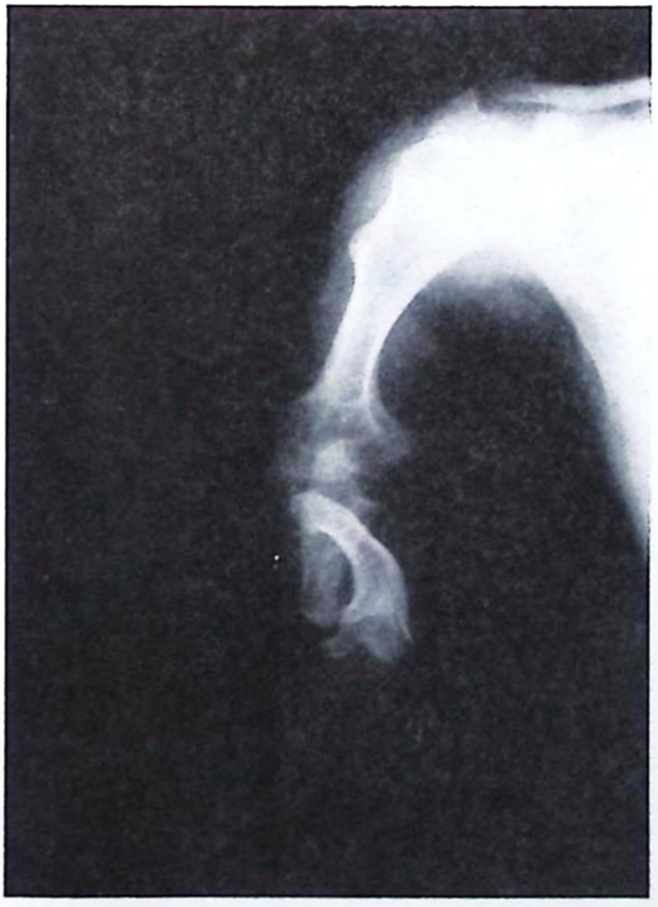

Fig. 3: Forearm, anteroposterior radiograph: shortening of he radius and the ulna and associated bowing of the radius. Proximal portion of the radius and distal portion of the ulna are hypoplastic. Slight broadening of the humenus.

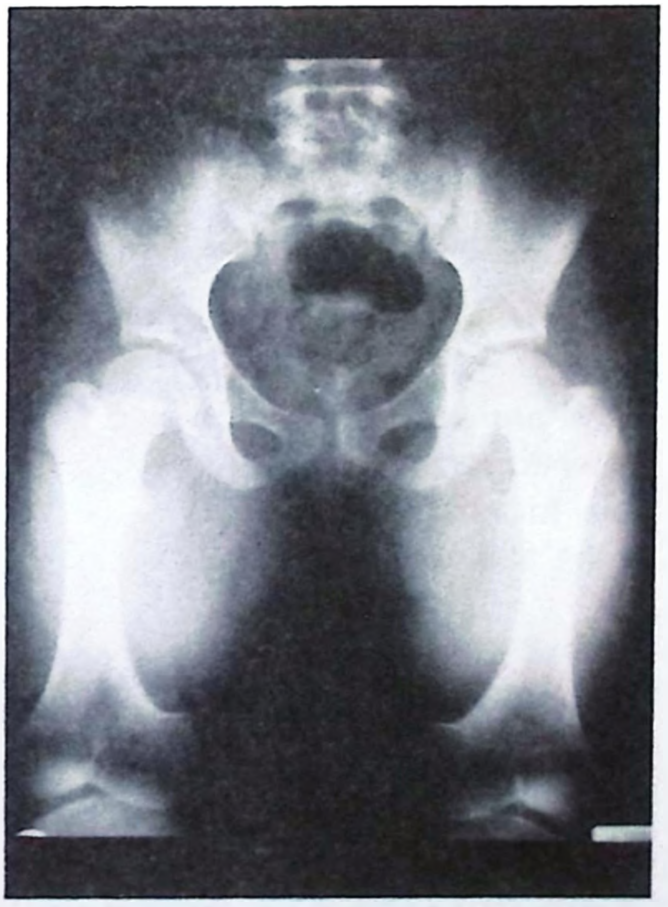

Fig. 4: Hip and thigh, anteroposterior radiograph: the femur is broad and short. Spina bifida malformation at 5th lumbar vertebra.

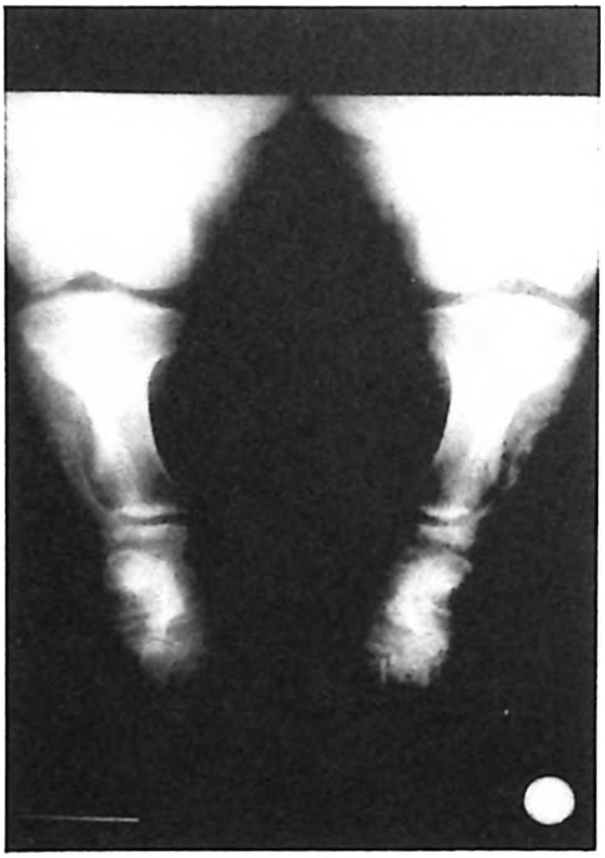

Fig. 5: Shank, anteroposterior radiograph: the tibia is broad and short. Early fusion in tibial proximal epiphyseal ossification center is observed. Proximal segment of the fibula is hypoplastic.

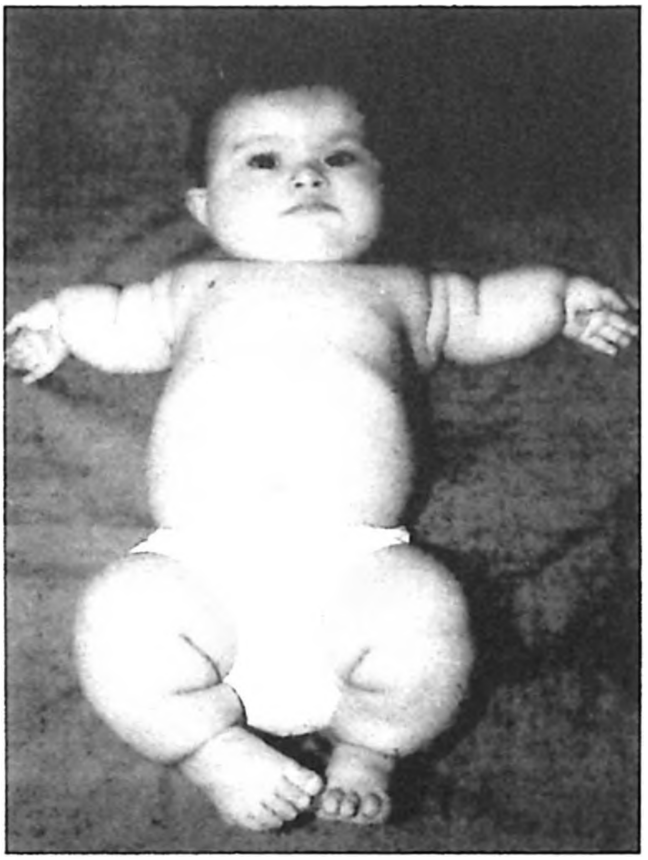

Fig. 6: The second case at six months. Limb shortening is most obvious in the forearms and shanks.

Hand and foot roentgenograms showed no abnormalities. Tibial plates were flattened, and eminentia intercondylaris was not clearly seen. Early fusion in tibial proximal epiphyseal ossification center was observed. Proximal segment of the fibula was hypoplastic (Fig. 5).

Case 2

When the first affected daughter was 14 years old, the mother became pregnant again. She was 37 years old and the father was 33 years old. Although the parents were invited to the hospital many times, financial problems prevented their coming. The family did not apply to our center for prenatal ultrasonography of this third pregnancy. Routine prenatal ultrasonography was performed by inexperienced staff. Unfortunately, the third child was born with similar findings. Birth weight was 3,700 g and length was 49 cm. After six months, her weight was 9 kg (90-97th percentile) and her length was 61.5 cm (3rd-10th percentile) (Fig. 6). On physical examination, bowing and shortness of upper and lower

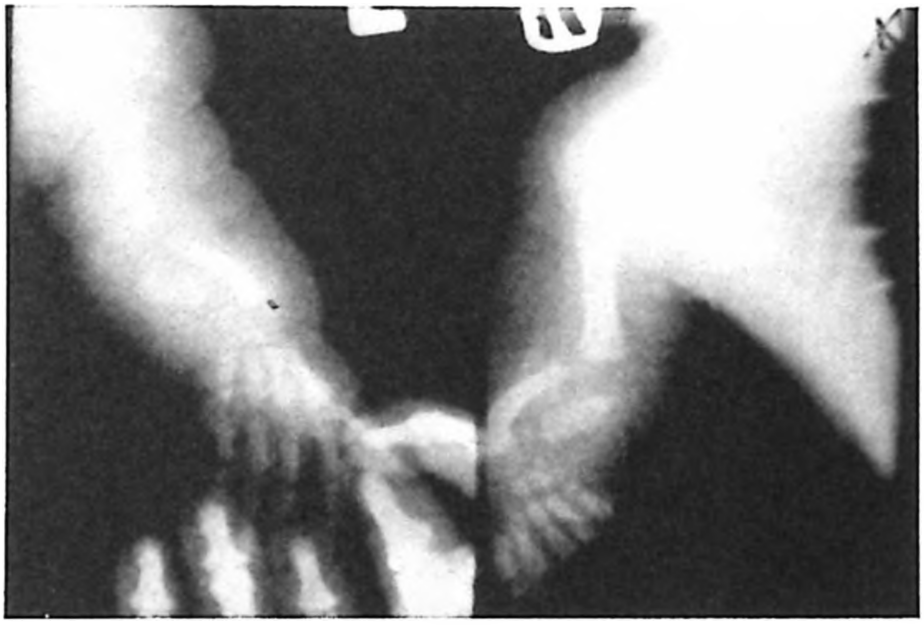

Fig. 7a

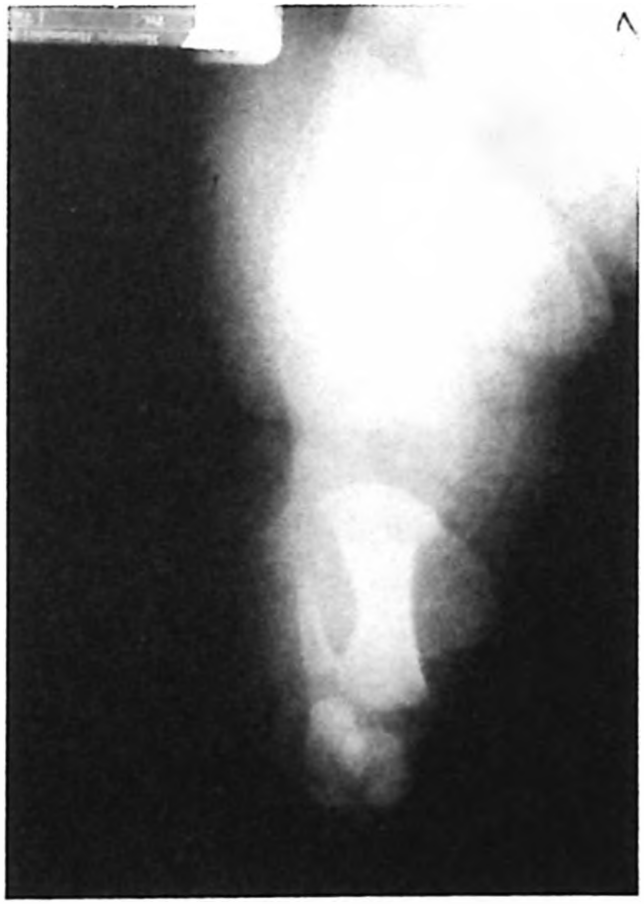

Fig. 7b

Fig. 7a-b: a: Right arm, anteroposterior radiograph: broadening of the humerus. Radial bowing of the radius and absence of the ulna including the epiphysis. b: Right leg, anteroposterior radiograph: shortened tibia with broad shaft, rudimentary fibula. Absent upper portion including the ossification center of the fibular head.

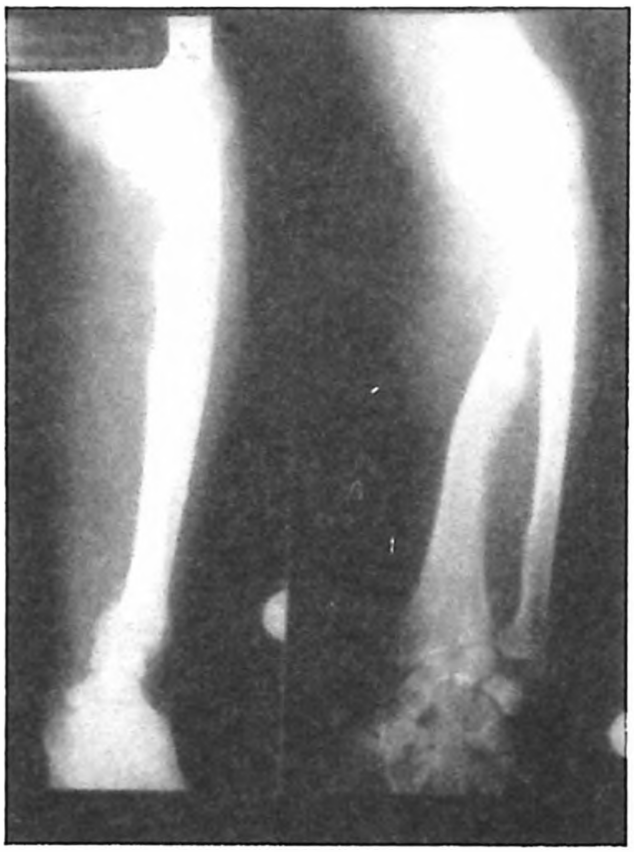

Fig. 8a

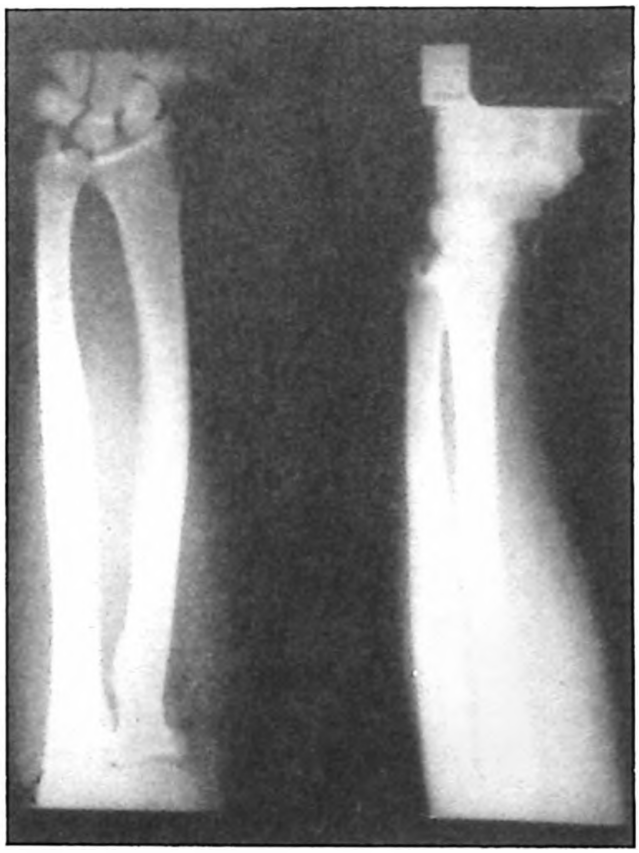

Fig. 8b

Fig. 8a and b: Right forearms of the parents with typical changes caused by Madelung deformity.

extremities were detected. Radiological examination: Roentgenograms obtained at six months showed symmetrical shortening of the radius and ulna with bowing of the radius. The humerus was short and thickened. The ulna was hypoplastic and the distal portion including the epiphysis absent. The middle segments of the lower extremities were considerably shortened. The tibia had a broad shaft and was shortened. The fibula was rudimentary: the upper portion, including the ossification centers of the tibia, was small and moderately deformed. The radiological findings of the second affected female patient are shown in Fig. 7a, 7b. The mother measured 141 cm and demonstrated typical Madelung deformity (Fig. 8a). The father measured 171 cm and had a slight deformity of both forearms (Fig. 8b). The maternal grandmother was 59 years old, and roentgenograms of her upper extremities showed slight bowing of the radius (Fig. 9). Figure 10 summarizes this current pedigree of kinship.

Discussion

Langer[1] described three families and referred to the entity as 'mesomelic dwarfism of the hypoplastic ulna, tibia, mandible'. Langer mesomelic dysplasia is characterized mainly by shortening of the long tubular bones, more markedly

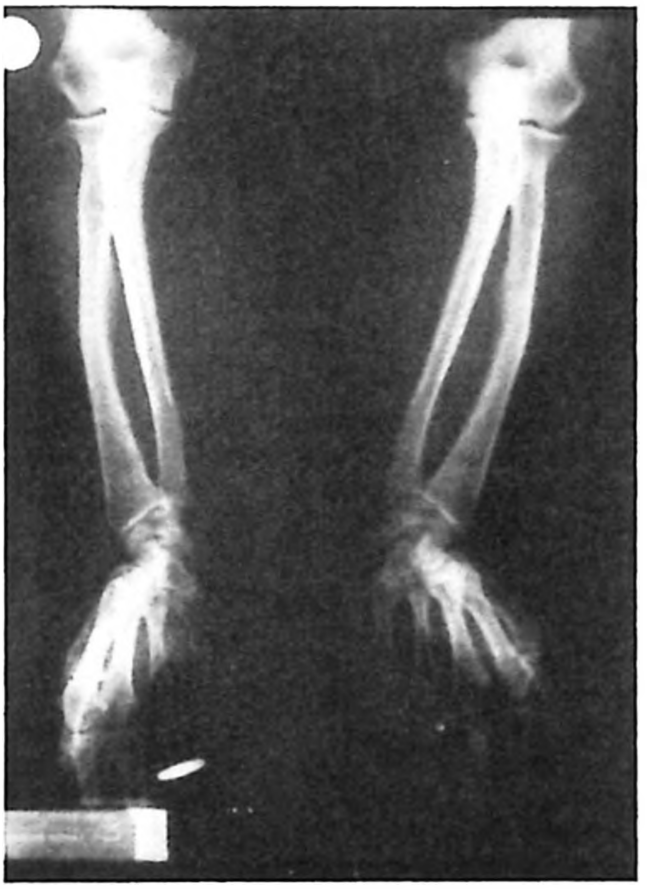

Fig. 9: Right forearms of the maternal grandmother showing slight bowing of the radius.

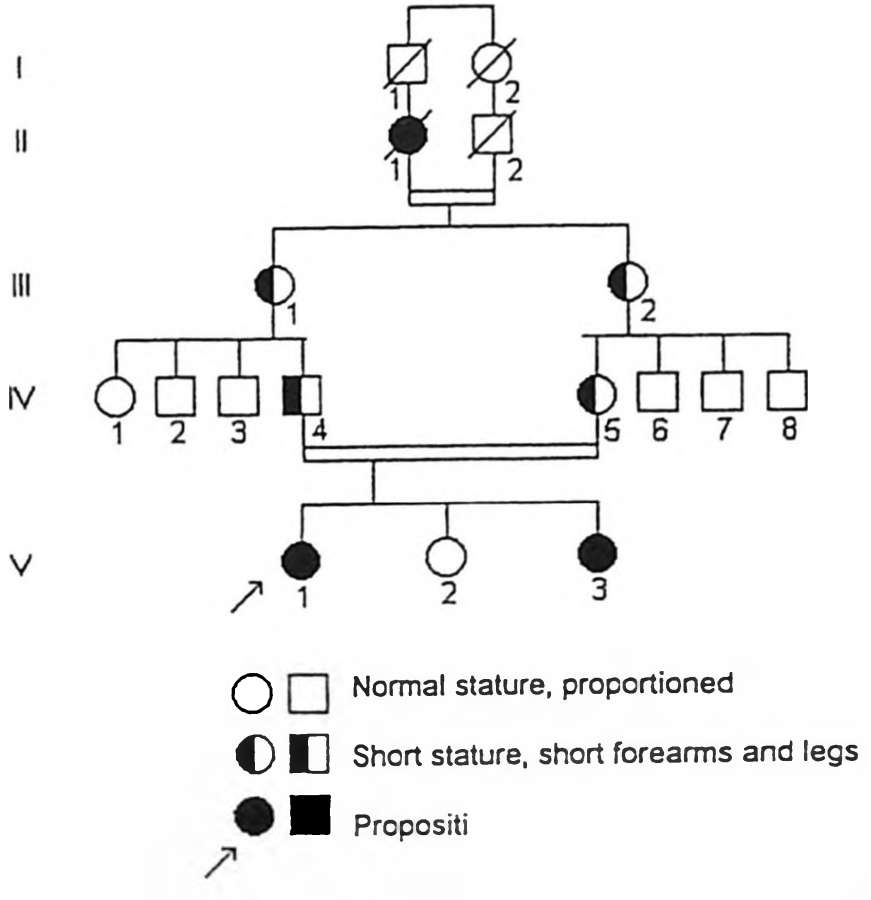

Fig. 10: Pedigree of kinship. Members IV-5, V-1 and V-3 have classical dyschondrosteosis.

in the middle than in the proximal and distal segments of the extremities. The second most common finding is radial bowing of the radius with additional radial and volar curvature, hypoplasia of the distal part of the ulna and absence of an ossified distal ulnar epiphysis, such as was seen in our case[3,4]. The third common finding is marked shortening of the tibia with lately developed, moderately deformed and prematurely fused epiphyseal ossification centers[3].

Mesomelic is a nonspecific term representing shortening, most apparent in the forearm and lower leg, and referred to as 'dyschondrosteosis'. In addition to these findings, a hypoplastic mandible and normal skull are the other characteristic features. There are no extraskeletal abnormalities and intelligence is normal[3].

The mesomelic dysplasias are a heterogeneous group of disorders characterized by shortness predominantly in the radioulnar and tibiofibular segments. Five have been delineated: Nievergelt, Langer, Robinow, Rheinhardt-Pfeiffer, and Werner[3].

Included in the differential diagnosis of mesomelic dysplasia is also Ellis-van Creveld syndrome. This syndrome can be differentiated from Langer mesomelic dysplasia by the presence of cardiac malformations, oligodontia with conical teeth, polydactly and nail dysplasia. The mode of inheritance of this syndrome is autosomal recessive. Heterozygote individuals show mild-to-moderate shortness of stature and relatively short forearms with Madelung deformity. The homozygous state is associated with a much more severe shortness of stature.

Book[6] described the first kindred with Langer type mesomelic dwarfism from northern Sweden in 1950. Heterozygotes in this family were short (the father was 160 cm and the mother 150 cm) and had relatively short fingers, and broad hands. Motor and mental development were normal. The adult height was approximately 130 cm. In our cases there was first-degree consanguinity between the parents, and there was a history of the maternal great grandmother being of short stature. Heterozygotes in the family were also short (the mother was 141 cm tall and the great grandmother was even shorter, with relatively short fingers and hands). The first Turkish case of Langer type mesomelic dwarfism was reported by Kunze and Klemm[2] in 1980. They reported two female cases of separate unrelated Turkish families. Both parents of affected females had signs of dyschondrosteosis, including Madelung deformity. The first three cases with Langer mesomelic dysplasia from separate unrelated families from Turkey were reported in 1985 by Diren[3] et al.

Goldblatt et al.[7] described a case of Langer type mesomelic dysplasia with a mild deformity of the forearms similar to but different from Madelung's deformity of the forearms.

Evans et al.[8] described the first prenatally diagnosed (2nd trimester) case of Langer type mesomelic dwarfism by sonography, and reported the pathologic findings in this case. The mother of this fetus also had Madelung deformity. Madelung deformity or mild deformity of the forearm may be an important sign for mesomelic dysplasias.

In this report two sisters from consenguineous parents with Langer type mesomelic dysplasia are presented. There is no report of this dysplasia occurring in a related family in the literature. This observation suggests that this dysplasia may be due to an autosomal recessive gene. In our cases despite the older sister's illness being well documented, prenatal diagnosis of the younger sister was not made, probably due to misinterpretation of the first and second trimester sonographics by inexperienced staff at a local urban hospital. This highlights the importance of early prenatal recognition of this rare dysplasia. We believe increased reporting of such cases will prompt early diagnosis.

Acknowledgements

The authors are grateful to Professor J. Spranger for evaluation of the patients' X-rays and confirmation of the diagnosis.

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The Turkish Journal of Pediatrics 1999; 41: 541-544 *Case*

HEPATITIS AS THE PRESENTING SYMPTOM OF CHILDHOOD SYSTEMIC LUPUS ERYTHEMATOSUS*

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SUMMARY: Apak RA, Beşbaş N, Özdemir S, Özen H, Bakkaloğlu A, Saatçi Ü. (Nephrology and Rheumatology, and Gastroenterology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Hepatitis as the presenting symptom of childhood systemic lupus erythematosus. Turk J Pediatr 1999; 41: 541-544.

We report in this article a girl with an initial diagnosis of autoimmune hepatitis who developed full-blown systemic lupus erythematosus (SLE) at her two-years follow-up. She was formerly considered as HBV-related chronic active hepatitis but due to the persistence of elevated liver enzymes, the reversal of the albumin and globulin ratio and abnormal HBV serology, she was later diagnosed as autoimmune hepatitis. With the clinical findings of arthritis, arthralgia and malar rash and supported by results of laboratory tests, she was diagnosed as a case of unusual SLE presenting with autoimmune hepatitis. We conclude, therefore, that each patient with a diagnosis of autoimmune hepatitis in childhood who exhibits abnormal HBV serology must be evaluated for a possible diagnosis of SLE. *Key words: systemic lupus erythematosus, autoimmune hepatitis, hepatitis B virus.*

Systemic lupus erythematosus (SLE) is a multisystem disease with a wide range of symptoms resulting from different antinuclear antibodies directed against one or more components of the cell nuclei. In these patients certain genetic markers are associated with distinct clinical manifestations[1].

Although 15 to 30 percent of patients have elevated liver enzymes during the course of the disease, hepatic involvement as the first manifestation is very rare in SLE[2]. More severe liver disease may also be associated, but it is almost always of infectious origin, due to misdiagnosed autoimmune hepatitis or primary biliary cirrhosis[3].

We report a girl diagnosed as autoimmune hepatitis with an abnormal hepatitis B virus serology who developed clinically full-blown SLE within three years.

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Case Report

A nine-year-old girl was admitted with a one-month history of fever, malar rash, and redness, swelling and pain on her finger joints, elbows and knees.

She was otherwise healthy until the age of six when she developed anorexia, fatigue and jaundice, and was diagnosed as hepatitis. Physical examination findings at that time revealed her height as 112 cm (25th centile), weight as 20 kg (25th centile), and blood pressure 120/70 mmHg. She had jaundice and hepatomegaly. The remainder of the examination was normal. On laboratory evaluation, hemoglobin was 6.5 g/dl, hematocrit was 22 percent, and white blood cell count was 4,500/mm^3 with normal differential and normochrome microcytic erythrocyte morphology. Urinalysis was unremarkable. Direct Coombs' test was negative. Serum AST and ALT levels were 773 IU/L and 486 IU/L, respectively. Serum total and direct bilirubin levels were 2.2 mg/dl and 1.4 mg/dl, total protein level was 8.6 g/dl and albumin level was 4.1 g/dl. Hepatitis A virus antibody (anti HAV Ab), hepatitis B virus surface antigen (HBs Ag), HBV enzyme antigen (HBe Ag) and antibody (anti HBe Ab), HBV core IgM and IgG antibodies (anti HBc IgM and IgG) and hepatitis C virus (HCV) antibody (anti HCV) were all negative. One month later, her complaints and physical findings had not changed but AST, ALT and total protein levels rose and HBs Ag and Anti HBc IgG became positive. Two months later, her liver enzyme levels dropped about 50 percent and only HBs Ag remained positive as a serological marker. Within one year of follow-up, AST and ALT levels fluctuated between 164 and 239 IU/L. Bilirubin levels remained within normal limits. Total protein level was always high despite a normal serum globulin level. All serological markers related to HBV, including HBc IgG, became negative during the consecutive three months.

On her first year of admission, a percutaneous fine needle liver biopsy was performed due to her persistently elevated liver enzymes. Histological examination revealed disorganized hepatic architecture exhibiting portoportal bridging and increased fibrous tissue invading the liver parenchyma with mononuclear cell infiltration in portal areas. There was also evidence of suspicious nodule formation. However, these changes were not associated with a specific clinical condition.

She remained stable clinically for two years without any treatment; however, during this period her liver enzymes were persistently elevated (two-fold). At the end of second year, she started complaining of fever over 38.5°, pain, swelling and redness on her finger joints, elbows and knees. On physical examination, she had a striking malar rash, livedo reticularis, hepatosplenomegaly and fusiform swelling on her proximal interphalangeal joints. On laboratory examination, hematocrit was 32.9 percent, white blood cell cound 7,300/mm^3 with normal

differential, thrombocyte count 380,000/mm^3, and sedimentation rate 88 mm/hour. Direct Coombs' test and C reactive protein tests were positive. Anti DNA antibody level was 145 IU/L (Normal: 0-7 IU/L), complement 3 and complement 4 levels were 35.4 mg/dl (Normal: 60-120 mg/dl) and 5 mg/dl (Normal: 30-60 mg/dl), respectively. With these clinical and laboratory findings, she was diagnosed as having SLE and corticosteroid treatment was initiated. Following her first month of therapy, her complaints subsided, hepatomegaly disappeared and liver enzyme levels dropped within normal limits.

Discussion

A hepatitis-lupus connection has been recognized in adults for more than 30 years. It has not previously been considered a significant problem and is thought to be surprisingly rare[4]. However, later reports state that subclinical liver disease may be a manifestation of SLE[5]. Harvey et al.[6] reported that 35 percent of patients with SLE had palpable livers. In another report by Kofman et al.[7], 52 percent of patients with SLE had hepatomegaly, 12 percent had jaundice and 31 percent had elevated liver enzymes.

In our case, determining the etiology of chronic hepatitis was more of a problem. The patient was not taking drugs which were potentially hepatotoxic and she had negative HCV serology. Similarly, cytomegalovirus. Epstein-Barr virus and toxoplasmosis, although not directly tested in her, do not usually produce chronic hepatitis; the biopsy did not present the typical histological picture of these infections. Initial clinical presentation with abnormal transaminases levels, positive HBs Ag and anti-HBc Ab, with the support of liver histology, suggested HBV related chronic active hepatitis. On the other hand, we considered the diagnosis of autoimmune hepatitis in view of the spontaneous serologic remission following an abnormal HBV serology, the increased globulin concentration and the reversal of the albumin/globulin ratio during her follow-up. The diagnosis of SLE was established after her 1.5 year follow-up when the joint complaints appeared. The question remains whether this patient with liver disease had a coincidental serious liver disease and SLE, serious liver disease as an initial manifestation of SLE, or autoimmune liver disease with associated autoimmune phenomena suggesting the diagnosis of SLE. Our patient fulfills the ARA criteria for the correct diagnosis of SLE. Previous reports state that 10 to 20 percent of patients diagnosed as autoimmune hepatitis fulfill the ARA criteria for SLE[8]. In addition, hepatic manifestations of SLE have been considered as a significant problem in the adult population as early as four years prior to the diagnosis of SLE[9]. These data show that the former clinical presentation in our patient was the presenting symptom of an existing SLE.

However, serological markers that would have supported the diagnosis of autoimmune hepatitis were not available at that time. In fact, in a recent report by Satoh et al.[10], two cases with SLE-associated autoimmune hepatitis were found to have negative serological markers. Novel antibodies which react with transfer RNA related antigens (anti-ribosomal P antibodies) are found to be more specific for the diagnosis of SLE-associated autoimmune hepatitis[11]. Therefore, an initial positive HBV serology) recalls false-positivity resulting from an autoimmune phenomena. False-positive HCV serology in patients with autoimmune hepatitis has been reported, but this is the first case exhibiting false-positive HBV serology with autoimmune hepatitis[12].

We conclude, therefore, that in children, liver disease as manifested by autoimmune hepatitis and liver cirrhosis, even with the presence of an abnormal HBV serology, may develop in the course of SLE, and that each patient with autoimmune hepatitis in childhood must be evaluated for a possible diagnosis of SLE.

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The Turkish Journal of Pediatrics 1999; 41: 545-549 Case

PSORIASIS IN A PATIENT WITH NEUROFIBROMATOSIS*

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SUMMARY: Çelebi S, Kılıç SŞ, Okan M. (Department of Pediatrics, Uludağ University Faculty of Medicine, Bursa, Turkey). Psoriasis in a patient with neurofibromatosis. Turk J Pediatr 1999; 41: 545-549.

A seven-year-old boy with neurofibromatosis who developed scalp psoriasis is presented. The clinical evaluation of the patient revealed multiple café au lait spots, axillary freckling, bilateral Lisch nodules and a psoriatic plaque on his scalp. Though there is no known direct relationship between neurofibromatosis and psoriasis, one is suggested in this patient, possibly related to a common genetic defect. *Key words: neurofibromatosis, psoriasis.*

Neurofibromatosis is a multisystem disease that is transmitted in an autosomal dominant pattern and seen with a frequency of about 1:3,000[1]. Approximately 50 percent of cases represent new mutations. There are seven distinct forms of neurofibromatosis. Neurofibromatosis type 1 (NF-1) is the most prevalent. The abnormal gene of NF-1 is the band q11,2 on the proximal long arm of chromosome 17[2,3]. Psoriasis is a common chronic hyperproliferative inflammatory papulosquamous dermatosis of unknown etiology. It tends to occur in families, although genetic transmission has not been clearly delineated[4]. When the onset occurs during childhood, about 50 percent have a positive family history of the disease[5].

It is rare for patients to have both disorders; we could find only two previous reports with this combination in the literature[6,7]. In this paper we report a male patient with neurofibromatosis who developed scalp psoriasis.

Case Report

A seven-year-old boy was admitted to emergency service because of a partial seizure attack. He had multiple brown papules on his trunk and extremities, reportedly present since birth. He had some learning problems. There was no family history of neurofibromatosis, psoriasis or consanguinity. On physical examination there were multiple café au lait spots and axillary freckling (Figs. 1-2). He had a dysmorphic facial appearance and hypertelorism. Examination

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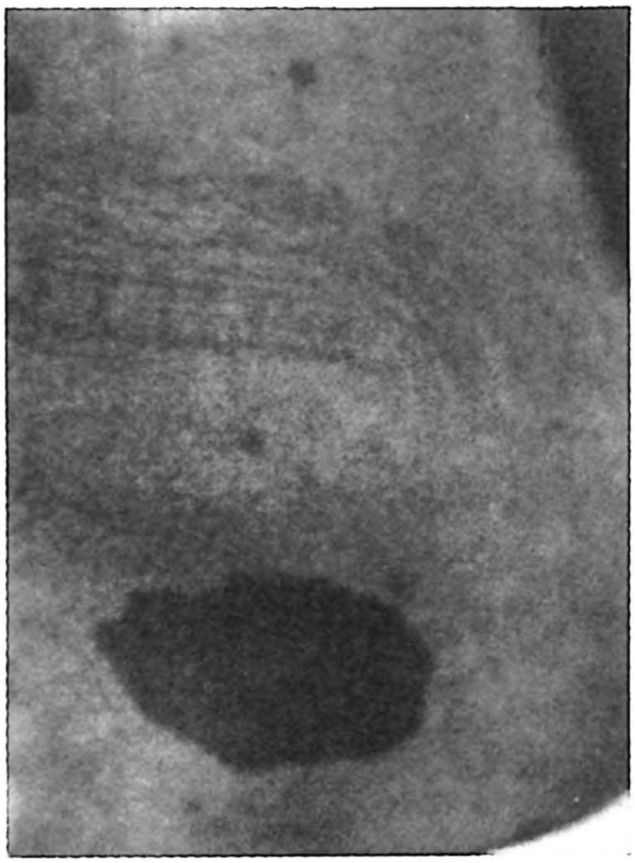

Fig. 1: Multiple café au lait macules in the patient with neurofibromatosis type 1.

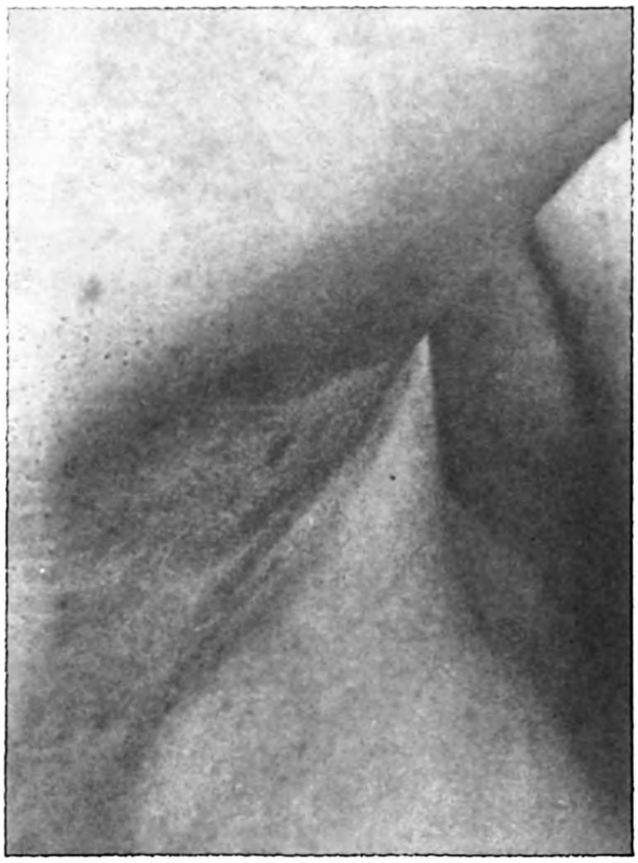

Fig. 2: Axillary freckling in the patient with neurofibromatosis type 1.

by an ophthalmologist revealed bilateral Lisch nodules. He had a large plaque of psoriasis on his scalp, varying in size from 2 to 3 cm in diameter and had erythema and scale. There was hair loss on the lesion. This plaque had been present on his scalp since the age of 6½.

Laboratory values were as follow: Magnetic resonance imaging (MRI) of the head demonstrated hyperintense fields and hamartomatous changes of basal ganglions, cerebellum and medulla oblongata. On electroencephalogram (EEG) there was active epileptiform abnormality originating from the right hemisphere. A biopsy specimen taken from the plaque on his scalp revealed hyperkeratosis with parakeratosis, acanthosis and neutrophilic infiltrates forming microabscesses, all characteristic for psoriasis. The chromosome analysis was normal. Treatment with topical steroid for his scalp lesion and with carbamazepine therapy for his convulsion was started.

Discussion

Neurofibromatosis (NF) is the consequence of an abnormality of neural crest differentiation and migration during the early stages of embryogenesis[8]. NF-1, von Recklinghausen's neurofibromatosis, includes the characteristic skin lesions

which occur in several forms, chiefly neurofibromas, but also café au lait macules, axillary freckles and giant pigmented hairy nevi. The café au lait macule is a hallmark of this disease[8]. The National Institutes of Health Consensus Development Conference[9] identified the following seven important components of the syndrome, two or more which must be present to establish the diagnosis of NF-1: 1) six or more café au lait macules, the greatest diameter of which is more than 5 mm in prepubertal patients and more than 15 mm in postpubertal patients (Crowe's sign), 2) two or more neurofibromas of any type, or one plexiform neurofibroma, 3) freckling in the axillary or inguinal regino, 4) optic glioma, 5) two or more Lisch nodules, 6) a distinctive osseous lesion such as sphenoid dysplasia or pseudoarthrasia, 7) a first-degree relative with NF-1 according to the preceding criteria. Our patient met these criteria for diagnosis of NF-1.

Children with NF-1 are susceptible to neurological complications. Neurological involvement may be focal or diffuse, central or peripheral. Mental retardation, epilepsy, brain tumors, and tumors of cranial nerves may occur[10]. MRI studies in selected children have shown abnormal signals in the globus pallidus, thalamus, and internal capsule, which probably represent low grade glioma or hamartoma that is not detected by computed tomography (CT) scanning[8]. Cranial MRI of our patient showed similar changes as above. These findings may account for the high incidence of learning disabilities. Complex partial and generalized tonic-clonic seizures are a frequent complication[10]. Our patient had some learning difficulties and partial seizure.

The Lisch nodules (hamartomas of iris) present as grayish-white spots, and are visible by direct examination. The optic nerve gliomas may be unilateral or bilateral and are observed in about 15 percent of patients; only about a third are associated with loss of vision[11]. Ophthalmologic examination of our patient disclosed bilateral Lisch nodules, but optic glioma was not detected.

Endocrine disorders such as acromegaly, cretinism, hyperparathyroidism, pheochromocytoma, or precocious puberty may be present. Bone changes (usually erosive) may produce lordosis, kyphosis, pseudoarthrosis, dislocations, and atraumatic fractures[11]. We did not detect any endocrinological abnormality or bone changes in our patient.

In most children within psoriasis of the scalp, other areas of the skin also are involved, showing the typical plaques set on an erythematous base. Occasionally, however, only the scalp may be affected. The base of a scalp lesion is always inflammatory, thus erythema underlies the whitish scale. The scale sometimes forms large plaques, resulting in hair loss[12]. Psoriatic plaque of our patient was only present on his scalp, with similar properties as above.

Although a variety of clinical presentations of NF-1 may occur and the disease can affect many organ systems, there has been suggestion of a direct relationship between NF and psoriasis. Previously there were two reported cases in which neurofibromatosis and psoriasis coexisted. In 1985, Roenigk et al.[6] reported a case with NF-1 and psoriasis. In 1990, Nishimura et al.[7] reported neurofibromas developed in a patient with psoriasis vulgaris during PUVA treatment. Whether or not there is a causal relationship between neurofibromatosis and psoriasis in an intriguing question. Neurofibromas with large numbers of mast cells may occur as a cardinal feature of a wide spectrum of disorders referred to collectively as neurofibromatosis. Mast cells directly or indirectly contribute to the origin and/or growth of neurofibromas; interference with neurofibroma growth might be possible though the use of agents that block mast-cell secretion[13]. Furthermore, mast cell densities are increased in lesional psoriatic skin compared with normal or involved psoriatic skin. If non-involved skin is traumatized, mast cell numbers increase before psoriatic lesinos appear. Whether the presence of neurofibromatosis might predispose a patient to flares of psoriasis is only speculative. In any case, neurofibroma was not present in our patient. Although neurofibromatosis and psoriasis are genetically transmitted diseases in which skin involvement is of primary importance, there have been only two cases in the literature to date. With this case report, we want to draw attention to this association, which may even occur more often than is reported in the literature.

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AUTHOR INDEX TO VOLUME 41

ACAR Yeşim 495
ACUNAS Betül 429
ADALIOĞLU Gönül 413
AHISKALI Rengin 495
AKALIN Figen 127
AKALIN Nursel 201
AKÇORAL Adnan 341
AKGÜN Nejat 447
AKINCI Ayşehan 381
AKSARAY Necmi 81
AKSOY Ayşe 253, 267
AKSU Aslan 473
AKSU Nejat 225
AKSUNGUR Pauline 81
AKTUĞ Tanju 139
AKYÖN Yakut 189
AKYÜZ Canan 161
ALAND Jenny S15
ALEHAN Dursun 395
ALİKAŞİFOĞLU Mehmet 287, 299
ALPARSLAN Bülent 375
ALTAY Çiğdem 323, 467
ALTUNKAYA Hanife 273
ANADOL Deniz 53
ANTMEN Bülent 81
APAK R. Anıl 521, 541
ARİ Nurcihan 253
ARIKAN Sevtap 189
ARISOY Nil 67
ARITÜRK Nurşen 43
ASLAN Yakup 253, 267
AŞAN Esin 353
AYDIN Abdullah 381
AYDIN G. Burça 201
AYDINLI Nur 121
AYDOĞDU Sema 335, 457
AZUMA Hiroshi S5
BABALIOĞLU Mustafa 509
BAKKALOĞLU Ayşın 277, 387, 541
BALAT Ayşe 381
BALCI Sevim 259, 525, 531
BALDINI Alessandra 421
BALKAN Emin 245
BALTA Günay 197
BAŞDEMİR Gülçin 139
BAYER Milan 151
BAYKAL-ERKILIÇ Aslı 473
BERKEL A. İzzet 369
BERTAN Vural 161
BEŞBAŞ Nesrin 387, 541
BİLAL Mehmet Salih 127
BISTRUP Marie Louse S45
BLAU Nenad 231
BODUROĞLU Koray 107, 259, 287, 299
BOZER Hasan Kani 413
BROWN Brett V. S. S51
BÜYÜKKALE Gökhan 277
BÜYÜKPAMUKÇU Münevver 161, 517
BÜYÜKPAMUKÇU Nebil 483
CANTEZ Talat 509
CEVİZ Naci 395, 403
CEYHAN Mehmet 37, 277, 421
COŞKUN Turgay 231, 501
ÇAKMAK Murat 147
ÇAKMAKÇI Handan 505
ÇALIŞKAN Mine 121
ÇELEBİ Solmaz 545
ÇELEBİOĞLU Bilge 273
ÇELİKER Alpay 395
ÇELİKKOL Bilge 341
ÇETİN Mualla 329, 353, 467
ÇİMŞİT Gülseren 253
ÇOKER Işıl 335, 457
ÇUHADAROĞLU Füsun 73, 307
ÇULLU Emre 375
ÇULLU Fügen 181
DEMİRCİN Metin 133
DEMİRHAN Beyhan 361
DERECİ Selim 267
DICKINSON Dee S127
DIMITRIOU Gabriel 429
DİNÇ Hasan 267
DİNDAR Hüseyin 147
DİNLER Gönül 91
DİZDARER Gülşen 173
DOĞAN Rıza 133
DOĞRUEL Nesrin 447
DOĞRUYOL Hasan 245
DÜZOVA Ali 239
ERBİL Mine 525
ERDOĞAN Hakan 225
ERGÖR Gül 413
ERGÜVEN Sibel 189
ERKAN Dilek 43
ERKAN Tülay 181
ERKILIÇ Metin 473
EROĞLU Ayşe Güler 399
ERSOY Fügen 369
ERTEM Deniz 495
ERTEM Ulya 219, 437
ETİKAN İlker 437
GAMSU Harold 429
GEDİK Yusuf 253, 267
GOH Denise Li Meng S13
GOONASEKERA Anura S61
GOUSSEINOV Afig 335, 457
GÖÇMEN Ayhan 53, 525
GONÜL Ali 329

GÖRE Oya 139
GREENOUGH Anne 429
GÜLAY Zeynep 283
GÜMRÜK Fatma 239, 323, 467
GÜNALP Ayfer 189
GÜNEL Ruhi 283
GÜR Deniz 37
GÜRAKAN Berkan 361
GÜRAKAN Figen 91, 99
GÜRGEY Aytemiz 197, 323, 467, 517
GÜRPINAR Arif 245
GÜVENER Murat 133
HALİLOĞLU Mithat 143
HAZAR Volkan 117
HİÇSÖNMEZ Akgün 143, 391
HİÇSÖNMEZ Gönül 219, 467
HIROSE Michitaka S73, S83
HO Hong-Fa S21
HÜDAOĞLU Suphi 341
İKİZLER Coşkun 403
İLHAN Hüseyin 365
İLYAS Can 133
İNGÖL Hüseyin 67
ISHI Takemochi S149
ISSHIKI Nobuo S73
IWATA Hiroo S73
KABUKÇUOĞLU Sare 365
KALAYCI Ömer 413
KALKANOĞLU Serap 231, 501
KANG Ellen S. 1
KANMAZ Turan 147
KANRA Berkand 37
KANRA Güler 37, 277, 421
KANRA Tekin 467
KANSOY Savaş 225
KARA Ateş 329, 421
KARA Ş. Pınar 329
KARAGÖZ Tevfik 231
KARAMAN Can 375
KASAPÇOPUR Özgür 67
KASIRGA Erhun 335, 457
KAYMAKOĞLU İbrahim 37
KILIÇ Fatma S. 447
KILIÇ S. Şebnem 545
KILIÇALP Alp 139
KILINÇ Yurdanur 81
KINIK Erol 315
KİPER Nural 53, 525
KİRAZLI Şerafettin 323
KIREL Birgül 447
KIRIŞTIOĞLU İrfan 245
KOBAYASHİ Noboru S1, 73
KOCABAŞ Emine 81
KOÇ Ahmet 517
KOÇAK Nurten 91, 99
KORKUSUZ Petek 353
KOVANLIKAYA Arzu 505
KÖSEOĞLU Vedat 161
KUTILEK Stepan 151
KUTLU Tufan 181
KUTLUK M. Tezer 161
KUYUCU Necdet 103
KUYUCU Semanur 413
MATSUDA Souhei S91
MELİKOĞLU Mustafa 473
MEŞE Timur 341
METİN Ayşe 369
MILJETEIG Per S45
MIZRAK Bülent 381
MUTAFOĞLU-UYSAL Kamer 207
MUTLU Belgin 421
NAKAGAKI Maki S99
NIHEI Kenji S73
OĞUZKURT Pelin 143
OKAN Mehmet 545
OKAR İmer 495
OKUR Hamza 219, 323, 437
OLCAY Lale 219, 239, 437
OLGAÇ Nihal 283
OLGUN Nur 207
OLGUNTÜRK Rana 201
ONGUN Hakan 117
ÖGE İhsan 43
ÖKTEN Ayşenur 253
ÖLMEZ Seval 113
ÖMEROĞLU EKER Rukiye 509
ÖNCÜLOĞLU Cem 61
ÖNDEROĞLU Lütfü 61
ÖNER Ahmet Faik 249, 323
ÖZALP İmran 231
ÖZBAKIR Fatma 67
ÖZBEK Namık 361
ÖZÇELİK Uğur 53, 525
ÖZDEMİR Sıla 387, 541
ÖZDOĞAN Huri 67
ÖZEN Hasan 91, 99, 541
ÖZEN Seza 387
ÖZER Erdener 139
ÖZER Sema 403
ÖZGÜVEN Evin 495
ÖZİNEL Sema 225
ÖZKAN Hasan 283
ÖZKAN İlhan 375
ÖZKAN Tanju 245
ÖZKUTLU Süheyla 349
ÖZMEN Meral 121
ÖZMERT Elif 421
ÖZSOYLU Şinasi 467
ÖZTUNÇ Funda 127, 399
PAPERT Seymour S137
PAŞAOĞLU Güven 283
PAŞAOĞLU İlhan 133

PAŞAOĞLU Özgül 365
PEHLİVANOĞLU Ender 495
PODDA Audino 421
POLAT Bülent 127
SAATÇI Işıl 387
SAATÇİ Ümit 387, 541
SAĞIN-SAYLAM Gül 341
SALMAN A. Bedii 483
SALTIK İrfan Levent 399
SANAL Özden 369
SARAÇLAR Muhsin 349
SARAÇLAR Yıldız 413
SARIALİOĞLU Faik 207
SARIHAN Haluk 267
SARIOĞLU Ayşe 399
SAVAŞ Çağrı 147
SEÇMEER Gülten 37, 277
SERDAROĞLU Piraye 121
SERİN Mehmet 81
SEZGİN Gülay 277
SHIMAUCHI Yukio S7
SHIRAKAWA Kimiko S73
SINMAZ Kutluğ 245
SOMEOKA Shinichi S35
SÖĞÜT Ayhan 509
SÖNMEZIŞIK Gülşen 103
SÖYLEMEZOĞLU Figen 161
SÜLLÜ Yüksel 43
ŞAHİN Kamil 509
ŞAHİN Mustafa 43
ŞEKEREL Bülent Enis 413
ŞENDUR Faruk 375
ŞENER Nuri 173
ŞENOCAK M. Emin 483
TAKEMURA Shinichi S141
TANELİ Baha 335, 457
TANYEL F. Cahit 143, 273, 391, 483
TANYELİ Atilla 81
TEKGÜL Hasan 173
TEKİN Neslihan 447
TEL Nilüfer 365
TEZCAN İlhan 353, 369
TEZİÇ Tahsin 103
THÖNY Beat 231
TOKATLI Ayşegül 231
TOPÇU Meral 259
TUNAOĞLU F. Sedef 201
TUNCEL Mürvet 525
TUNCER A. Murat ... S27, 219, 353, 437, 467
TUNCER Ayfer 413
TUNÇBİLEK Ergül 107, 287, 299
TURANLI Güzide 521
TURGUT Mehmet 381
TURGUT Melek 113
TÜMAY Güngör T. 181
TÜRELİ Cem 375
TÜRKEN Aydın 391
TÜRKER Meral 225
TÜTÜNCÜOĞLU Sarenur 173
UÇAR Birsen 447
UÇKAN Duygu 249
UEDA Nobuyuki S109
UZAMIŞ Meryem 113
UNAL Nurettin 341
ÜNSAL Metin 531
VARAN Ali 517
VARAN Birgül 361
VIVIANI Simonetta 421
WEINTRAUB Hillel S119
YAĞCI Raşit V. 335, 457
YALÇIN S. Songül S27, 315, 421
YALMAN Nevin 509
YALMAN Osman 173
YARALI Neşe 103
YARKIN Figen 81
YAZICI Hasan 67
YAZICI Kazım M. 307
YEĞİN Olcay 117
YEL Leman 525
YENİCESU İdil 467
YEŞİLİPEK M. Akif 117
YETGİN Sevgi 249, 329, 467
YILMAZ Erkan 181
YILMAZ Kutluhan 509
YILMAZ Sümer 103
YILMAZ Yüksel 121
YİĞİT Şule 489
YUNUS Şemsettin 341
YURDAKÖK Kadriye 189, 421
YURDAKÖK Murat 489
YÜCE Aysel 91, 99, 283
YÜCESAN Selçuk 147
YÜNTEN Nilgün 173
ZAFER Yeşim 531

ARTICLE INDEX TO VOLUME 41

A Case of Hemophilia A Associated with Hodgkin's Disease 517
A Case with Acute Leukemia Presenting with Cardiac Tamponade 509
A Turkish Family with Greig Cephalopolysyndactyly Syndrome 259
A Warm Antibody Mediated Acute Hemolytic Anemia with Reticulocytopenia in a Four-Month-Old Girl Requiring Immunosuppressive Therapy 239
An Uncommon Association of H-Type Tracheoesophageal Fistula with Infantile Hypertrophic Pyloric Stenosis 143
Associated Brain Abnormalities in Patients with Corpus Callosum Anomalies 173
Autoimmune Hemolytic Anemia with Warm Antibodies in Children: Retrospective Analysis of 51 Cases 467
Benign Rheumatoid Nodules of Childhood 365
Biphenotypic Characteristics, Cell Size and Prognosis in Childhood Acute Myeloblastic Leukemia 219
Blood Levels of Leukotrienes (LTC_4, D_4, E_4, B_4) and Synthesis of Leukotriene B_4 by Peripheral Leukocytes in Children with Acute A and B Hepatitis 457
Bronchiectasis due to Ciliary Aplasia in Turner's Syndrome 525
Cardiac Dysrhythmia that Simulates Seizure Disorder in Two Children 521
Cartilaginous Hamartoma of the Chest Wall with Secondary Aneurysmal Cyst-Like Areas in an Infant: A Case Report 139
CEA, CA125 and CA19-9 Levels in Congenital Gastrointestinal Anomalies 473
Cerebrospinal Fluid Pleocytosis in Acute Lymphoblastic Leukemia without Central Nervous System Relapse: A Report of Three Cases 249
Changes of Hemostatic Factors in Patients with Hemoglobinopathies 323
Clinical and Laboratory Approach to a Neonate Suspected of an Inborn Error of Metabolism 1
Congenital Malaria: A Case Report 103
Congenital Microvillus Atrophy in a 4-Month-Old Girl 495
Congenital Primary Hypoparathyroidism Presented with Extensive Cutaneous and Subcutaneous Calcifications 253
Cryptosporidium Parvum Prevalence in a Group of Turkish Children 189
Cyclosporin A Plus Prednisone Treatment of Steroid-Sensitive Frequently Relapsing Nephrotic Syndrome in Children 225
Echocardiographic Diagnosis of Sinus Valsalva Aneurysm Rupture in Two Pediatric Patients 127
Eosinophilic Fasciitis-Progression to Linear Scleroderma: A Case Report 381
Epidemiology and Antibiotic Resistance of Gram Negative Urinary Pathogens in Pediatric Patients 37
Failure of Granulocyte Colony-Stimulating Factor and Granulocyte-Macrophage Colony-Stimulating Factor in a Patient with Kostmann Syndrome 117
Fatal Acidosis in a Neonate with Pearson Syndrome 361
Four Different Hernias are Encountered in the Anterior Part of the Diaphragm 483
Frequency of Antinuclear Antibodies and Rheumatoid Factor in Healthy Turkish Children 67
Gastric Antral Stricture in a Patient with Chronic Granulomatous Disease 369
Gastric Perforation Presenting as Bilateral Scrotal Pneumatoceles 267
Guanosine Triphosphate Cyclohydrolase I Deficiency: A Rare Cause of Hyperphenylalaninemia 231
Hemostatic System in Early Respiratory Distress Syndrome: Reduced Fibrinolytic State? 489
Hepatitis as the Presenting Symptom of Childhood Systemic Lupus Erythematosus 541
Human Leukocyte Antigens in Turkish Pediatric Celiac Patients 181
Identity Confusion and Depression in Groups of Adolescents Having Psychiatric and Physical Symptoms 73
Increased Gastric Juice Leukotriene B_4, C_4 and E_4 Concentrations in Children with Helicobacter Pylori Colonization 335
Intermittent Chemotherapy for Miliary Tuberculosis in Children 53
Late Infantile Acid Maltase Deficiency: A Case Report 121
Leukocytoclastic Vasculitis in a Child with Epidermolysis Bullosa Simplex 277
Methylenetetrahydrofolate Reductase (MTHFR) 9C677T Mutation in Turkish Patients with Thrombosis 197

MRI of Fibromatosis Colli: Case Report 505
Neonatal Outcome Following Early Onset Preterm Premature Rupture of the Membranes-A Case Controlled Study 429
Neonatal Sublingual Traumatic Ulceration (Riga-Fede Disease): A Case Report 113
Neural Tube Defects in Turkey: Prevalence, Distribution and Risk Factors 299
Neurological Crisis Mimicking Acute Pancreatitis in Tyrosinemia Type I 501
Percutaneous Transvenous Balloon Valvuloplasty: Mid-term Results in Adolescents 341
Prevalence of Asthma Symptoms Among Turkish Cypriot Schoolchildren 413
Prevalence of GB Virus C/Hepatitis G Virus Infection in Pediatric Patients Receiving Multiple Transfusions in Southern Turkey 81
Primary Intracranial Germ Cell Tumors in Children: A Report of Eight Cases and Review of the Literature 161
Profile of Bone Marrow Iron Stores in Childhood Iron Deficiency Anemia 329
Psoriasis in a Patient with Neurofibromatosis 545
Psychiatric Symptoms Among Turkish Adolescents 307
Relationship between High Leukocyte Count and Cell Size in Childhood Acute Myeloblastic Leukemia 437
Respiratory Distress Due to Esophageal Perforation Caused by Ball Point Ingestion 391
Results of the Turkish Congenital Malformation Survey 287
Rh Disease: Intrauterine Intravascular Fetal Blood Transfusion by Cordocentesis 61
Rhabdomyosarcoma of the Biliary Tree Rheumatic Heart Disease Prevalence Among Schoolchildren in Ankara, Turkey 201
Sacroilitis in Familial Mediterranean Fever: An Unusual Presentation in Childhood 387
Safety, Tolerability and Immunogenicity of a Haemophilus Influenzae Type b Vaccine Containing Aluminum Phosphate Adjuvant Administered at 2, 3 and 4 Months of Age .. 421
Scimitar Syndrome with Absence of the Right Pulmonary Artery: A Case Report 399
Serum Leptin Levels During Childhood and Adolescence: Relationship with Age, Sex, Adiposity and Puberty 447
Stenotrophomonas Maltophilia Pneumonia in a Premature Infant 283
The Accuracy of Antenatal Fetal Echocardiography 349
The Evaluation of Ocular Trauma in Children Between Ages 0-12 43
The Impact of the Sexual Maturation Stage on Body Mass Index in Adolescent Girls 315
The Split Notochord Syndrome with Dorsal Enteric Fistula, Meningomyelocele and Imperforate Anus 147
Torsade de Pointes Associated with Encephalitis 395
Tracheal Rupture: A Rare Complication Related to Foreign Body Aspiration 273
Transient Hyperphosphatasemia-Where Do We Stand? 151
Tropisetron in the Prevention of Chemotherapy-Induced Acute Emesis in Pediatric Patients 207
Tumoral Calcinosis: A Case Report 375

Two Female Siblings from Turkey with Langer Mesomelic Dysplasia (Homozygous Leri-Weill Dyschondrosteosis Syndrome) 531
Two Siblings with Bloom's Syndrome Exhibit Different Clinical Features: Possible Effect of Sex 107
Ultrastructural Findings of Bone Marrow in a Case with Malignant Osteopetrosis Following Successful Allogeneic Bone Marrow Transplantation 353
Ursodeoxycholic Acid Therapy in Children with Cholestatic Liver Disease 91
Vascular Hamartoma of the Mediastinum: A Case Report 133
Vascular Ring Formed by Right Aortic Arch with Aberrant Left Subclavian Artery and Left Ligamentum Arteriosum 403
Wilson's Disease Patients with Normal Ceruloplasmin Levels 99

Supplement

Augmented Childhood. Evolution of Child Development in the Multimedia Environment. CRN International Symposium 98

A Country Report on Multimedia and Internet for Children and Youth in Taiwan 21
A Quick Overview of Present Technology in Japan 149
Awareness of the Earth and Possibilities for New Science Education in the Internet Age 141
Children are Born as Information Seekers, "Bright and Dark Sides of Multimedia for Child Development" 1
Children in Japan and Multimedia 7
Country Report: Singapore Designing, Constructing and Utilizing New Learning Spaces: Space as a Place to Think with 119
Digital Kids of the Heisei Era: Experiment at Toyonaka Bunka Kindergarten 91
Education in Australia 15
Expanding Concept of Learning to Learn 5
Implications for the Future 137
Introducing the Internet to Primary Schools in Japan: 100-School Networking Project, NTT Konet Plan, and Related Projects 35
Introduction of "Shinkenzemi 84 Net" Project 99
Multimedia and Children in Turkey 27
Multimedia for Edu-Tainment of Children in Asia: The Case of Television in Seven Asian Countries 61
Multimedia Unplugged: A Workshop on Learning Designs at the neoMuseum, Japan 109
New Tools for Learning 127
Social Indicators of Child Well-being and the World Wide Web: Considering Youth as Users 51
The Use of Multimedia in the Childwatch International Research Network 45
Virtual Reality-Current Media-the World of Virtual Reality for Children 83
Virtual Reality in a Children's Hospital 73

acid maltase deficiency 121
glycogen storage diseases 121
late-infantile form 121
acute emesis 207
adolescent 207
antiemetic 207
children 43, 53, 91, 127, 161, 189, 207, 225, 329
tropisetron 207
acute lymphoblastic leukemia 249, 509
central nervous system involvement 249
pericardial effusion 509
pleocytosis 249
acute myeloblastic leukemia 219, 437
biphenotypic expression 219
cell size 219, 437
childhood 219, 239, 277, 365, 387
high leukocyte count 437
acute viral hepatitis 457
leukotrienes 457
lipid mediators 457
adolescence 73
depression 73
identity 73
antinuclear antibodies 67
rheumatoid factor 67
Turkish children 67
autoimmune hemolytic anemia 239, 467
childhood 219, 239, 277, 365, 387
Coombs test 467
reticulocytopenia 239
warm antibody 239, 467
benign rheumatoid nodule 365
childhood 219, 239, 277, 365, 387
Bloom's syndrome 107
clinical heterogeneity 107
sex effect 107
sun-sensitive lesions 107
body mass index 315
adolescent girls 315
Tanner breast stage 315
bronchial asthma 413
ISAAC questionnaire 413
wheezing 413
bronchiectasis 525
ciliary aplasia 525
primary ciliary dyskinesia 525
Turner's syndrome 525
celiac disease 181
human leukocyte antigen types 181
Turkish children 181
benign mesenchymoma 139
cartilaginous hamartoma 139
rib 139
cholestatic liver disease 91
children 43, 53, 91, 127, 161, 189, 207, 225, 329
ursodeoxycholic acid 91
chronic granulomatous disease 369
complications 369
gastric antral stricture 369
congenital diaphragmatic hernia 483
Morgagni hernia 483
congenital gastrointestinal anomalies 473
RIA 473
tumor markers 473
congenital hypoparathyroidism 253
extensive calcifications 253
congenital malaria 103
newborn 103, 283
pregnancy 103
congenital malformations 287
abnormal pregnancy 287
neural tube defects 287
Turkey 287
ultrasonography 287, 289
congenital microvillus atrophy 495
infant 495
intractable diarrhea 495
corpus callosum 173
brain abnormalities 173
magnetic resonance imaging 173
cryptosporidiosis 189
children 43, 53, 91, 127, 161, 189, 207, 225, 329
eosinophilic fasciitis 381
childhood scleroderma 381
epidermolysis bullosa simplex 277
childhood 219, 239, 277, 365, 387
leukocytoclastic vasculitis 277
familial Mediterranean fever 387
childhood 219, 239, 277, 365, 387
computed tomography 387
sacroiliitis 387
fetal echocardiography 349
congenital heart disease 349
dysrhythmia 349
echocardiography 127, 349
fetus 349
fibromatosis colli 505
magnetic resonance imaging 505
frequently relapsing nephrotic syndrome 225
children ... 43, 53, 91, 127, 161, 189, 207, 225, 329
cyclosporin A 225
prednisone 225
gastric perforation 267
scrotal pneumatocele 267
GBV-C 81
hemoglobinopathy 81
pediatric cancer 81

Greig cephalopolysyndactyly syndrome 259
macrocephaly 259
polydactyly 259
prenatal diagnosis 259
syndactyly 259
guanosine triphosphate cyclohydrolase I deficiency 231
biopterin 231
hyperphenylalaninemia 231
neonatal screening 231
neopterin 231
neurotransmitters 231
Haemophilus influenzae type b vaccine ... 421
aluminum phosphate adjuvant 421
immunogenicity 421
safety 421
Helicobacter pylori 335
antral gastritis 335
gastric juice 335
leukotrienes 335
lipid mediators
hemoglobinopathies 323
activation in coagulation 323
coagulation inhibitors 323
sickle cell disease 323
thalassemia 323
hemophilia A 517
Hodgkin's disease 517
malignancy 517
hemostatic system 489
disseminated intravascular coagulation ... 489
fibrinolysis 489
newborn infants 489
respiratory distress syndrome 489
H-type tracheoesophageal fistula 143
hypertrophic pyloric stenosis 143
hyperphosphatasemia 151
alkaline phosphatase 151
transient 151
Inborn error of metabolism 1
clinical approach 1
laboratory approach 1
neonate 1
intracranial tumor 161
children 43, 53, 91, 127, 161, 189, 207, 225, 329
germ cell 161
iron deficiency anemia 329
bone marrow-stored iron 329
children 43, 53, 91, 127, 161, 189, 207, 225, 329
Kostmann syndrome 117
eosinophilia 117
rhG-CSF 117
rhGM-CSF 117
Langer mesomelic dysplasia 531
homozygous Leri-Weill dyschondrosteosis syndrome .. 531
hypoplastic ulna-fibula 531
hypoplastic mandible 531
prenatal diagnosis 531
leptin 447
childhood ... 219, 239, 277, 365, 387, 447
obesity 447
puberty 447
malignant-infantile osteopetrosis 353
allogeneic bone marrow transplantation .. 353
ultrastructure 353
mediastinum 133
hemangioma 133
vascular hamartoma 133
methylenetetrahydrofolate reductase 197
C677T mutation 197
hyperhomocysteinemia 197
thermolabile enzyme 197
thrombosis 197
miliary tuberculosis 53
children 43, 53, 91, 127, 161, 189, 207, 225, 329
intermittent chemotherapy 53
neural tube defects 299
prevalence 299
risk factors 299
Turkey 287, 299
neurofibromatosis 545
psoriasis 545
ocular injury 43
children 43, 53, 91, 127, 161, 189, 207, 225, 329
trauma 43
Pearson syndrome 361
lactic acidosis 361
neonate 361
percutaneous balloon mitral valvuloplasty ... 341
mitral stenosis 341
preterm premature rupture of the membranes .. 429
antenatal steroids 429
infection 429
pulmonary hypoplasia 429
psychiatric symptoms 307
Turkish adolescents 307
respiratory distress 391, 403
esophageal perforation 391
laryngomalacia 403
right aortic arch 403
vascular ring 403
Rh disease 61
cordocentesis 61
fetal blood transfusion 61
rhabdomyosarcoma 245
biliary tree 245
rheumatic heart disease 201
prevalence 201
Turkey 201
Riga-Fede disease 113, 287, 299
neonatal teeth 113
sublingual ulceration 113
scimitar syndrome 399

right pulmonary artery 399
seizures 521
cardiac dysrhythmia 521
sinus of Valsalva aneurysm rupture 127
children 43, 53, 91, 117, 127, 161, 189, 207, 225, 329
echocardiography 127, 349
subarterial ventricular septal defect 127
split notochord syndrome 147
anal atresia 147
dorsal enteric fistula 147
Stenotrophomonas maltophilia 283
newborn 103, 283
nosocomial infection 283
systemic lupus erythematosus 541
autoimmune hepatitis 541
hepatitis B virus 541
torsade de pointes 395
encephalitis 395
tracheal rupture 273
foreign body aspiration 273
intubation complications 273
rigid bronchoscopy 273
tumoral calcinosis 375
ectopic calcification syndrome 375
tyrosinemia type I 501
acute pancreatitis 501
neurologic crisis 501
urinary tract pathogens 37
antibiotic resistance 37
Wilson's disease 99
ceruloplasmin 99