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CONTENTS

ORIGINAL ARTICLES

CASE REPORTS

Analysis of the modifying effects of TAP 1/2 genes on cystic fibrosis phenotype

Filiz Özbaş-Gerçeker[1], Uğur Özçelik[2], Nural Kiper[2], Deniz Anadol[2], Ayhan Göçmen[2] Engin Yılmaz[3], Hayat Erdem-Yurter[3], Meral Özgüç[1,3]

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SUMMARY: Özbaş-Gerçeker F, Özçelik U, Kiper N, Anadol D, Göçmen A, Yılmaz E, Erdem-Yurter H, Özgüç M. Analysis of the modifying effects of TAP 1/2 genes on cystic fibrosis phenotype. Turk J Pediatr 2002; 44: 91-97.

Phenotypic variability has been reported in cystic fibrosis (CF) patients. TAP1 and TAP2 genes are encoding "the transporter associated with antigen processing" proteins. The aim of the present study was to analyze the frequency of TAP 1/2 variants in the Turkish population and to investigate a possible modifying role of these variants in CF phenotype. Sixty-three CF patients of known genotypes and 100 healthy control subjects were analyzed. There was a significant difference in the frequencies at positions 333 and 637 of TAP 1 gene and at position 665 of TAP 2 gene between patients and controls. Comparison of TAP gene polymorphisms in 36 CF patients homozygous for ΔF508 mutation with control subjects revealed a significant difference at position 665 of TAP 2 gene. These findings may be useful to assess the predisposition and to predict severity of the disease. We demonstrated that TAP genes might have modifying effects on the CF phenotype.

Key words: cystic fibrosis, TAP 1/2 genes, modifying genes.

Cystic fibrosis (CF) is an autosomal recessive genetic disorder with a prevalence of 1/2500 in the Caucasian population[1]. The disease manifests itself by pulmonary and pancreatic exocrine insufficiency, meconium ileus and pseudomonas infections. The mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) are known to be responsible for the disease[2]. These mutations may result in the lack of protein production, defective protein processing, defective regulation or in defective ion conduction[3]. CFTR was the first gene cloned by positional cloning[4] and the second chloride channel cloned[5]. CFTR gene was mapped to 7q31.2 and protein functions as a phosphorylation and nucleotide regulated small-conductance chloride channel[6], has been but is also regulates the epithelial sodium channels[7].

Phenotypic variability has been reported in CF patients[8]. It is known that CF patients are susceptible to different types of lung infections with some specific agents. Pier et al.[9] showed that CF patients were susceptible to Pseudomonas aeruginosa lung infection, and they reported that CFTR protein might have a host-defense function in the clearance of Pseudomonas aeruginosa from the respiratory tract. Pseudomonas aeruginosa lung infection primarily leads to a sustained immune response causing chronic inflammation and gradual tissue destruction resulting in premature death from respiratory insufficiency in CF patients[10]. However, the course of the disease differs between patients even if they have the same CFTR mutations. Mannose binding Lectin (MBL), a key factor in innate immunity which is thought to be associated with recurrent infections[11], and variant alleles in CF patients were analyzed and it was shown that lung function was significantly reduced in carriers of these variant alleles when compared to normal homozygotes. Therefore MBL deficiency might be a risk factor for CF patients[12]. Later it was concluded that CF and MBL deficiency results in a risk for Burkholderia cepacia colonization[13].

There may be other genes having modifying or modulating effects on the CF phenotype. TAP1 and TAP2 genes are such candidates located in the MHC class II region at 6p21.3 and encoding "the transporter associated with antigen processing" proteins[14]. TAP1 and TAP2 are half transporters[15] and they function in the ATP dependent transport of cytoplasmic peptides, which are generated from the degradation of cytoplasmic proteins by proteosome complexes, into the endoplasmic reticulum lumen[16]. TAP proteins are members of ATP-binding cassette (ABC) family of membrane translocators, which also include the CFTR[17].

Polymorphisms at positions 333 and 637[18], and 659[19] of TAP1 gene at 253[20], 379, 665 and 687[18], 565[21], and 577[22] of TAP2 gene have been reported. Some rare TAP2 polymorphisms were also reported in African populations[23]. It is thought that polymorphic variants of TAP genes can restrict the antigenic peptides that are bound and presented[24]. Individual variability in structure or expression of these genes can affect specificity and result in different sets of peptides derived from the same antigen being presented to T cells in different people, therefore TAP genes are attractive candidates of susceptibility/severity factors[25].

An association between TAP gene polymorphisms and many human diseases such as Graves' disease[26], rheumatoid arthritis[25], diffuse panbronchiolitis[27], hepatitis C virus infection[28], nickel allergy[29], juvenile onset psoriasis[30] and sarcoidosis[31] has been reported. The aim of the present study was to analyze the frequency of TAP1/2 variants in a healthy Turkish population and also to investigate a possible modifying role of these variants in the CF phenotype.

Material and Methods

Patients and Control Subjects

Sixty-three CF patients (mean age: 7.35, range 2-13 years, 31 male/32 female) of known genotypes were chosen from the total 443 CF patients followed at Hacettepe University Children's Hospital. Diagnosis of CF was established on the basis of abnormal sweat electrolytes and clinical features. Of the patients, 95.2% had pancreatic insufficiency and 17.5% were chronically infected, at least by one microorganism (S. aureus, P. aeruginosa) in the lower respiratory tract. In all CF patient, diagnosis was confirmed by mutation analysis. Thirty-six were homozygous for ΔF508 mutation. Eleven were homozygous for other CFTR mutations, and the remaining 16 patients were compound heterozygous.

One hundred unrelated healthy subjects were selected as a control group. Informed consent was obtained from the parents of the children and control subjects.

Genomic DNA Isolation

Blood samples from CF patients and control subjects were collected into EDTA tubes with verbal and written explanation and signed consent. Genomic DNA was isolated by standard salting out procedure[32]. The DNA concentrations were measured and DNAs were stored at –80°C until use.

Analysis of TAP Gene Polymorphisms and TAP Genotyping

The amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR)[23] was used to determine TAP1 and TAP2 polymorphisms. Two polymorphisms in the TAP1 gene (positions 333 and 637) and three polymorphisms in the TAP2 gene (position 379, 565 and 665) were analyzed in this study. The oligonucleotide primer sequences were as previously described[23]. Genomic DNA samples (0.25 μg) were amplified in 25 μl reaction mixtures containing 0.25 μg of each oligonucleotide primer, 200 μM dNTP's, 1Xtaq DNA polymerase buffer and 0.5 units of Taq DNA polymerase (Promega, Madison, USA) overlaid with mineral oil. Reaction conditions using a thermal cycler (MJ Research PTC-200, Massachusetts, USA) were 95°C for 5 min, 35 cycles of 94°C for 1 min, the appropriate annealing temperature for 2 min, 72°C for 2 min and 72°C for 10 min. Reaction products were separated on a 2% agarose gel containing 0.1% w/v ethidium bromide for visualization.

Flanking primers of each polymorphic site were used to have an "internal control" for each specific reaction. The variant amino acids for each position and the sizes of the resultant products are given in Table I.

Statistical Analysis

Since TAP genes can be found as heterozygous at more than one position, genotype and amino acid frequencies rather than allele or haplotype

frequencies were preferred. Both genotype and amino acid frequencies were calculated for CF patients and control subjects. Amino acid frequencies were compared using 2x2 chi-square test with df of 1, and genotype frequencies were compared by 3x2 chi-square test with df of 2 using the statistical program Stats version 1.1 (Decision Analyst Inc., Arlington).

Table I. The TAP variant amino acids for each position and the sizes of the ARMS-PCR products

Position	Control	Variant 1		Variant 2	
TAP 1-333	533 bp	Ile	241 bp	Val	351 bp
TAP 1-637	429 bp	Gly	180 bp	Asp	307 bp
TAP 2-379	427 bp	Ile	158 bp	Val	328 bp
TAP 2-565	400 bp	Thr	161 bp	Ala	298 bp
TAP 2-665	408 bp	Thr	141 bp	Ala	326 bp

ARMS-PCR: amplification refractory mutation system-polymerase chain reaction.

Results

The phenotype and genotype frequencies of all polymorphisms calculated for CF patients and control subjects are shown in Table II together with the χ^2 and P values. Statistically meaningful differences between Turkish CF patients and control subjects were found at TAP 1 positions 333 and 637 and TAP 2 position 665.

For TAP 1, at position 333 there was a significant increase in Val frequency in CF patients (χ^2=4.75, df: 1, p=0.046). The frequency of Ile and genotypes were not significantly different between the two groups.

At position 637, the frequency of Gly was significantly increased in CF patients (χ^2=4.08,

Table II. Comparison of TAP 1 and TAP 2 polymorphisms in Turkish control subjects and cystic fibrosis patients of known genotypes

	Control subjects		CF Patients			
TAP polymorphism frequencies	(n=100)	(%)	(n=63)	(%)	χ^2	P
TAP1 P333						
Phenotypes						
Ile	96	96	59	93.6	NS	
Val	25	25	26	41.3	4.75	0.046
Genotypes						
Ile/Ile	75	75	37	58.7		
Ile/Val	21	21	22	34.9	NS	
Val/Val	4	4	4	6.3		
TAP1 P637						
Phenotypes						
Asp	86	86	49	77.7	NS	
Gly	18	18	20	31.7	4.08	0.046
Genotypes						
Asp/Asp	82	82	43	68.2		
Asp/Gly	4	4	6	9.5	NS	
Gly/Gly	14	14	14	22.2		
TAP 2 P379						
Phenotypes						
Ile	26	26	16	25.4	NS	
Val	75	75	49	77.7	NS	
Genotypes						
Ile/Ile	25	25	14	22.2		
Ile/Val	1	1	2	3.2	NS	
Val/Val	74	74	47	74.6		

Table II. Continue next page.

Table II. Continued
Comparison of TAP 1 and TAP 2 polymorphisms in Turkish control subjects and cystic fibrosis patients of known genotypes

TAP polymorphism frequencies	Control subjects (n=100)	(%)	CF Patients (n=63)	(%)	χ^2	P
TAP 2 P565						
Phenotypes						
Thr	5	5	6	9.5	NS	
Ala	99	99	62	98.4	NS	
Genotypes						
Thr/Thr	1	1	1	1.6		
Thr/Ala	4	4	5	7.9	NS	
Ala/Ala	95	95	57	90.5		
TAP 2 P665						
Phenotypes						
Thr	100	100	60	95.2	4.85	0.046
Ala	1	1	9	14.3	11.8	0.001
Genotypes						
Thr/Thr	99	99	54	85.7		
Thr/Ala	1	1	6	9.5	12.03	0.003
Ala/Ala	0	0	3	4.7		

NS: not significant.

df: 1, p=0.046). The overall genotype frequency was not significantly different at this position.

When we examined phenotype frequencies of TAP 2 variants, no significant difference was observed in amino acid and genotype frequencies at positions 379 and 565; however, a significant difference was seen at position 665. Thr variant frequency at position 665 was significantly reduced in CF patients (χ^2=4.85, df: 1, p=0.046) but Ala frequency was increased (χ^2=11.8, df: 1, p=0.001). Comparison of the overall genotype frequencies at position 665 indicated a significant difference between CF patients and control subjects (χ^2=12.03, df: 2, p=0.003). Thr/Thr genotype was underrepresented while Thr/Ala and Ala/Ala genotypes were over represented in CF patients.

Furthermore, we analyzed homozygous patients with the genotype ΔF508/ΔF508. The comparison of these patients with control subjects revealed a significant difference only at position 665. The frequency of Ala was found to be higher in ΔF508/ΔF508 CF patients than the control subjects (χ^2=7.64, df: 1, p=0.008). When we compared the overall genotype frequencies in the two groups, a significant difference was observed (χ^2=7.89, df: 2,

Table III. Comparison of TAP 1 and TAP 2 polymorphisms in control subjects and ΔF508/ΔF508 CF patients

TAP polymorphism frequencies	Control subjects (n=100)	(%)	ΔF508/ΔF508 CF patients (n=63)	(%)	χ^2	P
TAP1 P333						
Phenotypes						
Ile	96	96	33	91.6	NS	
Val	25	25	15	40.6	NS	
Genotypes						
Ile/Ile	75	75	21	58.3		
Ile/Val	21	21	12	33.3	NS	
Val/Val	4	4	3	8.3		

Table III. continue next page.

Table III. Continued
Comparison of TAP 1 and TAP 2 polymorphisms in control subjects and ΔF508/ΔF508 CF patients

TAP polymorphism frequencies	Control subjects (n=100)	(%)	ΔF508/ΔF508 CF patients (n=63)	(%)	χ^2	P
TAP1 P637						
Phenotypes						
Asp	86	86	28	77.7	NS	
Gly	18	18	10	27.7	NS	
Genotypes						
Asp/Asp	82	82	26	72.2		
Asp/Gly	4	4	2	5.5	NS	
Gly/Gly	14	14	8	22.2		
TAP 2 P379						
Phenotypes						
Ile	26	26	8	22.2	NS	
Val	75	75	29	80.5	NS	
Genotypes						
Ile/Ile	25	25	7	19.4		
Ile/Val	1	1	1	2.7	NS	
Val/Val	74	74	28	77.7		
TAP 2 P565						
Phenotypes						
Thr	5	5	5	20.0	NS	
Ala	99	99	35	100	NS	
Genotypes						
Thr/Thr	1	1	1	2.7		
Thr/Ala	4	4	4	11.1	NS	
Ala/Ala	95	95	31	86.1		
TAP 2 P665						
Phenotypes						
Thr	100	100	35	97.2	NS	
Ala	1	1	4	11.1	7.64	0.008
Genotypes						
Thr/Thr	99	99	32	88.8		
Thr/Ala	1	1	3	8.3	7.89	0.031
Ala/Ala	0	0	1	2.7		

NS: not significant.

p=0.031). Thr/Thr genotype was underrepresented ΔF508/ΔF508 CF patients while Thr/Ala and Ala/Ala were overrepresented (Table III).

Discussion

In the present study, the frequencies of polymorphic variants of TAP genes were analyzed for a healthy Turkish population and CF patients. The frequencies of TAP gene polymorphisms differ among different populations. Table IV shows frequencies of TAP variants in several representative populations. It can be seen that the Turkish population has a very different frequency distribution than the others. The most striking observation was the underrepresentation of the Ala variant at position 665: 1% in the Turkish population versus 46.9% in Polish and 51.4% in British populations[31].

Since TAP genes are located within the MHC class II region and function in the processing of antigenic peptides, they are very good candidates for especially MHC-linked diseases and are also likely susceptibility/severity factors for other diseases. The polymorphisms of TAP genes affect the specificity of antigen binding and presentation. Therefore, they might have important roles in the course of some diseases. There are many studies on the association of TAP gene polymorphisms with different diseases.

It is known that CF patients are susceptible to lung infections with some specific agents. In a previous study Garred et al.[12] showed that there is an association between MBL variants and the severity of the lung disease and survival in CF. We thought that TAP 1 and TAP 2 genes might be other candidates having modifying effects on the CF phenotype. Our results indicate that TAP 1 gene polymorphism frequency at positions 333 and 637, and TAP 2 gene polymorphisms at position 665 are significantly different between CF patients and control subjects. There was an increased frequency of Val at position 333 and Gly at position 637 of TAP 1 and of Ala at position 665 of TAP 2 in CF patients.

Table IV. Distribution of TAP 1 and TAP 2 gene variants in three different populations

Position	Polish population* n: 128 %	British population* n: 290 %	Turkish population n: 100 %
TAP 1-333-Ile	98.7	97.9	96.0
TAP 1-333-Val	31.0	33.4	25.0
TAP 1-637-Asp	98.7	99.3	86.0
TAP 1-637-Gly	22.5	31.4	18.0
TAP 2-379-Ile	25.0	25.2	26.0
TAP 2-379-Val	97.5	99.7	75.0
TAP 2-565-Ala	100.0	99.7	99.0
TAP 2-565-Thr	23.1	19.0	5.0
TAP 2-665-Ala	46.9	51.4	1.0
TAP 2-665-Thr	96.2	95.5	100.0

n: number of subjects.
* Foley et al.[31]

In order to further analyze the role of TAP gene polymorphisms in the severity of the disease, we analyzed the ΔF508/ΔF508 CF patients (n: 36). From the comparison of this group with the control subjects, a significant difference in the overall genotype frequency of TAP 2 position 665 was observed. The frequency of Ala was also higher in this CF patient group when compared to control subjects. Thr/Ala and Ala/Ala genotypes again were overrepresented in ΔF508/ΔF508 CF patients.

This is the first study analyzing the association between TAP gene polymorphisms and CF phenotype. Furthermore, it is the first report of TAP gene polymorphism frequency in the Turkish population. We demonstrated that TAP genes in addition to other factors such as MBP variants might play a modifying role in the course of the CF phenotype. It will be interesting to see results from other populations to determine if TAP genes have a role in the modification of the CF disease phenotype.

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Clinical features of tuberous sclerosis cases

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SUMMARY: Söğüt A, Özmen M, Sencer S, Çalışkan M, Aydınlı N, Ertuğrul T, Peksayar G. Clinical features of tuberous sclerosis cases. Turk J Pediatr 2002; 44: 98-101.

Tuberous sclerosis (TS) is an autosomal dominant, multisystemic and neurocutaneous disease with high spontaneous mutation rate, and it mostly involves the skin, brain, kidneys, heart and the eyes. This study included 35 patients diagnosed with tuberous sclerosis and aged 6 months to 17 years, with a mean age of 6.5±4.8 years. The most frequently observed manifestations were those of the skin (97.1%) and of the central nervous system (seizures 94.2%, mental retardation 51.4%), followed by renal (32.2%), cardiac (25.8%) and ocular (22.5%) manifestations. Among cutaneous manifestations, hypomelanotic macules (94.3%), facial angiofibromas (40%), shagreen spots (20%), fibrous plaques on the forehead (5.7%) and ungula fibromas (5.7%) were observed. Tonic seizures (37.1%) and infantile spasms (21.2%) accounted for majority of seizures. Neurophysiological development was normal in 25.6% of cases, retarded in 51.4% and borderline in 23%. Thirty-four patients had typical pathological findings on magnetic resonance imaging (MRI).

In conclusion, the earliest and most frequent complaint is seizure in cases with TS. Careful investigation for hypomelanotic macules and other skin manifestations typical for TS in cases presenting with convulsion makes early diagnosis possible and obviates unnecessary investigations.

Key words: tuberous sclerosis, seizure, neurocutaneous syndrome.

Tuberous sclerosis (TS), known as Bourneville's disease, epiloia or Pringle syndrome, is a multisystemic and a neurocutaneous disease affecting mainly the skin, brain, kidneys, heart and the eyes. It is usually autosomal dominant, but the rate of spontaneous mutations is also very high[1-3]. The phenotypes vary widely, including seizures, mental retardation, functional abnormalities of the kidneys and the heart and dermatological manifestations[2]. The majority of clinical findings result from the hamartomas in the affected organs[3].

In this paper, we present the clinical features of 35 patients with TS.

Material and Methods

Ninety patients either diagnosed with TS or suspected of having TS presented to the Department of Pediatrics, Division of Pediatric Neurology, İstanbul University between January 1987 and December 1998. Thirty-five patients diagnosed as definite TS according to the criteria shown in Table I[4] who accepted to participate in the study were recruited. Initial complaints, history, type and frequency of seizures and age at the onset were recorded. According to the seizure frequency, patients with seizures every day were classified as having severe TS, those with seizures once a week or less as having moderate TS and those with no seizures for two years, whether they were on anticonvulsive medication or not, as having mild TS. Physical and neurological findings obtained from baseline examinations were compared with those from final examinations, and ophthalmologic examinations were repeated. Neuropsyschological development was investigated using Brune-Lezine (BL) test in children younger than six years old and Wechsler Intelligence Scale for Children-Revised (WISC-R) in older children. IQ or Developmental Quotient (DQ) above 90 was considered as normal, between 71 and 90 borderline, and 70 or lower retarded.

Cranial magnetic resonance imaging (MRI) and electroencephalography were performed in all patients. MRI was performed in the MRI unit with the power of 1 T magnet. T1 weighted (TR: 600, TE: 70 matrix 256 x 256, FOV: 200 cm) axial, sagittal and coronal planes and T" weighted (TR: 3000, TE: 300) spin density (TR: 3000, TE: 17) were used in all cases and multiple sequences in 3 planes in 26 cases following IV gadolinium DTPA (0.2 mg/kg) injection. Thirty-one cases underwent abdominal ultrasonography, echocardiography and retinal examinations.

Table I. Diagnostic criteria for TS according to tuberous sclerosis (TS) complex consensus conference (1998)

Major features	Minor features
1. Facial angiofibromas or forehead plaque	1. Multiple, randomly distributed pits in dental enamel
2. Cardiac rhabdomyoma, single or multiple	2. Hamartomatous rectal polyps
3. Nontraumatic ungual or periungual fibroma	3. Bone cysts
4. Hypomelanotic macules (three or more)	4. Radial migration lines in the cerebral white matter
5. Shagreen patch (connective tissue nevus)	5. Gingival fibromas
6. Multiple retinal nodular hamartomas	6. Nonrenal hamartoma
7. Cortical tuber	7. Retinal achromic patch
8. Subependymal nodule	8. 'Confetti' skin lesions
9. Subependymal giant cell astrocytoma	9. Multiple renal cysts
10. Lymphangiomyomatosis of the lungs	
11. Renal angiomyolipoma	

Definite TS complex : Either two major features or one major feature plus two minor features.
Probable TS complex: One major plus one minor feature.
Possible TS complex : Either one major feature or two or more minor features.

Results

This study consisted of 35 patients (22 boys, 13 girls) with a mean age of 3.1±3.2 years on baseline examination and of 6.6±4.8 years on final examination. Clinical manifestations included those of the skin, central nervous system, kidney, heart and the eyes, in order of decreasing frequency. Thirty of 35 cases (97.1%) had dermatological manifestations. Hypomelanotic macules and facial angiofibromas were the most common dermatological findings. Other cutaneous findings were shagreen patches, fibrous plaques on the forehead, ungula fibromas, light brown patches, skin tags and hemangiomas (Table II). On the final examination, the frequency of hypomelanotic macules, facial angiofibromas and shagreen patches increased.

The most common presenting symptom was seizures (94.2%) and they began within 18.3±29.2 months on average; 62.5% of cases had seizures beginning before their first birthday. Generalized seizures affected 72.7% of cases, whereas 27.3% suffered from partial seizures (Table III). The most common generalized seizures were of tonic type in 37.1% of cases and of infantile spasm type in 21.2%. Seizure frequency was mild in 34.2% of cases, moderate in 42.8% and severe in 23%.

Neuropsychological development of patients was retarded in 51.4% of cases, borderline in 23% and normal in 25.5%.

Abdominal ultrasonography was performed in 31 cases, of which five (16.1%) were found to have renal angiomyolipoma and another five (16.1%) renal cysts. None had cardiac complaints, but of 31 cases, seven (22.5%) patients had rhabdomyomas and one patient had mitral and aortic insufficiency on echocardiography. Six patients (19.3%) had retinal hamartomas and one patient (3.2%) iris coloboma on retinal examinations.

Table II. Skin lesions in our cases

	First exam		Last exam	
Lesion	n	%	n	%
Hypomelanotic macules	30	(85.7)	33	(94.2)
Facial angiofibromas	5	(14.2)	14	(40.0)
Shagreen patches	3	(8.5)	7	(20.0)
Fibrous plaques on the forehead	2	(5.7)	2	(5.7)
Ungual fibroma	2	(5.7)	2	(5.7)

Table III. Types of seizures

Type	No. of cases	%
Generalized	24	72.7
Infantile spasm	7	21.2
Tonic	13	39.4
Clonic	3	9.1
Atonic	1	3.0
Partial	9	27.3

Thirty-four cases were found to have pathological findings on cranial MRI. Thirty-four (97.1%) had cortical tubera, 34 (97.1%) subependymal nodules, 19 (54.2%) white matter involvement such as radial glial bands and heterotypes, and three cerebellar lesions. Since in nine patients subependymal nodules were localized in the foramen Monro and uptake of contrast medium was positive, they were suspected of giant cell astrocytoma.

Cortical tubera were counted in 26 of 34 cases who had pathological findings on MRI: 15.5% of cases were found to have less than 5, 53.8% of cases 5-10 and 30.7% of cases more than 10. The diameters of the tubera were less than 1 cm in 15.4% of cases, 1 2.5 cm in 50% of cases and more than 2.5 cm in 34.6% of cases. The tubera were found most often in the parietal region (58%) and least often in the median/ parasagittal region (50%).

Discussion

Clinical findings of TS, first described by Frederich Daniel von Reckinghauson in 1862[1], vary with age and manifest over the years. Dermatological signs, especially hypomelanotic macules, and central nervous system signs are commonly seen within the first year of life. Cardiac rhabdomyomas are usually seen in the newborn, but signs of disorders of the eyes and kidneys generally manifest after 2-3 years of age[5].

Among sings of central nervous system impairment, the most common and earliest one is seizures[1,6]. In our cases, the first complaint and presenting symptom was seizures in 94.2% of cases. Two newborns had been referred from the Cardiology Department due to cardiac rhabdomyomas.

The skin and the brain are the most frequently affected organs in TS[1,3,7,8]. Sings of skin impairment, including hypomelanotic macules, facial angiofibromas, shagreen patches fibrous plaques on the forehead and periungual fibromas may be seen in 96% of cases[1,9].

Hypomelanotic macules are the most common skin manifestation[1,10] and are seen in 80-100% of cases[1,11,12]. They may be detected at birth and they may disappear later in life[9]. Hypomelanotic macules may also be seen in normal individuals; for the diagnosis of TS, more than two lesions must be present[4]. In our cases, frequency of hypomelanotic macules was 94.2%. Frequency of facial angiofibromas is reported as 42-90%[1,2,9,13]. These lesions' are not present at birth and they appear at any time between five years and adolescence[14]. Their frequency increases with age[9]. The frequency in our cases was comparable to those reported in the literature and also increased with age.

Shagreen patches appear after puberty. They are diagnostic of TS and their frequency is between 20-54%[1,3,9,15]. Like facial angiofibromas, they become more common with increasing age[9]. The frequency in our cases was 20%, increasing with age.

Fibrous plaques on the forehead are usually seen in the newborn and in the early infancy period[1,4]. Their frequency ranges from 19-25%[13,16], but was 5.7% in our cases.

Rate of periungual fibromas ranges between 15% and 50%, and they usually appear after puberty[1,3,14]. However, it was 5.7% in our cases, which may be attributable to our cases being prepubertal.

Frequency of seizures in TS is about 80-92%[1,3,17,18]. Seizures may occur at any age but they usually begin within the first year of life[8,19]. They were the first presenting symptom in 94.2% of our cases and had begun within the first six months of life in 57.5% of cases.

Mental retardation is seen in 38-65% of TS cases[1,17]. In our cases, 51.4% had mental retardation, 17.3% had borderline IQ and 31.3% were considered normal. Jozwiak et al.[16] reported that in mentally retarded cases, the risk of seizures increased significantly within the first six months and was almost two and a half times higher than that in normal individuals. In our study, the seizures had started within sixmonths in six (33.3%) of 18 mentally retarded cases.

Cardiac rhabdomyomas (RM) are seen in 30-60% of TS cases[13,16]. RM may be detected in the prenatal or neonatal period[20]. Since these tumors may regress in time, they are usually treated in a conservative manner and followed by echocardiography[21]. RM in our cases also shrank in time. On the other hand, opthalmologic and renal signs may increase in time and, since the most common cause of mortality is renal pathology, these cases should be followed up carefully[22]. In our cases, rates of involvement of the eyes and the kidneys were very low, which was probably due to the younger ages of our cases.

Cortical tubera and subependymal nodules are typical lesions of TS. The rate of cortical tubera is about 88-95% and that of subependymal

nodules 80-95%[27-30]. The subependymal nodules in the foramen Monro region and holding contrast material need to be investigated for giant cell astrocytomas[26]. Rate of giant cell astrocytomas in TS is about 5-8.5%[5,26]. Rates of intracranial lesions in our series were comparable to those reported in the literature. However, rate of suspected giant cell astrocytoma (25.7%) was higher than that expected; the actual rates will be known only after the follow-up.

In conclusion, TS is a multisystemic disease affecting mainly the skin and the brain. The earliest and most frequent complaint is seizure. Therefore, investigation of TS specific skin lesions, especially in cases presenting with seizures, makes early diagnosis possible and obviates unnecessary investigations.

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Helicobacter pylori infection in Turkish children with gastrointestinal symptoms and evaluation of serology

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Helicobacter pylori infection is a common etiopathogenetic factor in children with gastrointestinal symptoms in the developing world. Although serology offers an easy noninvasive method of diagnosis, its sensitivity and specificity are reported to be low among children. In this prospective study, we investigated the frequency and endoscopical and morphological findings of H. pylori infection in 180 Turkish children who underwent upper gastrointestinal endoscopy either for peptic symptoms or on a routine basis and in asymptomatic pediatric patients who underwent endoscopy for other reasons, and then evaluated the diagnostic accuracy of serology in our population. Overall H. pylori infection was diagnosed in 77 of the 180 patients (42.7%) by histology and urease test. The sensitivity of H. pylori specific IgG antibody assay by ELISA was determined to be 100%, while the specificity was 98%, the positive predictive value 97.4%, the negative predictive value 100%. Frequency of H. pylori infection is high in Turkish pediatric patients without gastrointestinal symptoms as well as in children with gastrointestinal complaints. H. pylori specific antibody assay is a noninvasive and sensitive method for the diagnosis of H. pylori infection in the Turkish pediatric population.

Key words: Helicobacter pylori, children, symptoms, endoscopical findings, diagnosis, serology.

Marshall and Warren[1] reported an association between the presence of Helicobacter pylori (H. pylori) in the gastric mucosa and antral gastritis in adults for the first time in 1983. This association was also noted in children[2]. H. pylori infection is considered to be one of the most common chronic infections in humans and usually persists during a lifetime if left untreated[2-3]. Although the infection commonly remains asymptomatic, chronic inflammation can predispose to the development of gastric or duodenal ulcers and even gastric cancer[4]. H. pylori infection can be detected in children with chronic abdominal pain, dyspepsia, malnutrition, growth retardation, and chronic diarrhea[5-8]. H. pylori associated with peptic ulcer disease is less frequently seen in children compared to adults. In the pediatric patients, the histological response to H. pylori infection is also different from that seen in adult patients. In infancy and early childhood, lymphocytic infiltration is more common in gastric mucosa, whereas in adults neutrophils are also frequently observed.

In the diagnosis of H. pylori infection, serology is one of the most well known, inexpensive, noninvasive and simple techniques. For that reason, ELISA is being widely used particularly in epidemiological studies. Especially in developing countries, it is preferable to another noninvasive assay, the breath test, since it is cheaper, readily available and more practically applicable in children.

In this study, our goal was to evaluate the reliability of serology by ELISA test in the diagnosis of H. pylori infection in our pediatric population, by comparing it with the rapid urease test and histological examination. Furthermore, we aimed to determine the frequency of the

H. pylori infection and the relation between the symptoms and both the endoscopical and histopathological findings in Turkish children.

Material and Methods

This study was carried out at the İstanbul Faculty of Medicine Pediatric Gastroenterology Department during a three-year period between May 1996 and June 1999. We investigated prospectively 180 consecutive children (108 boys and 72 girls; mean age 10.8±3.7 years, range 1-18 years) who underwent upper gastrointestinal endoscopy for symptoms related to upper gastrointestinal tract (139 patients) or for intestinal biopsy in growth retarded children (11 patients) or on a routine basis in patients with chronic renal failure before renal transplantation (30 patients). Upper gastrointestinal symptoms leading to endoscopic investigation in our study group were recurrent abdominal pain, vomiting, melena/hematemesis and dyspepsia. Symptoms such as nausea without vomiting, and bloating and belching without definite abdominal pain were defined as dyspepsia. The patients having more than one symptom were grouped according to the predominant symptom which was most disturbing for the patient. Patients who had received antibiotics, anti-inflammatory drugs or drug therapy for peptic ulcer disease during the preceding month and patients with portal hypertension and/or coagulation disorders were excluded from the study.

Fiberoptic endoscopes (GIF-P20, Olympus, Tokyo, Japan and FG-100PE Fujinon) were used in all endoscopic procedures. During endoscopy, at least four mucosal biopsy specimens were obtained from the gastric antrum, gastric corpus and from all areas with a pathological appearance, for rapid urease test and histopathological examination. In every patient, one of the antral biopsy samples was placed into the rapid urease test plate (CLO test, Delta, West Beutley, Western Australia) at 25°C; other biopsy specimens were stained with hematoxylin-eosin and Giemsa for histological examination to determine the presence of H. pylori infection and the morphological changes.Each biopsy sample was examined under light microscope by the same pathologist who was unaware of the results.

During endoscopic examination, the presence of eosophagitis defined by hyperemia or erosions in the distal esophagus, gastritis defined by edematous and hyperemic gastric mucosa with or without superficial erosions, gastric ulcer, antral nodularity, duodenal ulcer and duodenitis defined by mucosal congestion with exudates were investigated in every patient. In histological examinations, chronic gastritis was defined by the infiltration of the lymphocytes, monocytes and/or plasma cells whereas chronic active gastritis was defined as the inflammation characterized by neutrophil infiltration. Additionally, the peresence of intestinal metaplasia and lymphoid follicles was recorded.

Children were considered to be H. pylori positive if urease test and histological examination were both positive and H. pylori negative if both tests were negative. If one of the two test results was negative, the patients were excluded from the study. Two of the 182 children studied were excluded since they had positive histological examination but negative urease test for H. pylori.

Venous blood samples were obtained from each child at the time of endoscopy. The serum was separated, divided into aliquots, and stored at –20°C before testing. Sera were assayed for H. pylori specific IgG antibodies (HP-IgG) using a commercial system, which is a qualitative enzyme-linked immunoassay kit (Biomerica Newport Beach). The assays were performed in the laboratory blinded to the children's H. pylori status. The concentrations of IgG antibody in the serum samples were determined by interpolation form a standard curve constructed by dilution of the positive control and by plotting absorbance values obtained for each standard against the corresponding anti-H. pylori concentrations in units per milliliter. A cut off value of 20 U/ml was accepted according to the manufacturer's instructions. Values above 20 U/ml were considered positive, and values below 12 U/ml were considered negative. Equivocal values between 12-20 U/ml necessitated repeat testing. One patient with an equivocal result at the initial assay had a H. pylori specific IgG level below 12 U/ml on the repeat testing and was considered negative for H. pylori serology. Sera that showed discrepant ELISA results with the histology and CLO test were also retested.

The performance of the test was evaluated by determination of the sensitivity, specificity and positive and negative predictive values.

Student's t test, χ^2 test and Fisher's exact test were performed for statistical analysis where appropriate. A value of $p \leq 0.05$ was considered as statistically significant.

Results

Among the 180 children participating in the study, 77 (42.7%) were H. pylori positive determined by the positive results of the histopathological investigation and the rapid urease test. The mean age of H. pylori positive children was significantly higher than that of the H. pylori negative group (11.3±3.1 years vs 9.2±4.0 years, $p<0.01$). H. pylori infection was more common in males (50.9% vs 30.6%, $p<0.01$). Incidence of H. pylori infection was significantly higher in children over 10 years (28.8% vs 54%, $p<0.01$) (Table I).

Table I. Frequency of Helicobacter pylori infection in different age groups

Age (years)	Number of patients	H. pylori positivity (%)
1-4	21	4 (19)
5-9	59	19 (32.2)
10-14	86	46 (53.4)
15-18	14	8 (57.1)

No significant difference existed between H. pylori positive and H. pylori negative groups with regard to family history of acid peptic disease or abdominal cancer (15.5% vs 12.6% and 2.5% vs 1.9%, respectively; $p>0.05$).

Abdominal pain was the main indication for endoscopic examination in our study group. Sixty-three patients had recurrent abdominal pain. Epigastric pain was the predominant type of abdominal pain (84.6% of the patients with abdominal pain). Frequency of H. pylori infection was highest (65.4%) in 26 patients with dyspepsia as the main symptom. Among 41 patients without gastrointestinal symptoms (11 with chronic diarrhea and growth retardation, 30 with chronic renal failure) who underwent upper gastrointestinal endoscopy either for intestinal biopsy or on a routine basis before renal transplantation, H. pylori was positive in 24.4% in total (18.2% and 27.6%, respectively), which was the lowest rate. Overall H. pylori positivity was significantly higher in children with gastrointestinal symptoms (48.2%, $p<0.05$). Distributon of different symptoms in H. pylori positive and negative groups are shown in Table II. Incidence of dyspepsia was significantly higher in the H. pylori positive patients compared to the H. pylori negative group (22% vs 8.7%, $p<0.05$).

Endoscopical findings were normal in 17 of 77 H. pylori positive children (22%). Incidence of endoscopical gastritis, antral nodularity, and duodenal ulcer were 33.8%, 27.3% and 13%, respectively, in the H. pylori positive group. Among these patients, only two had esophagitis (2.6%), and another one had gastric ulcer. Table III shows the incidence of different endoscopical findings in the H. pylori positive group compared to the H. pylori negative children. Incidence of gastritis, duodenal ulcer and antral nodularity was significantly higher in the H. pylori positive group ($p<0.01$).

In the context of endoscopical findings, H. pylori positivity was 91.3% in patients with antral nodularity, 71.4% in patients with duodenal ulcer, 61.9% in those with gastritis, 25% in

Table II. Helicobacter pylori infection rate according to clinical symptoms and frequency of clinical symptoms in Helicobacter pylori positive versus negative children

		H. pylori positive		H. pylori negative		
Symptoms	Percentage of H. pylori infected cases	Number of cases	%	Number of cases	%	P value
Recurrent abdominal pain	46%	29	37.7	34	33	NS
Melena/hematemesis	42%	14	18.2	19	18.4	NS
Dyspepsia	65.4%	17	22	9	8.7	0.017
Nausea/vomiting	41.2%	7	9.1	10	9.7	NS
Gastrointestinal	48.2%	67	87	72	69.8	
Growth retardation	18.2%	2	2.6	9	8.7	NS
Chronic renal failure	26.7%	8	10.4	22	21.4	NS
Extraintestinal	24.4%	10	13	31	30.2	

NS: not significant.

those with gastric ulcer and 16.6% in patients with esophagitis (Table III). Frequency of H. pylori infection among children with apparently normal mucosa on endoscopy was 20%.

Distribution of histopathological findings was also evaluated in the children participating in the study. Overall, in 122 (67.8%) of 180 patients, histological gastritis was observed (106 chronic gastritis and 16 chronic active gastritis). Intestinal metaplasia was seen in one patient and lymphoid follicles in four patients. Histopathological examination was normal in 58 patients who also did not have macroscopic abnormality on endoscopy. H. pylori frequency was 62.2% in the histological gastritis group, whereas it was 1.7% in patients with normal histology ($p<0.001$). H. pylori infection did not differ significantly in patients with chronic gastritis and chronic active gastritis (75% vs 65.5% $p>0.05$). Among 122 patients with histological gastritis, 42 (34.4%) had gastritis on endoscopy while 23 (18.8%) had antral nodularity.

Table III. Helicobacter pylori infection rate according to endoscopic findings and frequency of endoscopical findings in Helicobacter pylori positive and negative children

		H. pylori positive		H. pylori negative		
Endoscopical findings	Percentage of H. pylori infected cases	Number of cases	%	Number of cases	%	P value
Normal	20%	17	22	68	66	<0.01
Gastritis	61.9%	26	33.8	16	15.5	<0.01
Antral nodularity	91.3%	21	27.3	2	1.9	<0.01
Esophagitis	16.7%	2	2.6	10	9.7	NS
Duodenal ulcer	71.4%	10	13	4	3.9	<0.05
Gastric ulcer	25%	1	1.3	3	2.9	NS
Total	42.8%	77		103		

NS: not significant.

H. pylori specific IgG antibodies by ELISA method were positive in 79 of 180 patients. IgG antibodies were positive in all of 77 H. pylori infected patients as determined by CLO test and histopathological examination, whereas two of 103 noninfected patients were H. pylori IgG positive, which is considered to be false positivity. False negativity was not seen. By means of ELISA method, the sensitivity of H. pylori specific IgG antibody determination was found to be 100%, while the specificity was 98%, positive predictive value 97.4%, and negative predictive value 100%.

Discussion

In developing countries, H. pylori infection is generally acquired early in childhood, and frequency increases in line with low socioeconomic conditions, family lifestyle and low educational level of the family members[9-11]. Our findings indicate that Turkish children are more likely to be infected around the age of 10 years.

In our study population of 180 pediatric cases, frequency of H. pylori infection was 42.7%. H. pylori infection rate was determined to be 48.2% among 139 cases with gastrointestinal symptoms, whereas 24.4% of the 41 asymptomatic patients (those without gastrointestinal symptom) were infected. In two studies from Italy, H. pylori incidence in symptomatic children was reported as 52.3% and 56%, similar to our results[12,14]. Mitchell et al.[15] determined this ratio to be 14.1% in their study population in Australia. Our results are in accord with two other studies from our country in which H. pylori positivity was reported to be 41.3% and 53.1% in symptomatic patients[16,17].

Frequency of H. pylori infection was highest (65.4%) in 26 patients with dyspepsia as the main symptom. Association of nonulcer dyspepsia with H. pylori positivity has been shown previously[18]. Nevertheless, some authors have failed to demonstrate a relationship between dyspepsia and H. pylori status[19]. We believe that our sample size and characteristics are adequate to suggest a significant association. No significant difference existed between the groups with different gastrointestinal symptoms other than dyspepsia with regard to H. pylori infection rate.

Several previous studies indicate that the percentage of antral nodularity in children infected with H. pylori may vary between 30-100%[15,20,23]. In our study group this ratio was even lower (27.3%), suggesting a decreased sensitivity. In their prospective study, Ganga-Zandzou et al.[24] clearly demonstrated that the frequency of nodular gastritis increased significantly, from 11% at the baseline to 64%

at the end of one year and to 80% at the end of the second year, in parallel to the duration of H. pylori infection. Though not very sensitive, especially in the early periods of infection, antral nodularity was also a specific finding for H. pylori infection in our pediatric patients. Nevertheless, it may rarely be seen in the absence of H. pylori infection[23]. In our study, only two of the 23 patients with antral nodularity (8.7%) were H. pylori negative. It may be suggested that these patients had experienced H. pylori infection previously and that after antral nodularity developed, the infection spontaneously cleared, as has been reported by several investigators[24,25]. Reversal of antral nodularity might take some time after the microorganism is cleared.

When H. pylori infection rate was determined in children with different endoscopical findings, antral nodularity was the endoscopical finding most suggestive of H. pylori positivity; 91.4% of patients with antral nodularity were infected (Table III). The finding of such a high rate of infection among our pediatric patients with endoscopical gastritis suggests that H. pylori might be responsible for most cases of gastritis in Turkish children. Frequency of H. pylori infection among children with gastritis has been reported to vary between 41-82.4% in different populations[26,27]. H. pylori incidence in children with duodenal ulcer ranges between 33-100% in different studies[15,28]. H. pylori seems to contribute highly to the development of duodenal ulcer, evidenced by a high ratio of H. pylori infected children among those with duodenal ulcer in our pediatric population. Our finding of a significantly higher frequency of duodenal ulcer among H. pylori positive children compared to the H. pylori negative group is also supportive of this association (Table III). In our study group, 16.6% of children with esophagitis were infected whereas 20% of those with endoscopically apparently normal mucosa were found to be H. pylori positive.

Although the difference between these two groups is not significant, the low rate of infection among the children with esophagitis is suggestive of the negative correlation between H. pylori and esophageal inflammation reported in adults[29,30]. Our inability to demonstrate a significant difference between H. pylori positive and negative groups in terms of the incidence of esophagitis and gastric ulcer might be due to the limited number of children with these findings in our study group.

Distribution of histopathological findings and their association with H. pylori positivity were also evaluated in our pediatric population. Overall, histological gastritis was observed in 122 of 180 patients: 106 had chronic gastritis (86.8%), while 16 had chronic active gastritis (132.2%). In compliance with our findings, it has been previously shown that chronic active gastritis is more frequently encountered in adults and histological activity is minimal in children[15,22]. In our study group, frequency of H. pylori infection did not differ significantly in patients with chronic gastritis and chronic active gastritis, implying that histological activity of the gastritis was not suggestive of the presence of H. pylori infection (75% vs 65.5%, $p>0.05$). However, H. pylori infection rate was 62.2% in children with histological gastritis, whereas only 1.7% of children with normal histology were found to be H. pylori positive ($p<0.01$). Though rare, H. pylori positivity associated with normal gastric mucosa may be explained in two ways: infection can be at a very early phase during the endoscopic examination, or the bacteriological type of the H. pylori variant may affect the degree of mucosal damage[27,31]. Nevertheless, many investigators claim that H. pylori infection inevitably causes chronic inflammation and that the absence of antral inflammation should exclude H. pylori infection[32-34].

We found that determining the presence of H. pylori IgG antibodies in the sera had a high diagnostic yield in our pediatric population. H. pylori IgG were positive in all of the 77 infected patients as well as in all of the 10 H. pylori infected children with duodenal ulcer and in two of the 103 noninfected children, providing a sensitivity of 100%, specificity of 98%, positive predictive value of 97.4% and negative predictive value of 100%. Only two of the noninfected children who had not received antibiotics at least in the preceding four weeks had H. pylori IgG positivity (1.1%). This may be explained by false positivity due to cross-reaction with other IgG antibodies or failure to normalize of the high antibody levels after the infection was cleared.

Marked differences in both sensitivity and specificity of ELISA in children have been reported[35-37]. Oliveria et al.[12] found H. pylori IgG antibodies to be positive in all 20 infected children with duodenal ulcer while 54 of the

68 infected (79.4%) and five of the 62 noninfected children (8.1%) were determined to be antibody positive, suggesting lower sensitivity, specificity, and positive and negative predictive values (79.4%, 91.9%, 91.5% and 80.3%, respectively) in children without duodenal ulcer than those we observed. These findings have led us to conclude that though ELISA provides high sensivity and specificity in children with dueodenal ulcer in the diagnosis of H. pylori infection, it is not a reliable method in 2-11 year-old pediatric patients without duodenal ulcer. Because immune responses against H. pylori infection can take a few months, antibody test results might be false negative during the early periods of infection. Furthermore, the cutoff value of the antibody tests must be determined individually for each population. In our study, 20 U/ml was accepted as the cutoff value in accordance with the manufacturer's recommendations. Values higher than 20 U/ml were considered positive and values lower than 12.5 U/ml were considered negative. Our finding of H. pylori antibody positivity in all infected children in our study group can be associated with the long duration of the infection as a consequence of acquiring the H. pylori infection at an early age or with a high bacterial load. In light of the low rate of nodular gastritis in our patients, suggesting a shorter duration of infection, another possibility is that a more immunogenic strain, such as Cag-A protein, might have influenced the outcome. Unfortunately, we were not able to study H. pylori strains in our population. However, Mitchell et al.[38] indicated that particular ethnic or socioeconomic groups may be more susceptible to infection with Cag-A positive strains of H. pylori[38]. In this context, contribution of Cag-A and other specific H. pylori antigens to the pathogenesis of infection in Turkish children remains to be determined.

In conclusion, we found that ELISA assay provides a sensitivity of 100% and a specificity of 98% in the diagnosis of H. pylori infection in our pediatric population, independent of the age and the presence of duodenal ulcer, contradicting the results of Oliveria et al.[12] in 63 Brazilian children (79.4% and 91.9% respectively) and the results of Ni YH et al.[37] in 53 Taiwanese children (88.9% and 80.9%, respectively). We strongly suggest that every population must determine its own infection characteristics and accuracy of diagnostic tests. ELISA test for IgG antibodies against H. pylori proves to be an inexpensive and reliable method in the primary diagnosis of H. pylori infection in Turkish society regardless of the age. However, since serological investigation cannot predict the underlying gastrointestinal pathology, upper gastrointestinal endoscopy should be performed for final diagnosis in children with positive H. pylori IgG antibodies. In addition to revealing the mucosal lesions, the positivity of at least two of the CLO test, or histological or microbiological investigations in gastric biopsy samples obtained from antrum and corpus mucosa will be confirmatory in the diagnosis.

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Power spectral analysis of heart rate variability in children with aortic stenosis

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Aortic stenosis is a progressive disorder and can be the cause of serious arrhythmias and possibly sudden death. Evaluation and follow-up of the autonomic nervous system may provide some useful information for management of the disease. Our study aimed to examine heart rate variability in children with aortic stenosis in the supine position and to detect the changes in autonomic activity during head-up tilt testing. Sixteen patients and 11 healthy controls participated in the study. In the supine position, seven minutes of continuous echocardiographic (ECG) recording was performed, followed by four consecutive ECG recordings, each consisting of seven minutes in 70° tilt position. To obtain power spectrums, the tachograms were taken on the autoregressive mode. The mean RR interval duration, standard deviation of RR interval, central frequencies of low and high frequency oscillations, their powers, total power and percents of normalized low and high frequency powers were accepted for statistics. There were no significant differences between the groups in the supine position. In tilt position, mean RR interval and its standard deviation were decreased in both groups. The central frequency of low freuquency power significantly ($p<0.05$) shifted to left, normalized low frequency power increased and normalized high frequency power decreased in the control group at the beginning of tilt position, but at the second phase of tilt position in the patient group. We conclude from the results that children with mild-to-moderate aortic stenosis reflect delayed response to sympathetic provocation.

Key words: heart rate variability, aortic stenosis, tilt table testing, children.

Analysis of heart rate variability (HRV) is a reliable and noninvasive method for assessing cardiovascular autonomic control[1-4]. Reduced HRV has been found to be related with high cardiovascular mortality including sudden cardiac death in adult patients with myocardial infarction[5-6], chronic heart failure[7], left ventricular hypertrophy[8] and diabetic neuropathy[9]. While HRV became very popular in the field of adult cardiology, there are only a few reports available in pediatric literature[10-12]. Although impairment of HRV was reported in elderly patients with severe aortic stenosis[13,14], to our knowledge cardiovascular autonomic tone in children with mild-to-moderate aortic stenosis has not been studied. Because aortic stenosis is a progressive disorder and can be the cause of serious arrhythmia and possibly sudden death, evaluation and follow-up of the autonomic nervous system may provide some useful information for management of the disease. The aim of our study was to examine HRV in children with aortic stenosis in the supine position and to detect the changes in autonomic activity during the head-up tilt table testing. The head-up tilt table testing was used as a sympathetic provocative technique.

Material and Methods

Subjects

Sixteen patients with aortic stenosis and 11 healthy children participated in the study. One patient who developed syncope at 10th minute of head-up tilt testing was excluded. Therefore, data were analyzed from 15 patients (3 girls,

12 boys) ranging in age from 7 to 15 years (mean 9.7±3.7 years), and 11 healthy controls (4 girls, 7 boys; mean age, 9.3±2.1 years; range 7 to 15 years). All subjects were screened with a detailed history and physical examination. They were not taking any medication prior to the study. The procedures of the study were explained to each child and his or her family, and an informed consent obtained. The patients underwent echocardiographic study before echocardiographic (ECG) recordings and exercise testing afterwards. Echocardiographic study and exercise testing were not performed in the control group.

Echo-Doppler Study

Echo-Doppler studies were done using General Electric RT 6800 echocardiograph with 3.5 and 5 mHz transducers. End-diastolic and end-systolic diameters of the left ventricle and thickness of the interventricular septum and posterior wall were noted in parasternal long-axis window, then ejection fraction and fractional shortening were calculated. Left ventricular outflow tract and the structure of the aortic valve were evaluated by 2-D and M-mode echocardiography. Peak systolic pressure gradient between the left ventricle and aorta were determined by CW Doppler.

Recording and Analysis of the ECG Signals

All subjects were studied between 10:00 a.m.-12 noon after a light breakfast free of caffeine. The room was quiet, with dim lighting and a comfortable temperature (22°C). The subjects were loosely strapped to the tilt table that had a foot-broad support and was electrically driven. To avoid emotional stress, we did not perform any vascular intervention or blood pressure measurement during the study. The subjects were asked to breathe normally. After at least a 10-minute adaptation period in the supine position, seven minutes of continuous ECG recording was performed. At the end of supine recording, we rotated the table to head-up right tilt position with an angle of 70°, and four consecutive ECG recordings (tilt phase 1 to 4), each of seven minutes, duration, were done. Therefore, a total of 35 minutes continuous ECG recording (seven minutes in supine, 28 minutes in tilt position) were performed.

All recordings were done using a personal computer based high resolution ECG system (Kardiosis® Ars-LP). Bipolar X, Y and Z leads (0.5-340 Hz) and common-mode line interference signal on the body surface were recorded simultaneously. All signals were sampled at a rate of 1,000 samples/second and digitized using a 12-bit A/D converter. To obtain power spectrums, the tachograms were taken on the autoregressive mode with order 6, and power spectral densities were calculated. Very low frequencies (<0.03 MHz) were filtered before calculation. The mean RR interval duration, standard deviation of RR interval, central frequencies of low frequency (LF) and high frequency (HF) oscillations, their powers (LFP and HFP), total power (TP), normalized LFP and HFP were accepted for statistics.

Exercise Testing

After ECG recordings, all patient underwent exercise testing using Marquette 2000 tread-mill with Bruce Protocol. Tread mill stopped when desired maximal heart rate was attained or patient fatigued or when ST segment changes were observed on ECG monitor.

Statistical Analysis

Statistical analysis was done using SPSS for Windows Release 6.0 Data are presented as mean±SD. If the ±SD is near or higher than mean value, data are presented as mean ±SD (median). Wilcoxon matched-pairs signed-ranks test was used for comparing supine and tilt position results of each group. Mann-Whitney U-Wilcoxon rank sum W test was used for comparing counter groups. A p-value <0.05 was considered significant.

Results

The mean weights of the patient and control groups were found similar (29.4±11.2 kg vs 33.5±10.2 kg). There was also no significant difference in the mean heights of the patient and control groups (130.0±21.2 cm vs. 136.8±10.6 cm).

Ejection fraction and fractional shortening were found in normal ranges in all patients (74.0±3.9 and 42.5±3.7%, respectively). 2-D echocardiography showed subvalvular membranous aortic stenosis in two patients and valvular aortic stenosis in others. Color Doppler showed first-degree aortic insufficiency in five patients and second-degree in one patient. The peak systolic

transvalvular aortic gradients which were measured with CW Doppler were found ranging between 20-50 mmHg (mean 34.8±9.5 mmHg).

All patients completed at least three stages (nine minutes) of exercise testing with Bruce protocol. No patient developed chest pain or significant ST change on ECG monitor.

HRV Analysis

There were no significant differences between RR tachograms (mean RR interval and standard deviation of mean RR interval) and power spectral analysis (central frequency of LF and HF, their powers, total power, normalized LFP and HFP) results of patients and control groups in the supine position (Table I).

The changes in HRV parameters during tilt position are summarized in Table II (patient group) and Table III (control group). Figure 1 shows typical examples of power spectral analysis graphics of the supine position and first phase of tilt recordings.

The RR tachogram showed similar changes in both groups by tilting: mean RR interval and its standard deviation were decreased and remained stable during whole tilt testing.

The central frequency of LF shifted to left (decreased) by tilting in both groups. however, this shift became significant in the second period of tilt testing in the patient group, but in the first period of tilt testing in the control group.

The central frequency of HF did not change significantly during tilt testing in the control group, but it decreased in the fourth (last) period of tilt testing in the patient group.

In both groups, the power of LF decreased in the first period, then increased in the second period and remained stable, slightly under supine values.

Total power and the power of HF decreased at the beginning of tilt testing and remained stable until the end of testing in both groups. The percentage of normalized LFP increased significantly, while the percentage of normalized HFP was decreasing by tilting. These changes became significant in the second phase of tilt testing in the patient group, but in the first phase in the control group (Fig. 2). The LF/HF ratio was not significantly different in patient and control groups during supine position. In the first phase of tilt position, LF/HF ratio of the patient group was significantly lower than of the control group ($p<0.05$), but this difference disappeared in the 2nd, 3rd and 4th phases of tilt position.

Table I. HRV parameters of control and patient groups in supine position

Variable	Units	Patient mean±SD (median)	Control mean±SD (median)	p
CF1	Hz	0.102±0.027	0.105±0.018	>0.05
CF2	Hz	0.322±0.037	0.308±0.043	>0.05
LFP	ms^2	1907±1376 (1462)	1727±1185 (1433)	>0.05
HFP	ms^2	990±1232 (560)	1490±1986 (882)	>0.05
TP	ms^2	2898±2265 (2609)	3218±2279 (2316)	>0.05
NLFP	%	70±15 (73)	64±20 (63)	>0.05
NHFP	%	30±15 (27)	36±20 (36)	>0.05
MRR	ms	711.32±97.74	693.73±87.65	>0.05
MRRSD	ms	59.73±23.46	62.41±22.78	>0.05

HRV : Heart rate variability.
CF1 : Central frequency of low frequency oscillation.
CF2 : Central frequency of high frequency oscillation.
LFP : Low frequency power.
HFP : High frequency power.
TP : Total power.
NLFP : Normalized LFP.
NHFP : Normalized HFP.
MRR : Mean R-R interval.
MRRSD : Standard deviation of MRRR.
Hz : Hertz.
ms : millisecond.
* $p<0.05$.

Table II. Comparison of supine and tilt results of patient group [Mean±SD (median)]

Variable	Units	Supine	Tilt 1st phase	Tilt 2nd phase	Tilt 3rd phase	Tilt 4th phase
CF1	Hz	0.102±0.027	0.092±0.022	0.088±0.015*	0.086±0.013*	0.091±0.016
CF2	Hz	0.322±0.037	0.297±0.053	0.298±0.047	0.293±0.051	0.275±0.041*
LFP	ms²	1907±1376 (1462)	1159±808* (936)	1449±1173 (936)	1387±1012 (1085)	1480±1060 (1266)
HFP	ms²	990±1232 (560)	548±934* (166)	412±729* (164)	432±822* (195)	375±536* (174)
TP	ms²	2898±2265 (2609)	1708±1556* (1285)	1861±1805* (1100)	1819±1677* (1183)	1841±1492* (1269)
NLFP	%	70±15	75±15	84±10*	82±12*	84±10*
NHFP	%	30±15	25±15	16±10*	18±12*	15±10*
MRR	ms	711±97	632±82*	625±75*	616±67*	615±73*
MRRSD	ms	59±23	47±19*	47±19*	46±18*	46±18*

CF1 : Central frequency of low frequency oscillation.
CF2 : Central frequency of high frequency oscillation.
LFP : Low frequency power.
HFP : High frequency power.
TP : Total power.
NLFP : Normalized LFP.
NHFP : Normalized HFP.
MRR : Mean R-R interval.
MRRSD : Standard deviation of MRRR.
Hz : Hertz.
ms : millisecond.
* $p<0.05$.

Table III. Comparison of supine and tilt results of control group [Mean±SD (median)]

Variable	Units	Supine	Tilt 1st phase	Tilt 2nd phase	Tilt 3rd phase	Tilt 4th phase
CF1	Hz	0.105±0.018	0.088±0.019*	0.086±0.015*	0.087±0.009*	0.087±0.012*
CF2	Hz	0.308±0.043	0.293±0.045	0.294±0.053	0.277±0.043	0.281±0.037
LFP	ms²	1727±1185 (1433)	1063±687* (1048)	1212±617 (1096)	1379±505 (1443)	1262±914 (1115)
HFP	ms²	1490±1986 (882)	213±170* (164)	192±159* (146)	183±124* (186)	225±220* (104)
TP	ms²	3218±2779 (2316)	1267±808* (1238)	1405±715* (1267)	1562±589* (1556)	1488±1068* (1241)
NLFP	%	64±20	83±9*	86±8*	89±6*	86±10*
NHFP	%	36±20	16±9*	14±8*	11±6*	14±10*
MRR	ms	693±87	573±51*	573±40*	565±44*	564±41*
MRRSD	ms	62±22	42±11*	42±10*	43±9*	43±14*

CF1 : Central frequency of low frequency oscillation.
CF2 : Central frequency of high frequency oscillation.
LFP : Low frequency power.
HFP : High frequency power.
TP : Total power.
NLFP : Normalized LFP.
NHFP : Normalized HFP.
MRR : Mean R-R interval.
MRRSD : Standard deviation of MRRR.
Hz : Hertz.
ms : millisecond.
* $p<0.05$.

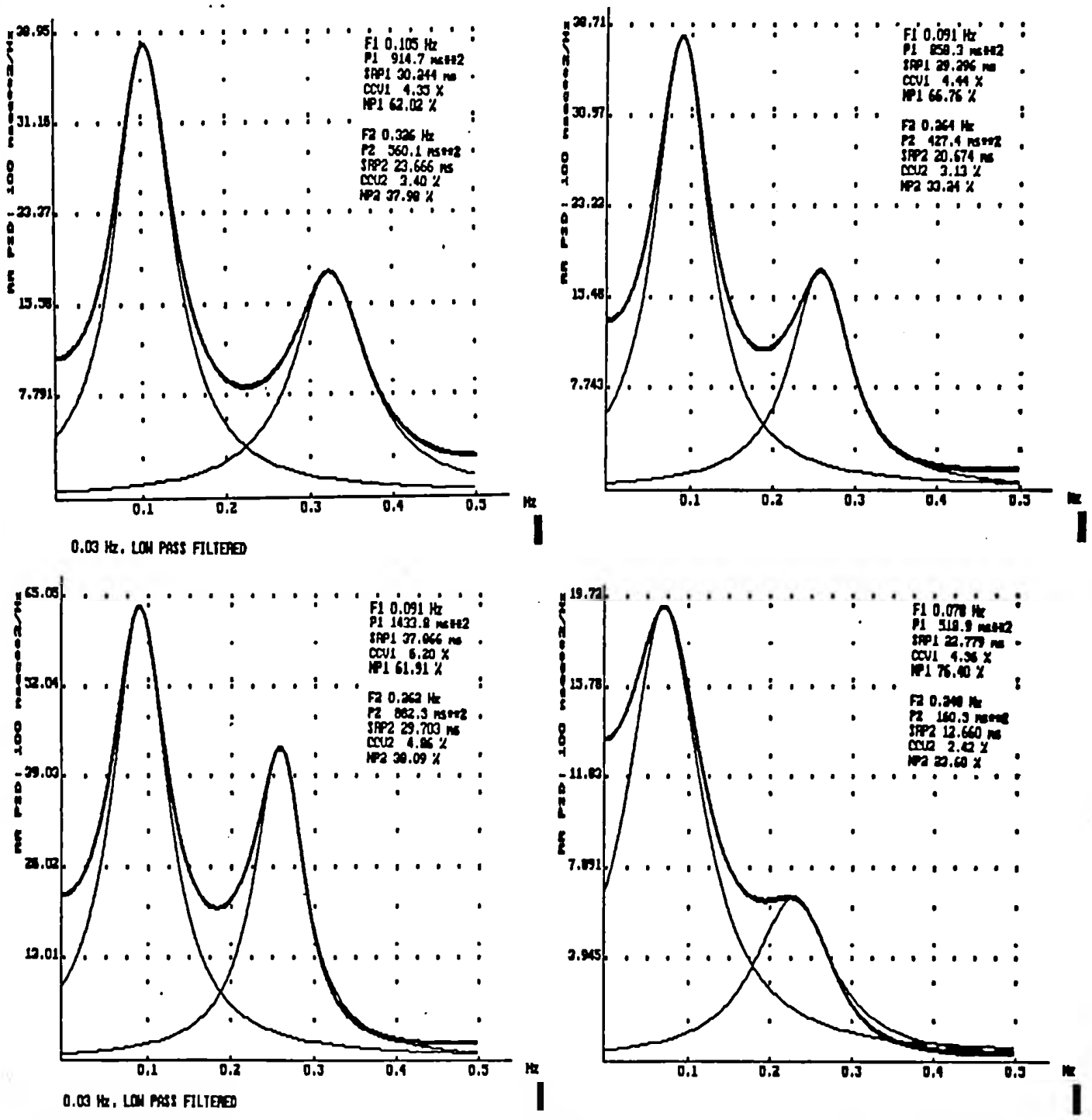

Fig. 1. Typical graphics of power spectral analysis; top left: patient supine, top right: patient first phase of tilt, bottom left: control supine, bottom right: control first phase of tilt. F1: Central frequency of low frequency, P1: Power of low frequency, NP1: % of normalized low frequency power, F2: Central frequency of high frequency, P2: Power of high frequency, NP2: % of normalized high frequency power.

Discussion

Power spectral analysis of heart rate variability has been accepted as a reliable, noninvasive tool for the assessment of sympathetic and parasympathetic control of the cardiovascular system in children as well as in adults[15-17]. To our knowledge, this is the first study which investigates HRV in children with aortic stenosis in supine and tilt positions. Dobutamine and isoproterenol are used in adults as sympathetic provocatives in stress echocardiography[18,19]. In the supine position, we did not find any significant difference between heart rate tachograms and power spectral analysis of HRV of children with aortic stenosis and of healthy controls. Some previous studies showed impairment of HRV in adult patients with severe aortic stenosis[13-14], and autonomic dysfunction tends to normalize within the first year of valve replacement[20]. Therefore, we can say that

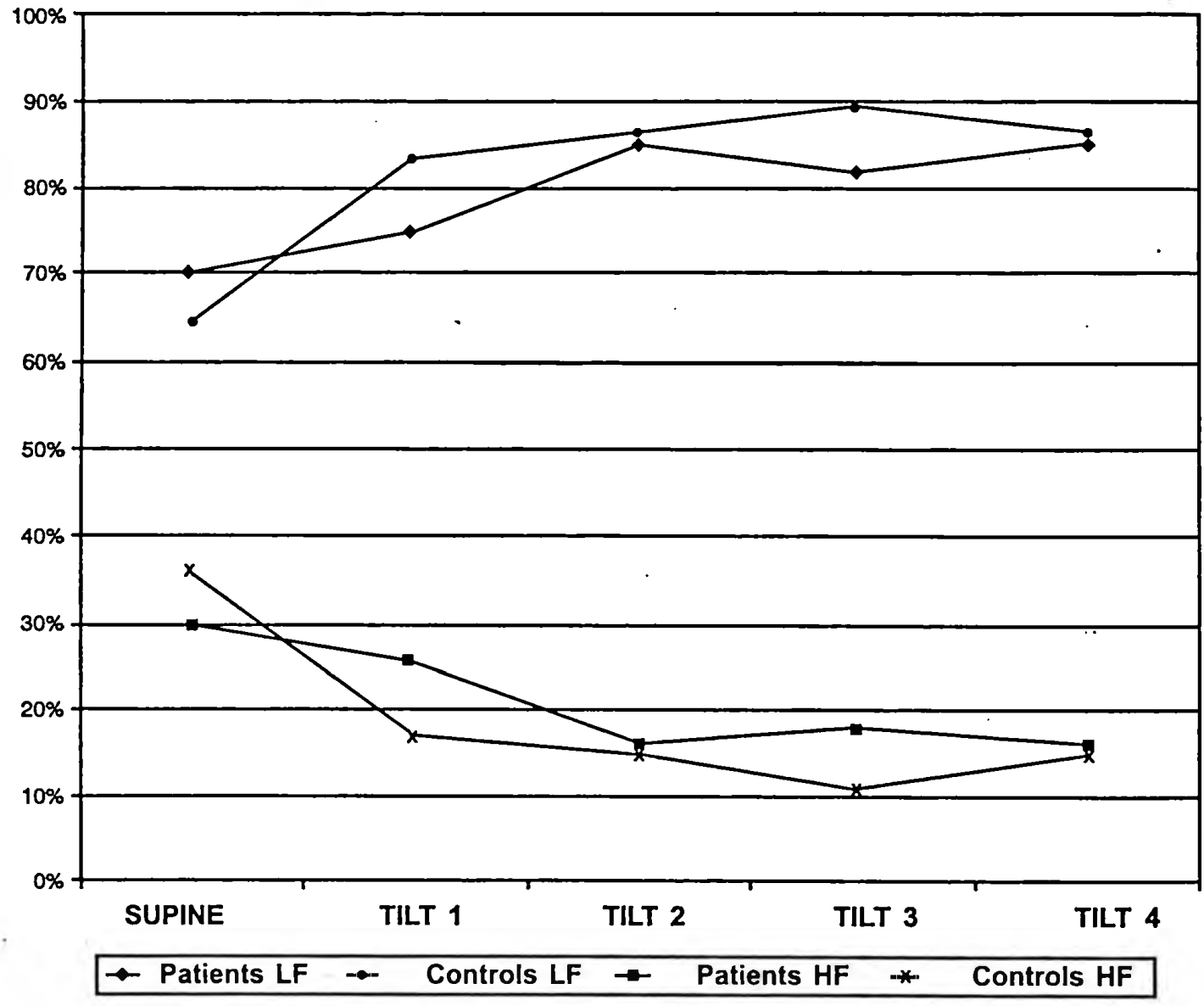

Fig. 2. Comparison of the percentages of normalized low frequency (LF) power and percentages of normalized high frequency (HF) power during tilt position phases. LF increased and HF decreased significantly by the first phase of tilt position in the control group and by the second phase of tilt in the patient group.

impairment of HRV is related to severity and duration of aortic stenosis.

Head-up tilt testing has become a widely accepted tool in the clinical evaluation of patients presenting with syncopal symptoms[21-23]. Head-up tilt position has been known to cause sympathetic excitation, vagal withdrawal and related heart rate changes[16-17]. In our study, both patients and controls showed similar response to tilt position: shifting of central frequency of LF to the left, decreased HF and total powers, and increase in normalized LF power and decrease in normalized HF power, thus an increase in normalized LF/HF ratio. However, there were some important differences between the groups in the timing of response to tilt testing. The patient group showed significantly delayed shifting of central frequency and also showed delayed increase in normalized F/HF ratio. It reflects that children with mild-to-moderate aortic stenosis have delayed response to sympathetic provocation. Previous studies have shown that LF/HF ratio is the best predictor of sympathovagal balance[2,4]. While LF has been found to be related with both the sympathetic and vagal limb of the autonomic nervous system, HF is accepted to be related only with vagal activity. The physiological interpretation of very low frequency component is not well known[2-3], thus we filtered very low frequency (<0.03 Hz) before power spectral analysis. In our study, we showed that augmentation of normalized LF/HF ratio is mainly due to decreased HF. The

major question that must be answered is why children with aortic stenosis showed a delayed response to sympathetic provocation. As is known, parasympathetic activity of the autonomic nervous system increases the fibrillation threshold and appears to protect against malignant ventricular tachyarrhythmias and related sudden cardiac death, while sympathetic activity decreases the threshold and predisposes to ventricular tachyarrhythmias[4]. We believe that in children with mild-to-moderate aortic stenosis, sympathovagal balance (LF/HF ratio) is well preserved and remains stable at the first phase of tilt position for protection against sudden predominance of sympathetic activity and related ventricular tachyarrhythmias. Although there was no statistically significant difference between control and patient groups in the supine position, a slight sympathetic predominance was detected in the patient group, and this chronic sympathetic activity could be the cause of delayed response to tilt position. When patients with aortic stenosis age, aortic stenosis becomes more severe and physical activity diminishes due to cardiac failure, rendering the protecting mechanism inadequate and increasing the risks of serious ventricular tachyarrhythmias and sudden cardiac death. Further studies with larger groups and wider symptomatology are needed to clarify our hypothesis.

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Proliferation of myeloid lineage cells and apoptosis of lymphoblastic leukemic cells induced by short-course high-dose methylprednisolone in patients with acute lymphoblastic leukemia

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SUMMARY: Yıldıran A, Erduran E, Tekelioğlu Y, Dilber E, Gedik Y. Proliferation of myeloid lineage cells and apoptosis of lymphoblastic leukemic cells induced by short-course high-dose methylprednisolone in patients with acute lymphoblastic leukemia. Turk J Pediatr 2002; 44: 116-121.

In this paper, we investigated the effects of short-course high-dose methylprednisolone (HDMP) treatment on the proliferation of myeloid lineage cells and on apoptosis of blast cells in eight children with acute lymphoblastic leukemia (ALL). The patients were given the HDMP treatment (30 mg/kg/d, perorally) before 9:00 a.m. for seven days. Bone marrow (BM) aspiration was done at days 0 and 3 of the HDMP treatment in all patients and at the 7th day of the HDMP treatment in six patients. Bone marrow blast cells had gradually decreased after the HDMP treatment by the 7th day. There were statistically significant differences between the mean percentages of BM blast cells at days 0 and 3, days 0 and 7, and at days 3 and 7 ($p<0.05$). The mean percentages of blast cell apoptosis at the 3rd day was significantly higher than at days 0 and 7, and apoptosis at day 0 was significantly lower than at the 7th day ($p<0.05$). The mean percentages of BM myeloid lineage cells at the 7th day was significantly higher than at days 0 and 3 ($p<0.05$), and the mean percentage at day 0 was significantly lower than at the 3rd day ($p<0.05$).

These findings indicate that short-course HDMP treatment causes apoptosis on lymphoblasts and increases the proliferation of myeloid lineage cells in children with ALL.

Key words: acute lymphoblastic leukemia, short-course high-dose methylprednisolone, proliferation of myeloid lineage cells, apoptosis of lymphoblastic leukemic cells.

High-dose methylprednisolone (HDMP) is used in the treatment of acute lymphoblastic leukemia (ALL) during remission induction chemotherapy[1-3]. HDMP during remission induction chemotherapy improves long-term event-free survival (EFS), especially for high-risk patients with ALL[1]. HDMP also causes acceleration of leukocyte recovery in children with ALL[4]. It was suggested that this effect of HDMP could be due to the increase of the serum granulocyte colony stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) levels[5]. On the other hand, HDMP causes apoptosis and differentiation of leukemic cells in patients with acute myeloblastic leukemia (AML)[6-10]. In this study, proliferation of myeloid lineage cells and apoptosis of blast cells induced by short-course HDMP was evaluated in patients with ALL.

Material and Methods

Eight patients with ALL were enrolled in the study with consent of their parents.

All patients received HDMP in a dose of 30 mg/kg/d perorally before 9:00 a.m. for seven days. Bone marrow (BM) aspiration was done at days 0 and 3 of the HDMP treatment in all patients and at the 7th day of the HDMP treatment in six patients for determination of the myeloid lineage cell proliferation and apoptosis of the blast cells. Flow cytometric analyses of BM aspiration materials were done at diagnosis, and CD_2, CD_3, CD_7, CD_{10} (CALLA), CD_{13}, CD_{14}, CD_{19}, CD_{20}, CD_{22}, CD_{33},

CD_{45}, HLA DR, and MPO monoclonal antibodies were used for the phenotyping of blast cells and of the myeloid population.

Bone marrow smears were stained with Wright's dye and 200 cells of bone marrow were counted under the light microscope for each patient. Differential counts of bone marrow cells were calculated as percentages. Apoptosis of the blast cells was determined by flow cytometric analysis. Two parents did not permit BM aspiration at the 7th day. Patients were treated with only HDMP 30/mg/kg/d for seven days, after which HDMP was given 20 mg/kg/d for seven days, and thereafter at 20 mg/kg/alternate day for 14 days in addition to vincristin (0.05 mg/kg in a week for 4 weeks), L-asparaginase (200 u/kg 9 times in 4 weeks), daunorubicin (1 mg/kg 3 times in 4 weeks), and cytosine arabinoside (10 mg/kg 3 times in 4 weeks) as remission induction treatment.

No side effects occurred from the administration of HDMP except for cushingoid appearance and weight gain in all patients.

Separation of Blast Cells and DNA Analysis

Obtained BM aspiration samples at days 0, 3 and 7 of the HDMP treatment were drawn into the tubes with EDTA. Lymphoblasts were obtained by lymphocyte separation medium (Gibco BRL 13010-012). 100 µl aliquots of lymphoblasts were put into the tubes and fixed in DNA prep (Coulter Epics Leukocytes Preparation Workstation) using DNA prepstain (Coulter PN 6604451) and DNA prep LPR (Coulter PN 6604454) kits. All tubes were kept at room temperature in a dark place for 20 minutes. Then, the tubes were treated with Coulter Epics Elite ESP Flow-cytometry. DNA analyses were done using Multicycle AV software (Advanced Version Cell Cycle Analysis, Phoenix Flow Systems, San Diego). Data on cell cycle (G_0/G_1, G_2/M and S phases) cytometry histograms of blast cells displayed a peak less than 2n DNA (Sub G_1), which generally accepted as an indication of cells undergoing apoptosis[11,12].

The proliferation of myeloid lineage cells was determined on gating of forward scatter and PMT_1 parameters in the same software. Gating on flow cytometry was performed according to total mononuclear cells indicating CD_{45} positivity.

Statistical Analysis

The percentages of blast cells of BM and apoptosis of blast cells were calculated as arithmetic mean±standard deviation (mean±SD).

The percentages of blast cells and myeloid lineage cells, and blast cell apoptosis were reciprocally compared for days 0, 3 and 7 of the HDMP treatment using Wilcoxon Rank Sum test.

Results

Bone marrow aspiration smears of the patients exhibited L_1 type morphology in four patients, L_2 morphology in two patients and L_3 type morphology in two patients according to French-American-British (FAB) system. According to ALL prognostic criteria, two patients had high-risk criteria and six patients had low-risk criteria[13]. Bone marrow karyotype analyses could not be performed because of technical limitations.

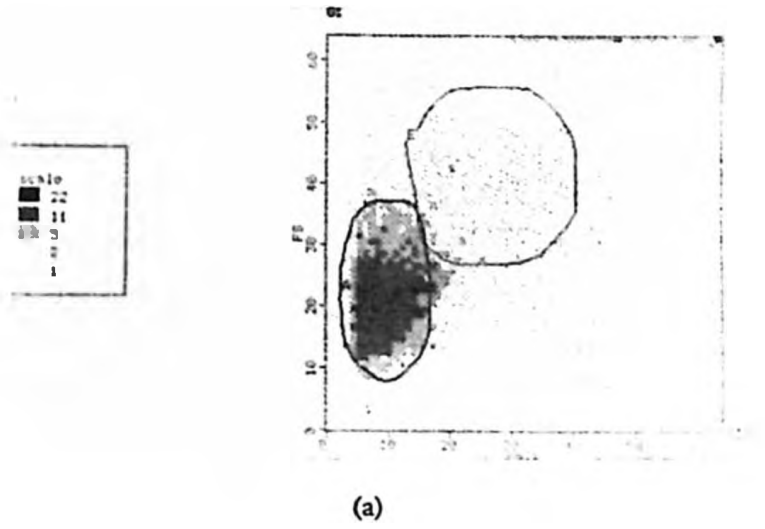
The characteristics of the patients are shown in Table I.

Apoptosis of blast cells was determined by flow cytometric analysis and according to the morphologic appearances of blast cells on light microscope. In addition to their morphologic changes, flow cytometric analysis of BM obtained at days 0, 3 and 7 after HDMP treatment disclosed the new cell population (myeloid lineage cells) (Fig. 1 a-c).

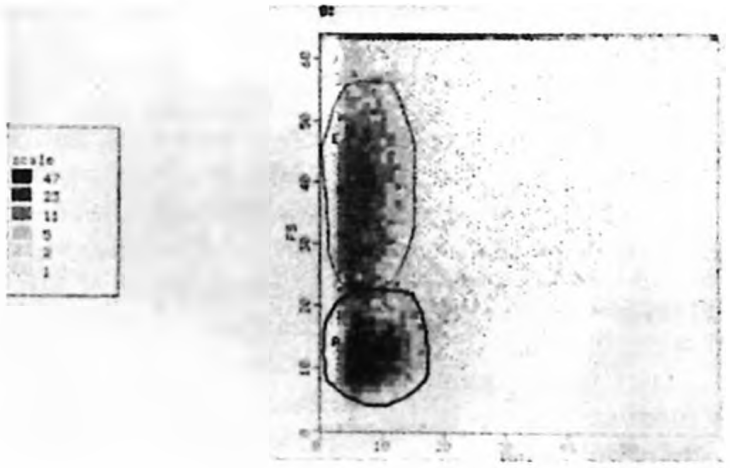
Table I. Patients' characteristics and diagnosis

Patients	Sex	Age	FAB	Leukocytes (/µl)	Diagnosis	Risk group
1	M	5	L3	12,600	B Cell ALL	Low
2	M	7	L1	11,00	B Cell ALL	Low
3	M	7	L1	9,200	Pre-B Cell ALL	Low
4	M	16	L2	38,500	Mixed ALL	High
5	M	7	L1	14,200	Mixed ALL	Low
6	F	8	L3	72,800	B Cell ALL	High
7	M	5	L2	13,700	B Cell ALL	Low
8	M	6	L1	18,900	Pre-B Cell ALL	Low

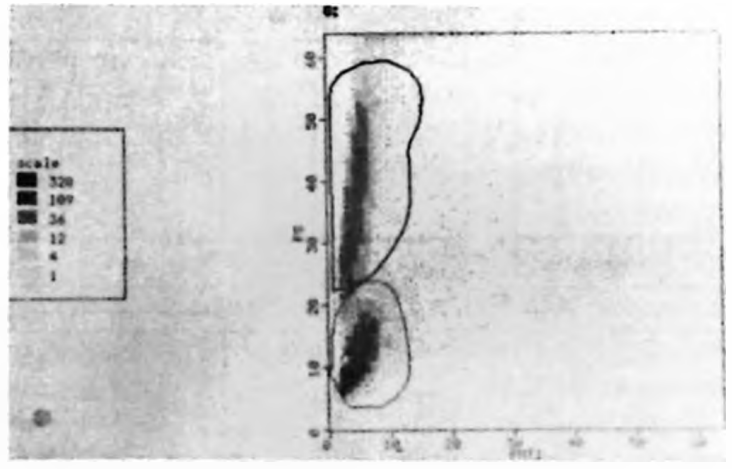
FAB: French-American-British classification. ALL: acute lymphoblastic leukemia.



(a)



(b)



(c)

Fig. 1. A flow cytometric profile of bone marrow of Case 3 on days, a) 0, b) 3 and c) 7 after HDMP treatment. Note the appearance of new cell population (myeloid lineage cells).

Flow cytometry histograms of blast cells displayed a peak less than 2n DNA (Sub G_1), which is indicative of cells undergoing apoptosis, at days 0, 3 and 7 of the HDMP treatment (Fig. 2 a-c).

The percentages of BM blasts, myeloid lineage cells, and apoptosis of blast cells according to flow cytometry histogram of the patients at days 0, 3 and 7 of the HDMP treatment are shown

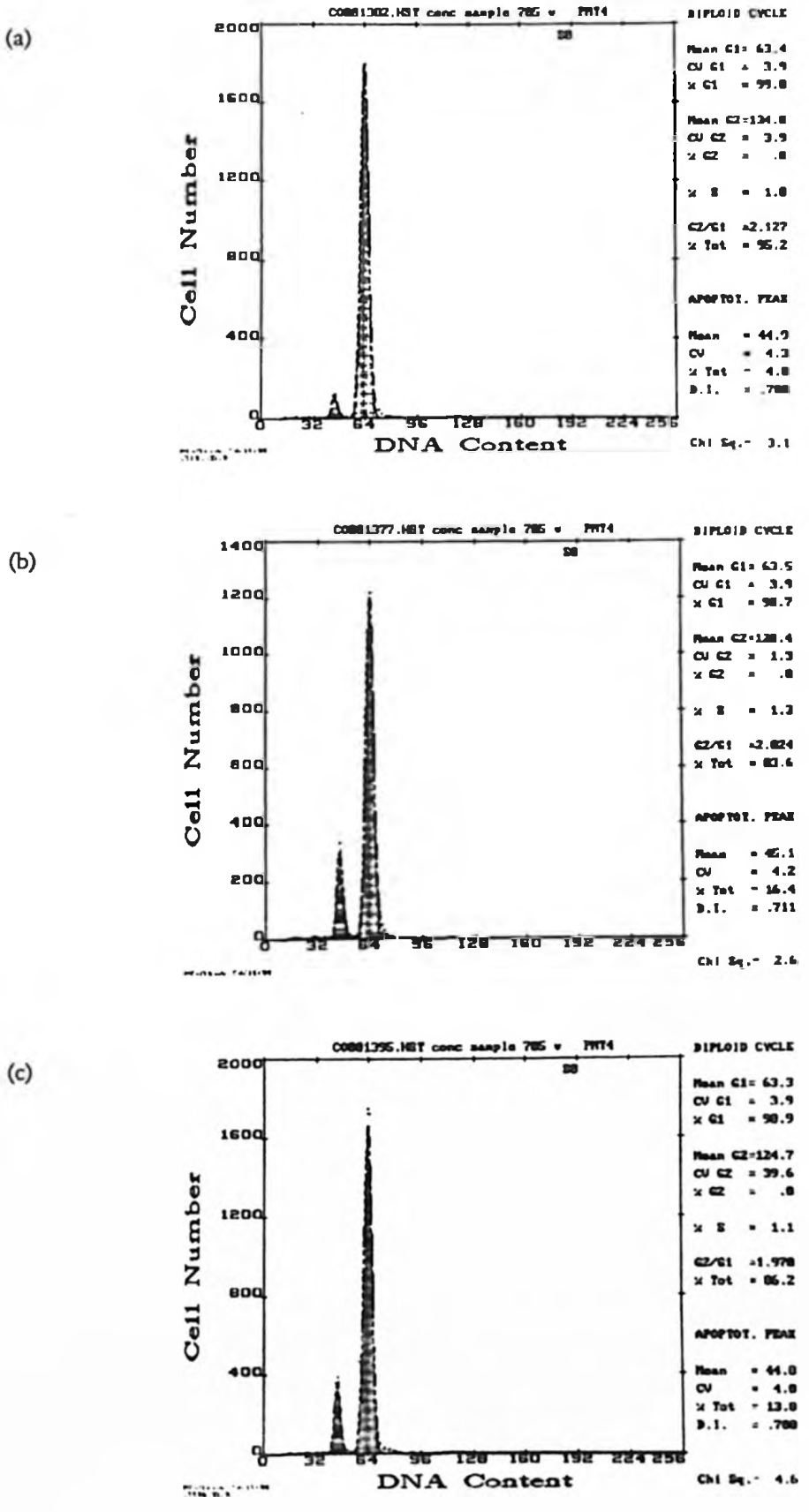


Fig. 2. The flow cytometry histogram of lymphoblasts depicts a peak less than 2n DNA (Sub G_1) on days a) 0, b) 3 and c) 7.

in Table II. BM blast cell percentages decreased and myeloid lineage cells increased by the 7th day, gradually. The percentages of blast apoptosis peaked at day 3.

Table II. Percentages of bone marrow blasts, myeloid lineage cells, and apoptosis of lymphoblasts according to flow cytometry histogram of patients on days 0, 3 and 7 after HDMP treatment

	Day 0			Day 3			Day 7		
Patients	BMB	Apo	Mye	BMB	Apo	Mye	BMB	Apo	Mye
1	95	3.3	1	84	26.3	5	72	17.8	12
2	82	1.6	5	80	15.5	8	66	5.6	11
3	67	2	5	48	7.9	10	26	4.5	32
4	97	4.5	3	85	24	5	18	9.1	5
5	95	1.3	2	83	10.2	4	69	8.7	13
6	93	4.8	3	83	16.4	8	65	13.8	10
7	95	1.7	2	19	3.5	10	ND	ND	ND
8	98	1.6	1	84	17	7	ND	ND	ND
Mean±sd	90.2±10.6[a]	2.6±1.4[b]	2.7±1.5[c]	70.7±24.3[d]	15.1±7.8[e]	7.1±2.2[f]	52.6±24.0[g]	9.9±5.1[h]	13.8±9.3[i]

BMB: bone marrow blasts (%).
Apo : apoptosis (%).
Mye : myeloid cells (%).
ND : not determined.
a-d, a-g, d-g, b-e, b-h, e-h, c-f, f-i, c-i $p<0.05$.

There were statistically significant differences between the mean percentages of BM blast cells at days 0 and 3, days 0 and 7, and days 3 and 7 ($p<0.05$). The mean percentage of blast cell apoptosis at the 3rd day was significantly higher than at days 0 and 7 ($p<0.05$).

There was significant difference between the mean percentages of blast cell apoptosis on days 0 and 7 ($p<0.05$). The mean percentage of bone marrow myeloid lineage cells at the 7th day was significantly higher than at days 0 and 3 ($p<0.05$). There was significant difference between the mean percentages of BM myeloid lineage cells at days 0 and 3 ($p<0.05$). All patients went into remission after remission induction treatment for four weeks.

Discussion

Two different cell deaths are described in vertebrates. Necrosis develops as the result of ischemia and physical and chemical traumas to the cells. In necrosis, all these events are accompanied by early membrane damage and cell disintegration. Apoptosis develops due to physiologic and immunologic factors. The concept of apoptosis as distinguished from necrosis was proposed about 27 years ago by Kerr et al.[14]. When undergoing apoptosis, cells decrease in size and their nuclei condense. Later in the course of apoptosis, the nuclei and the cells become fragmented, and cellular remnants are phagocytosed by macrophages. Fragmentation is double-stranded cleavage of DNA at internucleosomal sites[15,16]. This is determined by showing ladder-formation on gel electrophoresis of DNA extracted from cells.

Cohen et al.[17] suggested that ladder formation does not always indicate apoptosis. A peak less than 2n (Sub G_1) on flow cytometry is accepted as a typical pattern for cells undergoing apoptosis[11,12,18,19].

Fluorescence in situ TUNEL assay, trypan blue assay and flow cytometry detected DNA strand breaks occurring in apoptosis; flow cytometric detection of apoptosis was more sensitive than TUNEL assay[12]. We selected flow cytometry to look for the apoptotic effect of HDMP on lymphoblasts for this reason, and because ladder-formation on gel electrophoresis of DNA extracted from cells is not considered a diagnosis for apoptosis[17].

In this study, it was found that HDMP induced apoptosis of lymphoblastic leukemic cells and caused proliferation of myeloid lineage cells in children with ALL. It was suggested that HDMP caused differentiation and apoptosis of myeloid leukemic cells in children with AML[6-10]. It was found that the apoptotic effect of HDMP on lymphoblasts peaked at the 3rd day and decreased at the 7th day after beginning HDMP treatment ($p<0.05$). Myeloid lineage cells of BM in the patients gradually increased till the 7th day ($p<0.05$). The proliferative effect of HDMP

on myeloid lineage cells may be due to the increase of serum G-CSF and GM-CSF levels[5]. The serum G-CSF and GM-CSF levels were not evaluated in this study because the increase of serum G-CSF and GM-CSF levels following short-course HDMP treatment have been indicated in patients with ALL previously[5].

We believe that short-course HDMP treatment causes apoptosis on lymphoblasts and proliferation of myeloid lineage cells in children with ALL HDMP-induced apoptosis and the proliferative effect of HDMP on myeloid lineage cells should be evaluated in a larger series of patients with ALL.

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Treatment of developmental dysplasia of the hip before walking: Results of closed reduction and immobilization in hip spica cast

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SUMMARY: Aksoy MC, Özkoç G, Alanay A, Yazıcı M, Özdemir N, Surat A. Treatment of developmental dysplasia of the hip before walking: results of closed reduction and immobilization in hip spica cast. Turk J Pediatr 2002; 44: 122-127.

We retrospectively evaluated 200 hips of 129 patients with the diagnosis of developmental dysplasia of the hip treated with closed reduction and hip spica cast. There were 153 female and 47 male hips in the group. The mean age of the patients at the time of the reduction were six (range. 2-13) months and mean follow-up was 51 (range: 16-240) months. All the patients were treated with closed reduction and were immobilized in hip spica cast. The mean time of immobilization in the cast was 102 (range: 45-190) days. Avascular necrosis was observed in 15% of the hips. Clinical and radiological end results of the patients were evaluated with modified McKay criteria and Severin classification. Eighty-two percent of the patients had satisfactory results according to modified McKay criteria and 76% of the patients according to Severin classification. The most important parameters affecting the end result were pre-reduction location of the hip, pre-operative acetabular index values and avascular necrosis. Based on the results of this study, treatment of developmental hip dysplasia with closed reduction and hip spica cast is a relatively safe and effective method.

Key words: hip dislocation, closed reduction.

The term developmental dysplasia of the hip (DDH) refers to a spectrum of clinical and anatomical deformities ranging from capsular laxity to an irreducible dislocation. The pathological changes in a dysplastic hip in a newborn infant are generally reversible, with a 95% success rate with a simple means of treatment[1,2]. If the diagnosis is delayed, the obstacles to reduction become increasingly difficult to overcome, and recovery of the acetabulum after reduction is less predictable. The goal of the treatment of a dysplastic hip is to attain a concentric reduction to allow normal femoral and acetabular development[3]. It is very well known that a stable and concentrically reduced femoral head is the primary stimulus for the development of the acetabulum[4]. Closed reduction and immobilization of the hip in a spica cast is one of the most commonly used treatment methods of DDH. This method can be used before a child walks. But inappropriate use of the method commonly causes complications that affect long-term prognosis of the joint. Clinical results are generally better than radiological results in children. But the long-term prognosis of the joint is generally related to the radiological appearance[5]. Avascular necrosis, which increases delay in reduction, is the most important parameter affecting long-term clinical and radiological outcome of the disease. The aim of this study was to evaluate the functional and radiological results of closed reduction in one of the biggest series in the literature patients in mid-term.

Material and Methods

We retrospectively evaluated 200 hips of 129 patients with the diagnosis of developmental dysplasia of the hip (DDH) treated with closed reduction and hip spica cast. Arthrogrypotic patients and patients with chromosomal anomalies or multiple congenital anomalies were excluded from the study. The mean age of

the patients at the time of the diagnosis was six months [(range 2-13 and the mean follow-up was 51 months (range: 16-240)]. There were 153 female and 47 male hips in the group.

The position of the hip was evaluated according to Tonnis[6]. Tonnis divided the position of the hip into four groups: Type I describes dysplastic hips; type II, subluxated hips; type III, dislocated hips; and type IV, high dislocation. Radiographs of 184 hips before the treatment were evaluated with the radiological criteria as described above. According to Tonnis displacement grading system 56 hips had type I displacement, 116 hips had type II displacement, 10 hips had type III displacement, and 2 hips had type IV displacement in this study group[6].

The acetabulum was evaluated with acetabular index values. The mean acetabular index value was 35 degrees (range 15-52) before the treatment.

Home skin traction was applied for 155 hips for a period of 16 days (range 5-45) before the closed reduction. All closed reductions were performed under general anesthesia. Adductor tenotomy was necessary in only 76 hips. The reduction quality was evaluated with a direct radiograph in the operating room. If the femoral assific nucleus or shaft of the femur was directed to the triradiate cartilage, the reduction was accepted as a concentric reduction. Patients were immobilized in the human position, meaning 90-100 degrees flexion and 30-50 degrees of abduction. A secondary hip spica cast was applied for 58 hips. The mean time of the immobilization was 102 days (range 45-190). An abduction brace was used after the removal of the cast for an additional four months (range: 1-15).

Functional outcome of the patients was evaluated according to the modified McKay criteria[7,8] (Table I). Last radiographs of the patients were graded according to Severin classifications[9] (Table II). Severin class I and II hips and McKay type I and II hips were accepted as a good result. End results of the patients who needed surgical treatment after closed reduction were accepted as poor results. Avascular necrosis was classified according to Salter et al.[10] and Bucholz and Ogden[11]. Bucholz and Ogden identified four radiographic patterns of avascular necrosis in the proximal femur. Type I is characterized by either temporary fragmentation of the capital femoral ossified nucleus or delay in appearance of the ossified nucleus. The prognosis of type I avascular necrosis is good, and a skeletal maturity, it can be accepted as normal. In type II avascular necrosis damage to the lateral part of the physis causes premature fusion of the superolateral part of the physis. The outcome is a short femoral neck in valgus angulation with marked uncovering of the femoral head by the acetabulum. Type III avascular necrosis is whole head involvement. The femoral head is flattened and deformed and the hip joint is incongruous.

Type IV avascular necrosis affects the medial part of the capital femoral epiphysis and resulted in coxa magna and breva.

All the radiographic parameters were compared with the functional modified McKay criteria, Severin grade and avascular necrosis to determine the most important parameter affecting the end results.

Table I. Severin classification

		Center-edge angle	Radiographic appearance
Class I	Ia	>19⁰ (16-13 yrs) >25⁰ (>14 yrs)	Normal
	Ib	15-19⁰ (6-13 yrs) 20-25⁰ (>14 yrs)	Normal
Class II	IIa	>19⁰ (6-13 yrs) >25⁰ (>14 yrs)	Mild deformity in femoral head, neck or acetabulum
	IIb	15-19⁰ (6-13 yrs) 20-25⁰ (>14 yrs)	Same as IIa
Class III		<15⁰ (6-13 yrs) <20⁰ (>14 yrs)	Subluxation without dysplasia
Class IV	IVa	>0⁰	Mild subluxation
	IVb	<0⁰	Severe subluxation
Class V			Femoral head located at false acetabulum
Class VI			Re-dislocation

Table II. Modified McKay Criteria

Grade	Result	Description
I	Excellent	Painless, stable hip, normal range of motion, Trendelenburg's sign (–)
II	Good	Painless, stable hip, mild limping, mild loss of motion, Trendelenburg's sign (–)
III	Fair	Mild pain, mild loss of motion, Trendelenburg's sign (+)
IV	Poor	Severe pain

Results

Of the 200 hips 189 could be classified according to Severin classification in the latest follow-up[9]. There were 123 class I hips, 20 class IIa hips, 3 class IIb hips, 3 class III hips, 3 class IVa hips, 6 class IVb hips and 1 class VI hips. Thirty hips needed surgical treatment and were not graded according to Severin classification. Class I and II hips were described as good results and others were accepted as poor results according to Severin radiological classification (Fig. 1a, b). Seventy-six percent of the patients had good results and 23% of the patients had poor results.

One hundred and sixty-one hips could be classified according to modified McKay criteria[7,8]. There were 148 type I hips, 10 type II hips, and 3 type III hips. There were no type IV hips in the study group. Eighty-two percent of the patients had good results and 18% of the patients had poor results according to modified McKay criteria. Twenty-five hips 15% of the 164 for which sufficient radiographs were available had avascular necrosis. These hips were graded according to Bucholz and Ogden[11]. There were 11 type I, 5 type II, 5 type III, and 4 type IV avascular necrosis hips (Fig. 2a, b).

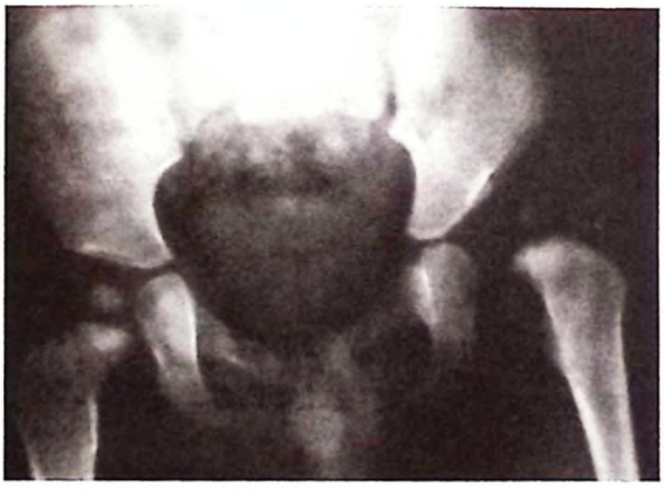

Fig. 1a: Nine-month-old female patient with left hip dislocation.

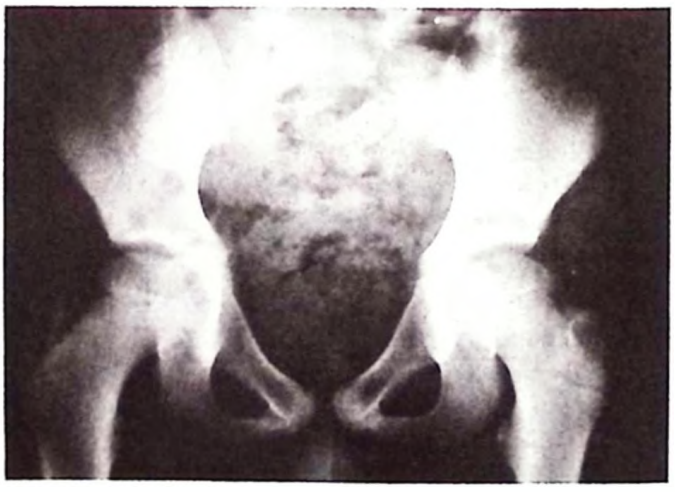

Fig. 1b: The same patients in Fig. 1a, 10 years after the closed reduction. The hip is normal.

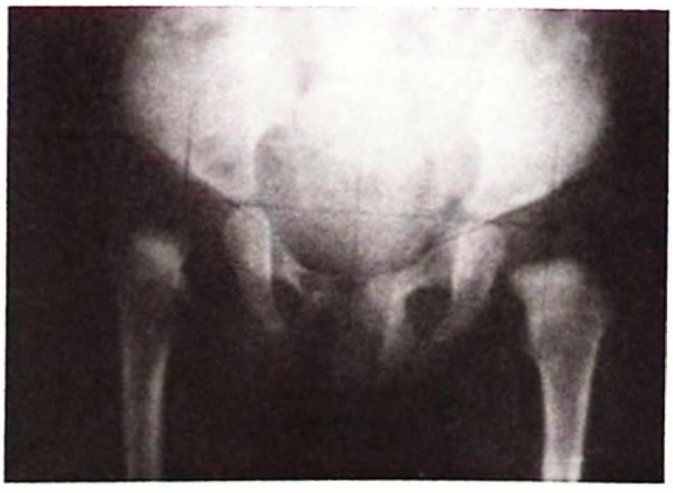

Fig. 2a: Seven month old female patient with right hip dislocation.

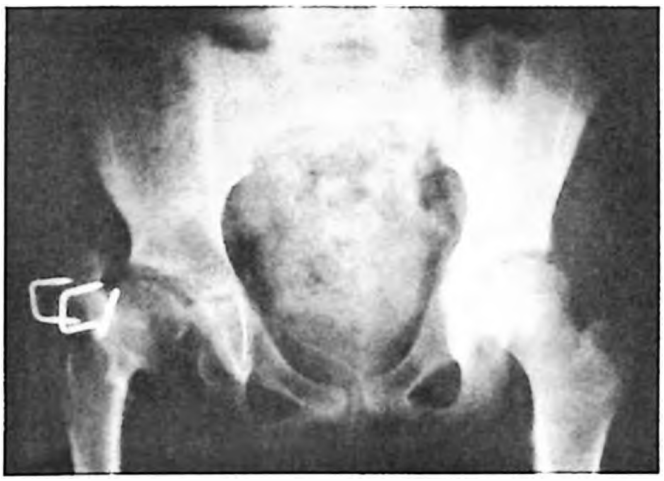

Fig. 2b: Same patient in Fig. 2a, 11 years after the closed reduction. There is a sequelae of whole head involvement avascular necrosis, and trochanteric overgrowth. Trochanteric apophyseodesis was performed to stop trochanter growth.

The mean acetabular index after removal of the cast was 23 degrees (range: 10-42) and the mean acetabular index at the end of the follow-up was 19.54 degrees. (range: 10-40). The mean center-edge angle was 20.51 degrees (range:15-48) after the treatment.

There was no statistically significant difference in the end results in terms of sex, or clinical and radiologic findings ($p>0.05$).

In the group for whom home skin traction was applied, there were 16 hips with avascular necrosis; in the group receiving no home skin traction, there were nine hips with avascular necrosis.

There was no statistically significant difference between home skin traction and avascular necrosis ($p=0.5$). There were statistically significant differences between the position of the hip before reduction and Severin anatomic classification ($p=0.001$); modified McKay criteria ($p=0.001$); and avascular necrosis ($p=0.001$). There were 106 hips treated before six months of age and 101 of them healed with satisfactory outcome. There were 83 hips treated after six months of age and 75 of them healed with satisfactory outcome according to Severin classification and McKay criteria. The difference between patients treated before six months of age and after six months of age was statistically significant ($p=0.006$); the end results of the patients were evaluated with either Severin classification or modified McKay criteria. The mean acetabular index before the treatment in the cases with good results (according to Severin) was 34.8 degrees. The mean acetabular index before the treatment in cases with poor results (according to Severin) was 38.5 degrees. The difference was statistically significant ($p=0.001$). There was no statistically significant difference between the acetabular index values and avascular necrosis.

There was a strong and statistically significant relationsip between avascular necrosis, Severin classification and modified McKay criteria ($p<0.001$), meaning if there were no avascular necrosis the result would be better.

Discussion

Treatment of DDH is often challenging, and evaluation of the results in the published series is difficult because there is no universally accepted, standard system for the assessment of function and of radiographs of patients who have hip disease[9,12,13]. Severin Class I and II are generally accepted as excellent and good results[5]. Similar diversity in the systems of classification for function also makes comparison of results difficult. There is a discrepancy between excellent clinical results and radiographic results that are not excellent anatomically, especially in children[14,15]. Radiologic results better predict the long-term prognosis of the joint[5].

Preoperative hospital or home skin traction is a debatable issue in the treatment of DDH. Fish et al.[16] surveyed the members of the Pediatric Orthopedic Society of North America on this topic. Most pediatric orthopedic surgeons thought that traction reduces the incidence of necrosis in the treatment of DDH. But contrary to that belief, several articles on closed reduction without use of preliminary traction report incidences of

proximal femoral damage comparable to those found in series in which traction was used[17,18]. The result of this study also indicates that there is no correlation between avascular necrosis and preliminary home skin traction.

Perereduction position of the hip was also found to be an important parameter related to the end result. In high dislocations the reduction may be more forceful, and this may explain this result. Tonnis[6] hip grading system is useful for predicting difficult and complication-prone cases and can be used in the preoperative evaluation of the patients as shown in this study.

Prereduction acetabular index values were found to be related with the end result. This illustrates that treatment of severe dysplasia is difficult and that these patients should be followed-up closely; secondary procedures may be necessary for further treatment. An insignificant relationship between avascular necrosis and severity of the disease supports the idea that avascular necrosis is an iatrogenic complication.

The most disastrous complication associated with the treatment of DDH involves various degrees of growth disturbance of the proximal femur, including the epiphysis and the physeal plate. In the current study avascular necrosis was found in 15% of the patients. The reported incidence of proximal femoral growth disturbance varies from 0 to 73% in the literature[5,8,19]. Different opinions exist about the reasons for this variation. The use of prereduction traction, adductor tenotomy, and open or closed reduction; the force of reduction; the position of the postoperative immobilization; and the age at reduction have been implicated as etiologic factors[19]. Prereduction traction was not found as an etiologic factor in that study. Adductor tenotomy was not evaluated because in this study group adductor tenotomy was performed only if it was necessary. Position in the cast after reduction may be a factor, but no patient was immobilized in an extreme position. The problem is that there is no universal position safe for every patient. We believe the most important factor affecting the rate of avascular necrosis is the force of reduction. The force of reduction is not a quantitative factor affecting results, and it can only be evaluated by position of the stable reduction. Reductions, which are stable in extreme positions, generally need much more force. In these hips, closed reduction is not an appropriate method of treatment. Age of the patient at the time of the reduction is also an important factor. The low rate of avascular necrosis in this group might be attributed to the low mean age of the patients, because younger patients have a lower rate of growth disturbances and because the incidence of avascular necrosis increases with delay in reduction[20]. The method of reduction may be related to the rate of avascular necrosis, but the method of reduction alone is not. We believe that open reduction is indicated if closed treatment fails, or if there is persistent subluxation, soft tissue interposition, or reducible but unstable reductions other than extreme positions of abduction. The avascular necrosis rate obtained in this series is one of the lowest rates published[19]. It is known that type I avascular necrosis has a good prognosis for the joint; if the hips with type I avascular necrosis were excluded, the rate of avascular necrosis would have been 7% in this group.

The results of the current study indicate that 76% of the patients had acceptable results with this method radiographically. According to modified McKay criteria, 82% of the patients had acceptable results. Patients with high dislocations and high acetabular index values, and patients older than six months of age at the time of the reduction had a poorer prognosis. There is a strong correlation between these criteria and avascular necrosis. As a result of this approach, the most important parameter affecting results is avascular necrosis of the femoral head.

The results of this present study showed that treatment of developmental dysplasia of the hip with closed reduction and immobilization in hip spica cast before a child walks is an effective and relatively safe method. Orthopedic surgeons should be very careful about patients with high acetabular index values and high dislocations, and who are older than six months of age at the time of reduction, as shown in this study. These cases should be evaluated very carefully under general anesthesia with the aid of dynamic arthrography in order to avoid avascular necrosis, and open reduction should be performed if forceful reduction is necessary.

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Factors influencing sleeping pattern of infants

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Many families apply to pediatricians with complaints of sleep problems of their infants. It is very important to inform families about the sleep pattern of infants and factors influencing it, and to answer their questions about infantile sleep.

A questionnaire was given to 165 families to elucidate the factors influencing continuous sleep pattern of the baby. Our study demonstrated that 50% of four-month-old and 96% of nine-month-old infants acquired continuous sleep pattern. Our figures were compatible and even higher than those in the literature.

None of the factors studied has an independent effect on infantile continuous sleep pattern in multifactorial analysis. Early weaning does not facilitate acquisition of continuous sleep pattern by the baby. This will help to support prolonged breast-feeding. It may be advisable not to keep children beside their mother during sleep for a long period of time. Care of the children by the maternal grandmother may facilitate their acquisition of continuous sleep pattern.

Key words: infants, sleep pattern.

Many families apply to pediatricians with complaints of sleep problems of their infants. Sleepless nights make the lives of parents less joyful and lead to loss of working power. The parents fell anxious about their baby because they think he is sick. Therefore, it is very important to inform families about the sleep pattern of infants and factors influencing it, and to answer their questions about infantile sleep.

There are several international studies about sleep pattern of babies[1-4]. However, there are no such studies in our country. In order to help parents deal with their infant's sleeping problems, one has to know when the infants will acquire continuous sleep and how long they will sleep every day normally.

There are many factors, especially those related to feeding pattern, influencing acquisition of continuous sleep pattern. Early weaning has been related to a prolonged nighttime sleep pattern; however, breast-fed babies also acquire continuous sleep pattern early[5-8].

This study was undertaken to investigate the possible factors influencing acquisition of continuous sleep pattern by infants.

Material and Methods

This study was conducted on 165 families that had children aged 9-15 months and who applied to the Department of Pediatrics at Başkent University between November 2000 and February 2001. One hundred and seventy-two mothers were asked to complete a questionnaire, and 165 (97%) consented. The questionnaire included questions about mother's and father's age, education level, employment, place of employment, maternal leave, birth weight, sex, number of other children under five years o age, breast-feeding duration (only & total), history of formula feeding, the place where the baby slept, person involved in day- and night-care of the baby, the methods used to put the baby to sleep, and the time the baby acquired continuous nighttime sleep.

'Continuous nighttime sleep' was defined as a continuous five hours of sleep at night[2,4,5]. Only breast-feeding time was defined as exclusive breast-feeding time and total beast-feeding time was defined as the period between the start of breast-feeding and its termination[9].

The feeding groups were defined as follows:

Group 1: Formula Group : Infants who received formula, whose only breast-feeding time was less than four months, and whose total breast-feeding time was less than six months.

Group 2: Beast-feeding Group : Infants whose only breast-feeding time was ≥4 months, whose total breast-feeding time was ≥6 months, and who started formula feeding at or after four months of age.

Group 3: Breast-feeding and Formula Group : Infants whose total breast-feeding time was ≥6 months, and who started formula feeding before 4 months of age.

Group 4: Babies who slept in mother's bed and baby's bed, and who never slept in separate room.

Group 5: Babies who slept in mother's bed, baby's bed, and separate room.

Group 6: Babies who slept in mother's bed and separate room, and who never slept in baby's bed.

Group 7: Babies who slept in baby's bed and who never slept in mother's bed or in separate room.

Lullaby, rocking on the legs/feet or swinging in a blanket are described as traditional methods, whereas mother's bosom and music are named as modern methods to put the baby to sleep.

The results were statistically analyzed using SPSS 10.0 Kruskal-Wallis analysis of variance, Mann-Whitney U test, chi-square test and multiple logistic regression were used for statistical analysis. The significance was taken as p=0.05.

Table I. Characteristics of mothers

Characteristics	n	%
Age (n=165)		
≤24	10	6
25-29	83	50
30-34	61	37
≥35	11	7
Education level (n=165)		
High school	65	39
University	100	61
Employment (n=165)		
Unemployed	74	45
Employed	91	55
Occupation (n=91)		
Private sector	50	55
Government sector	38	42
Private job	3	3
Maternal leave period (n=91)		
≤8 weeks	26	29
9-15 weeks	39	42
≥16 weeks	26	29

Results

The various characteristics of the mothers and fathers are given in Tables I and II. The characteristics of the infants are given in Table III. All babies were term infants. Feeding characteristics of babies are shown in Table IV.

Table II. Characteristics of mothers

Characteristics	n	%
Age (n=165)		
≤24	3	2
25-29	35	21
30-34	99	60
≥35	28	17
Education level (n=165)		
High school	50	30
University	115	70
Occupation (n=91)		
Private sector	48	29
Government sector	85	52
Private job	32	19

Table III. Characteristics of infants

Characteristics	n	%
Age (n=165)		
9 months	34	20
10 months	39	24
11 months	21	13
12 months	27	16
13 months	13	8
14 months	11	7
15 months	20	12
Birth weight (gr) (n=165)		
≤2800	8	5
2801-3500	105	64
3501-4000	43	26
>4000	9	5
Type of birth (n=165) cesarean section		
Cesarean section	98	59
Vaginal	67	41
Planned/Unplanned (n=165)		
Planned	144	87
Unplanned	21	13
Birth order		
First baby	82	50
Second baby	75	45
Third baby	8	5
Sibling <5 years of age (n=165)		
0	133	80
1	31	19
2	1	1

The time of acquisition of continuous nighttime sleep is given in Table V.

Mother's age, education, employment condition, maternal leave period, father's age, education, occupation, baby's sex, birth weight, type of birth, baby's being planned/unplanned, order of birth, or presence of sibling under five years of age did not significantly influence the acquisition of continuous sleep pattern by the baby (chi-square test, $p>0.05$).

Table IV. Characteristics of infants

Characteristics	n	%
Breast-feeding (n=165)		
Yes	140	85
No	25	15
Only breast-feeding (n=140)		
<4 months	29	21
≥4 months	111	79
Total breast-feeding (n=140)		
<4 months	29	21
4-6 months	29	21
>6 months	82	58
Formula (n=95)		
Yes	95	58
No	70	42
Start of formula		
<4 months	86	91
≥4	9	9

Table V. Distribution of the time of acquisition of continuous nighttime sleep

Continuous 5-hour sleep (months)	n	%	Cumulative %
2	13	7.9	7.9
3	35	21.2	29.1
4	34	20.6	49.7
5	17	10.3	60.0
6	18	10.9	70.9
7	23	13.9	84.8
8	14	8.5	93.3
9	5	3.0	96.4
10	1	0.6	97.0
12	1	0.6	97.6
13	3	1.8	99.4
14	1	0.6	100.0
Total	165	100.0	100.0

Forty-four percent (22/50) of infants of mothers working in private sector, 63% (24/38) of infants of mothers working in government sector and 0% (0/3) of infants of mothers working at a private job attained continuous sleep pattern at or before four months of age. The "private job" group was combined with the private sector group for statistical analysis. This difference was significant (chi/square test, $\chi^2=4.15$, $p=0.042$). The infants of mothers working in the government sector attained continuous sleep pattern significantly earlier than those of mother's working in the private sector.

Sixty percent (15/25) of non-breast-fed infants and 48% (67/140) of breast-fed infants attained continuous sleep pattern at or before four months of age. This difference was not significant (chi-square, $\chi^2=1.251$, $p=0.263$).

The differences between continuous sleep patterns of infants according to the sleep period in mother's bed were found to be statistically significant. The differences between sleep patterns of infants who never slept in their mother's bed and those who slept for four months or longer was significant ($p<0.05$), whereas the 1-3 month group was not significantly different from the other two groups ($p>0.05$) (Table VI).

The relation between acquisition of a continuous five-hour sleep pattern and only- and total breast-feeding times is shown in Table VI.

Fifty-three percent (37/70) of non-formula-fed infants and 47% (45/95) of formula-fed infants acquired a continuous sleep pattern at or before four months of age. This difference was not statistically significant (chi/square test, $\chi^2=0.486$, $p=0.486$).

The comparison of continuous sleep patterns of infants according to feeding groups was statistically nonsignificant ($p>0.05$) (Table VII).

The differences between continuous sleep patterns of infants according to the sleep condition were found to be statistically significant ($p<0.05$) (Table VIII). Mann-Whitney U test was used to analyze intergroup differences. The groups that were found to be significantly different from each other are: Group 1-Group 2, Group 1-Group 7, Group 2-Group 3, Group 3-Group 5, Group 3-Group 7 ($p<0.05$).

The differences between continuous sleep patterns of infants according to the day- and night-care were found to be statistically significant (chi-square test, $\chi^2=16.871$, $p=0.002$). Mother/mother group was significantly different from maternal grandmother (MG/mother and Mg/Mg groups; mother/MG group was significantly different from paternal grandmother (PG)/mother group; Mg/

Table VI. Relation between continuous sleep pattern and only and total breast-feeding times and sleep period in the mother's bed

		Continuous 5-hour sleep pattern			
		≤4 months	>4 months	Total	Statistics
Only breast-feeding time	<4 months	8 (28%)	21 (72%)	29	chi-square, χ^2=6.023, p=0.014
	≥4 months	59 (53%)	52 (47%)	111	
Total breast-feeding time	≥6 months	59 (54%)	51 (46%)	110	chi-square, χ^2=6.871, p=0.009
Sleep period in mother's bed	0	65 (56%)	51 (44%)	116	chi-square, χ^2=7.169, p=0.028
	1-3 months	13 (40%)	20 (60%)	33	
	≥4 months	4 (25%)	12 (75%)	16	

Table VII. Comparison of continuous sleep patterns of infants according to feeding groups (analysis of variance, F=0.773, p=0.463)

Feeding groups	n	Mean of acquisition of continuous 5-hour sleep (month)±SD
1	54	5.44±2.42
2	79	5.14±2.60
3	32	4.78±1.84
Total	165	5.17±2.41

SD : Standard deviation.
Group 1: Formula group.
Group 2: Breast-feeding group.
Group 3: Breast-feeding and formula group.

Table VIII. The effect of sleep conditions on continuous sleep pattern (Kruskal Wallis analysis of variance, χ^2=15.862, p=0.015)

Sleep contiditons (group)	n	Continuous 5-hour sleep (mean ranks)
1	7	122.93
2	83	75.09
3	5	137.50
4	14	96.00
5	23	85.89
6	7	88.86
7	26	75.88
Total	165	

mother group was different from PG/mother group; and MG/MG group was different from PG/mother group (p<0.05). PG/mother and mother/mother groups obtained the worst results regarding acquisition of a continuous 5-hour sleep pattern (Table IX).

The differences between continuous sleep patterns of infants according to the methods used to put the baby to sleep were not found to be statistically significant (Table X).

The following factors were included in the multifactorial analysis of continuous sleep pattern: 1. mother's age, 2. mother's employment, 3. maternal leave period, 4. planned baby or not, 5. only breastfeeding time. None of these factors significantly influenced continuous sleep pattern independently (p>0.05) (Table XI).

Table IX. Effect of day-and night-care on continuous 5-hour sleep (chi-square tes, χ^2=16.871, p=0.002)

Day/night care	Continuous 5-hour sleep ≤4 months	Continuous 5-hour sleep >4 months	Total
Mother/Mother	10	19	29
Mother/MG	7	5	12
Mother/PG*	1	2	3
MG/Mother	44	27	71
MG/MG	10	4	14
MG/PG*	0	1	1
PG/Mother	4	16	20
PG/MG*	2	1	3
PG/PG*	0	3	3
PG/Babysitter*	0	1	1
Babysitter/Mother*	4	3	7
Babysitter/Babysitter*	0	1	1
Total	82	83	165

* Groups were omitted during statistical analysis because of low numbers in groups. The possible groups without members were not tabulated.

MG: maternal grandmother. PG: paternal grandmother.

Table X. Effect of methods used to put baby to sleep on continuous sleep pattern (chi-square test, χ^2=4.66, p=0.198)

Traditional and/or modern methods	Continuous 5-hour sleep ≤4 months	Continuous 5-hour sleep >4 months	Total
Traditional (no) Modern (no)	15	10	25
Traditional (yes) Modern (yes)	15	8	23
Tarditional (no) Modern (yes)	19	22	41
Traditional (yes) Modern (no)	33	43	76
Total	82	83	165

Table XI. Results of multifactorial analysis of factors related to continuous 5-hour sleep pattern

Variable	B	Standard error	Significance	Exp (B)
1	-0.0008	0.0466	0.9856	0.9992
2	-0.2927	0.4825	0.5440	0.7462
3	0.0232	0.0280	0.4072	1.0235
4	-0.0005	0.4913	0.9992	0.9995
5	0.0360	0.0901	0.6896	1.0366
Constant	-0.1211	1.4530	0.9336	

Discussion

The acquisition of continuous nighttime sleep is an important developmental milestone for the infant. It is generally accepted that a regular sleep pattern has been attained by the baby when he/she sleeps continuously for 5-6 hours at night. As the diurnal sleep rhythm is acquired, daytime sleep moves towards the night and the expected sleep pattern is attained. Every baby reaches this stage at different times. Although some indicate that this rhythm can be acquired as early as two weeks after birth, the general acceptance is around six weeks[2].

According to one study, 35% of babies under three months of age could sleep continuously for five hours at night; this figure increased to 72% by 9-12 months of age. However, another study indicated that 60-70% of 3-4-month-old infants attained a continuous sleep pattern[10,11]. Our study demonstrated that 50% of four-month-old and 96% of nine-month-old infants acquired a continuous sleep pattern. Our figures were compatible and even higher than those in the literature.

It must be stressed that our study group included selected families, because these parents had a higher education level than seen in the rest of the country. Forty percent of the mothers had high school education and 60% had a university degree. Thirty percent of the fathers had high school education and 70% had university degree. It is obvious that our study group does not represent our population. However, the educational level of the mothers and fathers does not significantly influence the regular sleep pattern of infants. There are studies in the literature indicating the presence and absence of effect of parental education on infantile sleep pattern[12-14].

Fifty-five percent of our mothers were working mothers, and 55% of these worked in the private sector. In our previous study, we showed that mothers working in the government sector and in their own jobs had a longer maternal leave period and could breast-feed longer than those working in the private sector[15]. Since mothers working in their own job made up a very small group they were included in the private sector for statistical analysis. Mother's occupation significantly influenced acquisition of continuous sleep by the baby. The infants of mothers working in the government sector attained continuous sleep significantly earlier than those of mothers working in the private sector. However, maternal leave period did not significantly influence sleep pattern of infants.

Parallel to our study, several studies proved that sex of infant, birth weight, type of birth, order of birth, and presence of brother or sister younger than five years of age did not significantly influence infantile continuous sleep pattern[2,6,10].

Unplanned babies made up 13% of the study group. However, this factor did not significantly affect sleep pattern neither in uni- nor in multifactorial statistical analysis. It is well known that sensory stimuli by the parents and their close relationship with their baby is very important in acquisition of diurnal rhythm and sleep pattern[16]. This relationship may be weak in unplanned babies. Thus our figures may change by increasing the number of subjects in the study group.

The most important result of our study is the insignificant difference between sleep patterns of feeding groups (breast-feeding group, formula-fed group, and group with both breast-feeding and formula). Several uncontrolled studies in the literature have indicated that early weaning facilitated acquisition of continuous sleep pattern[5-8]. Clinical experiences show that many parents start supplementary food early with the expectation of early sleep pattern. However, the American Academy of Pediatrics and WHO suggest that supplementary food must be postponed until 4-6 months of age[9]. Furthermore, there are several studies indicating lack of effect of supplementary food upon sleep pattern[17-21].

Our study revealed that developmental and adaptive procedures rather than the nutritional composition are effective in determining the acquisition of a continuous sleep pattern. The most important factor determining nighttime awakenings of the baby has been demonstrated to be attitudes of the parents[22]. It has been

indicated that babies who are instantaneously handled by the parents upon awakening wake up more frequently, learn to wake up, and repeat it. The babies may wake up during REM sleep, but continue to sleep if not handled by the parents. It has also been shown that babies who lie next to their parents before falling asleep wake up more frequently, and that as the baby is handled by the parents with behaviors such as rocking, holding or feeding, they will be unable to fall asleep alone[23-24]. Because all mothers indicated that they were with their babies before they fall asleep, we could not investigate it as a possible factor in sleep pattern.

Our results also revealed that babies sleeping beside their mothers acquire continuous sleep pattern late. This appears to be due to the increased body temperature of the baby because of the presence of the mother[4,25].

Grandmothers are very important figures in childcare in our country. We could not find a study on breast-feeding times and sleep patterns of babies who were cared for by their grandmothers. However, the PG may increase the stress of the mother, compared to the MG. According to our results, babies cared for by the PG acquired continuous sleep pattern significantly later than those cared for by the MG. This subject is important in our country and requires further research.

There is a need for studies on continuous sleep patterns of babies in our country, which are conducted on a sufficient number of families of different sociocultural levels, where mothers are asked to fill in a 24-hour diary for their baby. Such studies give better results if they are prospective, rather than retrospective.

In conclusion, none of the factors studied has an independent effect on infantile continuous sleep pattern in multifactorial analysis. Early weaning does not facilitate acquisition of continuous sleep pattern by the baby. This will help to support prolonged breast-feeding. Furthermore, sleeping babies away from their mothers for a long period of time, and care by the MG may facilitate their acquisition of a continuous sleep pattern.

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Parapneumonic empyema in children: conservative approach

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SUMMARY: Yılmaz E, Doğan Y, Aydınoğlu AH, Gürgöze MK, Aygün D. Parapneumonic empyema in children: conservative approach. Turk J Pediatr 2002; 44: 134-138.

Forty-nine patients, aged 3 months to 13 years, were studied to determine the clinical presentation, bacteriology, treatment and outcome of empyema complicating pneumonia in children. There were 28 (57.2%) males and 21 (42.8%) females in the study, with a male/female ratio of 1.3/1. We found malnutrition in 15 (30.6%) patients. The most common symptoms at presentation were fever (93.8%) and cough (85.7%). Radiography demonstrated minimal effusions (6 patients, 12.2%), moderate effusions (23 patients, 46.9%), and massive effusions (20 patients, 40.9%). The pleural fluid was on the right side in 26 (53.1%) cases, the left side in 17 (34.6%) cases, and bilateral in 6 (12.3%) cases. Staphylococcus aureus was the most frequently isolated microorganism in pleural fluid. No organism was recovered in 33 (67.3%) patients. Most cases were treated with a combination of intravenous antibiotics and chest tube drainage. Decortication was carried out in only two patients. The hospitalization period was 28.02±10.18 days (11 to 57 days). There was one death due to widespread Staphylococcus aureus septicemia. All patients who were followed-up showed complete or near complete resolution of the chest radiography at six months, regardless of severity of disease or treatment modality. Children with pleural empyema can be successfully treated with appropriate antimicrobial therapy and adequate closed chest tube drainage. Further surgical intervention is rarely required.

Key words: parapneumonic empyema, chest tube drainage.

Pleural empyema is the presence of grossly purulent fluid in the pleural space. It is usually a result of an antecedent bacterial pneumonia. However, other etiologies including esophageal rupture, mediastinal or subdiaphragmatic disease, neoplasm, bacteriemia, chest trauma, thoracic surgery, collagen vascular disease, and immunodeficiency disorders should be considered[1]. Three distinct stages of progression of an empyema have been described. The first stage (stage I) is exudative and is characterized by free-flowing fluid that can easily be drained. The second stage (stage II) is fibrinopurulent with the formation of septations and loculations. Tube drainage would be very difficult in this stage. The final stage (stage III) is organization in which surgical debridement is considered by some to be the only treatment option[2,3].

Recent advances in imaging and instrumentation have facilitated the recognition and management of bacterial empyema[2,4,5]. Despite such rapid advances in diagnosis and therapy, it is still possible for an empyema to remain undetected unless the risks of this complication are appreciated and appropriate diagnostic measures are used. Although currently available antimicrobial agents can control some of the systemic manifestations of empyema, the morbidity and mortality caused by undrained pleural pus are still high[6-9]. Therapeutic decisions are made more difficult by wide variations in the disease spectrum, perhaps resulting from variables such as virulence of infecting organism, host resistance and timing of presentation for treatment[10].

In this study, we reviewed medical records of children with pleural empyema to examine the spectrum of the disease and to evaluate the role of surgical and nonsurgical management.

Material and Methods

The medical records of 49 children who had been discharged with the diagnosis of pleural empyema between January 1990 and January

2000 were reviewed. In the clinical setting of pneumonia, empyema was defined as pleural fluid demonstrated on chest radiography that contained >1000, white blood cells (WBC) count µl or from which an organism could be cultured.

After detailed history was obtained, physical examination was performed in all patients. The patients whose weights according to age were below 90th percentile were accepted as malnutrition[11]. Routine laboratory studies were performed for all patients including WBC count, sedimentation and blood cultures. C-reactive protein (CRP) was available in 42 (85.7%) patients. Fifteen patients were tested for the presence of IgM against Mycoplasma pneumoniae with ELISA (R-Biopharm GmbH, Darmstadt, Germany). Pleural fluid was analyzed for WBC count, glucose, protein and lactate dehydrogenase (LDH) concentrations and pH. Routine bacteriologic studies were also performed in pleural fluid. Anteroposterior and lateral chest radiographs were obtained. The degree of pleural involvement was classified into one of the following three categories: 1. Minimal: some costophrenic angle blunting or slight pleural thickening, 2. Moderate: opacity involving up to half of the hemithorax, or 3. Massive: opacity over more than half of the hemithorax[12]. Chest ultrasound was performed in 26 patients and computerized tomography (CT) in 18 patients. Most of the patients who had ultrasonography (USG) and/or CT admitted after 1995.

Data regarding hospital course included fever curve, total number of invasive procedures, duration of chest tubes, length of stay and complications. Statistical analysis was performed using Student's t test. A p valuve <.05 was considered significant.

Results

Of the 49 patients, 28 (57.2%) were boys, 21 (42.8%) were girls, and the ages of the patients ranged from 3 months to 13 years (mean age 4.5±3.4 years). The peak incidence was in the 0-4 years age group. The majority of patients were healthy children before the onset of their acute illness. We found malnutrition in 15 (30.6%) patients. Five patients had mild, six patients had moderate and four patients had severe malnutrition. Fourteen patients admitted directly to this hospital and 35 patients were transferred from other hospitals after a period of five to 15 days (mean 7.8±3.7 days). Children were ill on average for 8.3±2.1 days before hospital admission.

The most common symptoms at clinical presentation were acute illness with fever (93.8%) and cough (85.7%). Abdominal pain was seen in five (10.2%) patients and explained the misdiagnosis of an acute abdomen made for three of our patients. Dyspnea and tachypnea were found in 36 (73.4%) patients.

The mean peripheral WBC count was above 15,000/mm^3 in 31 (63.2%) patients. A moderate-to-marked degree of anemia was found in 24 (48.9%) patients. Increased erythrocyte sedimentation rate was present in all patients. Elevated values of CRP were found in 39 (92.8%) of 452 patients. Protein concentrations in pleural fluids obtained from patients ranged from 3.9 to 5.4 g/dl (4.7±0.8 g/dl). Glucose concentrations in pleural fluids ranged from 11 to 78 mg/dl (34.4±17.2 mg/dl), and LDH concentrations ranged from 450 to 1595 IU/L (710±230 IU/L). Total WBC counts in pleural fluids varied from 500/mm^3 to 26,000/mm^3 (12,500±4100/mm^3).

The effusions seen on initial chest X-rays were minimal in six patients (12.2%), moderate in 23 patients (46.9%) and massive in 20 patients (40.9 %). Right-sided effusions were most common. Empyema was right-sided in 26 (53.1%) children, left-sided in 17 (34.6%), and bilateral in six (12.3%). Four children had associated cardiomegaly. Chest USG revealed the following: non-loculated collections (n=19), pleural thickening (n=8), and loculated fluid (n=7). Computerized tomography showed non-loculated fluid (n=13), pleural thickening (n=5), loculated fluid (n=5), and lobar cavitation (n=3).

Gram strains of pleural fluid were positive in 13 (26.5%) patients. Eight of these were also culture positive. The most common bacterium isolated from the pleural aspirate was Staphylococcus aureus (Table I). The blood cultures were sterile in only three patients. In four of the patients with bilateral empyema, Staphylococcus aureus was isolated, and in two patients there was no microorganism in pleural fluid. Streptococcus pneumoniae was isolated from the blood cultures in two patients and Staphylococcus aureus was in one of the patients as well. Three patients had Mycobacterium tuberculosis. One patient had positive Mycoplasma pneumoniae serology. All patients whose pleural fluid and blood cultures were sterile had received antibiotics prior to thoracentesis.

Table I. Bacteriologic results in patients with pleural empyema

Organism	No. patients (%)
Staphylococcus aureus	6 (12.24)
Streptococcus pneumoniae	4 (8.16)
Mycobacterium tuberculosis	3 (6.12)
Staphylococcus epidermidis	1 (2.04)
Pseudomonas aeruginosa	1 (2.04)
Mycoplasma pneumoniae (serology)	1 (2.04)

All patients were treated intravenously with systemic antibiotics. A third generation cephalosporin or a ureidopenicillin with an aminoglycoside was the most frequently used combination in the patients. Three patients were treated successfully with antibiotics alone. These patients had minimal effusions on initial chest X-rays. Forty-six patients had chest tubes placed through a closed thoracotomy within one day of admission. The chest tubes functioned for 3 to 11 days (mean 7.1±2.4 days) and were removed after 6 to 19 days (mean 12.2±1.9 days). Only two patients required drainage beyond five weeks. WBC count decreased by the end of the first week. Inflammatory markers such as CRP and erythrocyte sedimentation rate remained elevated for up to three weeks despite treatment in four patients. They had persistent fever requiring a prolonged hospital stay. Two patients had decortication of thickened pleura, which prevented lung expansion. One patent underwent decortication on the 42nd day of admission and the other on the 45th day.

Hospitalization ranged from 11 days to 57 days (mean 28.02±10.18) days). The massive and moderate effusions presented earlier than minimal and, as might be expected, massive effusions led to longer hospitalization (Table II). There was a statistically significant difference in duration of hospitalization between patients with massive effusion and patients with minimal effusion ($p<0.001$). In seven patients who had loculated fluid demonstrated in the pleural cavity by USG and CT, the duration of hospitalization was also prolonged (mean 34.1±5.6 days).

Table II. Parapneumonic effusions data

Size of effusions	No.	Mean hospital stays (days)
Minimal	6	13±1.79
Moderate	23	27.91±7.04
Massive	20	32.65±10.47*

* $P<0.001$.

One patient with empyema with Staphylococcus aureus had broncho-pleural fistula and two patients had wound infections at the exit site of the chest tube. Only one patient died due to widespread Staphylococcus aureus septicemia. The patient had purulent pericarditis and renal abscess as well. Thirty-nine (81.25%) of 48 survivors were seen after discharge. Follow-up ranged from one month to three years (mean 1 year). Chest X-rays, performed at 3rd month after hospital discharge, were normal in only five patients. All patients showed complete or near-complete resolution of abnormalities related to the empyema in the 6th month after discharge in chest radiography, regardless of severity of disease or treatment option.

Discussion

Despite the availability of broad-spectrum antibiotics, empyema remains a significant problem in pediatrics[13]. Pleural empyema continues to contribute significantly to the mortality rates among the poor in developing countries[14]. In this study the maximum incidence of this disease was in the age group of 0.4 years. This agrees with the findings of the other authors[14-15]. The predominance of males has been described previously[12,16]. Our study also demonstrated predominance of the male gender.

The peripheral WBC count was elevated in most patients on admission. Increased erythrocyte sedimentation rate were observed in all patients. Increased CRP concentrations were found in 39 (92.8%) patients. These results agree with the findings of other studies[10,14,17].

Classically Staphylococcus aureus has accounted for the greatest proportion of cases of pediatric empyema[14,16,18]. Nelson[19] reported that 54% of the empyemas in children <6 months of age were caused by Staphylococcus aureus. Staphylococcus aureus was the most common organism isolated from the pus obtained at thoracentesis in our patients. Empyema resulting from pulmonary tuberculosis was found in three patients, and they were treated with antituberculous drugs. Purulent tuberculous empyema is rare and usually follows a long history of unsuccessful medical and/or surgical therapies[6]. Three patients with tuberculous empyema had malnutrition and had been managed with unsuccessful medical therapies before hospital admission. Protein-energy malnutrition is seen as associated

illness[20,21]. Ghosh et al.[22] described that a large majority of children with empyema were victims of malnutrition (73.2%) and mortality was high (17.1%). Malnutrition was detected in 30.6% of our patients.

Prior antibiotic therapy reduces the frequency of positive cultures[6]. Hoff and co-workers[10] reported that 71% of patients with sterile empyemas had received antibiotics before cultures were performed. Our results supported their findings.

When empyema is suspected, it is necessary to characterize the size and extent of the fluid collection and determine whether loculations and septations are present. Ultrasonography is more sensitive in characterizing the fluid density and detecting the presence of septations and loculations[1,2,6]. Computerized tomographic scanning has been shown to be invaluable in the evaluation of parapneumonic empyema[23-25]. In our study, we used CT if the patient's clinical condition failed to improve despite adequate therapy for a reasonable length of time (two weeks).

Appropriate antibiotics and closed tube drainage was the regimen most commonly used in the management of these patients[13,14,26]. Only three patients were successfully managed with antibiotics alone. These patients had minimal effusions on initial chest X-rays. The remaining 46 patients were treated with antibiotics, thoracentesis and closed-chest drainage. Two patients had decortication for thickened pleura. Tuberculosis-related pleural effusions respond well to the usual antituberculous regimens[6]. The pleura is usually quite thick, and often has high concentrations of mycobacteria. Tube drainage should be avoided in order to prevent secondary bacterial infection of a tuberculous empyema[27]. In this study, all patients with tuberculous empyema were managed with closed-tube drainage. But the chest tubes had been inserted within one day from the appearance of pleural effusion on chest X-ray. Tuberculosis was diagnosed by follow-up in these patients. Demonstration of high adenosine deaminase levels (>70 U/L) in pleural fluid supports the diagnosis of tuberculosis. However, we could not apply this.

The average duration of hospitalization in our study was 28.02±10.18 days. This is concordant with other reports[13,18]. In our series, the length of hospital stay increased with the size of effusion. Munglani and Kenney[12] confirmed this finding. Although resolution occurs in the majority of children with fibropurulent empyema, the hospitalization is prolonged[28]. and this is consistent with our results.

Fibrinolytic therapy has been useful and safe adjunctive tool to facilitate the drainage of pus in some children with pleural empyema[29,30]. Experience with intrapleural fibrinolytic therapy in children with empyema has been limited, and further studies are needed before these agents are routinely used[1,17]. Intrapleural fibrinolytic therapy was not used in this study.

Failure of medical management is often seen with pleural empyema that becomes organized, forming thickened pleural peels and loculation, for which chest drainage will most likely fail[17]. If the disease has presented with, or progressed to, the formation of multiloculated collections and fibropurulent exudates (stage II), video-assisted thoracoscopic adhesiolysis, or open surgery, should be considered. When stage III disease has developed, adhesiolysis has no role. Open surgery should be undertaken with confidence that meticulous technique results in early drain removal, rapid recovery, prompt hospital discharge, and complete resolution[31,32]. In the present study, two patients underwent decortication. The decision to operate was made on the basis of both the CT findings of a loculated collection and a pelural thickening, and failure to improve clinically despite appropriate antibiotic and tube drainage.

The morbidity and mortality associated with pleural empyema are affected by the microbial etiology, host defence defects, severity and duration of infection, and adequacy of antibiotic therapy and drainage[6]. Patients with nosocomial infections complicating severe underlying disease may die at rates in the range of 40%-70%[33]. Among otherwise healthy patients, mortality rates are 2%-15%, depending o n the duration and the severity of their infection[19]. In the present study, the mortality rate was 2%. In the patient who died, Staphylococcus aureus septicemia contributed to the fatal outcome.

We conclude that appropriate antibiotics with early closed-chest tube drainage is adequate to achieve clinical and physiologic resolution. A few patients may require further surgical intervention. The long-term prognosis is excellent.

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Neonatal tetanus in the middle Black Sea region of Turkey

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SUMMARY: Totan M, Küçüködük S, Dağdemir A, Dilber C. Neonatal tetanus in the middle Black Sea region of Turkey. Turk J Pediatr 2002; 44: 139-141.

We retrospectively analyzed 62 cases with neonatal tetanus (NT) seen in the Department of Pediatrics of Ondokuz Mayıs University from 1989 to 2001. Epidemiological characteristics and prognostic factors on survival were investigated. We grouped the patients into two groups, the survivors and those who died. All patients were born in non-hygienic conditions, coming from rural regions. None of the mothers had been immunized against tetanus. The mortality rate was 40% (25 of 62 cases) and the only two poor prognostic factors on survival younger were age younger than five days at onset and the presence of fever. There was no significant difference between the two groups regarding the other known prognostic factors. Although the NT rate is declining, we must continue to protect against it by strictly enforcing preventive policies, especially in rural regions.

Key words: neonatal tetanus, Turkey.

Neonatal tetanus (NT) is characterized by marked failure to suck, opisthotonus and spasms that occur thee to 14 days after septic delivery[1]. Although it can be prevented by immunization and aseptic delivery, it is a major cause of mortality in the neonatal period in developing countries, especially in Southeast Asia, Africa and the eastern Mediterranean region. Over 400,000 deaths are estimated to occur worldwide annually[1-3]. NT continues to occur in Turkey, despite the nationwide anti-tetanus immunization policy[4-8]. Here we report 62 cases with NT in our center, and analyze the possible factors affecting the prognosis and clinical features.

Material and Methods

We retrospectively analyzed the patients with NT who had been admitted to the Department of Pediatrics of Ondokuz Mayıs University Hospital, Samsun, Turkey, from 1989 to 2001. The diagnosis was based on the history of septic delivery and clinical observation of failure to suck, spasticity, trismus, opisthotonus or risus sardonicus.

Blood glucose, calcium and magnesium levels were determined. Lumbar puncture was performed to exclude central nervous system infection. The cords that were suspicious for the entry of microorganisms were excised. All patients were placed in silent and dark room to prevent contractions. We used diazepam, tetanus anti-toxin or tetanus immunoglobulin and benzylpenicillin as conventional treatment. Cases who showed evidence of secondary infection received appropriate antibiotic combinations. All patients were kept in a quiet, darkened room, and interventions were kept to a minimum.

We divided the patients into two groups: survivors and those who died of disease. We analyzed the prognostic factors on survival with the statistical program SPSS 6.0 on computer, and p value of <0.05 was considered significant.

Results

There were 62 patients with NT [40 (65% male, 22 (35%) female]. Most of the deliveries were at home in non-hygienic conditions, and none of the mothers had been immunized with tetanus toxoid or attended healthcare services during pregnancy. All of the families were of low socioeconomic level. Most of the mothers only attended primary school. Razor blade (65%), scissors (19%), and knife (16%) were used to cut the umbilical cord. Thirty-seven cases survived. The remaining 25 (40%) of 62 patients died of disease and formed the second group. Characteristics of the groups are

shown in Table I. The median hospitalization period was five days for fatal cases and 29 days for survivors. Clinical characteristics of the patients are shown in Table II.

The mean age of the cases who died was significantly lower than the survivors at admission ($p<0.05$). Birth weight and gender of two groups did not significantly differ. Although the duration of symptoms was longer in the survivors group, there was no significant difference between the two groups. We failed to find any specific symptom or sign affecting survival of patients, except fever at presentation.

Table I. Characteristics of cases with neonatal tetanus

	Survivors		Deceased	
	Mean±SD	Range	Mean±SD	Range
Age (days)	7.8±2.1	4-11	4.9±1.9	2-10
Birth weight (g)	3140±498	2000±4100	3020±448	1900-4200
Male/Female	24/13		16/9	
Age at onset of symptoms (days)	6.2±1.3	2-12	3.1±1.4	1-6
Duration of symptoms (days)	2.2±1.2	1-4	1.9±1.0	1-3

Table II. Symptoms and signs of cases with neonatal tetanus

	Survivors (37)		Deceased (25)	
	N	%	N	%
Spasticity	28	75	20	80
Poor sucking	26	70	16	64
omphalitis	23	62	14	56
Trismus	22	59	14	56
Fever	10	27	14	56
Risus sardonicus	10	27	6	24
Cyanosis	9	24	6	24
Other infections	14	37	10	40

Discussion

Tetanus occurs worldwide and is endemic in 90 developing countries. The most common form, neonatal tetanus, kills approximately 500,000 infants each year because the mother was not immunized. In addition, 15,000 to 30,000 non-immunized women worldwide die each year from maternal tetanus[1,2]. Although the incidence of NT is declining by widespread use of tetanus toxoid in pregnant women, an increasing number of hospital births and improvements in postpartum hygiene, it is still a problem, especially in the rural regions of Turkey[4-8]. There were 133 cases with NT in our hospital from 1978 to 1988[5]. On the other hand, we found only 62 cases with NT over the past 12 years, in spite of an increasing number of cases within the newborn period. Only two cases with NT have been detected in our clinic in the past year. This suggests the improvement of health conditions in the Black Sea region of Turkey. Other reports form Turkey also indicate a decreasing incidence of NT.

Neonatal tetanus occurs soon after the delivery in non-hygienic conditions. Immunization profile of the mother against tetanus is usually inadequate or absent. It is frequent in the rural regions in which the deliveries occur by untrained traditional birth attendants[1,2,9]. All of our patients were from the rural regions of the Black Sea and they were born in non-hygienic conditions. None of the mothers had been immunized against tetanus. Similar characteristics can be seen in the other reports from different parts of Turkey.

Neonatal tetanus occurs more in males than in females[4,5]. In our series, males were also seen more frequently (62%) than females. Incubation period, severity of illness, preterm birth, infant's weight, secondary infection, age at onset, fever, risus sardonicus, opisthotonus and mode of treatment affect the survival of patients with NT. In our study, mortality was significantly increased with age at onset under five days and the presence of fever. Our study did not

demonstrate that the previously reported prognostic indicators significantly affected NT survival in our region. The fatality rate was 40% in our cases in accordance with the other series[4-9].

Tetanus is one of the preventable diseases through widespread immunization. Additional precautions including the education of mothers, and hygienic delivery care and umbilical cord management will help prevent NT. Health care professionals dealing with preventive medicine should strictly carry out immunization against tetanus and educate the mothers.

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Conservative treatment of eminentia intercondylaris fractures of the tibia in children

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SUMMARY: Atay ÖA, Doral MN, Tetik O, Leblebicioğlu G. Conservative treatment of eminentia intercondylaris fractures of the tibia in children. Turk J Pediatr 2002; 44: 142-145.

Seventeen patients (16 children, 1 adolescent) were reviewed 31 months to 71 months after sustaining the common childhood fracture of the eminentia intercondylaris of the tibia. The aim was to assess long-term results and prognosis by clinical and radiological examination and to discover whether conservative treatment was adequate for type I and type II fractures according to Meyers and McKeever.

Early improvement occurred in all patients after conservative treatment, but long-term results were not satisfactory in displaced fractures,which were treated with closed reduction and immobilization in extension. Therefore, anatomic reduction and rigid fixation should be obtained for displaced fractures of the eminentia intercondylaris of the tibia.

Key words: fracture, pediatric, intercondylar eminence.

Avulsion fractures of the intercondylar eminence are mostly seen in children and adolescents and have been considered to be the childhood equivalent of anterior cruciate ligament (ACL) ruptures in adults[1-3]. This mode of ACL failure is thought to occur primarily in children because of greater elasticity of the ligaments in the pediatric population[4]. Instead of a tear of the ligament, there is an obvious fracture of the tibial intercondylar eminence through the cancellous bone located immediately beneath the cortical bone at the site of the insertion of the ligament[4].

Fractures of the intercondylar eminence of the tibia are enigmatic skeletal injuries. The mechanism of injury remains obscure, appropriate management is unclear and even the results are puzzling, especially in the pediatric age group. However, the classification bears consistency, since it relates to the degree of fragment displacement. Meyers and McKeever[3,5] have described a classification scheme for fracture of the intercondylar eminence of the tibia. In type I fractures, the fragment is non-displaced, whereas type II fractures are partial avulsions with an intact posterior hinge. Type III fractures are completely displaced and show no bony opposition.

Treatment methods for these injuries may be guided by the classification scheme. Most authors recommend treatment of type I and II injuries with cast immobilization for four to eight weeks[3,5-8]. A cylinder or long-leg cast may be used with the knee fully extended to maintain reduction. Type III injuries are generally treated with open or arthroscopic reduction, and internal fixation[2,3,5-7,9-16].

The purpose of this study was to review a group of pediatric patients who had sustained intercondylar tibial eminence fractures and were treated by conservative methods.

Material and Methods

Seventeen patients were reviewed who were treated with or without closed reduction and followed by cast immobilization of tibial intercondylar eminence fracture at our Department between October 1994 and February 1998. Sixteen were children (8-14 years), and one was an adolescent (16 years). All of the patients had a fresh injury and were seen within ten days after the trauma.

The most common complaints were an immediate and painful swelling and inability to move or walk on the injured extremity. The

findings were painful and severe effusion and lack of complete flexion and especially extension. Routine radiographs of the knees showed the fractures of the intercondylar eminence of the tibias and these were classified according to the system proposed by Meyers and McKeever, which was a three-type classification scheme based on the degree of fracture displacement[3,5].

Additionally, we investigated four knees using computed tomography (CT) for displacement and size of the fragment or fragments. It was not always easy using only plain radiographs to determine whether there was an intact posterior hinge or total displacement of the fracture fragments and to estimate the fragment size, which are important for choosing the type of treatment.

Afterwards, blood-stained fluid with droplets of fat was aspirated, and type I (non-displaced) fractures were treated by simple cast immobilization with the knee in slight flexion. Type II fractures, which were minimally displaced, were treated similarly to type I. 'Grossly displaced type II fractures were treated with closed reduction followed by cast immobilization with the knee in full extension. Hyperextension of the knee joint was avoided because of patient discomfort. The extended position of the knee joint allowed the femoral condyles to compress then fragment toward its fracture bed. All of the fractures were immobilized for a minimal of four weeks with a cast and then for at least two weeks in a long-leg hinge brace (Fig. 1a-d).

Type III and unreduced type II fractures of the intercondylar eminence, which were excluded from the study, were reducted and fixated with cannulated screws by arthroscopy.

After radiographs were taken and the fracture union was confirmed, the brace was weaned and, after 10 weeks, patients were allowed to return to their previous activities.

At the follow-up, subjective outcome was obtained using a standard questionnaire for patients regarding any knee symptoms and their level of activity. They also underwent a routine clinical examination of the knee with specific emphasis placed on objective clinical signs of ACL laxity. Clinical examination was performed by one of the authors (MND) in order to eliminate intra-observer error. All patients had anteroposterior and lateral radiographs of both knees. The height of the anterior tibial spine was measured on the lateral radiographs using a method described by Panni[17]. Measurement of the height of the anterior tibial spine was obtained and a ratio was determined using the other uninjured knee as a control (Fig. 2a-d). Statistical analysis of the differences in values between the two fracture type two fracture types was performed using Student's t test. Values of $P<0.05$ were considered statistically significant. All patients were rated using the criteria established by Lysholm functional rating system; Tegner activity levels were also utilized[18].

Results

Follow-up interval ranged from 31 months to 71 months (mean 47 months). The mean age of the patients was 12 years and 3 months. Eleven patients were male and six were female. The left knee was involved in nine patients and the right in eight patients.

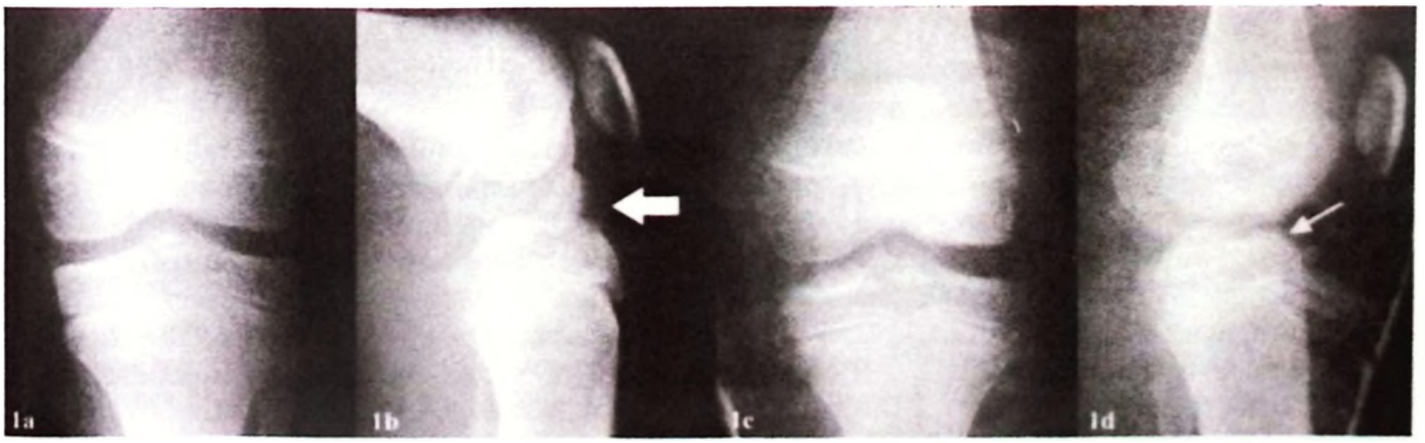

Fig. 1. Anteroposterior (a) and lateral radiographs of type II eminentia intercondylaris fracture, thick arrow revealing the displaced fracture fragment with an intact posterior hinge (b) Anteroposterior (c) and lateral radiographs with closed reduction followed by cast immobilizatilon with the knee in full extension, thin arrow revealing the adequate reduction of the fracture fragment (d).

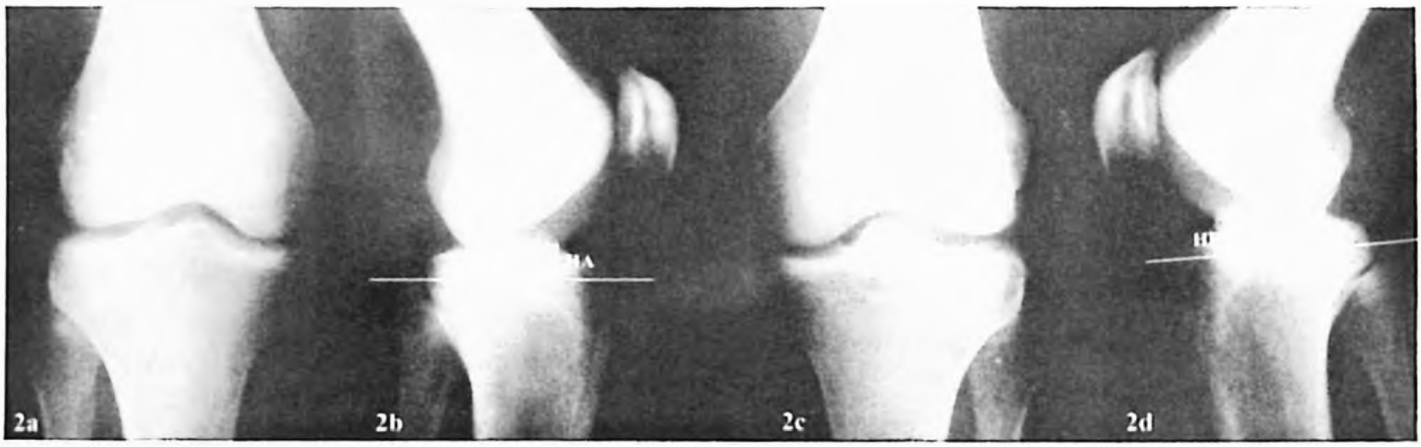

Fig. 2. Control anteroposterior and lateral radiographs of the contralateral knee (a, b) and injured knee after 59 months from the injury, showing malunion of the fracture in an elevated position and measuring the ratio (HB/HA) (c, d).

In 13 patients, the fracture had been caused by falling of a bicycle, in two by sports (while playing soccer) and in two in pedestrian-car collisions. In all cases the presentation had been with a painful hemarthrosis associated with decreased range of motion. In clinical examination lack of extension was revealed between 10-35° (mean, 24°).

In all patients, the avulsion fracture of the intercondylar eminence was the only injury to the knee. All of the fractures were diagnosed on initial radiographs and were classified according to Meyers and McKeever, but in four cases a definite decision could only be achieved using CT, which upgraded for type I minimally displaced fractures to type II[3,5]. In total, seven type I and to type II (four minimally displaced, six displaced) fractures were shown with radiologic evaluation.

Radiographic controls showed healing of the fractures within six to eight weeks for all patients. All of them had returned to regular activity and recovery seemed to be complete with no disability.

At the follow-up examination, no patients complained of residual pain but six patients who were displaced type II fractures, reported a history of giving-way episodes of anterior instability. Eleven patients were able to return to recreational sports. All patients regained the full flexion, but three patients who were displaced type II fractures had at least 5° of extension deficit; arthroscopic notchplasty was recommended to these patients[25]. All of the patients, other than six displaced type II fractures, exhibited a solid end point on the Lachman test with no positive pivot-shift tests. Six patients had moderate (grade II) Lachman tests and one had an obvious positive pivot-shift test, revealing a gross functional instability. Anterior cruciate ligament reconstruction was recommended near skeletal maturity for this case.

Follow-up radiographs showed union in all cases. Radiographical examination of the height of the anterior tibial spine showed a statistically significant difference ($P<0.05$) between type I (mean 0.13 ± 0.02) and type II fractures (mean 0.26 ± 0.06).

Functional assessment using the Lysholm scale was 92 (85-96) and 89 (86-93) for types I and minimally displaced type II, respectively. However, Lysholm functional scale was 78 (72-83) for displaced type II fractures of the eminentia intercondylaris. Tegner activity level was 9 in type I fractures and in four minimally displaced type II fractures, but was 6 in six displaced type II fractures.

Discussion

Most of the patients in this study were children under 14 years of age who had fractured the intercondylar eminence by falling off a bicycle. Our results in children agree with pervious reports regarding the most frequent causes of the fracture and the rarity of associated injuries[1,3,5,10,12,19-21].

There is no agreement in the literature about the treatment of these fractures. Some authors were in favor of operative treatment[1,2,10,13-16,19,22], whereas others advocated a more conservative approach[7,11]. Meyers and McKeever[3] stated in their classic article that the key to the choice of treatment should be based on a classification system. However, in clinical practice an accurate assessment of the fragment was not easy and the distinction between various grades of fracture was not always radiologically possible. In our study, CT proved to be a useful diagnostic tool

in distinguishing type II fractures. In four of our cases initially diagnosed as type I fractures, CT revealed the displacement of the avulsed fragment. We believe that the classification system described by Meyers and McKeever is complicated; it was often difficult to make a distinction between major types as well as between subgroups of the major type. We agree with the suggestion of Chandler and Miller[23] that the classification of the fractures could be simplified: displaced versus non-displaced.

The small number of reports of neglected fractures of the intercondylar eminence suggested that the untreated natural history of this injury either caused knee laxity and/or a loss of motion[1,9,11,12,15,21,24]. In our study, even if we performed conservative treatment, the results of obviously displaced type II fractures were nearly similar to neglected ones. Conservatively treated displaced type II fractures, even if successful closed reduction was performed, healed in a malunited. Malunion of the fracture in an elevated position of eminentia could (interfere with) knee extension, which was seen three patients, and could also be associated with ACL laxity (seen in six patients)[25]. In the present study, statistical analysis showed that the height of the tibial spine in the knees with a deficit extension and ACL laxity was significantly greater than that of the other knees. We found that especially in the displaced eminentia fractures, closed reduction and immobilization could not prevent unsatisfactory results such as ACL laxity and/or loss of motion of the knee.

Therefore, we recommend that non-displaced or minimally displaced fractures can be treated conservatively. For cases of an obviously displaced fragment, however, arthroscopic anatomic reduction and rigid fixation should be obtained instead of closed reduction and immobilization at extension.

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Circumcision in a combined factor V and factor VIII deficiency using desmopressin (DDAVP)

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SUMMARY: Devecioğlu Ö, Eryılmaz E, Çelik D, Ünüvar A, Karakaç Z, Anak S, Ağaoğlu L. Circumcision in a combined factor v and viii deficiency using desmopression (DDAVP). Turk J Pediatr 2002; 44: 146-147.

Combined factor V and VIII deficiency is a rare inherited autosomal recessive single gene disorder commonly seen in the Middle East. Although the factor levels are between 5-30%, several authors have reported that these patients are more prone to bleeding compared to those having an isolated factor deficiency with the same levels. We report an eight-year-old boy with factor V and VIII deficiency who underwent a successful circumcision using desmopressin (DDAVP).

Key words: combined factor V and VIII deficiency, desmopression, circumcision.

Combined factor V and VIII deficiency was first described in 1954 by Oeri et al[1]. The total number of cases was mentioned as 89 in 58 families in a review by Ginsburg et al.[2] in 1998. The disease is an autosomal recessive single gene disorder with factor levels ranging from 5% to 30%[2].

It has been demonstrated that this combined deficiency is due to the absence of an intracellular transport protein named ERGIC-53 which is responsible for transporting factors V and VIII from the endoplasmic reticulum to the Golgi apparatus. ERGIC-53 is encoded on chromosome18[3]. There is no clear evidence that the severity of bleeding is heightened by the concomitant presence of two coagulation defects[4].

Circumcision, as a traditional procedure for Muslims and Jewish people, carries major risk of bleeding in factor VIII-deficient patients[5]. We report an eight-year-old boy with combined factor V and VIII deficiency who underwent circumcision without any significant bleeding with appropriate usage of desmopressin (DDAVP).

Case Report

The patient was an eight-year-old boy diagnosed as mild hemophiliac at one year of age. Diagnosis of combined factor V and VIII deficiency was made after finding factor V level as 28% and factor VIII level as 32%, in order to explain mildly prolonged hematologic parameters (PT=15.8 sec, Control=12.2 sec; apt=46.1 sec, Control=26.7 sec). Before admission, desmopressin test was performed giving 0.3 μg/kg intravenously. After one hour, factor VIII level was 130% and factor V level remained the same. One day before operation tranexamic acid (3x250 mg) and thioridazine (2x10 mg) were started and continued until the 7th day postoperatively. Before the operation, 0.3 μg/kg DDAVP in 100 ml normal saline was given in 15 minutes and 10 ml/kg fresh frozen plasma in 30 minutes. The circumcision was then performed with no bleeding problem. Fibrin glue was not used. DDAVP (0.3 μg/kg IV) was continued every 12 hours for nine consecutive doses. One day after the operation, DDAVP was being given every 12 hours, resulting in a level of factor VIII of 65% on the 2nd day and of 41% on the 4th day. The DDAVP was stopped on the 4th day, and the other medications (tranexamic acid and thioridazine) on the 7th day. The patient had no problems on the 10th day and the operation site had sufficiently healed.

Discussion

Combined factor V and VIII deficiency is a rare inherited coagulation disorder. The factor levels range between 5 to 30%. These patients are more prone to bleeding compared to those

having an isolated factor deficiency with the same levels[2]. Desmopressin (DDAVP) has been used for several years in mild hemophilia and von Willebrand's disease. It acts by mobilization of factor VIII depots and is very useful, especially for minor procedures and hemorrhages[6,7]. Circumcision is a traditional procedure for Muslims and Jewish people. Currently worldwide, one in seven males is circumcised. To achieve safer hemostasis in circumcision of hemophiliac patients, factor VIII level concentration must be 100% and afterwards must be held >50% for several days[5].

Because of high cost and possible transmission of viral diseases, the use of human origin factor concentrates have to be limited, especially in mild cases. For these cases desmopressin is a good alternative, and has been used with success in mild hemophiliacs and in von Willebrand's disease. The factor VIII levels are 2-12 times higher (commonly 3-5) than basal levels after DDAVP administration. In our case the increase of factor VIII level from 32% to 130% was as expected. The gradual decrease after the first dose was due to depletion of the storage sites, but the wound healing was good because the factor VIII levels were still sufficient on the 4th day.

Tranexamic acid as a fibrinolysis inhibitor was used successfully in many hemorrhages of hemophiliacs It is especially useful for bleeding in the oral cavity and has also been used successfully for circumcision[3,8]. Thioridazine was used to prevent erection which is the critical problem after circumcision. No adverse effect was s been with use of these drugs together with DDAVP.

We performed a circumcision in combined factor V and VIII deficiency patient using desmopressin. The level of factor VIII after one hour of DDAVP administration was four times higher as expected. On the 2nd day and 4th day the decrease in factor VIII levels was due to depletion of stores. We think that four days of factor VIII coverage for combined factor V and VIII deficiency is enough in the case of circumcision. If the initial level of factor VIII is not very low, as in mild hemophiliacs, desmopressin is a good alternative.

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A case of purpura fulminans secondary to transient protein C deficiency as a complication of chickenpox infection

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SUMMARY: Canpolat C, Bakır M. A case purpura fulminans secondary to transient protein C deficiency as a complication of chickenpox infection. Turk J Pediatr 2002; 44: 148-151.

Purpura fulminans is a rare but dramatic disease which occurs most commonly during or after an infection. It is characterized by extensive involvement of the skin and extremities and involvement of visceral organs. Purpura fulminans, when occurring after a viral infection such as varicella, is usually characterized by purpuric lesions involving the trunk, usually with sparing of the visceral organs. In this report we describe a child with purpura fulminans due to a transient protein C deficiency as a complication of chickenpox infection.

A seven-year-old girl developed bruise-like lesions on her extremities on the fifth day after eruption of varicella exanthem. She had no previous history of bleeding tendency or thrombosis. Family history was also negative. On the seventh day of her illness she was admitted to Marmara University Hospital with widespread echymotic an partially crusted chickenpox lesions. CBC, urinalysis and blood chemistries were within normal limits. She had a prolonged aPT and apt with low serum fibrinogen and high D-dimers suggestive disseminated intravascular coagulation (DIC). Protein C activity was low. Punch skin biopsy was consistent with purpura fulminans. She was treated with heparin and fresh frozen plasma which helped her to recover clinically as well as hematologically. She was discharged with still low protein C activity that returned to normal by the next follow-up visit.

Key words: purpura fulminans, protein C deficiency, chickenpox.

Purpura fulminans (PF) is a descriptive term depicting a heterogeneous group of disorders characterized by rapidly progressive purpuric lesions, which may develop into extensive areas of skin necrosis and peripheral gangrene[1]. The disorder is associated with laboratory evidence of consumptive coagulopathy. The histopathologic features are widespread thrombosis of the dermal capillaries and venules with hemorrhagic infarction of the surrounding tissues[1,2]. The condition is often fatal, and survivors may have considerable morbidity related to loss of digits, limbs or areas of skin[3].

Purpura fulminans occurs most commonly in three different clinical settings. Transient or congenital deficiencies of protein S or protein C have been documented in cases of purpura fulminans in recent years[4]. The most common association is with sepsis with microorganisms such as Staphylococcus aureus[5], groups A and B beta hemolytic streptococci[6], Streptococcus pneumoniae[7], and Hemophilus influenzae[8]. It also is considered a cardinal feature of meningococcal septicemia[9]. The second groups of patients with PF are neonates and infants with inherited deficiencies of protein C and protein S. Recognition of these rare patients has drawn attention to the role of protein C anticoagulant pathway in the genesis of dermal vascular thrombosis[4]. The third group of patients includes those with PF that occurs a few days to weeks after the onset of a febrile illness[3,10]. Numerous infections have been reported to precede this disorder, the most common being varicella and streptococcal infections, which occur in 30% and 20% of patients, respectively[3]. The onset of the PF during the convalescent phase of the preceding febrile illness suggests that the disorder may be immunologically mediated. The overall mortality rate reported before 1964 was more than 50%[2]. In

more recent reports, the average mortality rate was 18% (14% in children)[3]. A wide range of therapies has been used, including clotting factor replacement[11], vitamin K[12], glucocorticosteroids[2,12], epsilon aminocaproic acid[13], hyperbaric oxygen[14], heparin[7,15], dextran[16], and exchange tranasfusion[17].

Case Report

A previously healthy seven-year-old girl developed pain in her knees on the fifth day after eruption of varicella exanthem. The pain was associated with bruise-like lesions on both her knees which was said to have spread to her thighs within 24 hours. She had been treated with acetaminophen for fever from the first to the fourth day of her exanthem. She had no previous history of excessive bleeding, easy bruisability or thromboses and no previous hospitalizations or serious illnesses. She also had no history of varicella infection or vaccine. There was no family history of bleeding disorder or thromboses.

On the seventh day of illness, when she was admitted to Marmara University Hospital for further evaluation, the physical examination showed an alert and oriented girl with normal vital signs for her age. She had widespread echymotic lesions on both sides of her knees and left thigh. The lesions were irregular in shape with well-demarcated borders and a surrounding rim of erythema. There was also widespread, partially crusted papulovesicular skin lesions typical for chickenpox (Fig. 1).

Her hemoglobin was 13.2 g/dl and hematocrit 40%. Platelet count was 186,000/mm^3 and the white blood cell (WBC) count was 16,200/mm^3 with differential of 60% segmented neutrophils, 35% lymphocytes, and 5% monocytes. Urinalysis was normal. Liver and kidney function tests as well as serum protein levels were within normal limits.

Her prothrombin time was 18.3 seconds with INR of 1.45, activated partial thromboplastin time 69.7 seconds, and fibrinogen 80 mg/dl (range: 180-350 mg/dl). Plasma levels of factors V, VII, IX and XI were normal. D-dimer test was positive at 1: 36,335. Protein C activity was 41.7% (70-140%) and protein S activity was 67.7% (60-130%) at the second day of admission. Lupus anticoagulant and anticardiolipin antibody tests were negative and antivaricella IgM and IgG were positive. Protein C activity of the patient

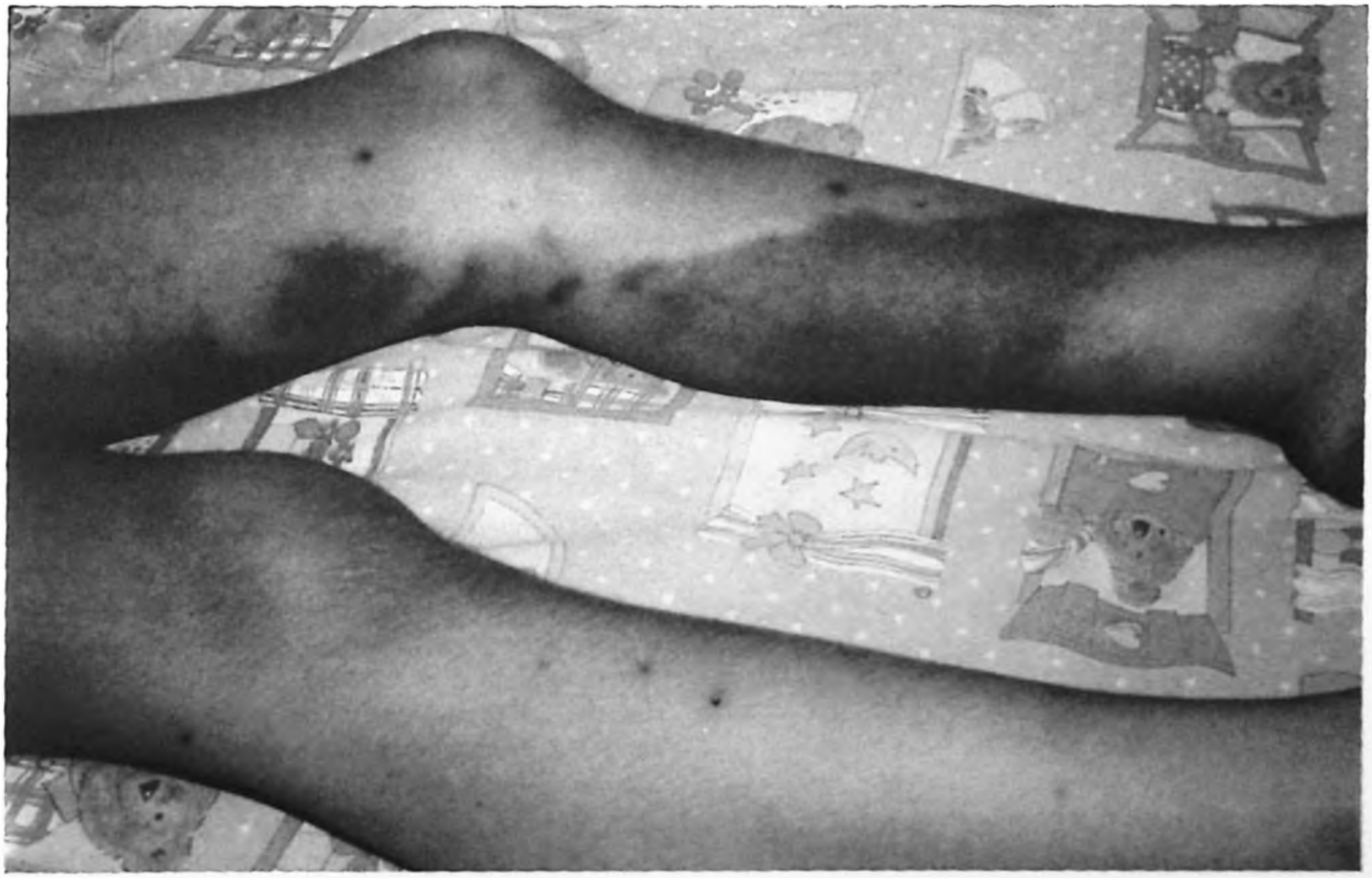

Fig. 1. Purpuric lesions involving medial sides of left knee and leg with typical papulovesicular skinlesions seen in chickenpox.

remained low after 1:1 mixing study with normal plasma. Protein C and protein S levels of the patient's parents and the first-degree relatives were within normal limits. Punch skin biopsy of the margin of the left thigh lesion showed numerous fibrin thrombi, without accompanying vasculitis, and epidermal and dermal necrosis consistent with purpura fulminans.

On the day of admission, she was started on continuous infusion of unfractionated heparin (100 U/hr) and received multiple 10 ml/kg of fresh frozen plasma infusions. During her hospital course, she had full correction of her prothrombin time, partial thromboplastin time, fibrinogen, and D-dimer test, but her protein C activity remained low. She remained afebrile and her skin lesions regressed. She was discharged from the hospital 13 days after admission with oral warfarin. Follow-up clinic visits showed full normalization of her purpuric lesions and correction of the protein C activity.

Discussion

Protein C is the best known among the identified anticoagulant factors in the delicate molecular balance of hemostasis. Activated protein C degrades activated coagulation factors VIIIa and Va, attenuating their procoagulant activation of factors X and prothrombin. Protein S, a vitamin K-dependent glycoprotein, functions as a nonenzymatic cofactor of activated protein C[18], accelerating the inactivation of factors VIIIa and Va, which accounts for the increased risk of thrombosis associated with protein S deficiency states[19]. Depletion of both protein C and protein S has been reported to occur in patients with septicemia-associated purpura fulminans[19,20]. In these cases laboratory evidence of consumptive coagulopathy was also present. It is not known whether low levels of protein C or protein S following such infections are of primary importance in inducing PF or are secondary to consumption coagulopathy caused by other mechanisms.

Although protein C may be depleted as part of disseminated intravascular coagulopathy[21], protein C levels in our patient remained low throughout her admission in the presence of normal levels of protein S and vitamin K-dependent clotting factors VII and IX, and normalizing levels of fibrinogen and D-dimer. Thus protein C deficiency was apparently central to the pathogenesis of the disease. Protein C and protein S levels in the first generation family members of the patient were normal. This, together with the transient nature of the protein C deficiency, excludes familial protein C deficiency as the cause of her disorder.

In 1993, D'Angelo[27] first described the association between transient protein S deficiency and circulating autoantibodies directed against protein S. These anti-protein C or anti-protein S type autoantibodies are IgM and IgG type and they persist for only a few months, after which time the activities of the proteins return to normal. The autoantibody appears to act by binding to and increasing the clearance of protein C or protein S. The frequency with which antibodies to protein C or protein S are induced in children with varicella infection is unknown. Because of the unavailability of the antibody tests in the hospital and outside laboratories, we were not able to confirm the presence of anti-protein C antibody, but we believe that the levels of protein C remaining low after 1:1 mixing test suggests its presence. .

Anticardiolipin antibodies have been associated with quantitative protein S deficiency[23] and functional impairment of protein C anticoagulant pathway[24]. An elevated IgG anticardiolipin antibody titer was also documented in the patient described by D'Angelo et al[22]. Because the titers were not consistently elevated, their contribution to the pathogenesis of the thromboemboli event was difficult to determine. In our patient, we failed to demonstrate any lupus anticoagulant or anticardiolipin antibodies.

It has not been clear whether post infectious PF is mediated by an inflammatory or a vasculitic process, by platelet microthrombi, or by activation of coagulation pathways. As a result, a wide range of therapies has been used. Recognition that the disorder is predominantly a thrombotic process resulting from the deficiency of antithrombotic factors suggests that immediate heparinization is the preferred treatment. Likewise, we believe that administration of heparin, together with large volumes of fresh frozen plasma, terminated the progression of the disease in our patient. Protein C concentrate has been used successfully in the treatment of purpura fulminans associated with disseminated intravascular coagulation and severe acquired protein C deficiency[25].

Based on the available case series of varicella-associated PF in children, it appears reasonable to evaluate children with any varicella-associated

thrombotic event for the presence of protein C or protein S deficiencies. Screening for lupus anticoagulant for anticardiolipin antibodies might also be considered. It should also be stressed that most PF cases secondary to varicella were associated with protein S deficiency rather than protein C deficiency[26,27]. This association of protein C deficiency with PF is, we believe, an interesting point of our case.

The rare but potentially serious occurrences of thrombotic events associated with varicella infection further support recommendations for universal vaccination of children for varicella.

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Hypomelanosis of Ito with trisomy 13 mosaicism [46, XY, der (13;13) (q10;q10), +13/46,XY]

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SUMMARY: Yakıncı C, Kutlu NO, Alp MN, Şenol M, Durmaz Y, Budak T. Hypomelanosis of Ito with trisomy 13 mosaicism [46, XY, der (13;13) (q10;q10), +13/46, XY]. Turk J Pediatr 2002, 44: 152-155.

The term hypomelanosis of Ito (HI) has been used as a diagnosis for individuals with swirly hypopigmentation or depigmentation distributed along the lines of Blaschko. HI should be appropriately evaluated for a possible association with chromosomal or genetic mosaicism or chimerism. We report a six-month-old severely motor and mental retarded boy with these typical cutaneous lesions associated with extracutaneous features, including facial dysmorphism, polydactyly, and inguinal hernia. The cytogenetic examination of lymphocytes demonstrated a mosaicism of 46, XY, der (13;13) (q10;q10), +13/46, XY. This is the first case reported in the literature showing an association between phylloid pigmentary pattern of hypomelanosis of Ito and trisomy 13 mosaicism.

Key words: hypomelanosis of Ito, trisomy 13 mosaicism, phylloid cutaneous pattern, West's syndrome.

A current debate is that certain genetic mechanisms leading to mosaicism are responsible for certain cutaneous patterns. Recent reports have shown that cutaneous symptoms of most of the congenital neurocutaneous syndromes were associated with an underlying chromosome mosaicism or chimerism[1].

Hypopigmentation along the lines of Blaschko is currently thought to be caused by mosaicism that is localized or generalized depending on the stage of fetal development at which the genetic mutation occurs[2]. The constancy of the Blaschko's lines in different dermatomes indicates a normal function rather than a pathologic effect. These lines reflect the streams of growth of embryonic tissues[1]. Chromosomal mosaicism and random distribution of two clones of cells with different pigment potentials with the capability of becoming melanoblasts are responsible in the pathogenesis[3].

In respect of pigmentary disturbance, mosaicism may cause different cutaneous patterns such as classic (lines of Blaschko), checkerboard, phylloid, or patchy patterns without midline separation[2]. The lines of Blaschko are well known to physicians but other patterns such as the phylloid type have so far been entirely ignored.

We present a case with phylloid cutaneous pattern and some extracutaneous manifestations accompanied by Patau's syndrome as well as chromosome 13 mosaicism.

Case Report

A six-month-old severely retarded Turkish boy with typical hypopigmented skin lesions along Blaschko's lines on his trunk noted at the age of one week (without previous history of inflammation, vesiculation, suppuration, or desquamation) was admitted for the evaluation of bilateral polydactyly and left inguinal hernia. His father was 48 and mother was 32 years old. He had two brothers and two sisters, all healthy. On physical examination, his length was 64.5 cm (25th centile) and his weight was 7.2 kg (50th centile), and his occipitofrontal head circumference was 40.1 cm (below 3rd centile). The hypopigmented lesions were bilateral, midline separated, asymmetric leaf-shaped, and

variable in size, found on the trunk and tending to run parallel to each other with spared extremities (Fig. 1a, 1b). He also had microcephaly, psychomotor retardation (two months according to Denver II Developmental Screening Test), coarse facial appearance, umbilical hernia, cryptorchidism, and seizure disorder which manifested as neck and whole body flexion. Routine laboratory and cranial computerized tomography findings were normal. No obvious cardiac, renal, skeletal, or other internal abnormalities were detected. For the chromosomal analysis, peripheral blood culture method[4] was used and slides were stained by trypsin G-banding technique. Peripheral blood of the patient and of his parents was taken and cultured.

The patient was found as mosaic. Some metaphases of the patient were found as normal and others were found trisomic for the long arm of chromosome 13. The breakage and reunion occurred at the band 13q10. The derivative chromosome replaced the long arm of two chromosomes 13. There was, however, one normal chromosome 13 as well. So, the karyotype of the patient was designated as: mos 46, XY, +13, der (13;13) (q10;q10) [27]/ 46, XY [23] (Fig. 2). The derivative chromosome was identified in 27 of 50 metaphases and 23 metaphases were found to have normal karyotype. Thus, the frequency of the trisomic cells was approximately 50%. Chromosomal analyses of parents were carried out and their karyotypes were found to be normal. Therefore, Robertsonian translocation between the chromosomes 13 of the cases was considered de novo.

Electroencephalogram demonstrated hypsarrhythmia. The characteristic clinical and electroencephalographic features favored the diagnosis of West's syndrome. The seizures responded to adrenocorticotropic hormone and valproate treatment. On his follow-up, he died from pneumonia at eight months of age.

Fig. 1a. Hypopigmented leaf-shaped patches with midline separation on the front of the patient (printed with written permission from the patient's parent).

Fig. 1b. Hypopigmented leaf-shaped patches on the lateral aspect of the case.

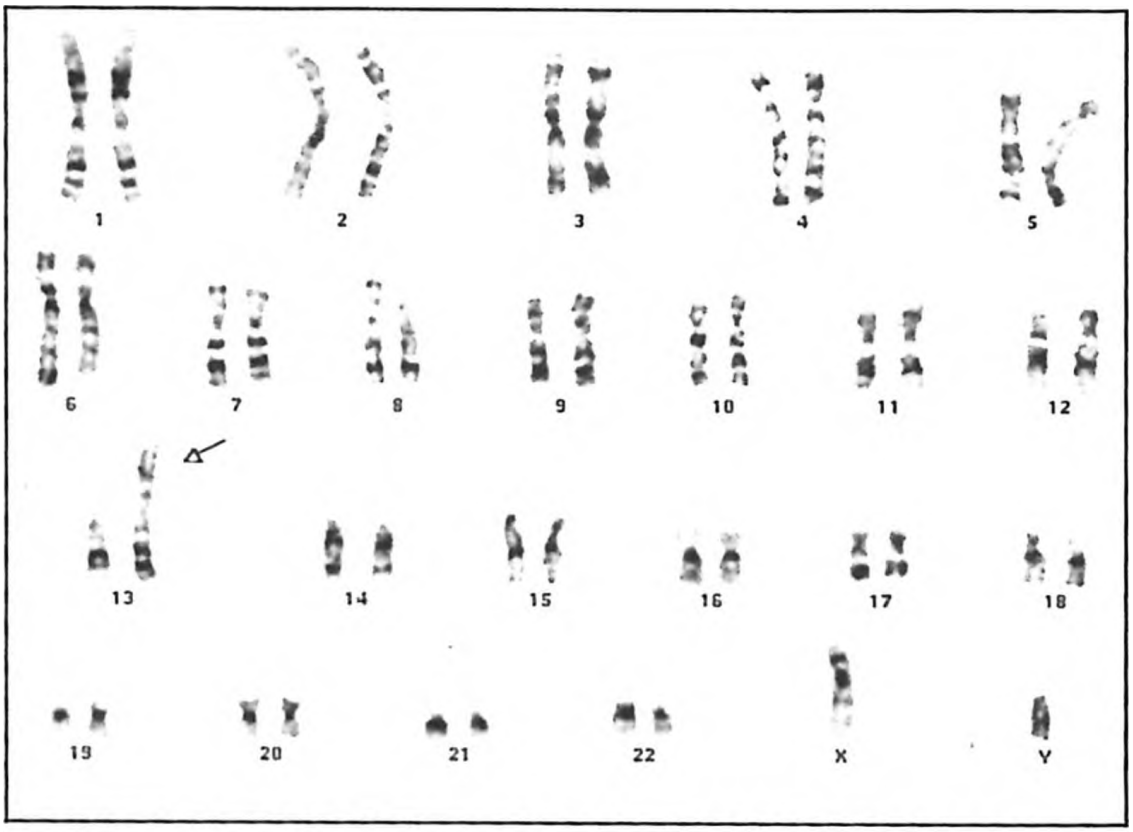

Fig. 2. Karyotype of the case: trisomy for the long arm of chromosome 13 mosaicism [mos 46, XY, +13, der (13;13) (q10;q10) [27]/46, XY [23]].

Discussion

Hypomelanosis of Ito (HI) is one of the well known syndromes in which chromosomal instability may be a component[5]. A number of studies have demonstrated miscellaneous chromosomal abnormalities in some but not all affected individuals[6]; however, there is still no consistent chromosomal finding, and a monogenic cause for HI seems so far unlikely[7]. Therefore, it has been suggested that HI is not a single condition but rather a nonspecific manifestation (i.e., phenotype) of chromosomal mosaicism.

Karyotyping of peripheral blood, and, if this is normal, of skin fibroblasts or better keratinocytes or melanocytes obtained from biopsies taken from affected and unaffected areas, should be performed in affected individuals to support the diagnosis[6]. Chromosomal alterations, however, have been documented in only half of the affected patients in the literature and can be currently classified into two groups: 1) various mosaicism for almost any autosome or sex chromosomes, including tetrasomy 12p, trisomy 18, diploidy/triploidy 45, X/46, X r(X) or 45, X/46, X, +mar and 46, XX/46, XY chimerism. The only consistent feature of this group is that at least two chromosomally different cell lines are present; 2) non-mosaic balanced X; autosome translocations with breakpoints in the juxtacentromeric region of the X chromosome at Xp11 found in a group of girls with skin pigmentary abnormalities in Blaschko's lines and multiple developmental defects including severe mental retardation[6].

What is still unclear in the pathogenesis of HI, however, is how such a variety of different karyotypes should result in a similar linear dyspigmentation, since the control of pigmentation is certainly complex and polygenic, but pigment genes are not likely to be at all of these loci[8]. Mosaicism may produce some kinds of cutaneous pigmentary patterns such as classic pattern through lines of Blaschko, checkerboard pattern, phylloid (leaf-shaped) pattern, and a patchy pattern[2]. So far, phylloid pattern has been described in five patients of whom two had trisomy 13 mosaicism[9] and one had pure trisomy 13[10]. In this patient the pigmentary disturbance did not follow the Blaschko's lines. This observation fits with type 3 (phylloid pattern) of Happle[9] who proposed classification of pigmentary patterns associated with mosaicism.

Associated systemic manifestations seen in HI are primarily neurological, musculoskeletal, and ocular, including mental retardation, seizures, macrocephaly or microcephaly, hemihypertrophy, kyphoscoliosis, hypertelorism, strabismus, and myopia. Other associated disorders occur inconsistently and include coarse facial features, syndactyly, clinodactyly, short stature, genital anomalies, inguinal hernia, congenital heart disease, and abnormalities of the teeth and feet[11]. In a series of 72 children with HI, Pascual-Castroviejo et al.[12] reported 57% with mental retardation, 10% with autistic behavior, 21% borderline and only 22% with normal mental status (IQ>85). Seizures in various types were present in 49% of cases consisting of infantile spasms in six cases (8%)[12]. The fact that there were no specific EEG or evoked potential findings for HI seems to be consistent with the pathogenetically heterogeneous nature of this disorder[13].

The differential diagnosis of HI therefore includes disorders of hypopigmentation that follow Blaschko's lines; the hypopigmented fourth stage of incontinentia pigmenti, Goltz syndrome, and the generalized forms of nevus depigmentosus[14]. Incontinentia pigmenti, like HI, is associated with ectodermal, neurologic and musculoskeletal abnormalities, and it has a hypopigmented fourth stag in approximately 14% of patients. These hypopigmented lesions usually appear on extremities, and are usually preceded by vesicobullous, verrucous, and hyperpigmented stages[14-16]. In our case, characteristic macules had been present since the age of one week, and there was no history of preceding vesiculobullous, verrucous, or hyperpigmented stages.

The features accompanying our case were West's syndrome, microcephaly, coarse facial features, polydactyly, cryptorchidism, and inguinal hernia. All of these clinical features are also the same clinical characteristics of trisomy 13 syndrome. Moreover, this finding supported the hypothesis that underlying chromosomal malformations, at least in some cases, play a definitive role in determination of the clinical appearance of HI, either with cutaneous or extracutaneous malformations.

We conclude that specific genetic anomalies, when present as a mosaic, can produce the same type of cutaneous pattern. We present a typical case of incontinentia pigmenti achromians with trisomy 13 mosaicism.

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A rare primary pulmonary tumor of childhood

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Pleuropulmonary blastoma (PPB) is known to be the pulmonary blastoma of childhood. It has a range of macroscopic and microscopic features which appear to correlate with eventual prognosis. Type 1, presenting as a multicystic lesion, occurs at an earlier age and has a more favorable prognosis than other types.

The presented case of type 1 PPB had a microscopic focus of rhabdomyosarcoma. Although this patient was disease-free one year after the initial diagnosis without chemotherapy, he presented at 14 months with local dissemination and cardiac metastasis, revealing the inevitable chemo-radiotherapy need in PPB.

Key words: blastomas, childhood tumors, histopathology, pleuropulmonary blastoma, prognostic factors.

Primary pulmonary neoplasms are rare in children and there are only sporadic examples of basically adult-type pulmonary tumors. Most neoplasms are metastatic in nature; more commonly there are some non-neoplastic conditions that may simulate a tumor, i.e. an abscess, intrapulmonary bronchogenic cyst, or solid adenomatoid malformation[1].

In the lung, mesenchymal tumors, with a clear antecedence of or concomitant with cystic lesions, have frequently been reported[2-4]. Rhabdomyosarcoma is the most common type of mesenchymal tumor associated with lung cysts[3,5,7]. We present a case of embryonal rhabdomyosarcoma arising within a pre-existing congenital lung cyst, namely a type 1 pleuropulmonary blastoma (PPB).

Case Report

A three-year-old boy was admitted to the hospital for tachypnea and shortness of breath. He had two previous similar attacks, the first at the age of three months and both resolving after chest tube placement. On these previous admissions, operation was recommended as chest X-rays suggested congenital lobar emphysema or adenomatoid cystic malformation, but the family refused. At the last admission, he was found to be afebrile and dyspneic. Breath sounds could not be heard on the upper sides bilaterally. A chest X-ray showed complete opacity in this area. The placement of a chest tube yielded partial expansion of the right lung but symptoms were not relieved. Culture from the outlet of the chest tube was negative. Thorax computerized tomography (CT) scan showed pneumothorax in the left lung field, and air-filled areas between the parietal and visceral pleura (Fig. 1). Thus, some kind of parenchymal or bronchial congenital abnormality characterized by a large cystic mass with questionable septa was proposed. Thoracotomy revealed a huge cyst based on the mediastinal surface of the left upper lobe and largely situated in the anterior mediastinum. The cyst could not be removed en masse and disintegrated when handled. The child was discharged two weeks later as both lungs expanded.

The surgical specimen was reddish-brown, fragmented, fibrotic and irregular tissue measuring 6x5x1 cm; some resembling cyst walls. The cuboidal epithelium could barely be seen lining the cyst wall with fibrosis, congestion and chronic inflammation. Neighboring lung tissue was unremarkable. Thorough sampling revealed that the cysts were mostly lined by respiratory epithelium and, in two examples, the cambium layer was present beneath the

This study was presented at the XXII International Congress of the International Academy of Pathology, October 18-23, 1998, Nice, France.

epithelium with deeply situated rahbdomyoblastic cells and rare bizarre giant cells (Fig. 2). Immunohistochemical staining using PAP method with anti-desmin and smooth muscle actin (SMA) antibodies (BioGenex) showed strong reactivity with desmin in most of the deeply situated cells, but no reactivity with SMA The pathologic diagnosis was type I PPB.

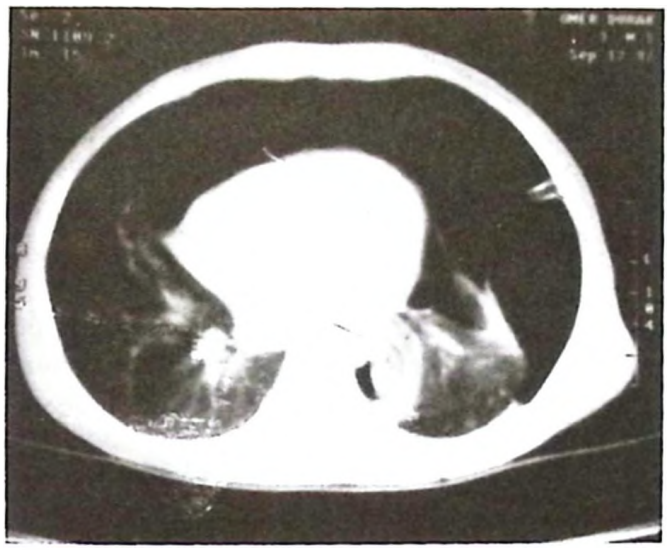

Fig. 1. Thorax CT image showing pneumothorax and air-filled areas between the parietal and visceral pleura in the left lung field.

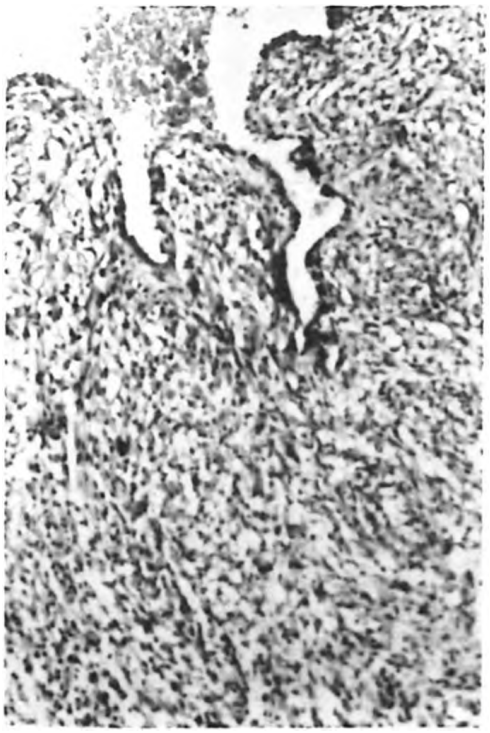

Fig. 2. Cells with rhabdomyoblastic differentiation beneath the surface epithelium of the cyst (H&E, x20).

After the pathologic diagnosis, thorough evaluation for metastases was negative and chemotherapy was planned. However, the family refused the medication. He was well 12 months later, but 14 months after the diagnosis, he presented with dyspnea, facial edema, prominence of jugular veins and a mass of 4x5 cm on the thoracic wall. The clinical picture was consistent with a superior vena cava syndrome. A chest X-ray and a CT of the thorax revealed the presence of a mass measuring 11.8x12x7.8 cm in the middle lobe of the right lung and pericardial effusion.

Echocardiogram showed a 1.5x1.5x1 cm mass in the vicinity of the tricuspid valve without disturbing its function. Investigations for metastases at other sites were negative. With these findings, he was accepted as PPB with tricuspid valve metastasis, and vincristine, Actinomycin D, cyclophosphamide polychemotherapy protocol was started. After five courses of chemotherapy, the tumor in the lung shrank to 6x5.3x4 cm and pericardial effusion regressed, but the mass in the heart remained the same. He then received radiation therapy to the primary tumor site, but died with disseminated metastasis 20 months after his initial diagnosis.

Discussion

Rhabdomyosarcoma (RMS), although a common mesenchymal tumor of childhood, rarely occurs as a primary pulmonary tumor[8]. Indeed, primary tumors of the lung are very uncommon in children. In a series of 43 childhood cases, Cohen and Kaschula[1] eight primary pulmonary tumors (18.6%), two (4.6%) of which were PPB, second only to plasma cell gronuloma.

In children, 10% or less of pulmonary blastomas (PB) have classic histological features with epithelium, blastema and stroma. PPB is accepted to be the PB of childhood and occurs in various anatomical locations in the thoracic cavity (intrapulmonary, mediastinal or pleural based), whereas classical PB is usually located in the lung parenchyma. In PPB only mesenchymal cells are neoplastic in contrast to classical PB defined as a distinctive group of carcinosarcoma[7,9-11]. There are also reported childhood cases of pulmonary adenocarcinomas of fetal type, but this entity bears no relationship to PPB[11].

Twenty-five percent of PPB occur in a constitutional/familial setting in which PPB patients themselves or young family members

have other dysplastic or neoplastic conditions, leading to the hypothesis that the PPB may arise in a precursor developmental abnormality[1]. There is preliminary evidence that PPB may be associated with loss of heterozygosity on chromosome 11p 15.5 in the region of Wilms' tumor gene; the rare association between PPB and Wilms' tumor has been implicated in RMS[7,12]. Cytogenetic findings suggest common genetic mechanisms between embryonal RMS and PPB[13,14]. We could not perform any cytogenectic investigations in our case. On the other hand, the child had an elder healthy sister, but the family history was questionable about presence of a similar disorder because another sister had died at the age of 40 days with cyanosis.

Pleuropulmonary blastomas (PPB) have a range of macroscopic and microscopic features which appear to correlate with eventual prognosis. Type 1 is the least complex pattern presenting as a multicystic lesion, occurring at an earlier age and having a more favorable prognosis than other types. Type 2 tumors have grossly visible cysts but also solid foci of blastematous islands; nodules of malignant appearing cartilage; small or large clusters of pleomorphic, anaplastic cells; and spindle cell sarcoma. Type 3 are exclusively solid tumors which are among the largest masses and are associated with the greatest degree of friability, hemorrhage and necrosis[9]. The macroscopic and microscopic features of our case were consistent with type 1 PPB with a focus of embryonal RMS.

There is a significant relationship between pulmonary cystic disease and PPB. In a series of 50 PPB cases, pulmonary cysts and/or pneumothorax had at some time been present in 19 cases (48%), and intracystic masses developed in 41.7% of the cysts observed before one month of age[7]. However, considering only congenital malformations, the frequency of pulmonary cyst-related PPB is not as high as expected[16,17]. It remains uncertain whether PPB arises in an underlying malformation of the lung or PPB has itself the potential to induce the formation of epithelial-lined cysts and to elicit cystic transformation as an initial manifestation of the neoplasm[7]. The presented case had two previous attacks of pneumothorax. The presence of cyst was first observed at the age of three months but it was probably congenital.

The probability of finding malignancies microscopically justifies prompt resection of pulmonary cysts shortly after diagnosis[16,17]. Other malignancies such as bronchoalveolar carcinoma, arising in congenital cysts, have also been infrequently reported[4,16].

Pleuropulmonary blastoma PPB is a disease with poor prognosis[3,5,7,10,11,18]. In a series of 50 PPB cases, event-free survival at two years was 83% for type 1, 49% for type 2 and 42% for type 3, and the overall survival at five years was 45%. Despite aggressive chemotherapy[18], and sometimes radiotherapy[3,7], some of the patients die with metastases especially to the brain[3,7,18], bones[7] and lymph nodes[3,5]. Local recurrences were also experienced[6,7].

The presented case was disease-free one year after the diagnosis despite no chemotherapy having been given. We were aware that this follow-up period was short for a distinct outcome, because recurrence after 60 months has been reported[7]. In fact, 14 months later local dissemination and cardiac metastasis were detected in this patient. Although CT and radiotherapy seemed to reveal the clinical symptoms and limit the disease, the patient died of disseminated metastasis at 20 months after the initial diagnosis.

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Congenital double-orifice mitral valve associated with atrioventricular septal defect: A case report

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SUMMARY: Küçükosmanoğlu O, Özbarlas N, Salih OK. Congenital double-orifice mitral valve associated with atrioventricular septal defect: a case report. Turk J Pediatr 2002; 44: 160-161.

The case of an eight-year-old girl with congenital double-orifice mitral valve associated with atrioventricular septal defect is reported. This rare mitral valve anomaly was diagnosed with two-dimensional echocardiography. The associated atrioventricular septal defect was repaired successfully. The central fibrous bridge between anterior and posterior mitral valve leaflets was left undivided to avoid iatrogenic mitral regurgitation. Preoperative diagnosis may provide some useful information to the surgeon. The surgical method can be individualized in each case.

Key words: double-orifice mitral valve, atrioventricular septal defect, echocardiography.

Double-orifice mitral valve (DOMV) is a rare condition and may be associated with other cardiac anomalies such as subaortic ring, coarctation of aorta, patent ductus arteriosus, pulmonary stenosis, ventricular septal defect, truncus arteriosus and primum type atrial septal defect[1-6]. The orifices are usually unbalanced in size and there is a central fibrous bridge between the anterior and posterior mitral valve leaflets. The medial and lateral orifices are equal in size in only 15% of patients[1].

Herein, we report an eight-year-old girl with balanced DOMV and primum type atrial septal defect who was diagnosed with two-dimensional echocardiography and was successfully treated with surgical intervention.

Case Report

An eight-year-old girl was referred to our department for echocardiographic study because of heart murmur. She had growth retardation with stature of 109 cm (3rd percentile), and weight of 17 kg (<3rd percentile). Physical examination revealed normal blood pressure, regular pulse of 88 bpm, fixed splitting of second heart sound at the upper left sternal edge and 3rd degree apical pansystolic murmur. The other findings were all normal. Chest X-ray showed marked cardiomegaly with a cardiothoracic index of 0.64 and increased vascularization. Echocardiogram (ECG) showed normal sinus rhythm, left QRS axis (-45°) deviation and incomplete right bundle-branch block. Two-dimensional echocardiography showed a large primum type atrial septal defect, small mitral cleft and balanced DOMV (Fig. 1), and color Doppler showed moderate mitral regurgitation. Cardiac catheterization revealed mild pulmonary hypertension with a mean pulmonary artery pressure of 27 mmHg and increased pulmonary flow with a Qp/Qs ratio of 3.2/1.

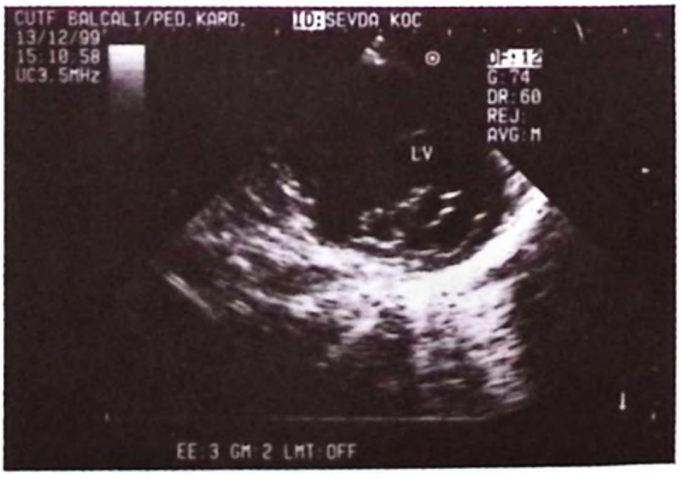

Fig. 1. Two-dimensional echocardiographic view of double-orifice mitral valve in parasternal short-axis window.

The primum atrial septal defect was repaired with pericardial patch without complication. The cleft was partially sutured to avoid creating mitral stenosis and the bridging tissue was left undivided to prevent more mitral regurgitation. After one year follow-up she caught up growth with a stature of 119 cm and a weight of 22 kg. Chest X-ray showed a normal cardiothoracic index and echo-Doppler study showed trivial mitral regurgitation.

Discussion

Congenital mitral valve anomalies are rare conditions and are usually associated with some other cardiac defects. Banerjee et al.[3] reported 65 children with congenital mitral valve anomalies in their study, which consisted of 13,400 new echocardiographic data during 7.5 years, and only seven of the children had DOMV.

As well as in other mitral valve anomalies, two-dimensional echocardiography is an extremely useful method for the diagnosis of DOMV. Two separate holes in the mitral valve can be identified in parasternal short-axis view. The color Doppler echocardiography provides an adequate anatomical and functional assessment of DOMV[3-4].

The diagnosis of DOMV prior to surgical intervention for associated defects is very important. If the central fibrous bridge between the anterior and posterior mitral valve leaflets causes functional mitral stenosis, it may require resection. But in patients without functional mitral stenosis it may be beneficial not to incise the bridging tissue to avoid iatrogenic mitral regurgitation[7]. Valve replacement should be the treatment of choice in severe mitral regurgitation with DOMV. Yurdakul et al.[8] reported successful valve replacement in an 18-month-old boy with severe mitral regurgitation associated with DOMV.

The DOMV may be an incidental echocardiographic finding but it is usually associated with another cardiac anomaly. Preoperative echocardiographic evaluation of the mitral valve may provide some useful information to the surgeon. The surgical method can be indivualized in each case.

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A case of severe Ebstein's anomaly with incompetent pulmonary valve

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SUMMARY: Özkutlu S, Ceviz N, Ayabakan C, Akçören Z. A case of severe Ebstein's anomaly with incompetent pulmonary valves. Turk J Pediatr 2002; 44: 162-167.

A case of Ebstein's anomaly with functional pulmonary atresia diagnosed in utero is presented. The diagnosis was confirmed by postnatal echocardiographic, angiographic, and postmortem pathologic findings. On echocardiography the septal leaflet of the tricuspid valve was displaced towards the right ventricular apex. The tricuspid valve was moderately regurgitant and the arterial duct was patent. Continuous wave or color Doppler revealed serious reduction in forward flow from right ventricle through the pulmonary arteries; however, massive pulmonary regurgitation was observed. Pulmonary circulation was dependent on the ductal flow due to functional pulmonary atresia. Angiography revealed the massively enlarged right atrium, the absence of forward flow through the tricuspid valve, transfer of contrast material through the atrial septal defect to the left atrium, and the retrograde inflow of the pulmonary arteries from the aorta via the patent arterial duct.

Ebstein's anomaly accompanied by functional pulmonary atresia is very rare. The fetal and neonatal presentation of this anomaly is associated with poor outcome.

Key words: Ebstein's anomaly, neonate, functional pulmonary atresia.

Ebstein's anomaly of the tricuspid valve is an uncommon malformation representing 0.3%-0.6% of all cases of congenital heart disease[1-3]. The basic defect involves a deformed and apically displaced tricuspid valve, resulting in symptoms that vary with age of the patient and the severity of the defect[1,4]. However, high pulmonary vascular resistance in the neonatal period may aggravate tricuspid regurgitation and lead to functional pulmonary atresia. Infants with severe Ebstein's anomaly and functional pulmonary atresia remain dependent on ductal patency. Hence this anomaly carries a high mortality rate in the neonatal period[1,2].

The purpose of this report is to describe and to discuss a neonate with severe Ebstein's anomaly, accompanied by functional pulmonary atresia. This iss a rare case, in which clinical, hemodynamic, angiographic, and pathological data are available.

Case Report

The mother of the patient was referred to the Pediatric Cardiology Unit in the 19th week of pregnancy with the suspicion of Ebstein's anomaly detected at routine fetal ultrasonographic evaluation. Fetal echocardiography revealed severe Ebstein's anomaly of the fetus.

The baby was delivered at the 35th week of gestation due to oligohydramnios and reduced fetal movements. The Apgar scores at 5 and 10 minutes postnatally were determined as 3 and 5, respectively. The baby was resuscitated because of absence of spontaneous respiration, severe cyanosis and bradycardia. Immediate postnatal echocardiographic study revealed displacement of the septal leaflet of the tricuspid valve towards the right ventricular apex. The distane between the tricuspid valve annulus and ventricular insertion of the septal leaflet was 11.8 mm (Fig. 1). The tricuspid valve was moderately regurgitant and the arterial duct was patent. Continuous wave or color Doppler revealed serious reduction in forward flow from right ventricle through the pulmonary arteries; however, massive pulmonary regurgitation was observed (Fig. 2). Pulmonary circulation was dependent on the ductal flow due to functional pulmonary atresia.

Cardiac catheterization via umbilical vein was performed; however, the catheter could not be advanced through the tricuspid valve to the right

ventricle and the pulmonary artery. Instead, it was advanced through the atrial septal defect to the left atrium and left ventricle. Right atrial and left ventricular angiograms were obtained. The right atrial angiogram (Figs. 3 and 3a) revealed the massively enlarged right atrium and the absence of forward flow through the tricuspid valve. All the contrast material passed through the atrial septal defect to the left atrium. The left ventriculogram (Fig. 4, 4a, 5 and 5a) demonstrated the retrograde inflow of the pulmonary arteries from the aorta via the patent arterial duct. Due to severe pulmonary regurgitation, contrast material reached the right ventricle and subsequently the right atrium via the regurgitant apically displaced tricuspid valve.

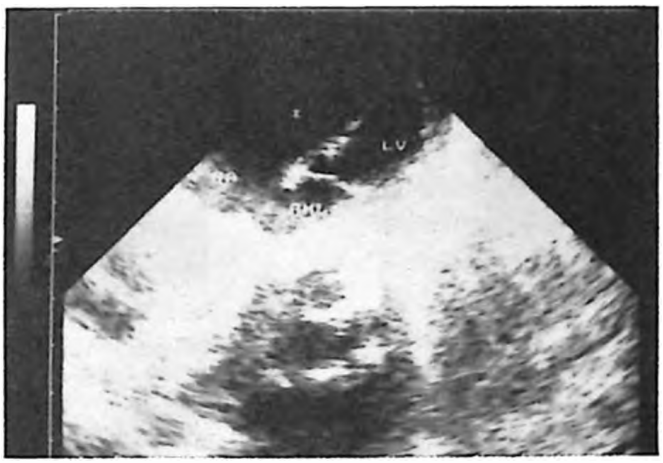

Fig. 1. The four-chamber view showing the mitral valve attachment. The right atrium is enlarged, and the tricuspid valve is greatly displaced towards the right ventricle and cannot be seen. The asterisk indicates the atrialized right ventricle. RA: right atrium, LV: left ventricle.

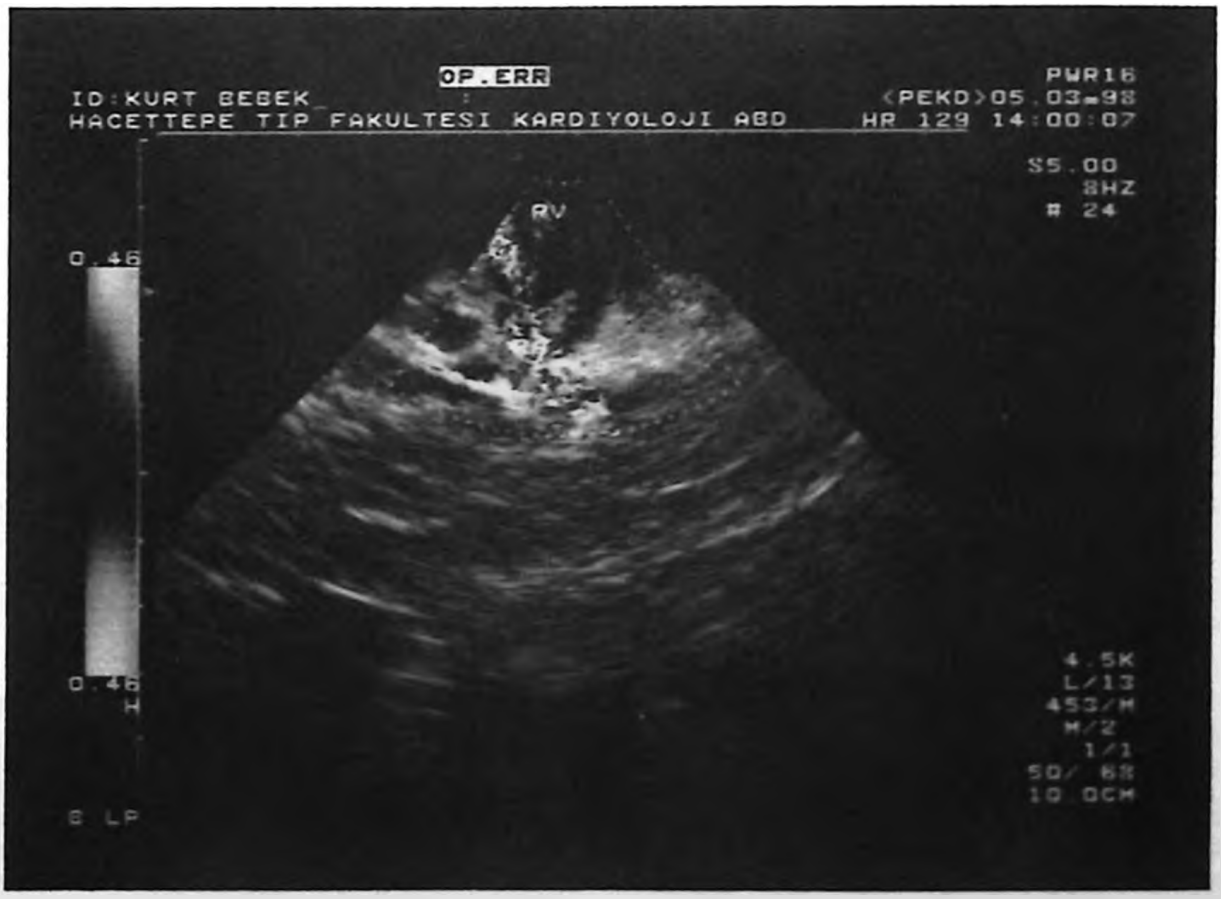

Fig. 2. A modified parasternal short-axis view showing severe pulmonary valve regurgitation. RV: right ventricle, PA: pullmonary artery.

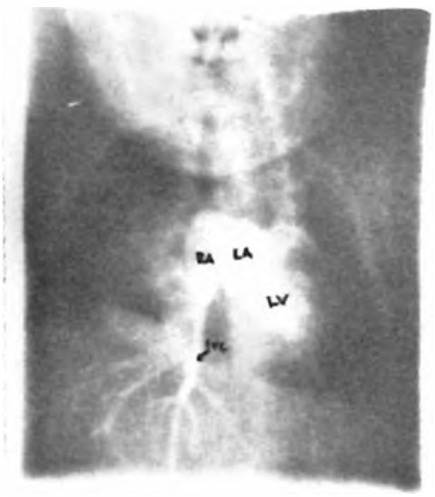

Fig. 3. The right atrial injection at antero-posterior position. Contrast material is seen in the right atrium. It travels to the left atrium and the left ventricle via the atrial septal defect. IVC: inferior vena cava, RA: right atrium, LA: left atrium, LV: left ventricle.

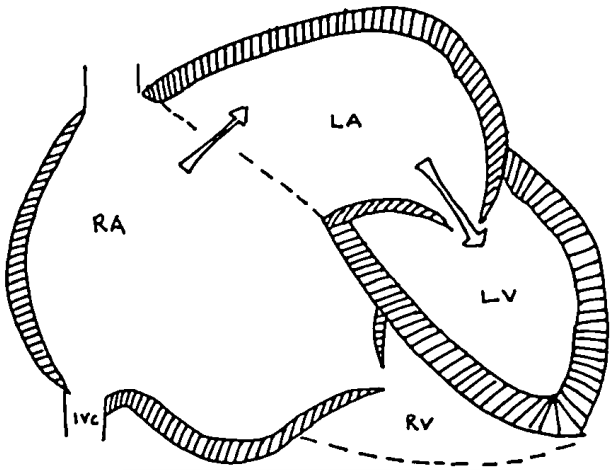

Fig. 3a: The right atrial injection at antero-posterior position (illustration of Fig. 3). Contrast materil is seen in the right atrium. It travels to the left atrium and the left ventricle via the atrial septal defect. IVC: inferior vena cava, RA: right atrium, LA: left atrium, LV: left ventricle.

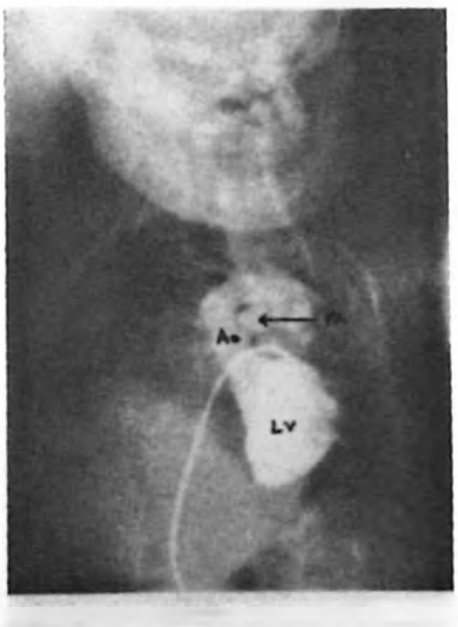

Fig. 4. The left ventricle injection in antero-posterior position (early phase). The left ventricle with intact ventricularseptum and the retrograde inflow of the pulmonary arteries from the aorta via the patent arterial duct are seen. LV: left ventricle, Ao: aorta, PA: pulmonary artery.

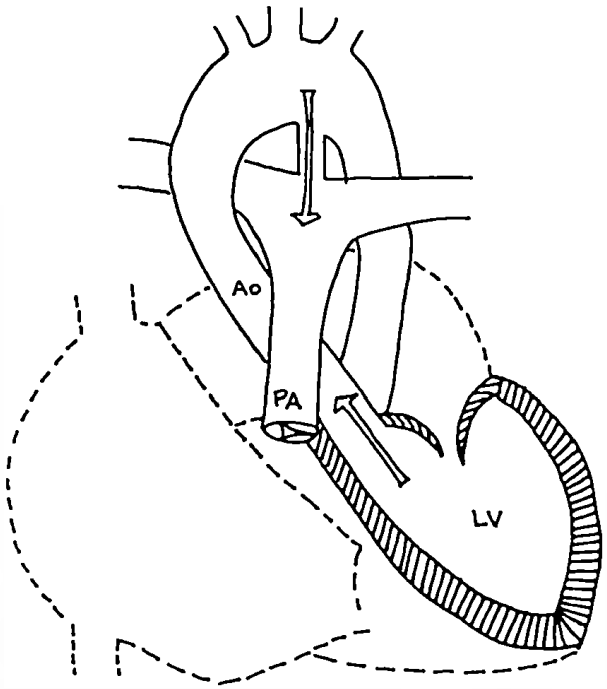

Fig. 4a. The left ventricle injection in antero-posteriorposition in early phase (illustration of Fig. 4). The left ventricle with intact ventricular septum and the retrograde inflow of the pulmonary arteries from the aorta via the patent arterial duct are seen. LV: left ventricle, Ao: aorta, PA: pulmonary artery.

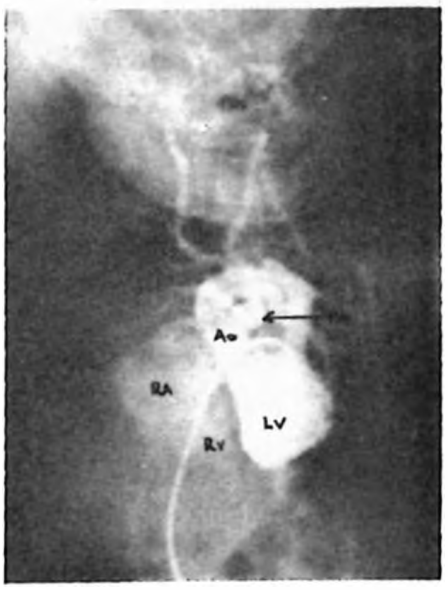

Fig. 5. The left ventricle injection in antero-posterior position (late phase). There is retrograde inflow to the pulmonary arteries from the aorta via the patent arterial duct. Due to severe pulmonary regurgitation, contrast material reaches the right ventricle and subsequently the right atrium via the regurgitant ricuspid valve. LV: left ventricle, Ao: aorta, PA: pulmonary artery, RV: right ventricle, RA: right atrium.

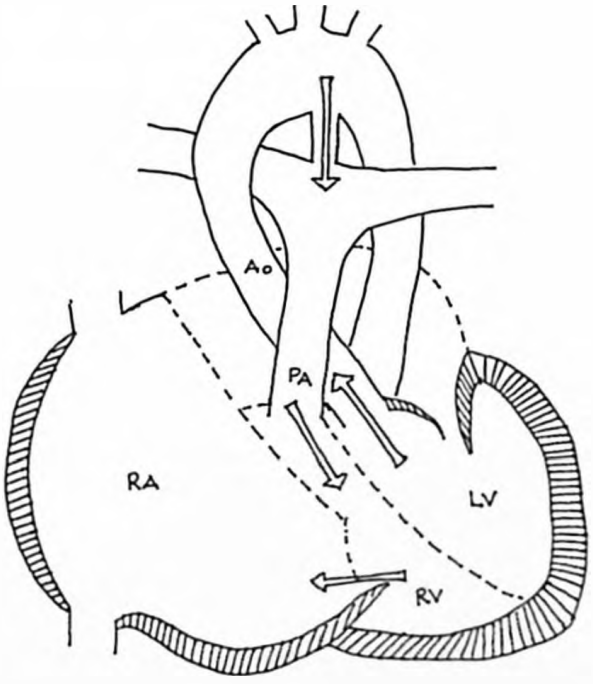

Fig. 5a. The left ventricle injection in antero-posterior position in late phase (illustration of Fig. 5). There is retrograde inflow to the pulmonary arteries from the aorta via the patent arterial duct. Due to severe pulmonary rergurgitation, contrast material reaches the right ventricle and subsequently the right atrium via the regurgitant ricuspid valve. LV: left ventricle, Ao: aorta, PA: pulmonary artery, RV: right ventricle, RA: right atrium.

The patient died of severe heart failure at the 9th hour of birth, despite aggressive resuscitation and prostaglandin E1 infusion. The postmortem examination revealed severe Ebstein's anomaly with a wide atrial septal defect, right atrial dilatation, a large patent arterial duct and a mild pulmonary valvular stenosis. ;Thickened and nodular leaflets were observed on the tricuspid and pulmonary valves. Microscopic examination of these leaflets demonstrated connective tissue without inflammatory reaction.

Discussion

Ebstein's anomaly of the tricuspid valve is a relatively uncommon congenital heart defect showing distinct clinical manifestations with a high mortality rate in the neonatal period[2,4]. The basic defect is marked by variable degrees of displacement of proximal attachments of the valve from the atrioventricular ring[5-9]. This is associated with considerable variability in clinical outcome, with mortality rates for neonatal presentation ranging from 27% to 48%[1,10-13]. The majority of these infants die within the first week of life, although there remains a significant risk of late sudden death with or without surgical intervention[5].

The natural history of this malformation was previously based on clinical and angiographic diagnosis of older children and adults; however, echocardiography facilitated fetal and neonatal diagnosis and has redefined the outcome[14]. In severe cases of Ebstein's anomaly, cyanosis that results primarily from a right-to-left shunt at the atrial level is a prominent finding. Presence of increased pulmonary vascular resistance is an additional problem in the neonatal period. As the right ventricle cannot generate forward flow through the pulmonary arteries with increased vascular resistance, it results in functional pulmonary atresia that worsens the clinical outcome[3,5]. Mortality rate for cyanotic neonates with Ebstein's anomaly (47%) has been reported to be significantly higher than in those without cyanosis (14%)[5]. Other predictors of neonatal mortality include tethered distal attachments of the anterosuperior tricuspid leaflet; right ventricular dysplasia; left ventricular compression by right heart dilatation; atrial septal defect of more than 4 mm; and the area of combined right atrium and atrialized right ventricle greater than the combined area of the functional right ventricle.

left atrium and left ventricle[11]. Our patient had massive right atrial dilatation and a wide atrial septal defect, causing the early mortality.

Early presentation is frequently associated with other cardiac lesions, usually pulmonary stenosis or atresia. Right ventricular outflow tract obstruction is a known risk factor for early death[14,15]. In patients with severe Ebstein's anomaly and functional pulmonary atresia, the clinical differentiation between functional and structural right ventricular outflow tract obstruction needs to be clarified, but it is frequently a difficult tast[16]. Pulmonary valve regurgitation as seen on aortography or left ventriculography does not ensure a non-obstructive pulmonary valve. Rarely regurgitation may be demonstrated across a severely stenotic pulmonary valve. However, significant pulmonary regurgitation (as assessed from opacification of the right atrium on aortography/left ventriculography) with normal or dilated pulmonary root precludes the diagnosis of structural atresia or severe obstruction, as in our patient[16]. The mechanism of regurgitation of contrast material across the pulmonary valve in patients with functional pulmonary atresia is uncertain. We think the thickened nodular leaflets and annular dilatation in our patient may be the most probable causes.

The postmortem pathologic examination of our patient revealed pulmonary valve stenosis with nodular thickening of the tricuspid and pulmonary valve leaflets. Pathologic findings mentioned in other studies included significant thinning of the right ventricular free wall distal to the tricuspid valve, reduced right ventricular fiber diameter, and increased fibrous tissue content of both right and left ventricular free walls[10]. The pulmonary valve anatomy may be variable in Ebstein's anomaly with functional pulmonary atresia. Among the necropsies of Freedom et al.[16], in some of the patients the pulmonary valve appeared entirely normal, whereas in others it was redundant. In one patient the valve was congenitally bicuspid but non-obstructive. Nodular changes on pulmonary or tricuspid valves were not mentioned. Therefore this morphology observed in our patient may be another variety of the pathology.

Grant[17] reported Ebstein's anomaly and tricuspid atresia in siblings. The presence of these two distinct pathologic variants of tricuspid valve malformations in siblings caused the authors to postulate that these malformations result from a common abnormality occurring during the development of the inlet portion of the ventricle. Bharati et al.[18], in an anatomic study of cases of pulmonary atresia with intact ventricular septum, found that the tricuspid valve and the right ventricular morphology differed greatly between type 1 and type II pulmonary atresium Type II (pulmonary atresia with tricuspid insufficiency) had a greater morphologic resemblance to Ebstein's disease with pulmonary atresia than type I (pulmonary atresia without tricuspid regurgitation). The variety of morphologies within a common embryological origin versus the morphological similarities between different embryological origin suggest that many aspects of these anomalies yet to be discovered and are imprecise. In conclusion, in Ebstein's anomaly, fetal and neonatal presentation is associated with poor outcome that can be predicted with the presence of associated lesions. It probably exhibits different undiscovered anatomical morphologies, which may, in the future, enlighten the developmental pathology linked to this anomaly.

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Renal tuberculosis mimicking xanthogranulomatous pyelonephritis: ultrasonography, computed tomography and magnetic resonance imaging findings

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The incidence of tuberculosis has been increasing in recent years, and its treatment has also become challenging. The diagnosis of renal tuberculosis is often difficult and delayed. Early and correct diagnosis of tuberculosis with different organ system involvement is very important and can be easier with ultrasonography, computed tomography and/or magnetic resonance imaging. Although renal tuberculosis is the result of hematogenous spread more commonly from the lungs, less than 5%, of patients with urinary tract tuberculosis have active pulmonary disease.

Renal tuberculosis may show variable radiological findings depending on the stage of the infection. Although an end-stage "autonephrectomized" kidey in tuberculosis is classically defined to be small in size, enlargement may on rare occasions be observed, which is the case in our patient. This form greatly mimics diffuse xanthogronulomatous pyelonephritis. Both diseases show thickening of the perirenal fasciae and spread of inflammation into the adjacent organs. Computed tomography and magnetic resonance imaging may show some specific features to differentiate these two entities.

Key words: kidney, tuberculosis, xanthogranulomatous pyelonephritis; ultrasound; computed tomography; magnetic resonance imaging.

Renal tuberculosis (TB) is almost always the result of hematogenous spread from the respiratory system, and occasionally from bones or the gastrointestinal tract. A spectrum o findings may be present depending on the stage of infection and the degree of host response. Advanced renal TB with fibrosis and stricture in the renal pelvis and total calyceal hydronephrotic/pyonephrotic appearance with renal calculi may mimic diffuse xanthogranulomatous pyelonephritis (XGP).

Case Report

A 16-year-old female patient, who was otherwise healthy, presented with intermittent left flank pain and a history of recurrent urinary tract infections. A tender mass in the left upper quadrant was palpated. Laboratory data revealed anemia (hemoglobin: 8.4 g/dl), leukocytosis (WBC count: $17x10^3/mm^3$), and urine culture positive for Proteus species and normal blood urea nitrogen, serum creatinine and liver function tests.

Scout kidney-ureter-bladder (KUB) film revealed multiple calculi and an irregular group of calcifications overlying the lower pole of the left kidney. Intravenous pyelogram (IVP) showed a nonfunctioning left kidney (Fig. 1). Ultrasonography (USG) revealed a large reniform shaped mass in the left renal bed with multiple variably echo-poor areas, probably corresponding to dilated calyces. Calculi and calcifications with distal shadowing were observed (Fig. 2). Anothner large heterogeneous mass posterior to the kidney extending into the left psoas muscle was identified. On computed tomography (CT), an enlarged kidney with a preserved reniform outline and marked peripelvic fibrosis was demonstrated. No calculus or calcification was observed in the renal pelvis but they were present in the lower pole calyces and parenchyma (Fig. 3).

Multiple low-density ovoid areas, consistent with a hydronephrotic pattern with a surrounding rim of tissue, that strongly enhanced with intravenous

contrast media, were observed. Perirenal and lateroconal fasciae were markedly thickened. The ureter and renal vascular pedicle could not be identified. CT demonstrated a huge left psoas abscess also involving the quadratus lumborum muscle. Dystrophic calcifications were observed within the abscess (Fig. 4). On CT scans the tail of the pancreas and spleen were displaced anteriorly and superiorly by the enlarged left kidney, and fat planes in between were obliterated. Lumbar vertebrae were normal. Magnetic resonance imaging (MRI) revealed an enlarged left kidney with prominent calyceal dilatation and cortical thinning. Corticomedullary differentiation was lost. On T2-weighted (TR/TE/NEX=2560/90/2) axial images, negative defects within some calyces corresponding to calculi were observed. Perirenal fasciae were thickened and showed a low signal on T2-weighted images, indicating fibrotic changes or thick fibrinous exudates. A mass lesion overlying the left psoas and quadratus lumborum muscles was observed. The contours of the pancreas anterior to the kidney were edematous, but the interface between the kidney and spleen was preserved. Postcontrast TI-weighted (660/21/1.5) images showed marked contrast enhancement of thinned cortex, thickened perirenal fasciae and psoas mass (Fig. 5).

Transabdominal nephrectomy was performed. The renal mass was yellowish in color, extremely firm and immobile. An abscess extending into the psoas and quadratus lumborum muscles was drained. Microscopic evaluation revealed giant granulomas with central caseification necrosis, peripheral giant cells, fibroblasts and lymphocytes consistent with tuberculosis.

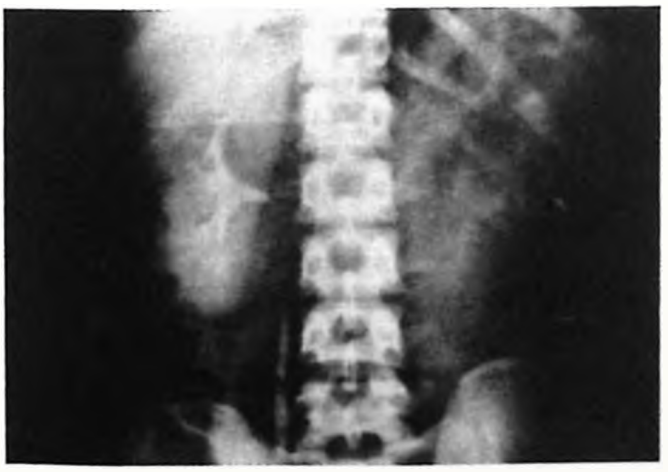

Fig. 1. Intravenous pyelogram shows nonfunctioning left kidney with multiple calculi and punctate calcifications overlying the lower pole (→).

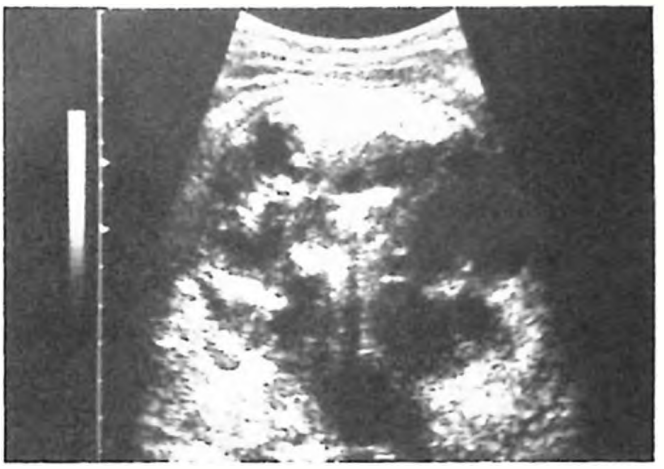

Fig. 2. Transverse sonogram of the left kidney shows diffuse renal enlargement with irregular contour and multiple hypoechoic areas probably corresponding to dilated calyces (→). Parenchyma-sinus differentiation is lost. Small calculi and calcifications with distal shadowing are observed (→).

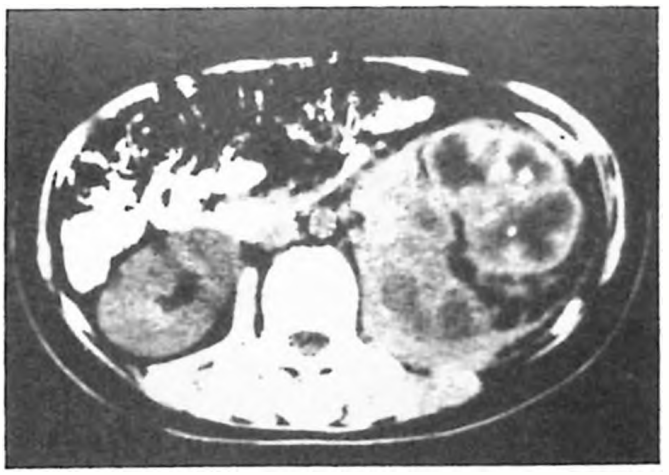

Fig. 3. CT reveals an enlarged left kidney with a preserved reniform outline and a hydronephrotic pattern (→). Small calculi and punctate calcifications are observed (→). Marked peripelvic, periureteric fibrosis; an abscess extending into the left psoas and quadratus lumborum muscles and thickened perirenal fasciae are domonstrated (→).

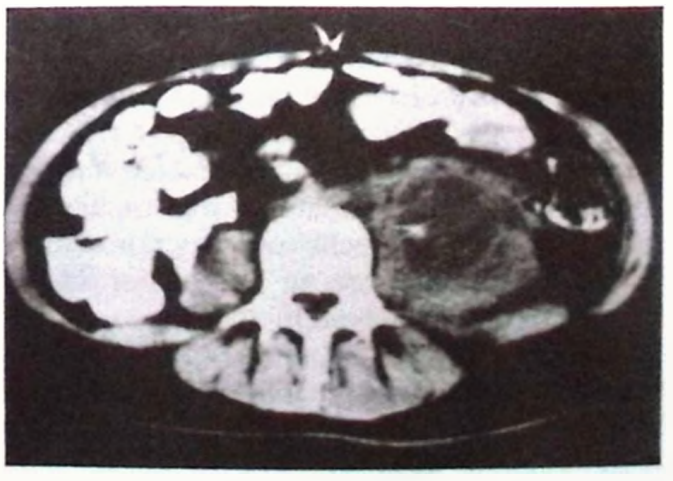

Fig. 4. CT shows calcification within the psoas abscess (→).

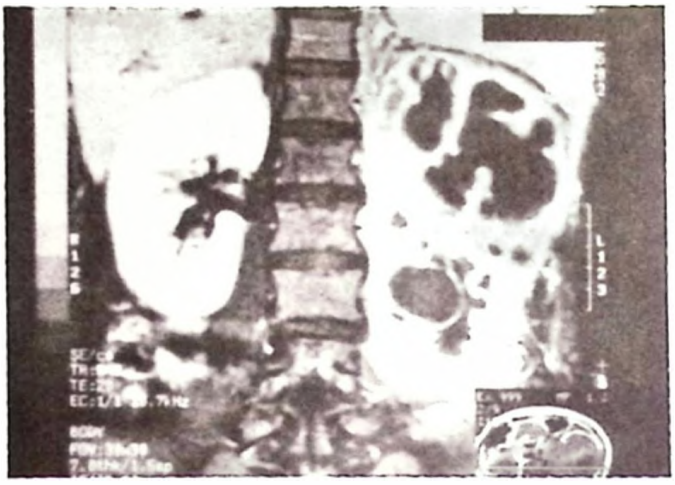

Fig. 5. Coronal postcontrast TI-weighted (660121/1.5) MR image demonstrates an upwardly and laterally displaced hydronephrotic left kidney with marked cortical thinning (→). Corticomedullary differentiation is lost. A hugee psoas abscess with hypointense necrotic foci and showing marked contrast enhancement is observed (→). Perirenal fasciae are thickened, but spleen, lumbar vertebrae and lateral abdominal wall are not involved.

Discussion

The diagnosis of renal TB is often difficult and delayed. Although renal TB is the result of hematogenous spread more commonly from the lungs, less than 5% of patients with urinary tract TB have active pulmonary disease. Radiographic evidence of past pulmonary TB is observed in less than 50% of patients[1]. No evidence of present or past pulmonary TB was observed in our patient. The median age of such patients is 50 years, and renal involvement is rare before the age of 20.

Early renal TB is usually bilateral, asymptomatic and stable. In more advanced disease, however, studies have shown that the macroscopic morphologic renal abnormalities visible on imaging modalities are almost always unilateral. The reasons for asymmetry of the gross findings in a disease in which early stages are symmetric remain unclear. Advanced disease is a result of focal disease progressing into tuberculous abscess (tuberculoma) that may rupture into the collecting system. A schirrous reaction will be stimulated and stenosis or obstruction of the involved collecting system will occur, which results in focal renal TB. If the disease process extends to involve the renal pelvis, total calyceal hydronephrosis will occur. The progressive granulomatous destruction of the kidney with fibrosis and subsequent obstructive uropathy will produce an "autonephrectomy" that is end-stage renal TB. At this stage, the nonvisualized kidney at urography which may be small, normal or enlarged in size is best evaluated with sonography or CT, which may show a hydronephrotic/pyonephrotic or solid granuloma pattern. Cortical thinning is observed overlying the affected calyces partly because of the resultant ischemia and partly due to an obstructive pyonephrosis[2]. Dystrophic parenchymal calcifications often develop. Involvement of the pararenal spaces with the spread of inflammation is an expected finding.

Xanthogranulomatous pyelonephritis is an uncommon chronic renal inflammation in which renal parenchyma are destroyed and replaced by lipid-laden macrophages. It is usually seen in middle-aged women, but pediatric cases have also been reported[3,4]. Unilateral diffuse involvement with urolithiasis is observed in 70-80% of then cases. Usually there is an enlarged nonfunctioning hydronephrotic kidney with pelvic calculi. Patients complain of recurrent urinary tract infections. Proteus mirabilis and Escherichia coli are the most commonly implicated organisms. Extrarenal extension to peri- and pararenal spaces is common. The process may involve more remote sites such as psoas muscles, subphrenic and pleural spaces, spleen, colon, abdominal wall and skin[5,6]. Anemia and leukocytosis were noted in most of the patients[7].

Our case, a 16-year-old female with left flank pain, showed anemia, leukocytosis and urine culture positive for Proteus mirabilis. IVP revealed multiple calculi and calcifications within a unilateral nonfunctioning kidney. USG, CT and MRI examinations demonstrated a diffusely enlarged kidney with contracted and fibrotic renal pelvis and multicystic cavities arranged in a hydronephrotic pattern. Perirenal extension and involvement of the ipsilateral psoas and quadratus lumborum muscles were also noted. Cortical thinning showing contrast enhancement was observed on both CT and MR scans. However, contrast material was not excreted into the hydronephrotic spaces. The psoas abscess also showed marked contrast enhancement. CT revealed nephrolithiasis and multiple small calcifications within the renal parenchyma. Punctate calcifications were also noted in the psoas abscess.

This case illustrates many radiologic and clinical characteristics suggestive of XGP. However, although calculous XGP may occur in up to 20%

of cases, complete or incomplete staghorn calculus within the renal pelvis is the most characteristic finding of XGP, and this was not present in our patient. In renal TB, dystrophic parenchymal calcifications often develop as a complication of renal papillary necrosis. Such calcific foci may detach from the papilla, or a completely necrotic papilla may slough into the pyelocalyceal system which then calcifies, resulting in calculus formation or putty-like calcification. Nevertheless, this process usually does not lead to the formation of staghorn calculi. Moreover, calcifications within an extrarenal inflammatory mass lesion is not noted in XGP, but it may be an expected finding in TB. In our patient, CT successfully demonstrated punctate calcifications within the psoas abscess. We suggest this finding may be helpful to consider a correct diagnosis of TB. Additionally, Goldman et al.[2] reported that in chronic inflammatory process of the kidney with periureteric or peripelvic fibrosis, TB must be considered. There was marked peripelvic and periureteric fibrosis in our patient, and the ureter could not be identified separately. Pelvic fibrosis may be observed in XGP but it usually accompanies the pelvic staghorn calculus.

Although an end-stage "Autonephrectomized" kidney in TB is classically defined to be small in size, enlargement on rare occasions may be observed, and this should be considered in the differential diagnosis. CT and MRI are very useful tools in addition to the IVP and USG and provide specific information aiding the correct diagnosis.

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Immune complex type crescentic glomerulonephritis and ANCA-positivity in a nine-year-old girl

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We report a nine-year-old girl who presented with the clinical and laboratory findings of rapidly progressive glomerulonephritis. She was found to be positive for both pANCA and cANCA. However, renal histopathology revealed immune complex type of crescentic glomerulonephritis. Thus, although testing for ANCA is an important tool in the prediction of the subtype of crescentic glomerulonephritis, a renal biopsy is still required to establish the diagnosis.

Key words: antineutrophil cytoplasm antibodies (ANCA), childhood, crescentic glomerulonephritis, immune complex.

Crescentic glomerulonephritis (CGN), characterized by extensive proliferation of cells within the Bowman's space, may be idiopathic or occur secondary to a wide variety of systemic diseases including vasculitides. Primary forms of CGN may be associated with anti-GMB autoantibodies, extensive immune complex deposition in the mesangium and capillary walls or with antineutrophil cytoplasm antibodies (ANCA) in the absence of glomerular immune complex deposition (pauci-immune)[1]. The latter subtype is the most common pattern in adults[2], whereas immune complex type of disease is the most commonly observed pattern in childhood[3]. The ANCA-associated CGN, on the other hand, has rarely been reported in children, and it may occur as an idiopathic variety without systemic manifestations or as part of a systemic vasculitis[4-7]. Thus, testing for ANCA has become an important tool in such diseases as Wegener's granulomatosis (WG), microscopic polyarteritis (MPA), and idiopathic CGN[4]. However, despite the usefulness of ANCA serologies, a biopsy, when clinically indicated, is still required to document the presence or absence of a pauci-immune CGN[8].

We report here a pediatric case who presented with a clinical course of rapidly progressive glomerulonephritis (RPGN) and had positive ANCA serology. However, renal biopsy revealed immune complex type CGN.

Case Report

A nine-year-old girl was admitted to the hospital with a history of cough, weakness, abdominal pain and pallor of 10 days' duration. She was reported to have an upper respiratory infection two weeks before admission.

Physical examination revealed pallor and increased pulse rate (110/minute). Other physical findings, including anthropometric measurements and blood pressure, were normal.

Urinalysis showed proteinuria (2+) and microscopic hematuria. Her hemoglobin was 6.2 g/dl, hematocrit 18.6%, mean corpuscular volume (MCV) 85.7 fl, leukocytes 10,900/μl, platelets 404,000/μl, and reticulocytes 1.8%. Peripheral blood smear revealed erythrocytes with mild anisocytosis and poikilocytosis, but no burr cells. Her serum creatinine was 7.9 mg/dl, blood urea nitrogen 101 mg/dl, uric acid 10.3 mg/dl, and albumin 2.8 g/dl. Serum glucose, cholesterol, electrolytes, bicarbonate, transaminases, bilirubin and blood gases were normal. Erythrocyte sedimentation rate was 150 mm/h, C-reactive protein 164 mg/L (range 0-5), rheumatoid factor 202 IU/ml (range 0-10), and ASO 258 IU/ml (range 0-200). Serum C3 and C4 levels were normal, and ANA and direct Coombs' tests were negative. Serum anti-MPO (40 U/ml, range 0-5) and anti-PR3 (29 U/ml, range 0-2) antibodies measured by ELISA were positive, while anti-GBM

antibody and cryoglobulin were negative. Random urinary protein/creatinine ratio was 3. She had been immunized against hepatitis B virus, (HBV) and her anti-HBs antibody level was 118 mIU/ml. Anti-hepatitis C virus (HCV) antibody was negative. Abdominal ultrasonography demonstrated kidneys of normal size with increased echogenicity of the parenchyma and normal pelvicaliceal structures. Chest x-ray was normal.

Histopathological examination of renal biopsy tissue revealed 16 glomeruli in light microscopy. Of these, 11 had cellular crescents, and two were globally sclerotic. Glomerular basement membranes were markedly thickened and there was patchy mesangial hypercellularity. There was no prominent polymorphonuclear leukocytic infiltration of the glomeruli. Interstitium showed marked inflammation and severe fibrosis (Fig. 1). Direct immunofluorescence microscopy exhibited diffuse peripheral granular capillary wall and patchy mesangial deposits of C3, membranous deposits of IgG, and small deposits of IgM, and to a lesser extent of IgA and C1q (Fig. 2).

Fig. 1. Vasculitic lesions involving two glomeruli. Interstitium is also densely infiltrated by lymphocytes (H&E, x200).

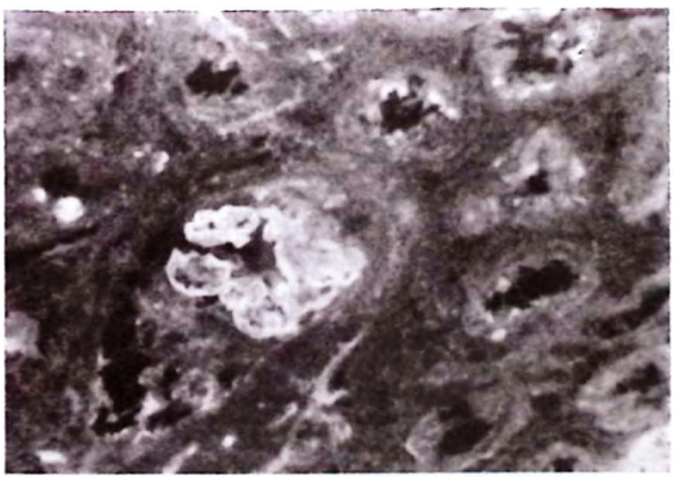

Fig. 2. A glomerulus having peripheral granular capillary wall staining pattern with anti-C3 antibody. Note crescentic proliferation in the Bowman's capsule (DIF, anti-C3, x200).

The patient was monitored for vital signs and given furosemide to increase urine output. However, on the second day of admission, acute peritoneal dialysis was initiated due to increasing BUN (117 mg/dl) and creatinine (10.2 mg/dl) levels. In addition, pulse methylprednisolone therapy (30 mg/kg/day for 3 days) was started. On the fourth day, percutaneous renal biopsy was performed, and histopathological examination was consistent with CGN. Thus, immunosuppressive treatment including cyclophosphamide 1 mg/kg/day prednisolone 2 mg/kg/day was instituted. As renal function did not improve, however, permanent peritoneal catheter was inserted and continuous ambulatory peritoneal dialysis was started on the 14th day of admission. In addition, plasma exchange therapy was added, and a total of six exchanges were performed. She was also given antihypertensive therapy, including a beta blocker and a diuretic, upon development of hypertension. After one month of treatment, anti-MPO and anti-PR3 were still positive (8 and 5 U/ml, respectively). Immunosuppressive therapy was continued for three months with no improvement in renal function. This treatment was stopped at this point due to recurrent peritonitis attacks, and the course of the kidney disease finally terminated with end-stage renal failure.

Discussion

A variety of disorders of different etiology and pathogenesis might result in crescentic glomerulonephritis that is characterized histologically by extensive proliferation of cells within the Bowman's space[2]. Crescentic glomerulonephritis may be a primary renal disease, termed idiopathic, or occur secondary to a wide variety of etiologic events and pathogenetic mechanisms. Primary forms of CGN have several pathogenetic subtypes. Type I is associated with anti-GMB autoantibodies. Type II is characterized by extensive immune complex deposition in the mesangium and capillary walls. These patients

usually do not have anti-GBM antibodies or ANCA. Type III is associated with the absence of glomerular immune complex deposition (pauci-immune) and is associated with ANCA[1]. The latter subtype is the most common pattern in adults[2]. On the other hand, the most commonly observed pattern in childhood is immune complex[3].

Pallor, abdominal pain, anemia, hematuria, proteinuria and impaired renal function following an upper respiratory tract infection in a nine-year-old girl might suggest some diagnoses as hemolytic uremic syndrome (HUS), systemic lupus erythematosus (SLE), or poststreptococcal acute glomerulonephritis (PSAGN). Absence of hypertension, thrombocytopenia, and microangiopathic hemolytic anemia excluded HUS as a diagnostic possibility in our patient. On the other hand, absence of hypocomplementemia, and negative Coombs' and ANA tests ruled out SLE. Anemia unrelated to the degree of renal failure, as in our patient, has been reported to be seen at presentation in 50% of the patients with membranoproliferative glomerulonehphritis (MPGN)[3]. However, hypocomplementemia, detected in most patients with MPGN, was not present in this case. Although ASO titer was only slightly elevated and serum complement levels were normal, PSAGN could not be exluded in this cases, since 11% of patients had normal complement levels[3]. In addition, presence of rheumatoid factor, although nonspecific, was reported in nearly all patients with PSAGN[3] as in our patient.

Recent onset of clinical symptoms, hematuria, proteinuria, and uremia along with rapidly increasing serum creatinine and BUN levels suggest, whatever the cause, RPGN[3]. This type of presentation is seen in any type of CGN[1]. The testing for ANCA has become an important tool in classification of idiopathic CGN[4]. Evaluation of the patients with CGN having positive ANCA serology reveals anti-MPO p-ANCA in 75-80% of individuals, with the remaining generally being positive for anti-PR3[9,10]. Our patient, on the other hand, had both anti-MPO and anti-PR[3] antibodies, anti-MPO being more pronounced. Thus, clinical and laboratory data of our patient were consistent with ANCA-positive idiopathic CGN, and the expected renal pathology was CGN with sparse or no immune deposition[9,10]. As such, light microscopic examination of renal biopsy specimen demonstrated crescents in 70% of then glomeruli examined. However, immunofluorescence microscopy findings were compatible with immune complex type CGN.

Crescentic glomerulonephritis with immune complex pattern could be primary or secondary to some multisystem diseases (like PSAGN, SLE, cryoglobulinemia, hepatitis C), or superimposed on another primary glomerular disease (like MPGN or IgA nephropathy)[1]. While clinical and serologic findings excluded multisystem diseases, histopathologic findings excluded other primary glomerular diseases in this patient. Thus, she was diagnosed as primary CGN. However, as stated previously, PSAG could not be excluded unequivocally.

Although ANCA were first described in a few patients with necrotizing glomerulonephritis and later gained interest due to their presence in Wegener's granulomatosis. (WG), it has been shown that ANCA are not specific for WG. Thus, diagnostic potential of these autoantibodies has been challenged. In addition to WG, MPA and idiopathic pauci-immune CGN, ANCA has been shown to be present in anti-GBM disease, Churg-Strauss syndrome, connective tissue diseases like SLE and rheumatoid arthritis, inflammatory bowel diseases, autoimmune liver disease, and some drugs and infectious diseases[9]. In addition, presence of ANCA has been demonstrated in PSAGN[11,12]. In fact, ANCA positivity, whether in a perinuclear or cytoplasmic pattern, has been reported to be present in approximately 60% of patients with immune complex glomerulonephritis[13].

The most important factor affecting prognosis is the speed with which treatment is instituted following recognition of disease; intravenous pulse methylprednisolone therapy can be initiated empirically while awaiting the results of diagnostic studies in CGN[1]. Most management protocols of CGN associated with ANCA include cyclophosphamide and prednisolone. In addition, plasma exchange therapy might be of value in those patients dependent on dialysis at presentation[1]. However, our patient did not benefit from these treatment protocols, and progressed to end-stage renal failure, The medications were stopped at the third month of treatment due to recurrent episodes of peritonitis.

In conclusion, this patient had ANCA-associated CGN with immune complex staining pattern and no finding of systemic vasculitis, making

her an interesting case. In addition, presence of ANCA in a child with the clinical course of RPGN is not a specific predictor of renal pathology; a renal biopsy is always needed to determine the nature of the disease and to select the appropriate treatment.

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The McKusick-Kaufman syndrome: report of a case with some associations

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McKusick-Kaufman syndrome (MKS) is a rare autosomal recessive condition consisting of congenital hydrometrocolpos, polydactyly and congenital heart defect. We present a female stillborn, the product of non-consanguineous parents, who presented postaxial polydactyly on both feet, micrognathia and marked abdominal distension. Postmortem examination revealed bicornuated cystic uterus and intestinal malrotation. She also had flat left kidney and left hydroureter due to compression by the cystic mass.

Key words: congenital hydrometrocolpos, bicornuated uterus, polydactyly, vaginal atresia, hydroureter, malrotation, abdominal cyst.

The McKusick-Kaufman syndrome (MKS) consists of congenital hydrometrocolpos with polydactyly and congenital heart malformation[1,2]. Hydormetrocolpos was found second to vaginal atresia and bilateral postaxial hexadactyly in an offspring of first-cousin parents by Dungy et al.[3] It is an autosomal recessively inherited condition of different ethnic backgrounds[4,5]. Other abnormalities reported in affected cases include several in other organs, especially the genitourinary system[6,8]. We report a new case of MKS syndrome with bicornuated uterus and intestinal malrotation.

Case Report

The patient was born to a 33-year-old G3, P1 woman and a 35-year-old father, both of Turkish descent. There was no consanguinity and other family history was not contributory. There was no prenatal care and the pregnancy was complicated at 33 weeks of gestation, when the mother presented with vaginal bleeding. No fetal heart beat could be heard and fetal ultrasonography revealed polydactyly, abdominal cyst, pes equinovarus deformity and possibly oligohydramnios. The gynecologist induced the delivery.

The birth weight was 2700 g (52% of the term, compatible with 35th gestational week). External examination showed CH (Crown-Heel), CR (Crown-Rump) and foot lengths as 40 cm, 30 cm, and 6 cm respectively (all compatible with 31-34 gestational weeks). There was postaxial polydactyly on both feet and marked abdominal distension with petechia around the umbilicus (Fig. 1a). Micrognathia and facial appearance were characteristic for Potter facies due to

Fig. 1a. Marked abdominal distension with petechia around umbilicus and polydactyly of the feet.

oligohydramnios sequences (Fig. 1b). There were overriding skull bones and scalp edema. The external genitalia was normal female but with a questionable short vagina.

Postmortem examination: Internal examination revealed a midline cyst measuring 6x4x3 cm (Fig. 2). The cyst was located just beneath the branching point of the aorta, between the two iliac arteries. There was a dilated bicornuated uterus with a blind vaginal orifice. Other findings included malrotation of the intestines, flattened left kidney and left hydroureter due mechanical compression by the mass, and a normal bladder. No other abnormalities were detected and the heart was normal.

Histopathological study of the cyst wall revealed endometrial epithelium, stroma and myometrium.

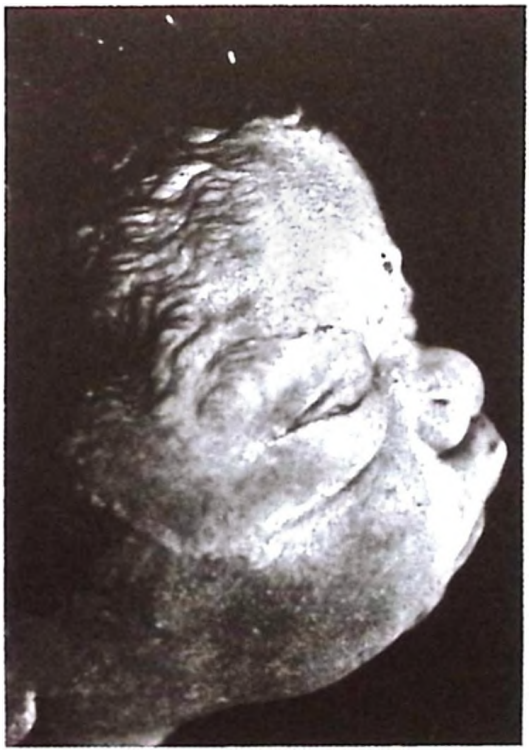

Fig. 1b. Micrognathia and characteristic facial appearance due to oligohydramnios.

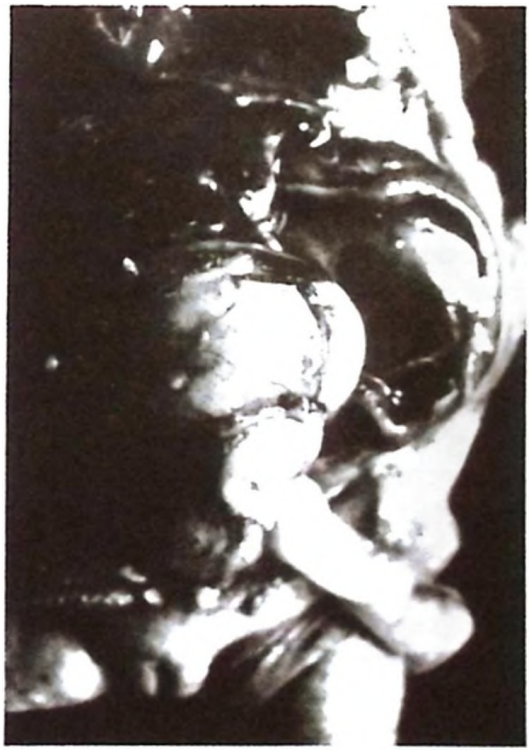

Fig. 2. The cyst measuring 6x4x3 cm in the midline.

Discussion

Sexual differentiation of the reproductive pathway begins early in the fetal period (35 mm embryo, 8 ½ postfertilization week) under the influence of the gonadal hormones. Arising as coelomic invaginations in the metanephros, the paramesonephric ducts grow caudally to form uterine tubes. Afterwards they approach each other and begin to fuse before reaching the urogenital sinus. The fusion is usually complete by 10 weeks and the cavities (uterus and vagina) become a single genital canal caudally. Incomplete fusion leads to many anomalies, e.g. septate uterus. At the end of the first trimester, the developing vagina becomes occludde by a cellular mass termed vaginal plate. The cells of the vaginal plate desquamate during the second trimester and the established vaginal lumen slides down along the urethra to its separate opening into the vestibule. The hymen is the partition that persists between sinovaginal bulbs and the urogenital sinus. A failure of the canalization leads to vaginal atresia, septa or stenosis. Vaginal atresia and/or imperforate hymen results in an accumulation of fluid within the reproductive organs, namely hydro(metro)colpos[9].

Hydro(metro)colpos was first reported in the mid 19th century, and towards the middle of the 20th century, efforts were aimed at classification according to the underlying anomaly. Vaginal atresia is seen rarely and is usually associated with other anomalies of the genitourinary tract such as fistulas[6], or is associated with Robinow's syndrome[10]. In the presented case, uterus septus bicollis was associated with mid-vaginal atresia.

Development of limbs occurs between the 5th and 8th gestational weeks. Although development and even contraction of the heart begin earlier,

development of valves and septa of the heart occurs when hand and foot plates form and finger and toe rays become visible[8]. Therefore, one can expect to see limb abnormalities together with heart and urogenital system malformations.

Hydrometrocolpos associated with polydactyly and congenital heart diseases are the cardinal feature of the MKS[1-4]. Kaufman et al.[4] noticed that postaxial polydactyly and/or congenital heart disease may sometimes be associated with hydrometrocolpos. Interestingly, in the kindred reported by McKusick et al.[1], one of the girls had hydrometrocolpos with polydactyly, while another girl in the same family had only congenital heart disease without hydrometrocolpos. Other reported associated anomalies are congenital heart diseases such as patent ductus arteriosus, ventricular septal defect, single atrium; urinary tract anomalies such as micropenis, glandular hypospadias, undescended testis, hydronephrosis, hydroureters, displaced urethral meatus, polycystic kidneys; Mullerian duct anomalies such as duplications of uterus or vagina; anorectal anomalies such as rectovaginal fistula, anal atresia; intestinal malrotation and Hirschsprung's disease; skeletal abnormalities such as syndactyly, cervical ribs, vertebral anomalies, retinal dystrophy; and pituitary diysplasia[5-8,9]. Non-immune hydrops in a MKS case has been also reported[10]. Intestinal malrotation and left hydroureter were present in our case. The fetal abnormalities resulting from hydrometrocolpos make it possible to diagnose MKS prenatally in female patients[5]. On the other hnand, in male patients who have an affected female relative, polydactyly is the only clue for diagnosis most of the time, although hypospadias, undescended testes or small penis may be associated[5,13].

The MKS is inherited as an autosomal recessive trait and parental consanguinity has been reported in all case when there are multiple affected siblings in the family[7]. Recently, using two pedigrees from the original work of McKusick et al. in the Old Order Amish population, short tandem repeat polymorphism screening showed homozygosity in 20p12 between D20S162 and D20S894. This region includes several genes including the jageed[1] gene. However, sequencing of the jagged1 gene in two unrelated individuals with MKS has not revealed any disease-causing mutations[14]. Therefore, other gene products may be involved in the pathogenesis of MKS in non-Amish populations, and sporadic cases, such as the one presented here, may attract the attention of research workers dealing with gene mapping. Indeed, a recent analysis in an Amish and a sporadic, non-Amish case revealed a frame shift mutation predicting premature protein truncation, and this protein showed structural similarity to the chaperonin family of pyroteins[15].

On the other hand, there are a few other syndromes with hydrometrocolpos associated with polydactyly, namely, Bardet-Biedl syndrome (BBS), Pallister-Hall syndrome, Ellis-van Creveld syndrome and orofaciodigital syndrome, type IV[16-17]. And recent data has revealed a significant overlap between MKS and BBS, in view of a series of patients having vaginal atresia and postaxial polydactyly in infancy and later developing obesity and retinal dystrophy[16].

In children with polydactyly and cystic masses in the lower abdomen, hydrometrocolpos should be considered and prenatal and postnatal ultrasound should be performed[18-19]. Hydrometrocolpos with polydactyly may easily lead to the diagnosis of MKS, but this should be done with caution in infancy. Systematic ophthalmological and neurodevelopmental follow-up should be established in order not to underdiagnose BBS[16].

Furthermore, Rosen and Bocian[12] reported a case of McKusick-Kaufman syndrome, but this case also had non-immune hydrops. A large pelvic mass was prenatally mistakenly attributed as due to inferior vena cava compression and lymphatic obstruction.

In conclusion, we presented a new McKusick-Kaufman syndrome form Turkey, which was characterized by congenital hydrometrocolpos, bicornuated cystic uterus, intestinal malrotation, and bilateral postaxial polydactyly.

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Klippel-Trenaunay-Weber syndrome with hydronephrosis and vesicoureteral reflux: an unusual association

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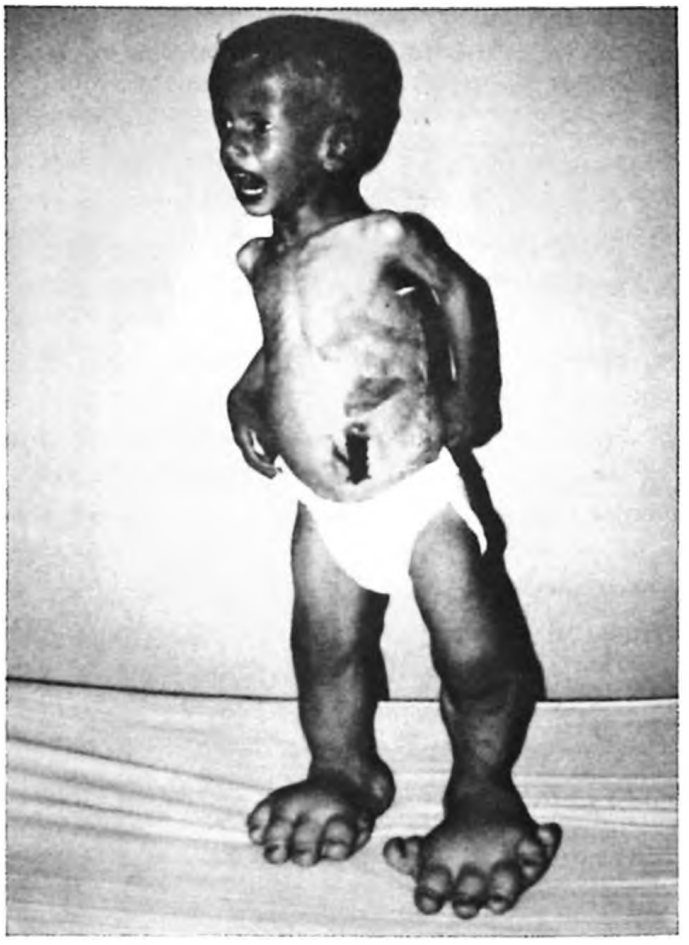
The Klippel-Trenaunay-Weber syndrome is a rare disorder characterized by congenital vascular hamartomas, limb hypertrophy, cutaneous manifestations, lymphangiomas and atresia of lymph vessels with non-pitting edema. A three-year-old boy was referred to our clinic for progressive hypertrophy of leg and feet with 32-month history. We diagnosed Klippel-Trenaunay-Weber syndrome, and determined vesicoureteral reflux in our patient. To our knowledge, hydronephrosis and vesicoureteral reflux have not been described previously in the KTWS.

Key words: Klippel-Trenaunay-Weber syndrome, hydronephrosis, vesicoureteral reflux.

The Klippel-Trenaunay-Weber syndrome (KTWS) is a rare disorder characterized by congenital vascular hamartomas, limb hypertrophy, cutaneous manifestations, lymphangiomas and atresia of lymph vessels with non-pitting edema. The basic defect is assumed to involve vascular embryogenesis and can result in malformations of almost any body area[1-3]. Mental retardation and/or convulsion, short stature, anomalies in genitalia, aberrant big vessels and hemimegalencephaly are associated in some rare cases with KTWS[4,5]. We report a child with KTWS and vesicoureteral reflux and hydronephrosis.

Case Report

A three-year-old boy was referred to our clinic for progressive hypertrophy of leg and feet with 32-month history. His height was at the 90th percentile weight at the 75th and head circumference at the 50th. The parents were not related, and his father was 47 years old and his mother 39 years old. Physical examination was remarkable for hyperpigmented vascular nevus in his left hypochondriac region, varicose veins and lymphedema in the legs and feet, polydactyly of feet, and bilateral diffuse hypertrophy of gluteal area (Fig. 1). There were no neurological disturbances. Laboratory data values at presentation vere as follows: WBC: 9,600/mm^3, Hct: 36%, Hb: 12 g/dl and platelet count: 277,000/mm^3. Pyuria was detected in urine and cultured P. aeruginosa. X-rays showed only phypertrophy of soft tissue of the legs and feet. Voiding cystography revealed grade V reflux in the left collecting system (Fig. 2). Computerized



Fg. 1. General appearance of the patient.

tomography (CT) of the abdomen showed a large, hyperdense, lobulated soft tissue mass in the mesentery of the small bowel (Fig. 3). Bowel loops were displaced anteriorly towards the abdominal wall. We did not detect any hemangiomatous involvement in the kidney. Dynamic DTPA scintigraphy of the right kidney revealed normal pattern of perfusion, uptake and excretion (Fig. 4); however, there was dilatation of the left collecting system and ureter with significant clearing following diuretic infection. DMSA scintigraphy revealed a normal right kidney but focal lesions in the upper and lower poles of the left kidney attributable to renal scarring (Fig. 5).

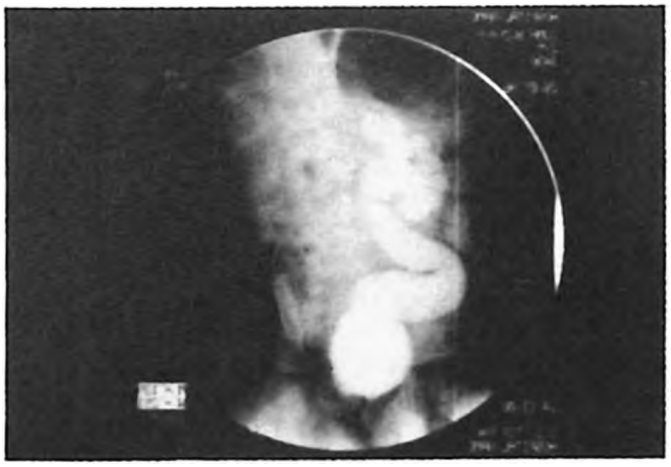


Fig. 2. Voiding cystography revealed grade V reflux in the left collecting systems.

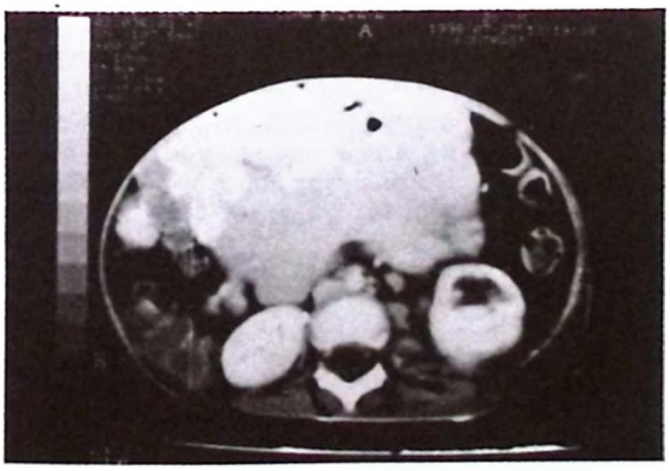


Fig. 3. CT of the abdomen showed a large hyperdense, lobulated soft tissue mass in the mesentery of the small bowel. Bowel loops are displaced anteriorly towards abdominal wall.

Discussion

The Klippel-Treaunay-Weber syndrome is a rare sporadic congenital malformation of the vascular system that was first described in 1900[6]. The

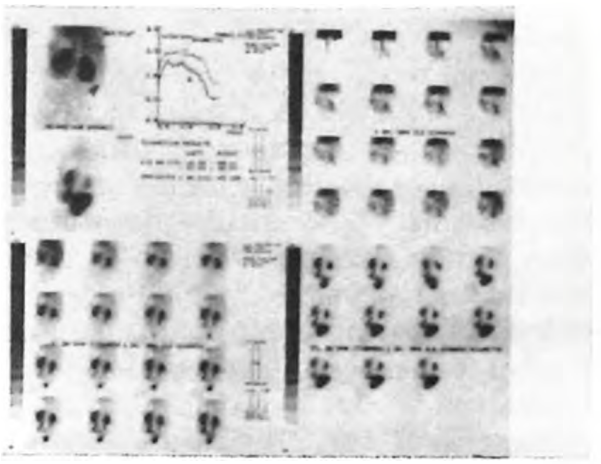


Fig. 4. Dynamic DTPA scintigraphy of right kidney revealed normal pattern of perfusion, uptake and excretion, but there was dilatation of the left collecting system and ureter with significantly clearing following diuretic infection.

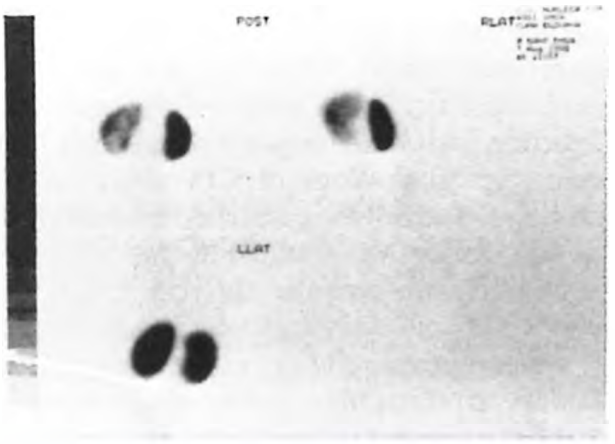


Fig. 5. DMSA scintigraphy revealed a normal right kidney and focal lesions in the upper and lower poles of the left kidney attributable to renal scarring.

current review was not undertaken as a population study. This syndrome is characterized by: 1) a more or less extensive nevus on the involved limb, 2) varicose veins on the involved limb arising in childhood, and 3) hypertrophy of usually one, but occasionally of more than one limb. There are well documented reports in which other congenital anomalies have been found to be associated with this syndrome[7]. Hamartomas and vascular malformations described in the KTWS are separate from the flat nevus and are usually localized to the affected limp. Cutaneous nodular hemangiomas consisting of capillary and cavernous elements together with lymph vessels may then be connected to a malformed venous system with reduplications, agenesis, arteriovenous shunts or

fistula[1,2,8]: Digital anomalies are well known and have been described in case reports or small series[9-11]. KTWS is also associated with lymphatic malformations resulting from the obstruction of the deep veins and with common lymphedema. The vascular malformations have been seen in different systems. Varicose veins and vascular anomalies have been described in different sites including vascular proliferative patches in the mouth[12]; varicosities in the small bowel, colon[13-14], and esophagus[15]; and ocular hemangiomas with glaucoma[16].

Although complications may develop, KTWS is generally a nonprogressive disorder. The problems, which include edema, stasis dermatitis, skin ulceration, cellulites, anemia[14], thrombosis, phlebitis, bone and joint abnormalities, scoliosis, and paresthesia, tend to be chronic in nature[17]. Reported life-threating complications include disseminated intravascular coagaulation[18], gastrointestinal bleeding as a result of hemangiomata in the intestine[18-20]. and systemic infection with Gram-negative bacteriemia[21]. In our case, typical findings of KTWS are described. During the screen for gastrointestinal involvement by sonography, we detected hydronephrosis. The hydronephrosis was also confirmed with CT. We performed voiding cystography and detected grade V vesicoureteral reflux in our patient. DTPA and DMSA scintigraphies demonstrated dilatation of the left collecting system and scars on the left kidney. To our knowledge, hydronephrosis and vesicoureteral reflux have not been described previously in the KTWS.

Our patient was followed closely. He had infections in the urinary tract and cultured. P. aeruginosa was cultured in urine. He was treated with ceftriaxone along with nutritional support. We consulted with pediatric surgeons on this case, and planned a surgical approach.

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SANTIAGO, 19 - 23 NOVEMBER 2002

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Inquiries to : III[rd] World Congress of Pediatric Infectious Diseases-WSPID
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web site : www.kenes.com/wspid

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WHY? UNDERSTANDING AND PREVENTING INFANT DEATH AND COMPLICATED GRIEF

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