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CONTENTS

ORIGINAL ARTICLES

CASE REPORTS

Polymorphisms of surfactant protein A genes and the risk of bronchopulmonary dysplasia in preterm infants

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SUMMARY: Weber B, Borkhardt A, Stoll-Becker S, Reiss I, Gortner L. Polymorphisms of surfactant protein A genes and the risk of bronchopulmonary dysplasia in preterm infants. Turk J Pediatr 2000; 42: 181-185.

The pathophysiology of bronchopulmonary dysplasia (BPD) as an inflammatory disorder secondary to neonatal respitaroy distress syndrome (RDS) is not yet fully understood and still represents a major complication of prematurity. The main pathophysiologic feature of RDS is a primary surfactant deficiency in a structurally immature lung. Pulmonary surfactant contains 90 percent phospholipids and 10 percent proteins (surfactant proteins A, B, C, and D). As surfactant protein A (SP-A) has several major immunological and metabolic intrapulmonary functions, we aimed at investigating an association of polymorphisms of SP-A1 and SP-A2 encoding genes and the risk of BPD.

We performed a case-control study exclusively including Caucasian preterm infants below 32 weeks of gestation matched for the degree of immaturity and the year of birth. Venous cord blood was taken prospectively and analyzed by polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP), cloning and sequencing. BPD was defined as oxygen dependency or need for mechanical ventilation at day 28.

Twenty-three infants with BPD were enrolled (mean gestational age 26.2 weeks; mean birth weight 760.4 g) and compared with 23 infants matched on the basis of gestational age (mean gestational age 27.9 weeks; mean birthweight 1015 g). We observed a significantly increased frequency of the SP-A1 polymorphism $6A^6$ in infants with BPD compared with controls.

In addition to previously established risk factors for BPD, $6A^6$ polymorphism for SP-A1 gene is an independent co-factor. We believe treatment of neonatal RDS should also include stratification according to genetic risk factors.

Key words: surfactant protein A genes, bronchopulmonary dysplasia, preterm infants.

Bronchopulmonary dysplasia (BPD) is a chronic inflammatory pulmonary disorder[1] resulting from neonatal respiratory distress syndrome (RDS) and is observed nearly exclusively in preterm babies ventilated artificially after birth. The main pathophysiological feature of RDS is a primary pulmonary deficiency of surfactant in a structurally immature lung. The number of babies surviving without developing BPD is increasing according to different European study populations, a finding attributed to improvements in perinatal care[2-4]. Mathematical models for the prediction of BPD never reached positive predictive values beyond 70 to 75 percent using multivariate regression analyses[2,3,5]. The key variables in the afore mentioned studies were the degree of immaturity, i.e. gestational age and birth weight, severity of respiratory failure, and in one study lack of prenatal glucocorticoid administration[3]. Thus, further factors, among then those influencing inflammatory intrapulmonary activity, must be operative.

Pulmonary surfactant containing around 90 percent phospholipids and 10 percent non-serum proteins is essential for maintaining physiological surface tension at the air-water interface. Surfactant protein A (SP-A) is the most abundant surfactant protein containing both collagenous and carbohydrate-binding domains[6,7]. It was shown to exhibit major effects on both surfactant metabolism and local

pulmonary immunologic functions, among them binding to different pathogens, regulation of alveolar macrophages and enhancement of neutrophil phagocytosis[8]. The human SP-A gene locus has been assigned to chromosome 10q22-q23, consisting of two very similar genes, SP-A1 and SP-A2[9]. Polymorphisms of the SP-A genes have been described[10], however a functional effect on long-term course of neonatal RDS, i.e. risk of BPD, has not been demonstrated yet.

It thus was the goal of the present study to prove the hypothesis that polymorphisms of SP-A genes are a significant cofactor in the pathogenesis of BPD.

Material and Methods

Patients

Preterm neonates of less than 32 completed weeks gestational age were enrolled prospectively after parental informed consent, from January to July 1998 (Lübeck) and from February to December 1999 (Giessen). For evaluation of SP-A genotypes, venous cord blood samples were taken and stored at -20 °C before analysis. Infants were classified as BPD if supplemental oxygen above room air was necessary for adequate oxygenation (transcutaneous arterial oxygen saturation > 90%) at day 28 following birth or if infants were still on mechanical ventilation[11]. Further prenatal and neonatal variables were defined as follows: a complete course of prenatal corticosteroids was diagnosed if two doses of betamethasone or four doses of dexamethasone were given > 24 hours before bith, or if preeclampsia existed on the basis of the criteria proposed by the American College of Obstetrics and Gynecology (ACOG)[12]. Radiographic grading of RDS was based on the criteria proposed by Couchard and co-workers[13]. Chronic lung disease was defined as supplemental oxygen or mechanical ventilation at 36 weeks postconceptional age.

Microbiological Analyses

DNA was extracted from blood samples using the QIAquick Blood Kit (Qiagen; Hilden/Germany). Reagents for the polymerase chain reaction (PCR) were purchased from Hybaid, (Heidelberg/Germany). Oligonucleotides used as primers for the PCR and the sequencing reactions were commercially synthesized by Roth (Karlsruhe/Germany). The 6-FAM and the HEX labeled primer used for PCR single-strand conformation polymorphism (SSCP) analysis were purchased from PE Biosystems (Weiterstadt/Germany).

Amplification of SP-A1 as well as of SP-A2 was performed on a programmable thermocycler (MJ Research, Massachusetts/USA) using 100 ng DNA in a 50 ml mixture of 400 pmol of each primer, 250 pmol deoxynucleotide triphosphates (dNTP), 1.75 mmol $MgCI_2$ and 1U Proof-Mix Taq/Pwo in buffer with 10 nM Tris, 50 mM KCI.

After initial denaturing at 94°C for 2 min, 34 cycles of annealing at 58°C for 30 sec, extension at 68°C for 3.5 min and denaturing at 94°C for 30 sec were performed, followed by a final extension at 68°C for 20 min. SP-A1 was amplified by oligonucleotides 469 FW1 and 4031 RV1; for amplification of SP-A2 we used oligonucleotide 441 FW2 and 4068 RV2 (Table I).

Table I. Primers Used to Identify SP-A2 Polymorphisms

Oligonucleotide	Sequence	Orientation	Product
469 FW1	5'-CCATGACTGACCACCTTGAG-3'	up	SPA-1
4031 RV1	5'-CACATCTGAAGGCGGCTCTAG-3'	down	SPA-1
441 FW2	5'ATCACTGACTGTGAGAGGGT-3'	up	SPA-2
4068 RV2	5'-TGTCTGCAGTGGGGGGCTCTTC-3'	down	SPA-2
1077 RV1	5'-GTGGCGTCTGCAGCACAGTA-3'	down	exon 1
1511 FW2	5'-GCTGACAGATCCTACACATC-3'	up	exon 2
1729 RV2	5'-TAAGTGACTTCAGGTCGCTG-3'	down	exon 2
3088 FW4	5'GTCCCAAGGAATCCAGAGGA-3'	up	exon 4
3026 RV4	5'-AGTCGGGAGTACAGGCAGTTC-3'	down	exon 4
971 FW-cc	5'-GCAGTATACTTCCTGAGTCCTGACAGAGC-3'	up	exon 1
3430 RV-Cla	5'-GCAATCGATCTAGCATCTCACAGACCAAG-3'	down	exon 4

An aliquot of the PCR product was used to evaluate the success of the PCR reaction by agarose gel electrophoresis. These PCR products were used as templates for a nested PCR amplifying exons 1, 2, and 4 of SP-A1 and SP-A2. Exon 1 was synthesized by oligo 971 FW1 and 1077 RV14, exon 2 by 1511 FW2 and 1729 RV2, and exon 4 by 3088 FW4 and 3026 RV4 (Table I) during an extension time of 60 sec in the same conditions as described above for PCR amplifying of SP-A1 and SP-A2.

To screen for variations in the nested PCR products, nonradioactive SSCP was performed on the Capillary Genetic Analyzer 310 (PE Biosystems; Weiterstadt/Germany). The nested PCR products were diluted 10 times and 1 µl of each of the dilution was mixed with 10.5 µl deionized formamide and 0.5 µl Genescan-350 ROX internal standard (PE Biosystems; Weiterstadt/Germany), heated at 90°C for 2 min and then placed into an ice bath for 2 min and further analyzed.

Each 20 µl ligation reaction contained 100-250 ng of linearized vector, with one third to one half of purified PCR amplification product insertion in the presence of 50 mM Tris, 10 mM $MgCl_2$, 2 mM ATP and 400 U of T4 DNA ligase at 16°C for 16 h. Cloning and sequencing were carried out as published previously[16,17].

Statistics

Infants classified as BPD were matched with controls on the basis of gestational age and year of birth. Basic prenatal and neonatal variables are given as mean and standard deviation (SD) or as percentages, respectively, in Table II. Analysis of continuous variables was performed on the basis of Mann-Whitney-U tests; categorical variables were compared by chi-square tests. Differences of statistical significance were assumed if p was < 0.05 (SPSS, version 6.12 for windows; Chicago, IL).

Interim analyses were scheduled after 20 patients in each group. The study was approved by the Committee on Investigation in Human Subjects.

Table II. Prenatal and Neonatal Characteristics of Study Infants

	BPD (n=23)	Controls (n=23)	p-value
Prenatal corticosteroids (n%)	20 (87%)	18 (78%)	n.s.
Cesarean section (n%)	22 (96%)	21 (91%)	n.s.
Gestational age (weeks)	26.2±1.3	27.7±1.3	p<0.001
Preeclampsia	6 (26%)	3 (13%)	
Birth weight (g)	760.4±140	971.6±175.5	p<0.005
Male/female	13/10	8/15	n.s.
Apgar < 7 at 5 min (n%)	2 (9%)	1 (4%)	n.s.
≥ III Respiratory distress syndrome	12 (52%)	9 (39.1%)	n.s.
Mean time of mechanical ventilation (days)	24.1±10.1	2.2±1.5	p<0.0001
Mean days on oxygen	69.9±29.2	9.27±10.4	p<0.0001
Chronic lung disease (36 weeks p.c.)	9 (39%)	0 (-)	p<0.001

Data are given as mean ± SD or percentages.
BPD: bronchopulmonary dysplasia; n.s.: not significant; p.c.: postconception.

The samples showing aberrant migration on SSCP were reamplified from genomic DNA, purified using the QIAquick kit (Qiagen; Hilden/Germany) and directly sequenced. In the case of multiple polymorphisms, cloning before sequencing was necessary.

A nested PCR amplified with primer 971 FW-Acc and 3430 RV-Cla 2559 yielded such products of the gene of the gene of interest bp including exons 1, 2 and 4. They were purified using the QIAquick Kit, digested with NOT I and SAC II (NEB; Beverly/USA) and used directly in ligation reactions. The linearized pBluescrip II+ vector was dephosphorylated (3 U alkaline phosphatase/µl at 37°C for 1 h).

Results

Basic prenatal and neonatal characteristics of the exclusively Caucasian study infants are given in Table II. Although matching was performed on the basis of gestational age, infants with BPD were less mature compared to controls, thus the differences in birth weight.

The overall distribution of polymorphisms of the genes encoding the SP-A is given as an overview in Table III. In addition to the polymorphisms primarily described by Floros and co-workers[10], we found silent polymorphisms in the SP-A1 gene which were observed in exon 2, amino acid 62: $6A^5$ corresponding to $6A^4$ (G→A), $6A^6$ corresponding to $6A^3$ (A→G), $6A^7$ corresponding

to 6A (G→A) and $6A^8$ corresponding to $6A^1$ (A→G), and in exon 1, amino acid 4 (T→C).

Table III. Distribution of SP-A1 and SP-A2 Gene Polymorphisms in Study Infants

SP-A1 Allele	BPD (n=23)	Controls (n=23)	p-value
6A	2/23	4/23	n.s.
$6A^2$	9/23	12/23	n.s.
$6A^3$	12/23	11/23	n.s.
$6A^4$	4/23	3/23	n.s.
$6A^5$	3/23	1/23	n.s.
$6A^6$	6/23	0/23	0.022
$6A^7$	3/23	3/23	n.s.
$6A^8$	1/23	1/23	n.s.
SP-A2 Allele	**BPD (n=23)**	**Controls (n=23)**	**p-value**
1A	4/23	1/23	n.s.
$1A^0$	14/23	17/23	n.s.
$1A^1$	4/23	5/23	n.s.
$1A^2$	12/23	6/23	n.s.
$1A^3$	3/23	0/23	n.s.
$1A^4$	3/23	2/23	n.s.
$1A^5$	1/23	0/23	n.s.

BPD: bronchopulmonary dysplasia; n.s.: not significant.

A difference between infants with BPD and controls in the allele distribution for the SP-A1 gene was demonstrated for the $6A^6$ polymorphism, indicating the latter to be a risk factor for BPD.

Discussion

This is to our knowledge the first report to suggest an association of a polymorphism of SP-A encoding genes and the risk of BPD in preterm infants. To date only an association of combined polymorphisms of genes encoding for surfactant associated proteins A and B has been described to modify the acute course during the first week of neonatal RDS[16]. Surfactant associated qrotein B is essential for immediate maintenance of lung function postnatally, whereas a complete lack of SP-A did not affect postnatal pulmonary adaptation in knock-out mice[17]. We therefore aimed to investigate a disorder like BPD in which the function of SP-A due to its inflammation-modulating properties is thought to be more relevant than in the acute phase of RDS. The functional role of SP-A in the development of BPD may be attributed to its innate immune response to microbiological challenge and regulatory immune effects[9].

We have established silent polymorphisms in the SP-A1 gene, the functional role of which may be questioned. Consequences of single nucleotide polymorphisms have been postulated for various cardiovascular, endocrine and psychiatric disorders, among them those affecting receptor expression and tertiary protein formation[20]. For example an association of two silent polymorphisms of platelet glycoprotein receptor with cardiovascular disorders has been established recently[21].

Our findings indicate a polymorphism of SP-A encoding genes to be a significant co-factor in the pathogenesis of BPD, in addition to the well-known risk factors such as the degree of immaturity, low birth weight and absent prenatal glucocorticoid administration in preterm infants with neonatal RDS. Studies enrolling preterm neonates from other ethnic origins will be necessary to further establish the described association.

Treatment of RDS in preterm infants requires stratification of the infants according to genetic risk factors.

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Dual-probe pH monitoring for the assessment of gastroesophageal reflux in the course of chronic hoarseness in children

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SUMMARY: Kalach N, Gumpert L, Contencin P, Dupont C. Dual-probe pH monitoring for the assessment of gastroesophageal reflux in the course of chronic hoarseness in children. Turk J Pediatr 2000; 42: 186-191.

The purpose of our study was to assess gastroesophageal reflux (GER) by dual-probe pH monitoring in children suffering from chronic hoarseness for more than six months.

Seventeen children (aged between 2 and 12 years, 10 boys and 7 girls) were enrolled. All children underwent a laryngoscopy and a 24-hour dual-probe pH monitoring. At both sensor, distal and proximal esophageal, a pathological GER was defined as the presence of episodes of acid reflux with pH<4 during a fraction of the total recording time greater than 5.2 percent. The computer considered the child was supine when asleep and upright when awake.

Laryngoscopy revealed interarytenoid erythema and/or edema with vocal cord nodules or granulomas in 13 cases (76.4%), isolated vocal nodules or granulomas in three cases (17.6%) and a normal appearance in one case (5.8%).

At both sensors, the majority of refluxes occurred when the child was upright, as analyzed by the percentage of time the intra-esophageal pH was below four (% time pH<4), number of refluxes, reflux episodes/hour and longest reflux episode, $p<0.05$ between upright and supine for each parameter. The median total % time pH<4 on the proximal and distal probes was respectively 1.62 percent (95% CI 1.50-3.79) and 11.49 percent (95% CI 8.81-27.17), $p<0.0003$.

Among the 17 hoarse children, a pathological GER was observed in 12 (70.5%) at the distal sensor and in three (17.5%) at both sensors.

Among the 16 hoarse children with abnormal findings on laryngoscopy, two (12.5%) had diagnosed pathological GER at the proximal and 11 (68.7%) at the distal sensor. The only child with normal findings on laryngoscopy exhibited a pathological GER at both sensors.

Our results suggest that chronic hoarseness is associated with a pathological GER. The majority of these documented refluxes ocurred when the child was awake.

Key words: hoarseness, dysphonia, gastroesophageal reflux, pH monitoring, child.

Hoarseness is a common complaint in both adults and children. The voice becomes hoarse and has diminished ability to change volume and pitch. It occurs when laryngeal function or anatomy is altered in such a way that proper apposition of the vocal cords for normal speech production is prevented. In children, the importance of "voice change" has not been stressed in the literature. However, Cohen et al.[1] highlighted that in children there is a tendency to postpone the examination merited by a hoarse voice, probably because of the rarity of malignancy in the child's larynx. Laryngeal structural anomalies, infection, inflammation and trauma, as well as neurogenic vocal cord dysfunction, can all result in hoarseness in children[2].

In adults, gastroesophageal reflux (GER) has been increasingly implicated as a cause of chronic laryngeal symptoms such as cough, sore throat, persistent throat clearing, globus sensation and hoarseness[3-5]. The incidence of hoarseness in association with GER ranges from 55 to 79 percent[5-7].

Limited data has been published on this subject in children. Pathogenic reflux in children has recently been associated with acute laryngitis, recurrent croup, stridor, obstructive sleep apnea, laryngospasm and cough[8]. To our knowledge, no correlation has been suggested between GER and hoarseness in this age group.

The purpose of our study was to assess GER by dual-probe pH monitoring in children suffering from chronic hoarseness.

Material and Methods

Between January 1995 and December 1996, 17 children (aged between 2 and 12 years, 10 boys and 7 girls) suffering from chronic hoarseness for more than six months were enrolled in the study. None of them were known to have classical symptoms of GER: emesis, dysphagia, rumination, belching, choking, gagging, failure to thrive, heartburn, indigestion, sour taste in mouth, and nocturnal dyspnea or cough. None were receiving any drugs which might affect gastrointestinal motility or gastric acid production.

All the children enrolled underwent a laryngoscopy and a clinical evaluation performed by the same Pediatric Otolaryngologist. All were then referred to the Pediatric Gastroenterology Department for 24-hour dual-probe pH monitoring. We used a "Digitrapper" recording device (Synectics, Stockholm, Sweden) and medical dual-channel pH catheter with two antimony pH sensors, model 91-9011, 2.1 mm diameter, 175 cm length (Ingold, Urdorf, Switzerland). The probe was introduced nasally. The distal sensor was placed at the level of the lower esophagus, 3 cm above the lower esophageal sphincter (LES), with the Strobel formula, length from nares to LES (cm): 5+0.25 height[9]. The proximal sensor, upper esophageal, was positioned either 10 or 15 cm above the distal sensor depending on the child's height. The position of both probe tips were checked radiographically. The system was calibrated before each recording. The probes registered every four seconds on each channel for between 18 and 24 hours.

Recording equipment was put in a rucksack on the child's back. The children received no food by mouth for two to three hours before the investigation and then ate a normal diet.

The parents recorded the child's actions such as eating and sleeping. The computer considered the child was supine when asleep and upright when awake. Each procedure was carried out by the same member of the staff. The pH monitoring tracings were analyzed by the same Pediatric Gastroenterologist to confirm the computerized calculations and to insure the quality of the recording.

The data from the "Digitrapper" were loaded into a computer and analyzed using an Esophagram program (Esophagram Software Package: Gastrosoft Inc., Chicago, IL). Computer analysis of the stored data was used to calculate at both levels, distal and proximal, the different parameters while the child was upright, supine and in total. The following parameters were: the percentage of time the intra-esophageal pH was below four (% time $pH<4$), the total number of refluxes, the number of reflux episodes per hour (reflux episodes/hr), the number of episodes longer than five minutes and the longest reflux episode.

At the distal sensor, a pathological GER was defined as the presence of episodes of acid reflux with $pH<4$ during a fraction of the total recording time greater than 5.2 percent[10]. Since there is no literature data concerning the acidic exposure of proximal sensor, we retained the same pathological criteria for pathological GER at the proximal sensor as for the distal one, thus probably underestimating the phenomenon. In addition, an upper esophageal reflux episode was considered secondary to GER only if associated with a lower esophageal reflux episode.

Calculation of median and 95% confidence interval (CI) of all quantitative parameters were done using the State View System. The data were non-parametrically distributed and were statistically analyzed using the non-parametric tests of Wilcoxon and Mann-Whitney ($p<0.05$).

Results

Fiberoptic laryngoscopy revealed interarytenoid erythema and/or edema with vocal cord nodules or granulomas in 13 cases (76.4%). Isolated vocal nodules or granulomas without interarytenoid erythema were observed in three cases (17.6%) and a normal laryngoscopy was found in one case (5.8%) (Table I).

At both sensors, the majority of refluxes occurred when the child was upright, as analyzed by % time $pH<4$, number of refluxes episodes/hr and longest reflux episode, $p<0.05$ between upright and supine for each parameter (Tables I and II). The median total % time $pH<4$ on the proximal

and distal probes was respectively 1.62 percent (95% CI 1.50-3.79) and 11.49 percent (95% CI 8.81-27.17), p<0.0003 (Table I).

Among the 16 hoarse children with abnormal findings on laryngoscopy, two (12.5%) had a pathological GER at the proximal and 11

Table I. Laryngoscopy and 24-hour pH Monitoring Findings for 17 Dysphonic Children

				Inferior probe			Superior probe		
Patients	Age (years)	Sex	Laryngoscopy	% time pH<4 total	pH<4 upright	pH<4 supine	% time pH<4 total	pH<4 upright	pH<4 supine
GER negative									
1	2	F	VCN	0.18	0.8	0	0.09	0.2	0
2	7	M	IEE+VCN	2.7	5.17	0.16	1.62	3.19	0
3	9	M	IEE+VCN	2.94	3.9	2.06	1.23	2.54	0
4	8	M	IEE+VCN	3.37	6.67	0.14	1.03	2.07	0
5	6	F	IEE	5.17	9.5	0.46	1.48	2.84	0
GER positive									
6	3	M	IEE	62.65	58.18	65.04	5.71	6.06	5.53
7	11	F	IEE+VCN	43.18	60.97	17.14	1.85	1.42	2.35
8	12	F	VCN	40.21	31.82	50.59	5.08	5.21	4.92
9	9	M	IEE+VCN	29.89	22.52	37.5	0.94	1.51	0.35
10	12	F	IEE	28.02	58.21	2.81	5.02	5	5.04
11	12	M	VCN	25.55	43.13	1.85	5.91	9.89	0.56
12	11	M	IEE	19.86	11.03	28.23	3.06	5.87	0.34
13	6	M	NL	13.8	21.49	3.58	7.18	4.72	10.41
14	8	M	IEE	11.49	19.13	0.94	0.4	0.68	0
15	10	F	IEE	6.38	13.39	0.66	0.8	1.77	0
16	7	F	IEE	5.22	8.67	1.46	2.02	1.94	2.11
17	5	M	IEE+VCN	5.27	8.99	0	1.59	2.71	0

GER: Gastroesophageal reflux. NL: Normal. IEE: Interarytenoid erythema and/or edema. VCN: Vocal cord nodules or granulomas.

Table II. Overall pH Monitoring Results for 17 Children

pH variables	Inferior probe median (95% CI)	Superior probe median (95% CI)
Total % time pH<4	11.49 (8.81-27.17)*	1.62 (1.50-3.79)
Upright % time pH<4	13.39 (12.05-33.06)*	2.71 (2.12-4.65)#
Supine % time pH<4	1.85 (2.01-22.9)*	0.34 (0.35-3.38)
Total number of refluxes	170 (108.6-335.02)*	24 (16.10-52.5)
Number of upright refluxes	154 (96.5-223.5)*#	20 (12.19-46.8)#
Number of supine refluxes	13 (6.9-130.5)*	1 (0.31-9.21)
Total reflux episodes/hr	8.6 (5.2-16.3)*	1.06 (0.73-2.60)
Upright reflux episodes/hr	14.8 (9.1-24.8)*#	1.82 (1.09-4.69)#
Supine reflux episodes/hr	1.11 (0.76-13.16)*	0.1 (0.02-0.92)
Longest reflux (min)	19 (16.60-39.98)*	7 (5.65-14.46)
Longest reflux while upright (min)	11 (10.87-30.86)*	6 (4.52-8.06)
Longest reflux while supine (min)	4 (2.51-22.89)	1 (1.14-11.4)

* Inferior probe vs superior probe, p<0.05 with Mann-Whitney test.
# Upright position vs supine position, p<0.05 with Wilcoxon test.

Among the 17 hoarse children, a pathological GER, according to the definition depicted in Methods, was observed in 12 (70.5%) at the distal sensor and in three (17.5%) at both sensors. The number of episodes greater than five minutes on the proximal and distal probes was respectively, one (95% CI 0.69-2.36) and two (95% CI 2.89-11.69), non-significant.

(68.7%) at the distal sensor. The only child with normal laryngoscopic exhibited a pathological GER at both sensors (Table I).

Discussion

Gastroesophageal reflux was first suggested as an etiological factor in laryngeal disease by Cherry and Margulies[11], who reported three

patients with contact ulceration of the larynx and significant GER on barium studies. Their theory was supported experimentally by the formation of granuloma at the site of daily application of gastric acid to vocal cords in two 2 dogs for six weeks[12].

Classically, GER is divided into uncomplicated, complicated and extraesophageal forms. Children with laryngeal symptoms fall into the latter extraesophageal group. The notion of silent GER is not a new one[13]. The data from our study help to emphasize that laryngeal symptoms with characteristic laryngeal lesions, particularly chronic hoarseness, may be secondary to occult GER. Classical symptoms of GER were found in 20 to 50 percent of patients with head and neck manifestations[4]. Thus, many otolaryngology patients have a pattern of GER disease that is distinctly different from the pattern seen in gastroenterology patients with esophagitis[4]. In patients attending ear-nose-throat disease clinics, GER is often occult, with a relative lack of esophagitis and its symptoms, making diagnosis of GER occasionally difficult.

There are two theories by which GER may cause laryngeal symptoms. The first is the "vagally mediated reflex" theory where the stimulus is acid in the lower esophagus and the response is chronic repetitive throat clearing and cough which would eventually lead to laryngeal lesions[5]. This neural mechanism is difficult to prove and there remains limited evidence for this theory. The second is the "direct acid injury" theory in which the refluxate must tranverse through the upper esophageal sphincter (UES). It was believed that pharyngeal pH monitoring might help to clarify this theory but unfortunately technical difficulties, such as intermittent drying out of the probe leading to pseudo-reflux, complicated matters[5]. However, the duration of acid refluxate contact with mucosa plays a key role in determining the degree of mucosal injury. As well as acid, refluxate contains pepsin, trypsin, bile and other gastroduodenal enzymes, all of which may damage laryngeal mucosa[14].

Unfortunately, there is surprisingly little data on laryngeal symptoms related to GER in either adults or children attending digestive clinics. In adults, two reports state that laryngeal symptoms occur in between six to 25 percent[15]. In children, a recent study has shown that GER was found in the presence of laryngeal symptoms: 61 percent laryngomalacia, 58 percent stridor, 56 percent laryngitis, 25 percent laryngeal papillomatosis and only 14 percent dysphonia[16]. However, we have already reported a pharyngeal acid reflux in a series of eight children with recurrent acute laryngitis who underwent two-channel pH monitoring for 24 hours[17]. That study suggests the role of GER in recurrent laryngitis in children[17]. We have also recently reported in three different series of children, using only distal probe 24-hour pH monitoring, that from 59 to 64 percent of those children suffering from chronic dysphonia had pathological GER[18-20].

Children in our study were selected for pH monitoring on the basis of their hoarseness and signs of "posteior acid laryngitis". These classical laryngeal changes are highly suggestive, but not pathognomonic, of GER. However, the posterior larynx is the area most often affected, probably due to positional and gravitational effects[3,21]. The causal relationship of these lesions with acid exposure is supported by canine studies in which similar lesions were reproduced by intermittent applications of gastric acid to the vocal cords or subglottic area, but not by applications of normal saline or saliva[12,22]. In the only known reported association between GER and hoarseness in children, Putman and Orenstein[23] described an eight-year-old girl who presented with hoarseness, night-time cough and gastrointestinal bleeding. No laryngoscopy was performed but pH monitoring disclosed a pH below 4 for nine percent of the total recording time. Postprandial reflux was greatly increased. Most of the refluxes occurred while she was supine[23].

In this study, the classical criteria for a pathological GER were retained at the distal sensor[10]. Since there is no literature data concerning the acidic exposure of proximal sensors, we retained the same criteria for pathological GER at the proximal sensor as for the distal one, thus probably underestimating the phenomenon at the proximal one. In addition, an upper esophageal reflux episode was considered secondary to GER only if associated with a lower esophageal reflux episode. In our study a pathological GER was found in 70.5 percent of cases at the distal probe and in only 17.5 percent of cases at the proximal one. Although the proximal probe has excellent specificity (91%) it has poorer sensitivity and

reproducibility (55%) for identifying abnormal amounts of proximal esophageal acid reflux[24]. Therefore, a negative test result does not exclude proximal reflux with microaspiration as a cause of atypical reflux symptoms[24].

In hoarse children without symptoms of GER, esophageal acid exposure time was much greater when the child was awake, the majority of refluxes were of short duration and their frequency was high. The reason for this is not known. It may be that these patients are repetitive air swallowers (aerophagics), a hypothesis currently under investigation[6]. The short duration and paucity of supine reflux may explain the low frequency of endoscopic esophagitis. This is supported by Katz[6] who studied 10 adults with hoarseness and GER, seven of whom had abnormal hypopharyngeal reflux predominantly in the upright position, of short duration and high frequency. These patients had no esophageal symptoms. Furthermore, Wiener et al.[5] studied 33 adults with hoarseness and signs of posterior laryngitis. GER, more than three times greater than the upper limit of the normal, was shown in 78.8 percent of patients. This GER was worse in the upright position but of long duration. Less than half of these patients had typical symptoms of GER. Of special interest is the fact that the severity of the GER noted in those patients was not proportional to the incidence of traditional symptoms of esophagitis (heartburn and chest pain). The fact that patients with reflux laryngitis had GER predominantly in the upright position may help to explain this disparity[5]. All these articles claim that the upright pattern of reflux seems to differentiate patients with hoarseness and GER from symptomatic patients with esophagitis. Why the characteristics of esophagitis are absent in these patients is not clear, but it may relate to the predominance of upright reflux, as opposed to supine GER. Koufman[4] explained this by the fact that clearance of refluxate is dependent on normal gastric motility and that esophageal dysmotility is uncommon in patients with otolaryngeal symptoms.

On the other hand, patients with both severe esophagitis and hoarseness apear to have a different pattern of reflux, being predominantly nocturnal supine refluxers[7,25]. In young infants, Vandenplas et al.[26] found that the acidity of long duration reflux episodes (the area under pH 4) is a determining factor in the prediction of esophagitis.

In our study, chronic hoarseness was associated with a pathological GER in 17.5 percent of cases at the proximal probe and in 70.5 percent of cases at the distal one. The pH traces highlighted that the majority of these documented refluxes were of short duration and occurred when the child was awake. Laryngoscopy is an essential investigation in the chronically hoarse child: it shows erythema, edema, nodules and granulomas, which are often attributed to vocal cord abuse. In these circumtances, GER should be considered, and 24-hour pH monitoring is indicated to determine appropriate treatment.

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Allogeneic bone marrow transplantation for children with myelodysplastic syndrome

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Six children with myelodysplastic syndrome underwent allogeneic bone marrow transplantation (BMT) from their HLA-identical siblings. Ages ranged from six to 16 years. French-American British (FAB) diagnosis was refractory anemia with excess blasts (RAEB) in three, RAEB in transformation (RAEB-t) in one and chronic myelomonocytic leukemia (CMML) in two cases. Two patients had progressed to leukemia before BMT. All patients received busulfan and cyclophosphamide as a conditioning regimen. Antithymocyte globulin (ATG) was administered to two of them due to the multiple transfusion history. Graft versus host disease (GvHD) prophylaxis consisted of cyclosporine-methotrexate. Engraftment was documented in all patients except one who underwent a second infusion of bone marrow cells. She died in the early post-transplant period with pancytopenia and veno-occlusive disease of the liver. Two patients died from disease recurrence. Three patients are alive >12 months post-transplant, two are in remission and one just relapsed at +16 months and is now being prepared for a second bone marrow transplant. The only significant factor for favorable outcome was short duration between diagnosis to transplant in the two patients in remission.

Key words: myelodysplastic syndrome, children, bone marrow transplantation.

Myelodysplastic syndrome (MDS) comprises a heterogeneous group of clonal stem cell disorders characterized by ineffective hematopoiesis and morphologic abnormalities usually involving all three blood lineages. Its occurrence is relatively unusual in childhood[1,2]. The reported incidence of MDS among all leukemias varies from one to 16 percent. In pediatrics, only three percent or less of hematological malignancies have been diagnosed as MDS; however, this figure is accepted to be an underestimated representation of its real occurrence[3,4].

The syndrome is highly aggressive in children and may progress to acute myelocytic leukemia (AML) in a short time period. The response to chemotherapy is limited, usually complicated by prolonged periods of aplasia, and most remissions are of short duration[1,2]. The goal of treatment is eradication of the preleukemic clone and reconstitution of normal hematopoiesis. Allogeneic bone marrow transplantation (alloBMT) has proved effective in this regard, representing the only curative treatment for MDS in young patients at present, with reported disease-free survival rates of 50-65 percent for patients <20 years of age[5-12]. However, a suitable bone marrow family donor is not available for the majority of patients. In such cases intensive chemotherapy followed by autologous stem cell rescue may also be considered[13]. Recent studies suggest that ineffective hematopoiesis, excessive bone marrow apoptosis, and an aberrant immune response play a role in the pathogenesis of MDS[14]. Therefore, in selected patients the use of trophic agents (amifostine); recombinant growth-factors; anti-apoptotic treatment; differentiation inducers such as hexamethylene bisacetamide, 5-azacytidine[14], and high-dose methyl prednisolone[15]; or immunosuppressive treatment (cyclosporine; antithymocyte globulin has been of benefit. Meanwhile, alloBMT from

a HLA-matched sibling remains the best treatment modality for children with MDS[7,8,10].

Here we describe six children with MDS who underwent alloBMT from tehir HLA-identical sibling donors at our institution, using a busulfan-based conditioning regimen.

Material and Methods

Patients' Characteristics

Between March 1995 and January 1999, six children with MDS underwent alloBMT from their HLA-identical, mixed lymphocyte culture (MLC) non-reactive siblings at our institution. Table I shows the patient characteristics including age, sex, clinical findings, peripheral blood and bone marrow blasts, karyotype analysis, and French American British (FAB) classification. Transplant characteristics are listed in Table II. The ages at time of transplantation ranged fram six to 16 years. Four patients were female and two males. None was diagnosed as sceondary MDS. Patients were classified according to the FAB cooperative group criteria. Diagnosis at the time of presentation was refractory anemia with excess blasts (RAEB) in three patients, RAEB in transformation (RAEB-t) in one and chronic myelomonocytic leukemia (CMML) in the remaining two children.

Pre-BMT cytogenetic studies were obtained from marrow aspirates in all patients. Trisomy 8 was detected in one patient with CMML, and 45,XX,-15,11(q21) in one.

Two out of six patients were in remission at the time of transplant. Four patients were treated with conventional chemotherapy before BMT. The remaining two patients (Case 3, Case 5) were not treated with chemotherapy before BMT due to the low number of BM blasts (<10%).

Table I. Patients' Characteristics at Diagnosis

Case	Age at diagnosis years/sex	FAB	Hepatomegaly/ Splenomegaly	EMD	Hb (g/dl)	WBC ($x10^9$/L)	Platelet ($x10^9$/L)	PB blast	BM blast	MCV (fl)	Cytogenetics
1	12/F	RAEB-t	13/7	Gingival	5.3	80	68	12	28	90	45,XX,-15,11 (q21)
2	9/M	RAEB	0/0	–	6.4	2.3	220	0	9	80	NA
3	7/F	RAEB	7/0	–	5.6	0.8	19	0	10	82	NA
4*	4/F	RAEB	2/0	–	5	2.3	180	2	4	90	46,XX
5	8/M	CMML	0/0	–	11	6.7	36	24 mono	4	112	47,XY,+8
6	14/F	CMML	0/splen.**	–	9	18.7	180	30 mono	7	–	NA

* syngeneic.

** splen: splenectomy, six months before the diagnosis of myelodysplastic syndrome.

FAB: French American British classification. EMD: extramedullary disease. RAEB: refractory anemia with excess blasts. RAEB-t: RAEB in transformation. CMML: myelomonocytic leukemia.

Table II. Patients' Characteristics at the Time of Transplantion

Case	Age at BMT (yrs)	Interval Dx-BMT (mo)	Disease status at BMT	Sex (donor/ recipient)	ABO (donor/ recipient)	Nucleated cell ($x10^8$/kg)	Conditioning regimen	GVHD prophylaxis	Engraft (+day) PNL/plt	Outcome (mo)
1	16	50	Disease present	M/F	A/A	4	BU/CY	CsA+Mtx	16/23	AML (+4) exitus
2	11	22	CR,	F/M	O/O	3.7	BU/CY	CsA+Mtx	14/17	AML (+16) alive
3	7	4	Disease present	F/F	AB/AB	4	BU/CY/ATG	CsA+Mtx	14/19	CR (+16)
4*	6	24	CR,	F/F	O/O	4	BU/CY	CsA	12/14	ALL (+12) exitus
5	8	3	Disease present	F/M	O/O	5	BU/CY	CsA+Mtx	16/18	Cr (+13)
6	16	17	Disease present	F/F	A/A	3	BU/CY/ATG	CsA+pred	–	exitus

* syngeneic.

BMT: bone marrow transplantation. Dx: diagnosis. GVHD: graft versus host disease. PNL/plt: polymorphonuclear leukocytes/ plateles. BU/CY: busulfan/cyclophosphamide. CsA+mtx: cyclosporin A+methotrexate. AML: acute mvelocytic leukemia. CR: complete remission. ATG: antithymocyte globulin. ALL: acute lymphocytic leukemia. pred: prednisolone.

Case 4 progressed to acute lymphocytic leukemia (ALL) 16 months after initiation of chemotherapy for MDS and underwent BMT from her twin sister following eight months of ALL treatment. Another child (Case 2) transformed to AML four months after diagnosis of MDS AlloBMT was performed from his HLA-identical sibling following 22 months of chemotherapy on AML protocol. One splenectomized patient with CMML (Case 6) was transplanted for chemoresistant disease; at the time of transplantation, she had been suffering from persistent fevers, pancytopenia and hemolytic anemia.

Conditioning Regimen

Conditioning regimen consisted of: busulfan (Bu) 16 mg/kg of total dose given orally in 16 divided doses every six hours from day -9 to day -6 and cyclophosphamide (Cy) 50 mg/kg/day given intravenously (i.v.) on days -5 to day -2. Antithymocyte globulin (ATG) was added to the conditioning regimen in two patients due to multiple transfusion history, at a dose of 30 mg/kg/day on days -5 to -3. Anticonvulsive therapy as seizure prophylaxis during Bu administration was employed in all patients. In addition, hyperhydration and administration of mesna were done according to the standard schedule to reduce the risk of hemorrhagic cystitis during Cy administration.

Marrow Transplantation

Donor marrow was infused on day 0, and median nucleated marrow cell dose was $4x10^8$/kg (range $3\text{-}5x10^8$/kg). Written consent was obtained from parents before transplant.

All donors were HLA-identical, MLC non-reactive siblings (one identical twin). There were two sex mismatched; none of the recipient-donor pairs showed ABO incompatibility.

Graft-Versus Host Disease (GvHD) Prophylaxis

Graft versus host disease (GvHD) prophylaxis consisted of cyclosporin A (CsA) administered i.v. starting on day -2, at a dose of 3 mg/kg/day for the first two to three weeks and subsequently perorally (p.o.) at a dose of 6 mg/kg/day for six months after transplant, and short-term methotrexate (MTX) at a dose of 10 mg/m^2 on days 1, 3 and 6. MTX was omitted from the protocol in a patient at high risk of developing hepatic venoocclusive disease (VOD). One patient who received syngeneic BM cells was not given GvHD prophylaxis.

Supportive Care

Patients were isolated in hepa-filtered rooms at day -10 and received oral antibiotics for enteric decontamination. For Pneumocytis carinii prophylaxis, all patients received TMX 3 days a week, starting a week after engraftment. Irradiated and filtered blood products were used in all. Other supportive care measures included weekly infusions of intravenous immunoglobulins (200 mg/kg), acyclovir for herpes simplex virus (HSV) and cytomegalovirus (CMV) prophylaxis, and fluconazole. In addition, low molecular weight heparin, vitamin E and ursodeoxycolic acid were used for VOD prophylaxis in one splenectomized chemoresistant patient.

Engraftment

Hematopoietic engraftment was documented by either karyotype analysis on peripheral blood (PB) or BM cells, or DNA analysis by polymerase chain reaction (PCR) amplification of STR regions. White blood cell enfragtment was defined as the first of three consecutive days when neutrophils reached $>0.5x10^9$/L and platelet engraftment as the first of three consecutive days when the unsupported platelet count was $>50x10^9$/L.

Results

Transplant characteristics and the outcome of six patients are reported in Table II.

Engraftment

Duration of cytopenia and blood requirements were unremarkable except in one case with graft failure. Five patients achieved prompt and complete engraftment as evidenced by rising blood counts (neutrophil engraftment at 12-16 days with a median of 14 days). Platelet engraftment took place at 14-23 days (median 18). Donor engraftment was documented in four patients by karyotype or DNA analysis.

GvHD

None of the patients developed >grade II acute or chronic GvHD.

Regimen Related Toxicity

Grade I-II mucositis was documented in five patients, and Case 1 developed necrotizing and hemorrhagic (grade IV) mucositis. Nausea and

vomiting were infrequent. No significant central nervous system or renal toxicity was observed. Moderate VOD occurred in one patient.

Patient Outcome

Of six patients, three are alive, two in complete remission with a follow-up period of 12 and 16 months and a Karnofsky score of 90-100 percent. One patient just relapsed at +16 months during the preparation of this manuscript. The patient has received remission-induction treatment and will be prepared for a second BMT. Serial karyotype and molecular analysis demonstrated a full chimerism of donor cells in two surviving patients; complete disappearance of the characteristic chromosome abnormality (Trisomy 8) was demonstrated in Case 5, following BMT.

Two of six patients died with recurrent disease at four and 12 months after BMT. Another case (Case 6) with CMML, who had resistant disease, pancytopenia, persistant high grade fevers and hemolytic anemia at transplant did not engraft. Not surprisingly, she had a very complicated post-transplant course with continuous fevers, worsening hemolytic anemia, CNS hemorrhage, VOD, platelet refractoriness, and graft failure necessitating a second bone marrow boost from the same donor at day +50. No conditioning regimen was used. She died of pulmonary compromise, infection and hemorrhage 32 days after her second transplant. BM aspiration at the time of death showed recurrent disease with infiltration of the marrow with >30% percent blasts. Postmortem examination of liver and lung tissues was consistent with VOD and hemorrhage.

Discussion

Myelodysplastic syndromes (MDS) run an aggressive course in children. Most pediatric cases show features associated with poor prognosis, and AML develops within a few months[16]. The syndrome comprises a heterogeneous group of clinical disorders such as monosomy 7 and juvenile chronic myelogeneous leukemia (JCML), in which clinical behavior can vary substantially with overlapping features and similarities to adult MDS[1,16]. BMT offers a good chance of cure for such patients if a suitable donor is present[7,8].

The effect of age on the outcome of transplantation in patients with MDS is well documented. In most disease categories, the survival after alloBMT is better in younger patients due to less GvHD and better ability to tolerate the toxicity of chemotherapy. Similarly, patients with MDS less than 20 years of age are reported to achieve a disease-free survival (DFS) of 50 to 65 percent[5-12].

The cohice of the conditioning regimen also has an impact on transplantation success. Conditioning regimens with busulfan have been used successfully for eradication of the malignant clone in MDS[7,8,17], and are generally preferred in children over total body irradiation to avoid late sequelae of irradiation. In the present study, the conditioning regimen was busulfan-based due to its strong efficacy and acceptable toxicity. All patients received busulfan and cyclophosphamide (BuCy); in addition, ATG was added to the regimen in two hypertransfused patients to prevent graft rejection. The regimen was generally well tolerated in all children. In three patients, the myeloablative regimen was not sufficient to eradicate the clone since two died of early recurrence, and one with resistant disease.

Several investigators have suggested the use of intensified conditioning regimens to potentially eradicate stem cells and the abnormal clone. The addition of melphalan to the classical BuCy regimen has resulted in >70 percent survival rates with acceptable toxicity[7]. Likewise, an Ara-C, busulfan and melphalan combination has also led to a favorable outcome after BMT in children[17]. However, large-scale randomized pediatric series are missing since the syndrome is rarely diagnosed in children.

Disease duration has also been suggested as a significant factor for transplant success although not as important as the impact of age[7,12]. It has been shown that patients transplanted after five years of diagnosis had a less favorable outcome than the patients transplanted at earlier phases of disease[12]. In the present study, in the patients with relapse and resistant disease, the interval from diagnosis to transplant was long (17-50 months), as opposed to that of relapse-free patients (3-4 months). Longer disease duration, and therefore, more advanced stages of the disease, may have contributed to the higher incidence of treatment-related mortality and the higher relapse risk. Two of our surviving patients were in the early stage of their disease suggesting the important role of transplant timing in patients with MDS as in other hematological malignancies.

Other factors that may contribute to the adverse effect on BMT outcome have been reported as the presence of BM fibrosis, an increased number of BM blasts and secondary MDS[7,8]. One of our patients with a fatal outcome had an increased number of blasts (Case 1) in the bone marrow; none had BM fibrosis. All of our patients were de novo cases with no secondary MDS.

Extramedullary involvement has also been suggested as a poor prognostic factor in MDS; however, its effect on BMT outcome is not well known. In a recent study, pretransplant diagnosis of extramedullary disease was associated with poor outcome in a group of patients less than two years old with AML (n=34) and MDS (n=6)[10]. One of our patients who presented with gingival involvement and massive organomegaly at initial diagnosis had a poor outcome with early BM relapse at +4 months. At the time of BMT and at relapse she was free of extramedullary disease. In another study, granulocytic sarcoma after BMT was demonstrated as a poor risk factor in a group of patients with AML, MDS and chronic myelocytic leukemia (CML)[18]. Thus, the role of extramedullary involvement on BMT outcome in patients with MDS has yet to be defined.

The role of cytogenetic analysis in prognosis determination in patients with MDS has been clearly demonstrated by the international prognostic scoring system[19]. Certain cytogenetic abnormalities such as del5(q), del(20q), -Y (as sole abnormalities), or normal cytogenetics are considered as good factors, whereas the presence of 3 karyotype abnormalities or monosomy 7 or del (7q) suggest a poor prognosis[19]. The application of this scoring system to pediatric cases is not defined. Numerical abnormalities, in which a whole chromosome is either lost or gained (monosomy 7, trisomy 8) are typically seen in pediatric ases[1]. In the present report, one of our patients carried trisomy 8 abnormality in his hematopoietic cells; he is currently in remission >12 months post-transplant. However, due to the small number of cases in pediatrics, the effect of cytogenetic abnormalities on BMT outcome is not clearly defined.

In the present report we have described the outcome of six children with MDS treated with alloBMT. The only significant factor was the disease duration between relapsed and relapse-free patients. Two patients alive in compelete remission were transplanted very early after diagnosis without previous chemotherapy. In spite of the small number of patients, the present study suggests early transplantation as a favorable prognostic factor.

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Fludarabine, cytarabine, G-CSF and idarubicin (FLAG-IDA) for the treatment of relapsed or poor risk childhood acute leukemia

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SUMMARY: Yalman N, Sarper N, Devecioğlu Ö, Anak S, Eryılmaz E, Can M, Yenilmez H, Ağaoğlu L, Gedikoğlu G. Fludarabine, cytarabine. G-CSF and idarubicin (FLAG-IDA) for the treatment of relapsed or poor risk childhood acute leukemia. Turk J Pediatr 2000; 42: 198-204.

The prognosis of relapsed acute leukemia or chronic leukemia in acute blast crisis is poor and new chemotherapeutic regimens could be useful for these patients. Six relapsed acute lymphoblastic leukemia (ALL), nine relapsed acute myeloblastic leukemia (AML), one chronic myelomonocytic leukemia (CMML) and one chronic myeloid leukemia (CML) in acute blast crisis between three to 18 years (median 10 years) received fludarabine, cytarabine, G-CSF and idarubicin (FLAG-IDA) chemotherapy (CT). Five of the AML relapses were after bone marrow transplantation (BMT) and four were recurrent relapses. At the end of the second course only three patients (2 AML, 1 ALL) were in complete remission (CR). Of the three patients in CR, one patient with AML had her first donor lymphocyte transfusion (DLT) on the 7th day of the second FLAG-IDA course and she is disease-free on the 30th month of the second remission. The remaining two patients were transplanted from unrelated donors in a BMT center abroad on the 5th and 8th month of the last remission and both died with BMT-related complications. Out of 25 courses, seven resulted in fatal infections. The regimen was ineffective in B-cell ALL as in acute blastic crisis of CMML and CML. We could not evaluate the remission-inducing effect accurately in most of the patients due to induction failure. FLAG-IDA appears to be a myelotoxic therapy for relapsed or poor risk leukemia in a developing country. It is not cost-effective; dose modifications or a regimen without IDA may be tried if there is an available marrow donor.

Key words: fludarabine, childhood, leukemia, FLAG, idarubicin.

Despite high remission rates in de novo childhood acute leukemia, relapse is still a serious problem and complete remission (CR) rates of relapse regimens are not satisfactory. In a recent report of the Children's Cancer Study Group, rates of six year survival in acute lymphoblastic leukemia (ALL) after isolated bone marrow (BM), central nervous system and testis relapse were 20, 48 and 70 percent, respectively. Rates of survival after isolated BM relapse at 0-17 months, 18-35 months and after 36 months were six, 11 and 43 percent, respectively[1].

Bone marrow transplantation (BMT) is an effective first line treatment in poor prognosis ALL, acute myeloblastic leukemia (AML) or in chronic myeloid leukemia (CML), but relapse after BMT is generally considered incurable and has not been standardized[2].

In the light of report that FLAG-IDA (fludarabine, cytarabine, G-CSF, and idarubicin) is effective in the treatment of children and adolescents with relapsed AML and chronic myelomonocytic leukemia (CMML) in acute blast crisis[3] and that a complete remission rate of 58-63 percent can be achieved in adult AML and myelodysplastic syndrome (MDS)/AML[4,5], we started FLAG-IDA chemotherapy (CT) for children and adolescents with poor prognosis leukemia (relapsed AML, ALL and CMML, CML in blast crisis). Visani et al.[6] and Montillo et al.[7] also used FLAG in relapsed or primary resistant ALL with a CR rate of 67 percent (8/12) and 83 percent (10/12), respectively.

Fludarabine (9-B-D-arbine-fluranosyl-2 fluoroadenine) is a synthetic purine analogue. Its anti-tumor activity is by termination of DNA and RNA synthesis, inhibiting DNA and RNA polymerases, DNA primase, DNA ligase and ribonucleated reductase, and by potentiation of deoxyctidine kinase activity. It also induces apoptosis[8] and potentiates the activity of anti-tumor agents by modulating metabolism of cytarabine[9]. Fludarabine increases the accumulation of Ara-CTP (active triphosphate metabolite) in leukemic blasts by a median 1.7 fold in vitro studies[9]. Co-administration of G-CSF to patients receiving fludarabine plus cytarabine (FLAG) was based on reports that G-CSF shortened the duration of neutropenia and reduced infection rates following completion of CT[10]. Additionally, in vitro studies suggest that G-CSF administered shortly before, during and a short time after Ara-C can make myelogenous leukemia cells more sensitive to the drug by recruiting quiescent cells into the cell cycle and increasing Ara-C phosphorylation[11,12]. An anthracycline is also combined to the FLAG regimen to increase response to CT. Besides its antileukemic activity, idarubicin is less cardiotoxic[13], which is an important factor for heavily pre-treated relapsed patients.

Material and Methods

Between March 1997-July 1998, six relapsed ALL, nine relapsed AML, one CMML and one CML in acute blast crisis between three to 18 years (median 10 years) were treated with FLAG-IDA regimen after informed consents of parents and/or patients were obtained. Two patients had B-cell ALL (L_3), three B precursor ALL, one T-cell ALL, one AML-M_0, two M_2 two M_4 and four M_5. Five patients with AML had post-BMT relapses (3 after allogeneic-BMT from matched siblings, 1 after auto-BMT, and 1 after autologous peripheric blood stem cell transplantation) at a median of six months (3-7 months). Three patients were in the second, one patient in the third and the rest in the first relapse. All patients had marrow relapse which was associated with extramedullary disease in five (2 testis, 2 central nervous system, 1 bone). Duration of first remission of patients with acute leukemia was ≤six months in three patients, seven to 17 months in seven patients, 18-35 months in four patients and ≥36 months in one patient. Patients with chronic leukemia were on the 24th and 48th months of hydroxyurea and interferon-alpha therapy at the time of acute blastic transformation. AML-MRC-10, BFM-86 ALL and BFM-90 B-cell ALL protocols were administered to patients as first line therapy. Busulfan-cyclophosphamide regimen without irradiation was used as conditioning for BMT.

Cardiac (left ventricle ejection fraction >60), renal (urea, creatinine, creatinine clearance) and hepatic functions (transaminases, bilirubin) were evaluated before CT. Patients were eligible if these functions were within normal limits. The patients received therapy in isolated rooms and all had central venous catheters (Hickmann). In addition to intravenous immunoglobulin (0.2 g/m^2/kg twice a week), amphotericin B and ceftazidime inhalation (2x4 mg/day and 2x1g/day, respectively) were also administered as prophylaxis in the last seven patients. An antiemetic drug (ondansetron) was also used throughout the chemotherapy courses. Total parenteral nutrition, intravenous antibiotics and antifungal agents were administered according to current protocols when necessary. Irradiated blood products were used. A bone marrow aspirate was performed upon hematologic recovery (neutrophils >1x10^9/L, platelets >100x10^9/L). CR was defined as evidence of normal hematopoietic regeneration (neutrophils >1x10^9/L, platelets >100x10^9/L), with <5% blasts), and partial remission was defined as reduction in marrow blasts to at least 50 percent. A second course of FLAG-IDA was planned after the first course. Patients in CR and with a suitable bone marrow donor underwent allogeneic BMT; donor lymphocytes were transfused to patients relapsing after allogeneic BMT.

Treatment Regimen: G-CSF beginning on day 0 was continued up to absolute neutrophil count (ANC)>1000 µl, with 400 µg/m^2/d administered intravenously over 30 minutes; fludarabine (30 mg/m^2) was administered intravenously over 30 minutes, daily for five consecutive days; high dose cytarabine (2000 mg/m^2) was administered for five consecutive days intravenously over four hours, starting four hours after fludarabine; idarubicin (10 mg/m^2) was administered on days two to four intravenously over 30 minutes. The idarubicin dose was reduced to 8 mg/m^2 in the last five patients to reduce myelosuppression.

Results

A total of 25 courses were administered to 17 patients. Patients' characteristics and outcomes are shown in Table I.

Table I. Patients' Characteristics and Response to FLAG-IDA Chemotherapy

Patient	Sex/age at FLAG-IDA, (year)	Diagnosis	Duration of first remission	Response to FLAG-IDA	Overall survival	Survival following 1 FLAG-IDA Outcome
1	M, 8	ALL-second marrow, testis relapse	36 mo.	CT 1: Persisting marrow aplasia	44 mo.	33 d-died with infection
2	M, 10	ALL-T-cell, first marrow, testis, CNS relapse	6 mo.	CT 1: Persisting marrow aplasia	8 mo.	47 d-died with infection
3	F, 3	ALL-L_3, first marrow relapse	16 mo.	CT 1: No reponse	19 mo.	80 d-died with resistant disease
4	M, 4	ALL-L_3, first marrow and bone relapse	6 mo.	CT 1: No response	7 mo.	30 d-died with resistant disease
5	F, 10	ALL-first marrow relapse	20 mo.	CT 1: Persisting marrow aplasia	21 mo.	26 d-died with infection
6	M, 15	ALL-third marrow relapse	48 mo.	CT 1: CR, CT 2: CR	12 years +	11 mo-died with BMT** related complications
7	F, 14	AML-M_2-marrow relapse after AlloBMT	13 mo.	CT 1: Persisting marrow aplasia	14 mo.	35 d-died with infection
8	F, 17	AML-M_4, first marrow relapse after auto PBSCT	14 mo.	CT 1: Persisting marrow aplasia	15 mo.	37 d-died with infection
9	M, 13	AML-M_2, second marrow relapse	6 mo.	CT 1: CR, CT 2: no response	36 mo.	6 d-died with resistant disease
10	M, 14	AML-M_5, second marrow relapse	20 mo.	CT 1: CR, CT 2: CR	39 mo.	7.5 mo-died with BMT*** related complications
11	M, 13	AML-M_5, first marrow relapse after AlloBMT	19 mo.	CT 1: Persisting marrow aplasia	20 mo.	35 d-died with infection
12	F, 3	AML-M_5, first marrow relapse after AlloBMT	13 mo.	CT 1: CR, CT 2: CR	39 mo.	30 mot-disease free after three donor lymphocyte transfusions
13	M, 15	AML-M_5, first marrow relapse	9 mo.	CT 1: CR, CT 2: Persisting marrow aplasia	11 mo.	63 d-died with infection
14	M, 8	AML-Mo, first marrow relapse after AlloBMT	11 mo.	CT 1: no response	13 mo.	60 d-died with resistant disease
15	M, 10	AML-M_4, first marrow and CNS relapse	7 mo.	CT 1: no response, CT 2: no response	10 mo.	85 d-died with resistant disease
16	F, 45	CMML-acute blast crisis	*24 mo.	CT 1: no response, CT 2: response	34 mo.	10 mo-died with resistant disease
17	F, 18	CML-acute blast crisis	*48 mo.	No response	54 mo.	6 mo-died with resistant leukemia

* duration of chronic phase.
** mis-matched unrelated donor.
*** MUD: matched-unrelated donor.

CNS : central nervous system.
PBSCT : peripheric blood stem cell transplantation.
d : days.
mo : months.
CT : chemotherapy.
CR : complete remission.
ALL : acute lymphoblastic leukemia.
AML : acute myeloblastic leukemia.
AlloBMT : allogeneic bone marrow transplantation.
CMML : chronic myelomonocytic leukemia.
CML : chronic myeloid leukemia.

Toxicity: Nausea and vomiting were not serious problems and were controlled with antiemetics. There was no acute neurological, cardiac or renal toxicity. Two patients developed hyperbilirubinemia and hepatic failure associated with severe infections. Recovery of blood counts and incidence of infection: duration of neutropenia (ANC<500x10^9/L) was a median of 32 days (18-42 days) and of thrombocytopenia (<30.000x10^9/L) 27 days (7-38 days) in eight courses resulting in CR. Patients experienced infection episodes in every course. Nine patients (9/17:52.9%) had severe mucositis and one had oral herpetic lesions. Infection episodes were febrile neutropenia (6/25), septicemia (8/25), pneumonia (10/25), enteritis (2/25), sinusitis (2/25) and cellulitis (1/25). Hemoculture isolates were Staphylococcus aureus (4 episodes), Enterobacter (1 episode), Escherichia coli and Candida spp. (1 episode). Pseudomonas aeruginosa (oral lesion), Staphylococcus aureus (throat culture, urine culture), and Candida spp. (throat culture) were other isolates. Two patients had Aspergillus antigenemia and one patient had radiological findings suggesting pulmonary aspergillosis. Seven courses (7/25: 29%) resulted in fatal infections without marrow recovery in a median of 35 days (26-47 days).

Treatment Outcome: Response to FLAG-IDA CT courses and post-CT BMT and DLT (donor lymphocyte transfusion) procedures are shown in Table II. At the end of two CT courses there were only three patients in CR (2 AML and 1 ALL). One patient with AML had DLT on the 7th day of the second FLAG-IDA course. After recovery she had one cycle of Capizzi II (cytosine arabinoside 3g/m^2x4 and L-asparaginase 6000 U/m^2x1) and a second DLT on the 7th day. Then maintanence therapy cycles (cytosine arabinoside 60 mg/m^2 for 4 days and 6-thioguanine for 28 days) were administered for 12 months; at the end of maintenance therapy, a 3rd DLT was given without any complication. She is disease free with a 100 percent Karnofsky score at the 15th month of the 3rd DLT. The remaining two patients who had BMT from unrelated donors in a BMT center abroad at the 5th and 8th months of the last remission died with BMT-related complications (Table II). The patient who had veno-occlusive disease (VOD) during BMT was treated with interferon-alpha for chronic hepatitis C infection and was cured before the leukemia relapse.

Cost of Therapy: Cost of therapy including chemotherapy, G-CSF and supportive measures (antibiotics, antifungal agents, intravenous immunoglobulin, parenteral nutrition and blood products) was a median of 20,400 U.S. dollars ($ 16,000-$ 116,000 dollars) per course.

Discussion

Experience with fludarabine in leukemia cases in children and adolescents is quite limited. In 1996, Fleischhack et al.[3] reported initial results of a pilot study with FLAG-IDA in the treatment of recurrent AML in children and adolescents. In 1997, Dinndorf et al.[14] and Leahey et al.[15] used idarubicin with fludarabine and cytarabine for refractory or recurrent pediatric acute leukemia. In 1998, Fleischhack et al.[16] reported that 17/23 patients with a median age of 7.7 years achieved CR with a median duration of 13.5 months. Eleven patients underwent BMT or peripheric blood stem cell (PBSC)

Table II. Summary of Response to FLAG-IDA Courses and Post-Chemotherapy BMT and Donor Lymphocyte Transfusion Procedures

Diagnosis	All patients n	Response to 1st FLAG-IDA	Response to 2nd FLAG-IDA CT	Post-CT BMT/DLT CT	Outcome
ALL	6	1 CR	1 CR	1 BMT*	BMT-related early death with CMV pneumonia
AML	9	4 CR	2 CR	1 BMT (MUD)	BMT-related early death with VOD
				1 DLT	Disease-free at 30 month of first FLAG-IDA
CMML	1	No response	No response	–	Died with resistant disease
CML	1	No response	–	–	Died with resistant disease

* one mis-matched unrelated donor.

DLT : donor lymphocyte transfusion.
CR : complete remission.
VOD : veno-occlusive disease.
MUD : matched unrelated donor.
AML : acute myeloblastic leukemia.
CML : chronic myeloid leukemia.
CMV : cytomegalovirus.
CT : chemotherapy.
BMT : bone marrow transplantation.
ALL : acute lymphoblastic leukemia.
CMML : chronic myelomonocytic leukemia.

transplantation following FLAG-IDA-FLAG regimen, and overall nine patients remained in CR with a median duration of 17.5 months (9.5-39 months). But eight of 23 patients received only a single FLAG-IDA course and two received FLAG alone. The initial recommendation of FLAG-IDA was changed to FLAG courses for myelotoxicity. Based on their data their conclusion was that a FLAG-IDA regimen with FLAG-IDA as a reinduction course and FLAG as consolidation therapy is an effective therapy prior to allogeneic and autologous BMT or PBSCT. In our study group, induction failure was higher and the outcome was disappointing. Five of nine AML patients had relapsed post-BMT and two patients had second marrow relapse. In their series, none of the patients were post-BMT relapse cases and only two patients were on second relapse. Additionally, neutropenia was shorter in that study (median 22.5 days, range 14 to 42 days). Our patients' longer marrow aplasia and higher CT-related deaths might be attributed to their heavily pre-treated state. In Fleischhack et al.'s[16] series, only three patients were treated with two courses of FLAG-IDA[16]. FLAG-IDA courses were more myelosuppressive than FLAG courses. In our series, CR was induced in patient 13 with one course of FLAG-IDA; however, he could not tolerate the second course. Fleischhack et al.[16] also reported pulmonary infections as the main non-hematological toxicity, and there were three therapy-related deaths.

The FLAG-IDA regimen seems ineffective in acute B-cell leukemia. Two patients with B-cell leukemia died with resistant disease. Two patients with B-precursor ALL and one with T-cell ALL died with infection before marrow recovery. Patient 6 completed two courses with CR without severe infections, but his previous relapses were all responsive to CT, with long remission duration. The regimen was also unsuccessful in inducing remission in acute blastic transformation of CML and CMML.

Time of relapse is an important prognostic variable in leukemia. In the MRC UKALL X study, only three of 106 children with marrow or combined relapse within two years or less of completing therapy were in second CR[17]. Four out of six ALL patients in our series relapsed within two years of remission.

In a more recent report on FLAG for therapy in refractory or high-risk relapsed ALL and AML in children, 13/16 (70%) patients achieved CR, and 4/16 partial remission (PR)[18]. Thirteen patients received BMT as consolidation, and seven patients were alive at 12 months post therapy. In that study, McCarthy et al.[18] preferred to avoid anthracyclines for inducing remission in heavily pre-treated children, and concluded that FLAG is an effective regimen. We did not observe acute cardiac toxicity, but Fleischhack et al.[16] reported cardiac toxicity in three of 24 FLAG-IDA courses. Acute cardiac toxicity is generally acceptable, but all authors emphasize pulmonary infections during CT or post-transplant[16,18]. The high rate of pulmonary infections could have been caused by toxic injury of the lung epithelial cells, by cytostatic drugs, or by the long-term neutropenia involving a risk for fungal infections[19]. Purine analogues such as fludarabine produce long term T-helper cell depletion involving a high risk for opportunistic infection[20]. Although we used liposomal amphotericin and ceftazidime inhalation for prophylaxis during CT of our last seven patients, only three of them completed CT without pulmonary infection. Neutropenia was quite long, despite G-CSF. Laminar air flow rooms may also reduce infections.

Other CT alternatives for primary resistant or relapsed AML also result in low CR rates but with lower induction death rates[21].

Second BMT or DLT must be pursued as soon as CR is achieved, because CRs after post-BMT relapses are generally short lived[22]. Second transplants with a different cytoreductive regimen can eradicate disease resistant to prior myeloablative treatment[22]. Outcomes of second transplants were better in AML compared to ALL[23]. Unfortunately, only one of our patients with post-BMT relapse could achieve CR; he had DLT, which is another alternative. Remission duration of this patient after DLT was longer than the first remission duration.

The FLAG-IDA regimen was used in Philadelphia chromosome positive relapsed ALL to induce a second remission followed by BMT or PBSCT. In this series, two of eight patients received second transplants from their original donors with no further conditioning[24]. This approach may be a safer procedure because our patients who had BMT from unrelated donors in their third and fourth remission could not tolerate the conditioning CT.

In relapsed patients this regimen may be administered if there is an available donor. It seems that stem cell rescue is necessary in heavily pretreated patients who have recurrent relapses or had a previous BMT procedure. In addition, patients stand the risk of another relapse while the search for a matched related donor is undertaken.

Costs of FLAG-IDA courses were very high. Besides hematopoietic growth factors, blood products and therapy of major bacterial and fungal infections increased the cost.

In conclusion, FLAG-IDA is a very intensified therapy causing prolonged neutropenia. Its major toxicity is fatal infections in previously heavily treated patients. We could not evaluate the remission-inducing effect because many courses resulted in fatal infections before marrow recovery. We consider it not to be a cost effective therapy in a developing country. New studies with dose modifications or with FLAG may be tried as pre-BMT CT in relapsed leukemia in children. Our results were disappointing although patients carried very poor risk criteria.

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Hemophilic arthropathy: evaluation of clinical and radiological characteristics and disability

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Hemophilia is an inherited bleeding disorder which produces its greatest morbidity in the musculoskeletal system. Musculoskeletal complications of hemophilia include acute hemarthrosis, chronic arthritis and hemophilic arthropathy. We studied the clinical and radiological characteristics of joint involvement in hemophiliacs. Functional loss was also demonstrated. There were 25 patients with a mean age of 17; the mean coagulant factor level was 4.2 percent. All the patients had hemarthrotic attacks. Knees were the most commonly affected joints followed by elbows, ankles and hips. The mean number of involved joints was 3.3. Even the patients with moderate disease had arthropathy. Sixty-four percent of the patients had pain and motion restrictions of the involved joints. Four patients developed intramuscular hematoma. We had patients at several radiological stages of severity. Radiological scores best correlated with age and the total number of involved joints. Various degrees of functional loss, namely disability, were observed. The disability score significantly correlated with the radiological score and age. The results of this study suggest that hemophilic arthropathy is an important problem and that multidisciplinary management is needed. Musculoskeletal care as well as appropriate and timely rehabilitation programs could prevent the development of sequelae.

Key words: hemophilic arthropathy, activities of daily living, rehabilitation, prevention.

The hemophilias are genetically transmitted disorders that are characterized by a quantitative and qualitative deficiency of circulating blood clotting factors. Hemophilia A is caused by a functional deficiency of factor VIII, or antihemophilic factor, and is the most common type, comprising approximately 85 percent of the total number of cases. Hemophilia A is a sex-linked recessive genetic disease affecting men, with women as carriers, although 25 to 30 percent of the cases may be due to new mutations. The severity of the disorder varies from patient to patient. Patients with hemophilia are classified as mild, moderate, or severe depending on the level of circulating coagulant factor. In the mild form, the patient has a functional plasma level of 26 to 60 percent of factor VIII and may bleed excessively only during surgery. In the moderate form, the plasma level is between six and 25 percent, and bleeding may occur during surgery or after trauma. Patients with moderately severe disease have plasma levels of factor VIII between one and five percent those with severe disease have levels below one percent. Patients with moderately severe to severe disease are at a greater risk for bleeding into joints, muscles, and vital organs with minimal or recognized trauma. Spontaneous bleeding into joints is limited to persons with severe hemophilia[1-4]. There appears to be a predilection for large joints, namely the ankles, knees, hips, elbows, and shoulders. The bleeding manifestation requiring treatment most often is acute hemarthrosis (extravasation of blood into joints) and, when frequent, these painful acute episodes may lead to a self-perpetuating synovitis and ultimately to a chronic, deforming arthropathy and disability[5,6].

In this study, our aim was to document the musculoskeletal problems and the disability of 25 hemophiliacs followed-up at the Pediatrics and Physical Medicine and Rehabilitation Departments, Ankara University Faculty of Medicine.

Material and Methods

Twenty-five patients with hemophilia were included in the study. Disease history, age, age at the onset of symptoms, coagulant factor levels, and the average number of yearly bleeding episodes were recorded for each patient. An extensive musculoskeletal evaluation of all joints and soft tissues was performed in all patients. Pain, tenderness, swelling, effusion, synovitis, crepitation, range of motion (ROM) limitation and contractures were assessed as a part of the follow-up protocol. The total number of affected joints in each patient was recorded.

Standard anteroposterior and lateral radiographs of the affected joints were taken for the roentgenographic classification of hemophilic arthropathy. All films were scored on a five point scale (Table I)[7]. This scale was derived from those of Arnold and Hilgartner[3] and Pettersson et al.[8] to provide a simple and reproducible measurement which particularly distinguished those appearances (including joint space narrowing and erosions) signifying irreversible damage to cartilage and bone. If there was a discrepancy, a consensus was reached after a revision of the radiograph by the authors. The scores of all joints were added to give a composite score for each patient.

Table I. Classification of Radiological Changes[7]

0 = Normal
1 = Soft tissue swelling, increased synovial density, juxta-articular osteoporosis
2 = Marginal erosions, bone cysts and joint space narrowing in non-weight bearing joints
3 = Severe joint space narrowing, erosions and cysts, osteophytes, growth anomalies and configurational abnormalities in weight bearing and non-weight bearing joints
4 = Disrupted joint, transarticular bony trabeculae

In addition, we measured the functional status of our patients, because the musculoskeletal impairments caused by hemophilic arthropathy might lead to disability. For this purpose, we used the Juvenile Arthritis Functional Assessment Report for Children (JAFAR-C)[9].

This functional assessment tool was primarily developed for patients with juvenile chronic arthritis and as designed has 23 items about the activities of daily living. From this tool, we chose the most suitable 16 items which could reflect the difficulties that hemophiliac[5] could face in their daily life. We also considered the fact that in using such scales the questions should be convenient and adaptable culturally. The revised assessment tool is shown Table II. The response to the each activity was scored on a scale of 0-2: a score of 0 was given if the action could be accomplished alone without any difficulty all the time over the past week, 1 if it could be done sometimes and 2 if it was almost never done by the patient alone. The total score was calculated as the sum of the scores of all items, assuming a range between 0 and 32.

Table II. Questions Chosen from JAFAR-C[9]

Can you
1. Pull on sweater over head
2. Turn on water faucet
3. Climb into bathtub
4. Wash face
5. Tie shoelaces
6. Pull on socks
7. Brush teeth
8. Stand up from chair without using hands
9. Get into bed
10. Use knife and fork
11. Walk 50 m without help
12. Walk up five steps
13. Reach above head
14. Pick up something from floor from standing position
15. Push open door after turning knob
16. Squat

* Responses are scored 0 for all the time, 1 for sometimes, and 2 for almost never.

JAFAR-C: Juvenile Arthritis Functional Assessment Report for Children.

Descriptive statistics and Spearman's correlation analysis were performed and the statistical significance was set at $p<0.05$.

Results

Demographics and other characteristics of our patients are provided in Table III. The mean age was 17.4 years and the average number of yearly bleeding episodes was 11. The mean coagulant factor level was 4.2 percent (range, 0 to 18). There was a weak association between the factor level and the average number of yearly bleeding episodes ($p>0.05$, $r=-0.22$). The mean number

of involved joints was 3.3 (range, 1 to 7). A total of 82 joints in the study group were found to have hemarthrotic attacks, and the knees (37/82, 45.1%) were the most commonly affected joints followed by elbows (18/82, 22%), ankles (17/82, 20.7%), hips (5/82, 6.1%), and the shoulders (4/82, 4.9%). One patient had involvement of the third proximal interphalangeal joint. Knee and ankle involvement was bilateral in 13 and seven patients, respectively. Moreover, four patients developed intramuscular hematoma (psoas, quadriceps, deltoid and forearm). The patient with psoas hematoma also developed entrapment of the femoral nerve causing severe axonal degeneration of the nerve shown by electroneuromyography. One patient had septic arthritis of the knee. Sixteen patients in this study group had pain and ROM limitations and contractures of the affected joints. Again, knees and elbows were the most commonly restricted joints.

Table III. Demographics and Other Characteristics of the Patients (n=25)

Variables	Min-Max	Mean	SD
Age (years)	4-46	17.4	9.9
Age at onset (months)	0.132	24.4.	34.6
Factor level (%)	0-18	4.2	4.5
# Yearly bleeding episodes	2-72	11	14.4
# Joints involved	1-7	3.3	2
Radiological score	1-16	6.8	4.7
Disability score	0-22	7.5	6.3

The mean radiological score was 6.8 (range, 1 to 16). There was a statistically significant correlation between the radiological score and age ($p<0.05$, $r=0.45$) and the total number of affected joints ($p<0.001$, $r=0.77$). Although it did not reach a significant level, there was an association between the radiological score and the average number of yearly bleeding episodes ($p>0.05$, $r=0.32$).

The patients of this study had various degrees of functional difficulties; the mean disability score was 7.5 (range, 0 to 22). Of the 16 functional activities, squatting, walking up five steps and picking up something from the floor from a standing position were the most difficult activities. The disability score correlated significantly with the radiological score ($p<0.01$, $r=0.69$) and age ($p<0.05$, $r=0.42$). There was a weak correlation between the disability score and total number of involved joints ($p>0.05$, $r=0.33$).

Discussion

Musculoskeletal complications of hemophilia include acute hemarthrosis, subacute or chronic arthritis and hemophilic arthropathy. Patients with hemophilia may also experience soft tissue and intramuscular hemorrhage and, rarely, pseudotumors, septic arthritis and nerve compressions[10]. Hemophilic arthropathy, the total destruction of the joint, develops due to the recurrent hemarthrosis and the specific changes occurring in the synovium and cartilage[4,11,12]. The pathophysiology of this blood-induced joint damage is not known in detail but it is thought to be multifactorial in origin and to include degenerative cartilage mediated and inflammatory synovium-mediated damage. A possible suggested sequence of events may be that intra-articular bleeding initially provokes a non-specific inflammatory response, macrophages accumulate around synovial iron deposits, release monokines, and stimulate production of latent collagenases and prostanoids. The continued leak of red blood cells from the hypertrophied, vascular synovium then produces a vicious cycle of synovitis-bleeding-synovitis[4]. The increased iron load results in further synoviocyte hyperplasia and macrophage accmulation. Degradation of the cartilage matrix through several cartilage-damaging mediators such as enzymes, cytokines, and oxygen metabolites might also start as a direct response to blood[4,11,12]. The clinical findings resemble both degenerative joint diseases and advanced rheumatoid arthritis. The involved joint is enlarged owing to osteophytic bony overgrowth, the range of motion is restricted and subluxation and instability are common. The hemophilic arthropathy is considered to be the most important cause of morbidity in patients with severe hemophilia[6,13]. In the present study, we tried to demonstrate the caharacteristics of joint involvement in hemophiliacs as well as the disability associated with it.

All patients in this study had hemarthrotic attacks; the mean number of affected joints was 3.3, and the most involved joints were the larger joints such as knees, elbows, and ankles, confirming the previous studies[6,7]. Seventy-eight percent of our patients had a plasma coagulant factor level below five percent, and the others were moderately ill. Even the patients with moderate disease had hemarthrotic attacks and various degrees of radiological findings, suggesting that mild or infrequent bleeding

might also start joint damage. Similar results were reported by Steven et al.[7] In fact, it is possible that these patients with moderate illness may not have been admitted to the hospital because of rather silent or less severe clinical presentation. Therefore, examination of all joints should be carried out, even in the absence of a history of hemarthrosis, to identify early cases of arthritis.

There was not a strong correlation between the total number of involved joints and the coagulant factor level. This could be due to the development of a frequently bleeding "target joint" in most patients[14]. Sixty-four percent of the patients had motion restrictions of the involved joints and this had a considerable impact on performing daily activities.

In hemophilic arthropathy, the earliest radiographic findings are seen at the soft tissue; the joint capsule is distended and the intraarticular bleeding demonstrates an increased density. With the progression of the proliferative synovitis, irreversible radiological changes occur. These are periarticular soft tissue thickening and demineralization, marginal erosions, subchondral irregularity and cyst formation, decreased joint space, osteophytes and chondrocalcinosis. The specific radiological features of hemophilic arthropathy are the widening of the femoral intercondylar notch, squaring of the distal patellar margin, enlargement of the proximal radius and flattening of the talus with or without ankle joint ankylosis[10]. Our patients were at several radiological stages. Radiological scores best correlated with age and the total number of affected joints, which we think could be primarily based on the disease duration and resultant increased recurrent hemarthrotic attacks. Moreover, although it was not statistically significant, an association existed between the radiological score and the average number of yearly bleeding episodes.

Despite advances in modern treatment having dramatically reduced the mortality associated with the bleeding episodes of hemophilia, it is not clear whether there has been any impact on the morbidity resulting from hemarthrosis and arthropathy. There have been few studies about the functional loss in hemophiliacs, although it seems to be the most important issue in these people's lives. Ahlberg[15] reported that over half of his group of 102 patients with severe hemophilia had marked-to-severe disabilities as a result of hemophilic arthropathy, eight had to use a wheelchair, and 20 percent required crutches, sticks or other orthopedic appliances. He also described a relation of disability to age. Recently, Iwata and his colleagues[16] studied the limitation of joint function in 97 helophiliacs in relation to functional limitations and/or activities of daily living. Their analysis showed that full flexion of the lower limbs was easily limited in these patients and that this deterioration was difficult to prevent, resulting in marked disability. They also concluded that the functional limitation level differed significantly by age group. Heijnen et al.[17] worked on the estimation of severity of joint movement limitation giving rise to disabilities. They found that many patients started to compensate with another joint motion if one joint in the chain of a limb was limited. They also emphasized that functional jüoint motion may differ between cultures and countries; slight restrictions in lower limbs are disabling in Asian countries where people squat and use an Eastern type of toilet, whereas in Western countries knee flexion of 100° might enable the patient to climb stairs and ride a bicycle. Mild-to-moderate disabilites were observed in the present study and the most difficulty activities for our patients were squatting, walking anda picking up something from the floor from a standing position. Disability correlated strongly with age and radiological score, indicating that severe arthropathy increased the functional loss. Multiple joint involvement increased the disability, and this condition was suggestive of the difficulty in compensation which has also been reported before[17].

Overall, our study and the others show that hemophilic arthropathy is an important problem, and that multidisciplinary management of this condition might provide good results. Although costly from an economic point of view, prophylaxis has effectively prevented joint and musculoskeletal disease in severe hemophilia[18-20].

Additionally, patient and family education, proper and efficient rehabilitation programs and the maintenance of physical activity are important preventive measures[13,21]. We believe that rehabilitation and musculoskeletal care could prevent the appearance of sequelae and improve the quality of life for hemophiliacs.

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Clinical features of 21 patients with lissencephaly type I (agyria – pachygyria)

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Lissencephaly (agyria-pachygyria) is the most severe neuronal migration disorder, characterized by total or partial absence of gyri. In this study, 21 patients with lissencephaly type I (9 girls, 12 boys) with a mean age of 19±21 months (2 weeks-8 years) were evaluated clinically and graded according to neuroradiological findings (19 patients by magnetic resonance imaging MRI and 2 by computed tomography CT). Three patients were classified as lissencephaly grade 2 and 18 patients as grade 3 or 4. Clinically, 12 patients (57%) had microcephaly, and eight (38%) had facial dysmorphism. All the patients had prominent psychomotor retardation, moderate to severe; the most frequent neurological findings were spastic guadriplegia (36.4%) and hypotonia with exaggerated tendon reflexes (27.3%). Seventy-eight percent of the patients had epileptic seizures resistant to conventional treatment. Lissencephaly is a cerebral cortical malformation that should be considered in children with developmental delay with or without microcephaly and facial dysmorphism. In addition, it should be investigated in the etiology of early-onset childhood epilepsy.

Key words: lissencephaly, neuronal migration disorders, childhood epilepsy, psychomotor retardation.

Neuronal migration disorders (NMD) are a rare group of malformations of the brain caused by insults to migrating neuroblasts between the second and sixth months of gestation[1-4]. Several different types of these disorders have been described, of which lissencephaly (agyria-pachygyria) is the most severe form, characterized by total or partial absence of cortical gyration[2,5-8].

Although lissencephaly was first described more than 100 years ago, there had been few reports until the introduction and widespread use of computed tomography (CT) and particularly magnetic resonance imaging (MRI)[5,8-10]. Pathologically the disorder consists of different subtypes, including type I or classical lissencephaly, type II or cobblestone lissencephaly, and other rare or atypical types[1,5,7,11,12]. Type I lissencephaly, the most common form, is characterized by a flattened and thickened cerebral cortex with absent or reduced cortical gyri. This type can occur as an isolated defect, which is referred to as isolated lissencephaly sequence (ILS) or as a part of a recognizable multiple congenital anomaly syndrome known as Miller-Dieker syndrome (MDS)[3,7,13].

Type I lissencephaly may manifest by variable clinical findings. In this paper we report the clinical features of 21 patients with lissencephaly type I.

Material and Methods

Twenty-one patients with lissencephaly (9 girls, 12 boys) admitted to the Pediatric Neurology Division were studied. The children were referred for evaluation of developmental delay and/or microcephaly, and/or seizures.

The diagnosis was made by MRI in 19 patients and CT in two patients. All patients had type I lissencephaly (isolated lissencephaly sequence) according to the criteria of Barkovich and Dobyns[1,3]. None of the patients had clinical findings, or muscular or ocular abnormalities consistent with type II lissencephaly. The severity of gyral malformation was graded according to

the grading system by Dobyns (grade 1: diffuse agyria; grade 2: diffuse agyria with some shallow sulci in the frontal regions; grade 3: mixture of agyria and pachygyria; grade 4: diffuse or widened pachygyria with no areas of agyria; grade 5: mixed pachygyria and subcortical band heterotopia; grade 6: subcortical band heterotopia)[7]. Chromosome analysis was performed in three patients with grade 2 lissencephaly to exclude Miller-Dieker syndrome and no chromosomal abnormality was detected.

Physical and neurological findings and types and frequency of the seizures were evaluated and developmental status tested with Denver II Developmental Screening Test (adapted to Turkish children) and Brunette Lezine test. The relationship between severity of lissencephaly and clinical status was assessed.

Results

Mean age of patients at first visit was 19±21 months, range from one month to eight years (77% under 2 years; 95% under 4 years of age). Mean age during the last follow-up visit was 31±22 months. Three patients (14%) were classified as lissencephaly grade 2 (Fig. 1), five patients (23%) grade 3 (Fig. 2) and 13 patients (61%) grade 4 (Fig. 3). Pachygyric lesions were located mostly in the frontoparietal regions in grade 3 patients.

Clinical findings of the patients are listed in Table I. Twelve patients (57%) had microcephaly and eight (38.1%) had facial dysmorphism consisting of bitemporal narrowing (n: 6), small jaw (n: 4), narrow forehead (n: 4), large ear lap (n: 2), and malformed low-set ears (n: 1). At the

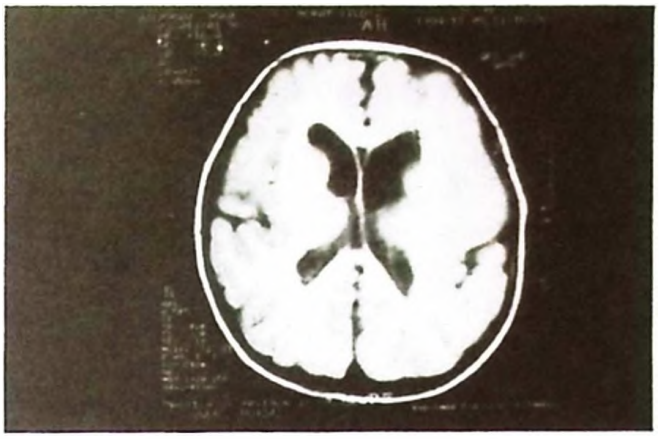

Fig. 2. Cranial MRI of Case 5 (grade 3 lissencephaly, axial view).

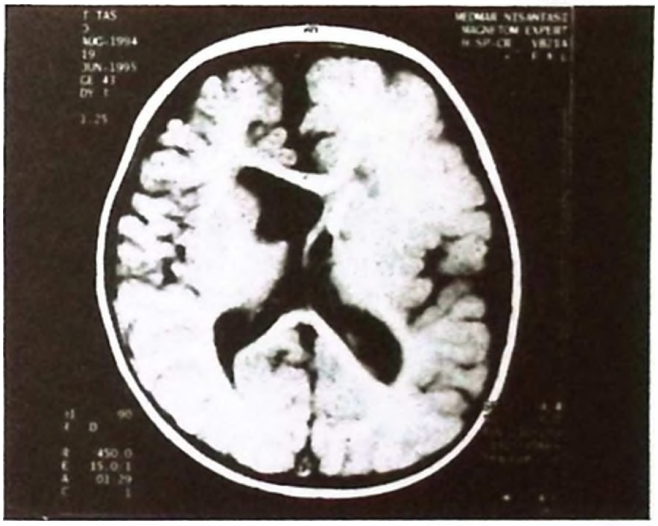

Fig. 3. Cranial MRI of Case 16 (grade 4 lissencephaly, axial view).

(a) (b)

Fig. 1. Cranial MRI of Case 1 (grade 2 lissencephaly) (A: axial view; B: sagittal view).

last examination, 11 patients were older than two years of age. Seven cases (64%) only had head control, one patient (9%) could sit without support, two patients (18%) were able to walk, and one patient (9%) had no motor development including no head control. The most common neurological findings were spastic quadriparesis (47%) and hypotonia with exaggerated tendon reflexes (28%).

lissencephaly. Similarly, seizures were observed in grades 2, 3 and 4 lissencephaly in 100, 80 and 69 percent of cases, respectively.

Discussion

Lissencephaly, meaning literally "smooth brain", refers to a genetically, clinically, neuroradiologically, and histologically heterogeneous group of conditions that are characterized by total or partial

Table I. Clinical Findings and Grades of Lissencephaly of the Patients

Patient	Age* (yrs; mths)	Age** (yrs; mths)	Sex	Grade of lissencephaly	Dysmorphism	Microcephaly	Neurological findings	Seizure/ epilepsy	Psycho/ motor retardation
1	3;7	6;3	F	2	–	+	Hypotonia	LGS	Severe
2	2;2	3;8	F	4	+	+	Hypotonia	LGS	Severe
3	0;5	1;3	F	3	–	+		IS	Severe
4	1;1	1;10	M	4	+	+	SQ	NS	Severe
5	3;2	4	M	3	–	–		LGS	Moderate
6	0;8	2;2	M	4	–	–	SQ	NS	Moderate
7	0;7	1;7	M	4	+	–	SQ	generalized	Moderate
8	1;7	1;8	M	4	–	+		NS	Mild
9	0;11	4;10	F	4	+	+	SQ	LGS	Severe
10	1;7	1;10	F	2	–	+		IS	Severe
11	0;6	2	M	3	–	–	Hypotonia SQ	IS	Severe
12	2;10	3;4	M	4	+	+		LGS	Severe
13	0;6	3;4	F	3	–	–	SQ	NS	Severe
14	8;0	8;0	M	4	+	–	Hypotonia	NS	Severe
15	0;3	2	M	4	–	–	SQ	Focal clonic, generalized tonic	Moderate
16	0;10	1;5	M	4	–	–	Spastic left hemiparesis	generalized clonic, tonic, atonic	Moderate
17	0;5	0;6	F	2	–	–	Hypotonia	IS	Severe
18	1;5	1;6	F	4	+	+	SQ	generalized tonic	Severe
19	1;8	1;8	F	4	–	+	Hypotonia	generalized tonic	Moderate
20	0;3	0;3	M	3	–	+	SQ	IS	Severe
21	0;11	2;5	M	4	+	+	SQ	IS	Severe

* At the first examination.
** At the last examination.
F: Female. M: Male. SQ: Spastic quadriparesis. IS: Infantile spasm. LGS: Lennox-Gastaut syndrome. NS: No seizure.

Seventy-six percent (n: 16) of the patients had epileptic seizures including infantile spasms (IS) (n: 6), Lennox-Gastaut syndrome (LGS) (n: 5), and secondary generalized and mixed type seizures (n: 5) resistant to conventional antiepileptic drugs. The age at onset of seizures ranged from two weeks to 22 months (mean 6±6 months). In 12 patients (57%) seizures occurred before six months of age, and in 14 (66%) before 12 months.

Table II presents the relationship between the clinical findings and grades of lissencephaly. All patients with grade 2 lissencephaly presented severe developmental delay and had seizures (one with IS, two with LGS), whereas severe retardation was found in 80 percent of cases with grade 3 and in 53 percent with grade 4

absence of gyration[1-3,5,6,14]. Several types of lissencephaly have been identified according to the clinical, morphologic, and genetic criteria. The most common types are type I (classic lissencephaly) and type II (Cobblestone lissencephaly)[7,11,12,15,16]. Type I lissencephaly occurs most commonly as an isolated defect (isolated lissencephaly sequence), but is may also be associated with MDS, in which case, it presents in a more severe form[3,7,13,17].

All our patients were diagnosed to have type I lissencephaly, since muscular and ocular anomalies as well as neuroradiological findings characteristic of type II lissencephaly were absent. Chromosomal analyses could be performed in three patients with severe lissencephaly to exclude MDS, and no deletion was found in chromosome 17. In addition, none

of the cases with severe lissencephaly had any facial dysmorphism that occurs in patients with MDS. Therefore, alll cases were classified as type I lissencephaly-ILS.

1990, de Rijk van Andel[17] postulated that grades 3 and 4 have been under-represented in the literature. Based on our findings, we also consider that lissencephaly grades 3 and 4 were more frequently seen than grades 1 and 2. In the near future, with the wide use of MRI, cases with mild and moderate lissencephaly may outnumber more severe forms.

Table II. Distribution of Clinical Findings According to the Grades of Lissencephaly Type I

	Grade 2 (n: 3)		Grade 3 (n: 5)		Grade 4 (n: 13)		Total	
Clinical Findings	n	%	n	%	n	%	n	%
Microcephaly	2	66	2	40	8	61.5	12	57
Facial dysmorphism	0	0	0	0	8	61.5	8	38
Psychomotor retardation							21	100
Severe	3	100	4	80	7	54		
Moderate	0	0	1	20	5	38		
Mild	0	0	0	0	1	8		
Seizures	3	100	4	80	9	69	16	76
Spastic quadriparesis	0	0	2	40	8	61.5	10	47
Hypotonia	2	66	1	20	3	23	6	28
Spastic hemiparesis	0	0	0	0	1	7.6	1	4.7

Patients with lissencephaly type I may present with variable findings including microcephaly, facial dysmorphism, motor deficits, seizures, and developmental delay[12,14,15,18,19]. Most of these patients have poor neurological prognosis. All our patients had psychomotor retardation, and 57 percent exhibited microcephaly. Of 11 patients older than two years, two were able to walk and seven only had head control. Spasticity and hypotonia resulting in severe motor retardation were the most common neurological findings in our series. In the literature, all of the 124 reported patients had developmental delay, and 71 percent were microcephalic[13,14,16-21].

Seizures were present in 76 percent of our cases and half of them had infantile spasms or LGS preceded by IS. Intractable seizures is one of the most common clinical manifestations of patients with lissencephaly. In other series, the seizures have been reported in 75-90 patients of the patients (IS 35-50%)[13-15,18,19]. According to these results, lissencephaly should be considered in children with psychomotor retardation with or without microcephaly and facial dysmorphism. In addition, it should be investigated in the etiology of some early-onset childhood epilepsies, particularly IS.

In our series the most common forms of type I lissencephaly were diffuse pachygyria (grade 4) (62%) and agyria-pachygyria (grade 3) (24%). As far back as 1956, Crome[22] suspected that pachygyria would be much more frequent than agyria, but this has never received much attention in clinical studies[13,17,21]. In

Is there any relationship between the severity of the clinical findings and the severity of cortical malformation? The number of patients in our series was not sufficient for statistical evaluation. However, we noticed that of our three cases with grade 2 lissencephaly all had profound psychomotor retardation and seizures, whereas 54 percent of the patients with grade 4 lissencephaly were severely retarded and 69 percent of these cases had seizure. In the series of de Rijk van Andel[17], all patients with grades 1 and 2 were bedridden and had little contact with their environment, whereas cases with grades 3 and 4 had some social development. Dobyns[13] reported that the developmental level did not correspond with the severity of the brain malformations in these cases. Similarly, Barkovich[14] postulated that there was no relationship between clinical manifestations and MRI findings. Larger series may be helpful to elucidate the relationship between the grade of lissencephaly and the clinical outcome.

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Microdeletion of 22q11 (CATCH 22) in children with conotruncal heart defect and extracardiac malformations

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SUMMARY: Alikaşifoğlu M, Malkoç N, Ceviz N, Özme Ş, Uludoğan Ş, Tunçbilek E. Microdeletion of 22q11 (CATCH 22) in children with conotruncal heart defects and extracardiac malformations. Turk J Pediatr 2000; 42: 215-218.

CATCH 22 is a medical acronym for cardiac defects, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcemia, and a variable deletion on chromosome 22q11. The deletion within the chromosome region of 22q11 may occur in patients with dysmorphologic and cardiological syndromes: DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), and conotruncal anomaly face syndrome (CAFS). In this study, using N25 (D22S75) DiGeorge chromosome region probe. fluorescence in situ hybridization (FISH) analyses were performed on 32 patients with congenital heart diseases. Twenty-nine of 32 patients had conotruncal heart disease. A 22q11 deletion was detected in two patients (6.9%) of the 29 patients with conotruncal heart disease. One of our 22qdel (+) patients had unilateral facial nerve palsy. Although it is not a frequent finding, unilateral facial nerve palsy will be included among the symptoms of CATCH 22 syndrome. After careful clinical evaluation of patients with conotruncal cardiac anomalies, only syndromic cases should be screened for this deletion.

Key words: CATCH22 cardiac defects, abnormal facies, thymic hypoplasia.

Despite recent advances in the diagnosis and treatment of children with congenital heart disease, we do not have enough knowledge on the etiology of these defects. One of the most dramatic relationships between genetic and congenital cardiac lesions is the finding of monosomy of a locus on chromosome 22 in patients with aortic arch and conotruncal cardiac defects. Chromosome 22q11 deletions have been demonstrated in patients with a spectrum of cardiac malformations, from severe lesions such as truncus arteriosus and interruption of the aortic arch, to the mildest form of conotruncal defects such as isolated subarterial ventricular septal defect.

Hemizygosity for the human chromosome region 22q11 is associated with a wide range of overlapping phenotypes including DiGeorge syndrome, velocardiofacial (Sprintzen) syndrome and conotruncal anomaly face syndrome (CAFS). The acronym CATCH22 (cardiac anomaly, abnormal facies, thymic hypoplasia, cleft palate, hypocalcemia) has been suggested to decribe the broad clinical spectrum of phenotypes with 22q11 deletions[1-3]. The aim of this study was to estimate the frequency of microdeletion of 22q11 in children with conotruncal heart defects and extracardiac malformations.

Material and Methods

This prospective study was based on 32 patients with cardiac defects who were investigated by the Pediatric Cardiology and Clinical Genetics Units of İhsan Doğramacı Children's Hospital of Hacettepe University Faculty of Medicine. Twenty-nine of the patients had conotruncal heart anomalies. In addition, two patients with tricuspid insufficiency and one patient with atrial septal defect were included in the study because of similarities of extracardiac anomalies.

All patients were evaluated by echocardiography or cardiac catheterization in respect to cardiological problems. A clinical geneticist obtained family history and evaluated dysmorphic features of the patients.

Chromosome preparations were obtained from peripheral blood of each patient and analyses were performed by conventional G banding and fluorescence in situ hybridization (FISH). Two Oncor N25 (D22539) chromosome 22 control probes, both labelled with dioxigen, were used for FISH analysis. Slides were denatured in 70 percent formamide/2xSSC at 70°C for 2 min, dehydrated by passing them for 3 min each into 70, 90, and 100 percent ethanol and air dried. Ten microliters of the combined probe were hybridized in situ to denatured chromosome preparations at 37°C overnight. After post hybridization washing at room temperature, the hybridizing probe was viewed using a detection kit (Oncor). The chromosomes were observed using Nikon E800 fluorescent microscope and Cytovision (ver. 2.1. Applied Imaging).

The serum calcium concentration was investigated in all patients and immunologic studies were performed in one of the FISH-positive patients.

Results

A total of 32 patients were studied. Of these, 15 (46.9%) were female and 17 (53.1%) were male. The youngest patient was 16 days old and oldest was 15 years of age.

Conotruncal cardiac anomalies were as follows: tetralogy of Fallot (19 cases; 65.5%), truncus arteriosus (3 cases; 10.4%), supracristal ventricular septal defect (2 cases; 6.9%), transposition of great arteries (2 cases; 6.9%), pulmonary atresia (2 cases; 6.9%) and vascular ring (1 case, 3.4%).

Tricuspid insufficiency in two patients and atrial septal defect in one were found as cardiac anomalies in an additional three patients.

The most common extracardiac features were prominent nose, squared nasal root, short stature, long slender fingers, micrognathia, mild mental retardation, microcephaly, malar hypoplasia, almond shaped palpebral fissure, minor auricular anomalies, and thin upper lip.

The less frequently observed findings were hypertelorism, undescended testes, cleft palate, retrognathia, and scoliosis.

Serum calcium concentrations were normal in all patients. All karyotypes were normal at the level of 400-600 band (ICSN) using G banding.

Two patients had a segmental monosomy 22q11 that could be detected by FISH analysis (6.3%). One was a 15-month-old girl with tetralogy of Fallot, patent foramen ovale and peripheral pulmonary hypoplasia. Dysmorphic features of this girl were unilateral facial nerve palsy at right side, prominent nose, squared nasal root, almond-shaped palpebral fissure, minor auricular abnormality, hypertelorism, long slender fingers, short stature and hypotonia (Fig. 1). Immunological studies of this patient were evaluated as normal.

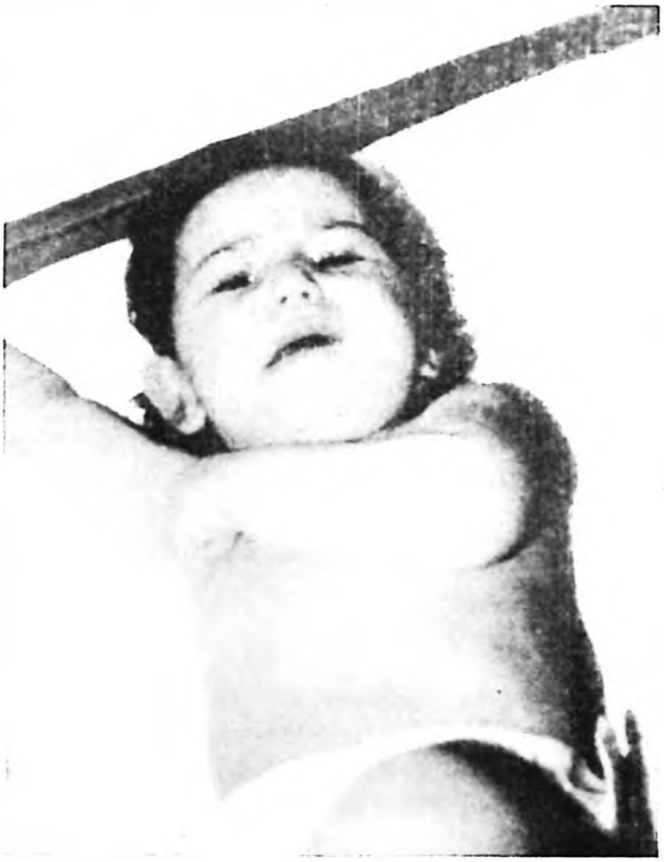

Fig. 1. One patient with segmental monosomy 22q11. Please note unilateral facial nerve palsy.

The second patient was a one-year-old boy with supracristal ventricular septal defect and pulmonary stenosis. Clinical findings of this child showed micrognathia, short neck, camptodactyly of the 5th finger of right hand and the 2nd, 3rd and 4th finger of the left hand, and hypotonia. Immunological investigations were not performed in this patient.

Serum calcium levels of both patients were within normal limits (9.2 and 9.8 mg/dl).

Discussion

Most previous studies revealed a high incidence rate of deletions in 22q11 among patients with

congenital heart defects. It is estimated that deletion of 22q11 may be involved in five percent of all newborns with heart defects, and that it is the single most important cause of heart malformation after Down syndrome[1]. But recent studies have shown that the incidence of deletion in 22q11 is lower than suspected by former investigators. Possibly, the observed frequency of deletions depends on the selection criteria.

We found the frequency of del 22q11 among 32 patients with cardiac defects and dysmorphic features as 6.3 percent. However, if only patients with conotruncal heart defects were evaluated, the incidence would be 6.9 percent.

Different researchers reported different frequencies of 22q del among different conotruncal heart defects and aortic arch anomalies:

Although the number of cases was not enough, the highest incidence of deletion (75%) was found in absent pulmonary valve syndrome by Johnson et al.[5] Interruption of the aortic arch type B (50%) and truncus arteriouss (33%) were also associated with a high frequency of del 22q11[6,7].

One of the highest figures belongs to Goldmuntz et al.[4] They studied 17 non-syndromic patients with one of the three most common conotruncal defects: truncus arteriosus, interruption of the aortic arch and tetralogy of Fallot. Out of these 17 patients, a 22q deletion was found in five (29%). They wrote that the 22q11 deletion-positive patients were mildly dysmorphic but did not have characteristics of a specific syndrome.

Webber et al.[15] stated that 22q11 deletions are important causes of selected malformations of the ventricular outflow tracts and aortic arch, and that they account for about 15-20 percent of cases.

Digilio et al.[8] evaluated 315 children with conotruncal heart defects and 22qdel was found in 11 percent of the patients. All the patients except one presented with one or more extracardiac anomalies, and were therefore considered as syndromic. The authors point out that five patients diagnosed as isolated in the beginning were subsequently included in the group of syndromic cases because of the presence of subtle facial dysmorphism which was previously overlooked.

Takahaski et al.[9] found a submicroscopic deletion in chromosome 22q11 in five out of 64 patients (7.8%) with a conotruncal heart disease.

Devriend et al.'s analysis of 150 patients with conotruncal anomalies found the incidence of del 22q11 to be 12.8 percent. They did not include patients with transposition of great arteries (TGA) in their group, since this is an uncommon heart defect in del 22q11[10].

It is clear from the above-mentioned studies that there are great variations in the reported incidence of CATCH 22 syndrome, even in study groups consisting only syndromic conotruncal heart anomalies, but a major limitation of many studies is the small sample size. These observations suggest the need for establishing the incidence of 22q11 deletions for individual anatomic diagnosis instead of for generic groups such as conotruncal anomalies.

General opinion is that facial appearance involves subtle changes and can easily be misinterpreted in young children. These dysmorphisms may barely be recognizable; a precise phenotypical evaluation is essential to distinguish syndromic from isolated conotruncal anomalies[8,9,13,14]. Different studies showed that non-syndromic conotruncal cardiac anomaly patients are not likely to have a 22q11 deletion[13,8,9,10]. Therefore, it is advisable that children with conotruncal cardiac anomalies should be carefully examined to detect dysmorphic features of DiGeorge, velocardiofacial and conotruncal anomaly face syndromes. After careful clinical evaluation, only syndromic cases should be screened for this deletion.

One of our 22qdel (+) patients had unilateral facial nerve palsy. Unilateral facial nerve palsy was described in a patient with CATCH 22 syndrome without cardiac anomaly, thymic hypoplasia cleft palate or hypocalcemia[12]. Tho the best of our knowledge, our patient is the first case showing unilateral facial nerve palsy in syndromic conotruncal heart anomaly. These observations suggest that although it is not a frequent finding, unilateral facial nerve palsy should be included among the symptoms of CATCH 22 syndrome.

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Serratia marcescens: an emerging microorganism in the neonatal intensive care unit

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As smaller babies survive in neonatal intensive care units, late-onset septicemia with unusual pathogens appears. Between 1 January and 31 December 1998, in Hacettepe University İhsan Doğramacı Children's Hospital Neonatal Intensive Care Unit, seven infants had S. marcescens isolates. Four babies had septicemia with the microorganism. The case fatality rate was 50 percent in infants with S. marcescens septicemia. The combination of ceftazidime or imipenem with amikacin appears appropriate for the treatment of newborns with Serratia infection.

Key words: Serratia marcescens, infection, neonatal intensive care unit.

In the 1990's Group B streptococcus and E. coli were the most frequent bacterial pathogens causing early neonatal sepsis. On the other hand, coagulase negative staphylococci, Klebsiella pneumonia, Pseudomonas aeroginosa, other Gram-negative bacteria and Candida species are the most frequently encountered microorganisms in late-onset sepsis of the newborn[1-3].

Serratia marcescens is a motile, non-sporulating, Gram-negative bacillus classified in the family Enterobacteriacea. It has been recognized as a cause of hospital-acquired infection for the last two decades[4]. These infections were mainly reported in association with altered host defenses. The immaturity of neonatal host defenses is a major risk factor predisposing to bacteremia and sepsis in the newborn. The lack of type-specific antibodies, decreased levels of immunoglobulin G, A and M levels, reduced neutrophil chemotaxis and defective oxidative burst in granulocytes are important factors that contribute to the neonate's (and especially of the preterm infant's) susceptibility to bacterial infection[1-3]. There are reports of Serratia species causing neonatal infection[4-12]. The first time Serratia was isolated in our Neonatal Intensive Care Unit (NICU) was January 1998. Since that time Serratia has been isolated from seven patients and all were S. marcescens. In this report we present our patients with Serratia isolation.

Material and Methods

In our NICU, every newborn with suspected sepsis undergoes a sepsis work-up which includes complete blood count with differential, urine analysis, C-reactive protein level, chest X-ray, and cultures o fblood, urine and cerebrospinal fluid. All infants requiring mechanical ventilation have routine endotracheal aspirate cultures when the tube is removed or changed and when there is a lower respiratory tract infection proven by chest X-ray. Routine cultures of humidifiers, incubators, ventilators, and disinfectant solutions are performed twice monthly, and cultures of intravenous fluids are performed once monthly.

Bacterial identification was performed by Sceptor System (Becton Dickinson). Antibiotic susceptibilities were determined using Microbroth dilution method, according to NCCLS gudelines (M7-A4).

Results

Between 1 January-31 December 1998 S. marcescens was isolated from ten cultures of seven infants. The clinical characteristics of these infants are summarized in Table I. Four isolates were from blood, four from tracheal aspirates, one from cerebrospinal fluid and one from bone marrow aspiration culture. All isolates were sensitive to amikacin, imipenem and ciprofloxacin (Table II).

Table I. Clinical Characteristics of the Patients

Case no.	1	2	3	4	5	6	7
Gestational age (week)	37	33	27	33	31	27	32
Birth weight (g) APGAR score	2300	1780	600	1900	1330	750	2400
(5 min)	5	9	2	9	8	6	2
Umbilical artery catheterization	-	-	+	-	-	+	+
Endotracheal intubation	+	-	+	-	-	+	+
Ventilator treatment (days)	22	0	3	2	0	20	2
Age at diagnosis of infection (days)	7	6	23	5	9	45	4
First sign of infection	Atelectasis of right lung upper lobe	Hypoactivity Abdominall distention	Bradycardia Abdominal distention	Apnea Hypoactivity	Tachypnea	Apnea Hypoactivity	Diffuse lung infiltrates
Blood culture	-	+	+	+	-	+	-
CSF culture	-	-	-	-	-	+	ND
Bone marrow aspirate culture	ND	ND	-	ND	ND	+	ND
Endotracheal aspirate culture	+	-	-	+	+	-	+
Total leukocyte count (/mm^3)	18800	14600	10100	19300	11500	2800	11800
Immature: Mature ratio	0.17	0.2	0.15	0.5	0.15	0.1	0.3
Thrombocytopenia	-	+	+	+	-	-	-
CRP level (mg/dl)	ND+	0.4	ND+	4.2	0.12	9.52	0.4
Hyperbilirubinemia	-	+	-	+	-	-	+
Antibiotic treatment	Imipenem+Amikacin	Cefotaxime+Amikacin	Ceftriaxone+Vancomycin	Ceftazidime+Amikacin	Ceftazidime+Amikacin	Imipinem+Amikacin	Ceftazidime+Amikacin
Outcome	Died at 24 days days due to HIE	13 months old, healthy	Died at 33 days	9 months old, healthy	7 months old, healthy	Died at 48 days	6 months old, healthy

• Not done due to the unavailability of CRP kit.

CSF : Cerebrospinal.
Fluid, HIE : Hypoxic-Ischemic Encephalopathy.
ND : Not Done.

Table II. Susceptibility Patterns of Different Isolates of S. Marcescens

Case	1	2	3	4	5	6	7
TMP/SMX	R	R	S	–	R	S	R
Sulbactam-Ampicillin	R	R	R	R	R	R	R
Ceftazidime	S	S	S	S	S	R	S
Cefotaxime/Ceftriaxone	S	S	S	R	R	R	R
Imipenem	S	S	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S	S	S
Amikacin	S	S	S	S	S	S	S
Netilmicin/Tobramycin	R	R	S	–	R	–	–

R : Resistant.
S : Sensitive.
TMP/SMX : Trimethoprim-sulfamethoxazole.

Discussion

Severe illness due to S. marcescens is generally seen in immunocompromised patients such as the elderly and neonates. In newborns the organism can be responsible for sepsis and meningitis, which have a high case fatality rate[10]. Studies in the 1970's suggested that neonatal colonization and infection were rare events, but from 1980 to 1999, reports of S. marcescens epidemics in NICU's have increased[4,9,10]. Before January 1998, Serratia had not been isolated from cultures in our NICU. At the time, neither overcrowding nor patient admission from other hospitals had ocurred in our NICU.

The symptoms of infection were non-specific, such as hypoactivity, abdominal distention and apnea (Table I). The day of onset of symptoms ranged between four to 45 days, which suggests late sepsis with acquisition of the microorganism from the NICU. Endotracheal intubation and mechanical ventilation were required in five of the cases. There was no growth in urine cultures. In one patient (Case 2), hemolysis with jaundice that could not be controlled by photoherapy and required two exchange transfusions was observed. Other reasons for hemolysis were excluded in that patient, thus, it was thought to be due to S. marcescens infection. To our knowledge, this symptom has not been observed before in newborns with Serratia septicemia.

It has been shown that Serratia is a human pathogen capable of causing significant mortality and morbidity, with the small premature infant being most at risk of serious infection[7]. In our series, three of the babies with Serratia infection died. One of them (Case 1) was a debilitated baby with severe neurological sequelae due to hypoxic-ischemic encphalopathy (HIE). This baby had S. marcescens grown only in endotracheal aspirate culture. The other two babies (Cases 3 and 6) were premature and extremely low birth weight babies, prone to nosocomial infections. Since two out of four of the babies with S. marcescens septicemia died, the case fatality rate attributable to S. marcescens septicemia was 50 percent. Zaidi et al.[10] reported a 60 percent mortality rate for newborns weighing 2500 g or less with S. marcescens infection. In the series of van Ogtrop et al.[9], septicemia had a mortality rate of 100 percent in preterm newborns. In our series, the surviving infants with S. marcescens septicemia had a greater birth weight and gestational age than the ones that died. At the moment, Case 2 is 13 months and Case 4 is nine months old. The other two babies with S. marcescens growth only in endotracheal aspirate cultures (Case 5 and Case 7) are seven and six months old, respectively. All the babies with S. marcescens infection are being followed up. No late complication of infection nor any neurological sequelae has been observed in these four babies.

After the emergence of Serratia strains, environmental samples were taken from incubators, humidifiers, suction jars, soaps, intravenous solutions, sinks and stethoscopes. Extensive investigation and cultures failed to identify any environmental reservoir of S. marcescens. Since colonization on hands of the personnel had been reported to be responsible for spread of the microorganism, strict handwashing policies were re-introduced. Since June 1998, only one case with S. marcescens growth has been identified, in August 1998. In our NICU for the last five years, the standard treatment protocol for late-onset neonatal sepsis has been the combination of cefotaxime or ceftriaxone with amikacin. As smaller babies began to survive in our NICU, combination of an antipseudomonal cephalosporin with vancomycin was introduced in April 1998 as an alternative empyric late-onset sepsis treatment protocol for catheter-related sepsis.

Serratia species are known to carry chromosomally inducible beta-lactamase. Exposure to a beta-lactam antibiotic results in induction of expression of the enzyme, which persists as long as the inducer is present. Depressed mutants secreting beta-lactamase may colonize in an NICU and then be transferred to the patients later. Since in our series none of the isolates was susceptible to sulbactam ampicillin, and only 40 percent were

susceptible to cefotaxime or ceftriaxone, we think that our isolates also carry the enzyme. When we consider the high resistance levels with sulbactam ampicilin and cefotaxime or ceftriaxone for S. marcescens, a combination of ceftazidime or imipenem with amikacin seems appropriate for the treatment of newborns with Serratia infection.

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Ventricular diastolic filling indices in pulmonary stenosis

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Children with valvar pulmonary stenosis have right ventricular diastolic filling abnormalities that may be due to either right ventricular hypertrophy or right ventricular outflow obstruction.

In order to investigate the reason for this abnormality, 23 consecutive cases with pulmonary stenosis (mean age 7.94 ± 3.33 years) undergoing transluminal pulmonary balloon valvuloplasty without significant tricuspid or pulmonary valvar regurgitation were studied prospectively. Right ventricular diastolic filling indices and pulmonary valvar systolic gradients were measured in these children one day before and after pulmonary balloon valvuloplasty and were re-examined six months later. Right ventricular diastolic indices based on rapid early diastolic filling peak velocity (peak E), peak velocity during atrial contraction (peak A), and ratio of E/A were determined by pulsed Doppler echocardiography.

In conclusion, right ventricular diastolic filling indices in patients with pulmonary stenosis did not improve after pulmonary balloon valvuloplasty in the first day but when re-examined by the sixth month there was a significant improvement. These data suggest that diastolic filling abnormalities are more likely a result of right ventricular hypertrophy than of right ventricular outflow obstruction.

Key words: pulmonary stenosis, balloon valvuloplasty, Doppler echocardiography, diastolic functions, children.

Left ventricular filling indices obtained from the transmitral flow velocities by echocardiography have been used in patients with a variety of cardiac diseases including systemic hypertension[1], left ventricular outflow tract obstruction[2], hypertrophic cardiomyopathy[3], coronary artery disease[4], dilated cardiomyopathy[5,6], constrictive pericarditis[7] and valvular heart disease[8]. Use of transtricuspid flow velocities as determined by Doppler echocardiography has been established as a valid method for assessing right ventricular filling[9]. Right ventricular diastolic function has been less extensively studied than diastolic function of the left ventricle[10]. Tricuspid valve Doppler indices were studied for normal values in children with a normal heart[9] and in children with valvular pulmonary stenosis. It is shown that right ventricle (RV) diastolic filling abnormalities in patients with pulmonary stenosis (PS) do not change immediately after successful relief of RV outflow tract obstruction[11].

The purpose of the present study was to determine whether there is any improvement via pulmonary balloon valvuloplasty (PBV), in the early and late phases, on diastolic filling of the RV, and to determine the mechanisms underlying RV diastolic filling abnormalities.

Material and Methods

Patients: The study investigated ventricular diastolic filling indices in patients with pulmonary stenosis and in 17 age-matched normal control subjects (1 females, 10 males). The patient group comprised 23 children (11 females, 12 males) who were randomly selected from all children with pulmonary stenosis undergoing cardiac catheterization in Hacettepe University İhsan Doğramacı Children's Hospital between April 1991 and May 1994. The patient group was 3.5 to 16.5 years old (mean 7.94±3.33 years) and the control group was 3.5 to 14 years old (mean 9.0±3.1 years).

Selection Criteria Included: a) Presence of PS severe enough to warrant treatment with balloon valvuloplasty, b) Absence of additional congenital defects such as tricuspid stenosis or left to right shunts that might alter the tricuspid valve Doppler recording, and c) Absence of significant tricuspid or pulmonary regurgitation by Doppler examination before and after valvuloplasty.

Cardiac Catheterization: The patient group underwent right-and left-sided cardiac catheterization after being sedated with a combination of morphine sulfate, diphenhydramine and diazepam. Peak to peak pressure gradient across the obstruction was measured before and after balloon dilatation.

Echocardiographic Examination: All study participants underwent a range-gated pulsed Doppler eşamination one day before and after pulmonary balloon valvuloplasty and were re-examined six months later. Tricuspid valve Doppler examination was from the apical four chamber view using Toshiba Sonolayer S-SSH 60-A. The sample volume was positioned so as to record the maximum velocities through the valve. Velocities through the tricuspid valve vary significantly throughout the respiratory cycle, with maximal velocities occurring at peak inspiration[5,10]. Therefore, to obtain all Doppler measurements at a standard time in the respiratory cycle, only those beats recorded at peak inspiration were used.

From the Doppler spectral recordings, the peak velocities during rapid ventricular filling (peak E) and during atrial contraction (peak A) were measured and the ratio of peak E to peak A velocities (E/A) was calculated.

Statistical Analysis: Using paired t tests, statistical comparisons were made between control and patient groups before and after PBV. A two tailed p value of <0.01 was used to indicate a significant intergroup difference. All values are presented as mean ± standard deviation.

Results

Patients: There were no significant differences in age, heart rate (93.4±13.3 beats/min versus 87±11 beats/min) or systolic blood pressure (112.78±13.2 mm/Hg versus 109±9.3 mm/Hg) between the pulmonary stenosis group and the control group, respectively. Clinical data of the patient group is illustrated in Table I.

Table I. Clinical Data of the Patients

Patient	Sex	Age (year)	Weight (kg)	Heart Rate (beat/minute)	Systolic Blood Pressure (mmHg)
1	F	16.5	52.6	82	146
2	M	4.5	17.4	122	110
3	F	6.3	22.7	106	120
4	F	14.7	36.3	80	134
5	M	7.6	18.3	95	108
6	M	9	22.8	90	120
7	F	6.5	17.1	110	110
8	M	5.1	14.6	100	85
9	F	10.8	33	80	115
10	M	4.5	13.9	98	100
11	F	11.2	37.5	85	110
12	F	6.7	18.1	74	105
13	F	7.4	24.8	78	105
14	M	5.7	15.5	102	95
15	M	3.6	9.2	90	115
16	F	4.4	13.9	106	96
17	M	5.3	14.7	94	120
18	F	6.6	16.5	110	115
19	M	7	17.1	83	120
20	M	9.7	28.9	92	115
21	M	10.3	24.3	110	130
22	F	11.4	24.6	72	105
23	M	7.9	22.7	90	115
Mean		7.94±3.3	22.45±9.86	93.43±13.28	112.78±13.21

Pulmonary balloon valvuloplasty (PBV) was carried out successfully in all patients without any complication. At cardiac catheterization, all of the patients had sufficient relief of pulmonary stenosis by balloon valvuloplasty. The peak to peak systolic pressure gradient before balloon valvuloplasty (87.3±33.0) was significantly higher than that after valvuloplasty (19.5±5.9) ($p<0.0001$) (Table II).

Table II. Mean Values for the Doppler Measurements of Patients Before and After Pulmonary Balloon Valvuloplasty

	Pre-PBV	Post-PBV (1st day)	p value	Post-PBV (6th month)	p value†
PS-gradient (mmHg)	87.3±33.0 (catheter)	19.5±5.9 (catheter)	0.0001*	23.1±7.5 (echo)	0.0001*
	91.4±22 (echo)	23.2±3.6 (echo)			
E wave (m/sec)	0.48±0.10	0.47±0.7	N.S.	0.51±0.10	N.S.
A wave (m/sec)	0.55±0.10	0.49±0.9	0.0136	0.41±0.09	0.0001*
E/A ratio	0.89±0.21	0.99±0.22	N.S.	1.3±0.2	0.0001*

* Statistically different between groups using paired t test at the 1% level.
† Comparison between pre-PBV and post-PBV (6th month).

PS : pulmonary stenosis.
E : velocity during rapid filling (m/s).
A : velocity at atrial contraction (m/s).
Pre-PBV : before pulmonary balloon valvuloplasty.
Post-PBV : after pulmonary balloon valvuloplasty.

Echocardiographic comparisons of patients with pulmonary stenosis prior to and after PBV: Although less significant differences were observed in Doppler velocities one day before and one day after PBV, A wave values in pre-PBV were much higher than those after six months ($p<0.0001$). E/A velocity ratios were not significantly different one day before and after PBV; however, there was a statistically significant difference in the values six months later, when compared with the one day before and after PBV values ($p<0.0001$) (Table II).

Echocardiographic comparisons of normal subjects and patients with pulmonary stenosis: Mean values of the Doppler measurements of the control group and those of the PS group one day before and six months after PBV are listed in Table III. The patient group had a much higher peak A velocity than the control group ($p<0.0003$) and a much lower peak E velocity than the control group ($p<0.0001$). Therefore, the ratio of peak E to peak A velocities was significantly lower in the patient group ($p<0.0001$). When we compared the results of the control group with results of PBV patients at the sixth month, we detected that there was no significant difference in A wave or E/A ratio (Fig. 1).

Discussion

The indices of right ventricular diastolic relaxation are related with many factors such as age, heart rate, and right ventricular preload (wall stress)[12-15]. Peak A velocity is higher then peak E velocity in the fetus and the newborn infant, but after this period, it returns to normal values and does not change during childhood[12,16-18]. The changes in preload and after load can affect the tricuspid valve diastolic filling indices. So in cases with pulmonary and tricuspid regurgitation, an increase in preload can change diastolic function of the tricuspid valve[5,19]. For these reasons we excluded the patients with pulmonary or tricuspid valve regurgitation. Normally, the peak E velocity of tricuspid valve diastolic indices increases up to 26 percent from expiration toward inspiration, but peak E velocity increases up to 18 percent and the E/A ratio is stable during respiration[12]. Therefore, measurements of the right ventricular diastolic filling flow velocities should be made in a standard time during respiration. We studied diastolic valve flows during peak inspiration.

Evaluation of the diastolic function of the left and right ventricle in congenital heart disease has been increasingly used in recent years[3,11].

Table III. Tricuspid Valve Doppler Measurements in Control Subjects and Patients with Pulmonary Stenosis Before and After PBV

	Controls	Pre-PBV	p value	Post-PBV (6th month)	p value
E wave (m/sec)	0.61±0.12	0.48±0.10	0.0001	0.51±0.10	0.0075
A wave (m/sec)	0.43±0.09	0.55±0.10	0.0003	0.41±0.09	0.5
E/A ratio	1.43±0.27	0.89±0.21	0.0001	1.3±0.20	0.296

* Statistically different between groups using paired t test at the 1% level.
PBV: pulmonary balloon valvuloplasty. E: velocity during rapid filling. A: velocity at atrial contraction.

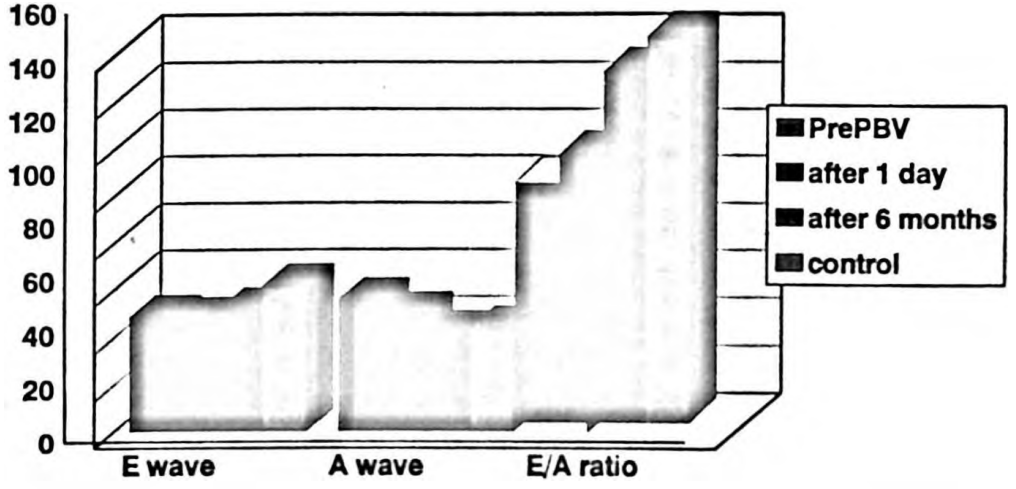

Fig. 1. Outcome of patients with pulmonary stenosis treated by balloon valvuloplasty. Doppler peak velocities before PBV, after 1 day and after 6 months.

It has been shown that diastolic functions worsened in children with right and left ventricular outflow tract obstruction and that they did not improve in the early stages after relief of the obstruction[11,20]. We detected that diastolic filling changes in patients with valvar PS do not improve after PBV, as shown in Table II. Six months after PBV, there was no significant difference in the pulmonary valve peak to peak systolic gradient, but the improvement in tricuspid diastolic indices was statistically significant. Diastolic filling abnormalities seen in patients with pulmonary stenosis did not improve immediately after PBV, as shown by Vermilion et al[11]. In this study, in patients having RV hypertrophy diagnosed by electrocardiographic criteria and echocardiographic findings, tricuspid valve diastolic functions partially improved six months after relief of obstruction.

Conclusion

The abnormalities of RV diastolic filling in patients with PS are not related with right ventricular outflow tract obstruction but are mainly due to right ventricular hypertrophy. As a result of our study, we recommend long-term echocardiographical follow-up for all patients with balloon valvuloplasty in order to evaluate future improvement of ventricular diastolic functions.

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Unguarded tricuspid orifice diagnosed by echocardiography: a clinical study

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Complete absence of tricuspid valve tissue and apparatus with a normal orifice between the right atrium and the right ventricle is defined as "unguarded tricuspid orifice". Very few case report of this anomaly have appeared in the literature. In this article, we present five cases of unguarded tricuspid orifice, isolated or in combination with other anomalies. All patients were males, aged six days to five years; only one case is alive at present. In our opinion, this anomaly is not so infrequent as it is believed to be, and the diagnosis can be made easily with echocardiography if it is kept in mind.

Key words: unguarded tricuspid orifice, echocardiography.

Complete absence of tricuspid valve tissue and apparatus with a normal orifice between the right atrium and the right ventricle was first described by Kanjuh et al.[1] in 1964 and named "unguarded tricuspid orifice". Very few cases have been reported in the literature since then; the authors are aware of only 10 case reports. In our Department, between December 1995 and February 1998; five patients have been diagnosed by echocardiography to have unguarded tricuspid orifice either as an isolated anomaly or in combination with various other congenital anomalies. The diagnoses were confirmed by catheterization in all five cases. Two of these cases have been published previously[2].

Material and Methods

The patients were all referred to the Department of Pediatric Cardiology with profound cyanosis present from birth and signs and symptoms of heart failure. The patients were all males, ranging in age from six days to five years. After initial routine evaluation with physical examination, telecardiography, electrocardiography and complete blood count, their echocardiographic examinations were made on an emergency basis. Two-dimensional, Doppler and color Doppler echocardiographic examinations were made by the same physician with a Toshiba Sonolayer SSH 160A system. The echocardiographic diagnoses were confirmed and hemodynamic measurements made by cardiac catheterization in all five cases. The diagnoses were further confirmed by necropsy in two cases.

Results

Physical examination was not contributory in any of the patients. Blood counts and electrocardiograms of the patients were nonspecific; telecardiography revealed marked cardiomegaly.

Echocardiography revealed complete absence of tricuspid valvular tissue and apparatus in all five cases (Fig. 1). Right atria and right ventricles were enlarged in all five patients. One patient (Case 5) had a single atrium.

One patient (Case 2), a newborn critically ill at presentation, died during catheterization; the diagnosis was confirmed by necropsy. One patient died awaiting shunt surgery. Three patients (Cases 1, 3 and 4) underwent surgery; two of these died and one (Case 4) is still alive and in good condition with a permanent transvenous pacemaker 18 months after surgery. Diagnoses, management and outcome of the patients are shown in Table I.

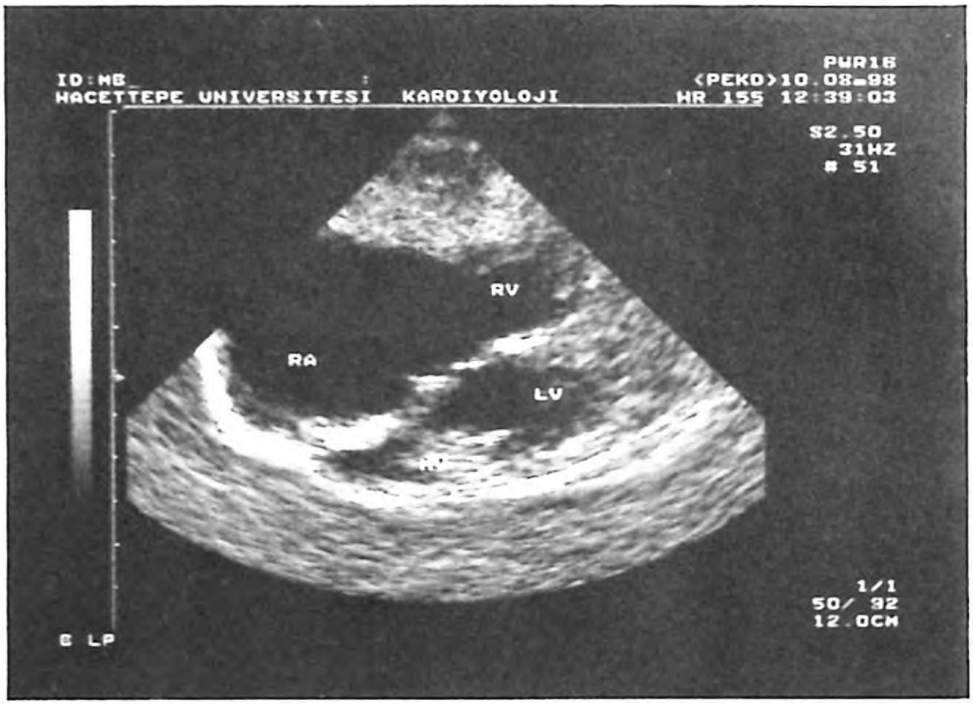

Fig. 1. Echocardiographic view of Case 4 showing complete absence of tricuspid valve tissue and a normal orifice between the right atrium and the right ventricle. RA: right atrium, RV: right ventricle, LV: left ventricle, MV: mitral valve.

Table I. Diagnosis, Management and Outcomes of the Patients

Case	Age	Diagnosis (echocardiography and catheterization)	Management and outcome
1	5 yr	UG	TVR, died postoperatively, necropsy
2	6 d	UG, isolated dextrocardia, pulmonary atresia, PDA, ASD	Died at catheterization, necropsy
3	1 mo	UG, Uhl's anomaly, pulmonary atresia, PDA, dextrocardia	Shunt, died at surgery
4	3 yr	UG, VSD, mild pulmonary stenosis	Alive, ASD and VSD closure, permanent pacemaker implantation
5	2 mo	UG, single atrium, pulmonary atresia, PDA/APCA, hypoplastic left pulmonary artery	Died awating shunt surgery

AG : unguarded tricuspid orifice.
TVR : tricuspid valve replacement.
PDA : patent ductus arteriosus.
ASD : atrial septal defect.
VSD : ventricular septal defect.
APCA : aortopulmonary collateral artery.

Discussion

Complete absence of tricuspid valvular tissue with pulmonary atresia-intact ventricular septum was first observed by Klein in 1938[3]. In this case, necropsy demonstrated almost complete absence of tricuspid valve tissue and rudimentary vestiges of valvular tissue attached to the posterior wall of the right ventricle. Kanjuh et al.[1] reported a second case of pulmonary atresia and intact ventricular septum and complete absence of tricuspid valve, which they termed "unguarded tricuspid orifice". Both of these cases had hypoplastic right ventricles and sinusoidal communications supplying the pulmonary circulation. Gussenhoven[4] reported this anomaly in a neonate with a double-chambered right ventricle.

Anderson et al.[5] reported 46 cases of pulmonary atresia and intact ventricular septum from the Cardiopathological Collection of the Children's Hospital of Pittsburg, of which three cases had unguarded tricuspid orifice and 17 cases had Ebstein's malformation. They observed that all the cases with unguarded tricuspid orifice, but only five of the 17 cases with Ebstein's malformation, had a dilated right ventricular cavity. They pointed out that the two conditions could be differentiated with the examination of the mural leaflet, which is absent when the orifice is unguarded, but displaced in association

with Ebstein's malformation. They suggested the use of cross-sectional echocardiography, especially the subcostal and parasternal long axis approaches, to recognize this feature. In their review, the variant of pulmonary atresia and intact ventricular septum with dilated chambers of the right heart was found to be a lethal combination, which should prompt ventricular decompression as soon as the diagnosis is made.

Magee et al.[6] reported an 11-month-old case of unguarded tricuspid orifice with preexcitation and successful radio frequency ablation of the accessory pathway.

Shahani et al.[7] reported surgical treatment of a seven-year-old girl with an unguarded tricuspid orifice and dilated right heart chambers. They inserted a prosthetic valve at the site of native tricuspid anulus, with prompt improvement in clinical status. After eight years' follow-up, the child is reported to be clinically well and the prosthetic valve functioning normally.

Hornberger et al.[8] and Achiron et al.[9] reported prenatal two-dimensional and Doppler echocardiographic diagnoses of unguarded tricuspid orifice.

In our Department, where approximately 4,500 echocardiographic examinations are made yearly, five cases have been diagnosed to have unguarded tricuspid orifice between December 1995 and February 1998. Although it is not possible with these data to predict the incidence of this rare congenital anomaly, it is the authors' impression that this anomaly is not so infrequent as it is believed to be and that it could be diagnosed easily with cross-sectional echocardiography if it is kept in mind.

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Hereditary angioedema: case report of a family

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Hereditary angioedema (HAE) is a rare disease resulting from deficiency of complement 1 esterase inhibitor (C1-INH). The clinical manifestations of this disease include recurrent attacks of self-limiting edema affecting face, extremities, gastrointestinal system and upper airways. In this report, we present eleven members of a family with HAE. Edema of the extremities was the most common symptom, occurring in ten patients. Three patients experienced severe laryngeal edema that required tracheotomy. Three patients developed facial and scrotal edema. Three patients experienced severe abdominal pain. The mean age at onset of symptoms was 11 years. C1-INH levels were undetectable in two patients and low in nine patients. CH50 was undetectable in all of the patients. C4 level for all patients was low. HAE in our first case, a 10-year-old boy, was diagnosed on the basis of low C1-INH, CH50 and C4, in addition to his familial history. Eleven members of this family, for whom laboratory studies could not be done, had similar symptoms and course. Two patients died as a result of laryngeal edema before establishment of dignosis. This case report indicates the importance of recognition and early treatment of HAE to prevent a potentially fatal outcome.

Key words: hereditary angioedema, complement 1 esterase inhibitor.

Hereditary angioedema (HAE) is a rare autosomal dominant disorder resulting from the absolute or the functional deficiency of complement 1 esterase inhibitor (C1-INH)[1,2]. The clinical manifestations of this disease are polymorphic and include recurrent non-pruritic and self-limiting soft tissue edema attacks that can involve face, extremities, and mucosal surfaces of the gastrointestinal tract and upper airways. The main cause of death in untreated patients is laryngeal edema[3,4].

Two types of HAE have been described. Type I HAE, the predominant form (85% of all patients), is characterized by decreased levels of C1-INH protein caused by a reduced synthetic rate of C1-INH. Type II (variant form) HAE is characterized by normal or raised levels of C1-INH that are functionally inactive[1,2]. Both types of HAE are characterized by low or absent levels of C2, C4, and CH50[1,3-5]. Less commonly, C1-INH deficiency occurs as an acquired form that may be associated with B-cell malignancies or with autoantibodies to C1-INH[6,7]. In this case report, we present the clinical features and course of a family with hereditary angioedema.

Case Reports

A 10-year-old boy (İ.A.) was admitted to our hospital with a history of intermittent edema attacks affecting mainly extremities, face and scrotum since he was six- months-old. There was no consanguinity. Laboratory studies were as follows: C1-INH 38 mg/dl, CH50 undetectable, C4 12 mg/dl, C3 114 mg/dl. His edema attacks were triggered after trauma. He reported that 21 members (11 males and 10 females) of his family suffered from episodic soft tissue edema. HAE was diagnosed on the basis of low C1-INH, CH50 and C4, in addition to his familial history. The pedigree of this family is given in Figure 1.

After the establishment of the diagnosis of HAE, we wanted to investigate other members of his family since they had shown similar symptoms. Blood samples could not be taken from 11

members: four had died and seven were either not available or did not give permission. Thus, including the case previously mentioned, C4, CH50 and CI-INH were studied in a total of 11 patients. The blood samples were immediately separated. C4 was measured by turbidimetric method (RA-XT, Technicon). The total hemolytic activity (CH50) and CI-INH were studied by standard radial immunodiffusion (The Binding Site Limited).

The age at onset of symptoms ranged from six months to 21 years (mean 11 years), and symptoms in most patients appeared in adolescence. The frequency of edema attack was usually four or five times a year.

None of the patients has been lost during the follow-up period of 10 years. Their history revealed that edema of the extremities was the most common symptom, occurring in 10 patients. Three of the patients experienced severe laryngeal edema

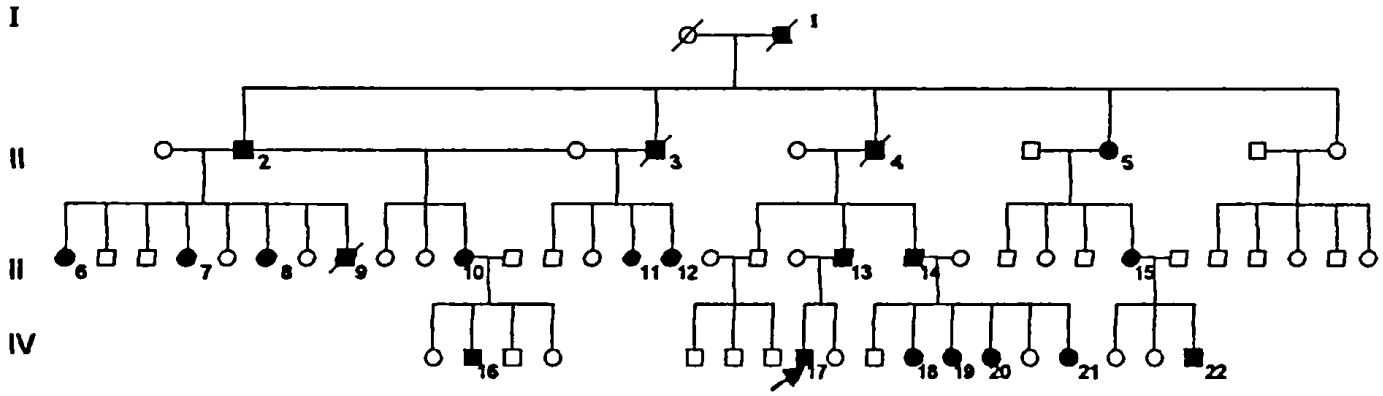

Fig. 1. Pedigree of the family. Circles denote females; squares, males. Open cricles and squares denote unaffected persons; closed circles and squares denote affected persons. Deceased persons are indicated by an oblique line.

The characteristics and laboratory findings of all the patients are given in Table I. C1-INH levels were undetectable in two patients, 12.1 mg/dl (36% of normal) in one patient, 38 mg/dl (11.5% of normal) in four patients, 39 mg/dl in one patient, 53.8 (15.9% of normal) in one patient and 76.6 mg/dl (23.3% of normal) in two patients. CH50 was undetectable in all patients. C4 level for all patients was low.

that required tracheotomy, three developed facial edema, and three experienced severe abdominal pain associated with vomiting and diarrhea related to gastrointestinal involvement. Two patients developed scrotal edema. Extremity, facial, and gastrointestinal system (GIS) edema were seen in 10, four, and three patients, respectively.

They reported that the symptoms lasted one to four days and then subsided spontaneously if

Table I. Characteristics and Laboratory Findings of the Patients

No	sex	Localization of edema	Age at onset of symptoms (year)	Number of attacks per year	C1-INH (mg/dl)	C4 (mg/dl)
6	female	extremity, face GIS	UN	UN	76.6	14
7	female	extremity, face, GIS	UN	UN	12.1	13
8	female	GIS	UN	UN	38	11
10	female	extremity, face, lrynx	10	3-4	38	10
13	male	extremity, larynx	10	8-10	38	9
14	male	extremity, larynx	10	3-4	UD	8
16	male	extremity	10	3-4	53.8	4
17	male	extremity, face, scrotum	1/2	3-4	38	12
19	female	extremity	11	5-6	39	4
20	female	extremity	10	5-6	UD	10
22	male	extremity, scrotum	21	8-10	76.6	14

UN: unknown.
ND: not done.
UD: undetectable.
GIS: gastrointestinal system.
The normal value C1-INH is 332±25 mg/dl.

there was no laryngeal edema. Symptoms were precipitated mainly by trauma, anxiety, cold weather, and tooth extraction, but sometimes developed without any triggering factors.

Five of the patients, whose ages ranged from 13 to 45 years, had received danazol therapy (400 mg daily) irregularly for a period ranging from one to three years prior to admission to our hospital but the drug had been discontinued because of its side effects, such as weight gain, hirsutism, and acne. There was not enough information about monitorization and treatment of the patients. These patients reported a reduction in both severity and frequency of edema attacks after this treatment, but one patient developed laryngeal edema.

We treated acute edema attacks with epinephrine, steroid, and antihistaminic drugs. Fresh frozen plasma (FFP) was used successfully to treat facial edema associated with buccal edema and life-threatening edema attacks in two patients. They reported that although there was no history of allergy, they had been diagnosed as allergic disease and had been treated accordingly.

Eleven members of this family had similar symptoms and course. Two patients died as a result of asphyxia secondary to laryngeal edema before the establishment of diagnosis.

Discussion

C1-INH is the main regulator protein of the activation steps of the complement pathway and it regulates inhibition of C1r, C1s, active Hageman factor, kallikrein, plasmin, and actived factor IX. A deficiency of C1-INH can result in a lack of regulation of the complement system and in the release of some mediators, leading to the increased vascular permeability responsible for clinical symptoms[3,4,8,10].

Diagnosis of type 1 HAE is usually established by positive family history and reduced serum C4, C1-INH, and CH50 levels, even when the patients are symptom-free[1-5]. The C1-INH level of patients with HAE is less than 20 to 30 percent of normal. The defect is transmitted as an autosomal trait, but can occur spontaneously as well, with no family history. These cases probably represent a newly acquired mutation[5]. About 15 to 25 percent of patients (type II HAE) have normal or elevated C1-INH levels. These patients have been shown to have non-functional protein[1,3,5]. There are a few patients with acquired angioedema (AAE). AAE usually starts after the fourth decade of life without family history. AAE type is characterized by the presence of antibodies to C1-INH and by the increased catabolism of C1-INH associated with benign or malignant B-cell lymphoproliferative disorders[6,7].

The clinical manifestations of HAE are polymorphic and include recurrent attacks of edema affecting the extremities, face, GIS, genitalia, and airways[3,4]. Edema of the extremities was the most common symptom in our cases. The edema is typically described as non-pruritic and painless. Laryngeal edema is the most feared complication. It can present rapidly and responds poorly to epinephrine, frequently requiring a tracheotomy to open the airway. Laryngeal edema influences the mortality rate, which may vary from 15 to 33 percent. The mortality rate in our cases was nine percent. Severe abdominal pain due to bowel wall edema is also common[4]. However, 18 percent of our patients had a history of severe abdominal pain. Sometimes the edema of the bowel wall may resemble an acute abdominal condition and many of these patients undergo an unnecessary laparotomy[4].

The first manifestations of HAE can appear at any age, but particularly at adolescence[3,4]. Mean age at onset of symptoms of our cases was 11 years. Although attacks may occur without any reason, trauma, stress, and cold are potential triggering factors. HAE usually lasts 24 to 72 hours and disappears spontaneously if there is no laryngeal involvement[3,4]. The patients should be warned about the triggering factors and avoid them when possible. The edema attacks of our cases were usually precipitated by trauma, anxiety, dental procedures or cold.

Treatment of the acute episode in patients with HAE remains the most difficult aspect in management. Epinephrine, corticosteroids, an antihistaminic, purified C1 inhibitor protein, or epsilon aminocaproic acid (EACA) may be partially effective in the management of acute attack. FFP has been used successfully. However, it should be noted that FFP could worsen the attack by supplying complement proteins; some authors recommend it not be used in the acute attack[3-5,8]. Long-term prophylaxis with the attenuated androgens (stanazol and danazol) has been used effectively daily or on alternate days in adults and children. The maintenance dose is

established by clinical response rather than by normalization of serum levels of C1-INH or C4. Long-term therapy depends on the severity and frequency of attacks[3,4,9]. We observed that the severity and number of attacks decreased in patients treated with danazol. The androgens have some side effects, such as hepatic damage and virilization, but these usually disappear after the drug is stopped[3,4]. Recently, interferon-λ has been shown to stimulate C-INH protein production[10].

Hereditary angioedema (HAE) is in fact poorly recognized by physicians, as in our cases. Most commonly, HAE is considered an allergic disease. Some physicians may not take into consideration the seriousness of this disease. Also, some physicians, frightened by the possibility of an allergic reaction, are hesitant to give the necessary antibiotic, analgesic or other drugs to such patients. Tho date only two drugs, estrogens and angiotensin-converting-enzyme inhibitors, have been proven detrimental in C1-INH deficient patients by facilitating attacks[4].

In this paper, we presented a family with type I HAE, eleven members of which were followed-up in our outpatient and emergency clinics. The main problem for patients with HAE is misdiagnosis due to the fact that the disease is relatively unknown. This case report also indicated the importance of recognition and early treatment of HAE to prevent a potentially fatal outcome.

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Ivemark syndrome: asplenia with kidney collecting duct cysts and polysplenia with cerebellar cyst

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SUMMARY: Krželj V, Kragić I, Glavina-Durdov M, Jakl R, Bucat M, Kuzmić-Prusac I. Ivemark syndrome: asplenia with kidney collecting duct cysts and polysplenia with cerebellar cyst. Turk J Pediatr 2000; 42: 234-238.

Two newborns, one male and one female, from two different families, with Ivemark syndrome proven at autopsy are reported. One of them had asplenia and another had polysplenia. Both newborns had complex cardiac defects with isomerism of the lungs. The newborn with asplenia had dextrocardia, transposition of the great vessels, stenosis of the pulmonary artery, common atrioventricular canal and patent ductus arteriosus. The newborn with polysplenia had a common atrium, hypoplastic left ventricle and patent ductus arteriosus. The patient with asplenia had cystic dilated collecting ducts of the kidney and the patient with polysplenia had cerebellar cyst. These associate malformations have not been reported previously. Both cases were sporadic.

Key words: cerebellar cyst, dextrocardia, Ivemark syndrome, kidney collecting duct cysts, transposition of great vessels.

Ivemark syndrome is a severe congenital malformation characterized by visceral heterotaxia, and cardiovascular and bronchopulmonary malformations[1]. This syndrome has been known by many names including asplenia/polysplenia syndrome, isomerism sequence, situs ambiguous and laterality sequence[2]. The etiology is not clearly defined yet, but exogenous factors between the 31st and 38th day of gestation are suggested as the main cause[3].

Ivemark syndrome results from bilateral right- or left-sidedness[2]. Bilaterally placed organs are identical, and unilateral organs may be duplicated or absent. Asplenia has been termed bilateral right-sidedness or right-isomerism. Hypoplasia of the spleen is sometimes the finding rather than aplasia. Asplenia is usually associated with severe atrioventricular canal malformations and marked deficiency of the interventricular septum, with bilaterally trilobed lungs, together with short eparterial bronchi and a heart with two right atrial appendages[2]. The liver remains in central position. The gallbladder is situated to the left near the obliterated umbilical vein. The stomach is tubular in shape and lies medially. The mesentery is aligned vertically rather than diagonally, with the result that malrotation of the bowel is common[4]. Other gastrointestinal malformations have often been described in association with congenital asplenia[5].

Polysplenia has been called bilateral left-sidedness or left isomerism. The atrioventricular defect associated with polysplenia is usually less severe and there are greater abnormalities of the interatrial septum[6]. Each lung has two lobes, there are two long hyparterial bronchi with distal branching and two atria with left atrial appendages[2]. The liver forms centrally and occasionally the gallbladder is absent[7].

Most cases are sporadic, but affected sibs are also reported[8]. The prognosis depends on the degree of the malformation of the heart, but other anomalies and sepsis complicate the clinical course.

Case Reports

Two cases of Ivemark syndrome are reported. In both, the syndrome was proven at autopsy. Both cases were sporadic.

Case 1

A ten-day-old male newborn was admitted to hospital because of severe cyanosis. He was born after an uneventful pregnancy with a birth weight of 3800 g. Parents were unrelated with no similar disorders in their families.

On examination the child had a systolic heart murmur. Echocardiography showed dextrocardia, transposition of the great vessels, stenosis of the pulmonary artery, common atrioventricular canal and patent ductus arteriosus. Acute omphalitis and bilateral pneumonia had developed. He died on the 13th day of life.

The autopsy documented dextroposition of the heart with cardiac defect, asplenia and bilateral trilobed lungs (Figs. 1 and 2). The liver was in a central position (Fig. 3). Histopathological finding of the liver was normal. There were no gastrointestinal malformations. The kidneys were of normal size and shape, with distinct border between cortex and medulla. Collecting system had no visible malformation or obstruction. Histological examination of the kidneys revealed some cystic-like dilated collecting ducts at the cortico medullary border and isolated dilated Bowman capsules (Fig. 4). Mildly dilated collecting ducts were lined by cuboidal epithelium (Fig. 5).

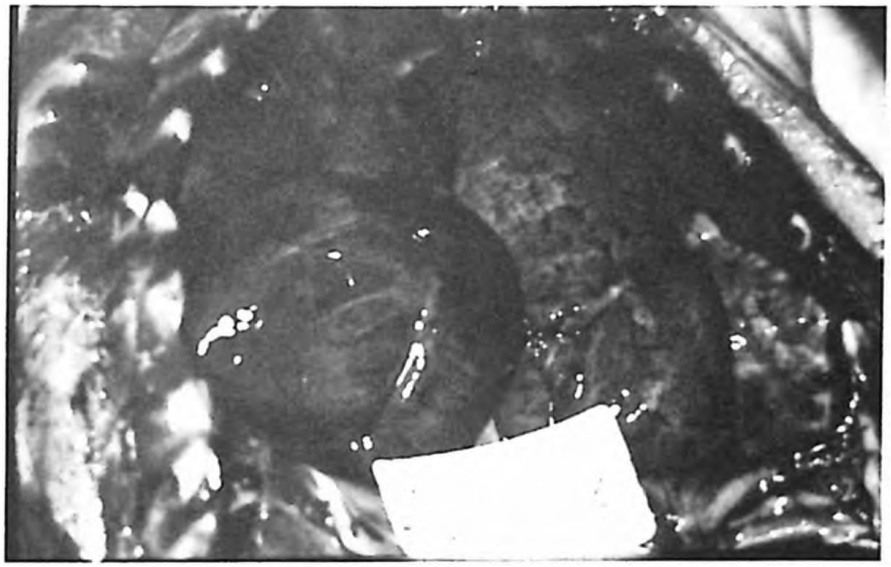

Fig. 1. Dextroposition of the heart (H) and trilobed left lung (LL).

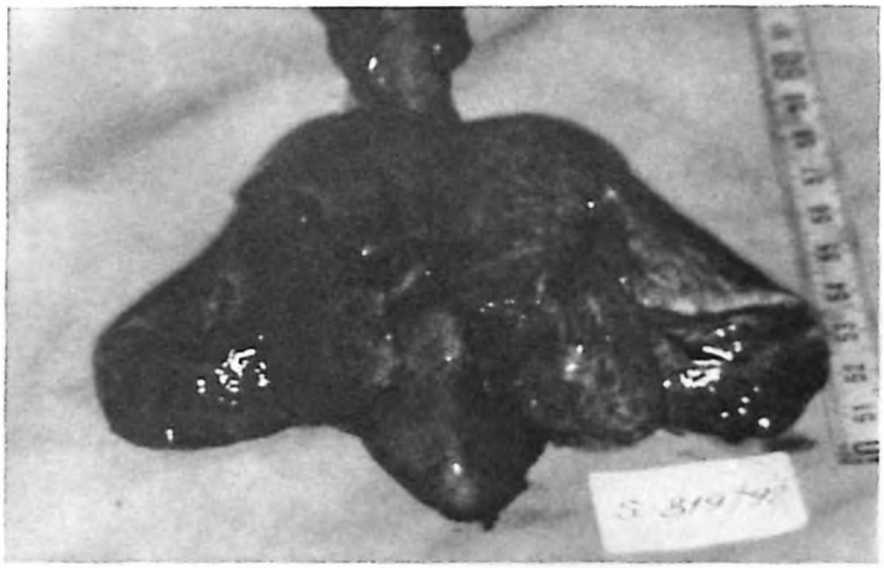

Fig. 2. Bilateral trilobed lungs (RL: right lung; LL: left lung).

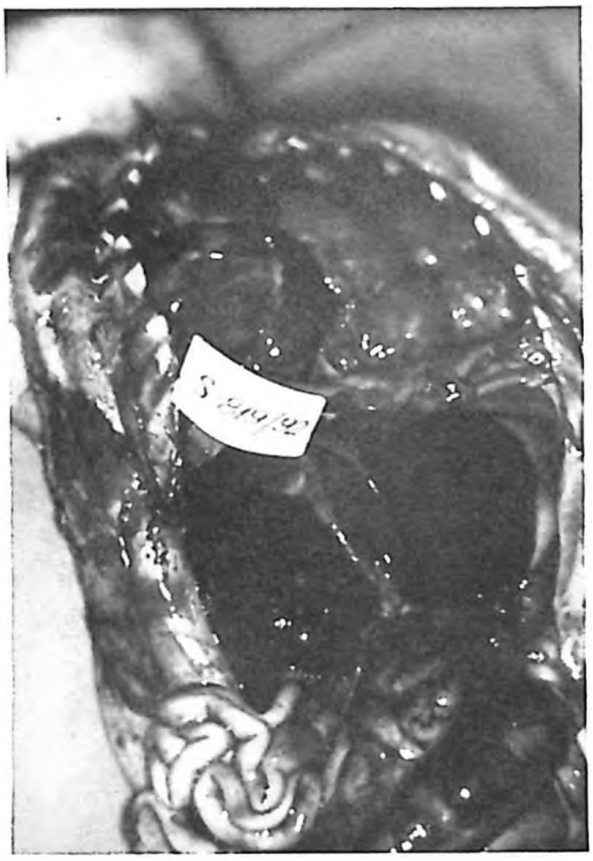

Fig. 3. Centrally situated liver associated with asplenia (a).

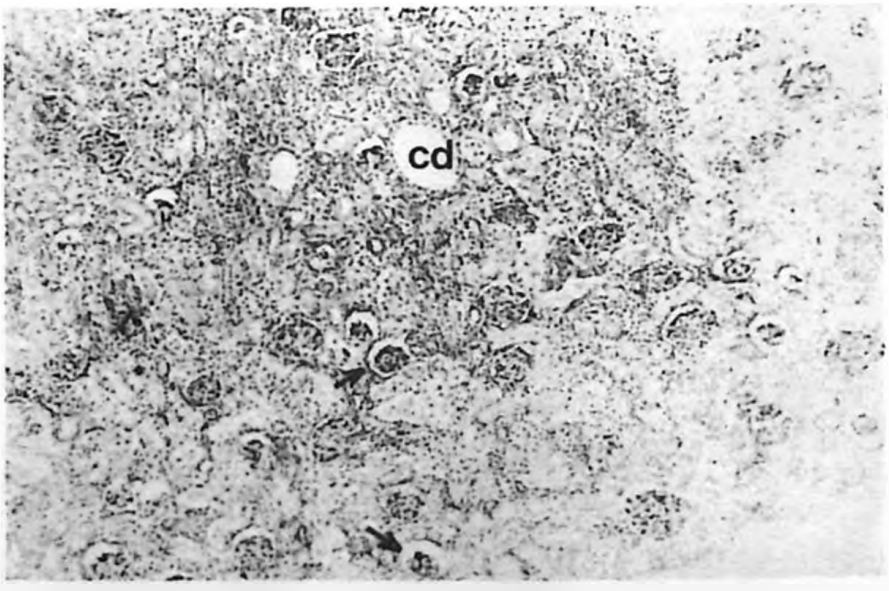

Fig. 4. Slightly dilated Bowman's capsules (arrow) and a few collecting ducts (cd) in the renal cortex (x 40).

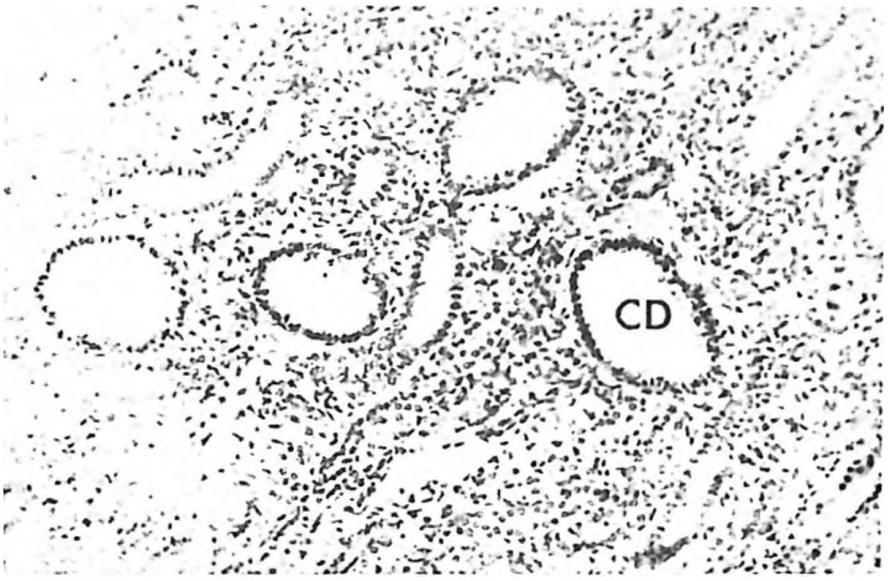

Fig. 5. Cystically dilated collecting ducts (CD) in the renal medulla are lined by cuboidal epithelium (x 200).

Case 2

Male newborn, 2900 g weight, with systolic heart murmur was admitted at the Intensive Care Unit. He was born after an uneventful pregnancy, from healthy and unrelated parents with unremarkable family histories.

Echocardiography revealed a common atrium, hypoplastic left ventricle and patent ductus arteriosus. Echosonography of the cerebellum showed one unilocular cyst, 2.5 cm in diameter. The cerebellum was smaller, with atrophic gyri. The patient became septic, developed heart failure and died at the age of 35 days.

The autopsy confirmed the described cardiac defects and a cerebellar cyst filled with clear liquid. The spleen was normal, but close to its hilus there were two smaller accessory spleens. Both lungs were bilobed. Histopathological findings of other organs were normal.

Discussion

Situs inversus is well described in humans and may be associated with defective cilia in Kartagener's syndrome. In just over one in 10,000 people situs inversus is present with no impairment of function[2]. Ivemark syndrome may be considered an example of damage to a polytopic morphogenetic field in the dorsal mesogastrium where the asymmetry center and the splenic anlage are located[9].

Ivemark also described a quite different syndrome of familial dysplasia of the kidneys, liver and pancreas. Renal-hepatic-pancreatic dysplasia (RHPD) also bears Ivemark's name, at the risk of being confused with asplenic-cardiac anomaly[10]. Ivemark reviewed RHPD four years later than asplenia/polysplenia syndrome[11].

Crawfurd[12] described two sibs with a disorder that appeared to combine features of the two Ivemark syndromes. Both had enlarged polycystic kidneys, a nodular and cystic pancreas associated with absence or hypoplasia of the spleen and cardiac anomalies. A similar case with pancreatic fibrosis, situs inversus, renal dysplasia and cardiovascular anomalies was also reported[13].

A new syndrome with situs inversus totalis, and a cystic dysplastic kidney and pancreas in two sib fetuses was recently reported[14].

Frequencies of the Ivemark syndrome in pediatric autopsies range from 0.02 to 0.38 percent. The male: female ratio is 5:4[9]. Most of cases are diagnosed for the first time at autopsy, as with both patients reported in this study.

Improvements in cardiac surgical techniques can lead to successful repair in some cases, if early cardiac surgery is done[15]. The case of a nine-year-old girl has been described[16].

Up to now there have been only a few reports about an involvement of the kidney[16]. Horseshoe kidney, hydronephrosis, urethral valves, renal hypoplasia, double collecting system, nephroptosis and polycystic kidneys have been reported[17]. A patient with Ivemark syndrome, horseshoe kidney and biliary atresia was also described[18]. The first case of this study had bilateral cystic collecting ducts at the kidney

corticomedullary border. There was no recent report of this kind of cyst associated with asplenia syndrome.

Abnormalities of the central nervous system have been reported in association with asplenia[17]. On the contrary, cerebellar cyst and cerebellar atrophy were associated with polysplenia in this study.

Polymalformation syndromes often include cystic changes. Asplenia syndrome with bilateral cystic collecting ducts at the kidney corticomedullary border and polysplenia syndrome with cerebellar cyst could be an isolated entity or a final pathway of response of these organs to a variety of developmental disturbances.

A sonographic examination of the abdomen should be done in any case of complex cardiac defect in order to diagnose possible asplenia or polysplenia. Similarly, in cases of malrotation of the intestines, special attention should be paid to the heart and spleen. We consider that such an approach could increase the number of cases with Ivemark syndrome diagnosed during their lifetime.

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A case of isotretinoin embryopathy with bilateral anotia and Taussig-Bing malformation

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SUMMARY: Ceviz N, Özkan B, Eren S, Örs R, Olguntürk R. A case of isotretinoin embryopathy with bilateral anotia and Taussig-Bing malformation. Turk J Pediatr 2000; 42: 239-241.

We report a newborn infant with multiple congenital anomalies (anotia and Taussig-Bing malformation) due to exposure to isotretinoin within the first trimester. In this paper we aim to draw to the fact that caution is needed when prescribing vitamin A-containing drugs to women of childbearing years.

Key words: maternal isotretinoin intake, anotia, Taussig-Bing malformation.

Although it has been reported that the use of low or moderate doses of vitamin A (<10,000 IU) during the first trimester of pregnancy is not teratogenic and provides some protection to the fetus[1], teratogenicity of both the lack of and excess of vitamin A during embryonic development has been proven by many studies[2-4].

In this report, we describe a newborn infant with multiple congenital anomalies who had been exposed to isotretinoin, a retinoid prescribed for severe recalcitrant cystic acne, in the first trimester of pregnancy. The case was reported to draw attention to prescription rules for vitamin A analogues in sexually active fertile women.

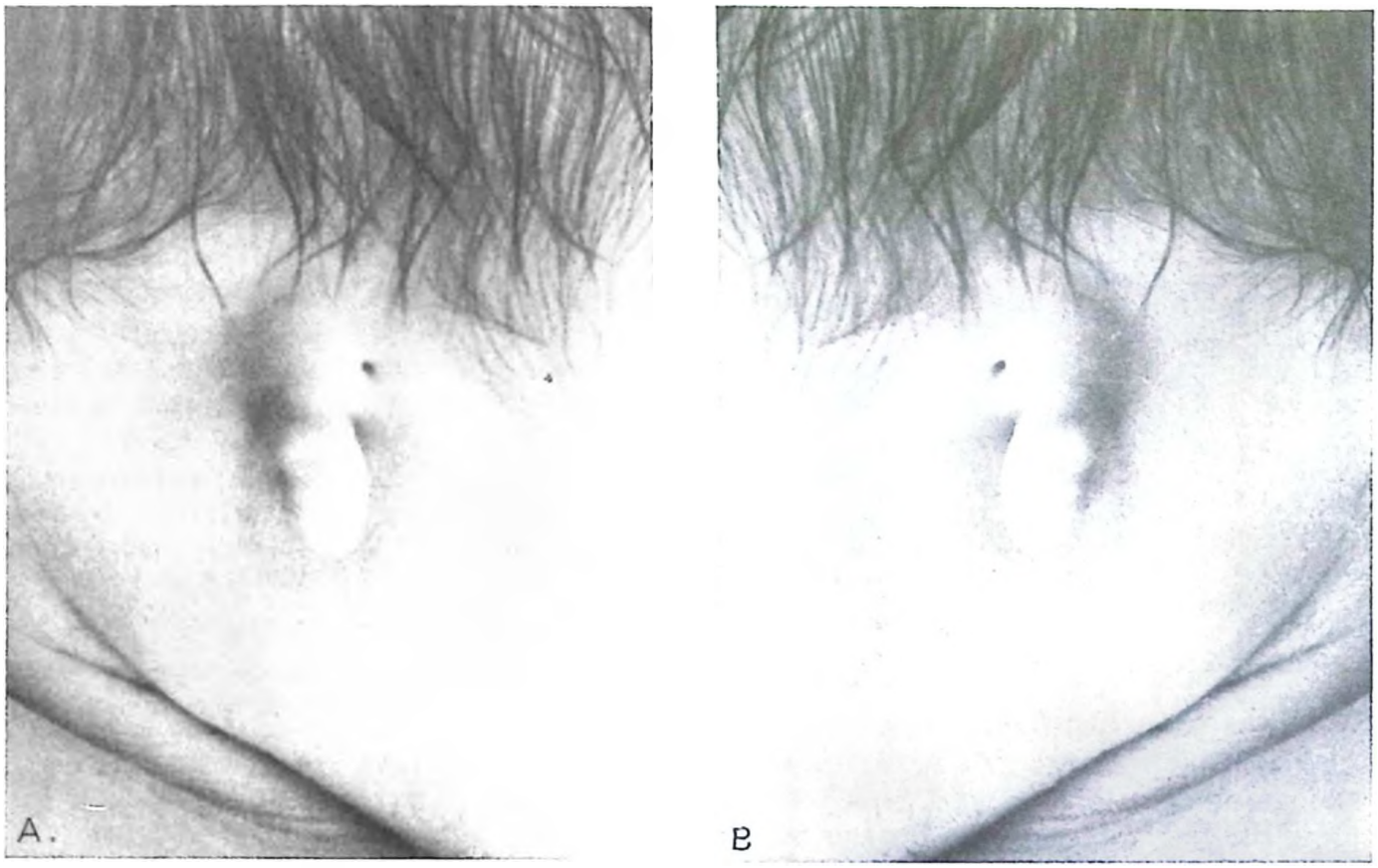
Case Report

A two-day-old term newborn infant was admitted to the Neonatology Outpatient Clinic with the complaints of absence of auricles, tachypnea, and feeding difficulties. The infant was a product of a pregnancy complicated by isotretinoin ingestion during the first two months (40 mg/day). The mother was well educated (engineer-argonomist). The preparation had been prescribed by a dermatologist, unaware of the pregnancy, for treatment of severe recalcitrant cystic acne. After a normal pregnancy, the newborn was born in a state hospital by normal vaginal delivery. The first problem noted was the absence of auricles bilaterally, and on the 2nd day of birth, tachypnea and tachycardia developed.

Physical examination revealed the bilaterally deformed and rudimentary auricles (Fig. 1). The patient had cyanosis in addition to the signs of heart failure (hepatomegaly, tachypnea, tachycardia). Increased precordial activity was apparent. Chest x-ray showed cardiomegaly and increased pulmonary vascular markings. On echocardiodiographic examination (Fig. 2), a) atrial situs was normal, b) a large subpulmonary ventricular septal defect (VSD) and a secundum atrial septal defect (ASD) were present, c) both great arteries originated from the anterior right ventricle, and d) tricuspid insufficiency jet velocity indicated approximately 70 mmHg of right ventricular pressure. Thus, the diagnosis was double outlet right ventricle, subpulmonary VSD (Taussig-Bing malformation), secundum ASD and pulmonary hypertension.

Ultrasonographic evaluation of cranium and abdomen showed no pathology. Cranial computed tomography (Fig. 3) showed atresia of the external ear canal, tympanic membrane, middle ear and antrum. Other structures (cochlea, semicircular canals and middle ear bones) were normal.

Digoxin and furosemide were administered for congestive heart failure. Cardiac catheterization and angiocardiography confirmed the diagnosis of Taussig-Bing malformation and ASD with pulmonary hypertension (systolic, diastolic and mean pulmonary artery pressures were measured as 64,13 and 34 mmHg, respectively). The family did not accept any surgical approach. The patient was discharged, and he died at home.



(a) (b)

Fig. 1. Figure depicts the bilaterally deformed and rudimentary auricles. a) Right, b) Left.

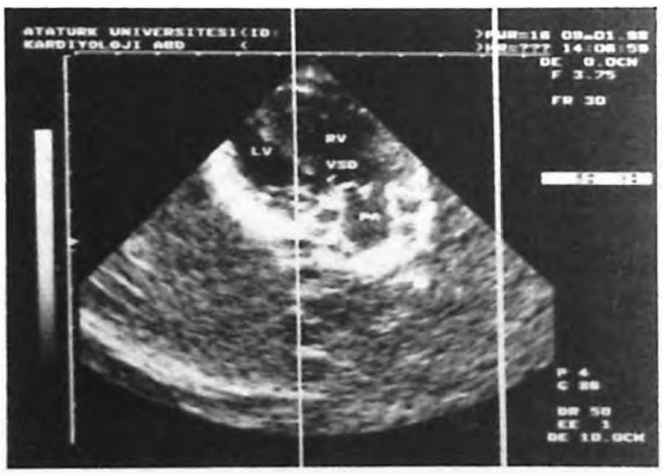


Fig. 2. A two-dimensional echocardiogram (parasternal long axis) shows the double outlet right ventricle and subpulmonary ventricular septal defect (VS). RV: right ventricle, LV: left ventricle, PA: pulmonary artery, AO: aorta.

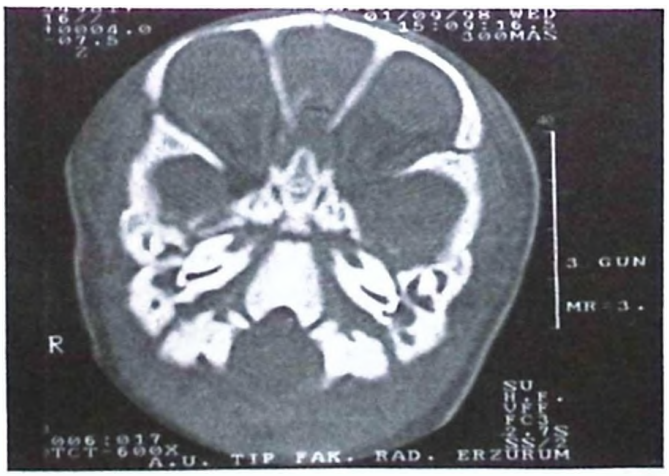


Fig. 3. Computed cranial tomography demonstrates atresia of the external ear canal, tympanic membrane, middle ear and antrum.

Discussion

Vitamin A analogues are known to be teratogenic in laboratory animals[5,6] and their teratogenicity has been reported in clinical case reports[4]. Exposure to isotretinoin has been reported to be associated with an unusually high relative risk for a group of select major malformations[5]. The most often reported malformations are related to craniofacial (microtia/anotia, micrognathia, cleft palate), cardiac (conotruncal heart defects, aortic-arch abnormalities), thymic and central nervous system structures (retinal or optic-nerve abnormalities, central nervous system malformations), suggesting the involvement of the cranial neural crest[6].

Our case had typical manifestations of fetal exposure to vitamin A derivatives (with craniofacial and cardiac defects).

In Turkey, isotretinoin can only be prescribed by dermatologists. Although an informed consent usually is obtained and a detailed explanation is given about the teratogenicity of vitamin A anologues, it is not mandatory to evaluate female patients in terms of possible pregnancy.

Our case with a well-educated mother suggests that the current application can be insufficient and can result in undesired outcomes. In conclusion, we propose that vitamin A analogues not be prescribed without obtaining a negative pregnancy test from fertile women. Also, in countries where adolescent pregnancies are frequent, women taking any teratogenic drug, like isotretinoin, should be actively practicing birth control while on this drug.

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Teratoid Wilms' tumor: a case report

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SUMMARY: Karaca İ, Şencan A, Ortaç R, Şencan-Bostancı A, Mir E. Teratoid Wilms' tumor: a case report. Turk J Pediatr 2000; 42: 242-245.

Teratoid Wilms' tumor is rarely seen and is a description used only recently. The term describes classical nephroma with a diversity of cell types and tissues. In this reported case, the epithelial component consisting of squamous areas made up 70 percent of the tumor; no criteria of dysplasia nor any nephroblastomatosis areas or endodermal elements were presented. Although it is reported that teratoid Wilms' tumor is not usually aggressive or metastatic, a case of unilateral teratoid Wilms' tumor in a 2.5-year-old-boy who died because of metastatic disease is presented and the literature reviewed.

Key words: pediatric "teratoid" Wilms' tumor, Wilms' tumor.

Wilms' tumor is the most common renal tumor of childhood. It is an embryonal neoplasm that is generally assumed to arise from metanephric blastema. Typically, it exhibits a triphasic histological pattern of blastema, epithelium and stroma[1-4]. In addition to these components, some small foci of heterologous elements may be encountered. The term teratoid Wilms' tumor was introduced by Variend et al.[5] to define a variant of nephroblastoma in which the heterologous tissue predominated. We report a child with unilateral Wilms' tumor which included teratoid as well as typical nephroblastic components.

Case Report

A 2.5-year-old boy was admitted to our clinic with hematuria and a right-sided mass noticed by his mother two years previously. The physical examination revealed a right-sided abdominal mass and right cryptorchidism. The laboratory findings were normal except for mild anemia and hematuria. Bone marrow aspiration was evaluated as normal. Vanillylmandelic acid was negative in 24-hour urine analysis. Ultrasonographic investigation showed anterior and inferior displacement of the right kidney and that the right echogenic mass was 10x15 cm. Intravenous urography showed a right-sided renal mass distorting the pelvicalyceal system, and hydronephrosis was present on the left kidney, resulting from extrinsic obstruction of the pelviureteric junction (PUJ). Computerized tomography (CT) confirmed that the mass arose from the right kidney, and it showed multiple periaortic lymph nodes. Chest x-ray was normal.

Exploratory laparotomy was performed. A firm well-encapsulated and lobulated renal tumor was found, completely excised, and a biopsy was taken from enlarged periaortic and mesenteric lymph nodes. Exploration of the contralateral kidney was evaluated as normal except for mild hydronephrosis. Hydronephrosis on the left kidney was assumed to be due to enlarged lymph nodes displacing and compressing the PUJ extrinsicly. The tumor was histopathologically diagnosed as teratoid Wilms' tumor.

The tumor was accepted as stage III because of periaortic and mesenteric lymph node metastases according to the National Wilms' Study Group 4. The child was given chemotherapy that included vincristine, dactinomycin, doxorubicin and whole abdomen irradiation (total 2,570 rad). Follow-up CT three months later revealed persistent multiple periaortic lymph nodes and a hydronephrotic left kidney. A second laparotomy was planned, but because of complications due to chemotherapy and irradiation, the operation was postponed. The patient was lost to follow-up and received no further chemotherapy. After an interval of six months, the patient was admitted with pulmonary

insufficiency. A computerized tomography examination of the abdomen and chest showed progressive hydronephrosis on the left kidney as well as pulmonary metastases. Although the chemotherapy protocol was changed, the patient died due to pulmonary and renal failure.

Pathology

Macroscopic Findings : The right residual normal kidney was compressed by a large, encapsulated tumor (13x13x9 cm). The kidney was seen as a band 3 cm in length under the capsule. The cut surface of the tumor was lobulated, pink, with scattered necrotic foci and lipomatous tissue. Some small cysts were also identified in the tumor. The renal artery and the ureter were grossly uninvolved.

Microscopic Findings : Microscopic examination of the resected specimen showed histology of classical triphasic Wilms' tumor which included blastematous, epithelial and stromal components. Solid nests of squamous epithelium were also present, some with central keratinization (Figs. 1 and 2). There was some mesenchymal differentiation showing the characteristics of striated muscle. The epithelial component consisting of squamous areas made up 70 percent of the tumor, and no criteria of dysplasia were identified. Although numerous samples were taken, there was no nephroblastomatosis area (Fig. 2). No endodermal elements were seen in the tumor tissue. There were metastases consisting of blastematous components in periaortic and mesenteric lymph nodes.

Fig. 1. A triphasic Wilms' tumor with solid nests of squamous epithelium (HEx40).

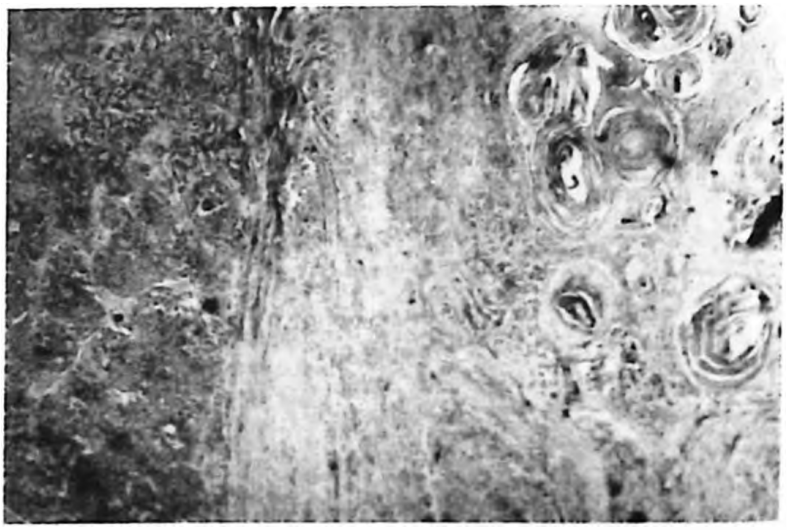

Fig. 2. High power view of Wilms' tumor with squamous nests (HEx100).

Discussion

Wilms' tumor is an embryonic tumor of mesodermal origin that contains blastematous, epithelial and stromal elements[6]. Embryonic tumors are totipotential in nature. Totipotency is variable and may depend on the stage of nephrogenesis at which tumor induction occurs[5,7]. Recently,. a rare variant of the typical triphasic Wilms' tumor, referred to as a teratoid Wilms' tumor, has been described, in which a more diverse differentiation causes a tumor that contains features of teratoma and nephroblastoma[5]. Although it is well known that nephroblastomas can include a diversity of cell types[2], in teratoid Wilms' tumor there is clear predominance of teratoid elements, comprising more than 50 percent of the tumor[3,5,8]. In our case, while the tumor histologically presented as classical triphasic Wilms' tumor, the epithelial component consisting of squamous areas made up 70 percent of the tumor. For this reason, it was accepted as a teratoid Wilms' tumor. Although many studies have described mucinous epithelium or an enteric-type epithelium including endodermal elements in the tumor tissue[9-11], no endodermal elements were seen in the tumor tissue of our case. Teratoid tumors of other organs, such as the intraocular teratoid medulloepithelioma, have also been described[12]. Ward et al.[13] reported a case of sacrococcygeal teratoma containing nephroblastoma in 1974. Teratoid Wilms' tumor differs from true intrarenal teratoma, the differential diagnostic criteria of which have been described by Beckwith[1] and Glazier[14]. According to them, epithelial and mesenchymal differentiation originated from the totipotent renal blastema, and "organogenesis" is the clue for differential diagnosis.

In our case, there was unilateral cryptorchidism as well. The presence of associated malformations (inguinal hernia, cryptorchidism, and club feet) suggests that there is a relationship between embryogenesis and carcinogenesis in this disease[8]. Among 290 patients admitted to St. Jude Children's Research Hospital with the diagnosis of Wilms' tumor, three of them were classified as teratoid Wilms' tumor. Their prominent clinical and histopathologic features were: 1) bilateral involvement, 2) tumor extension into renal pelvis causing ureteral obstruction, renal failure and hypertension, and 3) a close relationship between teratoid Wilms' tumor and the nephroblastomatosis complex. The well differentiated nature of the teratomatous elements may make this tumor somewhat resistant to chemotherapy and radiotherapy, depending on the extent of teratomatous involvement within the lesion[8]. In our case, there was unilateral involvement, and although there was no evidence of dysplasia, the patient died from metastatic disease. Hydronephrosis on the contralateral kidney in our case was thought to be due to enlarged lymph nodes around the PUJ.

To render optimal therapy for the teratoid type Wilms' tumor, consideration must be given to the clinical and histopathologic characteristics of the disease[8]. Inducing cellular modulations and nonepithelial differentiation of preoperative chemotherapy and radiotherapy have been reported, but the epithelial and rhabdomyoblastic components usually show no evidence of chemotherapeutic effect[15-17]. It is therefore not surprising that teratoid Wilms' tumor is resistant to chemotherapy. In the case reported by Kotiloğlu et al.[10], no regression in the volume of the tumor was observed after preoperative chemotherapy. In our case, neither chemotherapy nor radiotherapy was given preoperatively.

Teratoid Wilms' tumor is not usually aggressive or metastatic[3,4,5,8], if the proportion of blastemic elements in each tumor is considered. But, accompanying anaplastic elements may increase the likelihood of metastases and progression to death. Williams et al.[11] reported a case of teratoid Wilms' tumor who died because of metastatic disease, as in our case and it was reported that the major component of the tumor was adipose tissue. The interval time without chemotherapy and the postponed second look operation caused the bad prognosis in our case. In conclusion, teratoid Wilms' tumor is a rare variant of triphasic Wilms' tumor. Although it is reported that most of the teratoid Wilms' tumors are not usually aggressive or metastatic, our case died because of common metastatic disease without accompanying anaplastic elements. To our knowledge, our case is the twelfth case reported as teratoid Wilms' tumor and the seventh case reported as unilateral teratoid Wilms' tumor.

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Progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy (PEHO Syndrome) in a Turkish child

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SUMMARY: Tekgül H, Tütüncüoğlu S. Progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy (PEHO Syndrome) in a Turkish child. Turk J Pediatr 2000; 42: 246-249.

We report a Turkish boy with PEHO syndrome (progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy). He had generalized hypotonia and abnormal eye movements during early infancy. Infantile spasms were seen in the second year of life. Arrest of psychomotor development and blindness were noticed early in childhood. Serial magnetic resonance imaging revealed progressive infratentorial atrophy with association of cortical atrophy and corpus callosum hypoplasia. This is an additional case of PEHO syndrome, to our knowledge the first such case from Turkey.

Key words: PEHO syndrome, infantile spasm, optic atrophy, brainstem atrophy.

PEHO syndrome (progressive encepalopathy with edema, hypsarrhythmia, and optic atrophy) is a newly determined autosomal recessive disorder[1]. The clinical findings are infantile spasms with hypsarrhythmia, severe hypotonia, optic atrophy and early arrest of psychomotor development. Microcephaly and progressive cerebral and cerebellar atrophy are prominent features. PEHO syndrome was first presented in Finnish families[1-5]. and then a few patients were reported from non-Finnish populations[6,7]. We present here an additional case of PEHO syndrome, to our knowledge the first such case Turkey.

Case Report

A.T., a two-year-old boy, was referred for infantile spasms. He was the first child of healthy parents of Turkish descent who were cousins. He was delivered at 40 weeks of gestational age following an uneventful pregnancy.

Birth weight was 2750 g (10^{th} percentile) and head circumference was 35 cm (50^{th} percentile). He had generalized hypotonia with abnormal eye movements at one month of age. Bilateral narrow pupils were seen at the second month of age, and pupilloplasty operations were performed in the 2^{nd} and 4^{th} months of life.

Physical findings (at two years of age): Weight, height and head circumference were 9.5 kg ($<3^{rd}$ percentile) 78 cm, ($<3^{rd}$ percentile) and 43 cm (2^{nd} percentile), respectively. There was no progress of motor skills. He was hypotonic and did not have head control. Patellar reflexes were birsk. Bilateral optic atrophy was seen in the funduscopic examination. Epicanthal folds, midfacial hypoplasia (Fig. 1), high-arched palate, gingival hypertrophy and finger tapering were noted.

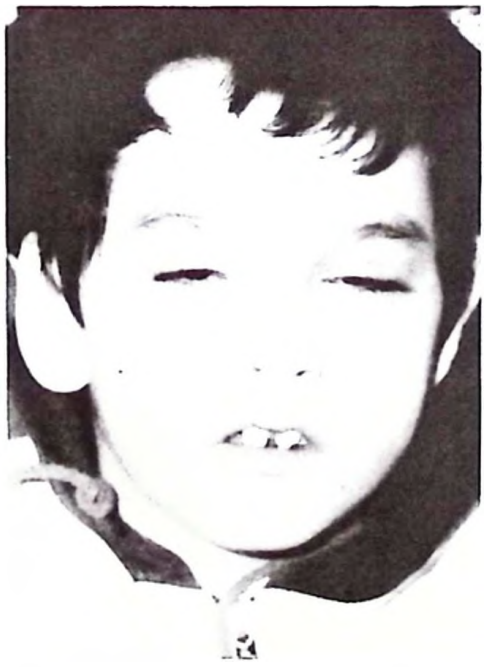


Fig. 1. Dysmorphic facial appearance (epicanthal folds, midfacial hypoplasia) of patient at five years of age.

Routine laboratory investigations of blood and cerebrospinal fluid, and extensive search for other metabolic, infectious or liver disease gave normal results (blood and urine amino acid chromatography; cerebrospinal fluid cell count, glucose and protein; urine organic acids; plasma lactate and pyruvate; ammonia levels; serum biotinidase activity; thyroid hormones; complement factors; and antibodies to cytomegalovirus and toxoplasmosis).

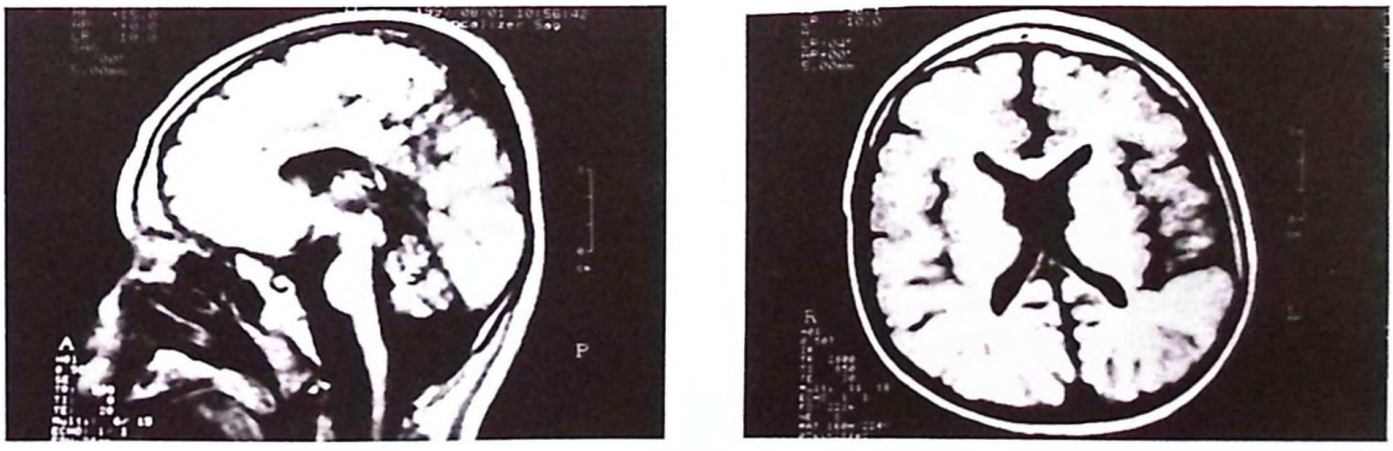
Serial EEG recordings first revealed hypsarrhythmia and then a slowing in background activity and generalized multiple slow spike-wave discharges. Cranial magnetic resonance (MR) images at two, four and six years of age revealed progressive atrophy of cerebellar vermis, slight progressive atrophy of the brainstem, diffuse corpus callosum hypoplasia, and cortical atrophy (Figs. 2a, 2b). Visual evoked potentials (VEP) were measured at five years of age with prolonged latency and abnormal wave configurations. Nerve conduction velocity (NCV) was within the normal range on the first examination done at three years of age. One year later, NICVs were compatible with polyneuropathy. Ulnar motor conduction velocity was reduced (29 m/s) and distal latency of median and ulnar nerves was prolonged. Motor conduction velocities at the lower extremities were not obtained, whereas sural nerve sensory conduction velocity was normal.

of age and ACTH treatment was repeated. After the second ACTH therapy, the patient became seizure free. At six years of age the patient had severe motor and mental retardation, hypotonia, visual failure and flexion contractures on wrists and ankles.

Discussion

In 1991, PEHO syndrome was defined by a rather nonspecific symptom complex characterized by progressive encephalopathy with edema, hypsarrhythmia, optic atrophy and dysmorphic features[1]. Neuroradiological and neuropathological studies disclosed cerebellar and brainstem atrophy as a prominent feature in PEHO syndrome[3,5].

In 1993 Somer[8] reestablished the following clinical criteria for the PEHO syndrome: 1) infantile hypotonia, 2) convulsive disorder manifesting with myoclonic jerking and infantile spasms, 3) profound psychomotor retardation with severe hypotonia, 4) absence or early loss of visual fixation with atrophy of optic disks by two years of age, and 5) progressive brain atrophy in neuroimaging studies, particularly in the cerebellum and brainstem. Supportive criteria for PEHO include: facial dysmorphic features, peripheral edema, brisk tendon reflexes in early childhood, abnormal auditory brainsten response (ABR), abnormal somatosensory evoked potential, abnormal NCV in late



(a) (b)

Fig. 2. MRI at six years of age. a) Midsagittal T1-weighted MRI demonstrates progressive atrophy of cerebellum, slight progressive atrophy of brainstem and diffuse corpus callosum hypoplasia.
b) Axial T1-weighted MRI shows cortical atrophy.

Flexor spasms were transiently controlled with adrenocorticotropic hormone (ACTH), valproate and lamotrigine between two and five years of age. Myoclonic seizures reappeared at five years

childhood and dysmyelination in MR imaging. Our patient had both the necessary criteria mentioned above and some of the supportive criteria such as facial dysmorphic features, brisk

tendon reflexes and abnormal VEP and NCVs (Table I). Progressive infratentorial atrophy, which is a prerequisite feature for PEHO syndrome, was seen in our patient. Our patient also had mild cortical atrophy. He had normal head circumference at birth but then microcephaly developed. Our patient also had narrow pupils, which has not been previously described in PEHO syndrome.

Table I. Clinical Criteria for the PEHO Syndrome

	Our patient
1. Infantile hypotonia	(+)
2. Infantile spasms	(+)
3. Profound psychomotor retardation with severe hypotonia	(+)
4. Absence or early loss of visual fixation with atrophy of optic disks by two years of age	(+)
5. Progressive brain atrophy in neuroimaging studies, particularly in the cerebellum and brainstem	(+)
6. Facial dysmorphic features (epicanthal folds, midfacial hypoplasia, gingival hypertrophy	(+)
7. Peripheral edema	(-)
8. Brisk tendon reflexes in early childhood	(+)
9. Abnormal VEP	(+)
10. Abnormal ABR	(ND)
11. Abnormal SEP	(+)
12. Abnormal NCV in late childhood	(+)

VEP : visual evoked potential.
ABR : auditory brainsten response.
SEP : somatosensory evoked potential.
NCV : nerve conduction velocity.
ND : not done.

On the basis of neuroradiological findings, Somer et al.[3] divided the patients with PEHO syndrome into two groups: 1) Group A patients (the true PEHO syndrome) mainly showed cerebellar and brainstem atrophy that appeared earlier and was more severe than supratentorial atrophy and 2) Group B patients had generalized, mainly supratentorial atrophy and abnormal gyral pattern. The patients in group B were clinically indistinguishable from group A patients, but generally showed no prominent cerebellar atrophy. Our patient had progressive infratentorial atrophy and mild cortical atrophy on his serial MR studies and was dignosed as the true PEHO syndrome.

There are some known disorders to be considered in the radiological differential diagnosis of the true PEHO syndrome. Some of the nonprogressive central nervous system malformations may resemble PEHO during the first year of life[9-10]. Dandy-Walker syndrome variants show inferior vermian aplasia and cerebellar hemispheric hypoplasia, but there is no progression like that in the PEHO syndrome[9]. In Joubert's syndrome, dysgenesis of the cerebellar vermis is typical. But these patients have a different clinical course and they show retinal dystrophy instead of optic atrophy[10].

Cerebellar hypoplasia is common in a variety of progressive disorders like pontocerebellar hypoplasia (type 1 and type 2) and carbohydrate-deficient glycoprotein (CDG)[11-13]. syndrome. Anterior spinal horn degeneration and chorea/dystonia are the hallmarks for pontocerebellar hypoplasia type 1 and type 2, respectively. These were not found in our patient.

In CDG syndromes, four types have been differentiated on the basis of the isoelectrofocusing pattern of the serum sialotransferrins[13]. In type I CDG syndrome (phosphomannomutase deficiency-PMM), abnormal slow rolling vertical or horizontal eye movements combined with slow head movements, alternating internal strabismus, and axial hypotonia with hyporeflexia are seen during the first months of life. Prominent psychomotor retardation, ataxia, deafness and retinitis pigmentosa are the late findings.

In type II CDG syndrome, the patients have stereotypic behaviors (tongue thrusting and hand-washing movements, head turning, knocking on cheeks and rocking) and facial dysmorphism (high forehead, long eyelashes, prominent base of the nose, beaked nose, upturned alae nasi, protruding upper gums, receding chin, and large dysplastic ears)[13]. Our patient did not display stereotypic movements and his facial dysmorphism was not compatible with CDG syndrome. Additionally, patients with CDG syndromes often have an extroversive and cheeful personality which was not present in our patient. On the other hand, serum levels of a number of glycoproteins (thyroid hormones, complement factors) which are expected to be low, were within normal limits.

A metabolic disorder, multiple carboxylase deficiency, is characterized by ketolactic acidosis and organic aciduria. Infantile spasms either

alone or with other neurological findings such as hypotonia, optic atrophy, developmental delay, ataxia, and hearing deficit are seen in the clinical presentation[18]. Additionally, more than 70 percent of the patients typically have cutaneous signs such as skin rash and alopecia, which were not seen in our patient. His serum biotinidase activity was also within normal limits.

Some of the neurometabolic diseases like glutaric aciduria type I and methylmalonic acidemia, and Krabbe's disease have pathognomonic radiographic features[14-16]. Besides cerebellar atrophy, white matter and/or basal ganglia involvement are prominent in those disorders. Infantile Refsum's disease may also show cerebellar atrophy[17], but cerebral changes are more severe and appear earlier in this disorder.

Aicardi's syndrome is an X-linked genetic disorder to be considered in the differential diagnosis of a female PEHO patient. The syndrome is characterized by agenesis of the corpus callosum, brain heterotopias, seizure disorder, chorioretinal lacunae, vertebral anomalies, and mental retardation[19]. Although infantile spasm with typical hypsarrhythmia may be seen in Aicardi's syndrome, it is the rule in the PEHO syndrome.

Serial neuroimaging studies indicate that the disease process of PEHO is operative during the postnatal period[3]. Therefore, a progressive metabolic disorder with unknown biochemical background is proposed in the PEHO syndrome. Further metabolic and genetic investigations are needed to explain the pathogenesis of the PEHO syndrome.

The syndrome is rare and had not previously been published from the Turkish population. For the differential diagnosis of patients with infantile spasm of unknown etiology, PEHO syndrome should be considered.

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Body stalk anomaly in monozygotic twinning: a case report

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SUMMARY: Aksoy F, Karayel FA, Ramazanoğlu R. Body stalk anomaly in monozygotic twinning: a case report. Turk J Pediatr 2000; 42: 250-252.

We describe a case of concordant body stalk anomaly in a monozygotic twin. Autopsy of the fetus showed abnormalities compatible with the maldevelopment of embryonic folding. Abdominal viscera were in a sac covered by the amnion and were attached directly to the placenta. The anus was not visible and no discernible external genitalia were noted. Other findings included a neural tube defect and a rectal duplication as an enteric cyst. Umbilical cord had only one vein and an artery. No abnormalities were found on pathologic examination of the placenta.

Although we encountered cases previously with gastroschsis and omphalocele, this was the first case of body stalk anomaly that we recognized as an enteric cyst, which is extremely rare in twins.

Key words: body stalk anomaly, monozygote twinning, embryonic body fold defect.

In early embryogenesis, maldevelopment of the body folding may cause a variety of abdominal defects such as pentalogy of Cantrell, gastroschisis, omphalocele, body stalk anomaly and cloacal extrophy.

The body stalk anomaly associated with agenesis of the umbilical cord or with a very short umbilical cord with multiple abnormalities is characterized by a large and irregular abdominal wall defect[1-3]. Two hypotheses have been postulated to explain this pathology. The first is maldevelopment of body folds when the trilaminal embryo is transformed into a cylindrical embryo, and the second is the syndrome complex resulting from mechanical teratogenesis following rupture of the chorion or yolk sac[3]. Body stalk anomaly in monozygotic twins is quite rare. There have been only three cases reported in the literature[3].

Because of its rarity and the lack of diagnostic criteria, prenatal diagnosis of body stalk anomaly is very difficult; however, there have been antenatally diagnosed cases with ultrasonography (USG)[4].

The body stalk anomaly, which is quite rare and lethal, may be associated with neural tube defects, intestinal atresia, genitourinary and skeletal defects, and chest wall abnormalities[1].

In our autopsy report, we tried to identify the features of the case and took a brief look at the literature.

Case Report

Our case was the second pregnancy of a 28-year-old female. A twin male fetus at 36th gestational week, clinically diagnosed as omphalocele, died within a few minutes after birth. No information was obtained about the prenatal period or family history. The analysis of this karyotype could not be done, nor was there ultrasonographic data concerning the follow-up of the prenatal period.

Autopsy Findings: The fetus had a large abdominal defect through which all the abdominal organs were exposed. The herniated organs were covered with a thin transparent membrane which was attached directly to the placenta without having a distinct border with the placental amniotic sac. The liver, small and large bowel, spleen and stomach were inside the herniated sac (Fig. 1). the anus was obliterated. External genitalia were not identified.

Placenta was diamniotic monochorionic, and had two centrally located umbilical cords, which were close to one another. The umbilical cord of the fetus in our case on to which the amnion was attached was 15 cm long and consisted of an artery and a vein. The other umbilical cord had 3 vessels.

There was marked kyphoscoliosis of the vertebra, and there was a cystic lesion, 15 cm in diameter, in the sacral region (including gluteal area). The lesion was covered by skin, and it was filled with a serous fluid. The interior of the cyst was smooth and the cyst was associated with the vertebral column (spina bifida) via a bone defect 3 cm in diameter (Fig. 2). The lungs were atelectatic. No other pathology was detected in the rest of the organs.

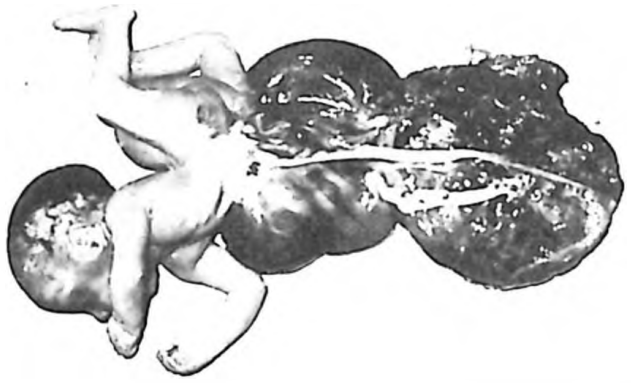

Fig. 1. The herniated organs were covered with a thin transparent membrane which was attached directly to placenta without having a distinct border with placental amniotic sac.

Fig. 2. There was a cystic lesion, 15 cm in diameter, in sacral region (including gluteal area). The lesion was covered by skin, and was filled with a serous fluid.

Discussion

The body stalk is closely involved in the process of lateral folding during the fourth to eighth week of embryonic life. It is derived from the umbilicus and remnant yolk sac and is covered by the amnion. Therefore, any deviation from the normal developmental process may cause severe abdominal wall defects, abnormal development of the hindgut and absence or very severe reduction of the umbilical cord[4].

Although the incidence of abdominal wall defects is said to be higher[1], the incidence of the body stalk anomaly was only six in 252,000. Lakshminarayana et al[2] determined a rate of one in 50,000, and in one other study it was found to be one in 14,273 births. Ours was the only body stalk anomaly previously reported[5].

Body stalk anomaly can be confused with short umbilical cord syndrome. Some believe that the latter is a variant of the stalk anomaly, while other authors say that it is a severe form of amniotic band syndrome, since amniotic bands are present in 40 percent of cases of body stalk anomaly[4].

Sacral vertebral anomalies, such as bifid vertebra in which the defect is large, and hemivertebra in which the defect is smaller, may be associated with hindgut enteric fistulas[6-8].

The cystic lesions of the sacral and coccygeal regions may derive from sequestered intestinal duplication or target remnants, or may occur as a part of the neuroenteric fistula complex. The sacral cysts are usually localized in presacral, intraspinal and postsacral regions; they may be large and multiloculated or multiple and small. The hindgut cyssts are surrounded by varying degrees of muscle layer, whereas tailgut cysts consist of isolated bundles of muscle in which no smooth muscle property is seen[8]. In our case, the cyst was lined by simple cuboidal epithelium and a small portion of the wall was made up of primitive nerve cells and nerve fibers (Fig. 3). For this reason it was accepted as neuroenteric cyst. The results of immunohistochemical stains in epithelial and nerve cells were S-100: positive (Fig. 4) and focal mucin positivity in epithelial cells (Fig. 5).

In our case, the umbilical cord was short and had one artery and a vein, which is not a common feature of body stalk anomaly in monozygotic twins. However, the presence of one artery accompanying some anomalies, such as ventral abdominal defect, has been reported.

Since body stalk anomaly is lethal, it necessitates eliminating omphalocele and gastroschisis in differential diagnosis[9].

In conclusion, although body stalk anomaly is rare, in order to exclude it, it is necessary to

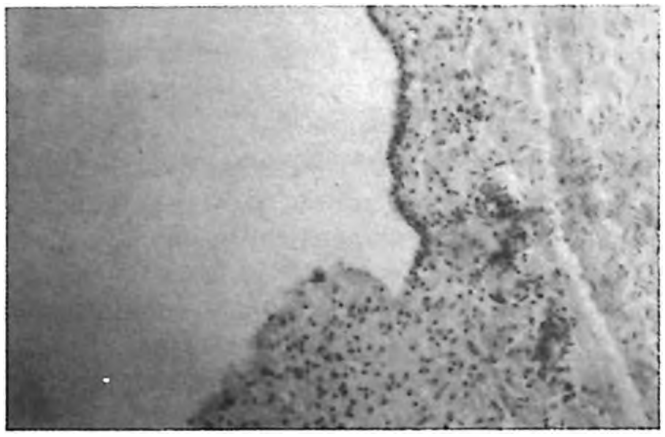

Fig. 3. The cyst was lined by simple cuboidal epithelium and a small portion of the wall was made up of primitive nerve cells and nerve fibers, (H+E X 200).

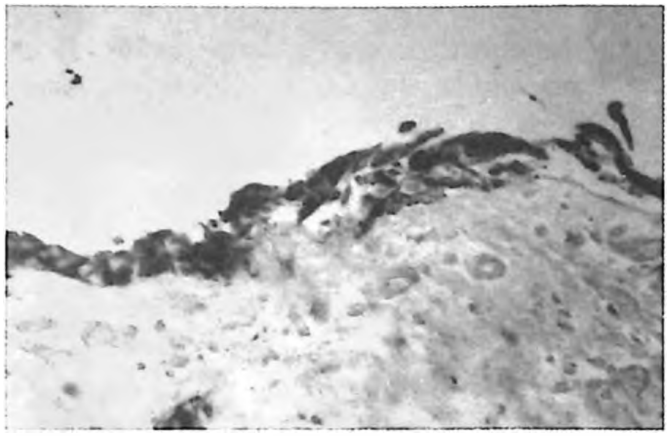

Fig. 4. S-100 positivity in epithelial cells and nerve cells, (S-100 X 400).

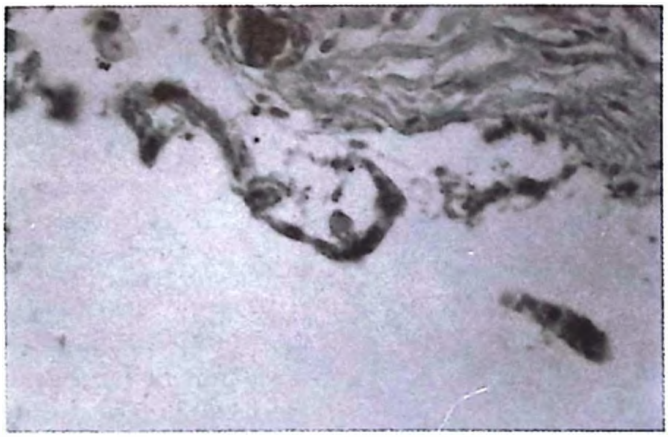

Fig. 5. Focal mucin positivity in epithelial cells, (Alcian Blue X 400).

identify the features of abnormalities in a fetus with an abdominal wall defect, especially omphalocele. The rarity of body stalk anomaly in monozygotic twins and association of neural tube defect and neuroenteric cyst are the particular features of our case.

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Thoracic ectopic kidney in a child: a case report

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SUMMARY: Aydın Hİ, Sarıcı SÜ, Alpay F, Gökçay E. Thoracic ectopic kidney in a child: a case report. Turk J Pediatr 2000; 42: 253-255.

Congenital thoracic ectopic kidney is a very rare developmental anomaly and the rarest form of all ectopic kidneys. It is usually asymptomatic and discovered incidentally on a routine chest radiography. We report a thoracic ectopic kidney in a 19-month-old boy, which initially presented as a well demarcated mass at the base of the right lung on chest x-ray. Intravenous pyelography (IVP) and thoraco-abdominal computed tomography (CT) demonstrated a normal functioning transdiaphragmatic thoracic ectopic right kidney, but technetium-99m DTPA and DMSA scintigraphy demonstrated pelvic stasis. We hereby discuss the features of congenital thoracic ectopic kidney and review the literature. Although it is extremely rare, thoracic ectopic kidney should be considered in differential diagnosis of a mass with a well demarcated superior margin in the lower part of the thorax, and renal scintigraphy must be performed even if CT and IVP results are normal.

Key words: thoracic ectopic kidney.

Congenital thoracic ectopic kidney is an uncommon developmental anomaly and the rarest form of all ectopic kidneys[1]. Of nearly 16,000 autopsies performed in a series, 22 ectopic kidneys were found, and only one was intrathoracic[2]. Wolfromm[3] reported the first case of clinically diagnosed congenital ectopic kidney by retrograde pyelography in a 43-year-old patient in 1940. It is generally asymptomatic and discovered incidentally on a routine chest radiography[1].

We herein report a thoracic ectopic kidney in a 19-month-old boy, which initially presented as a well demarcated mass at the base of the right lung on chest x-ray.

Case Report

A 19-month-old male infant had initially been examined for cough and wheezing of seven days' duration in a peripheral hospital. At that time, his chest x-ray had revealed a well demarcated homogeneous mass at the base of the right lung (Fig. 1). The patient was refererd to our hospital for evaluation of that mass. Prenatal, natal, postnatal and developmental history was unremarkable. Vital signs including body temperature, heart rate, blood pressure, and respiration rate and pattern were normal. Physical examination was normal except for his serous nasal discharge. Abdominal ultrasonography showed that the right kidney was located superior to its normal location (Fig. 2). Thoraco-abdominal computed tomography demonstrated a transdiaphragmatic thoracic ectopic right kidney (Fig. 3). Intravenous pyelography (IVP) revealed the mass in the right hemithorax to be an ectopic intrathoracic kidney with a normal collecting system and function (Fig. 4). Technetium-99m DTPA and DMSA scintigraphy demonstrated that the right kidney was superior to its normal location and draining spontaneously with pelvic stasis (Fig. 5).

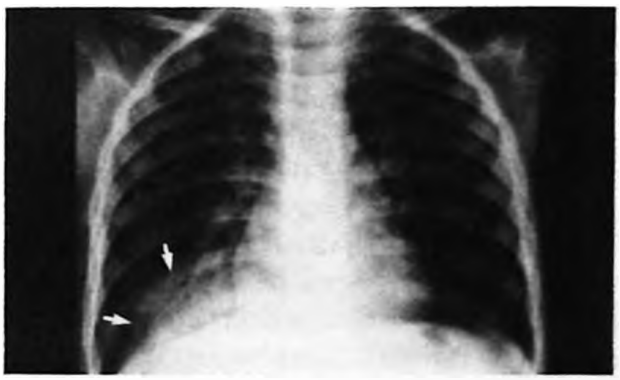

Fig. 1. Chest radiography of patient reveals a well demarcated homogeneous mass in the base of the right hemithorax.

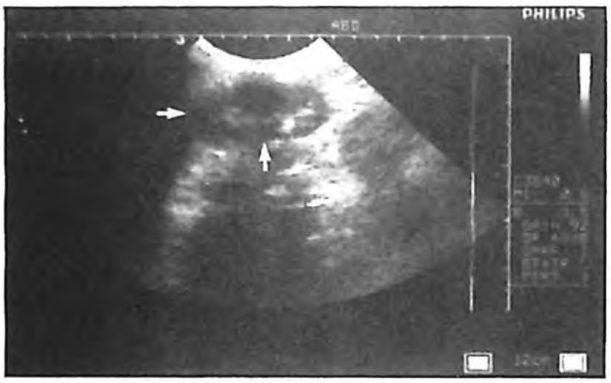

Fig. 2. Ultrasonography of the mass in the right hemithorax through posterior thoracic wall reveals right kidney.

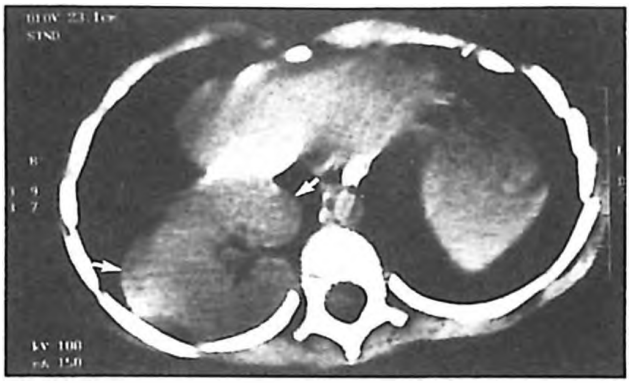

Fig. 3. Thoraco-abdominal computed tomography shows abnormal intrathoracic location of right kidney with remarkable calyceal system.

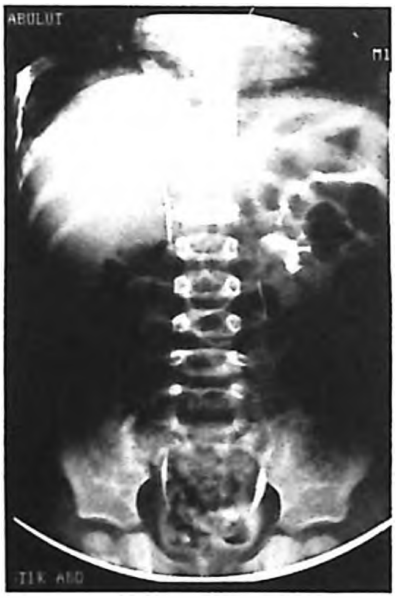

Fig. 4. Intravenous pyelography reveals the mass in the right hemithorax to be an ectopic intrathoracic kidney with a normal calyceal system, ureter and function.

Fig. 5. A postero-anterior view of technetium-99m DTPA scintigraphy shows that the right kidney is superior to its normal location and draining spontaneously with pelvic stasis.

Discussion

Ectopic thoracic kidney is an extremely rare congenital anomaly[1,2]. Usually, it is clinically asymptomatic and diagnosed as an incidental finding on a routine chest radiography[1]. There is a male preponderance and the majority of intrathoracic kidneys are located on the left side[3-5]. The location of thoracic ectopic kidney may be supra-, trans-, or infra-diaphragmatic[6]. The normal kidney lies beside T_{12}-L_3, its superior border ranges between T_{10} and L_1. Upper borders of thoracic kidneys range between T_7-T_{11} with transdiaphragmatic, T_7-T_9 with supradiaphragmatic, and upper pole in eventration may rise to T_5, with the kidney high but beneath the diaphragm[6]. In our case, the kidney was transdiaphragmatic and the upper pole of the kidney was at the level of T_{7-8}.

No consistent associated anomaly has been described with thoracic ectopic kidney. However, one child had trisomy 18 syndrome[3] and another patient had multiple pulmonary and cardiac anomalies in addition to the thoracic kidney[7]. Angulo et al.[2] reported a case of right intrathoracic kidney associated with a complex somite malformation that comprised vertebral fusion and right intrathoracic supernumerary ribs. There was no associated anomaly in our case.

Thoracic ectopic kidney may be a congenital anomaly or secondary to herniation through a congenital or acquired diaphragmatic defect[4,6,8].

We could not demonstrate any diaphragmatic defect in our patient.

Ebryologically, it is unclear whether the kidney ascends before the diaphragmatic leaflets close normally or if delayed diaphragmatic formation enhances the exaggerated renal ascent[8]. Initially, it was thought that intrathoracic kidneys resulted from maldevelopment of the pleuroperitoneal membrane, which caused a foramen in the posterior leaf of the diaphragm through which the kidney passed. This is now thought to be unlikely since the incidence of intrathoracic kidney with a diaphragmatic hernia is less than 0.25 percent[4]. The other possible mechanisms suggested are delayed development of the diaphragma, the influence of the developing adrenal gland and liver on renal position, and intrinsic factors in the kidney[4,6]. The synchronous association of congenital intrathoracic kidney and axial skeleton defects tends to favor the hypothesis that congenital intrathoracic kidney is secondary to persistence of the nephrogenic cord[2]. None of these mechanisms can be fully excluded and each may have a role in the development of thoracic ectopic kidney.

An intrathoracic kidney, although rare, should be considered in a child with a mass at the base of the lung on a chest x-ray. Differential diagnoses include posterior mediastinal masses such as neuroblastoma, ganglioneuroma, neurofibroma, neurogenic cysts, meningoceles, periardial cysts and Bochdalek's hernias[5]. The differential diagnosis can be made by a computed tomography (CT) that demonstrates the kidney, renal vascular pedicle and ureter[3]. IVP can demonstrate renal excretory system and ureters[3]. In our case, CT and IVP demonstrated that the right thoracic kidney was normal in size and function, but DTPA scintigraphy revealed pelvic stasis. Therefore, we suggest that DMSA and DTPA scintigraphy must be performed in cases of a thoracic ectopic kidney even if CT and IVP results are normal. No treatment is necessary once the diagnosis of congenital intrathoracic kidney has been confirmed[2].

In conclusion, although it is extremely rare, thoracic ectopic kidney should be kept in mind in differential diagnosis of a mass with a well demarcated superior margin in the lower part of the thorax.

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Acute lymphoblastic leukemia in a child with Wilson disease

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SUMMARY: Yüce A, Koçak N, Yetgin S, Özen H, Gürakan F, Yenicesu İ. Acute lymphoblastic leukemia in a child with Wilson disease. Turk J Pediatr 2000; 42: 256-257.

Wilson disease is an autosomal recessively inherited disease of copper metabolism and is characterized by liver and central nervous system dysfunction. The heterozygote carrier state rate is about one in 90 persons and the incidence of the disease is about 30 in 1,000,000. Although leukemia is the most common form of childhood malignancies, the probability of the presence of Wilson disease and acute lymphoblastic leukemia in the same patient is very low. We report an unusual case of a child with Wilson disease who developed acute lymphoblastic leukemia in three months.

Key words: Wilson disease, acute lymphoblastic leukemia.

Wilson disease (WD) is a rare inherited disease of copper metabolism, characterized by liver and central nervous system dysfunction[1]. It is transmitted through a recessive gene located on chromosome 13[2]. The heterozygote carrier state rate is about one in 90 persons and the incidence of the disease is about 30 in 1,000,000[1]. In certain countries, including Turkey, in which the number of consanguineous marriages is high, this rate may be higher than the estimation[3].

Leukemia is the most common form of childhood malignancies, and its incidence in white children younger than 15 years of age is approximately 31 per million per year[4]. The most common form of leukemia in childhood is acute lymphoblastic leukemia (ALL). The mortality rate is still high and it is inevitably fatal without treatment.

We report an unusual case of a child with WD having ALL. To our best knowledge, this is the first report of WD associated with ALL.

Case Report

A 12-year-old boy presented with walking and speech disorders. His parents were first-degree cousins. His physical examination showed ataxia and dysarthria. He had no hepatosplenomegaly. Kayser-Fleischer rings were positive on ophthalmologic examination. Liver function tests were normal and serum ceruloplasmin level was undetectable. Daily urinary copper excretion was 840 μg (normal <40 μg). He was diagnosed as WD with neurologic presentation. D-penicillamine, zinc sulfate and a low copper diet were started.

Three months after the diagnosis of WD, he was admitted to the hospital with the complaints of fever, pallor, fatigue, and night sweating. His body temperature was 38.2°C. On physical examination he was pale, had mask face, and bruises on extremities. Liver and spleen were palpable four and three cm below the right and left costal margins, respectively. His speech showed marked dysarthria.

Hematological studies showed anemia (hemoglobin level 8.2 g/dl), thrombocytopenia (platelet count 49,000/mm^3), and leukocytosis (white blood cell count 161,000/mm^3). All leukocytes were blasts on peripheral blood smear and bone marrow aspiration revealed that 95 percent of cells were CALLA positive B cell immunophenotype lymphoblasts. Alanine aminotransferase level was 62 IU/L, aspartate aminotransferase 54 IU/L, and total protein and albumin levels were normal. Serology of hepatitis A, B, C, cytomegalovirus, and Epstein-Barr virus were found negative. Abdominal ultrasonography showed hepatosplenomegaly and heterogeneity of the liver. Liver biopsy could not be performed due to severe thrombocytopenia. St. Jude total therapy study XI protocol was started[5]. Liver and spleen became smaller during chemotherapy. He

developed neutropenic sepsis during treatment and his clinical situation deteriorated. The parents insisted on discharging him, and he died at home.

Discusison

Most patients with WD present during childhood and the majority have hepatic presentation. Other presenting features are neuropsychiatric, hematological, endocrinologic, and renal abnormalities[1]. Although the diagnosis of WD may be problematic in some patients, in our case, the presence of a very low serum ceruloplasmin level, increased daily urinary copper excretion, and Kayser-Fleischer rings confirmed the diagnosis. ALL developed in three months and worsened his situation.

Although ALL is primarily a disease of bone marrow, any organ or tissue such as liver and spleen may be infiltrated by abnormal cells. On initial examination, most patients with ALL are likely to have pancytopenia[6]; anemia and thrombocytopenia were due to ALL in our patient. In fact, various factors may be responsible for anemia and thrombocytopenia in a patient with WD. Penicillamine therapy may cause pancytopenia. The other possibility is the presence of asymptomatic cirrhosis at the time of diagnosis, and the development of hypersplenism. Although a significant proportion of patients with ALL have leukocyte counts less than 3,000/mm^3, one-fifth of them have leukocytosis as in our patient[6]. Our patient had leukocytosis, and lymphoblasts were easily recognized on peripheral blood smear examination. Thus, the diagnosis of ALL was promptly established.

Wilson disease has been mapped to chromosome 13q14.3, and it has been shown recently that it encodes a copper transporting P-type ATPase[7,8]. Several chromosomal abnormalities, including hyperdiploidy, hypodiploidy, translocations, and deletions, have been shown in ALL, and they can help define the subtypes of leukemia[6]. Chromosomal deletion on chromosome 13 has been reported in patients with T-lineage ALL[9]. But, frequent deletions of the 13q14.3 region (D13S19), a tumor-suppressor gene, have been shown in patients with B-cell chronic lymphocytic leukemia[10]. The probability of the presence of WD and ALL in the same patient is very low. Even if we consider that all WD patients present during childhood, the presence of both diseases in one patient by coincidence is low. It is hard to speculate that there is an association between the two disease. It would be interesting if we could evaluate the DNA of our patient with B-cell ALL both WD mutations and D13S319 mutations.

The pyossibility of leukemia should be kept in mind in patients with WD in whom hepatosplenomegaly and pancytopenia developed or progressed within a short time. It may present a diagnostic challenge for the physician if the patient presents with pancytopenia but without abnormal cells on peripheral blood smear. In our case, because the patient had leukocytosis instead of leukopenia and blasts on his peripheral blood smear, we could make the diagnosis of ALL without delay.

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Biphasic pulmonary blastoma in a child

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SUMMARY: Uçar B, Akgün N, Bör Ö, Işıksoy S, Kuşku M, Dernek S, Paşaoğlu Ö. Biphasic pulmonary blastoma in a child. Turk J Pediatr 2000; 42: 258-263.

Pulmonary blastoma (PB) is a rare malignant pulmonary tumor composed of immature mesenchyme and/or epithelium that resembles an embryonic lung at 10-16 weeks gestation. PBs constitute only 0.25 to 0.5 percent of all primary malignant lung tumors. Approximately 20 percent of the reported cases have occurred in pediatric patients. A seven-year-old girl presented with fever, cough, respiratory distress and chest pain on the left side. An x-ray, ultrasonography and a computed tomographic scan of the chest showed a large mass consisting of solid and cystic components almost completely occupying the left hemithorax associated with pleural effusion. The diagnosis of biphasic PB was established by histological examination of thoracotomy material. The patient was considered inoperable due to tumor involvement of the mediastinum, and she died two days after the initiation of chemotherapy. We report this case of PB to raise attention to the clinical, radiological and pathological features of PB in childhood because of its rarity.

Key words: pulmonary blastoma, biphasic, child.

Pulmonary blastoma (PB) is a rare malignant pulmonary tumor composed of immature mesenchyme and/or epithelium. It was first described by Barret and Barnard in 1945[1-4]. The tumor was later named "pulmonary embryoma" by Barnard[5] in 1952 because of its microscopic resemblance to an embryonic lung at 10-16 weeks gestation. In 1961, Spencer[6] reported three cases and first used the term "pulmonary blastoma" believing that the tumor was the pulmonary analogue of nephroblastoma. PBs constitute only 0.25 to 0.5 percent of all primary malignant lung tumors[7]. However, 20 percent of the patients reported in the literature were younger than 20 years of age and 15 percent were younger than 10 years of age[2,4]. In 1988, Manivel et al.[8] described pleuropulmonary blastoma (PPB) in 11 children as a distinctive intrathoracic/pulmonary neoplasm whose blastematous and sarcomatous features differentiated it from the biphasic epithelial-stromal morphology of the classic adult type PB. We report a seven-year-old girl with PB because of its rare occurrence in childhood.

Case Report

A seven-year-old girl presented with fever, cough, difficulty in breathing and chest pain on the left side of one week duration. She had been hospitalized in a local hospital and administered nonspecific antibiotic therapy prior to her reference to our clinic, but she did not respond. During the physical examination her body temperature was 37.7°C, she was dyspneic and malaised. She had a respiratory rate of 42 breaths per minute, absence of breath sounds on the left chest and dullness to percussion on the left, and clubbing. Heart beats could be heard on the right chest. Laboratory investigation revealed Hb 10.3 g/dl, Htc 36%, MCV 76 fl, WBC 11,300/mm^3 with 72% neutrophils and 28% lymphocytes, platelet count 478,000/mm^3, ESR 68 mm/h, and CRP 24 mg/dl. PPD was negative, serum LDH level was 8,900 U/L, ALT 14 IU/L, and AST 26 IU/L. A chest x-ray showed complete opacification of the left lung field with rightward shift of trachea and mediastinum (Fig. 1). Ultrasonography (USG) demonstrated a mass consisting of solid and cystic components almost completely occupying the left hemithorax

This study was presented in the 40th National Congress of the Turkish Pediatric Association, 14-17 October 1996, Gaziantep.

associated with pleural effusion. A computed tomographic (CT) scan of the chest showed a large mass consisting of heterogeneous hyperdense and hypodense areas representing solid and cystic components associated with pleural effusion within the left chest region (Fig. 2). Skeletal survey, brain CT and abdominal USG findings were normal. The pleural fluid which was obtained by thoracentesis was serohemorrhagic; cytologic examination of pleural fluid revealed fluid revealed erythrocytes, and polymorphonuclear and mononuclear cells along with sparse plasma cells and mesothelial cells, but no malignant cell was observed. Pleural fluid density was 1026, protein concentration 7.5 g/dl, and LDH 139 U/L. No microorganism was seen with Gram staining; culture was negative.

Percutaneous needle biopsy of the left lung was performed, and a focal area suggesting a tumor consisting of blastematous features was seen microscopically. The patient then underwent a left thoracotomy. A large mucinous, fragile, necrotic and hemorrhagic tumor almost completely occupying the left hemithorax, invading the mediastinum and compressing the trachea was found. Pleura was thickened. The patient was considered inoperable and only a biopsy was performed. Histologically, the tumor included both epithelial and mesenchymal components. The epithelial component included tubular glands which were lined by pseudostratified ciliated cells; some were lined by columnar cells with clear cytoplasm (Fig. 3).

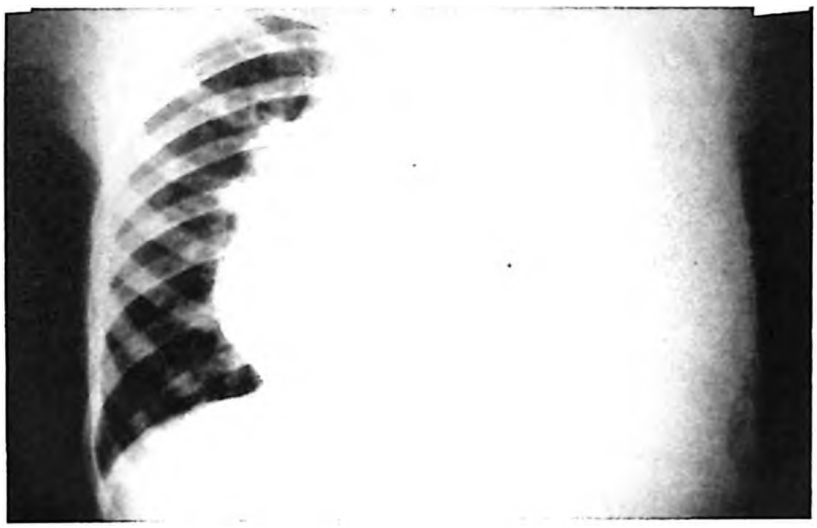

Fig. 1. Chest x-ray shows complete opacification of the left hemithorax, with rightward shift of the mediastinum.

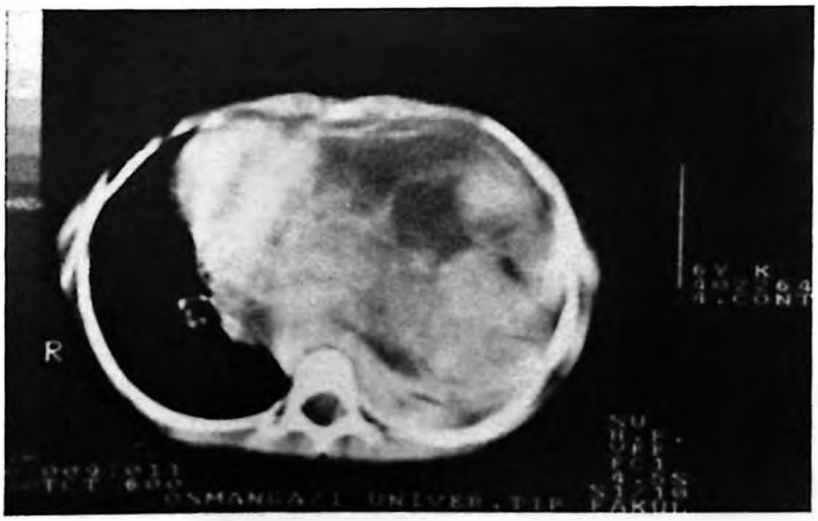

Fig. 2. CT scan of the chest shows a large mass consisting of heterogeneous hyperdense and hypodense areas representing solid and cystic components within the left chest region.

The stroma was embryonic or primitive blastematous with small, oval and spindled stromal cells and included the areas of immature striated muscle, cartilage and fascicles of fetal type smooth muscle (Fig. 4). A diagnosis of biphasic PB was made. Immunohistochemically, vimentin was negative, and desmin was positive in areas of rhabdomyoblastic differentiation. The patient was referred to an oncology center for chemotherapy and radiotherapy, but died two days after the initiation of chemotherapy.

Discussion

Most often PB in adults occurs in the fourth decade of life[2,7]. The age range at presentation for the pediatric population is newborn to 15 years[8-11]. No apparent gender difference has been observed in childhood PPB cases[9]. Recently, Priest et al.[12] reported that approximately 25 percent of PPB cases have a constitutional and familial predisposition to dysplastic or neoplastic conditions. Trisomy 2 and trisomy 8 were detected in some PPB cases and so were believed to be related to the development of PPB[13,14].

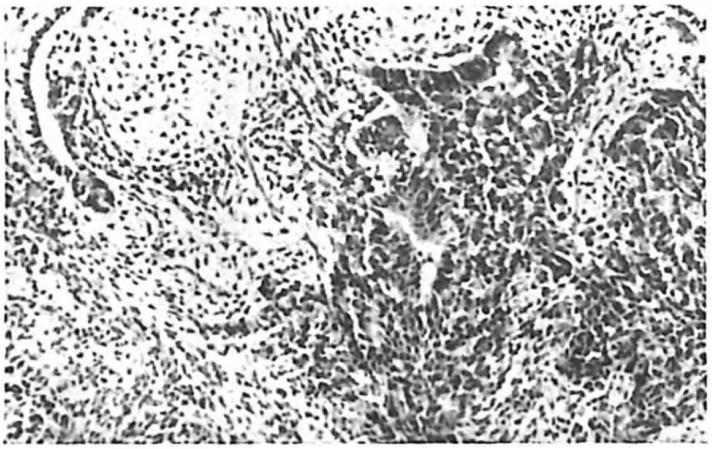

Fig. 3. Biphasic pulmonary blastoma. Tubular glands lined by pseudostratified columnar epithelial cells, some with clear cytoplasm are surrounded by embryonic and primitive blastematous stroma (H.E.x200).

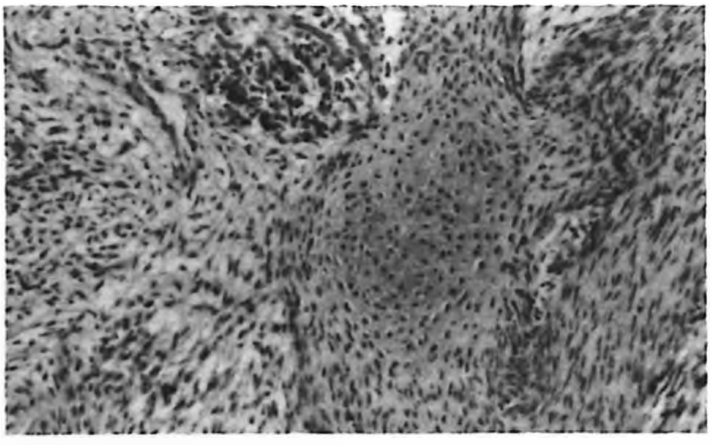

Fig. 4. Mesenchymal areas displaying rhabdomyoblastic, smooth muscle and cartilaginous differentiation (H.E.x200).

Forty-one percent of the patients are asymptomatic, and the tumor is generally found by routine chest radiography. The most common presenting symptoms in PB are respiratory distress, nonproductive cough, fever, and chest or abdominal pain[2,8,9]. Although our patient presented with cough, fever, chest pain and respiratory distress of one week duration, the presence of clubbing suggested that the tumor had been asymptomatic for a long time. Generally, the initial presentation suggests a respiratory infection unresponsive to antibiotic therapy[8,9].

Chest radiographs reveal a partial or total opacification of a lung field and mediastinal deviation to the contralateral side in almost all instances. A pleural effusion may be associated[2,3,8]. Noninvasive diagnostic tests and bronchoscopy have a low value in detecting blastomas. Percutaneous needle biopsies can establish the diagnosis, especially in the peripheral lesions[2,9,15]. Histological examination of the thoracotomy material is the most valuable method for definitive diagnosis[2,3,8,9]. Although the chest radiogram, USG and CT scan revealed a mass and a pleural effusion in the left chest region, and percutaneous needle biopsy suggested a blastematous tumor, the definitive diagnosis of PB was established in our patient by histological examination of the thoracotomy material. Cytologic examination of the pleural effusion did not assist in the diagnosis.

Pulmonary blastomas (PBs) are generally single tumors, well circumscribed and round, but sometimes are lobulated and multiple[2,3,7-9]. Tumors have well-delineated, gray to light yellow, solid, firm, friable areas which are focally rubbery or gelatinous with irregular foci of hemorrhage and necrosis[2,3,7-9]. At thoracotomy, a solitary, mucinous, fragile, necrotic and hemorrhagic tumor completely occupying the left hemithorax, invading the mediastinum and compressing the trachea was found in our patient.

In 1988, Manivel and associates[8] proposed that PPB -the so-called pulmonary blastoma of childhood- is a distinctive clinicopathologic entity from PB in adults because of its variable anatomic location, primitive embryonic-like blastema and stroma, absence of a carcinomatous component, and potential for sarcomatous differentiation, in contrast to PB in adults which consists of a mixture of immature mesenchymal and epithelial elements. These neoplasms may be intrapulmonary, mediastinal or pleural-based masses in contrast to PB in adults[7-9]. Because of the extensive nature of the tumor in the chest, it may not be possible to determine the precise site of origin in some instances[8,9], and pleural effusion may contribute[2,7-9] as in our patient. Manivel et al.[8] questioned whether truly biphasic pulmonary tumors occur in children, and they proposed that the previously reported cases of "pulmonary blastoma" in children were probably examples of the same tumor[8]. But in 1991, Koss et al.[2] reported that biphasic PBs do occur in children, although infrequently. Our patient is also a case of biphasic PB, since histologically the tumor included both epithelial and mesenchymal components. Koss et al.[2] suggested the separation of PBs into two major histological groupings, i.e., those composed solely of well-ordered neoplastic glands (well-differentiated fetal adrenocarcinoma -WDFA-) and those composed of a mixture of neoplastic glands and malignant stroma (biphasic PB). They reported that WDFA also occurs in children infrequently.

Pleuropulmonary blastoma (PPB) cases were subclassified into PPB type I, II, or III by Dehner et al.[16] based on the cystic (type I), cystic and solid (type II), or solid (type III) character of the tumor. The tumor of our case included both solid and cystic areas. Histologically, multiloculated cysts, separated by thin fibrous septa and lined by ciliated columnar respiratory epithelium is the characteristic feature in type I PPB. Beneath the respiratory epithelium, there is a continuous or discontinuous zone of condensed small, round-to-spindle-shaped immature or primitive tumor cells with the cambium layer-like appearance of sarcoma botryoids. Solid areas in type II and type III PBs consist of blastemal stromal cells, arranged in alternating bands of compact and loose cells in a myxoid matrix. There may be anaplastic and pleomorphic mesenchymal cells with numerous mitoses. Areas of chondrosarcoma, rhabdomyosarcoma, and smooth muscle-like spindle cells may be found[2,7-9]. In our case, the tumor also included rhabdomyoblastic, smooth muscle and cartilaginous differentiation areas within the malignant stroma.

Immunohistochemically, blastomas show expression of cytokeratin, epithelial membrane antigen, carcinoembryonic antigen, and often chromogranin in their epithelial elements;

however, the blastematous elements demonstrate vimentin, keratin, and actin expression[2,3,7,9]. In our case, vimentin was negative, and desmin was positive in areas of rhabdomyoblastic differentiation.

The association of PPBs with cystic lung lesions, including congenital cystic adenomatoid malformation, extralobar sequestration and bronchogenic cysts, has been reported, but it remains uncertain whether PPB arises in an uderlying malformation of the lung or whether longstanding lung cysts are themselves an initial manifestation of PPB[9,12].

Surgical excision, ranging from wedge excision to pneumonectomy, is the treatment of choice[2,3,8,9,11,17]. The duration of survival is quite different in operated cases, ranging from a few months to 24 years[2,3,8,9,17]. Chemotherapy and radiotherapy have been employed as either adjuvant to surgical treatment, or in patients whose condition is inoperable and in those with tumor recurrence. However, the effectiveness of these treatment modalities remains uncertain[3,8,9]. The most frequently used chemotherapeutic agents are vincristine, actinomycin D, cyclophosphamide, 5-fluorouracil, doxorubicin, and cisplatin[8,9]. Unusual therapeutic interventions such as intracavitary cisplatin and intracavitary ^{32}P appear worth considering[9]. Our patient was considered inoperable and died two days after the initiation of chemotherapy. recurrence of tumor may develop at the ipsilateral or contralateral lung, and distant metastases may occur chiefly to the central nervous system, involving brain and spinal cord, bone, liver, and soft tissue[3,8,9]. There was no distant metastasis in our patient.

Childhood blastomas especially have a very poor prognosis[8,9]. Priest et al.[9] reported that event free survival at two years from diagnosis was 49 percent, overall survival at two years 63 percent, and overall survival at five years 45 percent, even after multimodality therapy, in pediatric patients. A number of reports suggest that the presence of metastasis at initial presentation, tumor size greater than 5 cm, mediastinal or pleural involvement and tumor recurrence adversely effect the survival[2,4,9,17]. Koss et al.[2] reported that the prognosis of WDFA is better than for biphasic blastomas. However, Francis and Jacobsen[4] noticed that the prognosis of PBs is unpredictable based on their histological findings. In our patient, tumor size was greater than 5 cm, it was invading the mediastinum, there was no distant metastasis and the patient had a poor outcome.

We reported a seven-year-old PB case to give attention to the clinical, radiological and pathological features of PB in childhood because of its rarity. PB should be considered in differential diagnosis of patients who present with respiratory tract infection findings and whose symptoms do not improve with nonspecific antibiotic therapy in the presence of radiograms showing opacification of partial or total lung fields. Thorough family histories are essential on presentation of a child with a PB, and early surgical intervention is indicated for any pulmonary abnormalities in children from these families, in light of the literature knowledge[9,12].

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Juvenile hyaline fibromatosis in one Turkish child

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SUMMARY: Uğraş S, Akpolat N, Metin A. Juvenile hyaline fibromatosis in one Turkish child. Turk J Pediatr 2000; 42: 264-266.

We describe a case of juvenile hyaline fibromatosis (JHF) in a Turkish child. Only about 40 cases of juvenile hyaline fibromatosis had been reported in English literature as of March 1998, and it had not been reported in English literature from Turkey as of November 1998. Juvenile hyaline fibromatosis characterized by multiple cutaneous masses is a rare hereditary disorder. This disease is usually found in children, and a malfunction of collagen synthesis is considered as the pathogenetic cause. In the presented case, light microscopy demonstrated an abundance of a homogeneous, amorphous, eosinophilic extracellular matrix in which fibroblasts were embedded. Well-formed collagen fibers could not be demonstrated with Gieson's method or with reticulin preparation. The hayalin material periodic acid-Schiff-positive and diastase-resistant, whereas the Congo red method was negative. Immunohistochemically, the spindle-shaped cells were actin (smooth muscle) negative.

Key words: juvenile fibromatosis, hyaline fibromatosis, fibromatosis.

Juvenile hyaline fibromatosis (JHF) is a rare hereditary disease with only about 40 cases reported in English literature as of March 1998[1]. It had not been reported in English literature from Turkey as of November 1998. This disease is usually first noticed in children between two and five years of age and it typically consists of multiple cutaneous papules, nodules, or tumor masses that vary in size from 1 mm to about 5 cm, that grow slowly and are painless, and that are found mainly in the regions of the head, back, and extremities, with a predilection for the nose, ears, scalp, back, and knees[2]. This disease affects all races[3]. We describe a case of JHF that exhibited two small dermal nodules located exclusively on the occipital region and mental region which were characterized by painless and slow growth. This case seems to be the first one reported in English literature from Turkey, as of November 1998.

Case Report

A four-year-old boy presented in August 1997 with the diagnosis of tonsillitis and a five-month history of two painless, slowly growing tumors in the mental and occipital regions. The family history was negative. He was born of healthy but related parents. In the physical examination, a painless, firm mass 2 cm in diameter was found on the scalp in the occipital region. In addition, a firm, painless subcutaneous nodule 1.5 cm in diameter was found in the mental region. The skin covering the two masses was normal. Other findings of the general physical examination were unremarkable except for tonsillitis. There was no hyperplasia of the maxillary or mandibular gingiva. The complete blood count, chest radiography, abdominal ultrasound, biochemical data and radiological skeletal survey were within normal limits except for leukocytosis. The two masses in the mental and occipital regions were excised totally (14 August 1997). The postoperative course of the patient was uncomplicated. All the samples of the excised masses were fixed in 10 percent buffered formalin prior to the routine processing of the paraffin-embedded block. Multiple sections were obtained with use of a standard microtome and were stained with hematoxylin and eosin (H&E). Several sections were also stained with Congo red, reticulin, Van Gieson, and periodic acid-Schiff (PAS) with and without diastase. In addition, sections from formalin-fixed and paraffin-embedded tumor tissue were examined by a previously described avidin-biotin-peroxidase complex (ABC) method[4]

using actin [monoclonal mouse anti-actin (smooth muscle), clone:1A4, Cat.No.08-0106, isotype:IgG2a, ready-to-use, ZYMED, San Francisco, California, USA], which is an immunomarker of smooth muscle neoplasm.

Macroscopically, the mass on the scalp was firm, yellowish-white in color, with ill-defined margins, and measured 2 cm in diameter. The mass in the mental region was firm, yellowish-white in color, with ill-defined margins, and measured 1.5 cm in diameter. No focus of hemorrhage, necrosis, calcification, or areas of cystic change was observed in either mass. Light microscopy examination of both tumors showed identical histology: an abundance of a homogeneous, amorphous, eosinophilic extracellular matrix in which fibroblasts were embedded. They consisted of parallel-arranged cells with spindle nuclei and elongated cytoplasm, and epithelioid cells with a round, vesicular nucleus and clear cytoplasm that had a chondroid appearance (Fig. 1). Large amounts of hyalinized collagen-like material were recognized in some areas. Neither mitotic figure, hemorrhage, necrosis or calcification was seen. Well-formed collagen fibers were not demonstrated with the Van Gieson's method and reticulin preparation. The hayalin material was PAS-positive and diastase-resistant (Fig. 2), whereas results with the Congo red method were negative. Immunohistochemically, the spindle-shaped cells were actin negative. On the basis of the above gross findings, light microscopic findings, and histochemical and immunohistochemical findings, we considered the histopathologic diagnosis of these tumors to be JHF.

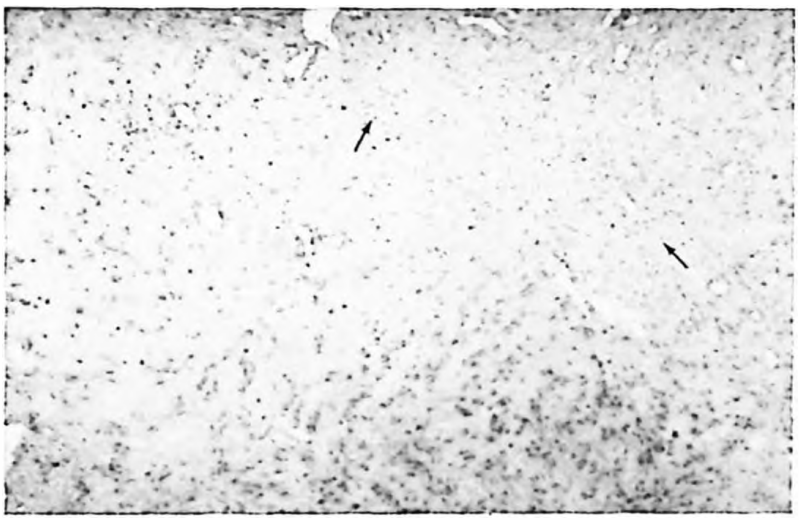

Fig. 1. Photomicrograph of the tumor characterized by scant strands of fibroblasts and epithelioid cells embedded in an abundant, homogeneous, amorphous matrix (H.E.X25).

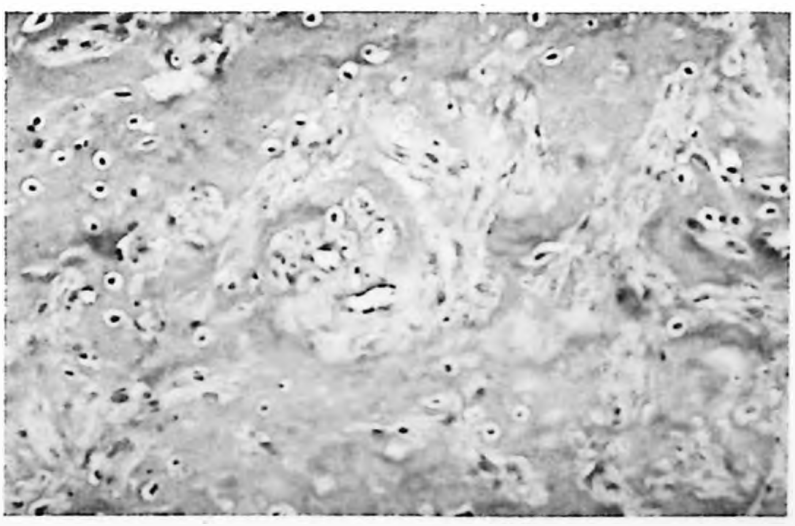

Fig. 2. The hyalinized collagen-like material is PAS-positive and diastase-resistant (PAS with diastase X 50).

Discussion

Juvenile hyaline fibromatosis is inherited as an autosomal recessive trait. It usually affects more than one sibling of the same family and there is no predilection for either sex[2]. Clinically, JHF is characterized by multiple or, rarely, single skin lesions. These lesions may appear as small, fleshy papules, translucent nodules, or as large subcutaneous masses of variable consistency. The largest masses are predominantly located on the scalp. Other frequent clinical manifestations of JHF are gum hypertrophy, flexion contractures of various joints, osteolytic lesions of bones, and undefined muscle illness[5]. These findings were not observed in our case. Histologically, the tumors consist of cords of spindle-shaped cells embedded in a homogeneous eosinophilic matrix in dermal deposition. Some cells have an epithelioid appearance with an oval or round vesicular nucleus and clear cytoplasm simulating a chondroid appearance. This appearance was observed in our case. In general, the smaller and younger lesions tend to be more cellular, while the larger and older lesions contain more ground substance[6].

The differential diagnosis of JHF includes neurofibromatosis, gingival fibromatosis, cylindromas, nodular amyloidosis, leiomyoma with sclerosis[6], infantile systemic hyalinosis, congenital generalized fibromatosis, multicentric infantile myofibromatosis, lipoid proteinosis (hyalinasis cutis et mucosae[2], and Winchester's syndrome[3], but particularly the latter five.

Multicentric infantile myofibromatosis is present at birth or occurs during the first year of life, and the lesions are better circumscribed, and are found not only in the subcutis but also in muscle, bone, and viscera, Microscopically, they consist of broad, interlacing bundles of plump myofibroblasts, often with pericytoma-like areas in the center of the lesion[2]. Congenital generalized fibromatosis is present at birth. Histologically, there is high fibroblastic cellularity and infiltrative borders. Infantile systemic hyalinosis appears at birth or during early infancy and invariably leads to death in early childhood. Winchester's syndrome is characterized by short stature, stiffness and contractures of small joints, and corneal opacities. The generalized osteoporosis with erosion of the metacarpal bones and progressive resorption of the carpal bones are characteristic radiological findings of Winchester's syndrome[3]. Lipoid proteinosis lacks the spindle cell proliferation and consists of hyalin material. In nodular amyloidosis, an abundant hyalin material is present in the dermis. This material stains positively for amyloid, whereas the same stain is always negative in JHF. In cutaneous leiomyomas, staining with smooth-muscle actin is positive, whereas the same stain is always negative in JHF[6]. In this disease, the basic defect appears to be a localized metabolic disturbance in the formation of collagen, presumably caused by incomplete alignment of precollagen or protocollagen, possibly due to increased or faulty synthesis of glycosaminoglycans by fibroblasts[2]. Mayerda-Silva et al.[7] suggested that JHF represents a disease of the connective tissue with progressive abnormal differentiation to chondroid tissue. Breier et al.[8] suggested that the band for type II collagen chain is absent in Western blot studies of clinically unaffected JHF skin. Type I collagen synthesis and degradation are increased in JHF fibroblasts compared with control fibroblasts. In contrast, type III collagen overall metabolism is significantly reduced (by 36%) compared with controls. The clinical course is variable: it may be characterized by the progressive appearance of new skin lesions or by progressive enlargement of old skin lesions over the years. The only effective treatment for JHF is surgery[6].

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Leukocytoclastic vasculitis associated with methotrexate therapy

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Sir,

Chemotherapeutic agents are associated with quite diverse mucocutaneous reactions, but vasculitis is rare[1]. The most common adverse effects of methotrexate (MTX) include bone marrow depression, gastrointestinal disturbances, and cutaneous and mucosal toxicity. MTX-induced hypersensitivity reactions were occasionally seen following intermediate or high-dose regimens: the usual manifestations have been urticaria, anaphylaxis and photosensitivity and, rarely, vasculitis[1-3]. We report a patient with acute lymphoblastic leukemia (ALL) who developed leukocytoclastic vasculitis following high-dose MTX infusion.

A nine-year-old boy was admitted to Hacettepe Children's Hospital in 1996 with a diagnosis of ALL. Complete remission was achieved following ALL induction chemotherapy which included prednisone, L-asparaginase, adriamycin, vincristine and intrathecal MTX (St. Jude Total XI Study Group Protocol).The patient experienced a bone marrow relapse during the intensification phase of the treatment. A second complete remission was achieved at the end of the induction phase. During the consolidation phase, high-dose MTX infusion (2 g/m^2) and calcium folinate rescue were instituted. Four days after the fourth course of high-dose MTX, diffuse palpable purpuric lesions were noticed on both upper and lower extremities (Fig. 1); the patient also suffered from abdominal pain and arthralgia. A skin biopsy showed leukocytoclastic vasculitis (LCV). No therapy was given; skin lesions and other symptoms resolved spontaneously within a few days.

Leukocytoclastic vasculitis (LCV) is an immune complex vasculitis that produces vascular damage and secondary ischemic manifestations. Small blood vessels, including the postcapillary venules, are affected. Polymorphonuclear leukocytes infiltrate the necrotic vessel wall, and scattered nuclear debris accumulates around the lesion. A number of agents have been postulated as etiologic factors of LCV, including infection, foreign proteins, chemicals and certain systemic disease such as chronic active hepatitis, ulcerative colitis and bacterial endocarditis[4]. Cutaneous and systemic vasculitis can be associated with malignant disorders, before, during or after their diagnosis[5-6]. The association with lympho- or myelo- proliferative disorders is significantly higher than that with solid tumors.

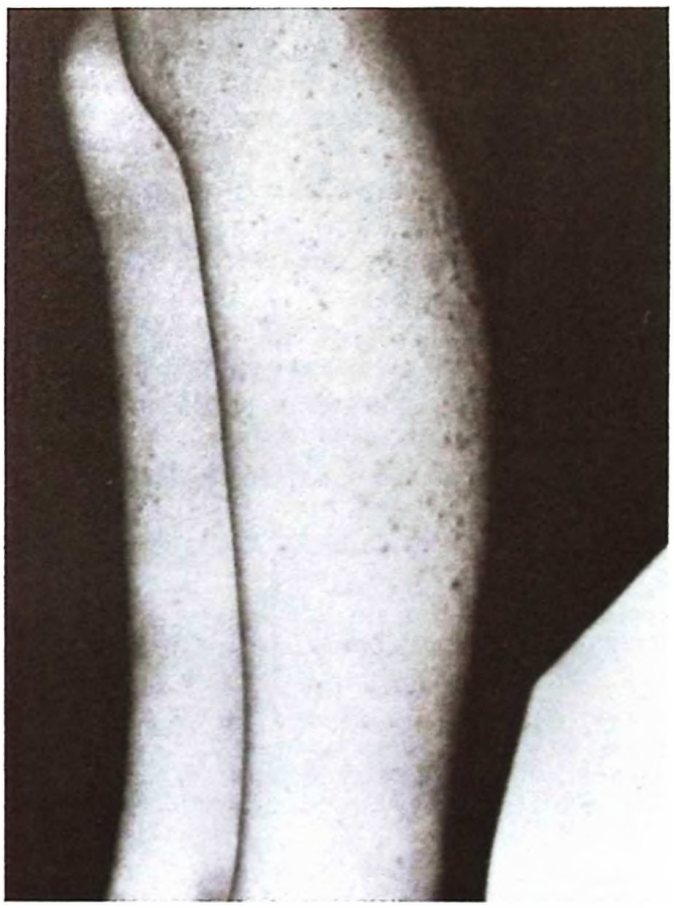

Fig. 1. Maculopapular rash in lower extremity.

The LCV of the presented patient, however, was not associated with leukemia, since the patient was in complete remission. Thus, MTX was thought to be the triggering factor in LCV, which has been reported to be associated with MTX even at low doses, suggesting a non-dose related association[3,7]. MTX is increasingly used in rheumatoid arthritis at low-doses (10-15 mg/m^2, weekly). We suggest that this should be a word of caution and that the drug history should be carefully investigated in similar cases.

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Concurrent presence of HBsAg and Anti-HBs in children

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To the Editor,

The presence of antibody (anti-HBs) against hepatitis B surface antigen (HBsAg) generally indicates the clearance of HBsAg and development of immunity to the hepatitis B virus (HBV). But, unusual serological findings, such as concurrent presence of HBsAg, anti-HBs and hepatitis B e antigen (HBeAg), are not uncommon[1]. This situation may be seen in one-third of patients in some reports[2].

We observed three children, aged six, 11 and 13 years, with concurrent HBsAg, HBeAg and anti-HBs among 184 consecutive HBsAg carriers. Two of them were brothers. All were HBsAg carriers for more than six months. Anti-HBs titrations were below 100 mIu/ml in all. Transaminase levels were normal in two patients. The other one had fluctuating transaminase levels. Liver biopsy of this patient revealed chronic persistent hepatitis. Transaminase concentration normalized in this patient after 24 months of follow-up, following HBeAg/anti-HBe seroconversion, and remained consistently normal thereafter. HBV DNA was negative in all during concurrence of HBsAg and anti-HBs. Anti-HBs became undetectable within four months in the two brothers, and within one year in the remaining patient. Immune complexes were negative in all and no extrahepatic finding was observed due to concurrence of HBsAg and anti-HBs.

In some areas, concurrent HBsAg and anti-HBs is a common serological pattern, affecting more than 30 percent of patients[2,3]. In our study, it was seen in 1.6 percent of the patients. Whereas there was no difference in HBV DNA-positive status between HBsAg carriers with and without anti-HB[2,3]. Concurrence was more common in patients with chronic active hepatitis than in those with acute hepatitis or chronic persistent hepatitis or in asymptomatic carriers[3]. In contrast, our two patients were chronic asymptomatic carriers and one had chronic persistent hepatitis. It has been shown that circulating complement-fixing immune complexes play an important role in the pathogenesis of extrahepatic symptoms, such as arthritis[4]. Our patients did not have extrahepatic symptoms and we could not detect circulating immune complexes. Although most patients remained positive for HBsAg and anti-HBs in other studies[3], anti-HBs disappeared in all our patients.

Infection with two different subtypes and inadequate host immune response are the most likely expanations for this phenomenon[1,3,5]. Mutations in the S region of HBV DNA may cause immune escape mutants of HBV and may explain why first contact with HBV leads to immune tolerance and a second to an immune response[6]. Adoptive immunity transfer with bone marrow transplantation has been reported to be effective in clearing chronic HBV infection[5]. In conclusion, concurrence of HBsAg anti-HBs occurs less frequently in children and it does not have an additional risk. Additionally, the presence of anti-HBs alone does not indicate a non-infectious serum.

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