PUBLISHED BY
Turkish National Pediatric Society,
Hacettepe University
Institute of Child Health and
The International
Children's Center

PRINTED IN
Meteksan Anonim Şirketi, Ankara

ISSN 0010-0161

This publication is included in the abstracting and indexing coverage of BIOSIS Previews, CAB Abstracts (Abstracts on Hygiene and Communicable Diseases, Public Health Bulletin, Tropical Diseases Bulletin), EMBASE/Excerpta Medica, IBIDS (International Bibliographic Information on Dietary Supplements), MEDLINE/ Index Medicus, MICROMEDEX for Health Care Series, Research Alert Direct (ISI), Science Citation Index Expanded (ISI), SERLINE and Turkish Medical Index.

EDITORIAL OFFICE
Prof. Dr. Turgay Coşkun
Editor
The Turkish Journal of Pediatrics
P.K. 66, Samanpazarı 06240 Ankara, Turkey
Fax: 90 (312) 324 32 84

SUBSCRIPTION ADDRESS
Prof. Dr. A. Murat Tuncer
Hacettepe University
İhsan Doğramacı Children's Hospital
06100 Ankara, Turkey
Fax: 90 (312) 324 32 84

THE TURKISH JOURNAL OF PEDIATRICS

VOLUME: 44 NUMBER: 1 JANUARY-MARCH 2002

CONTENTS

ORIGINAL ARTICLES

CASE REPORTS

The effect of short-course high-dose methylprednisolone on peripheral blood CD34+ progenitor cells of children with acute leukemia during remission induction therapy

Bahattin Tunç[1], Ahmet F. Öner[2], Gönül Hiçsönmez[3]

[1]Department of Pediatrics, Süleyman Demirel University Faculty of Medicine, Isparta, [2]Department of Pediatrics, Yüzüncü Yıl University Faculty of Medicine, Van and [3]Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Tunç B, Öner AF, Hiçsönmez G. The effect of short-course high-dose methylprednisolone on peripheral blood CD34+ progenitor cells of children with acute leukemia during remission induction therapy. Turk J Pediatr 2002; 44: 1-4.

This study was undertaken to determine the effect of short-course high-dose methylprednisolone (HDMP) treatment on peripheral blood (PB) CD34+ progenitor cells during remission induction treatment in 11 children with newly diagnosed acute leukemia (7 with ALL, 4 with AML) whose bone marrow (BM) cells expressed fewer than 5% CD34 at the time of diagnosis. All children who had no infection were given HDMP as a single daily oral dose of 30 mg/kg for the first four days of induction therapy. The number of CD34+ progenitor cells were determined by flow cytometry before and after four days of HDMP treatment. While the number of PB blast cells significantly decreased after only a four-day course of HDMP treatment, the number of PB CD34+ progenitor cells increased in all patients. In addition, after four days of HDMP treatment polymorphonuclear leukocytes (PMN) and mononuclear cells (MNC) increased significantly ($p < 0.05$). We suggest that the potential beneficial effects of HDMP in the induction treatment of acute leukemia may occur partly by the stimulation of PB CD34+ hematopoietic progenitor cells in a short period of time.

Key words: high-dose methylprednisolone, children, acute leukemia, CD34+ progenitor cells.

Although all hematopoietic progenitor cells in the bone marrow (BM) express the CD34 antigen, its expression decreases during the maturation of progenitor cells[1]. Only small number of CD34+ hematopoietic progenitor cells are found in peripheral blood (PB)[2]. These cells have been shown to increase during the period of hematopoietic recovery following myelosuppressive chemotherapy[3]. Treatment with recombinant hematopoietic growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) can increase PB CD34+ progenitor cells[4]. In addition, high-dose methylprednisolone (HDMP) combined chemotherapy has been shown to increase the number of BM CD34+ hematopoietic progenitor cells in children with acute lymphoblastic leukemia (ALL) during remission induction therapy. However, the increase in CD34+ cells was not significant in patients who received a conventional dose of steroid instead of HDMP[5]. Furthermore, short-course HDMP treatment has been shown to increase the circulating CD34+ hematopoietic progenitor cells during maintenance therapy in children with ALL and acute myeloblastic leukemia (AML) who had chemotherapy-induced neutropenia[6,7].

In the present study, we have evaluated the effect of short-course HDMP treatment of PB CD34+ cells during remission induction treatment to elucidate whether it could also increase the PB CD34+ progenitor cells in children with ALL and AML.

Material and Methods

Eleven children with newly diagnosed acute leukemia (7 with ALL, 4 with AML) were enrolled in this study. There were six boys and five girls with a median age of 5.6 years (range 3-14 years). None of them had infection.

Diagnosis was made by morphology according to the French-American-British (FAB) classification, by cytochemistry, and by cell surface marker analysis. With informed consent, methylprednisolone sodium succinate (Prednol-L 30 mg/kg/day) as a single agent was administered orally once a day to all children for the first four days of induction therapy. No other agent was given during that time. Treatment was then continued with HDMP containing chemotherapy regimens according to our institutional ALL and AML protocols.

Complete blood counts were performed using an automatic analyzer (STKS Coulter), and manual differential counts were performed on Wright-stained PB smears. Surface marker analysis was performed using a flow cytometry (FAC Scan, Becton Dickinson, San Jose, CA, USA) with a panel of monoclonal antibodies[8]. $CD34^+$ progenitor cells were measured in anticoagulated peripheral venous blood samples before and after four days of HDMP treatment. The patients were excluded if their BM blast cells expressed > 5% $CD34^+$ progenitor cells. The absolute number of circulating progenitor cells per mm^3 was calculated by multiplying the percentage of $CD34^+$ cells by the absolute number of mononuclear cells. The final results were expressed as mean ± SD. In addition, changes in the number of BM blasts, polymorphonuclear leukocytes (PMN) and mononuclear cells (MNC) were determined four days after HDMP treatment.

Statistical comparison was performed using the Wilcoxon test.

Results

Changes in the absolute numbers of PB $CD34^+$ cells before and after four days of HDMP treatment are shown in Table I. After four days of HDMP treatment, the number of PB $CD34^+$ progenitor cells increased significantly ($p < 0.05$) in children whose BM cells expressed < 5% $CD34^+$ progenitor cells. In addition, the absolute numbers of PB blast cells significantly decreased in all children with AML ($12.8 \pm 9.9 \times 10^9$/L vs $2.3 \pm 8.9 \times 10^9$/L) and ALL ($23.7 \pm 9.4 \times 10^9$/L vs $2.5 \pm 1.18 \times 10^9$/L) (Fig. 1). As also seen in Figure 1, significant increases were also observed in the number of PMN cells and monocytes in children with ALL and AML, four days after HDMP treatment.

Table I. Changes in Absolute Numbers of Peripheral Blood $CD34^+$ Progenitor Cells After 4 Days of HDMP Treatment in Children with Both ALL and AML whose Bone Marrow Cells Expressed < 5% $CD34^+$ Progenitor Cells

	Day 0	Day 4	p
PB $CD34^+$ cells/mm^3 n = 11	402±228	916±491	<0.05

PB: peripheral blood.

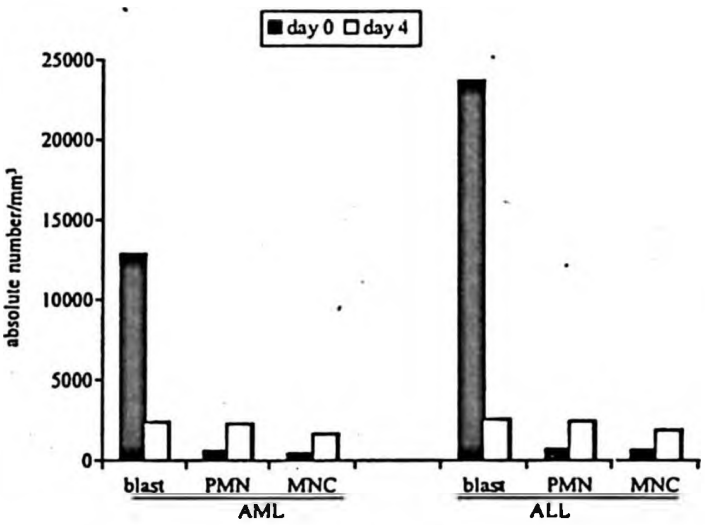


Fig. 1. Mean value of the absolute numbers of peripheral blood blast cells, PMN and MNC in children with AML (n = 4) and ALL (n = 7 before and after HDMP treatment. PMN: polymorphonuclear leukocytes; MNC: mononuclear cells; AML: acute myeloblastic leukemia; ALL: acute lymphoblastic leukemia.

Discussion

Although conventional doses of corticosteroids have long been used successfully for the treatment of ALL, more favorable results have been obtained by administration of higher doses of corticosteroid in children with ALL[9-13]. Administration of short-course (3 to 5 days) HDMP accelerates leukocyte recovery in leukopenic children with ALL and AML[14]. In addition, remarkable antileukemic effects of HDMP therapy through induction of terminal differentiation and apoptosis of myeloid leukemic cells have been shown in children with AML[15,16].

In the present study, we demonstrated that while PB leukemic cells decreased, PMN and MNC increased significantly, and PB $CD34^+$ progenitor cells increased in children with ALL and AML who were treated with short-course HDMP during the early phase of remission induction therapy. Although the mechanism of action of HDMP on PB hematopoietic

progenitor cells is not clear, the increase in the number of progenitor cells expressing CD34 antigen could be related to the stimulatory effect of HDMP on the production of some endogenous cytokines[17,18]. In addition, it has been reported that corticosteroids increased the levels of G-CSF and GM-CSF in normal subjects and in children with aplastic anemia and myelodysplastic syndrome[19,20]. Interestingly, the inhibitory effect of steroids on production of the leukemia-associated inhibitor (LAI) from human myeloid leukemic cells, which can have a myelosuppressive effect on normal progenitor cells, has been shown in vitro[21]. The inhibitory effects of high-dose steroid treatment on LAI could be another explanation for the stimulation of PB CD34+ progenitor cells.

Successful results with HDMP administration have also been reported in several hematologic diseases such as aplastic anemia[22], congenital hypoplastic anemia[23], and myelofibrosis[24]. The effect of HDMP in all these diseases might be related to its stimulatory effect on CD34+ hematopoietic progenitor cells.

In the present study, we have shown that administration of short-course HDMP treatment alone increases not only the number of PB CD34+ cells but also the PB PMN and MNC counts significantly during an early phase of remission induction therapy (Fig. 1). These results indicate that in addition to the remarkable antileukemic effects of HDMP in children with AML and ALL, short-course administration of HDMP can mobilize the CD34+ hematopoietic progenitor cells into PB and rapidly accelerate the hematopoietic recovery. Therefore, short-course HDMP treatment is recommended as an initial treatment of ALL and AML patients. Whether addition of HDMP alone or combined with chemotherapy is beneficial for the outcome remains to be determined. Its effects on hematopoietic recovery in children with other malignancies who developed myelosuppression should also be evaluated in further studies.

REFERENCES

1. Abrowitz MJ, Gockerman JP, Moore JO, et al. Clinicopathologic and cytogenic features of CD34 (My 10)/positive acute nonlymphocytic leukemia. Am J Clin Pathol 1989; 91: 265-270.
2. Bender JG, Unverzagt KL, Walker DE, et al. Identification and comparison of CD34-positive cells and their subpopulations from normal peripheral blood and bone marrow using multicolor flow cytometry. Blood 1991; 77: 2591-2596.
3. To LB, Shepperd KM, Haylock DN, et al. Single high doses of cyclophosphamide enable the collection of high numbers of hemopoietic stem cells from the peripheral blood. Exp Hematol 1990; 18: 442-447.
4. Baumann I, Testa NG, Lange C, et al. Haemopoietic cells mobilised into the circulation by lenograstim as alternative to bone marrow for allogeneic transplants. Lancet 1993; 341: 369.
5. Tuncer AM, Hiçsönmez G, Gümrük F, et al. The effect of high-dose methylprednisolone combined chemotherapy on CD34-positive cells in acute lymphoblastic leukemia. Hematol Pathol 1994; 8: 169-175.
6. Çetin M, Hiçsönmez G, Tuncer AM, Kansu E, Canpınar H. The effect of short-course high-dose corticosteroid therapy on peripheral blood CD34+ progenitor cells in children with acute leukemia. Exp Hematol 1996; 24: 1191-1194.
7. Özbek N, Yetgin S, Tuncer AM. Effect of high-dose methylprednisolone and G-CSF treatments on lymphocyte subtypes in neutropenic children with acute lymphoblastic leukemia: a pilot study. Pediatr Hematol Oncol 1998; 15: 539-544.
8. Rothe G, Schmitz G. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. Working Group on Flow Cytometry and Image Analysis. Leukemia 1996; 10: 877-895.
9. Shanbrom E, Miller S. Critical evaluation of massive steroid therapy of acute leukemia. N Engl J Med 1962; 266: 1354-1358.
10. Hiçsönmez G, Özsoylu S, Onat N. Zamani VP, Gümrük F, Tuncer AM. High-dose methylprednisolone in resistant and relapsed children with acute lymphoblastic leukemia. Med Pediatr Oncol 1994; 22: 68-69.
11. Hiçsönmez G, Gümrük F, Zamani PV, et al. High-dose methylprednisolone for children with acute lymphoblastic leukemia and unfavorable presenting features. Eur J Haematol 1997; 58: 26-31.
12. Yetgin S, Gürgey A, Tuncer AM, et al. A comparison of the effect of high-dose methylprednisolone with conventional-dose prednisolone in acute lymphoblastic leukemia children with randomization. Leuk Res 1998; 22: 485-493.
13. Rylalls MR, Pinkerton CR, Meller ST, Talbot D, McElwain TJ. High-dose methylprednisolone sodiumsuccinate as a single agent in relapsed childhood acute lymphoblastic leukemia. Med Pediatr Oncol 1992; 20: 119-123.
14. Hiçsönmez G, Onat N, Albayrak D, Yetgin S, Özsoylu S. Acceleration of leukocyte recovery by administration of short-course high-dose methylprednisolone in children with acute lymphoblastic leukemia. Pediatr Hematol Oncol 1991; 8: 193-197.
15. Hiçsönmez G, Tuncer AM, Toksoy HB, Yenicesu I, Çetin M. Differentiation of leukemic cells induced by short-course high-dose methylprednisolone in children with different subtypes of acute myeloblastic leukemia. Leuk Lymphoma 1999; 33: 573-580.
16. Hiçsönmez G, Erdemli E, Tekelioglu M, et al. Morpohologic evidence of apoptosis in children with acute myeloblastic leukemia treated by high-dose methylprednisolone. Leuk Lymphoma 1996; 22: 91-96.

17. Tuncer AM, Hiçsönmez G, Ertürk G, Gümrük F, Albayrak D, Oğuz H. The effect of high-dose methylprednisolone treatment on GM-CSF level in children with acute leukemia: a pilot study. Leuk Res 1992; 16: 615-619.

18. Tuncer AM, Hiçsönmez G, Gümrük F, et al. Serum TNF-alfa, gamma-INF, G-CSF and GM-CSF levels in neutropenic children with acute leukemia treated with short-course, high-dose methylprednisolone. Leuk Res 1996; 20: 265-269.

19. Nissen C, Moser Y, Speck B, Burgin M, Bendy H. Dexamethasone enhances "CSA" release and depresses "BPA" release. Br J Haematol 1983; 53: 301-310.

20. Bagby GC, Gabourel JD, Linman JW. Glucocorticoid therapy in the preleukemic syndrome (hemopoietic dysplasia): identification of responsive patients using in-vitro techniques. Ann Intern Med 1980; 92: 55-58.

21. Olofsson T, Sallerfors B. Modulation of the production of leukemia associated inhibitor (LAI) and its interaction with granulocyte-macrophage colony-forming cells. Exp Hematol 1987; 5: 1163-1167.

22. Özsoylu S, Coşkun T, Minassazi S. High dose intravenous glucocorticoid in the treatment of childhood acquired aplastic anaemia. Scand J Haematol 1984; 33: 309-316.

23. Özsoylu S. High-dose intravenous corticosteroid treatment for patients with Diamond-Blackfan syndrome resistant or refractory to conventional treatment. Am J Pediatr Hematol Oncol 1988; 10: 217-223.

24. Özsoylu S. High dose intravenous methylprednisolone for idiopathic myelofibrosis. Br J Haematol 1988; 70: 388.

Complications and outcome in left-sided endocarditis in children

Dursun Alehan, Süheyla Özkutlu, Canan Ayabakan, Arman Bilgiç, Şencan Özme Sema Özer, Alpay Çeliker

Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Alehan D, Özkutlu S, Ayabakan C, Bilgiç A, Özme Ş, Özer S, Çeliker A. Complications and outcome in left-sided endocarditis in children. Turk J Pediatr 2002; 44: 5-12.

We retrospectively assessed the clinical course and outcome of left-sided endocarditis in pediatric patients to find out the prognostic significance of the presence and size of echocardiographically detected vegatations.

Among the children admitted to our institution with endocarditis between January 1987 and October 1999, 16 patients (mean age 9.03 ± 4.95 years) who met the Duke criteria for the diagnosis of infective endocarditis (IE) were included in this study. Rheumatic valvular disease was the most frequent underlying heart disease (10 patients: 62.5%). Five patients were operated at a mean of 13.9 months before endocarditis, and all had residual defects. Vegetation was detected in 11 cases (69%). Ten patients had major complications (within 2 weeks in 6 patients). Three patients developed congestive heart failure (CHF), six had intracranial and one had lower extremity emboli. Among them four were operated because of complications (CHF: 3 cases, intracranial emboli: 1 case). All the operated cases are doing well. The association between intracranial embolic events and echocardiographically detected vegetations was determined by calculating specificity (40%), sensitivity (100%), positive predictive value (50%), and negative predictive value (100%). No intracranial embolism occurred in patients without vegetations. All vegetations were ≤ 6 mm in patients with systemic embolism. There were four deaths, three of which were because of intracranial embolism.

This study suggests that intracranial emboli have a major risk of mortality in left-sided endocarditis. The larger size of the vegetation is not a predictor of complications; furthermore, the absence of vegetations predicts that the patient is safe from embolic events. Therefore all patients with left-sided IE should be considered for earlier surgical intervention.

Key words: infective endocarditis, left-heart, children, systemic emboli.

In recent years, the prognosis of patients with infective endocarditis (IE) has improved with better antimicrobial therapy, advances in diagnosis, earlier detection, and urgent surgical management of complications[1,2]. Congestive heart failure, severe valvular regurgitation and uncontrolled infection are strongly associated with poor outcome when no surgical treatment is applied[2,3]. Echocardiography, beyond comparison, is the accepted procedure for the diagnosis of IE, with which assessment of valvular dysfunction, hemodynamic status, and direct visulization of endocarditis-induced lesions are possible. Echocardiography also aids in decision of the necessity and timing of surgical intervention[4-7]. On the other hand, echocardiographically demonstrated vegetations or occurrence of systemic emboli are less agreed upon indications for surgical intervention during active IE[8].

Although it has been reported lately that there is a moderate increase in the global incidence of IE, it is still a relatively rare disease in infancy and childhood, especially during the first two years of life[9,10]. Furthermore, most studies about the timing and necessity of surgical interventions in active IE are done on adult patients. In view of the conflicting reports, we reassessed

retrospectively the clinical course and outcome of left-sided IE in pediatric patients to find out the prognostic significance of size and the location of echocardiographically detected vegetations.

Material and Methods

We retrospectively reviewed the hospital records of 21 pediatric patients diagnosed with left-sided endocarditis between January 1987 and October 1999. Among these 21 patients, 16 who fulfilled the Duke criteria for the diagnosis of IE were included in this study[11]. All of the patients were evaluated with two-dimensional, M-mode, continuous wave and color Doppler transthoracic echocardiography for the site of vegetations, extension of intracardiac lesions, and presence of congestive heart failure. Each cardiac valve was especially evaluated for thickening, calcification, prolapse of leaflets and, in particular, for the presence or absence of typical vegetations. All echocardiographic examinations were performed with Toshiba Sonolayer SSH-160A using 5, 3.75 and 2.5 MHz transducers. The duration of medical treatment, the timing of the surgical treatment and the clinical outcome, emphasizing the systemic embolic events, were evaluated for all patients. Statistical analysis was done using frequencies of variables. The association between intracranial embolic events and echocardiographically detected vegetations was determined by calculating specificity, sensitivity, and positive and negative predictive values as follows:

$$\text{Sensitivity} = \frac{\text{Emboli with vegetations (veg)}}{\text{Emboli with veg} + \text{Emboli without veg}}$$

$$\text{Specificity} = \frac{\text{No emboli without veg}}{\text{No emboli without veg} + \text{No emboli with veg}}$$

$$\text{Positive predictive value} = \frac{\text{Emboli with veg}}{\text{Emboli with veg} + \text{No emboli with veg}}$$

$$\text{Negative predictive value} = \frac{\text{No emboli without veg}}{\text{No emboli without veg} + \text{Emboli without veg}}$$

Results

The age range of nine male and seven female patients was from 1 to 17 years (mean: 9.03 ± 4.95 years). All patients had preexisting cardiac abnormalities. Rheumatic heart disease (RHD), with occurrence in 10 patients (62.5%), was the most frequent underlying heart disease. Five patients with RHD had mitral regurgitation (MR), one had aortic regurgitation (AR), and four had both MR and AR. Two patients had primum atrial septal defect (ASD) and mitral cleft. Other pathologies included 1) Shone's anomaly (mitral stenosis-MS, aortic stenosis-AS, coarctation of aorta), 2) AS and secondary hypertrophic cardiomyopathy, 3) Truncus arteriosus type I, and 4) tetralogy of Fallot, with each seen in one patient. Five patients were operated at an average of 13.9 months before the diagnosis of IE, but all had residual defects (Table I).

Bacteriological Data

Blood cultures were positive in 10 patients (62.5%). Staphylococcus aureus (n = 5; 31.2%) and Streptococcus viridans (n = 4; 25%) species accounted for the majority of cases. One patient (6.3%) had Candida albicans growth in multiple blood cultures. Valve tissue was also culture positive in two patients who underwent surgery (Cases 9 and 14). Four of the remaining six patients, who were culture negative, had received empirical antibiotic treatment of various durations before presenting to our hospital.

Echocardiography

Vegetations were observed in 11 of the 16 patients (69%), and all were on native valves. Four patients had vegetations on the mitral valve (Fig. 1) (one of them also had vegetation on interatrial patch), three of them on the aortic valve (Fig. 2), and two of them on both aortic and mitral valves. There were extravalvular vegetations in the remaining two patients (1 in left atrium, and the other in left ventricular outflow tract). Size of the vegetations ranged from 3 mm to 17 mm in diameter (mean: 4.93 ± 5.05 mm). Among the six patients operated during active IE, four patients had vegetations at preoperative echocardiography. The

Table I. Details of the Study Group

Case	Age	Sex	Cardiac pathology	Operation/residual defect	Location/size of vegetation	Blood culture	Complication/day of complication	Surgery	Outcome
1	13	M	TOF	Total correct/VSD, PS	LV outflow tract 3 mm	Negative	Uncontrolled infection	Redo total correction	Cure
2	14	M	MR*	MVR/MR	Mitral 4 mm	C. albicans	Intracranial emboli/9	None	Died
3	12	F	MR*	None	Mitral 6 mm	S. aureus	Intracranial emboli/14	None	Cure
4	1.5	F	Mitral cleft, primum ASD	MVR, ASD closure/MR	LA 11 mm	S. viridans	None	None	Cure + elective surgery
5	1	M	Truncus type I	None	None	S. aureus	None	None	Cure + elective surgery
6	7	F	Shone's anomaly (MS, AS, coarctation (Ao)	Patch angioplasty of Ao/MS, AS, recoarctation	None	S. aureus	Lower extremity emboli/48 + CHF/69	None	Died
7	13	F	AR, MR+	None	None	Negative	None	None	Cure + elective surgery
8	17	M	AR*	None	Aortic 17 mm	Negative	Uncontrolled infection	AVR	Cure
9	7	M	MR*	None	None**	S. viridans	CHF/17**	MVR	Cure
10	9	M	AR, MR*	None	Aortic 3 mm	Negative	Intracranial emboli/27	None	Died
11	15	F	AR, MR*	None	Aortic-Mitral 3-13 mm	Negative	None	None	Cure
12	7	M	MR*	None	None**	S. aureus	CHF/35***	MVR	Cure
13	7	F	AS, HCMP	None	Aortic 5 mm	S. viridans	Intracranial emboli/1	None	Cure
14	4	M	MR*	None	Mitral 5 mm	S. aureus	Intracranial emboli/2	None	Died
15	13	M	MR, AR*	None	Aortic-Mitral 4-7 mm	S. viridans	CHF/10	MVR	Cure
16	4	F	Mitral cleft, primum ASD	Mitral cleft + ASD closure/MR	Mitral 5 mm	Negative	Intracranial emboli/1	Redo closure of mitral cleft and primum ASD	Cure

* Rheumatic heart disease.
** Perforation of anterior mitral valve leaflet.
*** Rupture of chordae tendineae of anterior mitral valve.

TOF : Tetralogy of Fallot.
HCMP: Hypertrophic cardiomyopathy.
Ao : Aorta.
MR : Mitral regurgitation.
AR : Aortic regurgitation.
AVR : Aortic valve replacement.
AS : Aortic stenosis.
LA : Left atrium.
MVR: Mitral valve replacement.
VSD : Ventricular septal defect.
MS : Mitral stenosis.
LV : Left ventricle.
CHF: Congestive heart failure.
ASD: Atrial septal defect.
PS : Pulmonary stenosis.

diagnosis was confirmed at surgery. Chordae tendineae rupture of the anterior mitral leaflet and perforation of the anterior mitral leaflet accompanying severe congestive heart failure were diagnosed echocardiographically in the remaining two patients.

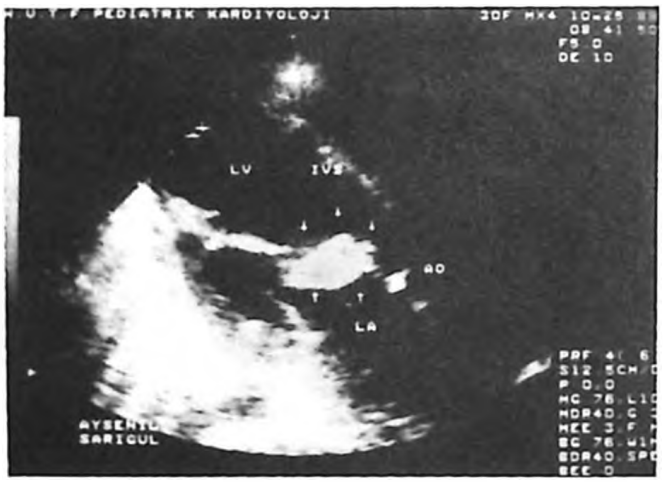


Fig. 1. A 13 mm vegetation (arrows) is observed on the anterior leaflet of the mitral valve of a 15-year-old female patient (Case 11) (long-axis view). LV: left ventricle, IVS: interventricular septum, AO: aorta, LA: left atrium.

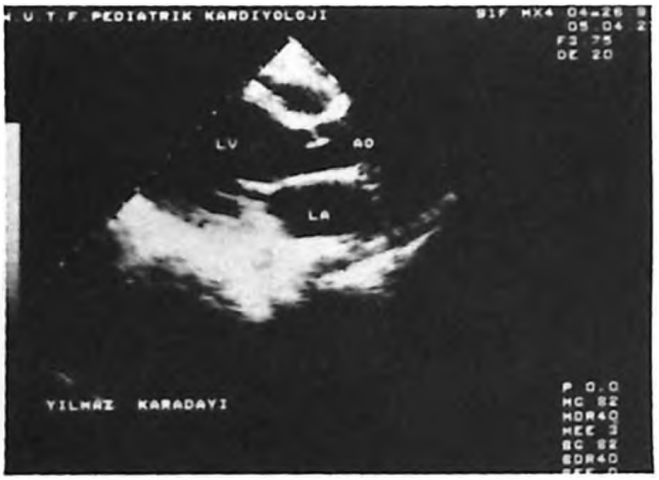


Fig. 2. A 3 mm vegetation is observed on aortic valve of a nine-year-old male patient (Case 10) (long-axis view). LV: left ventricle, LA: left atrium, AO: aorta.

Complications

All 16 patients were medically treated for a mean period of 32 ± 21 days (range: 2-69 days) after hospitalization for IE. During the in-patient follow-up period complications were observed in 10 patients (62.5%) on the 1st-69st days (mean: 20 ± 14 days) of the treatment. Clinical evidence of systemic embolism was present in seven patients (43.8%) (Table I). Intracranial embolism occurred in six patients, and one patient had lower extremity embolism. The association between intracranial embolic events and echocardiographically detected vegetations was determined by calculating specificity, sensitivity, and positive and negative predictive values. The sensitivity of occurrence of intracranial embolic events in the presence of vegetation was 100%. The specificity, positive predictive value, and negative predictive value were 40%, 50% and 100%, respectively. It is of note that no intracranial embolism occurred in patients without vegetations on echocardiographic examinations. However, a postoperative patient with Shone's anomaly had lower extremity embolism on the 48th day of treatment although no vegetation was detected on echocardiography. Among the patients with intracranial embolism, four patients had vegetations on the mitral valve, and two patients had vegetations on the aortic valve. The average size of the vegetations was 4.4 mm. An important finding of the study was that all vegetations were ≤ 6 mm in patients with systemic embolism. Two of these patients, however, had embolism before the initial echocardiographic examination (location: aortic valve in 1 patient, mitral valve in the other; vegetation size: 5 mm in both cases). One of them was treated only with medical therapy, while the other was treated with both medical and surgical therapy. Patients were discharged after six and five weeks of treatment, respectively.

There were three deaths among patients with cranial embolism. None of these patients was surgically treated during acute IE. Two of them died within 10 days of medical treatment, and one patient died on the 27th day of treatment. The patient with lower extremity embolism died on the 69th day of treatment because of severe congestive heart failure.

Three cases with severe congestive heart failure were operated during acute IE, and none of them died. One of these patients had vegetations at echocardiography (4 mm on aortic and 7 mm on mitral valve). The other two patients had perforation of the anterior mitral valve leaflet and rupture of the chordae tendinea, respectively. The echocardiographic diagnosis was confirmed histopathologically in these patients.

Surgical Treatment

Cardiac operation was undertaken in six (37.5%) of the 16 patients. The indications for cardiac surgery were severe congestive heart

failure (n = 3), intracranial embolism (n = 1), and uncontrolled infection (n = 2). There were no intraoperative or perioperative deaths among these patients. Three mitral valve replacements, one aortic valve replacement, and one mitral cleft surgery and interatrial patch revision were performed. Total correction for tetralogy of Fallot was redone in one patient. Vegetations were demonstrated surgically in all of the echocardiographically diagnosed patients.

In-Hospital Mortality

There were four in-hospital deaths (25%). Three patients died because of intracranial embolism. One patient (with lower extremity embolism) died due to severe congestive heart failure. This patient initially was operated for Shone's anomaly (MS, AS, coarctation of aorta). She had residual AS, MS, and recoarctation 4.5 months after that operation. None of the operated patients died intraoperatively or perioperatively.

Discussion

Infectious endocarditis remains a common and serious condition. Echocardiography is the only noninvasive method available today allowing direct visualization of endocarditis-induced lesions. The combination of M-mode and two-dimensional echocardiography imaging allows identification of vegetations in 13% to 78% of cases[12-16]. Transesophageal approach is important in patients whose transthoracic echocardiography fails to provide adequate imaging quality, as in patients who have advanced obesity, chest deformity or emphysema, or who are seen early after thoracic surgery or under artifical ventilation[4]. In our study group, vegetations were identified in 69% (11 patients) of the patients using the transthoracic approach. The presence of the vegetation was confirmed pathologically in all four patients who were operated during acute IE. Two other patients (Cases 9 and 12) without echocardiographically demonstrated vegetations were correctly diagnosed preoperatively by echocardiography as having mitral valve leaflet perforation and rupture of mitral chordae tendineae, respectively. Transthoracic echocardiography provided a reliable noninvasive diagnosis of IE-related lesions.

It has been suggested that the presence of echocardiographically identified vegetations may be associated with an impaired prognosis, based on a higher incidence of congestive heart failure, systemic embolism or death[12,13,17,18]. Recently, some studies have reported that prognostic implications are more dependent on vegetation size than on the presence or absence of vegetation[12-14,18-21]. In contrast, a relation between the size of vegetation and the incidence of complications has not been found in other studies[5,17,22,23].

Absolute indications for surgical treatment during active IE include severe heart failure, the presence of an infecting organism that is not susceptible to available antimicrobial agents, and unstable infected prosthetic valve[1,2,8]. Two or more embolic events and vegetations large enough to be demonstrated with echocardiography are less agreed upon, and are among the relative indications for surgical intervention during active IE. Valvular vegetations are hypothesized to increase the risk of systemic embolic events in left-sided IE[12,15,16]. The estimated cumulative incidence of such events ranges from 22% to 43%[21,24]. The neurologic complications due to cerebral embolism represent a major factor associated with an increased mortality rate in this disease[8,24]. If death does not occur after intracranial embolism, neurological deficit is usually the consequence. There is certain debate on the association of higher incidence of embolic events with larger vegetations (i.e. vegetations > 10 mm). Some of the studies did[12-14,18-21], and others did not[5,17,22,23], find an association with embolic events and vegetation size. Müge et al.[4] could not clearly associate the embolic events and vegetation size; on the other hand, when they considered the location of the vegetation, larger vegetations on mitral valves were significantly associated with increased risk of systemic embolism.

In our study, intracranial embolism was observed in six of the 11 patients with vegetations (54.5%) (Cases 2, 3, 10, 13, 14, and 16). None of these embolisms was recurrent and none of these patients had large vegetations by transthoracic echocardiography. The echocardiographic examination was performed after the embolic event in two patients (Cases 13 and 16). Although an embolic event might have reduced the size of the vegetations in these patients, all the remaining patients still had vegetations < 6 mm. Systemic embolic events other than cerebral embolisms were not considered to have increased mortality since the cause of death in the patient with lower extremity emboli was not the embolism itself, but congestive heart failure. The association between intracranial embolic events and echocardiographically detected vegetations

was determined by calculating specificity, sensitivity, and positive and negative predictive values. The sensitivity of occurrence of embolic events in the presence of vegetation was 100%. The specificity, positive predictive value, and negative predictive value were 40%, 50% and 100%, respectively. In other words, in our study, the absence of vegetations clearly predicted that the patient was safe from embolic events, and detection of vegetation by echocardiography was 100% sensitive for an embolic event. Intracranial emboli were significantly associated with mortality. Death occurred in three of the six patients (50%) with intracranial emboli on the 2nd, 9th, and 27th day of the medical treatment (Cases 2, 6, and 10). None of these patients was treated surgically for IE. Three patients with vegetations were operated for reasons other than intracranial embolism (Cases 1 and 8 because of uncontrolled infection and Case 15 because of congestive heart failure). If these patients had not been operated for other reasons, and if their vegetations had not been removed surgically, they might have been at increased risk of systemic or intracranial embolism.

Embolism occurs most commonly in patients infected with virulent organisms, an event more likely to be seen within two weeks in the course of this infection[5,24]. In our group the intracranial embolic complications were observed on the 1st to 27th day after the diagnosis (within 2 weeks in 5 of the 6 patients-83.3%). A lower extremity embolism was observed in one patient, on the 48th day. The microorganisms grown on blood cultures of these patients were as follows: S. aureus in three patients, Candida albicans in one patient, and S. viridans in one patient. No microorganism grew on blood cultures of the remaining two patients; both of them had received antibiotic treatment before admission and diagnosis of IE.

Blood cultures may be negative in 15% of all IE patients[25]. In our study group 37.5% of all the IE patients and 28.6% of the patients with embolism had negative blood cultures. Anerobic bacteria, fungi, or previously administered antibiotics may have been responsible for negative cultures.

The management of patients with clinical evidence of embolic disease remains unclear. There is no data to suggest that a patient with one major embolism is at an increased risk of having more[8]. As Lerner[24] states, one also must not forget that a single clinically evident embolus may be accompanied by clinically inapparent emboli, so a criterion of multiple emboli as indication for valve surgery seems to be inappropriate. None of our patients experienced recurrent intracranial embolism, but half of them died shortly after the first embolic event. On the other hand, all the patients operated during acute IE, including the patient with a single intracranial embolism episode, were successfully cured. There were four cases of severe congestive heart failure due to IE in our group (Cases 6, 9, 12, and 15). Two of these four cases had chordae tendineae, or mitral valve ruptures. Three of them were operated with no in-hospital mortality. Case 6 died on the 69th day of treatment due to congestive heart failure. Similar to recent reports, our study indicates that early operation during active infection may be safely performed before the classical antibacterial therapy is completed[1,26].

Chordal rupture due to IE has been reported in 8.5 to 45% of patients in various studies[28,29]. On the other hand Roberts and Buchbinder[27] state that cuspal perforations are always indicative of IE whenever they are seen. There is higher incidence of ruptured chorda to the posterior leaflet in the spontaneous group. In patients with floppy mitral valve, IE tends to results in chordal rupture of the posterior cusp as well. In IE involving normal or rheumatic valves, however, the anterior cusp is most commonly affected, with chordal rupture often occurring in association with cusp perforation[28]. In our study, one patient (Case 12) had rupture of the chorda to the anterior mitral valve, and another patient (Case 9) had perforation on the anterior mitral valve leaflet. Both patients developed severe congestive heart failure and were operated for this reason. No case of mycotic aneurism or ring abscess was observed.

One of the most striking changes that has occurred in infantile and childhood IE has been the virtual disappearance of rheumatic heart disease (RHD) as a predisposing factor. The introduction of antibiotics, especially penicillin, not only provided a most effective form of treatment for streptococcal infections, thereby dramatically reducing the incidence of RHD, but also provided an effective means of prophylaxis against IE in individuals with RHD. Congenital heart disease, on the other hand, has become the major predisposing factor in development

of IE during childhood[9,10]. In conflict with this data, our study disclosed that 10 of the 16 patients (62.6%) had valvular defects due to previous RHD as a predisposing pathology. We think that the changing patterns of IE cannot be applied globally. The clinical aspects and the prognosis of this disease may be different in developing countries like Turkey, where RHD is still considerably frequent. Furthermore, in a setting with higher incidence of RHD, IE involving mitral and aortic valves (i.e. left-sided IE) may be observed more frequently even among pediatric patients.

Limitations of the study

This study, like earlier reported studies, has some unavoidable limitations. There is remaining uncertainty regarding the true vegetation size when measurements are based on transthoracic echocardiography. The additional use of transesophageal echocardiography may improve detection rate and the accuracy of measurements. It is well known that some embolic events remain silent during the course of IE. This study was a retrospective one, with a relatively small study group, selected among the patients seen at a tertiary referral center.

In conclusion, the disease scope varies greatly in pediatric and adult patients as well as in different countries. Left-sided IE has a high rate of complications in children, which are mostly fatal or debilitating. RHD is still a frequent predisposing factor of IE in our population. There is limited information in literature focusing on pediatric patients, and collective information concentrating on left-sided IE in children is needed. Our study suggests that presence of an echocardiographically detected left-sided vegetation has high sensitivity and positive predictive value of an embolic event, irrespective of vegetation size. Therefore, we propose early surgical treatment in pediatric patients with echocardiographically discovered left-sided vegetations in an attempt to prevent systemic embolic events and, therefore, decrease mortality and morbidity of IE.

REFERENCES

1. Suryapranata H, Roelandt J, Haalebos M, Degener J, Bos E, Hugenholtz PG. Early cardiac valve replacement in infective endocarditis: a 10-year experience. Eur Heart J 1987; 8: 464-470.
2. Jaffe WM, Morgan DE, Pearlman AS, Otto CM. Infective endocarditis, 1983-1988: echocardiographic findings and factors influencing morbidity and mortality. J Am Coll Cardiol 1990; 15: 1227-1233.
3. Pelletier LL, Petersdorf RG. Infective endocarditis: a review of 125 cases from the University of Washington Hospitals, 1963-72. Medicine 1977; 56: 287-313.
4. Müge A, Daniel WG, Frank G, Lichtlen PR. Echocardiography in infective endocarditis: reassessment of prognostic implications of vegetation size determined by the transthoracic and the transesophageal approach. J Am Coll Cardiol 1989; 14: 631-638.
5. Steckelberg JM, Murphy JG, Ballard D, et al. Emboli in infective endocarditis: the prognostic value of echocardiography. Ann Int Med 1991; 114: 635-640.
6. Özkutlu S, Saraçlar M, Özme Ş, et al. Detection of right-sided endocarditis in children with congenital heart disease by two-dimensional echocardiography. Jpn Heart J 1990; 32: 175-182.
7. Özkutlu S, Saraçlar M, Özme Ş, et al. Çocukluk çağında sağ taraflı endokarditin 2 boyutlu ekokardiyografi ile tanısı. Turk J Cardiol 1991; 4: 41-45.
8. Alsip SG, Blackstone EH, Kirklin JW, Cobbs CG. Indications for cardiac surgery in patients with active infective endocarditis. Am J Med 1985; 78 (Suppl): 138-148.
9. Normand J, Bozio A, Etienne J, Sassolas F, Le Bris H. Changing patterns and prognosis of infective endocarditis in childhood. Eur Heart J 1995; 16 (Suppl): 28-31.
10. Mendelsohn G, Hutchins GM. Infective endocarditis during the first decade of life. Am J Dis Child 1979; 133: 619-622.
11. Bayer AS, Bolger AF, Tambert RA, et al. (Writing Group of the Committee on Rheumatic Fever, Endocarditis, and Kawasaki disease, American Heart Association). Diagnosis and management of infective endocarditis and its complications. Circulation 1998; 98: 2936-2948.
12. Mintz GS, Kotler MN, Segal BL, Parry WR. Survival of patients with aortic valve endocarditis: the prognostic implications of the echocardiogram. Arch Intern Med 1979; 139: 862-866.
13. Stafford WJ, Petch J, Radford DJ. Vegetations in infective endocarditis: clinical relevance and diagnosis by cross sectional echocardiography. Br Heart J 1985; 53: 310-313.
14. Bardy GH, Talano JV, Reisberg B, Lesch M. Sensitivity and specificity of echocardiography in a high-risk population of patients for infective endocarditis: significance of vegetation size. J Cardiovasc Ultrasonogr 1983; 2: 23-27.
15. Brandenburg RO, Giuliani ER, Wilson WR, Geraci JE. Infective endocarditis-a 25 years overview of diagnosis and therapy. J Am Coll Cardiol 1983; 1: 180-192.
16. O'Brien JT, Geiser EA. Infective endocarditis and echocardiography. Am Heart J 1984; 108: 386-394.
17. Stewart JA, Silimperi D, Harris P, Wise NK, Fraker TD, Kisslo JA. Echocardiographic documentation of vegetative lesions in infective endocarditis: clinical implications. Circulation 1980; 61: 374-380.
18. Çeliker A, Özme Ş, Paşaoğlu İ, Saraçlar M, Özkutlu S, Çakır S. Çocukluk çağındaki enfektif endokarditlerde erken cerrahi tedavinin önemi. Çocuk Sağ ve Hast Derg 1989; 32: 111-118.

19. Sheihk MU, Covarrubias EA, Ali N, Lee WR, Sheikh NM, Roberts WC. M-Mode echocardiographic observations during and after healing of active bacterial endocarditis limited to mitral valve. Am Heart J 1981; 101: 37-45.

20. Tischler MD, Vaitkus PT. The ability of vegetation size on echocardiography to predict clinical complications: a meta-analysis. J Am Soc Echocardiogr 1997; 10: 562-568.

21. Goldman ME, Fisher EA, Winters S, et al. Early identification of patients with native valve infectious endocarditis at risk for major complications by initial clinical presentation and baseline echocardiography. Int J Cardiol 1995; 52: 257-264.

22. Lutas EM, Roberts RB, Devereux RB, Prieto LM. Relation between the presence of echocardiographic vegetations and the complication rate in infective endocarditis. Am Heart J 1986; 112: 107-113.

23. Jung HO, Seung KB, Kang DH, et al. A clinical consideration of systemic embolism complicated to infective endocarditis in Korea. Korean J Intern Med 1994; 9: 80-87.

24. Lerner PI. Neurologic complications of infective endocarditis. Med Clin North Am 1985; 69: 385-398.

25. Mills SA. Surgical management of infective endocarditis. Ann Surg 1982; 195: 367-382.

26. Nihoyannopoulos P, Oakley CM, Exadactylos N, Riberio P, Westaby S, Foale RA. Duration of symptoms and the effects of a more aggressive surgical policy: two factors affecting prognosis of infective endocarditis. Eur Heart J 1985; 6: 380-390.

27. Roberts WC, Buchbinder NA. Healed left-sided infective endocarditis: a clinicopathologic study of 59 patients. Am J Cardiol 1977; 40: 876-888.

28. Oliveira DB, Dawkins KD, Kay PH, Paneth M. Chordal rupture. I: aetiology and natural history. Br Heart J 1983; 50: 312-317.

29. Potugese S, Amital H, Tenenbaum A, et al. Clinical characteristics of ruptured chordae tendineae in hospitalized patients: primary tear versus infective endocarditis. Clin Cardiol 1998; 21: 813-816.

30. Fernicola DJ, Roberts WC. Clinicopathologic features of active infective endocarditis isolated to the native mitral valve. Am J Cardiol 1993; 71: 1186-1197.

Biochemical and molecular analysis of mucopolysaccharidoses in Turkey

Serap Emre[1], Mügen Terzioğlu[1], Turgay Coşkun[2], Ayşegül Tokatlı[2], İmran Özalp[2] Vivien Müller[3], John Hopwood[3]

Departments of [1]Medical Biology, and [2]Pediatrics, Hacettepe University, Faculty of Medicine, Ankara, Turkey and [3]Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide S.A., Australia

SUMMARY: Emre S, Terzioğlu M, Coşkun T, Tokatlı A, Özalp İ, Müller V, Hopwood J. Biochemical and molecular analysis of mucopolysaccharidoses in Turkey. Turk J Pediatr 2002; 44: 13-17.

The mucopolysaccharidoses (MPSs) are a family of heritable disorders caused by deficiency of lysosomal enzymes needed to degrade glycosaminoglycans (GAGs). The undegraded or partially degraded GAGs are stored in lysosomes and/or excreted in urine. In our study, 118 patients seen over the past 20 years and suspected to have lysosomal storage disorders (LSDs) were subjected to clinical and biochemical analysis at Hacettepe University Children's Hospital. We analyzed urine and blood samples from 42 patients given a clinical MPS diagnosis. Using urine screening technique, we were able to show that 34 of the 42 patients had MPS condition. Further analysis of eight patients with normal urine MPS patterns revealed four patients as likely to have α-mannosidosis, fucosidosis, sialidosis, and aspartylglucosaminuria (one each). Four patients had normal oligosaccharide patterns. We were able to clearly identify 4 MPS I, 2 MPS II, 5 MPS IIIA, 8 MPS IIIB, 11 MPS IVA, 3 MPS VI, and 1 MPS IIIC patients. These results provided biochemical diagnosis for these 34 patients, and clearly show that Turkey has a higher incidence of MPS IVA, IIIB, and IIIA than of previously suspected MPS types. Molecular analysis of four MPS I patients revealed three polymorphisms which have been previously reported (A314, T388, and A461T). In MPS II patients, mutation analysis identified one previously detected (R172X) and one novel mutation (W109C).

Key words: mucopolysaccharidoses, lysosomal storage diseases, mutations, Turkish population.

Mucopolysaccharidoses (MPSs) are a large group of lysosomal storage disorders (LSDs) resulted from a deficiency in the enzymes required for the catalysis of the stepwise degradation of glycosaminoglycans (GAGs or mucopolysaccharide)[5]. For the degradation of mucopolysaccharides (or sulfated glycosaminoglycans-heparan, dermatan, keratan, and chondroitin sulfates), each step requires specific enzymes, the absence of which results in specific types of MPSs[5]. This group of diseases mainly follows an autosomal recessive inheritance pattern with a few exceptions, namely Fabry's disease and MPS type II, which are inherited as X-linked recessive[5,11].

The prevalence values for individual LSDs clearly define these cases as rare genetic disorders[9]. Their overall incidence is lower than 1:100,000[10-12]. In fact, there are some exceptions that show high incidence among some populations like Tay-Sachs and Gaucher's disease type I in Ashkenazi Jews and Salla disease in the Finnish populations[5].

The diagnosis and the differentiation of specific types among MPS can be made using urinary screening tests and molecular methods[14]. Early detection of MPSs would provide the option for prenatal diagnosis for many families carrying these disorders[20].

In our study, blood and urine samples from 42 clinically suspect patients were analyzed. For this purpose, biochemical and molecular methods were used in order to determine the specific types of MPSs.

Material and Methods

Patients: Over the past 20 years 118 patients from all over Turkey suspected to have LSD were

referred to İhsan Doğramacı Children's Hospital, Department of Pediatric Nutrition and Metabolism, Hacettepe University. Among these patients, 42 were clinically diagnosed with MPS. Blood and urine samples were collected from 38 and 42 patients, respectively.

Analysis of Urine Samples: All urine samples were analyzed first with semiquantitative Alcian blue procedure for mucopolysaccharides to detect the amount of MPS in urine at the laboratories of the Department of Chemical Pathology, Lysosomal Diseases Research Unit, Women's and Children's Hospital, North Adelaide, Australia. Then with high resolution electrophoresis, the migration patterns of MPS were detected. Each migration pattern is specific for each MPS type. Unfortunately, evaluation of the specific migration patterns with this technique only defects MPS type; further enzymatic analysis is required to identify subtypes.

Oligosaccharides Detection in Urine by Thin Layer Chromatography: Oligosaccharides excreted in urine at high levels can be detected by the thin layer chromatography technique[14].

Isolation of Lymphocytes and Analysis of Enzyme Activities: Specific radioactively labelled substrates were used to analyze the specific enzyme activities by high pressure liquid chromatography (HPLC). For this purpose, isolation of lymphocytes was done by Ficol-Hypaque method. The lymphocyte homogenates were prepared by freezing and thawing the cells 4-5 times. Protein determination in these cell homogenates was done according to Lowry et al[8]. For determination of enzyme activity, 50-100 μg of protein are needed.

Molecular Analysis of MPS I and MPS II Patients: In order to screen the mutations in α-L-iduronidase genes of MPS I and iduronate sulfatase genes of MPS II patients, single strand conformation polymorphism (SSCP) method was used. The fragments were then subjected to sequence analysis.

Allele Specific Oligonucleotide (ASO) Analysis: Biochemically diagnosed patients were first subjected to ASO analysis in order to screen the most common mutations as previously described[19].

Results

Over the past 20 years, 118 patients from different regions of Turkey suspected to have LSDs were referred to Hacettepe İhsan Doğramacı Children's Hospital, Department of Nutrition and Metabolism. Among these patients, 42 of them for whom blood and urine samples were available were evaluated for clinical, radiological, and simple biochemical findings. Skeletal deformities (25.4%) and developmental delay or mental retardation (23.7%) were two major presenting symptoms. Coarse faces with large nose and thick lips (87.3%) was noted in the majority of the patients on physical examination. In our study, once an MPS type was considered in a differential diagnosis, a laboratory evaluation was initiated in order to define the diagnosis biochemically. Two types of urinary screening tests were applied to look for the abnormal excretion of either mucopolysaccharides or oligosaccharides. Biochemical diagnosis of the MPS types was confirmed by measuring the specific enzyme activities. The urine screening test, enzyme activity, and the percentage of each MPS type results are shown in Table I. The MPS types of 42 patients according to urine screening tests and enzymatic activity analysis were detected. For all types, the enzyme activity range of the patients was far lower than the normal activity range, which was consistent with the clinical phenotypes. As a result of the urine glycosaminoglycan patterns, 11 patients were diagnosed as MPS IVA, 8 patients were MPS IIIB, 5 patients were MPS IIIA, 3 patients were MPS VI, 2 patients were MPS II, 4 patients were MPS I, and 1 patient was MPS IIIC. Four patients were found to have oligosaccharide patterns consistent with other lysosomal storage diseases (one each): α-mannosidosis, fucosidosis, sialidosis, and aspartylglucosaminuria. Oligosaccharidoses are also a group of LSDs characterized by the defects of glycoprotein degradation due to the deficiency of specific lysosomal enzymes[1]. The results in four patients were not consistent with either MPSs or oligosaccharidoses, and further analysis is needed to search for other types of LSDs.

Mutation screening was done for four MPS I and two MPS II patients. The results of the molecular analysis are shown in Table II. In MPS I patients, three previously reported polymorphisms were detected (A314, T388, and A361T)[18]. Mutation analysis of MPS II patients showed one previously reported (R172X) and one novel mutation (W109C)[7]. For the confirmation of the novel genetic defect as mutation, 100 alleles from unaffected individuals were tested and it was found that it is a disease-causing defect.

Table I. Enzyme Activity, Relative Frequency, and Urine Oligosaccharide Results of the MPS Patients

MPS type	Diagnosed patients	Deficient enzyme	Enzyme activity range (pmol/mg protein/min)	Normal enzyme activity (pmol/mg protein/min)	% of MPS
MPS I	4	α-L-Iduronidase	0.14-5.83	15-34	11.76
MPS II	2	Iduronate sulfatase	0.27-0.69	10.9-88	5.88
MPS IIIA	5	Sulfamidase	0.03-0.22	0.3-4.2	14.71
MPS IIIB	8	α-N-Acetylglucosaminidase	0.02-0.05	0.4-3.4	23.53
MPS IIIC	1	AcetylCoA: α-glucosaminidase-N-acetyl transferase			
MPS IVA	11	N-Acetylgalactosamine-6-sulfase	0.27-2.9	38.5-166	32.35
MPS VI	3	N-Acetylgalactosamine-4-sulfatase	0.04-0.08	5.8-18.9	8.82

Urine oligosaccharides results	
Diagnosed patients	Diagnosis
1	Fucosidosis
1	Sialidosis
1	Aspartylglucosaminidase
1	α-Mannosidosis
4	Normal*

* Normal for oligosaccharide pattern for MPSs but further analysis for other possible LSDs is needed.
MPS: mucopolysaccharidoses.

Table II. Results of the Polymorphism and Mutation Analysis of MPS I and MPS II Patients

Polymorphisms found in MPS I Patients		
Amino acid	Exon	Base change
A314	VII	GCG → GCC
T388	VIII	ACG → ACC
A361T	VIII	GCG → ACG
Mutations found in MPS II patients		
R172X	5	CGA → TGA
W109C*	3	TGG → TGC

* Novel mutation.

Discussion

Mucopolysaccharidoses are inherited diseases showing relevant clinical overlap with other LSDs, and they show a wide variety of clinical manifestations[1,3,11]. Differences in environmental and genetic backgrounds, in part, explain differences in the clinical phenotypes of the patients with the same disease genotype[6]. Due to the extreme phenotypic variability of many of these disorders, usually it is very difficult to discriminate between both types and subtypes without having detailed clinical, biochemical, and molecular data. As can be seen from Table I, enzyme activity ranges are lower than normal for all MPS types, and the enzyme results are consistent with the clinical phenotypes. However, it is very difficult to distinguish the clinical phenotype of the disease by evaluating only the enzyme activity, since there is a wide variety of heterogeneity among clinical phenotypes. Because of this variability, clinical, biochemical, and molecular findings together are very important in the differential diagnosis of MPS types. Analysis of undegraded and accumulated metabolites, demonstrating a profound deficiency of a specific enzyme, and finally the molecular analysis of the gene of interest are the basic approaches used to diagnose these diseases. The analysis of 42 patients' urine and blood samples using these approaches revealed that the most common MPS types in our population are MPS IVA and MPS IIIB, followed by MPS IIIA, MPS I, MPS VI, MPS II, and MPS IIIC, respectively. In their study, Ozand et al.[13] found the most common type in Saudi Arabia to be MPS IVA. It has been shown that the most common MPS types in the Russian population were types II, I, IIIA, and IVA[6]. Krasnopolskaya et al.[6] analyzed 363 patients, and 241 were found to have MPSs. Another research from Greece showed the MPS IIIB type as the most common among the Greek population[10].

Of the 42 clinical and biochemical diagnoses, there were two individuals who had one affected sibling each who were MPS IIIB, and MPS IVA, respectively. In our population, the consanguinity rate is very high (75%), and 37.5% of families have a history of MPS condition. Because of the high consanguinity rate in the population,

heterozygote screening is very important. This will help affected families in genetic counselling, eventually leading to a prenatal diagnosis in pregnancies at risk.

The molecular analysis of the α-L-iduronidase gene of four MPS I patients was done by SSCP/ sequence analysis. The MPS type I has a broad spectrum of clinical presentations, which are severe Hurler syndrome and the milder Scheie syndrome[5,18]. It has been difficult to predict the severity of a disease using only the biochemical techniques[18]. Before analyzing patients with SSCP, screening for common mutations in the iduronidase gene (W402X and Q70X for Hurler; R89Q and 678 7g→a for Scheie) was done using ASO method[18]. None of these mutations was detected in our patients. As a result of the molecular analysis of the four MPS I patients, three polymporphisms were detected in the α-L-iduronidase gene. In three patients, polymorphisms were detected in exons 7 and 8. Two of the polymorphisms in these exons were A314 and T388, respectively. They show no association with any specific mutations, but it is known that these polymorphisms seriously affect enzyme activity. The other polymorphism in exon 8 leads to the change of amino acid alanine[361] to treonine. This polymorphism is uniquely found in association with the R89Q mutation, and potentiates its effect, thus altering the clinical phenotype from mild to intermediate[18]. In our patient, we could not detect this mutation-polymorphism association, and the clinical phenotype was at the milder spectrum of the disease. In one patient with MPS type I we could not detect mutation by SSCP analysis; sequencing was required. In MPS II patients, molecular analysis revealed one previously reported (R172X) and one novel (W109C) mutation[7]. MPS II (Hunter) is an X-linked recessive disorder caused by various lesions in the iduronate sulfatase gene. Two clinical extremes of MPS II, mild and severe, have been recognized[5,7]. In general, the severe form has early onset at two to four years. In contrast, mildly affected patients preserve normal intelligence and survive into late adulthood. Our MPS II patients were both severely affected. The previously detected R172X mutation is the cause of the clinical pathology. It creates a stop codon at arginine residue position 172 and causes a truncated protein, which results in the defective activity. The other mutation is W109C, which causes the change of amino acid tryptophan to cysteine. This change is a nonconservative change of an aromatic tryptophan to polar cysteine and might have a serious effect on the activity of the enzyme (0.31 pmol/mg protein/min) (normal range in Table I). However, since our patient was mildly affected, the residual activity of the mutant enzyme keeps the patient in the mild spectrum of the disease. Since this mutation was not reported previously, the exact effect of the change on enzyme structure and expression remains to be analyzed.

The molecular genetic analysis of each MPS type remains to be done in the near future. Molecular analysis will reveal the exact mutations of the each type and the effects of these mutations on the activity and expression of each enzyme. Molecular genetic analysis of MPS IIIB, IIIA, I, and IVA is under investigation. In MPS IIIA and IIIB patients, novel mutations are found[3,4].

In this study, both biochemical and molecular defects in MPS types were revealed. The definition of more complete genotypes, and more detailed clinical descriptions of the patients will greatly assist the development of molecular analysis for MPS types and the establishment of genotype phenotype relationships. Recently, exciting progress has been made in the treatment of MPSs based on gene therapy, bone marrow transplantation, and enzyme replacement therapies. In this respect, as a result of biochemical and molecular diagnosis, any suspect patient is a potential candidate for new therapeutic strategies and deserves a precise diagnosis. On the other hand, molecular information regarding these families at risk would provide the option for prenatal diagnosis for many more families carrying these disorders.

REFERENCES

1. Andria G, Parenti G. Oligosaccharidoses and related disorders. Molec Med 1986; 19: 357-368.
2. Brooks DA. Immune response to enzyme replacement therapy in lysosomal storage disorder patients and animal models. Molec Genet&Metab 1999; 68: 268-275.
3. Emre S, Terzioğlu M, Müller V, et al. Lysosomal storage diseases in Turkey. 5th International Symposium on Mucopolysaccharide and Related Diseases, Vienna 1999; pl 32.
4. Emre S, Terzioğlu M, Tokatlı A, et al. Sanfilippo syndrome in Turkey: identificantion of mutations in subtypes A and B. 5th International Symposium on Mucopolysaccharide and Related Diseases; Vienna 1999; p147.

5. Hopwood JJ, Morris CP. The mucopolysaccharidoses: diagnosis, molecular genetics and treatment. Mol Biol Med 1990; 7: 381-404.
6. Krasnopolskaya KD, Mirenburg TV, Aronovich EL, et al. Diagnosis and prevention of lysosomal storage diseases in Russia. J Inherit Metab Dis 1993; 16: 994-1002.
7. Li P, Bellows AB, Thompson JN. Molecular basis of iduronate-2-sulphatase gene mutations in patients with mucopolysaccharidosis type II (Hunter syndrome). J Med Genet 1999; 36: 21-27.
8. Lowry OH, Rosebrough NJ. Protein measurement with the folic phenol reagent. J Biol Chem 1951; 193: 265-275.
9. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. JAMA 1999; 281: 249-254.
10. Michelakakis HP, Dimitriou E, Tsagaraki S, Giouroukos S, Schulpis K, Bartsocas CS. Lysosomal storage diseases in Greece. Genet Couns 1995; 6: 43-47.
11. Neufeld EF. Lysosomal storage diseases. Annu Rev Biochem 1991; 60: 257-280.
12. Neufeld EF, Muenzer J. The mucopolysaccharidoses. In: Scriver CR, Beaudet A, Sly WS, Valle D (eds). The Metabolic and Molecular Bases of Inherited Diseases (7th ed), Vol. II. New York: McGraw-Hill; 1995: 2465-2494.
13. Ozand PT, Gascon G, al Aqeel A, Roberts G, Dhalla M, Subramanyam SB. Prevalence of different types of lysosomal storage diseases in Saudi Arabia. J Inherit Metab Dis 1990; 13: 849-861.
14. Peelen GO, Jan GN, Wevers R. HPLC analysis of oligosaccharides in urine from oligosaccharidosis patients. Clin Chem 1994; 40: 914-921.
15. Sands MS, Vogler C, Kyle JW, et al. Enzyme replacement therapy for murine MPS type VII. J Clin Invest 1994; 93: 2324-2331.
16. Sands MS, Vogler C, Torrey A, et al. Long term therapeutic effects of enzyme replacement and bone marrow transplantation. J Clin Invest 1997; 99: 1596-1605.
17. Scott HS, Litjens T, Nelson PV, Brooks DA, Hopwood J, Morris CP. α-L-iduronidase mutations (Q70X and P553R) associated with a severe Hurler phenotype. Hum Mutat 1992; 1: 333-339.
18. Scott HS, Nelson PV, Litjens T, Hopwood JJ, Morris CP. Multiple polymorphisms with the α-L-iduronidase gene (IDUA): implications for a role in modification of MPS-I disease phenotype. Hum Mol Genet 1993; 2: 1471-1473.
19. Weber B, Guo XH, Wraith JE, et al. Novel mutations in Sanfilippo A syndrome: implications for enzyme function. Hum Mol Genet 1997; 6: 1573-1579.
20. Whiteman P, Henderson H. A method for the determination of amniotic fluid glycosaminoglycans and its application to the prenatal diagnosis of Hurler and Sanfilippo diseases. Clin Chim Acta 1987; 99: 99-105.

The prevalence and molecular basis of β-thalassemia in Isparta province and region

Bahattin Tunç[1], Hasan Çetin[1], Fatma Gümrük[2], Beysun İstanbullu[1], Hasan Yavrucuoğlu[1] Umran Kurt[1], Halide Genç[1]

[1]*Department of Pediatrics, Süleyman Demirel University Faculty of Medicine, Isparta, and* [2]*Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey*

SUMMARY: Tunç B, Çetin H, Gümrük F, İstanbullu B, Yavrucuoğlu H, Kurt U, Genç H. The prevalence and molecular basis of β-thalassemia in Isparta province and region. Turk J Pediatr 2002; 44: 18-20.

The prevalence and molecular basis of β-thalassemia in the district of Isparta were determined in a total of 6,054 healthy high school students who were recruited from 21 randomly selected high schools in the Isparta province and region. In 182 subjects, naked eye single tube red cell osmotic fragility test (NESTROFT test) was positive HbA_2 was measured by high-performance liquid chromatography (HPLC) in these subjects and was found to be high in 149 subjects. The incidence of β-thalassemia was 149 in 6,054 (25%). The β-thalassemia frequency was lower in the city center than in neighboring towns, 1.7% vs. 2.2%, respectively The most prevalent mutation of β-thalassemia in this region was IVS 1-110 (G-A), followed by Codon 39 (C-T) and IVSII-745 (C-G).

Key words: hemoglobinopathy, prevalence, β-thalassemia trait, screening, population.

The β-thalassemias are due to mutations of the beta globin gene that markedly decrease or completely prevent the production of beta globin chains[1-3]. Thalassemias are the most common inherited disorders in the world. A relatively high prevalence is also observed in Turkey. Although the overall estimated frequency of β-thalassemia in Turkey has been stated to be 2%[4], the frequency varies from 0.6% to 10.7% between regions[5-7]. Numerous up-to-date surveys have been conducted in various regions of Turkey, but there have not been any systematic studies in Isparta. We planned this screening program in order to determine the prevalence and to establish the molecular basis of β-thalassemia trait, and to estimate the magnitude of disease and requirements for its control in this district.

Material and Methods

An educational and screening program for the thalassemias was carried out in 21 randomly selected high schools with a total of 6,054 healthy students in different regions of Isparta. The ages of students ranged between 13 to 18 years. The number of male and female students were 3,360 (55.5%) and 2,694 (44.5%), respectively.

This screening program was structured in three steps: the first included information and educational aspects and the second involved blood sampling and detection of carriers. In the third phase, students were informed of the results, and genetic counselling was given.

During the second stage of the study, capillary blood was obtained from each participant's finger tip. The naked eye single tube red cell osmotic fragility test (NESTROFT test) (osmotic fragility of red blood cells in 0.4% saline solution) was applied as first-step screening test to detect β-thalassemia trait[8]. Further investigation was performed on subjects who were NESTROFT positive or doubtful. Their hemoglobin A_2 and hemoglobin F were then determined by high-performance liquid chromatography (HPLC) (Bio-Rad). For hemoglobin electrophoresis, 2 ml of venous blood sample anticoagulated with EDTA was obtained with parental consent.

We did not attempt to investigate other variant of hemoglobinopathy other than β-thalassemia. The students who were found to be carriers of thalassemia mutations were given an opportunity to receive additional information and counselling.

In addition, all carriers received a card printed with their thalassemia carrier status for their records and future reference.

DNA was extracted from the peripheral blood sample of the subjects collected in EDTA-vacutainer. Mutations in the beta globin gene were identified by polymerase chain reaction (PCR) based diagnostic strategies in Hacettepe University Faculty of Medicine and Faculty of Science[9].

Although the study was designed primarily for the students, 76 teachers also asked to be tested.

Results

A total of 6,054 blood samples were obtained and analyzed for β-thalassemia trait with NESTROFT test. Of 6,054 students 2,992 (49.4%) were from the center district of Isparta and 3,062 (50.6%) were from 11 different towns of Isparta. One hundred eighty-two out of 6,054 blood samples (3.12%) were positive for NESTROFT test. HbA_2 was found to be 2.5%. Distribution of carriers according to region is illustrated in Table I.

Table I. Distribution of β-thalassemia Traits

Region	Subjects	Traits	%
Center of Isparta	2992	52	1.74
Aksu	147	4	2.72
Atabey	151	5	3.31
Barla	87	2	2.29
Eğirdir	600	19	3.16
Gelendost	205	11	5.36
Gönen	179	12	6.70
Keçiborlu	342	9	2.63
Senirkent	208	9	4.32
Sütcüler	142	6	4.22
Şarkikaraağaç	542	12	2.21
Yalvaç	459	8	1.74
Total	6054	149	2.46

In order to establish the molecular basis of β-thalassemia, DNA samples of 14 carriers were analyzed. Three different point mutations were identified. These mutations, their frequency in this study and in Turkey overall are given in Table II.

Table II. Results of the Mutation Analysis

Molecular pathology	Number of % subjects	Overal frequency of mutation for Turkey (%)
IVS I-110 (G-A)	10	38.06
Codon 39 (C-T)	3	2.6
IVSII-745 (C-G9)	1	4.33

Discussion

β-thalassemia constitutes one of the most serious health problems in Turkey. It is preventable by carrier screening and prenatal diagnosis[10]. β-thalassemia major remains a serious physical, emotional, and financial burden for patients, their families and countries[11]. The related health care cost are very high. The success observed in some countries in reducing the prevalence of thalassemia major by genetic counselling shows that screening can have a major impact in communities in which the thalassemias are common. That is, screening of the population of thalassemia is the first step in eradication.

We determined the prevalence of β-thalassemia trait as 2.5% in the Isparta region. The prevalence of β-thalassemia in our study is slightly higher than that seen in Turkey as a whole, but is lower than that of neighboring provinces. Previous studies have reported the prevalence of thalassemia in Antalya[12], Konya[13], and Denizli[3] as 10.2%, 3.8%, and 3%, respectively.

It was noted that the prevalence in the city center is lower than that in neighboring towns and villages (Table I). This difference may be due to immigration to the city center from other cities or villages with low β-thalassemia frequency. It appears that heterozygotes for β-thalassemias are protected from the severe effects of falciparum malaria[14], and natural selection has increased and maintained their gene frequencies in these malarious regions as seen around Eğirdir Lake and some other towns (Table I).

We selected high school students as the target population for this survey. The ideal age for population screening for autosomal recessive disease is early adulthood, before marriage[15]. Therefore, screening of high school students for common genetically inherited disorders is generally recommended[16], The present study indicates that most high school students as well as their parents and teachers, after being given proper information, are willing to participate in a screening program for thalassemia.

The tests in developing countries for screening of hemoglobinopathies must be reliable, easy to perform, adaptable and cost-effective. It is recommended that 0.4% buffered saline solution be used as a first screening test for the detection of β-thalassemia trait in a population, as was used in this study[17-19]. However, this test is not

enough to differentiate hypochrome anemias of various etiology. Therefore, electronic whole blood count, electrophoretic studies and HbA_2 determination are recommended as a first step in many screening studies.

Our results show that in Isparta the IVS-I-110 (G-A) mutation is the most common β-thalassemia defect, followed by Codon 39 (C-T) and IVSII-745 (C-G). The frequencies of these mutations are higher than those reported for Turkey overall. However the number of carriers was too small to represent the thalassemic population[20-24].

In conclusion, the prevalence of β-thalassemia is relatively high in Isparta. Therefore, continuation of population screening programs and establishment of premarital screening programs, along with education and counselling, are necessary in this region.

Acknowledgements

We thank Çiğdem Altay MD for her support and encouragement, and we are indebted to all the students and their teachers for their participation in this project.

REFERENCES

1. Modell B, Mauzouras M, Ward RH, Fairwether DV. Population screening for carriers of recessively inherited disorders. Lancet 1980; 11: 806-809.
2. Lipkin MJ, Fischer L, Rwley PT, Iker HP. Genetic counselling of asymptomatic carriers in a pirimary care setting. The effectiveness of screening and counselling for β-thalassemia trait. Ann Intern Med 1986; 105: 115-120.
3. Davies SC, Wonke B. The management of hemoglobinopathies. Clin Haematol 1991; 4: 361-389.
4. Çavdar AO, Arcasoy A. The prevalence of β-thalassemia and abnormal hemoglobins in Turkey. Acta Haematol 1971;45: 312-317.
5. Kürkçüoğlu M, Dağcı A, Gencelli Y, Arcasoy A, Ağbaş A. Doğu Anadolu Bölgesinde β-thalassemia ve anormal hemoglobin taraması. Doğa Bilim Dergisi 1986; 8: 319-325.
6. Aksoy M, Kutlar F, Dinçol G, Erdem S, Baştanbilici S. Batı Trakya Türklerinde hemoglobin varyantları, β-thalassemia, G6PD eksikliği ve haptoglobin tipleri. Doga Bilim Dergisi 1985; 9: 45-49.
7. Altay Ç, Gürgey A. Distribution of hemoglobinopathies in Turkey. Turk J Pediatr 1986; 28: 219-229.
8. Kattamis C, Efromov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting β-thalassemia trait. J Med Gen 1981; 18: 266-268.
9. Pnocz M, Solowiejczyk D, Harpel B, Mory Y, Schwartz E, Surrey S. Construction of human gene libraries from small amounts of peripheral blood: analysis of beta-like globin genes. Hemoglobin 1982; 6: 27-36.
10. Cao A, Saba L, Galanello R, Rosatelli MC. Molecular diagnosis and carrier screening for beta thalassemia. JAMA 1997; 278: 1273-1277.
11. Modell B, Kuilev AM. A scientific basis for cost-benefit analysis of genetics services. Trends Genet 1993; 9: 46-52.
12. Bircan I, Sisli S, Güven A, et al. Hemoglobinopathies in the district of Antalya, Turkey. Ped Hematol Onco 1993; 10: 289-291.
13. Turan C, Topal B, Gürgey A, Altay Ç. Konya ve Denizli yöresinde beta talasemi sıklığı. Çocuk Sağ ve Hast Dergisi 1991; 34: 9-11.
14. Lilleyman JS. Hematologic effects of systemic disease and toxins. In: Lilleyman JS, Hann MI, Blanchette VS (eds). Pediatric Hematology (2nd ed) London: Churchill Livingstone; 1999: 771-789.
15. Altay C. Yılgör E, Beksaç S, Gürgey A. Premarital screening of hemoglobinopathies: a pilot study in Turkey. Hum Hered 1996; 46. 112-114.
16. Clayton EW, Steinberg KK, Khoury MJ, et al. Informed consent for genetic research on stored tissue samples. JAMA 1995; 274: 1786-1792.
17. Raghavan K, Lokeshwar MR, Birewar N, Nigam V, Manglani MV, Raju NB. Evaluation of naked eye single tube red cell osmotic fragility test in detecting beta thalassemia trait. Indian Pediatr 1991; 28: 469-472.
18. Manglani M, Lokeshwar MR, Vani VG, Bhatia N, Mhaskar V. "NESTROFT" an effective screening test for beta thalassemia trait. Indian Pediatr 1997; 34: 702-707.
19. Canatan D, Arcasoy A, Çavdar AO. Tek tüp osmotik frajilite test ile β-thalassemia trait taraması. Doğa Bilim Dergisi 1985; 9: 130-135.
20. Tadmouri GO, Tüzmen S, Özçelik H, et al. Molecular and population genetic analyses of beta-thalassemia in Turkey. Am J Hematol 1998; 57: 215-220.
21. Nişli G, Kavaklı K, Aydınok Y, Öztop S. Beta-thalassemia alleles in Aegean region of Turkey. Pediatr Hematol Oncol 1997; 14: 59-65.
22. Altay Ç, Öner C, Öner R, Mesci L. Genotype-phenotype analysis in Hb S-beta thalassemia. Hum Hered 1997; 47: 161-164.
23. Akar N, Cavdar AO, Dessi E, Loi A, Pirastu M, Cao A. β-thalassemia mutations in the Turkish population. J Med Gen 1987; 24: 378-379.
24. Gürgey A, Altay C, Diaz-Chico JC, Kutlar A, Huisman TH. Molecular heterogeneity of beta-thalassemia intermedia in Turkey. Acta Haematol 1989; 81: 22-27.
25. Altay Ç, Başak AN. Molecular basis and prenatal diagnosis of hemoglobinopathies in Turkey. Int J Pediatr Hematol Oncol 1995; 2: 283-290.

Analysis of thalassemia syndromes and abnormal hemoglobins in patients from the Aegean region of Turkey

Gülersu İrken[1], Hale Ören[1], Bülent Ündar[2], Murat Duman[1], Hüseyin Gülen[1]
Canan Uçar[1], Namık Şanlı[1]

Department of [1]Pediatrics, and [2]Internal Medicine, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey

SUMMARY: İrken G, Ören H, Ündar B, Duman M, Gülen H, Uçar C, Şanlı N. Analysis of thalassemia syndromes and abnormal hemoglobins in patients from the Aegean region of Turkey. Turk J Pediatr 2002; 44: 21-24.

Turkey is located in a geographic area of the world where thalassemia syndromes and abnormal hemoglobins are common. In this study we aimed to evaluate the thalassemia syndromes and abnormal hemoglobins in patients from the Aegean region of Turkey. Among the patients admitted to our Pediatric Hematology or Hematology Clinic between January 1997-September 1999, hemoglobin electrophoresis of 3,228 cases investigated for anemia was done using high performance liquid chromatography. Beta thalassemia trait was diagnosed in 21.1%, beta thalassemia major in 0.2%, S-beta thalassemia in 0.37%, Hb D in 0.37%, Hb S trait in 0.32%, Hb E in 0.18%, Hb O-Arab in 0.12%, Hb G-Copenhagen in 0.09%, Hb D-Iran in 0.06%, Hb Lepore in 0.06%, Hb Hasharon in 0.03%. Our results demonstrate that people in the Aegean region of Turkey have a wide spectrum of thalassemia syndromes and abnormal hemoglobins.

Key words: abnormal hemoglobins, chromatography, hemoglobinopathy, thalassemia.

Inherited abnormalities of hemoglobin synthesis may be divided into two groups: the first group includes the thalassemia syndromes in which one or more of the normal polypeptide chains of hemoglobin are synthesized at a reduced rate, and the second group is the abnormal hemoglobin group, those characterized by structurally abnormal hemoglobin variants[1]. Most abnormal hemoglobins differ from normal hemoglobin in the substitution of a single amino acid for another. Recently, 698 abnormal hemoglobins have been recognized[1]. The prevalence of the thalassemia syndromes and abnormal hemoglobins varies considerably with geographic location and racial group. Thalassemia is considered the most common genetic disorder worldwide. Turkey is located in a geographic area of the world where thalassemia syndromes and abnormal hemoglobins are common[1-8].

Disorders of globin chain synthesis constitute a significant public health problem. Diagnosis may be required to confirm a provisional diagnosis such as sickle cell disease or beta-thalassemia major; to explain a hematological abnormality such as anemia or microcytosis; to identify an abnormality in the presymptomatic phase, as in neonatal screening; to predict serious disorders of globin chain synthesis in the fetus and offer the option of termination of pregnancy; to permit genetic counselling of prospective parents; and as preoperative screening for the presence of sickle cell hemoglobin[9].

Until recently the identification and quantification of hemoglobin variants required a sequence of test, each with inherent problems of reproducibility, accuracy, labor intensity and cost[10]. improved fully automated systems and reagents for techniques such as high performance liquid chromatography (HPLC) and isoelectric focusing have led to their introduction in many laboratories[10-21]. HPLC system is a rapid, simple and reliable method for diagnosis of hemoglobinopathies[15-21].

In this study, we aimed to evaluate the thalassemia syndromes and abnormal hemoglobins in patients from the Aegean region of Turkey, who were admitted to our Pediatric Hematology or Hematology Clinic and needed to be investigated for anemia, using HPLC.

Material and Methods

Among the patients who were admitted to our Pediatric Hematology or Hematology Clinic between January 1997-September 1999, hemoglobin electrophoresis of 3,228 cases who needed to be invstigated for anemia was done using HPLC. Anticoagulated (EDTA) blood samples of all patients were analyzed by the Bio-Rad Variant Hemoglobin Testing System (Bio-Rad Laboratories, California, USA) which utilizes the principles of cation-exhange HPLC. All of the patients' blood samples were first analyzed by the Bio-Rad Beta-thalassemia Short Program, and if unknown hemoglobin was determined, the Bio-Rad Variant Hemoglobinopathy Program was then used to evaluate the abnormal hemoglobin.

For the analysis, 5 µl of EDTA whole blood was automatically diluted with 1 ml of hemolyzing reagent. Hemolyzed specimens were loaded into a 100-place sampler compartment maintained at 12 ± 2 °C. Specimens were sequentially injected into analysis stream at a certain interval. Two dual-piston pumps and a preprogrammed gradient controlled the elution buffer mixture passing through the analytical cartridge. A dual-wavelength filter photometer detected the absorbance of Hb at the primary wavelength (415 nm) and corrected for nonspecific absorbance at the secondary wavelength (690 nm). Changes in absorbance were monitored and displayed as a chromatogram of absorbance versus time. Analysis data from the detector was processed by the built-in integrator and printed on the sample report.

The Variant Hemoglobinopathy Program uzitilized a retention time marker with assigned windows for hemoglobins F, A_0, A_2/E, D, S, and C. Retention times of hemoglobins contained in patient samples were normalized relative to the retention time of hemoglobin contained in the retention time marker. Analyte identification "windows" were intended to assist the laboratory in the interpretation of normal and abnormal hemoglobins detected in patient samples. The "windows" were established time ranges in which common hemoglobins have been observed to elute using the Variant Hemoglobinopathy Program. The retention time was the center of the window. Retention time was measured from the time of sample injection to the maximum point of each peak. Analytes that were detected outside of a retention time window were labeled as "unknown x", which represented an abnormal hemoglobin. Interpretation of unknowns was done by hematologists by correlating the results with a previously studied and detected Hb specimen (Hb x was determined by comparing retention times with a known Hb x specimen).

Results

Beta thalassemia trait was diagnosed in 683 cases (21.1%), beta thalassemia major in eight cases (0.2%), and Hb H in five cases (0.15%). Abnormal hemoglobin variants detected in this study are shown in Table I.

Table I. Abnormal Hemoglobin Variants Detected in Our Patients

Hemoglobin variant	n (%)*
Hb S+beta thalassemia	12 (0.37)
Hb S D	12 (0.37)
Hb S trait	10 (0.32)
Hb E	6 (0.18)
Hb O-Arab	4 (0.12)
Hb G-Copenhagen	3 (0.09)
Hb D-Iran	2 (0.06)
Hb Lepore	2 (0.06)
Hb Hasharon	1 (0.03)
Hb Montgomery	1 (0.03)
Hb Constant Spring	1 (0.03)
Hb Köln	1 (0.03)

* Percentage of all patients.

Discussion

Beta thalassemia is the most common thalassemia syndrome in Turkey. It also occurs at high frequencies among individuals of Mediterranean, East Indian, Middle Eastern, African or Southeast Asian descent[22,23]. It is almost evenly distributed over Turkey, and heterozygous frequency is reported to be around 2%[2,24,25]. The incidence of beta thalassemia is higher (about 10%) in some regions like western Thrace and the Mediterranean coast[5,26]. In our study the prevalence of beta thalassemia traits with increased HbA2 was 21.1%. We can not compare our results with the above mentioned studies, since our patient population consisted of patients admitted to our Pediatric Hematology or Hematology Clinic who needed to be investigated for anemia.

The prevalence and the distribution of alpha thalassemia are not well known in Turkey, since alpha thalassemia traits can be demonstrated chromatographically only in the newborn period,

and very time-consuming and expensive techniques are needed for diagnosis afterwards. We found Hb H in 0.15% of our patients. Cord blood studies from different regions of Turkey demonstrated Hb Bart's incidence as 1.6% and 3.6%[6].

The prevalence of abnormal hemoglobins was found to be 1.7% in our survey. Among abnormal hemoglobins, sickle hemoglobin is the most frequently encountered variant worldwide[27]; heterozygous sickle hemoglobin was present in 0.32% of our patients. In previous population screening tests, differing carriage rates of the gene were found, from 0.3% to 37%[7,24,28-31]. While the frequency of Hb S is lower in the Aegean region, it is higher especially on the southeast coast of Turkey, where an ethnic group called Eti-Turks lives[29-31]. We also detected Hb S+beta thalassemia in 0.37% of our patients. In geographic areas where thalassemia mutations and structural variants of alpha and beta globin genes are frequent (such as Southeast Asia and Africa), compound heterozygotes with a thalassemia mutation and a structural variant are common[22].

In previous reports, following the higher frequency of Hb S, Hb E was reported as the second most common abnormal hemoglobin in Turkey, with an incidence of 0.2% in Turks and 1.37% to 2.43% in Eti Turks[28,29,32,35]. Similar to our study, Arcasoy et al.[36] found Hb E in 0.11% of 3,600 patients with hematological findings. Instead of Hb E, Hb D was the second most common abnormal hemoglobin in our study, detected in 0.37% of the patients. Hb D is also a well known abnormal hemoglobin in our country, and can be found throughout Turkey[36,40]. Most of the abnormal hemoglobins reported previously were detected with starch eletrophoresis, and since Hb D moves like Hb S and Hb E moves like Hb O-Arab in starch electrophoresis, it is possible that some Hb S cases eported previously may be Hb D, and some Hb E cases may be Hb O-Arab[37]. But those abnormal hemoglobins can be separated more distinctly with HPLC[17,20,21]. A Hb E variant, Hb E-Saskatoon, has also been detected in Turkey by Prozorova-Zamani et al. and Gürgey et al.[35,41]. Mild hypochromic microcytic anemia is reported in heterozygous Hb E cases, as was present in our patients, whereas those hematologic features are normal in cases with Hb E-Saskatoon. But the HPLC program we used could not demonstrate Hb E-Saskatoon. For further differential diagnosis, mutation analysis such as DNA-sequencing may be necessary.

Hb O-Arab and Hb Lepore can be found with decreasing frequency in the Turkish population, and Hb Köln, Hb Hasharon, Hb Montgomery, Hb G-Copenhagen, and Hb Constant Spring detected in our study are among the rare abnormal hemoglobin variants. There are more than 25 rare hemoglobin variants reported in Turkish patients[40,42-49].

Since Turkey is located in a geographic area of the world where thalassemia syndromes and abnormal hemoglobins are common, and because our results also demonstrate that people in the Aegean region of Turkey have a wide spectrum of thalassemia syndromes and abnormal hemoglobins, it is important to identify an abnormality in the presymptomatic phase and give genetic counselling when necessary in our country.

REFERENCES

1. Lukens JN.The abnormal hemoglobins: general principles. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer J, Rodgers GM (eds). Wintrobe's Clinical Hematology (10th ed). B.altimore: Williams & Wilkins; 1999: 1329-1345
2. Çavdar AO, Arcasoy A. The incidence of thalassemia and abnormal hemoglobins in Turkey. Acta Haematol 1971; 45: 312-318.
3. Arcasoy A, Çavdar A. Electrophoretically detectable abnormal haemoglobins in healthy Turkish population. New Istanbul Contrib Clin Sci 1978; 12: 258-263
4. Aksoy M. Hemoglobinopathies in Turkey. Hemoglobin 1985; 9: 209-216.
5. Aksoy M, Kutlar A, Kutlar F, Dinçol G, Erdem S, Baştesbihci S. Survey on haemoglobin variants, beta thalassemia, glucose-6-phosphate dehydrogenase deficiency, and haptoglobin types in Turks from western Thrace. J Med Genet 1985; 22: 235-244.
6. Özsoylu Ş, Malik SA. Incidence of alpha-thalassemia in Turkey. Turk J Pediatr 1982; 24: 235-244.
7. Koçak R. Hemoglobinopathies in Turkey. In: Uultin O (ed). Lectures of the XIIIth Meeting of the International Society of Hematology. İstanbul; 1995: 64-65.
8. Öner R, Altay Ç, Gürgey A, et al. β-thalassemia in Turkey. Hemoglobin 1990; 14: 1-13.
9. Working Party of the General Haematology Members of the British Committee for Standards in Haematology Guideline: the laboratory diagnosis of haemoglobinopathies. Br J Haematol 1998; 101: 783-792
10. Waters HM, Howarth JE, Hyde K, et al. An evaluation of the Bio-Rad Variant Haemoglobin Testing System for the detection of haemoglobinopathies.Clin Lab Haematol 1998; 20: 31-40.
11. Kutlar F, Kutlar A, Huisman TH. Separation of normal and abnormal hemoglobin chains by reversed-phase high-performance liquid chromatography. J Chromatogr 1986; 357: 147-153.

12. Kutlar A, Özcan O, Brisco JT, Ansley MC, Huisman TH. The detection of hemoglobin variants by iso electrofocusing using EDTA-collected and filter paper-dried cord blood specimens. Am J Clin Pathol 1990; 94: 199-202.
13. Cronin EH, Normand C, Henthorn JS, Hickman M, Davies SC. Costing model for neonatal screening and diagnosis of haemoglobinopathies. Arch Dis Child Fetal Neonatal Ed 1988; 79: 161-167.
14. Tan GB, Aw TC, Dunstan RE, Lee SH. Evaluation of high performance liquid chromatography for routine estimation of haemoglobins A2 and F. J Clin Pathol 1993; 46: 852-856.
15. Campbell M, Henthorn JS, Davies SC. Evaluation of cation-exchange HPLC compared with isoelecric focusing for neonatal hemoglobinopathy screening. Clin Chem 1999; 45: 969-975.
16. Galanello R, Satta S, Pirroni MG, Travi M, Maccioni L. Globin chain synthesis analysis by high-performance liquid chromatography in the screening of thalassemia syndromes. Hemoglobin 1998; 22: 501-508.
17. Lorey F, Cunningham G, Shafer F, Lubin B, Vichinsky E. Universal screening for hemoglobinopathies using high-performance liquid chromatography: clinical result of 2.2 million screens. Eur J Hum Genet 1994; 2: 262-271.
18. Papadea C, Cate JC. Identification and quantification of hemoglobins A, F, S, and C by automated chromatography. Clin Chemist 1996; 42: 57-63.
19. Rahbar S, Asmerom Y. Rapid HPLC techniques for globin chain synthesis studies. Hemoglobin 1989; 13: 475-487.
20. Wilson JB, Headlee ME, Huisman TH. A new high-performance liquid chromatographic procedure for the separation and quantitation of various hemoglobin variants in adults and newborn babies. J Lab Clin Med 1983; 102: 174-186.
21. Riou J, Godart C, Hurtrel D, et al. Cation-exchange HPLC evaluated for presumptive identification of hemoglobin variants. Clin Chem 1997; 43: 34-39.
22. Weatherall DJ, Clegg JB. The Thalassemia Syndromes (3rd ed). Oxford: Blackwell Scientific Publications; 1981: 16-34.
23. Baysal E, Carver MF. The beta- and delta-thalassemia repository (eighth edition). Hemoglobin 1995; 19: 213-236.
24. İrken G, Olgun N, Ünsal E, Coşkunkan S, Altıngöz O, Çevik N. İzmir'in Çeşme Alaçatı ve Urla ilçelerinde beta talasemi taşıyıcılığı. Dokuz Eylül Üniversitesi Tıp Fakültesi Dergisi 1995; 9: 26-31.
25. Aydınok Y, Öztop Ş, Nişli G, Kavaklı K. Prevalence of beta-thalassaemia trait in 1124 students from Aegean region of Turkey. J Trop Pediatr 1997; 43: 184-185.
26. Aksoy M. The history of beta-thalassemia in Turkey. Turk J Pediatr 1991; 33: 195-197.
27. Bunn HF. Human hemoglobins: normal and abnormal. In: Nathan DG, Orkin SH (eds). Nathan and Oski's Hematology of Infancy and Childhood (5th ed). Philadelphia: WB Saunders Company; 1998: 729-761.
28. Aksoy M. Hemoglobin S and E in Turkish people. Nature 1961; 193: 786-789.
29. Altay Ç, Yetgin S, Özsoylu Ş, Kutsal A. Hemoglobin S and some other hemoglobinopathies in Eti-Turks. Hum Hered 1978; 28: 56-61.
30. Aluoch JR, Kılınç Y, Aksoy M, et al. Sickle cell anemia among Eti-Turks: haematological, clinical and genetic observations. Br J Haematol 1986; 64: 45-55.
31. Koçak R, Alparslan ZN, Ağrıdağ G, Başlamışlı F, Aksungur PD, Koltaş S. The frequency of anemia, iron deficiency, hemoglobin S and beta thalassemia in the south of Turkey. Eur J Epidemiol 1995; 11: 181-184.
32. Aksoy M, Bird GW, Lehmann H, Mourant AE, Thein H, Wickremasinghe H. Hb E in Asia. J Physiol 1955; 130: 56.
33. Aksoy M. The hemoglobin syndromes I. Hb E in Eti-Turks. Blood 1960; 15: 606.
34. Okçuoğlu A, Minnich V, Arcasoy A. A further eşample of thalassemia-hemoglobin E disease in Turkey. Acta Haematol 1965; 34: 354-360.
35. Prozorova-Zamani V, Özsoylu Ş, Aksoy M, et al. Hb E and Hb E-like variants in individuals from Turkey. Hemoglobin 1981; 5: 743-748.
36. Arcasoy A, Çavdar A, Cin Ş, et al. Türkiye'de thalassemia ve abnormal hemoglobin insidansı. Ankara: Tübitak Yayınları; 1978.
37. Gürgey A. Talasemi ve hemoglobinopatilerde yeni görüşler. Ankara: Tübitak Yayınları: 1986.
38. Aksoy M, Lehmann H. A further example of Hb D in a Turkish family. Trans R Soc Trop Med Hyg 1956; 50: 178-179.
39. Özsoylu Ş. Homozygous hemoglobin D-Punjab. Acta Haematol 1970; 43: 353-359.
40. Bircan I, Şişli S, Güven A, et al. Hemoglobinopathies in district of Antalya, Turkey. Pediatr Hematol Oncol 1993; 10: 289-291.
41. Gürgey A, Sipahioğlu M, Aksoy M. Compound heterozygosity for Hb E-Saskatoon or alpha 2 beta 2 (22) (B4) Glu-Lys and beta-thalassemia type IVS-I-6 (T-C). Hemoglobin 1990; 14: 449-451.
42. Efremov GD. Beta-delta beta-thalassemia and Hb lepore among Yugoslav, Bulgarian, Turkish and Albanian. Haematologica 1990; 75 (suppl): 31-41.
43. Altay Ç, Gürgey A, Huisman TH. Homozygosity for hemglobin O-Arab (alpha 2 beta 2 121 Glu-Lys). Hb O-Arab disease. Turk J Pediatr 1986; 28: 67-72.
44. Akar N. Klinik Moleküler Patolojiye Giriş, 2. Baskı, Ankara: AÜTF Antıp AŞ; 1999.
45. Arcasoy A. Hemoglobinopathies in Turkey. In: Roath S (ed). Current Views on Thalassemia. London: Harwood Academic Publishers; 1992.
46. Arcasoy A, Casey R, Lehmann H, Çavdar AO, Berki A. A new hemglobin from Turkey: Hb Ankara (beta 10 (A7) Ala-Asp). FEBS Lett 1974; 42: 121-123.
47. Altay Ç, Kutlar A, Wilson JB, Webber BB, Huisman TH. Hb P-Nilotic or alpha Z (beta delta) 2 in a Turkish family. Hemoglobin 1987; 11: 395-399.
48. Gürgey A, Altay Ç, Gu LH, et al. Hb Hakkari or alpha Z beta Z 31 (B13) Leu-Arg, a severely unstable hemoglobin variant associated with numerous intraerythroblastic inclusions and erythroid hyperplasia of the bone marrow. Hemoglobin 1995; 19: 165-172.
49. Gürgey A, Altay Ç. Hemoglobin Köln (beta 98 (FG 5) Vol-Met) in a Turkish child. Turk J Pediatr 1982; 24: 271-273.

The effect of hand splints on stereotypic hand behavior in Rett's syndrome

Gonca Bumin[1], Mine Uyanık[1], Hülya Kayıhan[1], Tülin Düger[1], Meral Topçu[2]

[1]School of Physical Therapy and Rehabilitation and [2]Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Bumin G, Uyanık M, Kayıhan H, Düger T, Topçu M. The effect of hand splits on stereotypic hand behavior in Rett's syndrome Turk J Pediatr 2002; 44: 25-29.

The purpose of this study was to examine the effect of hand splints and one elbow restraint on persistent stereotypic hand movements of four girls with Rett's syndrome.

Among the most characteristic features of Rett's syndrome are stereotypic hand wringing and loss of previously acquired functional hand skills. Hand splints and one elbow restraint were used in this study. The subject's stereotypic hand behavior and functional hand use were calculated from five-minute segmental video tape recordings. The study consisted of three phases: baseline, intervention, and withdrawal.

All subjects demonstrated a decrease in stereotypic hand behavior after the application of hand splints. Although splints showed a positive effect on hand movements in Rett's syndrome, they could also lead to other, undesirable, movements. Whether splints have a positive effect on the functional use of the hand should be investigated in more subjects.

Key words: Rett's syndrome, stereotypic movements, hand splints.

Rett's syndrome is a progressive encephalopathy in females that appears during the first 18 months of life. It was described in Vienna in 1966 by Andreas Rett who noticed a similar appearance and complex wringing motions in the hands of several of his female patients[1,2]. Rett's syndrome is a neurological disorder affecting predominantly females and characterized by regression, and loss of speech and purposeful hand use after six to 18 months of almost normal development. Postnatal microcephaly, hand dyspraxia, stereotypic hand movements, ataxia, severe mental retardation and abnormal breathing are among the most characteristic features[3-8]. Naganuma and Billingsley[9] reported that bilateral hand splints significantly decreased stereotypic hand behavior in three cases with Rett's syndrome and increased finger-feeding skills in one of the cases. Sharpe and Ottenbacher[10] used an elbow restraint splint to improve the functional hand use in a child with Rett's syndrome, and stated only minimal support for its use in improving finger-feeding skills.

Aron[11] used elbow splint in eight children with Rett's syndrome in 1990, and studied the self-injuring behavior of children with their hands. Results were very positive and indicated an increased socialization and interaction with the environment, and decreased hand-mouth movements and hand wringing behavior.

The aims of our study were to investigate the effects of hand splints and one elbow restraint in preventing seterotypical hand movements and to determine functional hand use in four cases.

Material and Methods

Four subjects who were diagnosed at the Pediatric Neurology Department of Hacettepe University Hospital were assessed. All of them were from Turkey. They fulfilled the Rett's syndrome diagnosis criteria. Pregnancy, birth and psychomotor development during the first year of life were normal. The mean age at onset was 14 ± 10.95 months. Mental retardation, stereotypic hand movements and loss of

purposeful manual skills were noted in all cases. All children's parents were positive regarding splint application.

Subject 1 was a nine-year-old girl whose stereotypic hand behaviors began at 12 months of age. Before the study, her hand behaviors consisted of pill-rolling, hand-to-mouth, squeezing and wringing. Her previous levels of functional hand use were very limited.

Subject 2 was a 10-year-old girl who began to show seterotypic hand behavior at about 2.5 years of age. At the start of this study, her hand movements consisted of hand wringing, squeezing, and hand-to-mouth; grinding of the teeth was also present. Before the intervention, she could finger-feed herself independently and drink a from a cup.

Subject 3 was an 11-year-old girl who began to show seterotypic hand movements at about eight months of age. Before this study, her hand movements consisted of hand-to-mouth, pill rolling, wringing, and squeezing; trunk swinging and teeth grinding were also present. She was successful in her functional hand movements.

Subject 4 was an eight-year-old girl who began to show stereotypic hand behavior at about six months of age. Before this study, her hand behaviors consisted of hand clapping, hand-to-mouth, squeezing and hand wringing; trunk swinging was also present. On examination of hand function, she was able to hold an object (biscuit) with help, but would drop it in one or two seconds; failing to eat it. Characteristics of subjects are given in Table I.

patient needed a pair of elbow splints to avoid self injury. These restraints permitted only a few degrees of elbow flexion (Fig. 2). Application of splints was gradually increased to five hours per day.

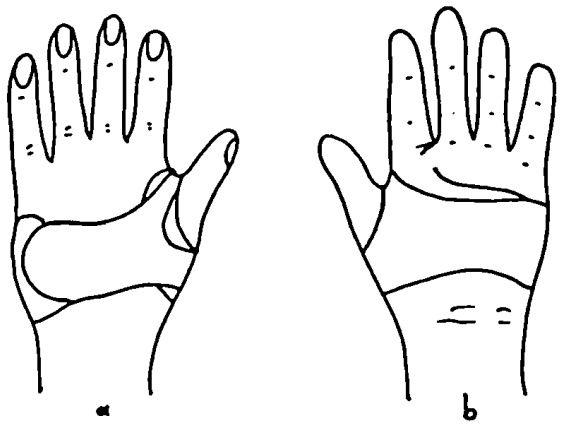


Fig. 1. Hand splint design a) dorsal view. b) palmar view.

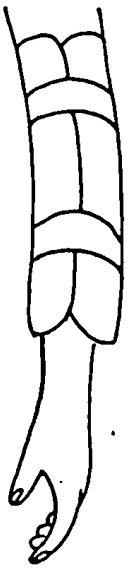


Fig. 2. Elbow restraint.

Table I. Characteristics of Subjects With Rett's Syndrome

Subjects	Age (years)	Age at onset (months)	Stereotypic behaviors before splints
1	9	12	Hand-to-mouth, pill rolling, wringing, squeezing
2	10	30	Hand-to-mouth, squeezing, wringing, tooth grinding
3	11	8	Hand-to-mouth, pill rolling, wringing, squeezing, trunk swinging, tooth grinding
4	8	6	Hand-to-mouth, pill rolling, clapping, squeezing, wringing, trunk swinging

Hand splints and elbow restraints were made at Hacettepe University School of Physical Therapy and Rehabilitation. The splints were fabricated of plastozoid materials. The hand splints resembled a cuff circling each palm and positioned the thumb out from the palm into abduction (Fig. 1). Three elbow restraints were constructed for nondominant arms but one

Procedure

Stereotypical hand behavior was defined as: 1) hands in contact with each other and moving in any way (washing, wringing or clapping); 2) hands not in contact but one or both hands engaged in repetitive squeezing, pill rolling, or writhing or hand-to-ear movements; 3) one or both hands in contact with the lips or tongue

aside from when self-feeding. In addition, anterior-posterior and lateral trunk movements and teeth grinding were observed. Stereotypical hand behaviors were scored separately, totaling number of each stereotypical hand movement observed.

Functional hand use was defined as the subject's hand successfully holding a cracker and moving it to her mouth. The definition excluded times when the subject held food but failed to bring it toward her mouth for more than two seconds. As long as the subject had one hand in the process of grasping a piece of food, and bringing it to her mouth or placing it in her mouth, functional hand use would be scored even if the other hand were engaged in stereotypic hand movements. For the functional use of the hands, every touch of the hand on the cracker was considered, and the number of successful results was scored. Successful finger feeding was defined as the patient's successfully picking up a cracker and putting it in her mouth. If the piece of cracker fell out of her mouth as she was chewing, it was still counted as a success. If a piece fell as the subject was holding it, was counted as a failure. Food which was carried to a patient's lips successfully but fell before entering her mouth was also counted as a failure. In addition, grasping and releasing movements were assessed.

The study consisted of three phases: baseline, intervention and withdrawal. During the baseline phase, subjects did not wear hand splints or elbow restraints, and could initiate functional hand use or perform stereotypical movements as they preferred while in the occupational therapy unit. During the intervention phase, subjects wore splints on both hands for increasingly longer periods of time. As during the baseline phase, hand behaviors (stereotypic or functional) were not interrupted nor was any attempt made to reinforce them during the intervention phase. A brief withdrawal phase (return to baseline condition) was conducted in all subjects at the completion of the study to identify any maintenance effects of the hand splints. Subjects did not wear their hand splints during the 3rd phase, and could perform whatever hand behavior they preferred. In keeping multiple-baseline requirements, hand splints were applied in a sequential fashion to each subject only after stability in a behavioral level or trend was established. After 10 days of the baseline phase, subjects began to wear their elbow restraints. Application was gradually increased to 10 days, after which subjects began to wear their hand splints. The subjects wore the splints for a month. In the withdrawal phase, subjects did not wear hand splints or elbow restraints.

Data Collection

Videotape recordings were made during the entire study: for 10 days of baseline phase, during the intervention phase of 10 days when only elbow restraints were used, during the second part of the intervention phase of 30 days when both elbow restraints and hand splints were used, and lastly during the withdrawal phase when both elbow restraints and hand splints were removed. During these recordings, subjects were positioned so that both of their hands were placed on the table. The number of stereotypical movements was obtained from the five minute video recordings made during the free time just before the assessment of functional hand use. During these recordings, subjects who permitted headphones listened to music. In the following five minutes, functional hand use was evaluated regarding ability to eat a cracker placed on the table. Thus, each time the camera obtained a 5 minute recording of both hands for each patient.

Results

In the first subject, the hand wringing movement disappeared with the use of the elbow restraint, but movements were observed to increase during the withdrawal phase. Hand squeezing appeared following the use of the splint and restraint, with a greater performance on the left hand. Pill rolling increased on the right with the elbow restraint, but on the left with the elbow restraint, it disappeared. However, with the use of splint and restraint, it appeared again, and at the with drawal phase, it continued at an increasing rate. Bringing of the left hand to mouth disappeared with both elbow restraint and hand splint, but the movement was observed to increase during withdrawal phase. The hand wringing movement appeared on the right with the use of elbow restraint and hand splints.

In the second subject, hand wringing movement decreased with the use of restraint, but with hand splint, it increased. The right hand

squeezing movement decreased during the intervention phase. The left hand squeezing movement when compared with the right decreased more at intervention phase; however, at withdrawal phase, it returned to that observed at the beginning phase. The movement of the right hand to the mouth decreased following the application of the elbow restraint. It decreased further with the application of restraint and splint, but returned to level observed at beginning phase during withdrawal phase. The bilateral hand-to-mouth movement disappeared during the intervention phase except on the 15th day, but it appeared again during the withdrawal phase.

In the third subject, pill rolling decreased at a greater rate on left and right sides during the application of elbow restraint in the intervention phase, and this decrease continued during withdrawal phase too. Bringing of the hand to mouth increased on the right with the application of the elbow restraint. The hand wringing movement on the right appeared during intervention, and continued at the same level during the withdrawal phase.

In the fourth subject, hand wringing movement disappeared during intervention and withdrawal phases. Pill rolling disappeared with the application of elbow restraint but it appeared again at withdrawal phase. Bringing of the hand to mouth disappeared on the left, and did not reappear during the withdrawal phase. Bringing of the hand to mouth decreased on the right during the withdrawal phase.

Discussion

In children with Rett's syndrome, many complicated pictures appear with a variety of clinical findings[12-17]. Hard work is necessary to investigate the effects of different rehabilitation approaches[18,21]. When considering the cases in our study, although stereotypical hand movements were observed in all, the most frequent movements observed were hand-to-mouth movement, unilateral hand squeezing and wringing motion. In addition, trunk movement was observed in two children and tooth grinding in one. The children did not show any significant reaction to splinting except unruliness on the first day. The elbow restraint applied on the nondominant side prevented hand-to-mouth movement on that side; however, withdrawal of the restraint resulted in a return to levels observed during initial stage. The hand wringing motion observed in four children decreased with splints in three children, but only one of them had a decrease at the withdrawal phase. Again, in three children the hand squeezing movement decreased with splinting. This decrease continued during the withdrawal stage. The pill rolling movement appearing in three children showed a decrease in two children. On withdrawal of the splint, the decrease continued with one child. Studies using the elbow restraint or the hand splints show a decrease in the stereotypical movements[1,9,10,11,20,21].

Based on our observations, the application of splints in general led to somewhat of a decrease in stereotypic movements. The decrease appeared with the hand-to-mouth motion unilaterally. Trunk movement was observed in two children. Following the application of the splint, this movement decreased in one child. This may be attributed to the synergic movement with the application of the splint. Tooth grinding was absent in two cases before the application of splints but it appeared after wards. This may reflect the children's reaction to the splingt. With the splint application a decrease was observed in other movements, too, but with the removal of the splint, many cases returned to levels observed during the initial stage. This may have been due to the withdrawal of the splint itself, to the short period of splint application. In further studies, more objective results could be obtained by extending the period of splint application. In addition, by separate application of splints, their effects could be compared.

Feeding activity and the functional use of the hand also showed an increase with splint application in one child. This continued during withdrawal phase. Stereotypical hand movements in this child showed a decrease. This result shows that the decrease in the stereotypical hand movements may results in an independence in daily living activities. The application of elbow restraint to the nondominant side also resulted in a decrease in stereotypical hand movements on the dominant side. The hand splints used on the dominant side produced a reflex-inhibitory posture, with the stabilization of these two effects. This was also found to be positive in increasing independence. Whether or not splints

have a positive effect on the function use of the hand should be investigated in more subjects. In conclusion, although splints showed a positive effect on the hand movements of children with Rett's syndrome, it can also lead to other, undesirable, movements. For this reason, the effect of the splints in decreasing the movements should be shown more precisely with many more subjects and by examining the causes bringing about undesirable effects. The use of other occupational therapy approaches together with the effect of the splints should also be investigated. Our researches on these subjects continues.

REFERENCES

1. Hagberg B, Aicardi J, Dias K, et al. A progressive syndrome of autism, dementia, ataxia and loss of purposeful hand use in girls: Rett's syndrome. Report of 35 cases. Ann Neurol 1983; 14: 471-479.
2. Shetty AK, Chatters R, Tilton AH, et al. Syndrome of microcephaly, mental retardation, and tracheoesophageal fistula associated with features of Rett syndrome. J Child Neurol 2000; 15: 61-63.
3. Hanks SB. The role of therapy in Rett syndrome. Am J Med Genet 1986; 24: 247-252.
4. Moodley M. Rett syndrome. S Afr Med J 1991; 80: 70-71.
5. Naidu SB. Rett syndorme. Indian Pediatr 1997; 64: 651-659.
6. Leonard H, Fyfe S, Dye D, et al. Family data in Rett syndrome: association with other genetic disorders. J Paediatr Child Health 2000; 36: 336-339.
7. Ghofrani M, Mahmoodian T. Rett syndrome. 2000; 67: 539-540.
8. Elian M, Rudolf N. EEG and respiration in Rett syndrome. Acta Neurol Scand 1991; 83: 123-128.
9. Naganuma G, Billingsley F. Effect of hand splints on stereotypic hand behavior of three girls with Rett syndrome. Phys Ther 1988; 68: 664-671.
10. Sharpe P, Ottenbacher K. Use of an elbow restraint to improve finger-feeding skills in a child with Rett syndrome. Am J Occup Ther 1990; 44: 328-332.
11. Aron M. The use and the effectiveness of elbow splints in the Rett syndrome. Brain Dev 1990; 12: 162-163.
12. Hanks SB. Motor disabilities in the Rett syndrome and physical therapy strategies. Brain Dev 1990; 12: 157-161.
13. Holm VA. Rett's syndrome: a progressive developmental disability in girls. J Dev Behav Pediatr 1985; 6: 32-36.
14. McIntosh RP, Simatos D, Weston HJ, et al. Rett syndrome: case reports and review. NZ Med J 1990; 103: 122-125.
15. Guidera KJ, Borrelli J, Raney E, et al. Orthopaedic manifestations of Rett syndrome. J Pediatr Orthop 1991; 11: 204-208.
16. Goodship S. Games and play in the Rett syndrome. Brain Dev 1990; 12: 164-168.
17. De Bona C, Zappella M, Hayek G, et al. Preserved speech variant is allelic of classic Rett syndrome. A Eur J Hum Genet 2000; 8: 325-330.
18. Triki C, Mhiri C. Rett's syndrome: report of 5 cases in Tunisia. Rev Neurol (Paris) 1999; 155: 955-959.
19. Hagberg B, Goutieres F, Hanefeld F, et al. Rett syndrome: criteria for inclusion and exclusion. Brain Dev 1985; 7: 372-373.
20. Kubas E. Use of splints to develop hand skills in a woman with Rett syndrome. Am J Occup Ther 1992; 46: 364-367.
21. Tuten H, Miedaner J. Effect of hand splints on stereotypic hand behavior in girls with Rett syndrome: a replication study. Phys Ther 1989; 69: 1099-1103.

Factors influencing breastfeeding for working mothers

Gonca Yılmaz[1], Berkan Gürakan[1], Seval Akgün[2], Namık Özbek[1]

Departments of [1]Pediatrics, and [2]Public Health, Başkent University Faculty of Medicine, Ankara, Turkey

SUMMARY: Yılmaz G, Gürakan B, Akgün S, Özbek N. Factors influencing breastfeeding for working mothers. Turk J Pediatr 2002; 44: 30-34.

In order to evaluate the relation between breastfeeding and working conditions a descriptive study was conducted on 301 working mothers. Seventy-seven percent of mothers breastfed their infants four months and longer, and the mean breastfeeding period was 6.2 ± 3.4 months. Forty-one percent of mothers started weaning before four months of age.

The multifactorial analysis of independent factors significantly influencing breastfeeding time were, in decreasing order of significance, breastfeeding conditions at work, maternal leave period, mother's smoking habit and the use of breastpump. For weaning period, these factors were the use of breastpump, breastfeeding leave at work and maternal leave period.

In conclusion, in order to support breastfeeding at work, maternal leave period must be prolonged, and breastfeeding conditions at work must be improved.

Key words: breastfeeding, working mothers, maternal employment.

Due to increasing urbanization and modernization, the number of working mothers has increased tremendously in our country in recent years. The mother's duties both at work and home may negatively affect her breastfeeding[1]. Mothers who work at private and government services are required to return to work at most eight weeks after delivery. This early return to work leaves breastfeeding mothers in a difficult position. Such women may start weaning earlier than the 4th month, and may prefer a formula diet more than expected.

The authors could not find a study in the literature investigation the relation between breastfeeding and maternal employment in Turkey. It appears that necessary precautions must be taken to ensure adequate breastfeeding by working mothers.

In this study the factors possibly influencing breastfeeding by working mothers were investigated through questionnaires.

Material and Methods

Working mothers, who applied to the Department of Pediatrics of Başkent University in Ankara between November 1998 and April 1999 and who had children aged 15 months to three years, were asked to complete a questionnaire about their breastfeeding history. Three hundred and one mothers out of 340 (88%) consented to answer the questionnaire. The questionnaire included mother's age; educational level; family income; mother's employement; maternal leave period; part-time vs. full-time employment; breastfeeding conditions at work; the use of breastpump; smoking habit; suport for breastfeeding by health care providers family and peers (bystanders); and the place of delivery. The mothers were divided into three groups according to their employment:

1. Independent jobs.
2. Government service.
3. Private service.

This study was presented as a poster in the 5th Regional Congress of Pediatric Societies of Turkish Speaking Countries with International Participation, Bishkek, 1999, and was awarded a group B award at the 12th Wyeth Scientific Research Competition.

The effect of these parameters on breastfeeding and weaning were investigated using unifactorial and multifactorial statistical analysis. For unifactorial analysis, Student's t test and chi-square test were used. For multifactorial analysis, regression was done. The significance was taken as $p = 0.05$.

Results

The age distribution, education and income of the study group are summarized in Table I.

Table I. Age Distribution, Education and Income of the Working Mothers

Age	≤ 25 years	141
	> 25 years	160
Education	University	158
	High school	143
Income	US $ 1000	43
	US $ 1000	258

Seventy-seven percent of the mothers breastfed their infants four months or longer. The mean breastfeeding time was 6.2 ± 3.4 months. Weaning before four months of age was determined in 40.5% of mothers.

One hundred and forty-one mothers were 25 years old or younger, and 160 were older than 25 years. The mean breastfeeding times were 6.0 ± 3.1 months and 6.4 ± 3.6 months, respectively. Weaning before four months of age was determined in 39% (55/141) and 41.9% (67/160) of mothers, respectively. According to the age groups, breastfeeding times and weaning periods were not significantly different (for breastfeeding, Student's t test, $t = 0.947$, $p = 0.344$; for weaning, chi-square test, $\chi^2 = 0.256$, $p = 0.613$).

One hundred and fifty-eight (52.5%) mothers had university degree and 143 (47.5%) were high school graduates. The mean breastfeeding times were 6.9 ± 3.4 months and 5.5 ± 3.2 months, respectively. Weaning before four months of age was determined in 37.3% (59/158) and 44.1% (63/143) of mothers, respectively. The mothers with a university degree breastfed their infants significantly longer than the others, whereas there was no difference between weaning periods of these groups (breastfeeding time: Student's t test, $t = 3.593$, $p = 0.000$; weaning period: chi-square test, $\chi^2 = 1.404$, $p = 0.236$).

Forty-three mothers (14.3%) had a monthly income of less than US $ 1000, whereas 258 (85.7%) earned more than $ 1000 monthly. Their mean breastfeeding times were 5.5 ± 3.4 months and 6.4 ± 3.4 months, respectively, with no significant difference found. However, 42% of mothers with a higher income and 63% of mothers with a lower income started weaning at the 4th month of age or later; this difference was statistically significant. Mothers with a high income started weaning significantly later than those with a lower income (breastfeeding: Student's t test, $t = 1.57$, $p = 0.116$; weaning: chi/square test $\chi^2 = 6.453$, $p = 0.011$).

Twenty-seven percent of mothers worked at private service, 18% worked independently, and 55% worked at government service. Table II shows the conditions at work according to employment groups. Part-time vs. full-time work, maternal leave period of longer than 16 weeks, and rate of breastfeeding at work are shown according to employment groups. With respect to employment groups, breastfeeding times and weaning periods were significantly different (Table III).

Table II. Conditions at Work According to Employment Gorups

Employment groups	n	Part-time (%)	> 16 weeks maternal leave	Breastfeeding at work
Independent jobs	53 (18%)	25 (49%)	35 (36%)	28 (47%)
2. Government	167 (55%)	18 (35%)	54 (56%)	21 (35%)
3. Private	81 (27%)	8 (16%)	6 (8%)	11 (18%)
Total	301 (100%)	51 (100%)	95 (100%)	60 (100%)
Statistics		Chi-square, $\chi^2 = 41.8$, $p = 0.000$. The 1st group is significantly different from both the 2nd and 3rd groups ($p < 0.05$).	Chi-square, $\chi^2 = 51.09$, $p = 0.000$. All groups are significantly different from each other ($p < 0.05$).	Chi-square $\chi^2 = 43.65$, $p = 0.000$. The 1st group is significantly different from both the 2nd and 3rd groups ($p < 0.05$).

Table III. Relation Between Breastfeeding times and Weaning Period According to Employment Groups

Employment groups	n	Mean breastfeeding time (months) + SD	Weaning before 4 months of age	Weaning at 4th month of age or later
1 Independent	53 (18%)	8.1 ± 3.6	16 (30.2%)	37 (69.8%)
2 Government	167 (55%)	6.3 ± 3.2	62 (37.1%)	105 (62.9%)
3 Private	81 (27%)	4.9 ± 3.0	44 (54.3%)	37 (45.7%)
Total	301	6.2 ± 3.4	122 (40.5%)	179 (59.5%)
Statistics	Analysis of variance, F = 15.572, p = 0.000. All groups are significantly different from each other (p < 0.05).		Chi-square test, χ^2 = 9.546, p = 0.008. The 3rd group is significantly different from the 1st and 2nd groups (p < 0.05).	

Mothers who could breastfeed at work breastfed significantly longer than those who could not (8.9 ± 2.8 months vs. 5.6 ± 3.2 months, Student's t test, t = 7.34, p = 0.000). Eighty-five percent of mothers who could not breastfeed at work and 53% of mothers who could breastfeed at work started weaning at the 4th month of age or later; this difference was statistically significant (chi-square test, χ^2 = 20.26, p = 0.000).

Forty-eight percent of mothers had a maternal leave period of 8 weeks or less, 20.9% had 9-16 weeks, and 31.6% had more than 16 weeks. Maternal leave period significantly influenced both breastfeeding time and weaning period (Table IV).

Seventy-one (24%) of the mothers were smokers. The mean breastfeeding time for smokers was 4.8 ± 3.1 months, and that for non-smokers was 6.7 ± 3.3 months. This difference was statistically significant (Student's t test, t = 4.252, p = 0.000). Forty-nine percent of smokers and 63% of non-smokers started weaning at the 4th month of age or later; this difference was statistically significant (chi-square test, χ^2 = 3.989, p = 0.046).

Table V shows the relation between the use of a breastpump and both breastfeeding time and weaning period.

Sixteen percent of mothers worked part-time. Part-time working mothers breastfed their infants significantly longer, and weaned significantly later than full-time working mothers (Table VI).

Table IV. Relation Between Breastfeeding Times and Weaning Period According to Maternal Leave Period

Maternal leave period	n	Mean breastfeeding time (months) + SD	Weaning before 4 months of age	Weaning at 4th month and later
≤ 8 weeks	143 (47.5%)	5.6 ± 3.6	73 (51%)	70 (49%)
9-16 weeks	63 (20.9%)	5.9 ± 2.6	18 (28.6%)	45 (71.4%)
> 16 weeks	95 (31.6%)	7.5 ± 3.2	31 (32.6%)	64 (67.4%)
Total	301	6.2 ± 3.9	122 (40.5%)	179 (59.5%)
Statistics	Analysis of variance, F = 9.769, p = 0.000. The > 16 wk group is significantly different from both ≤ 8 wk and 9-16 wk groups (p < 0.05).		Chi-square test, χ^2 = 12.761, p = 0.002. The ≤ 8 wk group is significantly different from both 9-16 wk and > 16 wk groups (p < 0.05).	

Table V. Relation Between Breastfeeding Times and Weaning Period According to the Use of Breastpump

	n	Mean breastfeeding time (months) + SD	Weaning before 4 months of age	Weaning at 4th month or later
Without breast pump	124	4.3 ± 3.4	88 (71%)	36 (29%)
9-16 weeks	177	7.6 ± 2.9	34 (19.2%)	143 (80.8%)
Statistics	Student's t test, t = 9.392, p = 0.000		Chi square test, χ^2 = 81.043, p = 0.000	

Table VI. Relation Between Breastfeeding Times and Weaning Period According to Part-time vs. Full-time Work

	n	Mean breastfeeding time (months) + SD	Weaning before 4 months of age	Weaning at 4th month and later
Full-time	250 (84%)	5.9 ± 3.4	109 (43.6%)	141 (56.4%)
Part-time	51 (16%)	7.8 ± 2.9	13 (25.5%)	38 (74.5%)
Total	301		122 (40.5%)	179 (59.5%)
Statistics	Student's t test, t = 9.392, p = 0.000		Chi square test, χ^2 = 81.043, p = 0.000	

Mothers getting breastfeeding leave at work had a mean breastfeeding time of 7.5 ± 2.8 months, whereas those without breastfeeding leave had a mean of 4 ± 3.5 months. This difference was found to be statistically significant (Student's t test, t = 6.907, p = 0.000). The rates of early weaning for mothers with and without breastfeeding leave were also significantly different (37% versus 81%, respectively) (chi-square test, χ^2 = 61.60, p = 0.000).

The mean breastfeeding time of mothers who received support for breastfeeding by "bystanders" was significantly prolonged when compared to those who did not (8.9 ± 4.6 months vs. 4.5 ± 4.1 months, Student's t test, t = 4.37, p = 0.000). The supported mothers also started weaning at a significantly later time than the unsupported ones (19% versus 57%, respectively) (chi-square test, χ^2 = 28.77, p = 0.000).

The following factors were included in the multifactorial analysis: mother's age, educational level, family income, mother's employment, maternal leave period, part-time vs. full-time employment, breastfeeding conditions at work, the use of a breastpump, smoking habit, support for breastfeeding "bystanders", and the place of delivery.

The multifactorial analysis of independent factors significantly influencing breastfeeding time were, in decreasing order of significance, breastfeeding conditions at work (p = 0.000), maternal leave period (p = 0.000), mother's smoking habit (p = 0.001), the use of breastpump (p = 0.008), and part-time work (p = 0.009).

The multifactorial analysis of independent factors significantly influencing weaning period were, in decreasing order of significance, the use of a breastpump (p = 0.000), breastfeeding leave at work (p = 0.000), maternal leave (p = 0.003), and support for breastfeeding by "bystanders" (p = 0.02).

Discussion

Mothers are active both at home and at work, and are unable to receive the necessary personal support due to fragmented city life in Turkey. Furthermore, in our country the mothers working at private and government services receive a maternal leave of only six weeks after normal delivery, and eight weeks after cesarean section. This maternal leave is insufficient for the exclusive breastfeeding period to continue. Our study demonstrated that 47.5% of mothers had maternal leave of eight weeks or less, and with a shortened maternal leave, the weaning period started significantly earlier and breastfeeding continued for a significantly shorter period. We also showed timing of return to work had a more negative effect than the number of hours worked. The 1988 National Mother and Child Health Study in the United States determined that working did not affect the decision by the mother to breastfeed, but that mothers returning to work early breastfed their infants for a shorter period and started weaning earlier than four to six months[2,3].

In this study we determined that 40.5% of mothers started weaning before four months of age. This figure is much lower than the rate determined by the Turkish Demographic and Health Survey[4,5]. The higher educational level of our patients versus the average seen in Turkey may have resulted in their weaning later. It has also been shown by others that as the length of maternal education increased, so did her breastfeeding time[3]. Additionally, our study group had a high ratio of high-income families (85.7% > $ 1000 monthly income) and older mother (47% > 30 years of age). Several studies have proven that higher income and older age of mother led to a higher rate of using breastmilk[3,6]. However, the 1993 Turkish Demographic and Health Survey revealed that breastmilk insufficiency and weaning were closely related to living area and maternal

education. It also showed that in urban areas where mothers had higher education and income, the ratio of breastfed children was low, early weaning was common, and there was a high rate of abandoning breastfeeding due to insufficient breastmilk[1].

The Baby-Friendly Hospital Initiative (BFHI) was launched by WHO and UNICEF in 1991 at the International Pediatric Association Conference in Ankara. Our results suggest that the decisions made in this conference with the objective of enabling mothers to breast feed exclusively for around six months and to continue breastfeeding for as long as the mother and baby want might have had positive impressions upon the medical staff of our hospital.

Mothers with independent employment had flexibility in terms of the time of returning to work and working hours; therefore, they had longer maternal leave, a higher rate of part-time work, and a higher rate of breastfeeding at work. These mothers breastfed their infants for a longer period of time than seen in the other employment groups. The shortest breastfeeding and highest rate of early weaning was determined in mothers working in private service. Higher pressure induced by the private service on the mother might provoke early cessation of breastfeeding and early weaning. Increasing control on the mother by her working environment may diminish the baby-mother relationship, and thus decrease breastfeeding. Our study revealed that breastfeeding conditions at work was the strongest parameter independently influencing breastfeeding time.

We also showed that mothers using a breastpump breastfed their infants longer and started weaning later than those who did not use breastpumps. A breastpump is a supplementary instrument that the mother uses to replace the missed feedings during work hours. It significantly prolongs the breastfeeding period[7,8].

A considerable percentage of our mothers (24%) were smokers. Smoking mothers breastfed significantly shorter and started weaning earlier than the non-smoking mothers. These results were comparable to previous reports in literature[9,10].

In conclusion, despite the lower mean breastfeeding time (6.2 ± 3.4 months) when compared to the mean of Turkey (12 months), our study group had a higher rate of late weaning compared to figures in the literature and the average of Turkey. This was thought to be related to the higher level of education and income, and to the higher rate of mothers above 30 years of age. Better conditions at work for breastfeeding, longer maternal leave period, and increased use of maternal leave without a salary will obviously support breastfeeding for working mothers.

REFERENCES

1. Taşkın L. Major barriers to breastfeeding: education and urbanization. Nüfusbilim Dergisi Turkish Journal of Population Studies 1998; 20: 31-41.
2. Visness CM, Kennedy KI. Maternal employment and breast-feeding: findings from the 1988 National Maternal and Infant Health Survey. Am J Public Health 1997; 87: 945-950.
3. Kurinij N, Shiono PH, Ezrine SF, Rhoads GG. Does maternal employment affect breast-feeding? Am J Public Health 1989; 79: 1247-1250.
4. Sağlık Bakanlığı (Türkiye), Hacettepe University Institute of Population and Macro International Inc. 1994, Turkish Demographic and Health Survey 1993, Ankara, Turkey.
5. Sağlık Bakanlığı (Türkiye), Hacettepe University Institute of Population and Macro International Inc. 1998, Turkish Demographic and Health Survey 1998, Preliminary Report 1998, Ankara, Turkey.
6. Ryan AS, Martinez GA. Breastfeeding and the working mother: a profile. Pediatrics 1989; 89: 524-531.
7. Bliss MC, Wilkie J, Acredolo C, Berman S, Tebb KP. The effect of discharge pack formula and breastpumps on breastfeeding duration and choice of infant feeding method. Birth 1997; 24: 90-97.
8. Auerbach KG, Guss E. Maternal employment and breastfeeding. A study of 567 women's experiences. Am J Dis Child 1984; 138: 958-960.
9. Sayers G, Thornton L, Corcoran R, Burke M. Influences on breastfeeding initiation and duration. Ir J Med Sci 1995; 164: 281-285.
10. Riva E, Banderali G, Agostoni C, Silano M, Radaelli G, Giovannini M. Factors associated with initiation and duration of breastfeeding in Italy. Acta Paediatr 1999; 88: 411-415.

Nutritional status of children with cancer and its effects on survival

Nilgün Yarış[1], Canan Akyüz[1], Turgay Coşkun[2], Tezer Kutluk[1], Münevver Büyükpamukçu[1]

Units of [1]Oncology, and [2]Nutrition and Metabolism, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Yarış N, Akyüz C, Coşkun T, Kutluk T, Büyükpamukçu M. Nutritional status of children with cancer and its effects on survival. Turk J Pediatr 2002; 44: 35-39.

In the present study we aimed to determine the nutritional status of our patients and to assess its relationship with survival. The nutritional status of 47 patients with cancer was evaluated at diagnosis, three months after initiation of the treatment and at the end of therapy. Weight for height, height for age, and weight for age of children were expressed as percent of standard. Values for each nutritional index were converted into standard deviation (Z) scores. Three-year overall survival (OS) and event-free survival (EFS) rates of patients were determined according to their nutritional status. The overall prevalence of malnutrition at diagnosis was 29.8%. Three months later the malnutrition ratio reached 38.3% and then decreased again to 18.5% at the end of the therapy. Although the prevalence of malnutrition at the third month of treatment was significantly higher from the prevalence at diagnosis (p: 0.001) and at the end of the therapy (p: 0.009), the mean Z scores of the nutritional indexes before and during the treatment were not significantly different. The survival rates of malnourished patients were not different from those of well nourished patients. In conclusion, malnutrition is one of the main problems in children with cancer; hovewer, nutritional status has no effect on survival.

Key words: malnutrition, cancer, children, survival, prognosis.

Malnutrition may be present at diagnosis or develop during the treatment of cancer. Its incidence reaches as high as 40%[1-4]. Malnutrition in cancer patients results from a combination of metabolic abnormalities and decreased food intake. Increased metabolic rates, increased nutritional and caloric requirements, chronic anemia, and protein loss may contribute to development of malnutrition[5-8].

Metabolic factors may include altered protein and carbohydrate metabolism, selective use and redistribution of nutrients by tumor cells, alteration of total lipid mobilization resulting in elevated free fatty acids, and abnormal synthesis of peptides which may distrub normal enzyme activity. The aggressive multimodal therapy (directly or indirectly) and/or the neoplastic process itself are responsible for the metabolic differences observed in cancer patients[5,6,9].

Decreased food intake mainly results from anorexia. Anorexia may be related to both psychological factors and to release of chemicals by the tumor or by the host immune system[10,11]. Patients with cancer have already been consuming less than that recommended and less than their own previous intakes. In addition, decreased taste, mucositis and stomatitis, emesis, intractable diarrhea and malabsorption, dysphagia, pain and psychological factors may interfere with the maintenance of adequate oral intake[5,6,12-15].

Malnutrition is an important health problem in children. It may be suggested that malnutrition causes an increase in morbidity and mortality when it occurs in a patient with cancer. In the present study we aimed to determine the nutritional status of our patients with cancer and to assess its relationship with survival.

Material and Methods

From December 1996 to July 1997, 47 (20 female, 27 male) newly diagnosed patients with cancer were evaluated prospectively. The mean age of patients was 107 ± 49 months (range: 36-190) The diagnosis of patients were lymphoma in 25 (53.2%), brain tumor in five (10.6%), soft tissue

and bone sarcoma in seven (14.9%), and other solid tumor in 10 (21.3%) patients. The stage of disease was early stage (stages I and II) in 14 patients (29.8%) and late stage (stages III and IV) in 33 patients (70.2%). Nutritional status of patients was evaluated at diagnosis and three months after initiation of the treatment in all cases and at the end of therapy in 27 patients. Height was measured to the nearest 0.1 cm, and weight to the nearest 0.1 kg. Measurements were made by one observer according to the standard techniques. The serum albumin, prealbumin and transferrin levels of patients were measured at diagnosis, and during and at the end of the treatment. The normal levels of albumin, prealbumin and transferrin levels were considered as 3.2 g/dl, 0.2-0.34 g/dl and 212-360 mg/dl, respectively.

Assessment of nutritional status was performed using recommended techniques[16,17]. Weight for height (WFH), height for age (HFA), and weight for age (WFA) of children were expressed as percent of standard. Standard was chosen as median value of age and sex-matched reference population[18]. Nutritional status of patients was classified according to Waterlow[19] and Gomez[20] systems. Values for each nutritional index were converted into standard deviation (Z) scores. Standard deviation scores of WFH could not be calculated in 12 patients whose heights were outside the range of the WFH table. The mean values of Z scores were used to compare anthropometric index of patients before and during the treatment. No comparison of Z scores obtained at the end of therapy was done because the treatment periods and consequently time of last evaluation varied widely according to diagnosis and stage of the patients.

The median follow-up time was 39 months (range: 2-44). The follow-up time was short in some patients because of death. The patients diagnosed as malnourished were treated with administration of a high calorie and protein diet, and vitamin and mineral supplements. Total parenteral nutrition was not routinely given in those children. To analyze the effect of nutritional status on prognosis, three-year overall survival (OS) and event-free survival (EFS) rates of patients were determined according to their nutritional status. The patients with lymphoma were also analyzed separately, since they were the largest and unique group.

Student's t test was used for comparison of mean values. Frequency of malnutrition before, during and at the end of the therapy was compared using the chi-square and Fisher's exact probability tests. Frequency of malnutrition according to the patient's characteristics was also compared using the chi-square and Fisher's exact probability tests. Kaplan-Meier method was used for survival analysis, and curves were compared with long-rank test[21].

Results

The overall prevalence of malnutrition at diagnosis was 29.8% (14 patients). While 16 (34%) patients lost 2% to 17.2% (mean: 7.4 ± 5.2%) of their initial weight throughout three months, conversely 10 patients (20.0%) gained weight. Three months after initiation of the treatment the nutritional status of 14 patients malnourished at diagnosis was as follows: malnutrition completey resolved in three patients, the degree was decreased in three patients, malnutrition was still present to the same degree in five patients and the degree was increased in three patients. Seven patients who were well nourished at diagnosis became malnourished by the third month of the treatment. Malnutrition ratio reached 38.3% after three months of treatment, and decreased again to 18.5% at the end of the therapy. The degree and type of malnutrition at diagnosis, three months later and at the end of the therapy are shown in Table IA-IB. Although the prevalence of malnutrition at the third month of the treatment was significantly higher than that at diagnosis (p: 0.001) and that at the end of the therapy (p: 0.009), the mean Z scores of the HFA, WFA, WFH before and during the treatment were not significantly different (Table II). We could not find any relationship between the nutritional status and sex or age of patients, or type of cancer at any time of the evaluation. Ten of 14, 15 of 18 and four of five malnourished patients had advanced stage disease at diagnosis, at the third month, and at the end of therapy respectively. However, the difference between the patients with early and advanced stage disease in malnutrition ratio was not statistically significant. There was no relationship between the serum albumin levels and nutritional status. The prealbumin and transferrin levels of patients were significantly related with presence of malnutrition on admission and during the treatment (p: 0.05 for prealbumin, p: 0.001 for transferrin at diagnosis;.

p: 0.006 for prealbumin, p: 0.02 for transferrin at third month of the treatment). There was no relationship between the serum prealbumin and transferrin levels and nutritional status of patients at the end of the treatment.

found in OS and EFS rates between patients with malnutrition at diagnosis which improved partially (degree of malnutrition improved) or completely at the third months, patients with malnutrition at diagnosis who remained

Table I A-B. Nutritional Status of Patients

Table I A

Type of malnutrition	At diagnosis	At third month	At the end of therapy***
A* Wasted	9 (19.2%)	13 (27.7%)	3 (11.1%)
C* Wasted + stunded	4 (8.5%)	4 (8.5%)	1 (3.7%)
Stunted	1 (2.1%)	1 (2.1%)	1 (3.7%)
Total malnutrition	14 (29.8%)	18 (38.3%)***	5 (18.5%)
Normal	33 (70.2%)	29 (61.7%)	22 (81.5%)

Table I B

Degree of malnutrition	At diagnosis	At third month	At the end of therapy***
0°, Normal	33 (70.2%)	29 (61.7%)	22 (81.5%)
1°, Mild	6 (12.8%)	9 (19.23%)	5 (18.5%)
2°, Moderate	6 (12.8%)	7 (14.9%)	0
3°, Severe	2 (4.2%)	2 (4.2%)	0

* A: Acute, C: Chronic.

** The prevalence of malnutrition at the third month was significantly higher than that at diagnosis (p: 0.001) and at the end of therapy (p: 0.009).

*** 27 patients were evaluated atthe end of therapy.

Table II. Z Scores of Anthropometric Indexes of Patients

Z scores	At diagnosis	At third month
Z score for WFA	-0.63 ± 0.99 (-3.47; + 0.78)	-0.72 ± 0.93 (-3.30; + 1.26)
Z score for HFA	-0,55 ± 1.10 (-2.90; + 1.60)	-0.52 ± 1.14 (-3.07; + 1.50)
Z score for WFH	-0.23 ± 1.34 (-2.60; + 3.4)	-0.22 ± 1.22 (-2.97; + 1.9)

* mean ± SD (minimum; maximum).

WFA: Weight for age.
HFA : Height for age.
WFH: Weight for height.

Three-year OS and EFS rates were 70% and 65%, respectively, for the whole group. There was no significant difference between the survival rates of malnourished and well nourished patients neither at diagnosis nor during or at the end of the therapy (Table III). When the patients were separated into two groups according to the stage of their disease (early or late stage), the OS and EFS rates of malnourished and well nourished patients were also not significantly different at any time of the evaluation. No significant difference was

malnourished to the same or a higher degree at the third month, and patients who were well nourished at diagnosis but became malnourished by the third month. When patients with lymphoma were evaluated separately, no difference was found in OS and EFS rates of patients according to their nutritional status at diagnosis, nor during or at the end of the treatment.

Table III. Three Year Overall (OS) and Event-Free Survival (EFS) Rates of Patients According to Nutritional Status

	Patients with malnutrition			Patients without malnutrition		
	No. of Pts.	OS (%)	EFS (%)	No. of Pts.	OS (%)	EFS (%)
At diagnosis	14	69	83	33	71	97
At third month	18	70	83	29	70	96

Discussion

Malnutrition is one of the main problems in children with cancer. Malnutrition is reported to be relatively uncommon at diagnosis[1,2,5,22]. We found a significantly higher incidence during the treatment, although we found its incident as high as 29.8% at diagnosis. This result

suggests that the metabolic changes resulting from the therapy and the decreased oral intake due to chemotherapy complications such as mucositis and vomiting were the major reasons for malnutrition. It is reported that malnutrition is more frequent with advanced stage disease and recurrent tumor. Children with advanced stage Wilms' tumor and Ewing's sarcoma have a particular risk for malnutrition[1,2,22-24]. We could not find any relationship between the stage of disease and nutritional status of patients, nor between the type of cancer and nutritional status of patient, perhaps because of the small number of patients in each cancer group.

Malnutrition increaes morbidity and mortality in pediatric cancer cases. Organ systems such as hematological system, gastrointestinal system and immune system are affected by malnutrition[24,25]. Malnutrition immunocompromises the host and increases his/her susceptibility to infections[24-26]. Malnutrition is significantly associated with increased physiological instability and quantity of care[27]. Chemotherapeutic toxicity is correlated with poor nutritional status of the patients. Nutritional support improves treatment tolerance and prevents treatment delay and dose reduction[23,25,28-30]. Besides these, the pharmacokinetics of several anticancer drugs have been shown to vary with changes in body fat, consequently nutritional status may change the effects of drugs on tumor tissue[31,32].

Although there are some reports showing that nutritional status has a prognostic effect on children with cancer, we could not find any relationship between the nutritional status and survival. It was reported that risk of death was high in undernourished patients with ALL in remission-induction phase[33]. Similarly a significant influence of weight-for-height standard deviation scores on relapse time of newly diagnosed ALL patients was observed[32]. It was reported that well nourished patients with stage IV neuroblastoma had better one-year event-free and overall survival rates than malnourished patients[23]. However, van Eys[34] observed only slightly improved survival in well nourished patients with stage IV neuroblastoma, and showed that nutritional support had no effect on survival. In another study children with neuroblastoma and Wilms' tumor were compared. In the Wilms' tumor group the anthropometric measurements correlated with subsequent development of complications, although the neuroblastoma group had significantly lower anthropometric measurements[35]. Donaldson et al.[22] showed that malnourished patients with localized disease had a poorer survival rate; however, survival of patients with advanced tumors did not differ significantly according to nutritional status. In our study most of the patients with malnutrition had advanced stage disease, although it was not statistically significant. There are a lot of factors affecting prognosis and survival. Characteristics of the host and tumor which affec survival may also affect the nutritional status of patients. We could suggest that the type of cancer, stage of disease, and specific molecular and genetic factors might be more important than nutrition in determing survival of our patients.

In conclusion, malnutrition is an important problem in children with cancer. The highest ratio is obtained during the treatment. It can be suggested that cancer treatment and its complications are the major factors causing malnutrition. Although it is known that malnutrition causes some secondary problems and increases morbidity, we could not determine an effect on survival.

REFERENCES

1. Van Eys J. Malnutrition in children with cancer. Cancer 1979; 43: 2030-2035.
2. Carter P, Carr D, Van Eys J, Coody D. Nutritional parameters in children with cancer. J Am Diet Assoc 1983; 82: 616-622.
3. Smith DE, Stevens MC, Booth IW. Malnutrition at diagnosis of malignancy in childhood common but mostly missed. Eur J Pediatr 1991; 150: 318-322.
4. Oğuz A, Karadeniz C, Pelit M, Hasanoğlu A. Arm anthropometry in evaluation of malnutrition in children with cancer. Pediatr Hematol Oncol 1999; 16: 35-41.
5. Van Eys J. Pathology of undernutrition in the child with cancer. Cancer 1986; 58: 1874-1880.
6. Vigano A, Watanabe S, Bruera E. Anorexia and cachexia in advanced cancer patients. Cancer Surv 1994; 21: 99-115.
7. Hyltander A, Drott C, Körner U, Sandström R, Lundholm K. Elevated energy expenditure in cancer patients with solid tumours. Eur J Cancer 1991; 27: 9-15.
8. Young V. Energy metabolism and requirements in cancer patient. Cancer Res 1977; 37: 2336-2347.
9. Lazo PA. Tumour-host metabolic interaction and cachexia. FEBS 1985; 187: 189-192.
10. Beck S, Mulligan H, Tisdale M. Lypolytic factors associated with murine and human cancer cachexia. J National Cancer Inst 1990; 82: 1922-1926.
11. Gelin J, Moldawer LL, Lonnroth C, Sherry B, Chizzonite R, Lundholm K. Role of endogenous tumor necrosis factor alpha and interleukin 1 for experimental tumor growth and the development of cancer cachexia. Cancer Res 1991; 51: 415-421.

12. Ohnuma T, Holland JF. Nutritional consequences of cancer chemotherapy and immunotherapy. Cancer Res 1977; 37: 2395-2406.

13. Donaldson SS. Nutritional consequences of radiotherapy. Cancer Res 1977; 37: 2407-2413.

14. Hyams JS, Batrus CL, Grand RJ, Sallan SE. Cancer chemotherapy-induced lactose malabsorption in children. Cancer 1982; 49: 646-650.

15. Bernstein IL. Physiological and psychological mechanisms of anorexia. Cancer Res 1982; 715s-720s.

16. Jeejeebhoy KN, Meguid M. Assessment of nutritional status in the oncological patient. Surg Clin North Am 1986; 66: 1077-1090.

17. Motil KJ. Sensitive measures of nutritional status in children in hospital and in the field. Int J Cancer Suppl 1998; 11: 2-9.

18. World Health Organization. Measuring change in nutritional status. Geneva: WHO; 1983.

19. Waterlow JC. Classification and definition of protein calorie malnutrition. Br J Med 1972; 3: 566-569.

20. Gomez F, Galvan RR, Frank S, et al. Mortality in second and third degree malnutrition. J Trop Pediatr 1956; 2: 77-81.

21. Peto P, Pike MC, Armitage P, et al. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. Br J Cancer 1977; 35: 1-39.

22. Donaldson SS, Wesley MN, De Wys W, Suskind RM, Jaffe N, Van Eys J. A study of the nutritional status of pediatric cancer patients. Am J Dis Child 1981; 135: 1107-1112.

23. Rickard KA, Detamore CM, Coates TD, et al. Effect of nutrition staging on treatment delays and outcome in stage IV neuroblastoma. Cancer 1983; 52: 587-598.

24. Coates T, Rickard RD, Grosfeld JL, Wetman RM. Nutritional support of children with neoplastic diseases. Surg Clin North Am 1986; 66: 1197-1211.

25. Rickard KA, Coates TD, Grosfeld JL, Weetman RM, Baehner RL. The value of nutrition support in children with cancer. Cancer 1986; 58: 1904-1910.

26. Taj MM, Pearson AD, Mumford DB, Price L. Effect of nutritional status on the incidence of infection in children with cancer. Pediatr Hematol Oncol 1993; 10: 283-287.

27. Pollack MM, Ruttiman UE, Wiley JS. Nutritional depletions in critically ill children: associations with physiologic instability and increased quantity of care. J Parenter Enteral Nutr JPEN 1985; 9: 309-313.

28. Obama M, Cagir A, Van Eys. J. Nutritional status and anthracycline cardiotoxicity in children. South Med J 1983; 76: 557-578.

29. Shamberger RC, Pizzo PA, Goodgame JT Jr et al. The effect of total parenteral nutrition on chemotherapy-induced myelosuppression. A randomized study. Am J Med 1983; 74: 40-48.

30. Tyc VL, Vallelunga L, Mahoney S, Smith B, Mulhern R. Nutritional and treatment-related characteristics of paediatric oncology patients referred or not referred for nutritional support. Med Pediatr Oncol 1995; 25: 379-388.

31. Zuccaro P, Guandalini S, Pafici R, et al. Fat body mass and pharmacokinetics of oral 6-mercaptopurine in children with acute lymphoblastic leukemia. Ther Drug Monit 1991; 13: 37-41.

32. Reilly JJ, Odame I, McCooll JH, et al. Does weight for height have prognostic significance in children with acute lymphoblastic leukemia? Am J Pediatr Hematol Oncol 1994; 16: 225-230.

33. Mejia-Arangure JM, Fajardo-Gutierrez A, Reyes-Ruiz NI, et al. Malnutrition in childhood lymphoblastic leukemia: a predictor of early mortality during the induction-to-remission phase of the treatment. Arch Med Res 1990; 30: 150-153.

34. Van Eys J. Effect of nutritional status on response to therapy. Cancer Res 1982: 42 (Suppl): 747s-753s.

35. Lahorra JM, Ginn-Pease ME, King DR. The prognostic significance of basic anthropometric data in children with advanced solid tumors. Nutr Cancer 1989; 12: 361-369.

The effects of gemfibrozil on hyperlipidemia in children with persistent nephrotic syndrome

Mithat Büyükçelik[1], Ali Anarat[1], Aysun Karabay Bayazıt[1], Aytül Noyan[1], Ahmet Özel[3] Rüksan Anarat[4], Handan Aydıngülü[2], Nurten Dikmen[2]

Departments of [1]Pediatric Nephrology, Çukurova University Faculty of Medicine, [2]Biochemistry Laboratory, [3]Department of Pediatrics, Selçuk University Faculty of Medicine, Konya, and [4]Düzen Laboratory, Adana, Turkey

SUMMARY: Büyükçelik M, Anarat A, Karabay Bayazıt A, Noyan A, Özel A, Anarat R, Aydıngülü H, Dikmen N. The effects of gemfibrozil on hyperlipidemia in children with persistent nephrotic syndrome. Turk J Pediatr 2002; 44: 40-44.

Persistent nephrotic syndrome is frequently accompanied by severe hyperlipidemia, and this may pose a substantial risk for cardiovascular disease. Lipid-lowering drugs are prescribed by many nephrologists for adult patients but rarely for nephrotic children. The present investigation was designed to evaluate the safety and efficacy of gemfibrozil in nephrotic children. Eight girls and four boys aged from 5 to 17 years were enrolled in this study. They were all steroid and immunosuppressive resistant patients with nephrotic range proteinuria. Placebo was administered to five patients and gemfibrozil was administered to seven patients for four months. Blood samples were taken for the determination of cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), BUN, serum creatinine (S_{cr}), ALT, AST, CPK, apolipoprotein A (apo A), apoliporotein B (apo B), and serum albumin levels during the initial and subsequent examinations. At the end of the fourth month, gemfibrozil reduced total cholesterol by 34%, LDL by 30%, apo B by 21% and triglycerides by 53% ($p < 0.05$). HDL cholesterol and apo A levels were not significantly altered. Renal function and urine protein excretion were not affected by gemfibrozil. In this study gemfibrozil therapy had no side effects and had favorable effects on the lipoprotein profile of nephrotic patients.

Key words: antilipid therapy, childhood, gemfibrozil, nephrotic syndrome.

Corticosteroids and cytotoxic drugs have dramatically improved the outlook for children with nephrotic syndrome, but resistance to treatment has raised interest in the short-and long-term effects of some important biochemical abnormalities that are secondary to primary disease. At this stage, attention is focused on hyperlipidemia, which is an important component of nephrotic syndrome[1]. This study was undertaken to investigate the safety and efficacy of gemfibrozil, one of the lipid-lowering agents, in nephrotic children resistant to therapy.

Material and Methods

Patients: This study took place in the Department of Pediatric Nephrology, Çukurova University Faculty of Medicine. Twelve patients between the ages of five and 17 (8 girls, 4 boys) were included in the study. All patients were resistant to prednisone, cyclophosphamide and dipyridamole treatment, subsequently received cyclosporine or chlorambucil, and showed proteinuria in the nephrotic limit (> 40 mg/m^2/hour). The patients were randomly divided into two groups. The average ages of Group I and II were 10.0 ± 3.9 years and 13.8 ± 2.5 years, respectively; the difference between these ages was not statistically significant. During the study period, the patients in Group I were treated with gemfibrozil which reduces the plasma cholesterol levels. The patients in Group II received a placebo instead. Group I consisted of seven children. These patients had received clinical treatment and follow-up for nephrotic syndrome for six to 36 months. One patient received levamisole as a third course of treatment. Renal biopsies were diagnosed as membranoproliferative glomerulo-nephritis in all patients. The children above seven years of age received 300 mg gemfibrozil twice daily

and younger patients received 150 mg gemfibrozil twice daily. During the study period, no special diet was followed; however, the patients were requested to stay within their regular diets. The patients in Group II (5 children) received a placebo. They were followed for nephrotic syndrome for 21 to 50 months under the regular therapy. As in the first group, all patients were resistant to prednisone, cyclophosphamide and dipyridamole treatment, and received cyclosporine or chlorambucil. Renal biopsies from four patients showed membrano-proliferative glomerulonephritis, and one showed focal segmental glomerulosclerosis. A placebo was administered at the same dose and at the same time as gemfibrozil. These patients were also requested to stay within their regular diets. The clinical characteristics of patients and controls are shown in Table I.

Prior to the start of the study, all patients and their parents were informed of the study goals, protocol and methodology, and their permissions were obtained. The duration of the study was set at four months.

Methods: During the study period, all patients were subjected to monthly examinations at the clinic for laboratory tests, and to determine adherence to the treatment course and side effects of the medication administered. Blood samples were taken for the determination of cholesterol, triglyceride, high-density liporotein (LDL), low-density lipoprotein (HDL), BUN, serum creatinine (S_{cr}) ALT, AST, CPK, apoliporotein A apolipoprotein B (apo A), (Apo B) and serum albumin levels during the initial and the subsequent examinations. A total of five sets of biochemical parameters were

Table I. Clinical Characteristics of Patient and Control Groups

Patient age (year)/sex	Antilipid treatment	C_{cr} (ml/min/1.73 m²)	Proteinuria (mg/m²/h)	Medications prior to antilipid treatment	Side effects of antilipid treatment	Other medications with antilipid treatment
10/M	gemfibrozil	55	200	prednisone, cyclophosphamide, dipyridamole, cyclosporine	None	None
9/M	gemfibrozil	60	45	prednisone, cyclophosphamide, dipyridamole, cyclosporine	None	captopril
5/F	gemfibrozil	60	61	prednisone, cyclophosphamide, dipyridamole	None	None
13/F	gemfibrozil	80	60	prednisone, cyclophosphamide, dipyridamole	None	prednisone
5/M	gemfibrozil	60	80	prednisone, cyclophosphamide, dipyridamole, cyclosporine, levamisole	None	prazosin, dipyridamole
13/F	gemfibrozil	60	47	prednisone, cyclophosphamide, dipyridamole, cyclosporine	None	dipyridamole, captopril
15/F	gemfibrozil	75	28	prednisone, cyclophosphamide, dipyridamole, chlorambucil	None	captopril
13/F	placebo	80	150	prednisone, cyclophosphamide, dipyridamole	None	captopril
13/M	placebo	61	94	prednisone, cyclophosphamide, dipyridamole, cyclosporine	None	prednisone
10/F	placebo	68	108	prednisone, cyclophosphamide, dipyridamole, chlorambucil	None	dipyridamole, cyclosporine
17/F	placebo	145	128	prednisone, cyclophosphamide, dipyridamole	None	None
14/F	placebo	70	115	prednisone, cyclophosphamide, dipyridamole, chlorambucil, cyclosporine	None	cyclosporine

C_{cr}: creatinine clearance.

obtained from each patient during the study period: at initial period and at first, second, third and fourth months. In addition, timed urine samples, taken over a 24 hr period, were analyzed for creatinine clearance (C_{cr}) and quantitative proteinuria.

Statistics: All biochemical values were determined as mean and standard deviation. The statistical evaluations were performed using Wilcoxon matched-pairs signed ranks test. Values of $p < 0.05$ were considered statistically significant.

Results

The statistical summary of the biochemical parameters obtained from blood and urine tests for each group is given in Table II. At the end of the fourth month gemifibrozil reduced total cholesterol by 34% from 378.1 ± 152.2 mg/dl to 247.0 ± 71.0 mg/dl, LDL by 30% from 266.0 ± 15.3 mg/dl to 161.7 ± 80.4 mg/dl, apo B by 21% from 266.0 ± 122.3 mg/dl to 210.0 ± 76.9 mg/dl, and triglycerides by 53% from 390.3 ± 280.5 mg/dl to 181.7 ± 68.8 mg/dl ($p < 0.05$). HDL cholesterol and apo A levels were not significantly altered. All other parameters for Group I and all parameters for Group II showed no statistically significant variations. All parameters in Group I showed statistically significant decreases during the course of treatment. The medication caused no significant side effects. There were positive correlations between cholesterol and LDL and apo B, and between LDL and apo B and HDL in Group I patients ($r = 0.9$, $r = 0.7$, $r = 0.5$, $r = 0.4$ respectively, $p < 0.001$).

Discussion

Hyperlipidemia is one of the common findings in children with nephrotic syndrome[1,2]. It may increase the cardiovascular risk and contribute to atherosclerotic complications and accelerated glomerular damage[3-6]. It has been demonstrated that reduction of hyperlipidemia by dietary or pharmacological means was protective in models of spontaneous and experimental glomerulosclerosis[7-9]. But, there is limited data in nephrotic children with lipid-lowering drugs.

Table II. Biochemical Parameters at the Beginning and End of Treatment

	Group I (n = 7)		Group (n = 5)	
	Month 0	Month 4	Month 0	Month 4
BUN (mg/dl)	19.4 ± 8.7	20.5 ± 9.2	22.2 ± 5.7	29.0 ± 15.2
S_{cr} (mg/dl)	0.9 ± 0.4	0.9 ± 0.5	0.8 ± 0.1	1.3 ± 0.5
C_{cr} (ml/min/1.73 m^2)	92.0 ± 21.9	87.7 ± 30.2	100.0 ± 22.4	80.2 ± 41.3
Total protein (g/dl)	5.0 ± 0.9	5.1 ± 1.2	5.6 ± 1.0	5.9 ± 1.2
Albumin (g/dl)	2.6 ± 0.6	2.5 ± 0.6	2.8 ± 0.5	3.0 ± 0.5
AST (U/L)	17.5 ± 14.3	18.8 ± 9.3	16.6 ± 3.9	20.8 ± 5.8
ALT (U/L)	7.5 ± 2.9	6.7 ± 3.3	8.4 ± 2.1	10.8 ± 2.1
CPK (U/L)	77.7 ± 50.2	86.7 ± 35.9	99.2 ± 61.4	93.4 ± 27.9
Apo A (mg/dl)	150.6 ± 22.3	156.5 ± 17.1	159.4 ± 36.7	147.0 ± 32.0
Apo B (mg/dl)	266.0 ± 122.3*	210.0 ± 76.9*	200.0 ± 83.0	173.6 ± 46.4
Cholesterol (mg/dl)	378.1 ± 152.2*	247.0 ± 71.0*	227.4 ± 52.1	205 ± 62.0
Triglyceride (mg/dl)	390.3 ± 280.5***	181.7 ± 68.8**	459.4 ± 281.3	413.0 ± 260.9
LDL (mg/dl)	266.0 ± 15.3*	161.7 ± 80.4*	140.4 ± 49.4	142.7 ± 34.9
HDL (mg/dl)	29.1 ± 8.3	43.1 ± 16.7	29.2 ± 11.6	31.0 ± 7.3
Proteinuria (mg/m^2/h)	156.0 ± 73.4	144.6 ± 73.0	161.4 ± 78.9	156.2 ± 56.0
U_p/U_{cr}	11.7 ± 7.9	11.4 ± 7.9	8.3 ± 4.8	7.0 ± 5.0

S_{cr} : Serum creatinine.
C_{cr} : Creatinine clearance.
Apo A: Apolipoprotein A.
Apo B: Apolipoprotein B.
LDL : Low-density lipoprotein.
HDL : High-density lipoprotein.
U_p/U_{cr}: Urinary protein/creatinine.
* $p < 0.05$.
** $p < 0.005$.
† All values are shown as mean ± one standard deviation.

Gemfibrozil is widely used in the treatment of nephrotic hyperlipidemia in adults but is sparingly used in children with persistent nephrotic syndrome[10,11].

Several clinical and experimental studies have reported that hypolipidemic drugs may cause improvement in deteriorated renal function[9,12,13]. However, some clinical studies demonstrated that the antilipidemic drugs have no effect on renal function[2,14]. In our study we could not find any significant difference between pre- and post-treatment values of BUN, S_{cr}, and C_{cr} in Group I and Group II patients. As in our study, some studies have demonstrated no regression in proteinuria with the treatment of hyperlipidemia[10,14].

Groggel et al.[10] demonstrated in adult nephrotic patients with gemfibrozil treatment that triglycerides decreased by 51%, plasma total cholesterol by 15% and LDL by 13%. HDL increased by 18%, apo A was unchanged, and apo B decreased by 26%. In our study, there were no significant differences in mean apo A levels before and after treatment in Groups I and II, but mean apo B, triglycerides and LDL values were significantly different at the beginning and end of the treatment in Group I. Mean apo B, cholesterol, triglycerides, LDL and HDL levels were not significantly different in pre- and post-treatment periods in Group II. There were no significant differences in mean HDL levels in both groups in pre- and post-treatment periods. Although mean HDL values were increased by 48% at the end of the treatment in Group I, the increase was not statistically significant. This effect is probably due to a stimulation of lipoprotein lipase, resulting in an increase in the clearance of triglyceride rich lipoproteins, as shown in previous studies[15-18]. Recently, it has also been revealed that fibrates activate peroxisome prolifetor-activated receptor alpha and there by alter the transcription of genes controlling lipoprotein metabolism[19,20]. Cholesterol level was significantly reduced by gemfibrozil in our study. Since all of the studies with this drug have been performed in adult patients, it is not appropriate to compare our results with them, because of the changes in lipid profile according to the age. Querfeld et al.[2] demonstrated that the mean concentration of triglycerides was reduced by 15%, plasma total cholesterol by 25%, LDL by 25%, LDL by 27% and HDL by 24%, as well as apo A by 19% and apo B by 21% with probucol, another hypolipidemic drug, in persistent childhood nephrotic syndrome.

Recent studies with lipid-lowering drugs have been successful without major side effects[5,11]. Groggel et al.[10] showed that gemfibrozil treatment caused no myalgia or muscle weakness. A temporary increase in mean CPK levels were found in only one patient, and this increase had improved with the continuation of the therapy; however, the changes in LDH, AST and ALT levels were not reported. We did not observe any side effects in our patients. There was no difference in CPK, AST and ALT levels before and after the treatment in either group.

In conclusion, this study shows that gemfibrozil therapy in children with corticosteroid and/or immunosupressive resistant nephrotic syndrome decreases the levels of cholesterol, triglycerides, LDL and apo B without significantly impacting renal functions and proteinuria. Since no changes were observed in the glomerular pathology that causes proteinuria, the improvement in hyperlipidemia can be attributed to the impact of gemfibrozil on the lipoprotein lipase enzyme. We still need a large multicenter and long-term trial to assess benefits and side effects of this type of therapy in children.

Acknowledgement

This study was supported by a grant from Çukurova University Research Fund and was presented at the 31st Annual Meeting of the European Society for Pediatric Nephrology, in Athens, Greece.

REFERENCES

1. Thabet MA, Salcedo JR, Chan JC. Hyperlipidemia in childhood nephrotic syndrome. Pediatr Nephrol 1993; 7: 559-566.
2. Querfeld U, Kohl B, Fiehn W, et al. Probucol for treatment of hyperlipidemia in persistent childhood nephrotic syndrome. Report of a prospective uncontrolled multicenter study. Pediatr Nephrol 1999; 13: 7-12.
3. Querfeld U. Should hyperlipidemia in children with the nephrotic syndrome be treated? Pediatr Nephrol 1999; 13: 77-84.
4. Mallick NP, Short CD. The nephrotic syndrome and ischaemic heart disease. Nephron 1981; 27: 54-57.
5. Moorhead JF, Chan MK, El-Nahas M, Varghese Z. Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. Lancet 1982; 2: 1309-1311.
6. Diamond JR. Hyperlipidemia of nephrosis: pathophysiologic role in progressive glomerular disease. Am J Med 1989; 87: 25-29.

7. Grone HJ, Walli A, Gron E, et al. Induction of glomerulosclerosis by dietary lipids. A functional and morphologic study in the rat. Lab Invest 1989; 60: 433-446.
8. Diamond JR, Karnovsky MJ. Exacerbation of chronic aminonucleoside nephrosis by dietary cholesterol supplementation. Kidney Int 1987; 32: 671-677.
9. Kasiske BL, O'Donnel MP, Cleary MP, Keane WF. Treatment of hyperlipidemia reduces glomerular injury in obese Zucker rats. Kidney Int 1988; 33: 667-672.
10. Groggel GC, Cheung AK, Ellis-Benigni K, Wilson DE. Treatment of nephrotic hyperlipoproteinemia with gemfibrozil. Kidney Int 1989; 36: 266-271.
11. Appel GB, Appel AS. Lipid-lowering agents in proteinuric diseases. Am J Nephrol 1990; 10 (Suppl 1): 110-115.
12. Kasiske BL, O'Donnel MP, Schmitz PG, Kim Y, Keane WF. Renal injury of diet induced hypercholesterolemia in rats. Kidney Int 1990; 37: 880-891.
13. Gentile MG, Fellin G, Cofana F, et al. Treatment of proteinuric patients with a vegetarian soy diet and fish oil. Clin Nephrol 1993; 40: 315-320.
14. Olbricht CJ, Koch KM. Treatment of hyperlipidemia in nephrotic syndrome: time for a change? Nephron 1992; 62: 125-129.
15. Miller DB, Spence JD. Clinical pharmacokinetics of fibric acid derivatives (fibrates). Clin Pharmacokinet 1998; 34: 155-162.
16. Framer JA, Gotto AM Jr. Choosing the right lipid-regulating agent. A guide to selection. Drugs 1996; 52: 649-661.
17. Matsuoka N, Jingami H, Masuzaki H, et al. Effects of gemfibrozil administration on very low density lipoprotein receptor RNA levels in rabbits. Atherosclerosis 1996; 25; 126: 221-226.
18. Farmer JA, Gotto AM Jr. Currently available hypolipidaemic drugs and future therapeutic developments. Bailliers Clin Endocrinol Metab 1995; 9: 825-847.
19. Kinoshita M. Fibric acid derivates. Nippon Rinsho 1999; 57: 2826-2830.
20. Gervois P, Torra IP, Fruchart JC, Staels B. Regulation of lipid and lipoprotein metabolism by PPAR activators. Clin Chem Lab Med 2000; 38: 3-11.

Meningitis due to Salmonella in preterm neonates

Mehmet Totan, Şükrü Küçüködük, Ayhan Dağdemir, Cengiz Dilber

Department of Pediatrics, Ondokuz Mayıs University Faculty of Medicine, Samsun, Turkey

SUMMARY: Totan M, Küçüködük Ş, Dağdemir A, Dilber C. Meningitis due to Salmonella in preterm neonates. Turk J Pediatr 2002; 44: 45-48.

Meningitis due to Salmonella is a rare condition. Here we report seven cases with neonatal Salmonella meningitis diagnosed and treated in the Pediatric Neonatology Unit of Ondokuz Mayıs University Faculty of Medicine between January 1985-February 2001. Five cases were cured with appropriate treatment; in two of them no neurological sequelae were seen. Of the remaining three, reversible hydrocephalus occurred in two cases and subdural empyema was found in one. Two patients died on the 30th and 40th days of hospitalization: one of them had hydrocephalus and the other ventriculitis. In this report we point out the importance of neonatal meningitis due to Salmonella serotypes, though it rarely occurs.

Key words: Salmonella, meningitis, newborn.

Salmonella are motile, Gram-negative, non-encapsulated, nonsporulating rods of the Enterobacteriaceae family. Salmonella can localize in any organ or tissue. Salmonella serotypes (Salmonella typhimurium, Salmonella heidelberg, Salmonella enteritidis, Salmonella saint-paul, Salmonella newport and Salmonella panama) which cause neonatal meningitis are usually associated with bacteriemia[1,2]. Salmonella meningitis (SM) is a disease with high relapse and mortality rate. Antibiotic therapy should be given at least for four weeks in order to decrease the mortality rate. If the antibiotic therapy is discontinued before the third week of therapy, relapse is observed in 64% of cases[3,4]. In the survivors of neonatal meningitis with Gram-negative bacteria, complications such as hydrocephalus, convulsion, abscess formation and subdural empyema are frequently observed[5,6]. The aim of this study was to review cases with SM in our clinic together with the literature.

Material and Methods

This retrospective study was based on seven preterm infants with SM who were diagnosed in the Neonatology Unit of Ondokuz Mayıs University Faculty of Medicine between January 1985-February 2001. The diagnois of SM was established on the basis of positive cerebrospinal fluid (CSF) and/or blood culture results in addition to CSF findings, which were correlated to bacterial meningitis. Complete blood count, ALT, AST, total bilirubin, direct bilirubin, total protein and albumin levels were measured. Antibiotic susceptibilities were determined using Microbroth dilution method. Gruber-Widal agglutination test and cranial computerized tomography (CT) were performed in all patients.

Results

Four hundred and forty newborns with bacterial meningitis were hospitalized in our unit during the study period. Seven (4 female, 3 male) of them had SM (1.6%). All babies were preterm and birth weights ranged from 1,700 to 2,700 g. The mean age of the cases was 13.1 ± 3.1 days (6 to 27). There was no finding of infection in the parents, and cultures were negative for Salmonella.

All patients were born in different centers and transferred to our clinic because of meningitis, developed during hospitalization. Diarrhea (86%), convulsion (71%), fever (57%), vomiting (57%), respiratory distress (43%), jaundice (43%) and peripheral facial paralysis (14%) were observed in the patients. Meningitis was associated with acute gastroenteritis and sepsis in five patients (71%). Esophageal atresia was diagnosed in one patient with sepsis (Case 2) and myelomeningocele was found in another (Case 4).

Complete blood count revealed mean hemoglobin levels, and white blood cell and platelet counts as 11.2 ± 2.1 g/dl, 15.1 ± 4.3 X 10^9/L and 82.3 ± 21.6 x 10^9/L , respectively. On CSF examination, cell counts were within 380-9,200/ mm^3 and the mean CSF protein and glucose levels were 210 ± 36.4 mg/dl and 24.6 ± 5.1 mg/dl, respectively, before the initiation of therapy. All Salmonella serotypes were sensitive to cefotaxime, ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole (TMP/SMX) and ciprofloxacin according to the results of culture and susceptibility tests. In addition to the antibiotic therapy, intravenous immunoglobulin (IVIG) was given to four cases (Cases 3, 4, 6, 7). Five cases were cured (Cases 1, 3, 5-7). Two patients died on the 30th and 40th days of hospitalization, one with hydrocephalus (Case 2) and the other with ventriculitis (Case 4).

Gruber-Widal agglutination test was negative in all cases. CSF findings of the patients with SM showed improvement on the 14th day of the treatment except for two infants (Cases 2, 4). The clinical and laboratory characteristics of these infants are summarized in Tables I and II.

Discussion

Although the involvement of the central nervous system is a rare complication of Salmonella infections, it forms a serious problem in the newborn and early infancy periods[5,7]. Salmonella meningitis accounts for 0.8 to 6% of bacterial meningitis cases, and is usually seen during infancy period[8,9]. It affects neonatal babies and those under four months of age. In the newborn period, our cases with SM totalled 1.6% of all cases with meningitis in the same age range. Though the mortality rate in SM was 94% before antibiotic therapy, it decreased to 59-62% between 1960 and 1970. Recently, the mortality rate has been decreased to 37% in developed countries[9]. It was determined as 29% in our study.

Salmonella typhimurium is the most commonly encountered Salmonella in neonatal meningitis[10,11], but other serotypes can be isolated[2,5]. The most frequent serotype of Salmonella was also Salmonella typhimurium (4 of 7 cases) in our study.

West et al.[3] reported that acute neurological complications consisting of ventriculitis, subdural empyema and hydrocephalus were

Table I. Clinical Findings in Cases with Salmonella Meningitis

Case No.	Sex	GA	AD	BW	Treatment	TD	IVIG	Outcome	NS
1	F	33	8	2300	CF+AMP	30	–	Recovered	–
2	M	30	6	1700	CF+AMP	30	–	Died	HD
3	F	34	14	2600	CF+AMP, CIP	32	+	Recovered	Subdural empyema
4	M	32	11	2200	CF+AMP, CIP	40	+	Died	Ventriculitis
5	F	34	12	2300	CF+AMP, CIP	31	–	Recovered	HD
6	F	35	14	2700	CF+AMP, CIP	30	+	Recovered	HD
7	M	34	27	2600	CF+AMP, CIP	30	+	Recovered	–

F : Female.
M : Male.
GA : Gestational age (week).
AD : Age at diagnosis (day).
BW : Birth weight (g).
TD : Therapy duration (day).
IVIG : Intravenous immunoglobulin.
CF : Cefotaxime.
AMP : Ampicillin.
CIP : Ciprofloxacin.
NS : Neurological sequelae.
HD : Hydrocephalus.

Table II. Laboratory Findings of Cases with Salmonella Meningitis

Case no.	Hb	WBC	PLT	CSF findings: Cell count (mm^3)	CSF findings: Protein (mg/dl)	CSF findings: Glucose (mg/dl)	Gruber-Widal test	Culture	Salmonella serotypes	CT
1	11.1	14.2	57.6	380	200	26.4	Negative	Blood	S. typhimurium	N
2	10.8	8.2	76.4	9200	270	10.2	Negative	Blood+CSF	S. enteritidis	A
3	11.2	14.6	92.6	4000	160	24.4	Negative	Blood	S. typhimurium	A
4	9.2	19.2	61.3	3200	220	13.2	Negative	CSF	S. enteritidis	A
5	10.6	16.7	98.6	960	200	36.1	Negative	Blood+CSF	S. species	A
6	14.2	15.6	94.2	1400	190	28.4	Negative	CSF	S. typhimurium	A
7	11.3	17.2	95.4	670	230	33.5	Negative	Blood	S. typhimurium	N

Hb : Hemoglobin (g/dl).
WBC : White blood cell count (10^9/L).
PLT : Platelet count (10^9/L).
CSF : Cerebrospinal fluid findings.
CT : Computerized tomography.
N : Normal.
A : Abnormal.

found in 43% of the cases, and they observed relapses in 64%. Dunn et al.[12] erported 12 cases of subdural empyema, two of them associated with SM. Krcmery et al.[8] reported reversible hydrocephalus that responded to intraventricular punctures in two of the 12 cases with SM. Hansen et al.[13] reported two cases of SM; one died six days after admittance to the hospital and the other recovered without sequelae. In our study, five of the seven cases were cured and two of them had no neurological sequelae (Cases 1, 7). Reversible hydrocephalus that responded to treatment was seen in two cases (Cases 5, 6). One case had subdural empyema (Case 3). Two patients died on the 30th and 40th days of hospitalization, one with hydrocephalus (Case 2) and the other with ventriculitis (Case 4).

Over the last 20 years, Salmonella meningitis was often treated with ampicillin and chloramphenicol with high relapse and mortality rates. Third generation cephalosporins such as cephotaxime and ceftriaxone, with a good penetration to CSF, were found more effective. Children with septicemia, enteric fever, or metastatic sites of infection should be treated initially with systemically administered cephotaxime and ceftriaxone. Ciprofloxacin and TMP/SMX are alternative agents. It is known that ciprofloxacin is effective in the treatment of SM. After the negative effect of ciprofloxacin on the development of cartilage was demonstrated in animal models, the use of ciprofloxacin under the age of 18 was limited[8,14]. However, this effect has not been supported in clinical tirals. Krcmery et al.[8] reported that 10 of 12 cases of neonatal and infant nosocomial meningitis treated with ciprofloxacin were cured. In our study, ciprofloxacin was given to the patients (5 of 7 cases) who did not show any sign of clinical improvement on the 7th day of treatment, which included a third generation cephalosporin antibiotic; even the susceptibility tests revealed no resistance. Four of the five cases recovered (Cases 3, 5-7), but one died (Case 4) on ciprofloxacin therapy.

Intravenous immunoglobulin has been proposed recently for neonatal SM. Brandun et al.[15] determined that there were antibodies against the D antigen of Salmonella in certain IVIG production pools and declared that IVIG could be used in serious Salmonella infections. In a study which was performed by Gökalp et al.[16], IVIG was given to three preterm newborns with Salmonella typhimurium infection. One of them recovered without sequelae, another had hydrocephalus and the other had subdural empyema. In our study, four cases received IVIG; three of them recovered (Cases 3, 6, 7) and one of them died (Case 4). On the other hand, one of the three cases who did not receive IVIG died (Case 2), while the other two recovered. It seems there is no additional effect of IVIG on the clinical improvement of the cases with Salmonella meningitis in the neonatal period. Our results may confirm the idea of Harry[17] on IVIG use in the neonatal period.

We conclude that SM should be considered among the causes of bacterial meningitis in the newborn period. Immediate suitable treatment is essential to obtain satisfactory recovery.

REFERENCES

1. Makin G, Abu-Harb M, Finn A. Salmonella durban in an infant. Lancet 1996; 348: 200-206.
2. Vazguez-Lopez ME, Pego R, Somoza C, Garcia-Plata C, Morales-Redondo R. Meningitis due to Salmonella in the neonatal period. Rev Neurol 1998; 27: 986-987.
3. West SE, Goodkin R, Kaplan AM. Neonatal Salmonella meningitis complicated by cerebral abscesses. West J Med 1977; 127: 142-148.
4. Sam WI, Mackay AD. Salmonella meningitis and a green inguana J R Soc Med 2000; 93: 318-319.
5. Udani RH, Kabra NS, Nanavati RN, Baweja S. Outbreak of Salmonella worthington meningitis in neonatal intensive care unit. Indian Pediatr 1999; 36: 300-303.
6. Kinsella TR, Yogev R, Shulman ST. Treatment of Salmonella meningitis and brain abscess with the new cephalosporins: two case reports and a review of the literature. Pediatr Infect Dis J 1987; 6: 476-480.
7. Lee WS, Puthucheary SD, Parasakthi N. Extra-intestinal non-typhoidal Salmonella infections in children. Ann Trop Paediatr 2000; 20: 125-129.
8. Krcmery VJ, Filka J, Uher J, Kuvak H, Sagat T, Tuharsky J. Ciprofloxacin in treatment of nosocomial meningitis in neonates and infants: report of 12 cases and review. Diagn Microbiol Infect Dis 1999; 35: 75-80.
9. Quagliarello V, Scheld W. Teatment of bacterial meningitis. N Engl J Med 1997; 323: 708-711.
10. Moyneux E, Walsh A, Phiri A, Molyneux M. Acute bacterial meningitis in children admitted to the Queen Elizabeth Central Hospital, Blantyre, Malawi in 1996-1997. Tropical Med Int Health 1998; 3: 610-618.
11. Vahapoğlu H, Hall LM, Mulazimoğlu L, Dodanli S, Yıldırım I, Livermore DM. Resistance to extended-spectrum cephalosporins, caused by PER-1-beta-lactamase, in Salmonella typhimurium from Istanbul, Turkey. J Med Microbiol 1995; 43: 294-299.

12. Dunn DW, McAllister J, Craft JC. Brain abscess and empyema caused by Salmonella. Pediatr Infect Dis J 1984; 3: 54-57.

13. Hansen LN, Eschen C, Bruun B. Neonatal Salmonella meningitis: two case reports. Acta Paediatr 1996; 85: 629-631.

14. Ragunathan PL, Potkins DV, Watson JG. Neonatal meningitis due to Salmonella typhimurium treated with ciprofloxacin. J Antimicrob Chemother 1990; 5: 727-729.

15. Brandun S, Imbach P, Kidt H. Der klinische einsatz von immunoglobulin. Basel, Switzerland: Sandoz; 1981.

16. Gökalp AS, Toksoy HB, Türkay S. Intravenous immunoglobulin in the treatment of Salmonella typhimurium infections in preterm neonates. Clin Pediatr 1994; 6: 349-352.

17. Hill HR. Additional confirmation of the lack of effect of intravenous immunoglobulin in the prevention of neonatal infection. J Pediatr 2000; 137: 595-597.

Phenylketonuria and glycogen storage disease type III in sibs of one family

Tuncay Yılmazer[1], Albert H. van Gennip[2], Nico G.G.M. Abeling[2], İmran Özalp[3]
Turgay Coşkun[3], Henk D. Bakker[1]

[1]Emma Children's Hospital, and [2]Laboratory of Metabolic Diseases, University of Amsterdam, Academic Medical Center, Amsterdam, The Netherlands, and [3]Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Yılmazer T, van Gennip AH, Abeling NGGM, Özalp İ, Coşkun T, Bakker HD. Phenylketonuria and glycogen storage disease type III in sibs of one family. Turk J Pediatr 2002; 44: 49-53.

Hyperphenylalaninemia result from a block in the conversion of phenylalanine into tyrosine due to a defect in either the enzyme phenylalanine hydroxylase (98% of subjects) or in the metabolism of the cofactor tetrahydrobiopterin. Phenylalanine hydroxylase deficiency is the most common form of inherited hyperphenylalaninemia disorders, with a prevalence between 1/4,000-1/40,000.

Glycogen storage disease (GSD) type III is caused by debranching enzyme deficiency of glycogen degradation. The clinical features vary in relation to the localization of the enzyme defect. Two clinical entities exist: a combined hepatic myogenic form (GSD IIIa) and a purely hepatic form (GSD IIIb). The inheritance is autosomal recessive.

We describe a Turkish family in which two girls were found to have phenylketonuria, while in two other sisters glycogen storage disease type III was diagnosed. The parents of these children are cousins and they have had 12 children.

Key words: phenylketonuria, debranching enzyme deficiency, consanguinity, glycogen storage disease III.

Hyperphenylalaninemia results from a block in the conversion of phenylalanine into tyrosine due to a defect in either the enzyme phenylalanine hydroxylase (98% of subjects) or in the metabolism of the cofactor tetrahydrobiopterin. Phenylalanine hydroxylase deficiency is the most common form of inherited phenylalanine disorders, with a prevalence of between 1/4,000-1/40,000[1-3]. The gene for hyperphenylalaninemia is mapped to chromosome 12q22; multiple mutations have been identified.

Glycogen storage disease (GSD) type III is caused by a debranching enzyme deficiency of glycogen degradation. The clinical features vary in relation to the tissue localization of the enzyme defect. Two clinical entities exist: a combined hepatic myogenic form (GSD IIIa) and a purely hepatic form (GSD IIIb). The inheritance is autosomal recessive. The gene for the debranching enzyme is mapped to chromosome 1p21[4-6].

We report a Turkish family in which two girls were found to have phenylketonuria (PKU), while in two other sisters GSD type III was diagnosed. The question arises as to whether or not these enzyme defects are associated in some way.

Case Report

We describe a Turkish family in which two girls were found to have phenyketonuria, while in two other sisters GSD type III was diagnosed. The parents of these children are cousins and they had eight more children. Of these, three male children had died: two due to neurologic deterioration and another due to birth trauma. One girl had died as well because of bronchopneumonia complicating measles. They have four healthy daughters who are now married. The family immigrated to the Netherlands 15 years ago.

Patient So. U., nine years old at admission and the ninth child of consanguineous and healthy parents, was admitted for metabolic investigation

because of hepatomegaly, cardiomegaly and attacks of hypoglycemia. Physical examination revealed a girl with thin extremities, height of 126 cm (3rd percentile), weight of 30 kg (weight for height > 90th percentile) and a head circumference of 52 cm (50th percentile). There was a systolic murmur of grade III/VI and the liver was palpable 12 cm below the right costal margin. Laboratory studies showed highly elevated liver enzyme activities (AST, ALT, γ-GT, LDH) and very high CK activity, indicating muscle wasting. Electron microscopy of a liver biopsy revealed massive amounts of intrahepatocytic glycogen.

She is now 22 years old. Physical examination is normal except for two-year history of supraventricular tachycardia, but we consider this problem to be independent of GSD. Abdominal USG and cardiac ECHO are normal. Her psychomotor development appears to be normal. She has been married for two years. She suffers from hypoglycemic symptoms, and thus has to eat every 2-3 hours.

Patient P. U., eight years old on admission and tenth child of the same family, was admitted for the same reasons as her one year older sister. She also had hepatomegaly, cardiomegaly and attacks of hypoglycemia. On physical examination thin extremities were also seen, and a systolic murmur noted. Her height was 116 cm (3rd percentile), weight was 25.5 kg (weight for height > 90th percentile) and she had a head circumference of 51 cm (50th percentile). The liver enzyme activities were highly elevated and CK activity was also high. As in her sister, glycogen storage was confirmed in a liver biopsy.

She is now 21 years old. Physical examination and biochemical findings are normal. There is no hepatomegaly or other pathological findings. She has been married for two years. In both sisters, GSD was diagnosed in Turkey. Type identification was performed in the Netherlands.

In both patients, glucose and fasting glucagon tests were performed. The glucose curves after glucose loading were clearly abnormal in both patients. Zero time values were below normal and the high, biphasic, prolonged curves were suggestive of a glycogen storage disease.

The lactate curves after glucose laoding pointed towards GSD III or VI. The fasting glucagon test excluded phosphorylase b kinase deficiency. The postprandial glucagon test showed an almost flat curve, so we were unable to discriminate between a debranching enzyme or phosphorylase deficiency.

The enzyme determinations in leukocytes, muscle, liver and fibroblasts of the two patients revealed a deficiency of a debranching enzyme consistent with GSD III (Table I), while phosphorylase activity was found to be normal in their erythrocytes (Table II).

Table I. Debranching Enzyme Levels

	Debranching enzyme			
	(nmol/min/mg)			nmol/hr/mg protein
	Leukocytes	Muscle	Liver	Fibroblasts
So.U.	0.4	0.16	ND	1.1
P.U.	0.4	0.13	0.08	1.6
Si.U.	–	–	–	12
Control (s)	1.7	1.76	2.7	13.-40

ND: not determined.

Table II. Phosphorylase Enzyme Levels in Erythrocytes

	Phosphorylase in erythrocytes		
	(nmol/min/mg protein)		(μmol/min/g Hb)
	A	a+b	b kinase
So.U.	0.018	0.036	0.95
P.U.	0.022	0.033	1.1
Control (s)	0.018-0.066	0.035-0.117	0.36-2.78

The enzyme assays in leukocytes, erythrocytes, muscle and liver were performed by Dr. R. Berger, Academic Hospital Groningen and in fibroblasts by Dr. O. P. van Diggelen, Erasmus University, Rotterdam.

Patient E.U., a girl aged four years, was found to have PKU by metabolic investigation after PKU was established in her sister. The girl was admitted to our hospital at the age of five years. Her weight was 18.5 kg (90th percentile), height 101 cm (< 3rd percentile), and head circumference 50.5 cm (50th percentile). She appeared to have psychomotor retardation. She is now 19 years old. She is able to do daily activity and to speak some sentences. She is going to a special school for the mentaly retarded.

Patient Si.U., aged 10 months, was admitted to our hospital for the differential diagnosis of PKU, which was found shortly after birth by routine neonatal screening in Turkey. She had been treated ever since with a phenylalanine restricted diet. On admission she weighed 12 kg (> 95th percentile), her length was 71 cm (10th percentile), and head circumference 46 cm

(75th percentile). Physical examination was normal. She is now 15 years old and a second-year student at college. She has normal mental and neuromotor development.

BH_4 loading test was performed in both patients on admission, and plasma phenylalanine levels did not decrease; six hours after loading the concentrations were similar to starting levels. The concentration of tyrosine did not increase in patient Si.U. and increased only slightly in patient E.U. (Tables III, IV, V, VI).

The biochemical findings indicated PKU caused by liver phenylalanine-hydroxylase deficiency in both patients.

Discussion

Turkey has a high rate of consanguineous marriages (21.1%). Social and cultural factors are especially important in marriages between first and second cousins[7]. Thus, a high prevalence of inherited metabolic diseases is present in Turkey[8]. Despite the lack of offical data, we also assume a high consanguinity ratio in the Turkish population living in the Netherlands. The parents of our patients are first cousins and they have been living in the Netherlands for 15 years.

The clinical manifestations of glycogenosis III tend to be milder than those of type I, but the diseases cannot reliably be distinguished without laboratory procedures. The most consistent clinical feature is an enormously enlarged liver, and it may be the only clinical abnormality at the time of presentation. Hypoglycemia is usually not a prominent feature of this disease, but fasting concentrations of glucose are usually moderately reduced and some patients, especially in infancy, have severe hypoglycemia and even convulsions[4-6]. Our patients had hepatomegaly and hypoglycemic

Table III. Phenylalanine and Tyrosine Concentrations in Plasma and Urine on Admission (in PKU Sibs)

	Plasma (μmol/L)		Urine (μmol/g creatinine)	
	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine
Si.U.	1442	36	3461	236
E.U.	1345	34	1021	83

Table IV. Abnormal Phenylalanine Metabolites in Urine on Admission (in PKU Sibs)

	(μmol/g creatinine)			
	Phenyl-pyruvate	phenyl-lactate	phenyl-acetate	O-OH-phenyl-acetate
Si.U.	1160	1967	2360	460
E.U.	4685	5149	1531	220

Table V. BH_4 Loading Test

		Si.U.		E.U.	
Sampling material	Sampling period (in hours)	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine
Urine (μmol/g creatinine)	12-0 before BH_4	5332	221	–	–
	0-4 after BH_4	5944	435	1827	101
	4-8 after BH_4	6117	387	2344	158
Plasma (μmol/L)	0	2131	87	1440	54
	2 after BH_4	2282	114	1431	45
	4 after BH_4	2238	111	1548	92
	8 after BH_4	2144	91	1571	76

Table VI. Urinary Pterins* (mmol/mol. creatinine) Before BH_4 Loading

	Si.U.	E.U.
Neopterin	4.4	3.2
Monapterin	0.3	0.3
Biopterin	4.5	10.4
Pterin	1.5	0.9
Mol % biopterin	49	75

* This assay was performed by Dr. M. Duran, University Children's Hospital, Het Wilhelmina Kinderziekenhuis, Utrecht.

attacks and patient So.U. also had convulsions. On the other hand, both sisters have normal mental and neuromotor development.

A variety of functional studies, especially glucose tolerance and postprandial glucagon tests, have been employed to document the presence of type III glycogenosis and to distinguish it from types I and VI[9]. However, in both sibs the two-hour postprandial glucagon test gave only a slight glucose response, which is difficult to interpret. In patients with a debranching enzyme deficiency, a higher response is expected. However, another possibility might be a shortage of glycogen containing enough outer branches for conversion to glucose by phosphorylase. Nevertheless, despite these results, the results of other tests were consistent with debranching enzyme deficiency.

The diagnosis of GSD type III could be established by determination of the debranching enzyme in our patients' cells and tissues[10].

At present only limited information is available about the long-term outcome of patients with GSD type III. In one study on 50 patients, hepatomegaly was found to be the most prominent sign in 68% of cases, followed by myopathy in 63%, cardiomyopathy in 50% and hypoglycemia in 8%. Mental development was reported to be normal in 93% of the patients[11]. In our patients, physical and routine laboratory examinations were normal, although patient So.U. suffered from intermittent hypoglycemic symptoms.

Analysis of urine and plasma samples from the GSD III patients for PKU showed no abnormalities. The PKU sibs were not extensively investigated for GSD as they were clinically nonsuspect for this disease. However, debranching enzyme activity in the fibroblasts from Si.U. showed a borderline value (Table I).

Phenylketonuria, an autosomal recessive disorder, occurs in one in 15,000 births and is most common among persons of Western European background. It is probably best characterized by a ratio of blood phenylalanine to tyrosine persistently greater than 3. Phenylketonuria results from a block in the conversion of phenylalanine into tyrosine due to a defect in either the enzyme phenylalanine hydroxylase (98% of subjects) or in the metabolism of the cofactor tetrahydrobiopterin.

The most important and sometimes the only manifestation of PKU is mental retardation. Behavioral difficulties, seizures, rashes, and an unusual body odor may occur[1-3]. Patients with PKU are fair haired, fair skinned and have blue eyes in over 90% of the untreated or later-treated cases, as was seen in the late-treated patient E.U.[12].

All subjects with hyperphenylalaninemia should be screened for disorders of biopterin metabolism[2]. After BH_4 loading test, our patients' plasma phenylalanine levels did not decrease; six hours after loading the concentrations were similar to starting levels. The concentration of tyrosine did not increase in patient Si.U., and only slightly increased in patient E.U. BH_4 loading test results in both patients were consistent with classic PKU.

In Turkey, PKU neonatal screening started 15 years ago. Patient Si.U. was diagnosed after the first neonatal screening in Turkey. The different clinical course seen in patients Si.U. and E.U. dramatically highlights the importance of PKU screening.

The incidence of PKU in Turkey is the highest recorded in any country (1:6,000)[13]. GSD is also frequently seen in Turkey due to the high consanguinity rate, but the distribution of various types of GSD is unknown[14]. The overall frequency of all forms of GSD based on European data is approximately 1 in 20,000 to 25,000 live births[4]. Because the incidence of GSD is also estimated to be high in Turkey, the existence of both diseases in one family could have been coincidental.

Association of PKU with scleroderma, Duchenne muscular dystrophy, Charcot-Marie-Tooth disease, Down syndrome, cystinuria, homozygous hypobetalipoproteinemia, and bilateral iris coloboma and optic atrophy has been reported previously[15]. Coşkun et al.[15] also described a three-year-old Turkish girl with PKU and hereditary fructose intolerance. As far as we know the combination of PKU and GSD III in one sibship has not been reported in the literature. The clinical history was reported without typing the GSD[16], thus this is the first full report on the combination of PKU and GSD in one sibship.

Every person is a carrier of at least six-to autosomal recessive disorders. Therefore the chance of having two inborn errors in the offspring of a consanguineous couple is not negligible. It is in this light even suprising that so few "double inborn errors" occur in highly in bred populations.

REFERENCES

1. Scriver CR, Kaufman S, Eisensmith RC, Woo SL. The hyperphenylalaninemias. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Bases of Inherited Disease (7th ed) Vol. I. New York: McGraw Hill, Inc.; 1995: 1015-1075.
2. Smith I, Lee P. Hyperphenylalaninemias. In: Fernandes J, Saudubray JM, van den Berghe G. (eds). Inborn Metabolic Disease (3rd ed). Berlin, Heidelberg, New York: Springer-Verlag, 2000: 170-184.
3. Wappner R, Cho S, Kraonmal R, Schuett V, Seashore MR. Management of phenylketonuria for optimal outcome: a review of guidelines for phenylketonuria management and report of surveys of parents, patients, and clinic directors. Pediatrics 1999; 104: 68.
4. Chen YT, Bruchel A. Glycogen storage disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Bases of Inherited Disease (7th ed) Vol. I. New York: McGraw Hill, Inc.; 1995: 935-966.
5. Nyhan WL, Ozand PT. Glycogenosis type III. In: Nyhan WL, Ozand PT (eds). Atlas of Metabolic Disease. London: Chapman Hall Medical; 1998: 348-355.
6. Fernandes J, Smit GP. The glycogen storage diseases. In: Fernandes J, Saudubray JM, van den Berghe G. (eds). Inborn Metabolic Disease (3rd ed). Berlin, Heidelberg, New York: Springer-Verlag; 2000: 86-101.
7. Tunçbilek E, Koç I. Consanguineous marriage in Turkey and its impact on fertility and mortality. Ann Hum Genet 1994; 58: 321-329.
8. Özalp I, Coşkun T, Tokol S, Demircin G, Monch E. Inherited metabolic disorders in Turkey. J Inherit Metab Dis 1990; 13: 732-738.
9. Perkoff GT, Parker VJ, Hahan RE. The effects of glucagon in three forms of glycogen storage disease. J Clin Invest 1962; 41: 1099.
10. Shin YS. Diagnosis of glycogen storage diseases. J Inherit Metab Dis 1990; 13: 419-434.
11. Smit GP, Fernandes J, Leonard JV, et al. The long term outcome of patients with glycogen storage disease. J Inherit Metab Dis 1990; 13: 411-418.
12. Kaufman S, Holtman NA, Milstien S, Butler LJ, Krumholz A. Phenylketonuria due to deficiency of dihydropteridine reductase. N Engl J Med 1975; 293: 785-790.
13. Özalp I, Coşkun T, Tokatlı A, et al. Neonatal PKU screening in Turkey: 7 years experience in a developing country. Screening 1995; 4: 139-147.
14. Saltık IN, Özen H, Ciliv G, et al. Glycogen storage diseases type Ia: frequency and clinical course in Turkish children. Indian J Pediatr 2000; 67: 497-501.
15. Coşkun T, Özalp I, Tekinalp G. Hereditary fructose intolerance in a patient with phenylketonuria. Turk J Pediatr 1991; 33: 181-184.
16. Coşkun T, Özalp O, Koçak N, Tekinalp G. Aynı ailede fenilketonuri ve glikojenozis. Çocuk Sağlığı ve Hastalıkları Dergisi 1986; 29: 233-237.

Acute isoniazid neurotoxicity in childhood

Agop Çıtak, Özlen Kaya, Raif Üçsel, Metin Karaböcüoğlu, Nedret Uzel
Department of Pediatric Emergency, İstanbul University Institute of Child Health, İstanbul, Turkey

SUMMARY: Çıtak A, Kaya Ö, Üçsel R, Karaböcüoğlu M,Uzel N. Acute isoniazid neurotoxicity in childhood. Turk J Pediatr 2002; 44: 54-57.

Acute isoniazid (INH) poisoning is uncommon in children. Although most physicians are aware of INH hepatotoxicity, acute INH poisoning and its treatment are not well recognized. INH is increasingly being used to control the spread of tuberculosis, and physicians should know its potentially fatal effects. INH overdose is known to result in rapid onset of seizures, metabolic acidosis and prolonged obtundation. We report two cases of obtundation secondary to INH overdose that was immediately reversed by pyridoxine. Parenteral pyridoxine administration is an effective method in INH intoxication. The intravenous form of pyridoxine must be available in the emergency care units, and INH toxicity should be suspected in any patient with refractory seizures and metabolic acidosis.

Key words: isoniazid neurotoxicity, pyridoxine.

The incidence of tuberculosis has increased dramatically in the world over the past decade as a consequence of poverty, immigration from high prevalence countries, the HIV epidemic, and limitations in health care services to high-risk populations[1]. Since introduced in 1952, isoniazid (INH) still remains as a first line agent in the treatment and prophylaxis of tuberculosis. INH-related acute poisoning, either intentional or accidental, can be fatal if not diagnosed and treated properly[2-6].

Most physicians are aware of the INH hepatotoxicity and the importance of liver function tests. However, acute INH poisoning and treatment are not very well known. The clinical triad of acute neurotoxicity includes seizures resistant to anticonvulsants, metabolic acidosis, and coma[1,3-8]. Although INH-related neurotoxicity is usually seen in ingestion of doses greater than 200 mg/kg, a less than 40 mg/kg dosage of INH may be symptomatic[4,7,8].

In this article, our experience with two INH intoxication cases is presented, and the treatment is discussed.

Case Reports

Case 1

A previously healthy 14-year-old girl with generalized seizures lasting 15-20 seconds presented to the nearest hospital, where she was given 10 mg of diazepam intravenously, due to repeated generalized seizures. Upon cessation of seizures, she was transported to our pediatric emergency department with the diagnosis of acute encephalitis.

Her history was unremarkable, with no previous seizures and no medication use. However, her sister had been receiving isoniazid for tuberculosis lymphadenitis for the last three months.

At admission to our pediatric emergency department, she was found to be lethargic. Neurologic examination showed no lateralizing signs. Her pulse rate was 104 beats/minute, and respiratory rate was 20 breaths/minute. Other vital signs were in normal limits.

Laboratory findings on arrival were as follows: WBC: 28,200/mm^3, Hb: 128 g/dl, Htc 39%, PLT: 309,000/mm^3, glucose 181 mg/dl, sodium 138 mmol/L, potassium 3.6 mmol/L, urea 14 mg/dl, SGOT 26 IU/L, SGPT 14 IU/L, pH: 7.40, pCO_2 34 mmHg, pO_2 74 mmHg, and HCO_3 21.6 mmol/L. Chest roentgenogram, computerized cranial tomography, and electroencephalographic investigations were all normal.

It was learned that the patient had ingested 2 g of her sister's isoniazid (30 mg/kg) after a dispute between them. She was treated with

multiple doses of activated charcoal and received 2 of pyridoxine intramuscularly (equivalent to the amount of isoniazid ingested) eight hours after INH ingestion. Since the intravenous form was not available, we used the intramuscular form of pyridoxine. Full conciousness was noted after six hours of the therapy. She did not become lethargic again or require further pyridoxine. After the resolution of intoxication, a psychiatric consultation was obtained.

The following hospital course was complicated by transaminase elevation on the 4th day after INH ingestion, measured as SGOT: 331 IU/L and SGPT: 114 IU/L. Other laboratory findings (GGT, NH_3, PT, PTT) were normal.

After SGOT and SGPT levels had dropped below 100 IU/L, the patient was discharged on the 7th day, with follow-up planned by our pediatric gastroenterohepatology and psychiatry outpatient clinics.

Case 2

A 20-month-old boy weighing 10 kg was well until two hours prior to admission when he became somnolent and developed generalized tonic-clonic seizures. He was brought to the nearest hospital where 5 mg rectal diazepam was administered. He was then transferred to our hospital with the diagnosis of acute encephalitis.

At admission to the pediatric emergency department, he was comatose, afebrile, and breathing spontaneously. Neurologic examination revealed generalized hypertonicity of the extremities, and hyperactive deep tendon reflexes. The heart rate varied between 100-120 beats/minute, respiratory rate was 28 breaths/minute, and blood pressure was 100/60 mmHg. The pupils were myotic and responded to light. Meningeal signs were negative, and there were no findings suggesting trauma.

Results of initial laboratory studies included the following: WBC: 20,700/mm^3, Hb: 11.5 g/dl, Htc 34%, PLT: 221,000/mm^3, glucose 119 mg/dl, sodium 139 mmol/L, potassium 4.0 mmol/L, urea 17 mg/dl, SGOT 31 IU/L, SGPT 16 IU/L, pH: 7.14, pCO_2 28 mmHg, pO_2 80 mmHg, HCO_3 9 mmol/L, and ABE-18 mmol/L.

One hour after arrival he developed clonic seizures marked on the right arm and right side of his face. Seizure activity stopped in a minute without any medication, but 10 minutes later a generalized seizure activity was observed which was treated with phenobarbital (10 mg/kg) intravenously. His cerebrospinal fluid examination, EEG, and cranial computerized tomography (CT) findings were normal. A review of his history revealed that he had been playing with his father's INH bottle 30 minutes before the seizures. INH toxicity was suspected. The patient's father checked the bottle and remarked that three tablets of INH were absent. After intravenous administration of 1 g pyridoxine [4 hours after INH ingestion, equal to the amount of isoniazid ingested (100 mg/kg)], no further seizure occurred.

Additionally, gastric lavage was performed, and activated charcoal and sodium bicarbonate were administered. He was also given 20 mmols sodium bicarbonate. After the therapy, his blood gas results were as follows: pH: 7.37, pCO_2 31 mmHg, Hg, pO_2 84 mmHg, Hg, HCO_3 28.0 mmol/L, and ABE-6 mmol/L.

Six hours after pyridoxine ingestion the patient was fully alert with no further seizure activity. He was followed for possible transaminase elevations. On the 4th day after INH ingestion, he was discharged and no enzyme elevation was observed.

Discussion

Isoniazid is widely used in the management and prophylaxis of tuberculosis. INH intoxication can occur either as an accident or with the intent to commit suicide, and is not frequent in childhood. The signs of intoxication can be observed if INH is consumed in doses of more than 1.5 g at once, or 30 mg/kg[4,7,8].

Isoniazid is rapidly absorbed following oral ingestion, and the first signs of intoxication can occur 30-45 minutes after ingestion[4,7], although this period is sometimes prolonged to two hours. In our first case, the first sign was observed within two hours, and in the second case this period was 30 minutes. The intoxication symptoms are nausea, vomiting skin rashes, fever, ataxia, speech disorders, peripheral neuritis, and alterations in consciousness. These symptoms are usually followed by seizures and coma. The seizures are often refractory to anticonvulsants. Rerspiratory failure and death can follow. The mortality rate due to INH intoxication is significantly high if it is not treated. Death occurs rapidly if the amount of ingestion is approximately 80-150 mg/kg[4,7-12]. In

our first case INH was consumed at a dose of 30 mg/kg, and in our second case the total amount of ingested INH was 100 mg/kg. The laboratory findings of acute INH intoxication were elevated anion gap, metabolic acidosis, hypokalemia, hyperglycemia, ketonemia, transient elevation of liver enzymes, leukocytosis, positive disseminated intravascular coagulation panel, glucosuria, ketonuria and cerebrospinal fluid pleocytosis[2,6,8,9,11,12].

Pyridoxine (B_6) has a significant role in the function of more than 60 enzymes. One of these important roles is the synthesis of γ-amino-butyric acid (GABA), a significant inhibitory neurotransmitter in the brain. The active form of pyridoxine, pyridoxine 5 phosphate, is the cofactor of the two enzymes that are responsible for the degradation and the synthesis of GAB INH binds with the active form of pyridoxine to produce INH pyridoxal hydrasone. This molecule is excreted in the urine and results in the pyridoxine decrease. Due to the decrease in GABA synthesis, seizures can occur. As seen in our cases, these seizures are usually resistant to anticonvulsive agents[1,2,5,6,11,12].

Altered consciousness observed in INH toxicity has a wide range, from lethargy to coma. The etiology is thought to be multifactorial. The use of anticonvulsive agents and the postictal period are generally blamed. In our cases, acute alternations in consciousness were observed and this led us to believe that there might be other responsible factors.

Wason et al.[5] and Brent et al.[13] observed an early recovery in altered consciousness following the administration of pyridoxine. In our cases, consciousness improved rapidly after pyridoxine administration. In our first case, altered consciousness totally recovered six hours after pyridoxine administration; in our second case this period lasted three hours.

Severe metabolic acidosis can occur in INH intoxication[2,3,6,8,11-14]. Lactic acidosis due to resistant seizures and inhibition of lactate dehydrogenase results in metabolic acidosis. Generally it is not necessary to manage this condition with sodium bicarbonate treatment, but severe acidosis is an exception[7,8,12-14]. In our second case we observed severe metabolic acidosis which was responsive to sodium bicarbonate.

In 1963, Starke and Williams[11] suggested that high-dose pyridoxine administration is an effective therapy in INH intoxication. The authors observed that the administration of pyridoxine equal in dose to the ingested INH might be more effective in controlling seizure activity and improving clinical prognosis[5,12]. Due to the similarity of pyridoxine and INH molecules, the "gram for gram" principle has taken its place in the management of INH intoxication[5]. Brown[12] and Katz et al.[15] noted that if the dose of INH ingestion was unknown, 5 g of pyridoxine should be administered. In our cases, data about INH ingestion was collected from the parents. Pyridoxine was administered with an equal dose to INH, and no seizure activity was observed following treatment.

In the literature, intravenous administration is usually recommended, but it can be given orally if the intravenous form is not available[2-5,7,8]. In our first case, the intramuscular form of pyridoxine was used as the intravenous form was not available, and this treatment was also effective. In our second case, the intravenous form of pyridoxine was administered.

Due to high-dose pyridoxine, side effects such as tachypnea, postural reflex disorders, paralysis and seizure can be observed. A peripheral neuropathy has also been noted. However, there have been several cases with no side effects, even with the administration of 52 g pyridoxine[3-5,8,15-18].

It is suggested that repeating pyridoxine might be useful if the seizure activity continues after the first administration. In our cases, there was no need for a second administration[3,7,8].

In Turkey, and in the world at large, tuberculosis is still an important health problem. INH is the most frequently used agent in the treatment. Therefore, physicians should be aware of the management of INH intoxication and of the side effects of INH.

In conclusion, parenteral pyridoxine administration is an effective method in INH intoxication, so the intravenous form must be available in emergency care units. The toxic effect of INH should be explained to all patients. Keeping the drug away from children must be stressed, and manufacturers should be encouraged to imprave the packaging and labelling of their products.

REFERENCES

1. Starke JF. Tuberculosis. In: Nelson WE, Behrman RB, Kliegman RM, Arvin AM (eds). Textbook of Pediatrics (15th ed). Philadelphia: WB Saunders Company; 1996: 834-846.

2. Alvarez FG, Guntapalli K. Isoniazid overdose. Four case reports and review of the literature. Intensive Care Med 1995; 21: 641-644.

3. Black LE, Ros SP. Complete recovery from metabolic acidosis associated with isoniazid poisoning in a young boy. Pediatr Emerg Care 1989; 5: 257-258.
4. Clari F. The paradoxical anticonvulsive and awakening effect of high dose pyridoxine treatment for isoniazid intoxication. Arch Intern Med 1992; 152: 2346-2347.
5. Wason S, Lacouture PG, Lovejoy FH. Single high dose pyridoxine treatments for isoniazid overdose. JAMA 1981; 246: 1102-1104.
6. Miller J, Robinson A, Percy A. Acute isoniazid poisoning in childhood. Am J Dis Child 1980; 134: 290-292.
7. Olson KR, Kearney TE, Dyer JE, Benowitz NL, Blanc PD. Seizures associated with poisoning and drug overdose. Am J Emerg Med 1993; 11: 565-568.
8. Siefkin AD, Albertson TE, Corbett MG. Isoniazid overdose: pharmacokinetics and effects of oral charcoal in treatment. Hum Toxicol 1987; 616: 565-568.
9. Sullivan EA, Geoffroy P, Weisman R, Hoffman R, Frieden TR. Isoniazid poisoning in New York City. J Emerg Med 1998; 16: 57-59.
10. Shah BR, Santucci K, Siner R, Steiner P. Acute isoniazid neurotoxicity in an urban hospital. Pediatrics 1995; 95: 700-704.
11. Starke H, Williams S. Acute poisoning from overdose of isoniazid: a case report. Lancet 1963; 83: 406-408.
12. Brown CV. Acute isoniazid poisoning. Am Rev Respir Dis 1972; 105: 206-216.
13. Brent J, Vo N, Kulig K, Rumarck BH. Reversal of prolonged isoniazid induced coma by pyridoxine. Arch Intern Med 1990; 150: 1751-1753.
14. Romero JA, Kuczler FJ. Isoniazid overdose: recognition and management. Am Fam Physician 1998; 57: 749-752.
15. Katz BE, Carver MW. Acute poisoning with isoniazid treated by exchange transfusion. Pediatrics 1956; 18: 72-76.
16. Sievers ML, Herrier RN, Chin L, Picchioni AL. Treatment of isoniazid overdose. JAMA 1982; 247: 583-584.
17. Coyer JR, Nicholson DP. Isoniazid induced convulsions. South Med J 1976; 69: 294-297.
18. Albin RL, Albers JW, Greenerg HS, et al. Acute sensory neuropathy from pyridoxine overdose. Neurology 1987; 37: 1729-1732.

Lamellar ichthyosis: a case report

Hamit Özyürek[1], Ayşe Kavak[2], Murat Alper[3]

Departments of [1]Pediatrics, [2]Dermatology, and [3]Pathology, Abant İzzet Baysal University, Düzce Faculty of Medicine, Düzce, Turkey

SUMMARY: Özyürek H, Kavak A, Alper M. Lamellar ichthyosis: a case report. Turk J Pediatr 2002; 44: 58-60.

Ichthyoses are divided into four groups according to clinical, histopathologic and genetic findings. Lamellar ichthyosis is one of them. The incidence of lamellar ichthyosis is believed to be approximately 1 per 100,000 to 300,000 live births. It is characterized by large, polygonal, grayish brown, and tightly adherent scales. We report a four-year-old boy with desquamative lesions since birth who had six-year-old sister with similar lesions, suggesting an autosomal recessive inheritance. His skin biopsy revealed hyperkeratosis with lamellae. There were no associated hair or neurological abnormalities. His clinical and histopathological findings were typical for isolated lamellar ichthyosis. Because of its rare occurrence, we report this case with a review of the literature.

Key words: lamellar ichthyosis, treatment, ectropion.

Lamellar ichthyosis (LI) is a rare autosomally inherited disorder of keratinization. The incidence is believed to be approximately 1 per 100,000 to 300,000 live births. Males and females are affected equally, with no ethnic preponderance. The major distinguishing feature is clinically the presence of thick hyperkeratotic scaling all over the skin surface[1,2]. Here, we discuss an uncommon case of ichthyosis and review the literature.

Case Report

A four-year-old boy suffered from desquamative lesions since birth. His past history was nonsignificant except for repair of ectropion when he was two years old. He is the product of a consanguineous marriage. His parents have two sons and one daughter. The six-year-old daughter has a similar skin problem.

On admission, his physical examination revealed a height of 102 cm (50p), weight of 15 kg (25p), and large, brown, centrally attached scales covering the entire body including the flexural folds and nonpalpable left testis (Figs. 1 and 2). All other findings on physical examination were normal. His hematological and biochemical tests were within normal limits. In skin biopsy, hyperkeratosis with lamellae was found. The patient was diagnosed as LI with clinical and histopathologic findings.

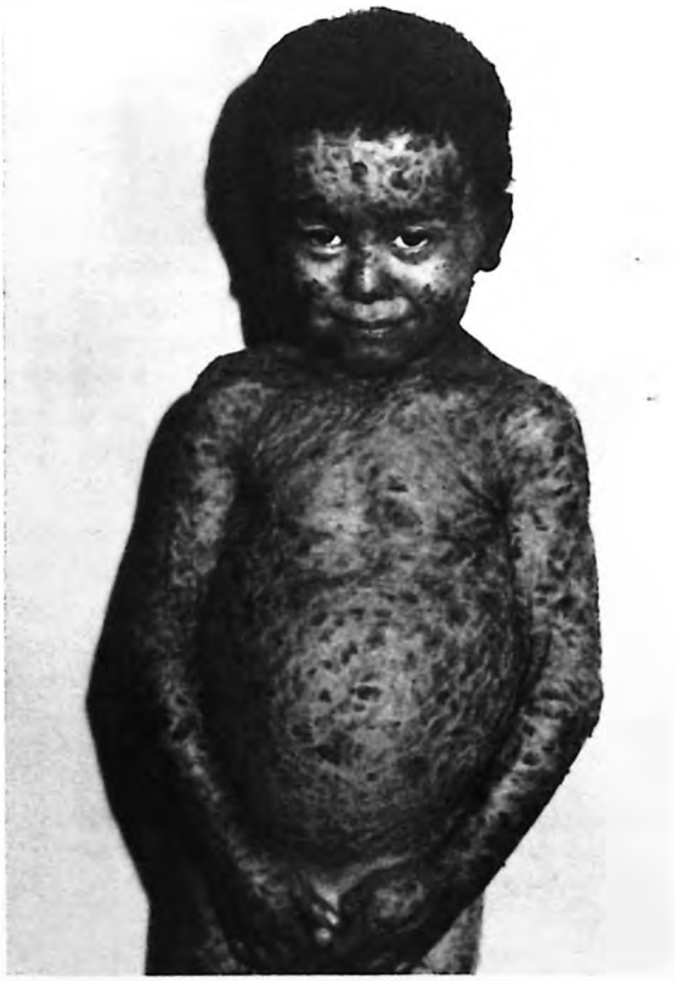


Fig. 1. General view of the patient.

Fig. 2. General view of the patient.

Discussion

The ichthyoses are a heterogeneous group of diseases that represent abnormalities in the formation and desquamation of the keratinocytes. There are four types, with different clinical, histopathologic and genetic findings (Table I). LI is one of them. LI is present at birth with collodion baby or becomes apparent soon thereafter and almost always involves the entire cutaneous surface. It is characterized by large, polygonal, grayish brown, and tightly adherent scales. Nails can be dystrophic. Palms and soles show thickening. Persistent ectropion of the eyelids is almost always present and is a helpful diagnostic sign. LI is generally inherited by an autosomal pattern[1-3].

The histologic findings of LI show proliferation, hyperkeratosis with acanthosis, hypergranulosis, and ortho- and parakeratosis. In the autosomal-dominant form, some ultrastructural similarities to the autosomal-recessive form are found, but it can be distinguished by a prominent regular keratin pattern of horny cells and the presence of only a few lipid inclusions in the corneocytes[1].

The mechanism of the disease is not yet known. Epidermal proliferation rates are normal or slightly elevated. In some families, transglutaminase 1 gene mutations have been identified as causative genetic defects[4,5]. This gene encodes the keratinocyte transglutaminase which is responsible for cross-linking epidermal proteins during formation of the stratum corneum. But the transglutaminase activity measured in some patients was within the normal range[6]. A new gene of lamellar ichthyosis was identified in a 6-cM interval on chromosome 19 during the examinations of nine large consanguineous families[3]. These results indicate that LI is a genetically heterogeneous disorder.

Alpha-hydroxy acids, urea-based creams, and propylene glycol are used for the aggressive moisturization which is the mainstay of treatment. Oral retinoids provide relief for LI, but the adverse effects of long-term maintenance therapy do not allow for prolonged use. Therefore topical retinoid appears to be an alternative approach in the treatment of lamellar ichthyosis[7]. Clinical trials with retroviral vectors expressing

Table I. Classification of Ichthyoses[2,8]

Type	Incidence	Genetics	Onset	Cause	Ectropion	Flexural affection	Prognosis
Ichthyosis vulgaris	1:250 (common)	Autosomal dominant	1-4 years	Profilaggrin expression ↓	No	No	Improves
Sex-linked ichthyosis	1: 2,000-6,000 males	Sex-linked recessive	Birth-1 year	Steroid sulfatase ↓	No	Occasional	Stable
Lamellar ichthyosis	1: 200,000-300,000	Autosomal recessive-dominant	Birth	Defect in transgluta-minase gene	Common	Always	Worsens
Epidermolytic keratosis	1: 300,000	Autosomal dominant	Birth-6 months	Defect in keratin 1 or 10	No	Always	Improves

transglutaminase 1 have been successful in the laboratory. Therefore gene therapy is the great hope of the future. Prenatal diagnosis can be done early in the first timester via chorionic villous sampling or prenatal ultrasonography. Characteristically, the ultrasonography demonstrates the collodian baby appearance during the intrauterine period. Intrauterine biopsying of the fetal skin is not useful because of false-positive and false-negative skin biopsy results[3,8].

In our case, the presence of ectropion, the persistence of skin lesions since birth, the autosomal recessive genetic pattern of inheritance as suggested by the parental consanguinity and histopathological findings revealed the diagnosis of LI. Characteristic hair findings (trichorrhexis invaginata) and neurological abnormalities which are seen in Netherton's syndrome and Sjögren-Larsson syndrome, respectively, which are associated with ichthyosis, were absent in our patient. For this reason, we considered the patient as isolated LI.

REFERENCES

1. Kolde G. Happle R. Traupe H. Autosomal-dominant lamellar ichthyosis: ultrastructural charcteristics of a new type of congenital ichthyosis. Arch Dermatol Res 1985; 278: 1-5.
2. Mansour AM, Traboulsi AI, Frangieh GT. Jarudi N. Unilateral magalocornea in lamellar ichthyosis. Ann Ophthalmol 1985; 17: 466-470.
3. Fischer J, Faure A, Bouadjar B, et al. Two new loci for autosomal recessive ichthyosis on chromosomes 3p21 and 19p12-q12 and evidence for further genetic heterogeneity. Am J Hum Gen 2000; 66: 904-913.
4. Tok J, Garzon MC, Cserhalmi-Friedman P, Lam HM, Spitz JL, Christiano AM. Identification of mutations in the transglutaminase 1 gene in lamellar ichthyosis. Exp Dermatol 1999; 8: 128-133.
5. Hennies HC, Raghunath M, Wiebe V, et al. Genetic and immunohistochemical detection of mutations inactivating the keratinocyte transglutaminase in patients with lamellar ichthyosis. Hum Genet 1998; 102: 314-318.
6. Huber M, Rettler I, Bernasconi K, Wyss M, Hohl D. Lamellar ichthyosis is genetically heterogenous-cases with normal keratinocyte transglutaminase. J Invest Dermatol 1995; 105: 653-654.
7. Steijlen PM, Reifenschweiler DO. Ramaekers FC, et al. Topical treatment of ichthyoses and Darier's disease with 13-cis-retinoic acid. A clinical and immunohistochemical study. Arch Dermatol Res 1993; 285: 221-226.
8. Shwayder T. Ichthyosis in a nutshell. Pediatr Rev 1999; 20: 5-12.

Oral acitretin treatment in severe congenital ichthyosis of the neonate

Z. Nurhan Saraçoğlu[1], Neslihan Tekin[2], Selim M. Ürer[1], İlham Sabuncu[1], Arif Akşit[2]

Departments of [1]Dermatology, and [2]Pediatrics, Osmangazi University Faculty of Medicine, Eskişehir, Turkey

SUMMARY: Saraçoğlu ZN, Tekin N, Ürer SM, Sabuncu İ, Akşit A. Oral acitretin treatment in severe congenital ichthyosis of the neonate. Turk J Pediatr 2002; 44: 61-64.

Two newborn infants with ichthyosis, one with lamellar ichthyosis and one with nonbullous ichthyosis form erythroderma, who presented at birth with a collodion baby appearance, were treated with acitretin (1 mg/kg/day). Clinical improvement was achieved shortly after treatment. The second case received oral retinoid for 3.5 months and was followed for nine months. The result was excellent. The treatment resulted in a satisfactory improvement in the skin condition of the first case. The tolerance to the drug was good. Side effects were not observed. It was concluded that early management of severe ichthyosis cases could prevent life-threatening events such as hyperthermia, disturbance in electrolyte and fluid balance, and infection.

Key words: congenital ichthyosis, collodion baby, acitretin, retinoids.

Ichthyosis is regarded as a disorder of keratinization or cornification, and is due to abnormal epidermal differentiation or metabolism[1]. Infants with this disorder may die of complications such as sepsis, or protein and electrolyte loss in the first months of life. For this reason, temperature control, electrolyte and fluid balance, caloric intake, and the prevention of infection are very important in newborn infants affected with severe generalized ichthyosis[2,3]. Since beneficial effects of systemic retinoids have been shown in the treatment of many disorders of keratinization[1], we tried acitretin in two newborn infants, one with lamellar ichthyosis and the other with nonbullous ichthyosiform erthyroderma (NBIE). They presented at birth as collodion babies and were treated with acitretin beginning soon after birth in addition to supportive treatment. The efficacy of treatment and the course of the disease are discussed.

Case Reports

Case 1

A three-and-a-half-hour-old male infant was admitted with erythema and abnormal appearance of the face. He was born at term as the first child of a 23-year-old mother and 25-year-old father with first-degree consanguinity. On physical examination weight was 2600 g (25^{th} percentile), length 50 cm (50^{th} percentile), and head circumference 34 cm (50^{th} percentile). His skin was parchment-like, with a yellowish film stretched over it, ectropion and eclabium were present, and nasal passages were obstructed. He was born as a collodion baby (Fig. 1). After shedding of the collodion membrane, generalized erythroderma with fine, white scales was evident. Laboratory findings were as follows: Hb 19.5 g/dl, WBC count 22,160/mm^3, platelet count 261,000/mm^3. Biochemical values of urine and blood were within normal limits. He was diagnosed as lamellar ichthyosis. Acitretin (1 mg/kg/day) in addition to supportive treatment in a humidified incubator with vaseline ointments and a prophylactic regimen of antibiotics were started. One month of follow-up resulted in improvement in skin lesions and he was discharged from the hospital (Fig. 2).

Case 2

An hour-old female infant was admitted with collodion baby appearance. She was the second child of a 27-year-old mother and 29-year-old father with first-degree consanguinity. On

physical examination weight was 3,480 g (50th percentile), length 50 cm (50th percentile), and head circumference 34 cm (50th percentile). Plate-like lesions covered the whole body, and ectropion and eclabium were also present. Laboratory findings were as follows: Hb 19.03 g/dl, WBC count 19,900/mm^3, and platelet count 244,000/mm^3. Biochemical values of urine and blood were within normal limits. After shedding of the collodion membrane, generalized scaly erythroderma was apparent (Fig. 3). She was diagnosed as NBIE. She was placed in a humidified incubator and treated with a prophylactic regimen of antibiotics. Artificial tears were used because of ectropion. Ointments containing vaseline for moisturization were used to reduce scaling. Oral retinoid treatment with acitretin (1 mg/kg/day) was administered. She was discharged from the hospital at nine days of age with topical vaseline application and oral retinoid treatment. At one and a half months of age oral retinoid treatment was discontinued because only slight erythematous areas were left on the trunk. At two months of age she was admitted with an increase in lesions with lamellar scaling of the skin. Oral retinoid treatment was restarted. She was hospitalized twice: first for septic arthritis and then for pneumonia. Oral retinoid treatment was discontinued at four and a half months of age. To date liver function tests have been within normal levels, and radiological evaluation was normal. On her last visit she was eight months old. Her skin was smooth (Fig. 4). Her mother was using vaseline ointment over her body twice daily. She could sit up straight with no support. Her weight was 6,400 g, length 67 cm, and head circumference 43.2 cm.

Fig. 1. Collodion membrane with eclabium, ectropion and obstructed nasal passages (Case 1).

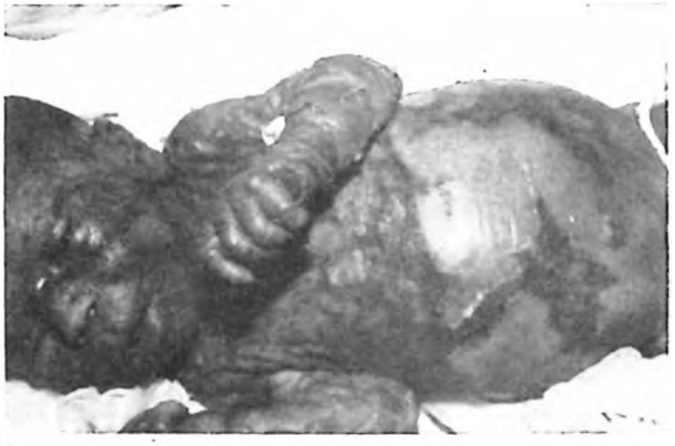


Fig. 2. Generalized scaly erythroderma at one month of age (Case 1).

Fig. 3. Generalized scaly erythroderma most prominent on the trunk (Case 2).

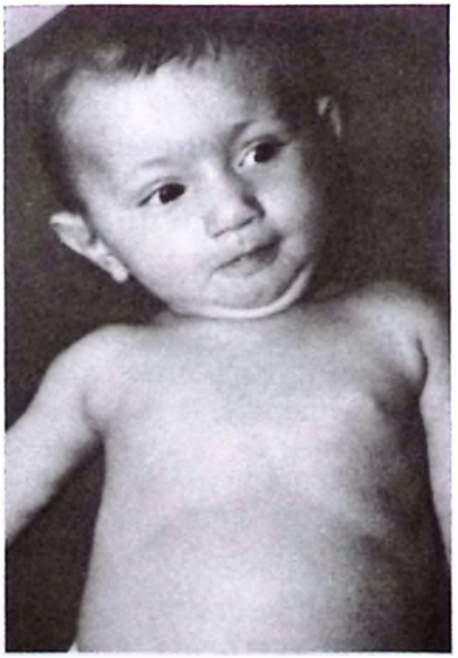


Fig. 4. After the treatment (at eight months of age) (Case 2).

Discussion

Severe generalized ichthyosis seen at birth is a life-threatening condition. Mortality is high due to complications such as marked temperature instability, skin irritation, water loss via the epidermis predisposing to hypernatremic dehydration, pyoderma, septicemia and pneumonia secondary to aspiration of squamous material in the amniotic fluid[4]. Cutaneous infections are common problems. Larreque et al.[5,6] reported a mortality rate as high as 33% in 267 collodion babies in 1976 and as 11% in 1984. Öztürk et al.[3], in their patient group of 16 collodion babies, reported a mortality rate of 25% and major complications were hypernatremia, cutaneous infection and sepsis.

These two infants were born as collodion babies. After shedding of the membranes in the second case, scaling affected the whole skin surface. Ectropion and eclabium were noted as severe as in the first infant. In the first case, after shedding of the collodion membrane, generalized scaly erythroderma was apparent and affected all areas including scalp, ears, face, flexures, palms and soles.

In severe congenital ichthyosis cases, general principles of treatment are maintainance of electrolyte and fluid balance, control of body temperature, and prevention of infection with a prophylactic regimen of intravenous antibiotics[1-3]. Management of ichthyosis primarily consists of daily hydration and lubrication of the skin. Erdem[7] reported five successfully treated cases with a topical preparation containing 5% lactic acid. Use of 12% ammonium lactate lotion, or a lactic, citric or ureic ointment base were also recommended[4]. Both patients presented here were placed in a humidified incubator. Daily sponge baths and application of emollients containing vaseline were introduced.

Systemic retinoids are effective in many disorders of keratinization, and have been shown to be helpful in reducing scaling, pruritus and erythema in most patients with severe congenital ichthyosis[1]. Oral retinoid treatment with isotretinoin, etretinate, or acitretin may be required for some patients. El-Ramly et al.[8] presented eight cases of ichthyosis treated with retinoids; the results were responses in five lamellar ichthyosis cases, while none of the patients with nonbullous or bullous congenital ichthyosiform erythroderma gave more than a slight response. On the other hand it was reported that aromatic retinoid treatment resulted in a satisfactory improvement in the skin conditioning of three patients with nonbullous congenital erythroderma[9]. Tamayo et al.[10] also recommended oral retinoid treatment after their experience in eight children with lamellar ichthyosis. In our two cases clinical course was impressive. After induction of oral retinoid in the early neonatal period, collodion membranes detached completely in a few days.

Long-term therapy with oral retinoids has provoked anxiety regarding the adverse effects on bone mineralization. In humans, the most commonly reported effect is the production of osteophytes and the calcification of ligaments. Other studies in humans have reported decreases in the radiologic appearance of bone[11,12]. Paige et al.[13] observed no evidence of skeletal toxicity in 42 children treated over an 11-year period. Usage of aromatic retinoid for more than two years in three lamellar ichthyosis cases resulted in a satisfactory improvement without any major side effects. Cheilitis, mild dryness of mucous membranes, slight hair loss, and pruritus are other side effects which were not detected in our cases[12]. In this report the result was satisfactory in the first case and excellent in the second case. Although some severe congenital ichthyosis cases heal completely in time, mortality due to the complications is high in the neonatal period. Because of its efficacy, good tolerance and easy administration, the oral retinoid acitretin is a treatment of choice for severe congenital ichthyosis forms.

REFERENCES

1. Griffiths WA, Judge MR, Leigh IM. Disorders of keratinization. In: Champion RH, Burton JL, Burns DA, Breatnach SM (eds). Textbook of Dermatology (6th ed) Vol II. London: Blackwell Science Co; 1998: 1483-1529.
2. Akiyama M. Severe congenital ichthyosis of the neonate. Int J Dermatol 1998; 37: 722-728.
3. Öztürk A, Çaksen H, Çetin N, Kurtoğlu S. A retrospective study on 16 collodion babies. Turk J Pediatr 1997; 39: 55-59.
4. Margileth AM. Dermatologic conditions. In: Avery GB, Fletcher MA, Macdonald MG (eds). Neonatology, Pathophysiology and Management of the Newborn (5th ed). Philadelphia: J.B. Lippincott Co; 1999: 1323-1360.
5. Larreque M, Bressieux JM, Fournet JP. Collodion baby. Mod Probl Paediatr 1976; 20: 40-49.
6. Larreque M, Ottavy N, Bressieux JM, Lorette J. Collodion baby: 32 new case reports. Ann Dermatol Venereol 1986; 113: 773-785.

7. Erdem G. Treatment of collodion babies with a topical preparation containing lactic acid. Turk J Pediatr 1982; 24: 97-101.

8. El-Ramly M, Zachariae H. Long-term oral treatment of two pronounced ichthyotic conditions: lamellar ichthyosis and epidermolytic hyperkeratosis with aromatic retinoid, Tigason (Ro 10-9359). Acta Derm Venereol 1983; 63: 452-456.

9. Frenk E. Oral treatment of lamellar icthyhyosis (non-bullous congenital ichthyosiform erythroderma) with an aromatic retinoid. Dermatologica 1981; 162: 91-103.

10. Tamayo L, Ruiz-Maldonado R. Oral retinoid (Ro 10-9359) in children with lamellar ichthyosis, epidermolytic hyperkeratosis and symmetrical progressive erythrokeratoderma. Dermatologica 1980; 161: 305-314.

11. Margolis DJ, Attie M, Leyden JJ. Effects of isotretinoin on bone mineralization during routine therapy with isotretinoin for acne vulgaris. Arch Dermatol 1996; 132: 769-774.

12. DiGiovanna JJ, Sollitto RB, Abangan DL, Steinberg SM, Reynolds JC. Osteoporosis is a toxic effect of long-term etretinate therapy. Arch Dermatol 1995; 131: 1263-1267.

13. Paige DG, Judge MR, Shaw DG, Atherton DJ, Harper JI. Bone changes and their significance in children with ichthyosis on long-term etretinate therapy. Br J Dermatol 1992; 124: 387-391.

A huge gastric stromal tumor in a 13-year-old girl

Pelin Oğuzkurt[2], Zuhal Akçören[2], M. Emin Şenocak[1], Melda Çağlar[2], Nebil Büyükpamukçu[1]

Departments of [1]Pediatric Surgery and [2]Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Oğuzkurt P, Akçören Z, Şenocak ME, Çağlar M, Büyükpamukçu N. A huge gastric stromal tumor in a 13-year-old girl. Turk J Pediatr 2002; 44: 65-68.

A 13-year-old girl presenting with severe anemia was diagnosed to have a large gastric tumor protruding toward the antrum with two central ulcerations. Partial gastrectomy including antrectomy and gastroduodenostomy were performed. Histologic and immunohistochemical studies revealed one of the most uncommon gastric tumors in children; a gastrointestinal stromal tumor. Close follow-up of the patient with endoscopy, abdominal ultrasonography and/or computed tomography in three to six month intervals revealed no recurrences or metastasis of the tumor following its complete excision.

Key words: gastrointestinal stromal tumor, stomach, immunohistochemistry, childhood.

Gastrointestinal stromal tumors (GIST) constitute the most uncommon category of primary nonepithelial tumors of the stomach and small bowel. They are supposed to arise from the cells located in the walls of the organs[1]. GIST have a wide variability in their clinical behavior, and malignant potential is often difficult to predict. Although there are no exact criteria for determining the malignant condition and prognosis of GIST, there are some variables that affect the prognosis[2].

To the best of our knowledge the presented case is the youngest patient with gastric stromal tumor[2]. Although the patient did not fulfill the criteria for a manifest malignancy, the tumor seemed to have had a malignant potential. Because of the rarity of GIST in childhood and limited information about the long-term survival and life expectancy, periodic endoscopy and radiologic investigations seem to be important in the follow-up of these patients.

Case Report

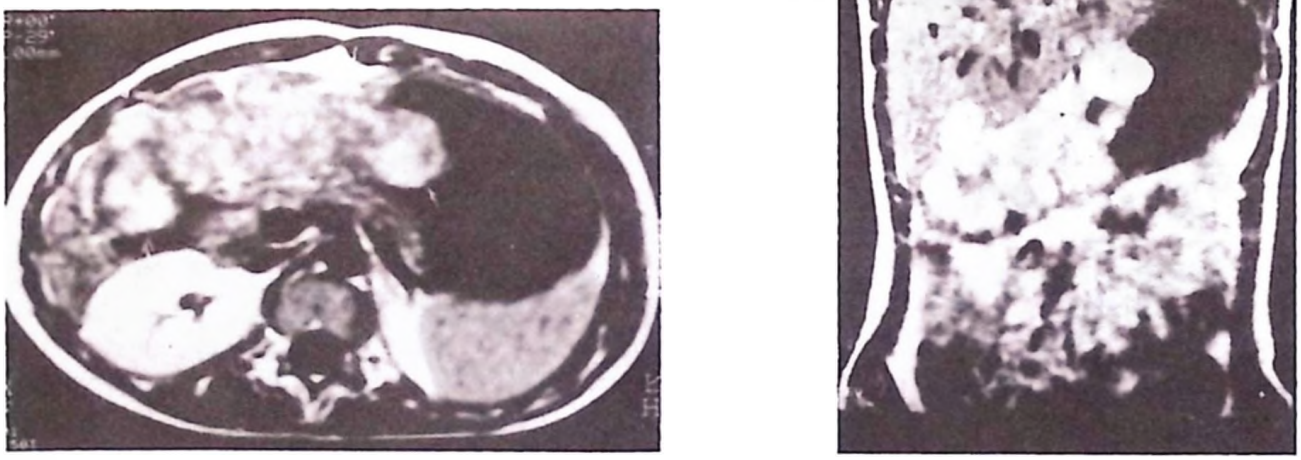
A 13-year-old girl presented with easy fatigability, paleness and anemia of two years' duration. Laboratory investigation revealed low Hb and Htc levels (6.0 g/dl and 19.9%, respectively), a moderate deficiency of serum iron (38 µg/dl) and ferritin (8.5 µg/dl) with normal WBC and platelet counts. The occult blood in stool was strongly positive. Abdominal ultrasonography (US) showed a tumor diffusely infiltrating the stomach from the lesser curvature through the antrum, extending into the gastric lumen and exophytically outside the gastric wall. No other metastatic lesions or lymph nodes were identified. Magnetic resonance imaging (MRI) confirmed the mass (Fig. 1a and b). Gastroscopy that was performed just before the operation revealed an ulcerated mass protruding into the gastric lumen which bled easily on touch. During laparotomy, a multilobulated exophytically developed large mass originating from the lesser curvature was found. The tumor mass extended through the antrum and protruded into the lumen but did not obstruct the first part of the duodenum (Fig. 2a). Two deep ulcerations were present on the mucosal surface (Fig. 2b). Frozen section performed from the serosal nodules revealed a benign tumor suggestive of leiomyoma. Partial gastrectomy including antrectomy with a safe margin was performed. The corpus was tubularized and gastroduodenostomy was done. Microscopic examination of the tumor with H&E staining showed fusiform cells with acidophilic cytoplasm and round-to-polygonal cells with central nucleus in most of the areas, resembling a leiomyoma (Fig. 3a). Mitotic activity was low, less than 5 per 50 high-pover fields. Some areas showed features of neural differentiation and were composed of palisading spindle cells (Fig. 3b). Immunohistochemical staining with antibodies to S-100 protein

(DAKO, antiserum), neuron specific enolase (DAKO, clone: BBS/NC/VI-H 14), and smooth muscle actin (DAKO, clone: 1A4) were all negative. These histopathological and immunohistochemical findings were consistent with GIST, lacking differentiation toward any cell type.

Postoperative course of the patient was uneventful. Three months after the operation the patient had a normal Hb level and had no complaints with a normal gastroscopic examination. The abdominal US and computed tomography findings did not reveal any mass one year after the operation.

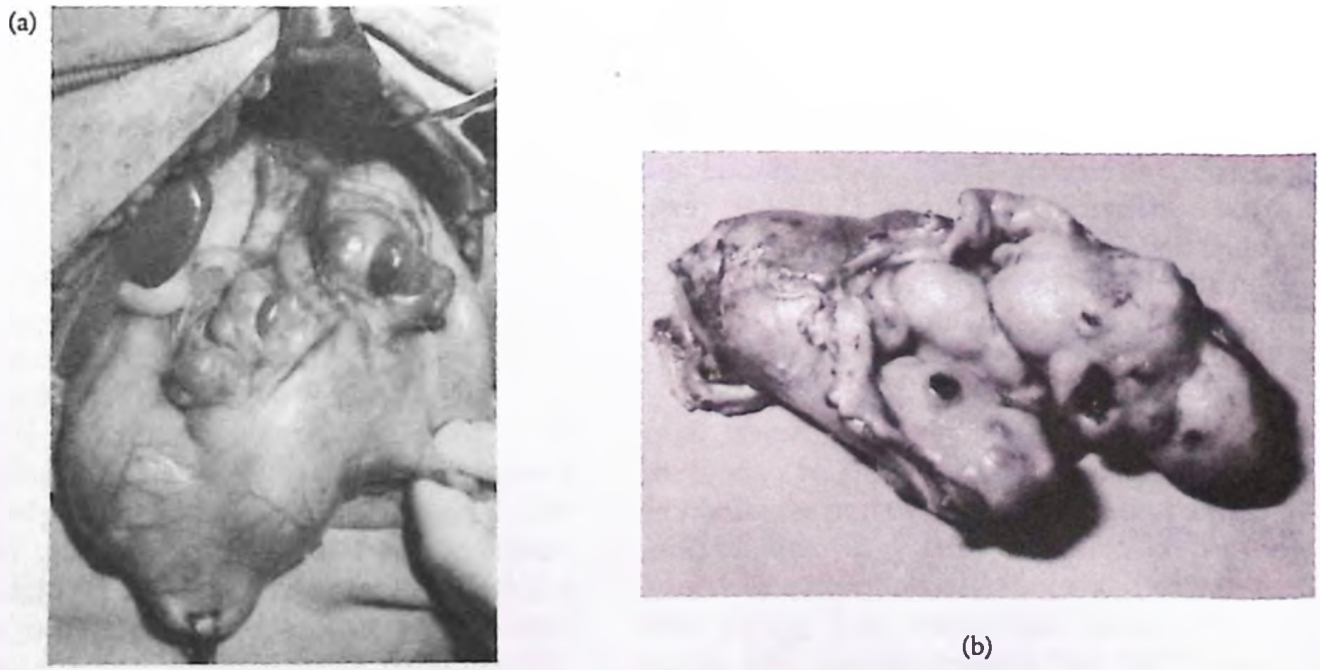
Discussion

Mesenchymal tumors of the gastrointestinal system arise from the cells located in the wall of the stomach and small bowel[1]. They are a heterogeneous group of tumors with unclear cell lineage[3,4]. However, immunohistochemical studies using S-100 protein, desmin, vimentin and smooth muscle actin show that gastrointestinal



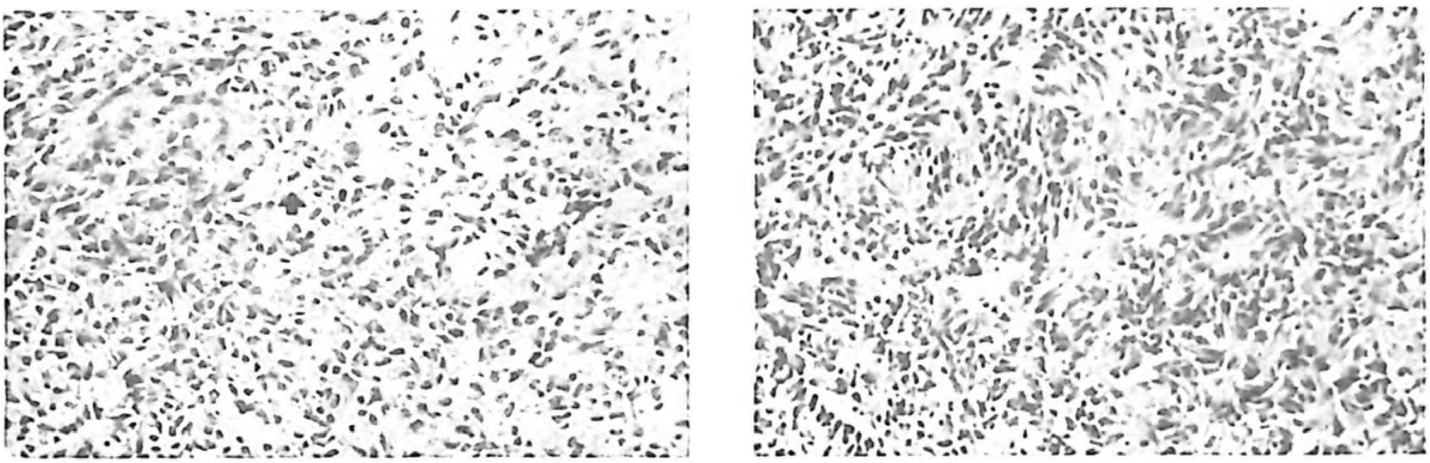
(a) (b)

Fig. 1. a) Transverse section of magnetic resonance image showing the huge tumor (arrows) originating from the lesser curvature of the stomach b) The contour of the mass (arrows) extending exophytically outside the stomach wall and protruding into the gastric lumen in the coronal section.



(a) (b)

Fg. 2. a) Gross appearance of the large tumor mass. b) The mucosal surface of the mass showing deep ulcerations (arrows) protruding into the gastric lumen.



(a) (b)

Fig. 3. a) Tumor area resembling leiomyoma with round-to-polygonal cells and some fusiform cells with scanty mitoses (arrow) (H&E stain, original magnification x 66). b) Tumor area with palisading spindle cells resembling neural differentiation (H&E stain, original magnification x 66).

mesenchymal tumors may be composed of cells showing differentiation toward smooth muscle cells and neural elements, dual differentiation toward both elements or showing no differentiation toward any cell type[1,3]. Tumors in the last category are undifferentiated tumors and are referred to as GIST[3].

Gastric stromal tumors (GST) may be asymptomatic for a long period of time. Symptomatic stromal tumors usually present with occult bleeding or severe anemia[5]. Neither malignant nor benign GST cause obstructive symptoms or symptomatic metastasis causing pain or weight loss[5]. Although endoscopy is indicated and demonstrate[5] the tumor mass, diagnostic yield of endoscopic biopsy is low because the mucosa may only show nonspecific pathologic changes[6]. Upper gastrointestinal series, abdominal US or computed tomography indicates the gastric tumor[5]. A spherical gastric filling defect with rounded regular edges and a smooth overlying mucosa suggest GST[5]. These tumors most frequently metastasize to omentum, liver or peritoneum. The above-mentioned features of GST differentiate them from other gastric lesions such as lymphoma and adrenocarcinoma[5].

Tumors smaller than 5 cm in diameter confined to the stomach have been reported to have a favorable outcome[3,6,7]. A high mitotic rate (over 5 mitoses per 50 high power field), cellularity and nuclear atypia are classifed as malignant but a low mitotic index does not indicate a benign course[5,6]. It is extremely difficult to distinguish benign lesions from malignant ones either by frozen section at the time of surgery or by postoperative routine histopathologic and immunohistochemical analyses[5,6]. Other factors in determining malignancy are resectability and/or presence of metastasis[6]. Tumor necrosis and mucosal ulcerations have also been stated to have a worse prognosis[3]. The tumor should be completely resected with negative tumor margins. These tumors have not been proven radiosensitive and no efficacious chemotherapy has been documented[5,8].

Although microscopic examination of the tumor with H&E revealed a leiomyoma-like pattern, some areas showing features of neuronal differentiation necessitated immunohistochemical staining with antibodies to S-100 protein, neuron specific enolase and smooth muscle actin, which were all negative, and the tumor was diagnosed as GIST of the stomach. In our case although the huge tumor size, serosal invasion and mucosal ulcerations were factors indicating poor prognosis, absence of high mitotic rate and nuclear atypia, total resection of the tumor with safe margins and absence of metastasis were more promising for a better outcome.

Gastric stromal tumors are uncommon in children. Because of the rarity of the disease, particularly in childhood, the evaluation of malignant potential, surgical treatment, adjuvant therapies and prognosis of the disease depend on the experiences of adult series. An upper abdominal mass with severe anemia in an otherwise healthy individual should suggest a gastric stromal tumor. An aggressive initial surgical approach seems to be the best treatment of these lesions. The unpredictable clinical and

histological behavior of the tumor necessitates a long-term follow-up of these patients with endoscopy and radiologic investigations.

REFERENCES

1. Rosai J. Ackerman's Surgical Pathology. Vol I. (8th ed) St Louis: Mosby; 1996: 645-647.
2. Ueyama T, Guo KJ, Hashimoto H, Daimaru Y, Enjoji M. A clinicopathologic and immunohistochemical study of gastrointestinal stromal tumors. Cancer 1992; 69: 947-955.
3. Ishii T, Kuyama Y, Obara M, Yamanaka M, Imamura TH. Gastrointestinal stromal tumor of stomach. Intern Med 1997; 36: 392-397.
4. Kodet R, Snajdauf J, Smelhaus V. Gastrointestinal autonomic nerve tumor: a case report with electron microscopic and immunohistochemical analysis and review of the literature. Pediatr Pathol 1994; 14: 1005-1016.
5. Basson MD, Modlin IM, Flynn SD. Current clinical and pathologic perspectives on gastric stromal tumors. Surgery 1992; 175: 447-489.
6. Sanders L, Silverman M, Rossi R, Braasch J, Munson L. Gastric smooth muscle tumors: diagnostic dilemmas and factors affecting outcome. World J Surg 1996; 20: 992-995.
7. Valente PT, Fine BA, Parra C, Schroeder B. Gastric stromal tumor with peritoneal nodules in pregnancy: tumor spread or rare variant of diffuse leiomyomatosis. Gynecol Oncol 1996; 63: 392-397.
8. Honda K, Mikami T, Ohkusa T, et al. Gastrointestinal autonomic nerve tumor with giant abscess. J Clin Gastroenterol 1997; 24: 280-285.

Familial secundum atrial septal defect with dysrhythmia associated with web neck

Zübeyir Kılıç, Birsen Uçar, Ferruh Baş, Ener Çağrı Dinleyici, Ersin Sarı

Department of Pediatrics, Osmangazi University Faculty of Medicine, Eskişehir, Turkey

SUMMARY: Kılıç Z, Uçar B, Baş F, Dinleyici EÇ, Sarı E. Familial secundum atrial septal defect with dysrhythmia associated with web neck. Turk J Pediatr 2002; 44: 69-72.

Most cases of atrial septal defect occur sporadically, but a few families have the defect as a genetic abnormality. A family having familial type secundum atrial septal defect with dysrhythmia associated with web neck is reported. In this family, two female siblings aged 11 (Case 1) and 4 years (Case 2) and their father had secundum atrial septal defect. Case 1 presented with two year history of syncope attacks and Case 2 with easy fatigability since early childhood. Both sisters also had web neck as a solitary anomaly. Electrocardiograms revealed prolonged PR interval and right bundle-branch block in both cases. In Case 1 first-degree atrioventricular block and Mobitz type I and II block were observed in Holter monitoring. Echocardiographical examination showed secundum atrial septal defect in both sisters. A permanent pacemaker was implanted in Case 1, and then atrial septal defects in both patients were surgically repaired; no postoperative complaints were observed. The father had been diagnosed as having atrial septal defect when he was 35 years sold, and first-degree atrioventricular block and atrial flutter developed after open heart surgery. In conclusion, the association of secundum atrial septal defect and prolongation of PR interval should be considered as familial occurrence of atrial septal defect. Identification of atrial septal defect in more than one family member should prompt clinical evaluation of all relatives.

Key words: dysrhythmia, familial atrial septal defect, web neck.

Secundum atrial septal defect (ASD) is one of the more common congenital cardiac defects to occur as an isolated lesion. It represents about 6 to 10% of all cardiac anomalies encountered and is more frequent in females than in males (2:1). It is estimated that an ASD occurs in 1:1,500 live births[1]. Most cases of ASD occur sporadically; however, a few families have the defects as a genetic abnormality. Autosomal dominant inheritance is possible for ASD with or without atrioventricular block[2,3]. Familial forms are characterized by the same type of ASD, and are frequently associated with other cardiac, osteoarthricular (Holt-Oram syndrome) or atrioventricular conduction abnormalities[4].

The electrocardiogram (ECG) in ASD usually reveals normal sinus rhythm; however, in a small number of patients, usually older, atrial dysrhythmias including atrial fibrillation, atrial flutter, conduction abnormalities, and sick sinus syndrome may be observed in the natural history of ASD, as well as after open heart surgery[1,5,6].

Herein, we report a family having familial type secundum ASD with conduction defects and atrial dysrhythmia associated with web neck.

Case Reports

Case 1

An 11-year-old girl was admitted with syncope attacks and palpitation. She had two-year history of palpitation attacks during effort and excitement followed by unconsciousness lasting for aproximately five minutes. She was the first child of a nonconsanguineous 41-year-old mother and 37-year-old father. Her father's past history revealed that he had been diagnosed as having secundum ASD when he was 35 years

old, and his defect had been repaired by open heart surgery. Complete atrioventricular block, atrial fibrillation and atrial flutter had developed one year after the operation, and a permanent pacemaker was implanted. Case 1 had a four-year-old sister with secundum ASD and web neck and a 10-year-old healthy sister.

On physical examination, she was conscious, and her general condition was well, with a heart rate of 96/min and blood pressure of 90/60 mmHg. Web neck deformity and low hair-line were present (Fig. 1). On cardiac auscultation, fixed splitting of the second heart sound in addition to the grade 2/6 systolic ejection murmur at the second left intercostal space was heard.

Chest X-ray showed a prominent pulmonary trunk. ECG showed sinusal bradycardia, first-degree atrioventricular block with a PR interval of 0.28 s, and incomplete right bundle-branch block (Fig. 2). On echocardiographic examination, an ASD of secundum type with a dimension of 12 mm at the region of fossa ovale, paradoxal septal movement, and left-to-right shunt at the atrial level were detected. Sinusal bradycardia (minimum heart rate 45/min), first-degree atrioventricular block and Mobitz type I and type II blocks were observed in Holter monitoring. Ultrasongraphic examinations of the abdomen and pelvis were normal. Chromosome analysis showed normal 46, XX pattern.

Syncope attacks of the patient were controlled with the implantation of a permanent pacemaker, and then her ASD was surgically repaired. Now the patient is well with no recurrence of her complaints.

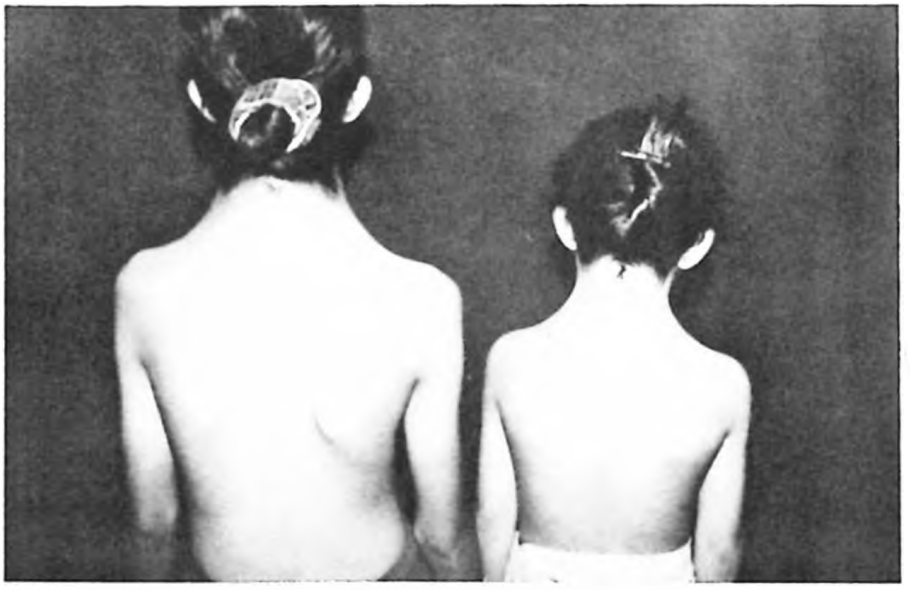


Fig. 1. Web neck deformities and low hair lines of the patients.

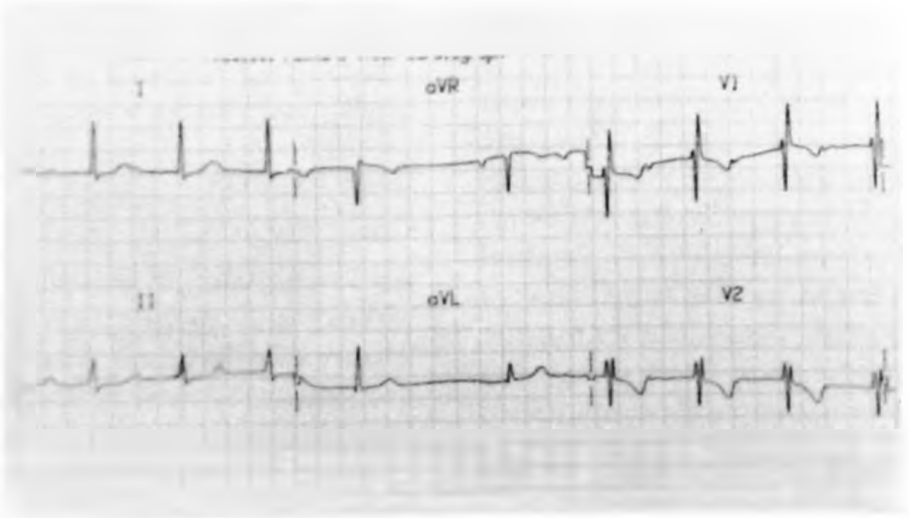


Fig. 2. Electrocardiogram (ECG) of Case 1 showing the prolongation of the PR interval and incomplete right bundle-branch block.

Case 2

A four-year-old female patient and the sister of Case 1 was admitted with easy fatigability recognized since early childhood. On physical examination, she was conscious, and her general condition was well with a heart rate of 92/min and blood pressure of 100/60 mmHg. Web neck deformity and low hair line were present (Fig. 1). On cardiac auscultation, fixed splitting of the second heart sound in addition to the grade 2-3/6 systolic ejection murmur at the second left intercostal space was heard.

Chest X-ray showed increased pulmonary vascular marking. ECG revealed incomplete right bundle-branch block and prolonged PR interval of 0.22 s. On echocardiographic examination, an ASD of the secundum type with a dimension of 13-14 mm at the region of fossa ovale, paradoxal septal movement, dominance of the right atrium and right ventricle, and left-to-right shunt at the atrial level were detected. Chromosome analysis showed normal 46, XX pattern. ASD of the patient was surgically repaired and no postoperative complaints were observed.

Discussion

Although most cases of ASD occur sporadically, the defect may show a familial pattern[1,2]. Autosomal dominant inheritance is possible for these cases[2,3]. The presence of the defect in both the father and his two daughters in the presented family suggests an autosomal dominant trait. Only a few families have been reported in whom the ASD is transmitted according to an autosomal dominant inheritance[2,3,6].

The ECG in ASD usually reveals normal sinus rhythm; however, in a small number of patients, usually older, a junctional rhythm or a supraventricular tachyarrhythmia, such as atrial flutter, can be seen. In most patients, the mean frontal plain QRS axis is to the right, from +90 to +170 °. The PR interval may be prolonged, especially in older patients, because of intraatrial and sometimes H-V conduction delay that results in first-degree atrioventricular block[1]. Prolonged PR intervals have been found in 5-15% of cases of ASD[2]. However, the incidence of PR prolongation in familial cases of ASD has been reported to be between 75-100%[8-11]. According to this literature knowledge, and from our findings, it seems that prolongation of atrioventricular conduction is more common among familial cases of ASD. Thus, the association of secundum ASD and prolongation of PR interval should be considered as familial occurrence of ASD. This point should be considered in genetic counseling. In contrast, some authors have reported some families with secundum ASD without PR prolongation[3,7]. Lynch et al.[12] suggested the existence of at least two distinct hereditary varieties of ASD, one with and one without a prolonged PR interval. Both of our cases and their father had prolonged PR interval in ECG, and one of the siblings (Case 1) also had Mobitz type I and type II blocks. In about half the cases, P wave changes suggest right atrial enlargement. There is also some variant of the rsR' or RSR' pattern ("incomplete right bundle-branch block" pattern) in lead V_1, consistent with right ventricle volume overload, as seen in our two both patients. The duration of the QRS complex is less than or equal to 0.1 second, and R' in lead V_1 is somewhat prolonged[1].

Atrial arrhythmias are the most common late problems following closure of the defect. But unrepaired ASDs are also associated with atrial dysrhythmia, particularly in older patients. Atrial fibrillation and atrial flutter are not common complications of ASD in children but are seen often in adult patients[5]. The father of our patients had experienced complete atrioventricular block, atrial fibrillation and atrial flutter during the postoperative period.

Sinus node dysfunction is one of the complications following surgery for ASD[1,2]. In the past, it has been thought that this arrhythmia was the consequence of either the surgical trauma to the artery supplying blood to the sinus node or damage to the sinus node area[13]. However, the sinus node function of these patients has rarely been investigated preoperatively. Some studies based on a standard ECG were able to detect sinus node dysfunction preoperatively in a limited number of patients[14]. However, preoperative electrophysiologic measurements in patients of all ages detect conduction abnormalities in as many as 40% of patients[1,5,15,16]. Apparent sinus node dysfunction has been reported as a result of finding abnormal corrected sinus node recovery times and sinoatrial conduction times; however, clinically, only rare patients have abnormal findings in resting ECGs or on 24-hour ambulatory monitoring. Perhaps these abnormal electro-

physiologic findings are due to an imbalance of the autonomic nervous system control of the sinoatrial and atrioventricular nodes. Intraatrial conduction time is prolonged in the older patient, and right atrial effective refractory periods are increased in some patients. Patients with both findings may be predisposed to atrial arrhythmias[1].

In addition, both of our cases had web neck deformity which might have been a solitary anomaly or associated with a genetic syndrome such as Ullrich-Turner, Noonan or Down syndromes[17]. Since our cases did not have other phenotypic features of these syndromes, was believed that they had web neck deformity as a solitary anomaly. It has been reported that 60% of infants with web neck had congenital heart defect, with a high incidence of flow-related defects such as hypoplastic left heart, coarctation, and secundum ASD[17]. This association implies a pathogenetic relationship and appears to be independent of causal factors[17]. Therefore, the finding of web neck on a prenatal ultrasound or newborn examination should prompt a search for congenital heart defect.

Familial secundum ASD is probably more frequent than commonly reported since cardiological examination of the relatives is not routinely performed in every case of apparently sporadic ASD. In conclusion, identification of prolongation of the PR interval in a case of secundum ASD should prompt clinical evaluation of all relatives. In addition, all patients with ASD should be carefully evaluated for conduction defects and atrial dysrhythmias.

REFERENCES

1. Porter CJ, Feldt RH, Edwards WD, Seward JB, Schaff HV. Atrial septal defects. In: Emmanouilides GC, Riemenschneider TA, Allen HID, Gutgesell HP (eds). Moss and Adams Heart Disease in Infants, Children, and Adolescents (5th ed) Vol. 1. Baltimore: Williams&Wilkins; 1995: 687-703.
2. Günal N, Gül S, Kahramanyol Ö. Familial atrial septal defect with prolonged atrioventricular conduction. Acta Paediatr Jpn 1997; 39: 634-636.
3. Li Volti S, Distefano G, Garozzo R, Romeo MG, Sciacca P, Mollica F. Autosomal dominant atrial septal defect of ostium secundum type. Report of three families. Ann Genet 1991; 34: 14-18.
4. Cachat F, Rapatsalahy A, Sekarski N, Hurni M, von Segesesr L, Payot M. Three different types of atrial septal defects in same family (Abstract). Arch Mal Coeur Vaiss 1999; 92: 667-669.
5. Fyler DC, Atrial septal defect secundum. In: Fyler DC (ed). Nadas' Pediatric Cardiology. Philadelphia: Hanley&Belfus, Inc.; 1992: 513-524.
6. Friedli B. Arrhythmias in adolescent and adult with a congenital heart defect. Schweiz Med Wochenschr 1993; 123: 2065-2071.
7. Tsuchioka Y, Kawagoe T, Hondo T, et al. Secundum atrial septal defect in two families (Abstract). Kokyu To Junkan 1990; 38: 93-96.
8. Bizarro RO, Callahan JA, Feldt RH, Kurland LT, Gordon H, Bradenburg RO. Familial atrial septal defect with prolonged atrioventricular conduction: a syndrome showing the autosomal dominant pattern of inheritance. Circulation 1970; 41: 677-683.
9. Weil MH, Allenstein BJ. A report of congenital heart disease in five members of one family. N Engl J Med 1961; 265: 661-666.
10. Amarasingham R, Heming HA. Congenital heart disease with arrhythmia in a family. Br Heart J 1967; 29: 78-80.
11. Kahler RL, Braunwald E, Plauth WH. Familial congenital heart disease: familial occurrence of atrial septal defect with atrioventricular conduction abnormalities; supravalvular aortic and pulmonic stenosis and ventricular septal defect. Am J Med 1966; 40: 384-390.
12. Lynch HT, Bachenberg K, Harris RE, Becker W. Hereditary atrial septal defect. Update of a large kindred. Am J Dis Child 1978; 132: 600-604.
13. Tung KS, James TN, Effler DB, McCormack LJ. Injury to the sinus node in open heart operations. J Thorac Cardiovasc Surg 1967; 53: 814-829.
14. Siltanen P. Atrial septal defect of secundum type in adults. Clinical and hemodynamic studies of 129 cases before and after surgical correction under cardiopulmonary bypass. Acta Med Scand 1968; 183-184 (Suppl): 497-520.
15. Bolens M, Friedly B. Sinus node function and conduction system before and after surgery for secundum atrial septal defect: an electrophysiologic study. Am J Cardiol 1984; 53: 1415-1450.
16. Karpawich PP, Antillon JR, Cappola PR, Agarwal KC. Pre- and postoperative electrophysiologic assessment of children with secundum atrial septal defect. Am J Cardiol 1985; 55: 519-521.
17. Berdahl LD, Wenstrom KD, Hanson JW. Web neck anomaly and its association with congenital heart disease. Am J Med Genet 1995; 56: 304-307.

Peripheral facial paralysis as initial manifestation of hypertension in a child

F. Müjgan Aynacı, Yaşar Şen

Department of Child Neurology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey

SUMMARY: Aynacı FM, Şen Y. Peripheral facial paralysis as initial manifestation of hypertension in a child. Turk J Pediatr 2002; 44: 73-75.

Hypertension is one of the rare causes of peripheral facial paralysis in children. The unawareness of this association at presentation may cause serious medical errors and result in delays in the diagnosis of hypertension, which may worsen with corticosteroid therapy given for Bell's palsy. We describe a severely hypertensive child who was first seen with peripheral facial paralysis and given corticosteroid therapy in another hospital. She presented to our clinic during the second facial paralysis attack with hypertensive pontine hemorrhage.

Key words: peripheral facial paralysis, hypertension, pontine hemorrhage.

Facial paralysis can be a rare initial feature of severe hypertension in children[1]. Even so, practitioners, pediatricians, neurologists and otorhinolaryngologists may be unaware of this association which may result in delay of the diagnosis of hypertension. Our case is an example of this problem which resulted in delayed treatment of hypertension and pontine hemorrhage.

Case Report

A nine-year-old female patient was first seen in another hospital with right peripheral facial paralysis. The diagnosis of idiopathic peripheral facial paralysis (Bell's palsy) was established and short-term methylprednisolone therapy was applied. Facial paralysis resolved completely in several weeks but headache did not. Four months later, she was admitted to our clinic with severe headache, vomiting, right peripheral facial paralysis and convulsion. Her headache became diffuse and continuous. Her blood pressure was 200/150 mmHg in the emergency department. On neurologic examination she was drowsy. Right peripheral facial palsy, pinpoint pupils, and gaze palsy together with internuclear ophthalmoplegia (one-and-a-half syndrome) were detected. Deep tendon reflexes were brisk on both sides and Babinski's sign was positive bilaterally. Fundoscopy showed bilateral severe hypertensive retinopathy and papilledema. Cranial magnetic resonance imaging (MRI) revealed basotegmental pontine hemorrhage (Fig. 1).

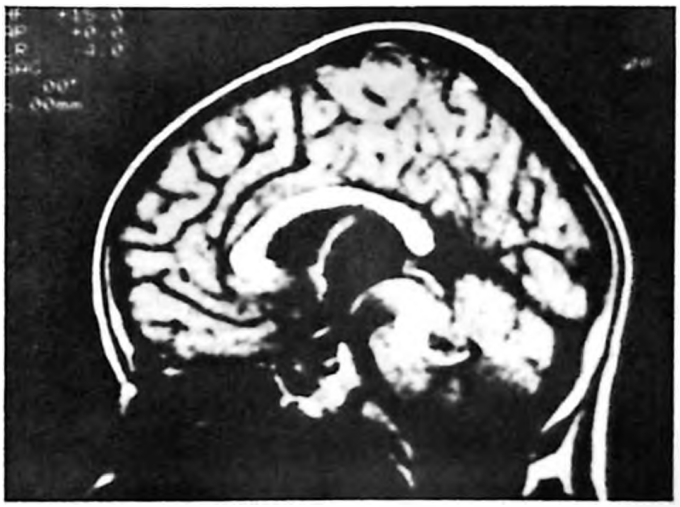


Fig. 1. T2-weighted magnetic resonance imaging (MRI) of the patient showed pontine hemorrhage.

Urine analysis showed mild proteinuria. On urine microscopy 4-5 leukocytes were seen and the urine culture grew E. coli. Creatinine clearance was within normal limits (80 ml/min). Other hematological and biochemical tests including urea nitrogen and creatinine, glucose, electrolytes, transaminases, creatine kinase, alkaline phosphatase, complete blood count, erythrocyte sedimentation rate, C3, C4, ANA,

and anti-DNA were normal. Blood renin, angiotensin and aldosterone levels were elevated. The renal ultrasonography revealed a mild degree dilatation of the left renal calices. Intravenous pyelography, renal angiography, renal scintigraphy with 99Tc-DTPA and 99Tc-DMSA, and voiding cystourethrography were performed. Bilaterally decreased renal vascularization, pyelonephritic scars, and cortical deformation (more prominent on the left than right), slow output function of the left kidney, and bilateral grade-I reflux were detected. Echocardiography showed left ventricular hypertrophy and mild aortic insufficiency. Cerebral angiogram revealed no pathology.

After admisison, she was treated with captopril, prazosin, atenolol and nitroprusside. Satisfactory and rapid control of her blood pressure was obtained. Her neurologic deficits resolved gradually. On the 18th day of admission, facial palsy recovered completely. She was discharged with 4 mg/kg captopril therapy after 33 days of hospitalization.

Discussion

Secondary hypertension is more common than essential hypertension in infants and children. Approximately 75-80% of children with secondary hypertension have a renal abnormality. Chronic pyelonephritis or reflux nephropathy with pyelonephritic scars, glomerulonephritis, renal obstructive disease and polycystic kidney disease are among the most common etiologies[2]. In our patient, pyelonephritic scars and activated renin-angiotensin system were present.

There is no specific symptom of hypertension. Headache is the most common complaint, and nausea and vomiting are initial complaints in many patients. Seizures occur more frequently in children[2,3]. Facial paralysis is a rare finding of hypertension. The most common causes of facial paralysis in children are otitis media and idiopathic Bell's palsy[4]. In addition, it can be secondary to trauma; skull diseases such as osteomyelitis and osteopetrosis; toxins; metabolic causes (hyperparathyroidism, hypothyroidism); neck lesions; infections (especially otitis media); intracranial space-occupying lesions; genetic, autoimmune, and muscular disorders and, rarely, hypertension[5,6]. The paralysis can be intermittent and independent of blood pressure control[1]. In 10 patients described in the literature, the facial paralysis was intermittent in six, as it was in our case[1,7,8]. Recurrent hemorrhage within the facial canal could account for the intermittent nature[1].

The cause of facial paralysis in the hypertensive child is unclear[6], but hemorrhage or edema in the facial canal may be important factors. As in our case, the prognosis in children is good[1,3,6,9]. The duration of palsy varies from days to weeks. Recovery begins when the pressure is reduced[6]. Our patient's paralysis resolved within 18 days following antihypertensive therapy.

The therapeutic effect of corticosteroids in acute idiopathic peripheral nerve paralysis is controversial. Some authors support early steroid treatment[10,11], others suggest that steroid therapy initiated at an early stage of childhood Bell's palsy does not significantly improve the outcome[12]. In contrast to cases of Bell's palsy, in hypertensive cases the use of glucocorticoids seems to be steroids[1]. It appears that unawareness of the fact that facial palsy can be the presenting sign of severe hypertension is aggravated by the use of steroids. In our case, failure to diagnose hypertension resulted in pontine hemorrhage.

In children with facial paralysis, blood pressure determination should be conducted repeatedly. This is particularly important in patients prescribed steroids. In patients who are normotensive on first evaluation, a careful history, and clinical and basic laboratory examination for hypertension should be performed. After the diagnosis of Bell's palsy was established, the follow-up of blood pressure should not have been ignored.

REFERENCES

1. Syegler RL, Brewer ED, Corneli HM, Thompson JA. Hypertension first seen as facial paralysis: case reports and review of the literature. Pediatrics 1991; 87: 381-389.
2. Londe SB. Causes of hypertension in the young. Pediatr Clin North Am 1978; 25: 55-65.
3. Trompeter RS, Smith RL, Hoare DR, et al. Neurological complications of arterial hypertension. Arch Dis Child 1982; 57: 913-917.
4. Lloyd AV, Jewitt DE, Still DL, Facial paralysis in children with hypertension. Arch Dis Child 1966; 41: 292-294.
5. Paire RS. Facial paralysis in children: review of the differential diagnosis and report of ten cases treated with cortisone. Pediatrics 1957; 19: 303-316.
6. Fenichel GM. Lower brainstem and cranial nerve dysfunction. Clinical Pediatric Neurology. Philadelphia: W.B. Saunders; 1993: 339-360.

7. Griffith JQ. Involvement of the facial nerve in malignant hypertension. Arch Neurol Psychol 1933; 29: 1195-1202.

8. Zeis PM, Rao S, John EG, Aschinberg LC. Stress polycythaemia and peripheral facial palsy compications of severe hypertension. Acta Paediatr Scand 1975; 68: 287-289.

9. Moore P, Fyddler GI. Facial palsy in an infant with coarctation of the aorta and hypertension. Arch Dis Child 1980; 55: 315-316.

10. Hurtado Garcia JF, Talavera Sanchez J, Lopez Rico JJ. [Early corticoid treatment of idiopathic facial palsy (Bell)]. Acta Otorrinolaringol Esp 1997; 48: 177-181.

11. Santos-Lasaosa S, Pascual-Millan LF, Tejero-Juste C, Morales-Asin F. Peripheral facial paralysis: etiology, diagnosis and treatment. Rev Neurol 2000; 30: 1048-1053.

12. Ünüvar E, Oğuz F, Sıdal M, Kılıç A. Corticosteroid treatment of childhood Bell's palsy. Pediatr Neurol 1999; 21: 814-816.

Congenital mediastinal immature teratoma: a case report with autopsy findings

Figen Aksoy[1], Cihat Şen[2], Nur Danişment[3]

Departments of [1]Pathology, [2]Obstetrics and Gynecology, and [3]Pediatric Surgery, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey

SUMMARY: Aksoy F, Şen C, Danışment N. Congenital mediastinal immature teratoma: a case report with autopsy findings. Turk J Pediatr 2002; 44: 76-79.

A full-term newborn with karyotype 46, XX was delivered by cesarean section. She had severe respiratory distress and substernal retraction, and underwent emergency operation, but she died on the same day due to respiratory failure.

The mother, 26-year-old prima gravida with no history of twinning, had been examined with ultrasonography at the 34th week of her pregnancy, which revealed a fetus with edema of head and neck region, a probable diaphragmatic hernia, polyhydramnios, and a large mediastinal mass with solid and multicystic parts with hypoplasia of the lungs.

Autopsy revealed a 9 x 5 x 3 cm lobulated mediastinal mass with both solid and cystic areas, displacing the lungs and the heart postero-inferiorly and thymus anteriorly. The lungs were hypoplasic. Microscopically, the mass showed mature epithelial and mesenchymal tissues with primitive mesenchyme and immature neuroepithelium. All these findings led to the diagnosis of an immature teratoma.

Mediastinal teratomas are rare and life-threatening, but early diagnosis and surgical intervention in a newborn with sufficient lung maturation may provide a long survival.

Key words: mediastinal teratoma, extragonadal teratoma, immature teratoma.

Mediastinal teratomas account for up to 20% of mediastinal masses in children. However, presentation in the neonate is rare, with only five cases reported in the literature before 1980[1-3].

The anterior mediastinum, the pericardium and, rarely, the lungs are principal sites of teratomas in the thoracic cavity. Respiratory distress and chest pain are the common presenting symptoms. Unusual manifestations include hemoptysis and hypoglycemia. Some affected children have no symptoms[4-8].

Mature and immature teratomas are the basic pathologic types of teratomas which mostly occur in the mediastinum and pericardium[3-4].

Here we present the clinical features and autopsy findings of a newborn with congenital mediastinal immature teratoma which caused a fatal respiratory distress.

Case Report

A 26-year-old prima gravida with no family history of twinning underwent cordocentesis at 34 weeks' gestation, and fetal blood sampling revealed karyotype of 46, XX. During this procedure polyhydramnios was noted and 1000 ml of amniotic fluid was evacuated.

A term baby girl with an APGAR score of 4 at 5th minute was delivered by cesaren section. There was severe respiratory distress and substernal retraction at the time of birth. She was duly transferred to the Pediatric Surgery Ward of the hospital for an emergency operation, but died on the same day due to respiratory failure.

Ultrasonographic Findings: An ultrasonographic examination at 34 weeks' gestation showed edema in the head and neck region, a probable diaphragmatic hernia, polyhydramnios, and a large solid multilocular cystic mass in the mediastinum with hypoplasia of the lungs.

Autopsy Findings: A full-term baby girl with normal growth and development was seen. No gross abnormality was observed, except for focal edema in the head and neck.

When the thorax and abdomen were dissected, a huge, lobulated, solid, partially cystic mass was seen, filling the mediastinum and displacing

the lungs and heart postero-inferiorly and the thymus anteriorly. The lungs were hypolasic. The rest of the organs were found to be hyperemic and showed no special features.

Gross Appearance : A 9 x 5 x 3 cm large mass was found with a multilobular outer surface, covered by a thin capsule. Cut surface showed multiple cysts with shiny solid and soft mucoid areas. The cysts varied in size from a few milimeters to several centimeters in diameter and were filled either with clear-mucinous or with brown-red fluid (Fig. 1).

Microscopic Findings : Small cystic and solid areas were quite distinct in the tumor. Cysts, in general, were lined by either single layered or stratified, columnar and mucin-secreting epithelia. The walls of some of the cysts resembled intestinal and bronchial mucosa (Fig. 2). In between and around the cysts were mature mesenchymal tissue, glandular elements, islands of cartilage, primitive brain tissue and scattered immature neuroepithelium (Figs. 3, 4). The tumor, which showed features of immature teratoma, contained mature tissue, immature mesenchyme and neuroepithelia tissues from all three germ layers; hence, our case was diagnosed as immature teratoma.

Discussion

Mediastinal teratomas are rare neoplasms in children, accounting for only 7% of all germ cell tumors. The presentation of mediastinal teratoma in the newborn is sparsely documented. In three series there were only four neonates in a total of 28 children. In the Lakloo[1] series, 40% (6/15) of the patients were neonates. In our series of 47 teratomas, 19 were in neonates and only one had mediastinal localization[9].

Teratomas are complex tumors composed of tissues originating from all three germinal layers[4,8,10-13].

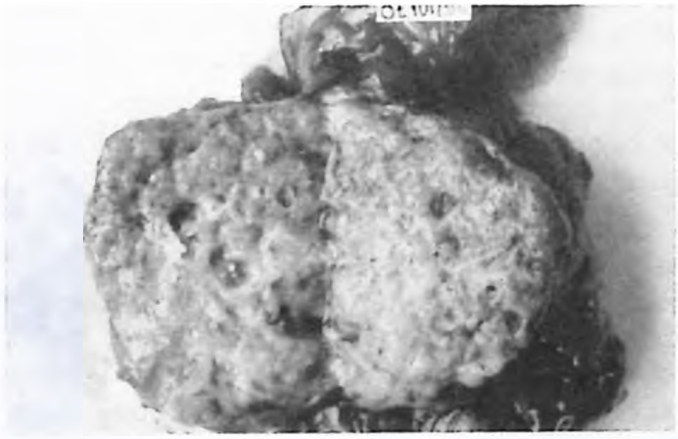
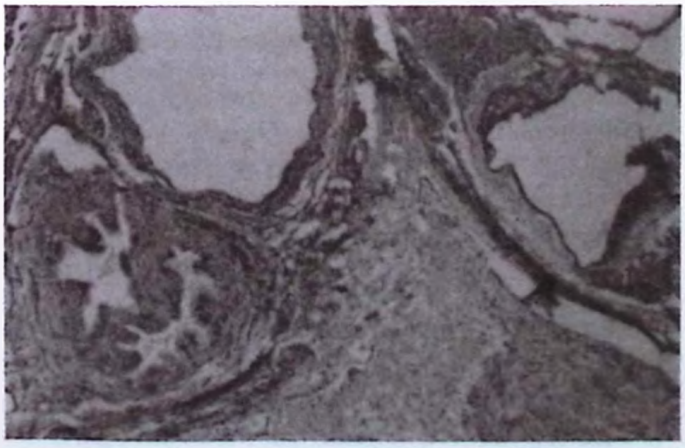
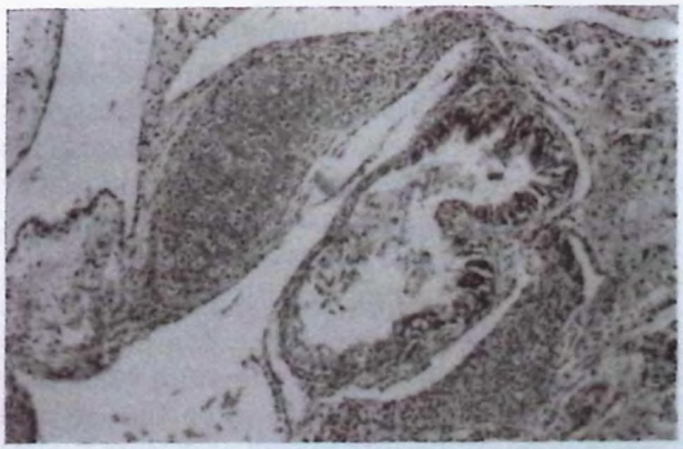


Fig. 1. The multilobulated with small cystic areas containing fluid and shiny solid fibrous areas with soft mucoid regions.



(2)



(3)

Fig. 2, 3. The histological appearance: The tumor contained various epithelial components such as cubic, stratified squamous epithelium, and bronchial mucosa type epithelium lining the cysts. Surrounding these cysts were mature cartilage, immature neuroepithelium and primitive mesenchyme.

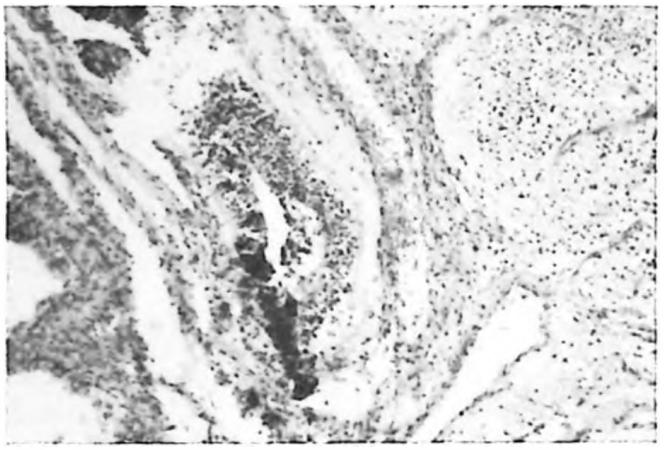


Fig. 4. Primitive mesenchyme and embryonic neuroepithelial tissue (primitive neural tissue component) were noted in other areas.

They usually occur in a para-axial or mild-line location from the brain to the sacral area and in gonads[8,12]. Less common sites include the posterior mediastinum, pericardium, heart, and lung parenchyme[1].

Mediastinal teratoma in a newborn may cause severe respiratory distress which is the most important clinical finding and requires immediate intubation and surgical intervention[6-8,12,14]. Respiratory distress was the main clinical presentation in our case but the time was insufficient for surgical intervention.

The congenital teratomas, if diagnosed and treated shortly after, birth, are usually benign regardless of the somatic immaturity; on the other hand, if the tumor has a critical site such as the central nervous system, the pericardium or mediastinum, it may cause death by local effects[4].

The prenatal diagnosis of a mediastinal teratoma ultrasonographically is based on a tumor composed of cystic and solid structures in the region of the upper mediastinum[8].

Differential diagnosis should include all intrathoracic malformations such as bronchogenic cysts, congenital adenomatoid malformation of the lungs, hamartoma of the lungs, bronchopulmonary sequestration, diaphragmatic hernias, thymoma, lymphoma and cystic hygroma[1,7,8].

Mediastinal teratomas have been classified as mature when there is histologically well differentiated tissue and as immature when they contain so-called immature epithelial and mesenchymal elements as well as mature tissues (especially tissues of neuroepithelia). The malignant group of immature teratomas consists of embryonal carcinomas, yolk sac tumor, seminoma and choriocarcinoma[1,2,10].

In the present case, we observed foci of mature elements containing primitive mesenchyme and primitive neuroepithelial tissues scattered through the mass (Fig. 4).

In such cases α-fetoprotein has been indicated as a good marker, which is especially valuable in the follow-up of patients for tumor recurrence[1].

The origin of the extragonadal teratoma is considered to be different from that of the gonadal. Extragonadal teratomas arise from early embryonic cells of primordial germ cells in the course of migration during embryogenesis. One hypothesis suggests that extragonadal lesions are often congenital and are typically misplaced in the midline in conjoined twin pregnancies, whereas gonadal teratomas may arise from sequestered haploid germ cells[10].

Since the teratomas show rapid growth in early gestational periods, they are responsible for the compression of the neighboring organs. Mediastinal teratoma, depending on the size, might cause polyhydraminos or fetal hydrops, because of a depressed esophagus or the obstruction of the venous return[15].

Diffuse edema in the head and neck region and polyhydramnios may indicate a mediastinal mass and, although rare, teratomas in this location should be included in the differential diagnosis when these findings are present in ultrasonography.

These masses are life-threatening, but early diagnosis and immediate surgical intervention may provide long survival in a newborn with sufficient lung maturation.

REFERENCES

1. Lakloo K, Boyle M, Drake DP. Mediastinal teratomas. J Pediatr Surg 1993; 28: 1161-1164.
2. Carter D, Bibro MC, Touloukian RJ. Benign clinical behavior of immature mediastinal teratoma in infancy and childhood: report of two cases and review of the literature. Cancer 1982; 49: 398-402.
3. Weidner N. Germ-cell tumors of the mediastinum. Semin Diagn Pathol 1999; 16: 42-50.
4. Liang RI, Wang P, Chang FM, et al. Prenatal sonographic characteristics and Doppler blood flow sudy in a case of a large fetal mediastinal teratoma. Ultrasound ObstetGynecol 1998; 11: 214-218.
5. Dehner LP. Gonadal and extragonadal germ cell neoplasia of childhood. Hum Pathol 1983; 14: 493-511.
6. Pate JW, Buker P, Korones SB. Mediastinal teratoma in the newborn. J Pediatr Surg 1963: 533-535.
7. Kenny JB, Carty HM. Infants presenting with respiratory distress due to anterior mediastinal teratomas. A report of three cases and a review of literature. Br J Radiol 1988; 61: 241-244.

8. Weinraub Z, Gembruch U, Fodisch M. Intrauterine mediastinal teratoma associated with non-immune hydrops fetalis. Prenat Diagn 1989; 9: 369-372.

9. İlvan Ş, Aksoy F, Dervişoğlu S. Kongental tümörler. Türk Onkoloji Dergisi 1996; 56-59.

10. Saiga T, Osasa H, Hatayaca H. The origin of extragonadal teratoma. Case report of an immature teratoma occurring in a prenatal brain. Pediatr Pathol 1991; 11: 759-770.

11. Whittaker LD, Jr, Lynn HB. Mediastinal tumors and cysts in the pediatric patient. Surg Clin North Am 1973; 53: 893-904.

12. Billmire DF, Grosfeld JL. Teratomas in childhood. Analysis of 142 cases. J Pediatr Surg 1986; 21: 548-551.

13. Topper D, Lack E. Teratomas in infancy and childhood. A 45 year experience at the children's hospital medical center. Ann Surg 1983; 13: 398-410.

14. Mahour GH, Wooley MM, Triveoli SN. Teratomas in infancy and childhood: experience with 81 cases. Surgery 1974; 76: 309-318.

15. Moermari P, Fryus JP, Goddeenis P. Non-immunologic hydrops fetalis. Arch Pathol Lab Med 1982; 106: 635-640.

An unusual case of esophageal and laryngotracheal atresia

Ayşe Korkmaz[1], Beril Talim[1], Gülsevin Tekinalp[1], Didem Aliefendioğlu[2]
Atilla Şenaylı[3], Safiye Göğüş[1]

Department of [1]Pediatrics, and [3]Pediatric Surgery, Hacettepe University Faculty of Medicine, and [3]Neonatology Unit, Social Security Children's Hospital, Ankara, Turkey

SUMMARY: Korkmaz A,Talim B, Tekinalp G, Aliefendioğlu D, Şenaylı A, Göğüş S. An unusual case of esophageal and laryngotracheal atresia. Turk J Pediatr 2002; 44: 80-82.

Esophageal atresia with or without tracheoesophageal fistula is a relatively common congenital anomaly. However, esophageal atresia with associated laryngotracheal atresia, double tracheoesophageal fistula and cardiac malformations is an extremely rare condition. In this article we report a newborn infant with severe respiratory distress at birth who had both esophageal and laryngotracheal atresia with congenital cardiac malformations, in an attempt to bring attention to the clinical presentation, and emergent diagnostic and therapeutic approaches.

Key words: esophageal atresia, laryngeal atresia, tracheal atresia, congenital cardiac malformations.

Esophageal atresia (EA) with or without associated tracheoesophageal fistula (TEF) is a relatively common congenital anomaly. However, EA in combination with laryngotracheal atresia and double TEF is an extremely rare congenital anomaly and it is almost always fatal[1,2]. It may be found in association with other congenital anomalies. Rapid evaluation of the clinical signs and suspicion of the diagnosis at birth are of great importance for acute management[3]. In this article we report a preterm newborn infant with esophageal and laryngotracheal atresia with double TEF, in an effort to call attention to the clinical symptomatology and physical findings and to the importance of differential diagnosis and urgent management procedures.

Case Report

A preterm male infant of 32 weeks' gestation and birth weight of 1000 g was delivered by urgent cesarean section because of fetal distress to a 24-year-old gravida 1 para 0 mother. The pregnancy was complicated with polyhydramnios, and amniocentesis was performed for reducing amnionic volume a few hours before delivery. The Apgar scores were 2, 5 and 6 at 1, 5 and 10 minutes, respectively. The infant was noted to have an absent cry, cyanosis and respiratory distress. The attempt to intubate the infant failed but the lungs could be ventilated using bag-valve mask. The infant was immediately taken to the Neonatal Intensive Care Unit but attempts for intubation by senior neonatologists and then by the anesthesiology and otolaryngology consultants also failed. Efforts to place a nasogastric tube into the stomach were also unsuccessful. The infant was taken to the operating room for laryngoscopy. Direct laryngoscopy revealed complete occlusion of the airway immediately after the subglottic area, and esophagoscopy revealed EA. A contrast material was given to the esophageal pouch to show the relation between the trachea and esophagus radiologically. The X-ray showed a proximal EA with a thin TEF to the distal trachea and bronchi continuing with distal esophagus and stomach. A 2 FG endotracheal tube was inserted by tracheotomy to the distal trachea and the infant was transported back to the Neonatal Intensive Care Unit under mechanical ventilation. The X-ray examinations were also consistent with severe neonatal respiratory distress syndrome. Oxygen saturations by pulse oximetry were consistently low although maximum ventilatory support was applied. The infant died at the sixth hour of life. Postmortem X-ray examination confirmed the diagnosis (Fig. 1).

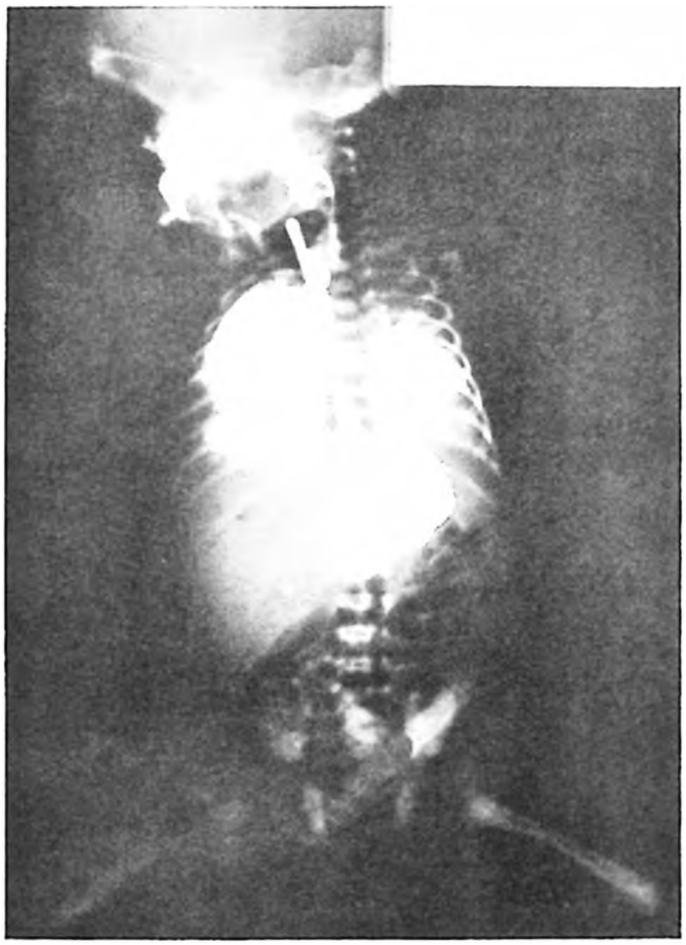


Fig. 1. Postmortem esophagography and bronchography of the newborn.

At autopsy, the larynx was normal until the level of vocal cords, after which the lumen was completely occluded as a consequence of laryngeal atresia. After an atretic segment of proximal trachea which was 1 cm in length, the distal trachea was normal. As for the esophagus, proximal atresia with proximal and distal TEF was present: the atretic proximal esophagus (3 cm in length) was connected to the distal trachea through a TEF of 0.1 cm in diameter. The tracheal bifurcation and main bronchi were opened to the distal esophagus by a very large fistula as if the esophagus was in continuity with the trachea (Vogt type IIc). The heart showed a complex combination of congenital malformations: left persistent superior vena cava, dilated right atrium, primum type atrial septal defect 0.4 cm in diameter and total anomalous pulmonary venous return. Left atrium was very small owing to total anomalous pulmonary venous return.

Discussion

Esophageal atresia with or without TEF is a relatively common congenital anomaly. However, EA with associated laryngotracheal atresia and with congenital cardiac malformations is an extremely rare condition[1,2]. Laryngotracheal atresia is almost always incompatible with life, more than 80 cases have been reported in the literature with only a few showing long-term survival[3].

Esophageal atresia can be found in association with laryngeal anomalies such as laryngeal stenosis and atresia, especially in Vog type IIb. The type of EA in our case was classified as Vogt type IIIc as it was associated with an extended form of laryngeal atresia and double TEF[4].

Experimental studies show that EA is probably caused by disorders in a system of folds in the tracheoesophageal space rather than abnormalities of a tracheoesophageal septum. In the past it was generally accepted that embryological differentiation of the esophagus from the trachea occurs when lateral ridges fuse in the midline forming a septum. But it has been shown that the differentiation of the foregut into the esophagus and trachea is a process of reduction in size of a foregut region called the tracheoesophageal space. This reduction is caused by a system of folds that develop in the primitive foregut. These folds approach but do not fuse. On the basis of this data it was concluded that malformations of the trachea or esophagus with fistula can be explained by abnormalities in the formation of the folds or their developmental movements[5]. In an adriamycin-treated animal model it was concluded that failure of the tracheal bud to develop normally from the primitive foregut is the main event which leads to the tracheoesophageal anomalies[6]. In another fetal rat model, exposure of fetal rat embryos to adriamycin has led to abnormal development of the notochord, including prolonged attachment to or fusion with the foregut and abnormal branching. Traction on the foregut by the notochord produces occlusion of its lumen and may result in its complete interruption. Separation of the notochord from the foregut would appear to be a prerequisite for the normal development of the foregut into its derivatives: the esophagus and trachea[7].

The prenatal presentation of infants with esophageal and laryngotracheal atresia has ranged from high-risk complicated pregnancies to completely uneventful ones. Polyhydramnios is one of the most significant prenatal findigs[3,5].

Although laryngotracheal atresia is a very rare congenital anomaly, this diagnosis should be suspected in any newborn with respiratory distress, absence of audible crying and difficult or impossible endotracheal intubation in the delivery room. Attempts at intubation will reveal an absent glottic or subglottic opening. No air movement with respiratory efforts is typically present and should alert the clinician to the possibility of laryngeal or tracheal atresia (TA). If the esophagus is normal and is intubated by accident when it is assumed that endotracheal intubation has been performed, insertion of the suction catheter to a greater depth than expected can be an indication of esophageal intubation. But, esophageal intubation generally allows relatively adequate ventilation in the short term through a TEF. Stabilization may also be achieved by bag-valve mask as in our case. Further respiratory difficulty or suspicion of the diagnosis generally leads to endoscopic examination of the infant or emergent neck exploration to identify the trachea and attempt a tracheotomy. Laryngoscopy, broncoscopy and esophagoscopy often confirm the diagnosis. More information can be obtained by a contrast study through the esophagus[3,5,8].

Esophageal and laryngotracheal atresias are usually complicated by many other congenital anomalies which worsen the prognosis. In a review of 89 patients with EA and TEF, genitourinary anomalies were present in 21%, cardiovascular in 19%, gastrointestinal in 10%, central nervous system in 9%, musculoskelatal in 8%, chromosomal in 5% and head and neck in 6% of cases. It is concluded that the survival rate of newborns with EA/TEF is high, especially in the absence of associated anomalies[9]. In another review, 52.4% of the patients with EA had associated congenital malformations. Early gestational age and lower birth weights are significantly correlated with higher rates of malformations[10]. Investigations for possible associated anomalies should be considered for all patients with EA, TA and TEF[11].

Infants with EA, with or without a TEF, are frequently of low birth weight. With advances in neonatal, respiratory, surgical and anesthetic care, more infants with very low birth weight are surviving. The most important risk factors are respiratory distress syndrome, pneumonia and major cardiac anomalies[12]. The therapy of the neonate with EA includes primary or delayed end-to-end anastomosis and different esophageal substitutes such as gastric, small intestinal and colonic transpositions, with good results[13,14]. Unfortunately, however, further management of laryngotracheal atresia has yielded no long-term survival. Intubation of the esophagus with either an endotracheal or tracheostomy tube provides a temporary airway. An attempt for a definitive repair is difficult none of the surgical approaches previously described has achieved adequate survival since a satisfactory tracheal prosthesis has not yet been developed[3,15].

REFERENCES

1. Sankaran K, Bhagirath CP, Bingham WT, Hjertaas R, Haight K. Tracheal atresia, proximal esophageal atresia and distal tracheoesophageal fistula: report of two cases and review of the literature. Pediatrics 1983; 71: 821-823.
2. Paes BA, De Sa DJ, Hitch DA. Fatal malformations of the larynx and upper trachea. Laryngoscope 1984; 94: 1477-1481.
3. Kerschner J, Klotch DW. Tracheal agenesis: a case report and review of the literature. Otolaryngol Head Neck Surg 1997; 116: 123-128.
4. Kluth D. Atlas of esophageal atresia. J Pediatr Surg 1976; 11: 901-919.
5. Van Veenendaal MB, Liem KD, Marres HAM. Congenital absence of the trachea. Eur J Pediatr 2000; 159: 8-13.
6. Merei JM, Farmer P, Hasthorpe S, et al. Timing and embryology of esophageal atresia and tracheo-esophageal fistula. Anat Rec 1997; 249: 240-248.
7. BQ, Beasley SW. Relationship of the notochord to foregut development in the fetal rat model of esophageal atresia. J Pediatr Surg 1999; 34: 1593-1598.
8. Peison B, Levitsky E, Sprowls JJ. Tracheoesophageal fistula associated with tracheal atresia and malformation of the larynx. J Pediatr Surg 1970; 5: 464-467.
9. Rejjal A. Congenital anomalies associated with esophageal atresia: Saudia experience. Am J Perinatol 1999; 16: 239-244.
10. Rokitansky A, Kolankaya A, Bichler B, Mayr J, Menardi G. Analysis of 309 cases of esophageal atresia for associated congenital malformations. Am J Perinatol 1994; 11: 123-128.
11. Saing H, Mya GH, Cheng W. The involvement of two or more systems and the severity of associated anomalies significantly influence mortality in esophageal atresia. J Pediatr Surg 1998; 33: 1596-1598.
12. Yagyu M, Gitter H, Richter B, Booss D. Esophageal atresia in Bremen, Germany-evaluation of preoperative risk classification in esophageal atresia. J Pediatr Surg 2000; 35: 584-587.
13. Dave S, Bajpai M, Gupta DK, Agarwala S, Bhatnagar V, Mitra DK. Esophageal atresia and tracheo-esophageal fistula: a review. Indian J Pediatr 1999; 66: 759-772.
14. Chahine AA, Ricketts RR. Esophageal atresia in infants with very low birth weight. Semin Pediatr Surg 2000; 9: 73-78.
15. Hicks BA, Contador MP, Perlman JM. Laryngeal atresia in the newborn: surgical implications. Am J Perinatol 1996; 13: 409-411.

Isolated noncompaction of the ventricular myocardium

Nazmi Narin[1], Alpay Çeliker[2], Kazım Üzüm[1], M. Hakan Poyrazoğlu[1], Musa Karakükçü[1]

[1]Department of Pediatrics, Erciyes University Faculty of Medicine, Kayseri, and [2]Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Narin N, Çeliker A, Üzüm K, Poyrazoğlu MH, Karakükçü M. Isolated noncompaction of the ventricular myocardium. Turk J Pediatr 2002; 44: 83-85.

Noncompaction of the ventricular myocardium is a rare congenital disorder characterized by the presence of numerous prominent trabeculations and deep intertrabecular recessess which communicate with the left ventricular cavity. The disease uniformly affects the left ventricle, and sometimes also affects the right ventricle. Echocardiographic findings are important clues for the diagnosis. Clinical symptoms include signs of left ventricular systolic dysfunction even to the point of heart failure, ventricular arrhythmias, and embolic events. We describe an illustrative case of isolated noncompaction of the left ventricular myocardium in a two-year-old child with the typical clinical and echocardiographic features of the disease. The literature on the topic is reviewed.

Key words: noncompaction, myocardium.

Noncompaction of ventricular myocardium (NCVM) is a rare disorder of endomyocardial morphogenesis[1]. NCVM refers to the arrest of compaction of loosely interwoven meshwork of myocardial fiber during embryogenesis[2]. CVM is normally more complete in the left ventricle than in the right ventricular myocardium[3]. This congenital disease is a distinct entity and should be classified as noncompaction among the unclassified cardiomyopathies[2]. Clinical manifestations include depressed left ventricular function, ventricular arrhythmias, and systemic embolization. We describe herein a child with NCVM and review the literature.

Case Report

A two-year-old infant was admitted to our hospital with heart failure. The medical and family histories were unremarkable. On physcial examination, his weight was 12,600 g and his height was 85 cm. He had normal facial features. Heart rate was 110 beat/min and blood pressure was 110/70 mmHg. The cardiac examination revealed a slightly increased apical impulse. There were soft holosystolic murmurs at tricuspid and mitral areas. The electrocardiogram (ECG) demonstrated biatrial enlargement and left ventricular hypertrophy. Chest roentgenography showed cardiomegaly and prominent ventricular markings. An echocardiographic examination revealed massive dilatation of the left atrium with normal left ventricular size and function. There was prominent trabeculation of the left ventricular wall with deep intertrabecular spaces and mild concentric left ventricular hypertrophy. There was second degree mitral regurgitation. The right ventricle was hypertrophic and there was second degree tricuspid regurgitation (Fig. 1). In Doppler echocardiographic evaluation, we found short mitral deceleration time, pulmonary vein atrial reversal consistently lasting longer than the mitral. A wave, and increased velocity and duration of pulmonary vein atrial reversal. The ventricular filling patterns were consistent with restriction, and the diagnosis of a restrictive cardiomyopathy of unknown etiology was made. Cardiac catheterization disclosed increased left ventricular end-diastolic pressure and pulmonary hypertension. Left ventriculography demonstrated the sponge-like appearance of the noncompacted ventricular wall during the diastolic phase and masked retention of the contrast medium in the trabecular recesses during the systolic phase (Fig. 2).

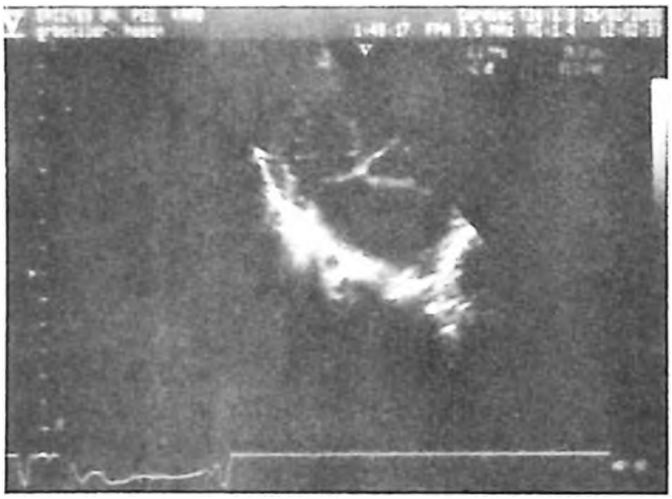


Fig. 1. Apical long axis view of the left ventricle (transthoracic echocardiography). Spongy myocardial appearance resulting from abundant myocardial trabeculations and intertrabecular recesses.

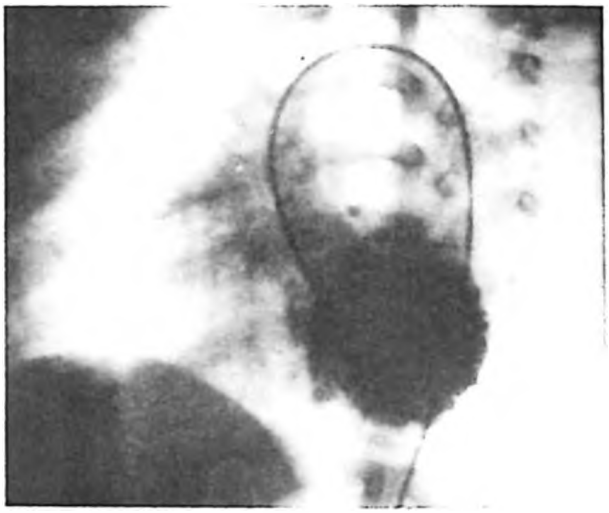


Fig. 2. Left ventriculography showing the sponge-like appearance of the noncompacted ventricular wall during diastolic phase.

Discussion

Isolated NCVM is a rare entity of unknown etiology that is characterized by numerous trabeculations within the ventricular walls. It has been previously described as involving either both ventricles or the left ventricle alone but never just the right ventricle[3,4]. Relatively rare in any case, ventricular compaction has almost invariably been associated with other congenital cardiac malformations, including anomalous origin of the left coronary from the pulmonary trunk and obstruction to right or left ventricular trunk[3]. Our patient had isolated noncompaction of the left ventricular myocardium, not associated with other congenital heart diseases.

The ECGs of the previously reported patients showed abnormal right or left frontal QRS axis deviations, first degree atrioventricular A-V block, abnormal p waves, intraventricular conduction defects of the left ventricle, right bundle branch block, and various arrhythmias. Ventricular conduction abnormality may develop later in life and could lead to progressive endocardial fibrosis in NCVM[3-5]. There were abnormal p waves in our patient.

Familial recurrence has been reported to occur more often in the pediatric population than in adults. A large family with six patients with isolated NCVM was reported by Bley et al.[6]. All reported cases were male, strongly suggesting x-linked recessive inheritance of this disorder[6,7]. In our patient, familial recurrence was not present.

Although the echocardiographic characteristics of numerous trabeculations and deep intertrabecular recesses have been well described and confirmed by necropsy, comparative hemodynamic properties assessed by cardiac catheterization have been reported in children[3]. Hook et al.[8] reported an exceptional case of NCVM presenting as restrictive cardiomyopathy, showing similarities to our data. Patients who are symptomatic at presentation and who follow a rapidly progressive clinical course may show hemodynamic properties similar to dilated cardiomyopathy, whereas asymptomatic patients may follow a slowly progressive course of the restrictive hemodynamic physiology, as our case demonstrated[3,4,8].

The differential diagnosis of NCVM includes the following: (a) Prominent normal myocardial trabeculation, commonly observed by echocardiography as a normal variant; (b) Hypertrophic cardiomyopathy in which ventricular hypertrophy may resemble the trabeculated myocardium of the noncompaction, as in our patient. The deep intertrabecular recesses characteristic of NCVM are, however, typically absent in hypertrophic cardiomyopathy; (c) Dilated cardiomyopathy, which may be accompanied by prominent myocardial trabeculations but to a lesser degree than in NCVM[3-5,8].

The treatment of patients with NCVM does not differ specifically from that of patients with other cardiomyopathies and is directed by the patient's symptoms and specific complications[3,4].

The prognosis of NCVM may range from a prolonged asymptomatic course to a fulminant course of progressive heart failure, leading to heart transplantation or death. Prognosis is worse in patients with symptoms than in patients without symptoms[3,4].

We conclude that isolated NCVM is a rare if not unique disorder with characteristic morphological features that can be identified by two-dimensional echocardiography. Proper recognization of this pathological entity is mandatory for adequae diagnosis and appropriate management and follow-up of this infrequently recognized cardiomyopathy.

REFERENCES

1. Agmon Y, Connolly HM, Olson LJ, Khandheria BK, Seward JB. Noncomaction of ventricular myocardium. J Am Soc Echo 1999; 12: 859-863.
2. Jenni R, Rojas J, Oechslin E. Isolated noncompaction of myocardium. N Engl J Med 1999; 340: 966-967.
3. Chin TK, Perlott JK, Williams RG, Jue K, Mohrman R. Isolated noncompaction of left ventricular myocardium. Circulation 1990; 82: 507-513.
4. Ichida F, Hamamichi Y, Miyawak T, et al. Clinical features of isolated noncompaction of the ventricular myocardium: long term clinical course, hemodynamic properties, and genetic background. J Am Coll Cardiol 1999; 34: 233-240.
5. Robida A, Hajar HA. Ventricular conduction defect in isolated noncompaction of the ventricular myocardium. Pediatr Cardiol 1996; 17: 188-1991.
6. Bley SB, Mumford BR, Thompson V, et al. Neonatal, lethal compaction of the ventricular myocardium is allelic with Barth syndrome. Am J Hum Genet 1997; 61: 868-872.
7. Kurosaki K, Ikeda U, Hojo Y, Fujikawa H, Katsuki T, Shimada K. Familial isolated noncompaction of the ventricular myocardium. Cardiology 1999; 91: 69-72.
8. Hook S, Rattiff NB, Rosenkranz E, Sterba R. Isolated noncompaction of the ventricular myocardium. Pediatr Cardiol 1996; 17: 43-45.

Letter to Editor

The second case with 47, XY, + 8 [38] / 45, X0 [12] karyotype

Özgür Çoğulu, Cengiz Tırpan, Ferda Özkınay, Cumhur Gündüz, Cihangir Özkınay
Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey

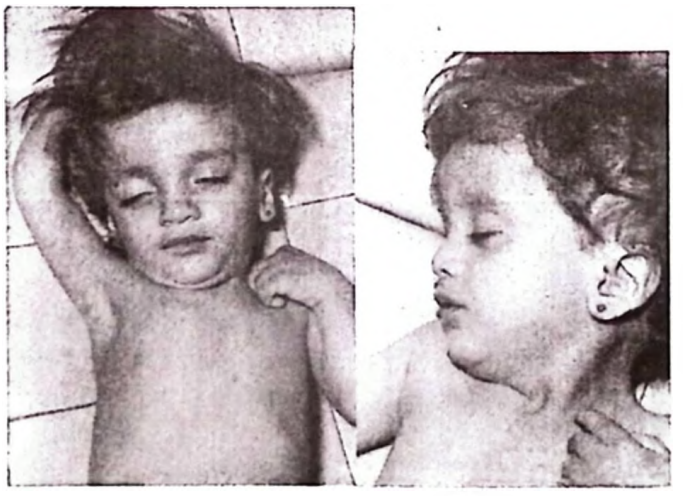
Trisomies are the cause of more than 50% of all spontaneous abortions[1]. In the literature, trisomy 8 is more frequently reported as mosaic cases with normal karyotype[2,3]. Features of mosaic trisomy 8 show great variability such as mental retardation in 90%, malformed pinnae, and contractures of the fingers and toes in 70%, vertebral anomalies in 55%, (which show progression), as the individual gets older, hypertelorism and strabismus in 50%, broad and upturned nose with anteverted nostrils and high palate in 60%, micrognathia, and everted lower lip in 40%, deep furrows on palms and soles in 75%, urogenital anomalies in 40%, and congenital cardiac anomalies in 25%[4,5].

Turner syndrome is characterized by short stature, primary amenorrhea, webbed necked, cubitus valgus in postpubertal females and sexual infantilism. Mental status is expected to be normal. Other common findings are epicanthal folds, ptosis of upper eyelids, prominent ears and micrognathia, and low-set hair-line in 75%; wide-spaced, hypoplastic nipples in 60%; small for gestational age in 50%; excess skin on the nape and peripheral lymphedema in infancy in 40%; hypoplastic, hyperconvex and deep-set toenails in 75%; increased numbers of cutaneous nevi in 60%; aortic stenosis and idiopathic hypertension in 25%; renal anomalies in 40%; and deafness in 50%[4].

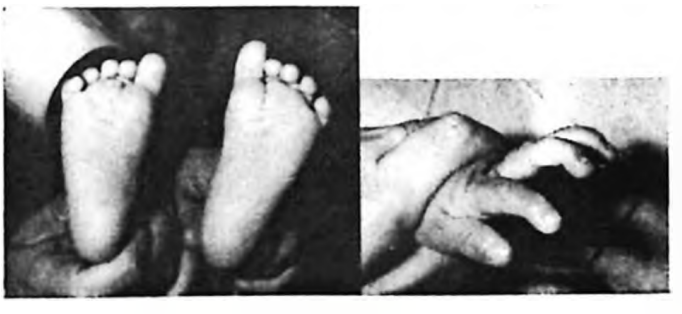
Turner syndrome and mosaic trisomy 8 have been reported in the literature by DeBrasi et al.[6] in 1995; we present the second case with those cytogenetic anomalies.

A twenty-three-month-old female case was admitted to the hospital because of delayed motor and mental development. She was born at term to non-consanguineous parents after a normal vaginal delivery. Her birth weight and height were not recorded. She had meningitis in the first year of life and chronic otitis.

On admission, she was 7,400 g (< 3rd centile), and 72 cm (< 3rd centile). Her head circumference was 46 cm (2-50 centile). She had protruding forehead, long face, hypertelorism, broad, upturned, and bulbous nose, long upper lip, thick and everted lower lip, micrognathia, low-set and deformed ears, high palate, short and broad neck, wide-spaced nipples, arachnodactyly, camptodactyly, deep furrows on palms and soles, and severe motor and mental retardation (Fig. 1a and 1b). Her blood analysis showed hypochromia and microcytosis. Her urine analysis, liver and renal functions were normal.



(a)



(b)

Fig. 1. a) Features of case at 23 months of age. b) Fingers and soles of case.

Her ECG, telecardiography and echocardiography were normal. Abdominal ultrasound (US) examination revealed ovarian agenesis. X-ray examination showed broad ribs and bone age delay. Her karyotype was 47, XY,+8 [38] / 45,X0 [12] (Fig. 2a and 2b). Parents' karyotypes were normal.

DeBrasi et al.[6] reported two cases with mosaic trisomy 8 syndrome and sexual chromosome aneuploidies. Karyotype of the first case was 45,X[59.2%] / 46,X,+8[1.2%] / 47, XX, + 8 [39.6%] and of the second case was 47, XX, + 8 [61.7%] / 47, XXY [38.3%]. They studied molecular analysis to explain the mechanism of those complex karyotypes and found postzygotic mitotic errors.[6] Karadima et al.[7] showed postzygotic mitotic errors by molecular analysis in cases with autosomal trisomies contrary to the common view that maternal meiotic errors cause autosomal trisomies.

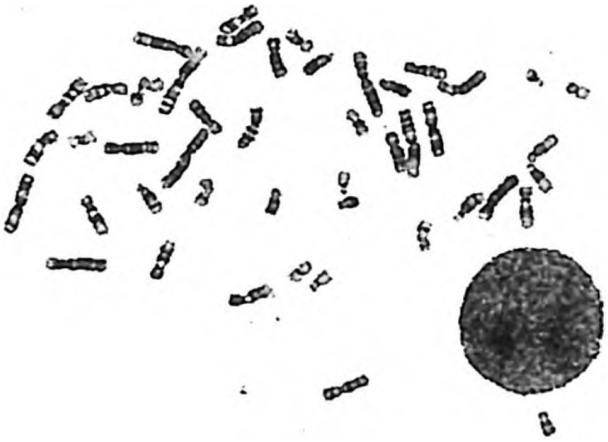


Fig. 2a. Trisomy 8 cell line of case.

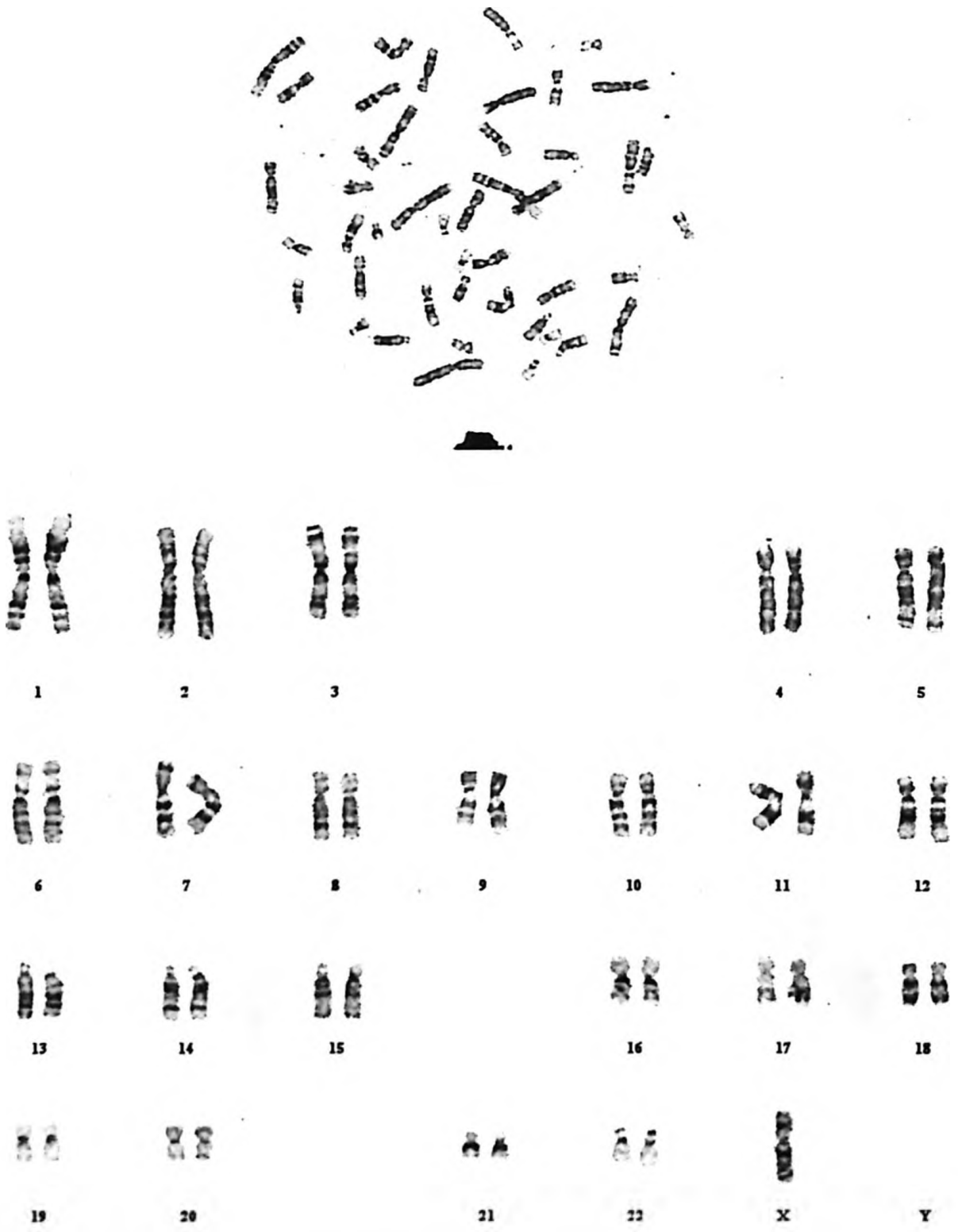


Fig. 2b. 45, XO cell line of case.

The most common findings of mosaic trisomy 8 such as protruding forehead, long face, hypertelorism, anteverted nostrils and broad nose, long upper lip, thick and everted lower lip, micrognathia, dysplastic and low-set ears, high palate, arachnodactyly and camptodactyly, and deep furrows on palms and soles were detected in our case as well. Short, broad neck, wide-spaced nipples and ovarian agenesis detected by US can be considered as the features of Turner syndrome. No cardiac anomaly was detected in our case; however, it can be seen in both trisomy 8 and Turner syndrome cases.

Cardiac and renal anomalies are the major factors that affect the prognosis in both trisomy 8 and Turner syndrome. The patient presented here is the second case in the literature with trisomy 8 and Turner mosaicism showing the most common features of both cytogenetic abnormalities.

REFERENCES

1. Lomax B, Tang S, Separovic E, et al. Comparative genomic hybridization in combination with flow cytometry improves results of cytogenetic analysis of spontaneous abortions. Am J Hum Genet 2000; 66: 1516-1521.
2. Tuncbilek E, Halicioglu C, Say B. Trisomy-8 syndrome. Humangenetik 1974; 23: 23-29.
3. Tunçbilek E, Atasu M, Say B. Dermatoglyphics in trisomy 8. Lancet 1972; 2: 821.
4. Goodman RM, Gorlin RJ. The malformed infant and child. London: Oxford University Press; 1983: 102, 128.
5. Barakat AY, Butler MG. Renal and urinary tract abnormalities associated with chromosome aberrations. Int J Pediatr Nephrol 1987; 8: 215-226.
6. DeBrasi D, Genardi M, D'Agostino A, et al. Double autosomal/gonosomal mosaic aneuploidy: study of nondisjunction in two cases with trisomy of chromosome 8. Hum Genet 1995; 95: 519-525.
7. Karadima G, Bugge M, Nicolaidis P, et al. Origin of nondisjunction in trisomy 8 and trisomy 8 mosaicism. Eur J Hum Genet 1998; 6: 432-438.

ANNOUNCEMENTS

İZMİR, 1 - 7 MARCH 2002

EUROPEAN SOCIETY FOR PEDIATRIC ENDOCRINOLOGY (ESPE) WINTER SCHOOL

Inquiries to : Prof. Dr. Atilla Büyükgebiz
Dokuz Eylül Üniversitesi
Tıp Fakültesi
Pediatrik Endokrinoloji ve
Adolesan Ünitesi
35340 İnciraltı, İzmir
TURKEY
Phone : +90 (232) 278 84 13
Fax : +90 (232) 278 84 11
e-mail : atilla.buyukgebiz@deu.edu.tr

İZMİR, 8 - 10 MAY 2002

IVth NATIONAL PEDIATRIC NEUROLOGY CONGRESS

Inquiries to : Pia Tour Turizm ve Ticaret Ltd. Şti.
Cumhuriyet Bulvarı, 123/1
Mayıs İş Merkezi Büro: 202
35220 Alsancak - İzmir
TURKEY
Phone : +90-(0) 232-464 23 21
Fax : +90-(0) 232-464 23 21
e-mail : piatour@superonline.com
web site : www.piatour.com.tr/cocuknorolojisi.htm

İSTANBUL, 22 - 25 MAY 2002

XIInd TURKISH PEDIATRIC ONCOLOGY GROUP CONGRESS ROUND CELL TUMORS

Inquiries to : Prof. Dr. Sema Anak
İstanbul University
İstanbul Faculty of Medicine
Department of Pediatrics
Division of Pediatric Hematology/Oncology
Çapa-İstanbul
TURKEY
Phone : +90 (0212) 635 20 40
635 11 89
Fax : +90 (0212) 631 13 12
e-mail : sanak@tpog2002.org

ANTALYA, 26 - 39 MAY 2002

XVIIth ANKEM:
CLINICAL AND MEDICAL SCIENCES CONGRESS

Inquiries to : SymCon Turizm ve Tic. Ltd. Şti.
Şemsettin Günaltay Caddesi
Osmanlı Sitesi No: 213 K: 2 D: 13
81080 Erenköy-İstanbul
TURKEY
Phone : +90 (0216) 478 40 40
Fax : +90 (0216) 478 40 50
e-mail : symcon@symcon.com.tr

LJUBLJANA, 13 - 15 JUNE 2002

XIIIth ANNUAL CONGRESS OF THE EUROPEAN SOCIETY OF PEDIATRIC AND NEONATAL INTENSIVE CARE (ESPNIC) ESPNIC NURSING SYMPOSIUM

Inquiries to : Mrs. Karmen Boh
Administrative Manager
Division for Pediatric Surgery and Intensive Care
Zaloska 7
SI-1525 Ljubljana
SLOVENIA

GENOVA, 26 - 29 JUNE 2002

Vth MEETING OF THE INTERNATIONAL SOCIETY FOR NEONATAL SCREENİNG "NEONATAL SCREENING FROM THE SPOT TO DIAGNOSIS AND TREATMENT"

Inquiries to : Top Congress Public Relations
Piazza G. Alessi, 2/1
16128 Genova
ITALY
Phone : +39 010 589 753/4/5
Fax : +39 010 532 858
e-mail : Topcong@tin.it

ALICANTE, 19 - 21 SEPTEMBER 2002

XVIIth UNION OF MIDDLE EASTERN AND MEDITERRANEAN PEDIATRIC SOCIETIES (UMEMPS) CONGRESS

Inquiries to : Viajes Pcifico, S.A.
E. Granados, 44
08008 Barcelona
SPAIN
Phone: +34 934 54 54 00
Fax : +34 932 38 74 88
e-mail: gp@pacifico-meeting.com

BEIJING, 20 - 25 SEPTEMBER 2002

JOINT CONGRESS OF ICNA AND AOCNA 2002 THE IXth INTERNATIONAL CHILD NEUROLOGY CONGRESS THE VIIth ASIAN AND OCEANIAN CONGRESS OF CHILD NEUROLOGY THEME: CHILD NEUROLOGY IN NEW LIFE SCIENCE MILLENIUM

Inquiries to : Yu-Wu, Jiang, MD
Xin-Hua Bao MD
Secretariat of ICNC-AOCCn
Department of Pediatrics
Peking University 1st Hospital
No. 1, Xi'anmen Dajie, Beijing 100034
CHINA
Phone: +86 10 661 342 61
e-mail: icnc@public3.bta.net.cn

MERSİN, 15 - 19 OCTOBER 2002

XLVI[th] NATIONAL PEDIATRIC CONGRESS
XX[th] NATIONAL PEDIATRIC SURGERY CONGRESS
VI[th] PEDIATRIC SURGERY NURSING CONGRESS
II[th] NATIONAL PEDIATRIC NURSING CONGRESS

Inquiries to : Necdet Kuyucu, MD
Öznur Düzovalı, MD
Dinçer Avlan, MD
Mersin University
Faculty of Medicine
Department of Pediatrics
33070 Mersin
TURKEY
Phone : +90 (0) 324 336 35 40
337 29 25
Fax : +90 (0) 324 361 00 43
e-mail : nkuyucu@yahoo.com
pediatri2002@yahoo.com

SANTIAGO, 19 - 23 NOVEMBER 2002

III[rd] WORLD CONGRESS OF PEDIATRIC INFECTIOUS DISEASES - WSPID

Inquiries to : III[rd] World Congress of Pediatric
Infectious Diseases-WSPID
c/o Kenes International
17 Reu du Cendrier
PO Box 1726
CH-1211 Geneva
SWITZERLAND
TURKEY
Phone : +41 229 08 04 88
Fax : +44 870 121 34 74
(International/European number)
e-mail : wspidqkenes.com
web site : www.kenes.com/wspid

BUENOS AIRES, 30 MARCH - 3 APRIL 2003

IV[th] WORLD CONGRESS ON PEDIATRIC INTENSIVE CARE

Inquiries to : PIC 2003 Congress Secretariat
c/o Events International Meeting Planners Inc.,
759 Square Victoria, Suite 300
Montreal, Quebec, H2Y 2J7
CANADA
Phone : (514) 286 08 55
Fax : (514) 286 60 66
e-mail : pic03abs@eventsintl.com
web site : www.pic2003.com

OSLO, 20 - 24 MAY 2003

X[th] CONGRESS OF THE EUROPEAN SOCIETY FOR THE STUDY AND PREVENTION OF INFANT DEATH (ESPID)
WHY? UNDERSTANDING AND PREVENTING INFANT DEATH AND COMPLICATED GRIEF

Inquiries to : The Norwegian SIDS Society
Arbinsgt. 7, 0253 Oslo
NORWAY
Phone : (+47) 22 54 52 00
Fax : (+34) 22 54 52 01
e-mail : sids-norge@sids.no
web site : www.sids.no

NOTICE TO AUTHORS

The Turkish Journal of Pediatrics is an English publication of original articles, case reports, reviews of the literature, case of the month, short communications, clinicopathological exercises and letter to the editor in the field of pediatrics, and will consider papers from authors of all countries. Original articles should report scientific findings within the pediatric field of interest. Case reports should contain accounts of rare syndromes, new diagnostic tools and methods, new kinds of treatment and laboratory research with foreseeable practical application. Review articles should contain an objective account of different points of view and an internationally representative bibliography. Authors considering submission of review articles of medical progress are encouraged to consult with the Editor before submission. Papers should in general not exceed 15 written pages for original articles or 7 for case reports and reviews of the literature, including tables, figures and illustrations. All papers are subject to editorial revision for the purpose of conformity to the style adopted by *The Turkish Journal of Pediatrics,* and authors may be requested to shorten or revise their papers.

Statements and opinions expressed in the articles are those of the author(s) and not necessarily those of the Editor or publisher; the Editor and publisher disclaim any responsibility or liability for such material. Neither the Editor nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication; neither do they guarantee any claim made by the manufacturer of such product or service.

We recommend that all manuscripts be approved for submission by the department chairman or head of the institution in which the study has been done. All manuscripts must be accompanied by the following **written statement,** signed by the author(s): "The author(s) transfer(s) all copyright ownership of the manuscript entitled (title of article) to *The Turkish Journal of Pediatrics* in the event the work is buplished. The author(s) warrant(s) that the article is original, is not for consideration by another journal, has not been previously published (except in a congress report), and has been prepared according to the manuscript rules" (as described below). Papers describing research involving human subjects should indicate that informed consent was obtained from the parents or guardians of the children who served as subjects and, when appropriate, from the subjects themselves.

Preparation of Manuscript

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles and may result in rejection of otherwise acceptable manuscripts. Manuscripts should be sent in triplicate (original and two photocopies acceptable) to the Editorial Secretary, *Turkish Journal of Pediatrics,* P.K. 66, 06240 Samanpazarı, Ankara, Turkey. Manuscripts, whether rejected or published, are not returned to the authors, but original illustrations which are rejected are returned upon request of the author.

Format Manuscripts should be typed, double-spaced, on one side of the paper only, with wide margins.

Title Page A separate title page should include authors' names and academic degrees; departmental and institutional affiliations of each author, and sources of financial assistance, if any. Designate one author as the correspondent, and provide an address as well as business and home telephone numbers. Galley proofs and order forms for reprints will be sent to the corresponding author, at a later date.

Contents Each original article should contain the following sections: Introduction, Material and Methods, Results, Discussion and Summary, and each Case Report should contain an Introduction, Case Report, Discussion and Summary.

Abbreviations Abbreviations must conform to accepted standards. Laboratory slang, clinical jargon and colloquialisms must be avoided.

Tables Tables should be numbered with Roman numerals according to the order of mention in the text. Each table must be typed double-spaced on a separate sheet of paper, and a concise title supplied for each. Tables should be self-explanatory and supplement, not duplicate the text. If a table or any data therein have been previously published, a footnote must give full credit to the original source.

Figures Figures and illustrations should be of direct interest to the subject of the paper and numbered with Arabic numerals according to order of reference in the text. Figures should be drawn on white paper and photographs must be clear prints. The name of the first author and the number of the figure or illustration must be written in pencil on the back of each, with an arrow indicating the top side. Original drawings or graphs should be prepared in black India ink or typographic (press-apply) lettering. Typewritten or freehand lettering is unacceptable. All lettering must be professional and in proportion to the drawing, graph, or photograph. Do not send original art work, x-ray films or ECG strips. A list of legends for the figures and illustrations must be provided on a separate page.

Summary / Abstract An informative summary not exceeding 150 words must accompany each manuscript, typed on a separate sheet. Unexplained abbreviations and references are not allowed.

References References to the literature should be limited to those cited by the author, arranged and numbered according to order of mention in the text. All authors of an article or book should be listed where there are six or fewer. If more than six, the first three names should be given followed by the indication "et al". In a manuscript underlining indicates italics.

References to journal articles:

Example : 1. Bier DM, Fulginiti VA, Garfunkel JM, et al. Duplicate publication and related problems. Pediatrics 1990; 86: 997-998.

Names of journals are to be abbreviated in accordance with the style of *Index Medicus.*

References to books:

Example : 2. Praat RTC. *The Genetics of Neurological Disorders.* London: Oxford University Press; 1967: 173-174.

References to chapters in books:

Example : 3. Kissane JM. Development of the kidney and congenital malformations. In: Heptinstall RH (ed). Pathology of the Kidney (2nd ed) Vol. 1. Boston: Little, Brown and Co; 1974: 69-109.

Key Words Authors are requested to list on a separate sheet of paper key words related to their article. These will bu used in compiling an index for the volume of the journal in which the article appears.

Proofs and Reprints Galley proofs will be sent to the corresponding (or principal) author by the editor. The master proof should be returned to the Managing Editor together with the manuscript within two days, and by air mail if received by air mail.

Announcements Announcements of scheduled meetings, symposia, or postgraduate courses may be sent to the publisher for consideration at least 5 months in advance of the meeting date.

Books for Review Books and monographs, domestic or foreign, may be sent to the Editor for consideration for review. No books are returned, nor acknowledgement made of books received.

Advertising Communications of a business nature and all advertising communications should be addressed to the Journal's Editor.

Checklist

Please check your article as described below before sending it to the Editor.

- Letter of submission
- Signed "Statement" (which is written on a separate paper) as described above
- Three copies of article (one original and two photocopies will be acceptable).
- Title page
  - Title of article
  - Short running title
  - Name(s), academic degrees, and affiliations of author(s)
  - Name, address, and business and home telephone numbers of corresponding author
- Article proper
  - Original articles: Introduction, Material and Methods, Results, Discussion, Summary
  - Case Reports: Introduction, Case Report, Discussion, Summary
  - Key words
- References on a separate sheet
- Tables on separate sheets
- Legends on separate sheets
- Illustrations properly labelled (one set of glossy prints and one set of photocopies).