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DIAGNOSIS OF CHILDHOOD TUBERCULOSIS BY POLYMERASE CHAIN REACTION*

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SUMMARY: Ceyhan M, Kanra G, Seçmeer G, Erdem G, Ecevit Z, Yılmaz E, Tuncer S. (Infectious Disease Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Diagnosis of childhood tuberculosis by polymerase chain reaction. Turk J Pediatr 1996; 38: 399-405.

The polymerase chain reaction (PCR) using oligonucleotides based on the repetitive sequence (IS6110) of Mycobacterium tuberculosis as a primer was evaluated for detection of M.tuberculosis in clinical samples. We tested 55 clinical specimens from patients suspected of having tuberculosis and 71 specimens from control subjects. PCR was more sensitive than culture (positivity rate was 14.5% and 36.3%, respectively, in suspected patients). This approach to the diagnosis of tuberculosis is a valid diagnostic alternative to classical procedures. *Key words: polymerase chain reaction, M.tuberculosis, children.*

Tuberculosis continues to be one of the most important causes of death in developing countries and has made a dramatic comeback in developed countries, in part because of the acquired immune deficiency syndrome (AIDS) pandemic[1]. Since clinical diagnosis alone is unreliable, it must be confirmed by demonstration of Mycobacterium tuberculosis (MT)[2]. Although early diagnosis is very important in control with isolation precautions and treatment of the disease, cultures are cumbersome and time-consuming for diagnosing tuberculosis[3]. Microscopic examination of smears of acid-fast bacteria by Ziehl-Neelsen staining is a rapid but insensitive and nonspecific method for detection of MT. Immunological and serological methods, such as latex agglutination, radioimmunoassay, and enzyme-linked immunosorbent assay (ELISA) are not sensitive or specific[3]. The radiometric BACTEC system has shortened the time for detection of MT, but still requires 10 to 12 days for growth of the bacteria and completion of susceptibility tests[4,5].

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One diagnostic difficulty specific to the pediatric age-group is related to the type of infection in children. Since primary tuberculosis is the prominent type in childhood, miliary and meningeal tuberculosis are common and bronchial involvement is rare; also, the number of microorganisms in the clinical specimens, such as sputum and gastric lavage, is low[1]. Thus, a rapid, sensitive and specific diagnostic test is needed, especially in childhood tuberculosis.

Recently, several attempts have been made to diagnose MT infection specifically through biochemical or molecular biological techniques. Among them, polymerase chain reaction (PCR) has been shown to be rapid and reliable test[2, 3, 6-9]. Several sets of primers have been used in MT PCR. Of these, the IS6110 primer has been used with high sensitivity and specificity in many studies[10-12]. In this report, we describe the application of the PCR technique with the IS6110 primer for the detection of MT DNA in the clinical specimens of children with the clinical diagnosis of tuberculosis.

Material and Methods

Patients: Specimens were obtained from patients admitted to Hacettepe University İhsan Doğramacı, Dr. Sami Ulus and SSK Children's Hospitals in whom tuberculosis was suspected during the course of routine diagnostic evaluation. The age of the patients ranged from six months to 15 years. There were 55 girls and 71 boys. Among the 126 patients, 55 patients had proven or suspected tuberculosis and 71 cases were non-tuberculosis controls. Laboratory diagnosis was made with positive MT cultures in eight patients. The criteria used for the diagnosis of tuberculosis were prolonged fever, radiographic findings, cerebrospinal fluid findings (more than 0.02×10^9 cells/L with lymphocytes predominating, protein greater than 1 g/L, glucose less than 50% of blood glucose) in cases with meningitis, sterile routine bacterial and fungal cultures, a positive history for exposure to an adult who had MT in sputum, a positive tuberculin test, and a clinical response to antituberculous therapy.

The tuberculous cases were classified as being proven tuberculous (a positive culture for MT), highly probable tuberculous (met all the clinical and laboratory criteria), probable tuberculous [met at least 5 of the 6 or 7 (6 criteria plus cerebrospinal fluid findings in tuberculous meningitis) criteria]. The non-tuberculous control group consisted of 36 cases of meningitis with etiology other than tuberculosis and cases with non-tuberculous infections in whom the etiology was proven by microbiological cultures.

Clinical Specimens: The type of specimens submitted were as follows: 57 cerebrospinal fluid, 22 sputum or gastric lavage, 14 pleural fluid, seven pericardialea fluid, seven peritoneal fluid, nine blood, five skin and tissue biopsies,

two urine, two infectious aspirate, and one bone marrow aspirate. All specimens were cultured for MT and were subjected as appropriate to lysis to prepare the DNA for amplification.

Sample Preparation: For extraction of DNA from pleural, pericardial and peritoneal paracentesis materials and cerebrospinal fluid, a 400 µl sample was incubated with 10 mM Tris-HCl pH 8.0, 0.5% SDS, 100 mM NaCl, 25 mM EDTA and 50 µl proteinase K (10 mg/ml) at 37 °C for 24 hours. Samples were bolied for 10 minutes, and phenol-chloroform extraction and ethanol precipitation were performed. The precipitates were dissolved in 30 µl distilled water, and 5 µl was used for amplification. After mincing 10-50 mg biopsy specimens, DNA was extracted as described above. For sputum and fasting gastric lavage fluids, a simplified procedure was performed[13]. Samples were incubated in 1 M NaOH for 30 minutes at room temperature on a shaker. After neutralizing with 1 M HCl, samples were centrifuged for 5 minutes at 5,000 rpm. Sediments were washed twice with TE (10 mM Tris pH 8.0, 1 mM EDTA) buffer. Suspension of the sediment in 500 µl TE was bolied for 10 minutes, and 5 µl supernatant was used for PCR.

Primers: A primer pair was selected to yield a final amplification product of 123 base pairs of the insertion element IS6110, a DNA sequence specific for the MT complex.

The sequences of the primers were:

MT1: 5' CCTGCGA GCGTAGGCGTCGG 3" and

MT2: 5' CTCGTCCAGCGCCGCTTCGG 3'[14]

Amplification: A suspension of MT isolate in TE buffer was used as positive control. PCR was carried out in 50 µl volumes. The reaction mixture contained 10 mM potassium chloride, 10 mM tris-HCl pH 8.3, 2.5 mM magnesium chloride, 100 µM of each dNTP, 50 pmol of each primer and 1.5 units of taq polymerase (Cetus stoffel fragment). After five minutes of denaturation at 94 °C for one cycle, 35 amplification cycles were performed with an automated DNA thermal cycler (Model 9600, Perkin Elmer Cetus). Each cycle consisted of 15 seconds of denaturation at 94 °C and 100 seconds of annealing and extension at 68 °C. The reaction mixture without DNA was used as a negative control. The peresence of the 123-bp amplification product was analyzed by electrophoresis of 10 µl amplified mixture on two percent agarose gel. The gel was stained by ethidium bromide and photographed on a UV transilluminater (Fig. 1).

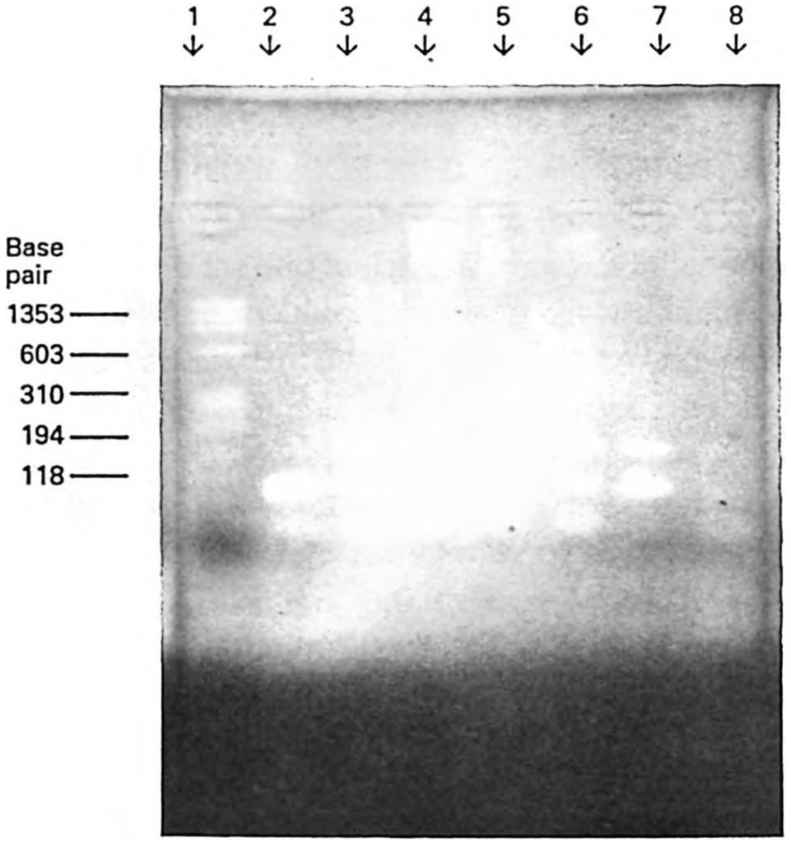

Fig. 1: Analysis of PCR products by agarose gel electrophoresis. PCR was based on the amplification of a 123-bp fragment of the insertion element IS6110 of M.tuberculosis.
Lane 1: ØX174 DNA marker. Lane 2: positive control.
Lanes 3-7: Positive cases. Lane 8: Negative control.

Results

Cultures for MT were not as sensitive as PCR. Cultures were positive in only eight of 55 (14.5%) cases of suspected tuberculosis. We were able to detect eight of eight (100%) proven cases eight of 18 (44.4%) highly probable cases, and four of 29 (13.7%) probable cases (Table I). In the non-tuberculous control group, the PCR was positive in one cerebrospinal fluid sample. In this case, a second DNA preparation was made from the remaining sample, which had been stored elsewhere. This case was PCR-negative on retesting. The first positive result in PCR was evaluated as cross-contamination. The positivity rate was highest in culture-proven cases.

Table I: Results of PCR in Tuberculous and Control Children

	No (%) of Patients with Positive Results					
	Tuberculosis					
Sample	Proven	Highly Probable	Probable	Total	Control	Total
Cerebrospinal fluid	2/2 (100)	2/8 (25.0)	2/11 (18.2)	6/21 (28.5)	1/36 (2.7)	7/57 (12.2)
Sputum-gastric lavage	1/1 (100)	1/3 (33.3)	1/10 (10.0)	3/14 (21.4)	0/8 (0)	3/22 (13.6)
Peritoneal fluid	1/1 (100)	2/2 (100)	–	3/3 (100)	0/4 (0)	3/7 (42.8)
Pleural fluid	1/1 (100)	2/2 (100)	1/3 (33.3)	4/6 (66.6)	0/8 (0)	4/14 (28.5)
Pericardial fluid	–	1/2 (50.0)	0/2 (0)	1/4 (25.0)	0/3 (0)	1/7 (14.2)
Urine	–	0/1 (0)	0/1 (0)	0/2 (0)	–	0/2 (0)
Biopsy specimen	3/3 (100)	–	0/1 (0)	3/4 (75.0)	0/1 (0)	3/5 (60.0)
Blood	–	–	–	–	0/9 (0)	0/9 (0)
Bone marrow	–	–	0/1 (0)	0/1 (0)	–	0/1 (0)
Infectious aspirate	–	–	–	–	0/2 (0)	0/2 (0)
Total	8/8 (100)	8/18 (44.4)	4/29 (13.7)	20/55 (36.3)	1/71 (1.4)	21/126 (16.6)

Discussion

The diagnosis of pediatric tuberculosis is dependent on a high index of suspicion based on history, physical examination and initial laboratory examination of the samples and culture. However, smears are usually negative and cultures take too long and frequently are negative in pediatric cases; generally the clinical findings alone lead to initiation of antituberculous therapy. Thus, diagnosis of tuberculosis in childhood requires a reliable and fast technique. However, many tests developed for this purpose have been found to be lacking in either specificity or sensitivity. PCR is a promising test in the diagnosis of tuberculosis with its high sensitivity and specificity. In this technique, following amplification, mycobacterial DNA can be detected in clinical samples from many children with primary tuberculosis, despite the fact that the same samples are negative by culture. The sensitivity of PCR is related to the type of mycobacteria. M.tuberculosis and M.microti harbor 10 to 20 copies of IS6110, which was the primer used in our study, while M.bovis and M.bovis BCG appear to contain a single copy[15].

We found that 21 of the 126 samples were positive in the PCR (Table I). Only eight of the samples were also culture-positive.

We performed PCR on cerebrospinal fluid, sputum or gastric lavage, and eight other samples from 126 children and found a significant number of PCR-positive

(13 of 118), culture-negative and smear-negative samples from different sites. Other investigators have reported similar high positive rates[16-18].

The specificity of the PCR was excellent. There was only one sample with an amplicon of 123 bp in the control group. It is not possible to exclude the possibility that this case had a subclinical tuberculous infection. If it was a false-positive result, it could have been caused by true contamination or carry-over (contamination by the products of the amplification which can serve as a substrate for the generation of more product)[19]. The proportion of false-positive results observed here (1.4%) is comparable to that seen in other series[20].

Although the sensitivity of PCR in this study was 100%, with eight positive results in eight culture-positive samples, false-negative results have been reported previously[21, 22]. These false-negative results could have resulted from 1) The presence of inhibitors not detected by the control amplification; 2) non-homogeneous distribution of bacteria in the specimen such that the fraction tested did not contain mycobacteria; or 3) low numbers of bacilli in the specimen, which decreases the probability of mycobacteria being found in the fraction analyzed by PCR[17].

Detection of M.tuberculosis by PCR has previously been shown to be more sensitive than microscopy and much faster than culture[16, 17, 18, 23]. We believe that PCR is a definitive diagnostic alternative for tuberculosis.

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MICROORGANISMS INVOLVED IN ACUTE BACTERIAL MENINGITIS IN CHILDREN AND THE ROLE OF HAEMOPHILUS INFLUENZAE*

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SUMMARY: Kanra G, Akan Ö, Ecevit Z, Ceyhan M, Seçmeer G. (Infectious Disease Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Microorganisms involved in acute bacterial meningitis in children and the role of Haemophilus influenzae. Turk J Pediatr 1996; 38: 407-412.

Acute bacterial meningitis (ABM) is an important cause of mortality and neurological damage in children. Documentation of the etiological agents is very important both for the treatment of patients and for prophylactic approaches. H.influenzae, N.meningitidis and S.pneumoniae are the three major pathogens involved in ABM. In Turkey for many years H.influenzae has not been isolated in cerebrospinal fluid (CSF) specimens. In order to show the bacteria involved in ABM in our hospital and to see the role of H.influenzae, we investigated the CSF of 59 patients with bacterial meningitis using Gram and Wayson stains, culture and latex agglutination techniques. The agents were determined in 38 (64.4%) specimens by using culture positivity in 30 (50.8%), and latex or stain positivity in eight (13.6%) specimens. The microorganisms causing ABM included S.pneumoniae (25.6%), gram-negative enteric bacilli (17.9%), N.meningitis (12.8%), alpha hemolytic streptococci (10.3%), H.influenzae (10.3%), nonfermentative gram-negative bacilli (5.1%), candida spp. (5.1%), group B streptococci (2.6%), coagulase negative staphylococci (2.6%), S.aureus (2.6%) and pseudomonas spp. (5.1%). In this study it has been shown that H.influenzae can cause ABM in Turkish children. Multicentric studies from different parts of Turkey will be helpful in showing the real incidence in our country. *Key words: acute bacterial meningitis, bacterial pathogens, Haemophilus influenzae.*

Despite research in diagnosis, prevention and therapy, acute bacterial meningitis (ABM) in children is still an important cause of death and major neurological damage[1]. ABM is caused by a variety of microorganisms. Age and underlying conditions of the patients as well as seasonal and geographic differences influence the types of microorganisms involved in these infections. Together with N.meningitidis and S.pneumoniae, H.influenzae is one of the three most common organisms causing ABM[2]. Infants who have medical devices or underlying illnesses can be infected with less virulent microorganisms[3]. Documentation of

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the etiological agents involved in ABM is very important not only for the treatment of patients but for prophylactic approaches as well. Development of immunization policies depends on a reliable bacteriological analysis showing the prevalence of microorganisms in cerebrospinal fluid (CSF) specimens. In the USA, vaccination against H.influenzae, which is the most common agent in ABM, has decreased the incidence of H.influenzae type b (Hib) meningitis[1]. In Turkey H.influenzae has been isolated very rarely in CSF specimens in recent years[4-7]. It is believed that H.influenzae is not a danger for Turkish children.

This study was performed to investigate the types of bacteria involved in ABM and the role of H.influenzae in childhood meningitis in our hospital.

Materials and Methods

CSF specimens were obtained from children with clinically suspected meningitis in the emergency ward of Hacettepe Children's Hospital. Specimens from hospitalized patients who developed ABM during their hospital stay were also included in the study. Quantitative cell counts were immediately performed in the counting chambers, and CSF specimens lacking polymorphonuclear leukocytes and those with predominant lymphocytes were excluded from the study. Culture, stain and latex agglutination techniques were performed 30 minutes after the lumbar puncture.

Culture Technique: Two to three ml of the CSF specimen was centrifuged for 15 minutes at 2000-3000 rpm. The supernatant was poured in another test tube for other analysis, and the sediment was vortexed for 10 minutes. Using a sterile Pasteur pipette, inoculations on GC-chocolate agar with 10 percent horse blood, five percent sheep blood agar and thioglycollate broth were performed. Agar plates were incubated for 48 hours in a CO_2 incubator at 35 °C by daily examination. Broth was observed for five days in ambient air at the same temperature. Bacterial identification was performed using standard techniques in case of any growth. X, V and XV factor discs (Difco) were used to identify H.influenzae. The colonies of H.influenzae were serotyped using H.influenzae antiserum poly a-f (Difco)[8].

Stain: A few drops of sediment were placed on alcohol-soaked microscopic slides and stained using Gram and Wayson stains[9].

Latex Agglutination: Supernatant fluid was used for this procedure. Latex agglutination was performed using kits by Biomerieux (France) in only half of the specimens according to the instructions.

Results

Over a 16-month period, a total of 59 specimens were obtained from 32 boys and 22 girls between four days and 14 years of age (mean age 2.5 ± 3.3 years).

Thirty (50.8%) of the specimens revealed growth of various microorganisms. In eight (27.6%) of 29 culture-negative specimens, the etiological agent was determined by either direct smear or latex agglutination tests. Using the three methods, causative agents were determined in 38 (64.4%) of the 59 specimens. The types of microorganisms are listed in Table I. Among 29 patients who had no growth, six of them had Gram (+) diplococci on Gram stain and one had latex positivity against group B streptococci. Fifty-nine percent of the culture-negative patients had a history of prior antibiotic usage. Table II shows the age distribution of the patients whose etiological agent was documented by at least one of the three methods.

Table I: Microorganisms Causing ABM

Microorganisms	n	%	Culture (+)	Culture (-) stain and/or latex (+)
N.meningitidis	5	12.8	5	0
S.pneumoniae	10	25.6	4	6
Gram (-) enteric bacilli	7	17.9	7	0
H.influenzae	4	10.3	4	0
Alpha hem. strep.	4	10.3	4	0
Candida spp.	2	5.1	2	0
Nonferm. gram (-) bacilli	2	5.1	2	1
Pseudomonas spp.	2	5.1	2	0
Group B strep.	1	2.6	0	1
Staph. aureus	1	2.6	1	0
Stap. coag. (-)	1	2.6	1	0

Table II: Age Distribution of Patients Whose Microorganisms were Documented

0-1 Month	n	1 Month-3 Years	n	> 3 Years	n
Gr. B strep.	1	N.meningitidis	2	N.meningitidis	3
K.pneumoniae	3	S.pneumoniae	5	S.pneumoniae	4
E.coli	1	H.influenzae	2	Staph coag. (-)	1
S.bovis + proteus spp.**	1	Alpha hemolytic streptococci**	3		
Pseudomonas spp.	1	K.pneumoniae	1		
S.aureus	1	Erwinia spp.	1		
		Candida spp.	1		
		Pseudomonas spp.	1		
		Nonfermentative bacilli	2		

* mixed growth.
** Streptococcus intermedius (2), nontypable streptococcus (1).

Discussion

In surveillance studies from developed countries, H.influenzae has been reported to be the most common agent (45%) causing ABM after the neonatal period[10]. In many other countries, though isolation rates are lower, H.influenzae is one of the three most common isolates[11-13]. In our country there has been no isolation of H.influenzae in CSF specimens in most studies[5-7]. In one study performed in our hospital between 1977 and 1980, H.influenzae represented only one percent of 950 CSF isolates[4]. The isolation of H.influenzae from other specimens such as throat cultures revealed isolation rates of 2.96 and 5.2 percent, which are lower than those in the literature[14, 15]. Given these findings, for years it has been believed that H.influenzae did not cause invasive infections in Turkish children because of antigenic cross-reactivity between H.influenzae and E.coli. The capsular antigen of E.coli K100 is immunochemically related to one of H.influenzae type b. Bacterial antibodies against this microorganism are also believed to protect children from Hib infections[16, 17]. In our study, the isolation rate of 10.3 percent shows that H.influenzae may cause ABM in Turkish children. It is the second most common isolate in the one-month to three-year age-group. Why it has not been isolated for years may be explained by the inoculation techniques and the media used in other studies. H.influenzae requires X and V factors for growth, and special culture media are necessary for isolation. This may be supported either by adding these factors to the culture medium or by using chocolate horse-blood agar[8]. In our study S.pneumoniae was the most common isolate, followed by N.meningitidis. Our isolation rate of N.meningitidis was lower than in previous studies from our hospital[4, 5], possibly because most of our patients had previous antibiotic usage. Premature infants with immature immunity, broad-spectrum antibiotic usage or invasive medical approaches are at increased risk of acquisition of meningitis with more noninvasive microorganisms, such as candida or coagulase (-) staphylococci[18]. Both our candida and coagulase (-) staphylococcus isolates were from patients with ventriculoperitoneal shunts. Alpha haemolytic streptococci rarely cause ABM. They mostly infect children with underlying diseases[19]. In our series, three of the four isolations were from children who had brain abscesses ruptured into the meningeal space. The fourth patient had a ventriculoperitoneal shunt, as mentioned before.

The changing pattern of ABM in the neonatal period is noted in some studies, but gram (-) enteric bacilli, reported to be the most common pathogens of the neonatal period, were isolated more frequently in our study[2, 20]. Bacterial culture is inexpensive and still accepted as the "gold standard" for the diagnosis of bacterial meningitis[9]. With the standardized, routine isolation technique, there is still a 49.2 percent rate of culture negativity in our series. More sensitive and rapid techniques such as PCR are being worked on. PCR gives promising results in the early laboratory diagnosis of bacterial meningitis[21].

In this study it has been shown that H.influenzae can cause ABM in Turkish children. A special culture medium, standardized techniques and good cooperation between microbiologists and clinicians can solve the problem of isolation. Multicentric studies from different parts of Turkey covering more patients will be helpful in documenting the real incidence in Turkey.

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A LINKAGE ANALYSIS IN TWO FAMILIES WITH BILATERAL RETINOBLASTOMA*

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SUMMARY: Emre S, Sungur A, Hazar V, Bilgiç S, Büyükpamukçu M, Günalp İ. (Departments of Medical Biology, Pathology and Ophthalmology, and Oncology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). A linkage analysis in two families with bilateral retinoblastoma. Turk J Pediatr 1996; 38: 413-417.

Using polymerase chain reaction (PCR) amplification from blood samples, XbaI and BamHI polymorphisms were analyzed in two families with bilateral retinoblastoma. In one of the families it was predicted using the BamHI polymorphism that the 200 bp allele co-segregates with the disease. This family was uninformative for XbaI polymorphism. The second family was uninformative for both XbaI and BamHI polymorphism. *Key words: retinoblastoma, polymorphism, polymerase chain reaction.*

Retinoblastoma (RB) is the most common intraocular tumor in children. It may affect one or both eyes and occurs in both familial and sporadic forms. In familial cases the inheritance follows an autosomal-dominant pattern[1]. Sporadic cases represent the majority of both unilateral and bilateral forms of the disease. Random inactivation or loss of both alleles at the RB locus are thought to cause unilateral unifocal cases[2,3]. Individuals with bilateral sporadic disease are known to have new germline mutations and frequently have affected offspring. In other words, they carry the mutation in their germline. Familial cases are more frequently bilateral and multifocal and are believed to be either the result of inheritance of one defective RB allele from an affected or carrier parent or the somatic inactivation or loss of the remaining functional allele[4,5]. The RB gene has been mapped to chromosome region 13q14. The RB gene contains 27 exons and spans 180 kb of genomic DNA[6]. The message (4.7 kb) contains a relatively short 5' untranslated sequence followed by a coding region of 2.7 kb which encodes 928 aminoacids. The sequence for the entire RB cDNA is now available, and

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the sequences of XbaI and BamHI polymorphic sites have also been determined; thus linkage analysis in RB families can be performed using the polymerase chain reaction (PCR).

In this study, XbaI and BamHI polymorphic sites were analyzed and the segregation of the alleles was detected by PCR in two families with bilateral retinoblastoma.

Material and Methods

Patients and DNA Preparation: Patients were identified through the Department of Ophthalmology at Hacettepe University and Ankara University. The diagnosis of retinoblastoma had been established by current Ophthalmologic criteria[9]. DNA from whole blood samples was prepared using standard phenol/chloroform extraction followed by ethanol precipitation[10].

PCR-Based Detection of Polymorphic Sites

BamHI Site: Two primers (Table I), one from exon 1 and the other from intron 1 of the human RB gene, were used to amplify a genomic DNA fragment containing the polymorphic BamHI site. For the BamHI polymorphism an approximately 200 bp genomic DNA fragment containing the intron 1 splice donor site was amplified. Fragments of 140 bp and 60 bp were generated after BamHI digestion of the PCR product. PCR was carried out in a total volume of 50 ml containing approximately 1 µg DNA and buffer consisting of 50 pmol of each dNTP (dATTP, dTTP, dCTP, dGTP), 50 mmol/l KCl, 10 mmol/l TrisHCl, (pH 9.0 at 25 °C) 1.5 mmol/l MgCl, 10 percent dimethyl sulphoxide (DMSO), and two units Taq DNA polymerase (Promega).

Table I: Sequence of PCR Primers Used to Amplify Polymorphic Restriction Enzyme Sites within the RBI Gene

	Primers	
Polymorphic Site	Sense	Antisense
BamHI	5'-CAGGACAGCGCCCGGAG-3	5'-CTGCAGACGCTCCGCCGT-3'
XbaI	5'-TTCCATGAAGAACAAATGG-3	5'-GCAATTGCACAATCCAAGTT-3'

Amplification was performed using a programmable thermal cycler (Perkin Elmer Cetus 480). Amplification consisted of three steps (after an initial 15 minute denaturation step at 96 °C): denaturation at 96 °C for 20 seconds, annealing at 60 ° for 20 seconds followed by an extension step at 72 °C for 60 seconds. After 30 cycles of amplification, the amplified product was digested overnight in restriction enzyme BamHI. DNA fragments were resolved by electrophoresis through two percent agarose gels.

XbaI Site: A genomic DNA fragment containing the polymorphic XbaI site (21.8 kb downstream from exon 17) was amplified using primers (Table I) from intron 17 of the human RB gene. XbaI digestion of this fragment identifies two alleles: an allele 945 bp and an allele consisting of two fragments 630 bp+315 bp long. The PCR conditions were the same as those used for the BamHI site except that the PCR mix did not contain DMSO and the annealing temperature was 50 °C. The amplification product was digested overnight with XbaI. DNA fragments were resolved by electrophoresis through one percent agarose gels.

Results

XbaI and BamHI polymorphisms were analyzed using PCR amplification, from blood samples in two families with bilateral retinoblastoma.

The first family (RB-1) pedigree is shown in Figure 1. The father developed tumors in both eyes, and one of the eyes was enucleated. There was no history of RB on the maternal side of the family. At the age of 14 months, the firstborn child, a boy, was diagnosed with bilateral RB. Blood samples were taken from all of the family members. Using BamHI polymorphism, the mother was found to be homozygous for the 140/60 bp allelle. The father and proband were heterozygous for the 200/140-60 bp allele (Fig. 1). Homozygous individuals are described as 140/60 bp. Heterozygous individuals are described as 200/140-60 bp. Using the other set of primers (Table I), an approximately 945 bp genomic DNA fragment containing the polymorphic XbaI site was amplified. At the XbaI locus, the mother, father and proband were all heterozygous for the 945/630-315 bp allele (Fig. 2). Heterozygous individuals are described as 945/630-315 bp.

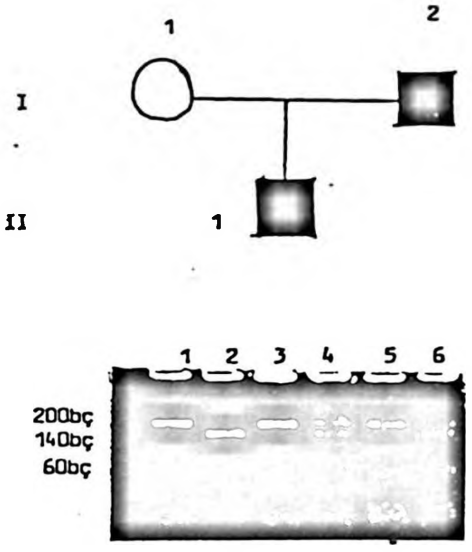

Fig. 1: Pedigree of family RB-1. PCR amplification of the polymorpic BamHI site from DNA samples from the members of family RB-1. In all cases a dominant band of the expected size, ± 200 bp long was observed. The amplified DNA from each patient was digested with BamHI. lane (1, 3, 5): PCR products (± 200 bp). The mother (lane 2) is homozygous for the 140/60 allele, but the father (lane 4) is heterozygous. The proband (lane 6) is also heterozygous. The RB gene was therefore segregating with the 200 bp allele from the father.

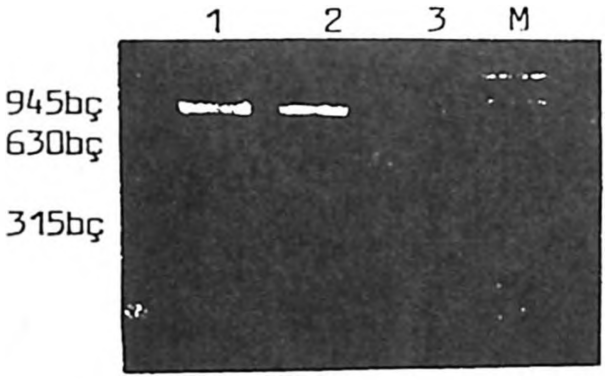

Fig. 2: Segregation of the alleles for the polymorphic Xbal site in family RB-1. The father (lane 1), mother (lane 2) and proband (lane 3) here heterozygous for the 945/630-315 allele. M: ØX174 HaeIII digested. (Marker lane).

In the second family (RB-2), three affected siblings were born to unaffected parents. This family pedigree is shown in Figure 3. There was no previous history of RB in this family.

All three children were boys, ages 8, 9 and 11, with bilateral RB. All the children had one enucleated eye. Blood samples were obtained from all the family members, including healthy individuals, and analyzed for the polymorphisms. All the affected and unaffected individuals were found to be heterozygous for BamHI and Xbal polymorhism (Fig. 3).

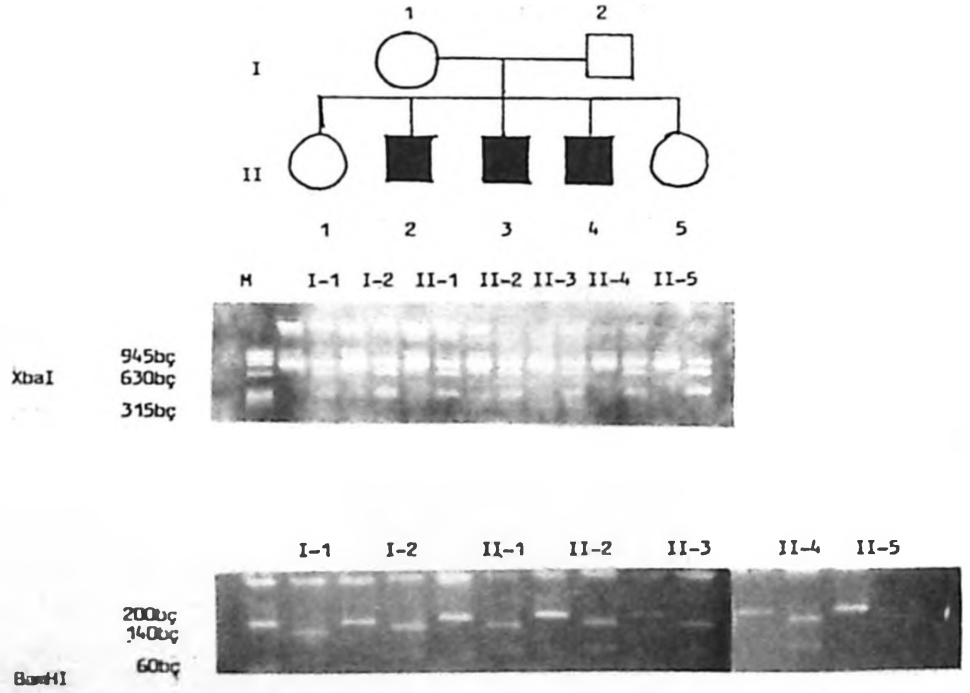

Fig. 3: Pedigree of family RB-2. Segregation of the alleles for the polymorphic Xbal and BamHI site in family RB-2. The father, (I-2), mother (I-1), all probands (II-2, II-3, II-4) and healthy children (II-1, II-5) are heterozygous for Xbal and BamHI sites. Thus the family was non-informative for mutant allele segregation. M: ØX174 HaeIII digested (marker lane).

Discussion

Predisposition to cancer can be assessed by identifying mutant alleles in affected pedigrees. By identifying carriers of the mutant gene, management of the disease and early intervention become possible. Now with the use of various DNA markers, linkage analysis allows prenatal diagnosis in RB families[11, 12].

In this study, two families identified as RB-1 and RB-2 were analysed. No tumor tissue had been saved from enucleated eyes; only blood samples were obtained and analyzed from all of the members of the two families.

Family RB-1 with hereditary retinoblastoma was investigated by linkage analysis using the BamHI polymorphism. Our prediction is that it is the upper allele (200 bp) which cosegregates with the disease. This test would allow counseling of this family in the future. This family was uninformative for the Xbal polymorphism. The family RB-2 was uninformative for both the Xbal and BamHI polymorphisms. In this family it was not possible to determine whether one of the parents was a true case of incomplete penetrance or whether one or the other was a germline mosaic for the mutation.

This segregation analysis with polymorphic DNA markers is often not feasible. Therefore, direct detection of the germline defect is the method of choice for identifying individuals at risk.

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EFFECTS OF METHYLPREDNISOLONE PULSE THERAPY ON INSULIN INJECTIONS IN PATIENTS WITH INSULIN – DEPENDENT DIABETES MELLITUS*

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SUMMARY: Satman İ, Fıçıcıoğlu C, Karşıdağ K, Yılmaz T, Dinçdağ N, Koca F, Odabaşı F, Aydın A, Devrim S, Haktan M. (Metabolism-Nutrition Unit, Department of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine, and Diabetes Unit, Department of Internal Medicine, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey). Effects of methylprednisolone pulse therapy on insulin injections in patients with insulin-dependent diabetes mellitus. Turk J Pediatr 1996; 38: 419-429.

In this study we evaluated 31 insulin-dependent diabetes mellitus (IDDM) patients (ages 12.1 ± 3.4 years, 18 males/13 females) who started on multiple subcutaneous insulin injections (MSII) within six weeks of diagnosis and achieved either complete (CR: no insulin requirement and near-normoglycemia for at least two weeks) or incomplete (ICR: minimum 50% decline in insulin requirement while maintaining near-normoglycemia for two weeks or more) remissions within the first 12 weeks of the MSII trial. Methylprednisolone pulse therapy (MP) was administered four times per day by IV bolus at a dose of 30 mg/kg (max. 1000 mg) on alternate days.

Eleven patients did not accept "MP-pulse" therapy; therefore, we followed these cases (7 males/4 females) as the control group. During the first year of follow-up, 13 patients from the "MP pulse" group achieved CR (3 males/1 female) or ICR (5 males/4 females) in 3.5 to 14 months. Remission occurred in only two of the control group cases (1 male CR for 17 days and 1 female CR for 7 months). Of those with CR in the "MP-pulse" and control groups, all were greater than 12 years of age, and all but one in the "MP-pulse" group were males. The stimulation capacity of beta cells (as defined by percentage increase in serum C-peptide levels after glucagon injection) among CR

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cases was found to be higher than that of non-remitted (NR) cases ($p < 0.05$ at onset, $p < 0.001$ during MSII-induced remission and $p < 0.05$ at the end of the first year of follow-up). Although patients with CR or ICR had higher beta cell reserves than NR cases at onset, only CR cases could sustain this capacity during the MSII-induced remission phase and one year after "MP-pulse" therapy. From this preliminary study, we conclude that "MP-pulse" therapy, may lead to prolonged near-normal beta cell function or partly preserved residual beta cell reserve during the MSII-induced remission phase of IDDM. The beneficial effects of MP could be seen clearly in patients diagnosed during the late childhood years. *Key words: insulin-dependent diabetes, methylprednisolone immuno therapy.*

Insulin-dependent diabetes mellitus (IDDM) is thought to result from the auto-immune destruction of beta cells in genetically susceptible individuals[1]. Beta-cell destruction is mediated by cells from the immune system and their products due to loss of self-tolerance[2,3].

Life prolongation achieved by the introduction of insulin has not changed the mostly unavoidable face of long-term complications[4]. The ability to secrete even a small amount of insulin seems to have a favorable influence on metabolic control and on the delay of onset of microvascular complications. As a logical approach, several immuno-interventions, ranging from aggressive immunosuppression to mild immunomodulation, have been tried in the past 15 years[5]. Immunointervention trials during the early clinical phase have failed because the pathological process has started months or even years before clinical onset, and many of the patients have only a limited beta-cell reserve at diagnosis[6]. On the other hand, 10 to 30 percent of patients may experience a naturally occurring complete remission as defined by clinical and metabolic characterizations[7]. Interestingly, in approximately half of new-onset patients, remission can be induced by intensive insulin therapy within the first few weeks or months of the disease[8]. Over the last 10 years our group has been involved in studying the potential value of the remission period for immunotherapeutic interventions.

The aim of this study was to prolong the remission period with the use of an immunosuppressive agent, methylprednisolone. Our study is the first to consider the effects of methylprednisolone pulse (MP-pulse) on multiple subcutaneous insulin injection (MSII)-induced remission in patients with IDDM.

Material and Methods

Selection of Patients

The study group consisted of 31 patients ranging in age from six to 19 years. Subjects were considered for recruitment in the study if they were six to 20 years of age, were of normal body weight, had IDDM diagnosed according to the WHO criteria[9] within the preceding six weeks, and developed a remission phase within 12 weeks of MSII therapy. Patients were started on 55 percent carbohydrate, 30 percent fat and 15 percent protein diets. Twenty patients were enrolled in

the "MP-pulse" group. Eleven patients who did not accept "MP-pulse" therapy were followed as the control group. Patients were admitted to the hospital during MSII therapy and were not discharged until their metabolic control was assessed after MP-pulse therapy. After discharge they self-monitored their blood glucose were contacted frequently by telephone for adjustments in insulin doses.

The study was approved by the local Ethical Committee, and an informed consent was obtained from patients or their parents.

MSII Therapy

Therapy consisted of regular human insulin injections before each main meal (3 doses) plus an intermediate-acting (NPH) insulin injection at bedtime for up to 12 weeks. Insulin doses were adjusted, aiming for preprandial glucose level of less than 140 mg/dl. Glucose profiles were monitored at least four times daily using a portable glucometer and glucose strips.

Remission

Remissions were defined according to the following criteria:

1. Compelete Remission (CR):
   - Preprandial glucose levels less than 140 mg/dl.
   - HbA1c less than 7.5 percent.
   - No insulin requirement for at least two weeks.
2. Incomplete Remission (ICR):
   Less than 50 percent of initial insulin requirement in spite of near-normal metabolic control for two weeks or more.

MP-Pulse Therapy

Methylprednisolone (MP) was administered four times per day on alternate days at a dose of 30 mg/kg (max. 1000 mg/day) by intravenous bolus.

Methods

Plasma glucose was measured by glucose oxidase and HbA1c was determined by a column chromatographic method. A commercially available RIA kit (DPC) was used for C-peptide determinations. Stimulated C-peptide levels were defined as a peak increase within 10 minutes of glucagon (30 µg/kg, max. 1000 µg I.V. bolus) injection. ICA (islet-cell antibody) was assayed by Botazzo's indirect IF technique, while CD4 (helper-inducer T cells) and CD8 (suppressor-cytotoxic T cell) evaluations were performed by EPICS CS apparatus (Coulter Electronics Inc. U.K.).

Statistical Evaluation

Student's t-test (paired or unpaired) and chi-square test were used for statistical analyses whenever required. A p value of less than 0.05 was accepted as significant.

Results

The clinical characteristics of the "MP-pulse" and control groups at entry (at clinical onset) listed in Table I.

Table I: General and Laboratory Characteristics of Patients at Entry*

General Characteristics	"MP-pulse" Group	Control Group
	n: 20	n: 11
Age (years)	11.7 ± 3.6	11.8 ± 3.1
Sex (m/f)	11/9	7/4
BMI (kg/m^2)	16.9 ± 2.9	16.3 ± 2.2
Occurrence with DK/DKA (%)	50	45
Family history of diabetes (%)	35	36
Laboratory Characteristics		
Blood glucose (mg/dl)**	338.4 ± 124.3	343.0 ± 119.3
HbA1c (%)	121.1 ± 4	12.0 ± 3.5
FCR (ng/ml)	0.51 ± 0.36	0.47 ± 0.25
SCR (ng/ml)	1.13 ± 0.78	1.11 ± 0.38
ICA positivity (%)	45	64
CD4/CD8 (n: 16)	1.9 ± 0.8	–
Insulin requirement (IU/kg/day)	0.73 ± 0.29	0.69 ± 0.13

* Results given as mean ± SD. (No significant difference other than ICA was found between the groups).
** Mean preprandial blood glucose level.

DK : Diabetic ketosis.
DKA: Diabetic ketoacidosis.
BMI : Body mass index.
FCP : Fasting C-peptide level.
SCP : Peak stimulated C-peptide level within 10 minutes of 1 mg glucagon injection.

Participants in the "MP-pulse" therapy included 11 males and nine females, while the control group consisted of 11 patients (seven males and four females). All differences between groups were insignificant at entry except for ICA. The ratio of CR/ICR after MSII therapy in the "MP-pulse" group was 10/10 (7 males and 3 females/4 males and 6 females) and 5/6 (2 men and 3 women/5 men and 1 woman) in the control group (Table II). The mean duration from disease onset to remission was 8.8 and 8.9 weeks in the "MP-pulse" and control groups, respectively. Laboratory characteristics during MSII-induced remission were not significantly different between the two groups, except for ICA positivity.

No side effects were noted during MP-pulse therapy except for a temporary increase in blood sugar which was normalized by insulin adjustments.

Table III shows the remission results and laboratory findings 12 months after "MP-pulse" therapy and among controls. Sixty-five percent of patients underwent

clinical remission during the first year of followup (4 CR and 9 ICR) in the "MP-pulse" group and 18.2 percent (1 CR and 1 ICR) in the control group ($p < 0.01$).

Table II: Patient Characteristics During MSII-Induced Remission Phase

	"MP-pulse" Group	Control Group
Mean time from onset to remission (weeks)	8.8 ± 26	8.9 ± 1.4
Remission status		
– CR (m/f)	7/3	2/3
– ICR (m/f)	4/6	5/1
total (CR/ICR)	10/10	5/6
Blood glucose (mg/dl)	121.8 ± 50.3	107.5 ± 17.6
HbA1C (%)	7.1 ± 2.3	7.6 ± 1.1
FCP (ng/ml)	0.68 ± 0.45	0.53 ± 0.31
SCP (ng/ml)	1.20 ± 0.68	1.14 ± 0.52
ICA positivity (%)	33	62
CD4/CD8 (n: 15)	1.5 ± 0.5	
Insulin requirement (IU/kg/day)	0.31 ± 0.12	0.29 ± 0.17

* No significant difference was defined between groups except ICA positivity.

CR : Complete remission.
ICR: Incomplete remission.

Table III: Patient Characteristics After "MP-Pulse" Therapy

	"MP-pulse" Group	Control Group
Duration of remission (months) (Mean total CR and/or ICR) Range	6.6 ± 4.6 (3.5-14 months) $p < 0.01$	3.1 ± 2.3 (17 days-7 months)
Remission status		
– CR (m/f)	3/1	1/10
– ICR (m/f)	5/4	0/1
Total (CR/ICR)	4/9 $p < 0.01$	1/1 1/1
Laboratory findings (at 12th month)		
Blood glucose (mg/dl)	121.8 ± 50.3	178.0 ± 40.6 $p < 0.01$
HbA1c (%)	9.2 ± 3.6	10.5 ± 1.9
FCP (ng/ml)	0.64 ± 0.29	0.36 ± 0.23
SCP (ng/ml)	1.17 ± 0.60	0.68 ± 0.43 $p < 0.02$
ICA positivity (%)	3.5%	5.5% $p < 0.01$
CD4/CD8 (n: 10)	1.7 ± 0.3	–
Insulin requirement (IU/kg/day)	0.31 ± 0.14 (n: 16)	0.66 ± 0.28 $p < 0.01$ (n: 11)

* NS: Not significant.

The ratio of helper-inducer to suppressor-cytotoxic T-cells (CD4/CD8) in the "MP-pulse" group increased at onset, markedly declined during the MSII-induced remission phase, and although slightly increased at the first year, remained lower than the initial value ($p < 0.01$ onset vs. MSII remission, $p < 0.05$ MSII remission vs. "MP-pulse", and $p < 0.05$ onset vs. "MP-pulse"). Unfortunately, we were not able to follow-up CD4/CD8 profiles of control patients because of limited resources.

At the end of the first year, the mean insulin requirement of patients in the "MP-pulse" group was 0.31 IU/kg/day, and 0.66 IU/kg/day in the control group ($p < 0.01$).

Another reflection of this difference appeared in the serum C-peptide levels. Patients in the "MP-pulse" group had higher beta-cell reserves than control cases, as indicated by both fasting and stimulated C-peptide levels ($p < 0.01$ and $p < 0.02$, respectively).

Discussion

The ultimate aim of the ongoing research in the field of immunotherapy for IDDM is to provide non-insulin dependence and prevention of long-term complications without upsetting patients' quality of life[7]. Early immunosuppressive therapy with azothioprine may prevent the rapid decline of endogenous insulin secretion and increase the remission rate during the first year[11]. No long-term benefits of either azothioprine or cyclosporin therapy have been reported, and after discontinuation of the drug the insulin dose is similar in treated and non-treated groups[12-15]. This fact is more or less true for other immunotherapeutic modalities, such as plasmapheresis[16], prednisone[17], antithymocyte globulin[18], anti-CD5[19] and nicotinamide alone or in combination with steroids or cyclosporin[20, 21]. Therefore, researchers have addressed the problem at the preclinical phase, aiming at prevention of IDDM before marked beta-cell loss[22-28].

On the other hand, intensive insulin therapy (IIT) either with continuous subcutaneous insulin infusion (CSII) or MSII started very early after the onset of IDDM (within the first few weeks) may induce remission in approximately 50 percent of patients[6, 7, 29]. The beta-cell recovery leading to a full remission with IIT depends not only on the magnitude of improved endogenous insulin secretion due to removal of the suppressive effect of glucotoxicity on the intact beta cells and allowing them to rest but also on the relieved body's sensitivity to insulin[30, 31].

The diagnosis of IDDM is very easy; careful follow-up and awareness of the possibility may be sufficient to notice the remission phase. Finally, patients in remission are destined to be "definitive" diabetics if left without intervention. For this reason, the ethical argument for intervention, mostly at the preclinical stage, is not fair[32].

Because autoimmune diseases in general are favorably influenced by a course of corticosteroids, it seemed reasonable to investigate whether steroids may

improve residual beta-cell function and prolong the remission phase in IDDM. One can expect increased insulin resistance during steroid therapy, but slowed down or halted beta-cell destruction at remission may suggest that this is not as severe a problem as suspected to warrant intervention with corticosteroids soon after clinical onset[32].

The increased CD4/CD8 ratio in our "MP-pulse" group, which indicates active autoimmunity, declined at remission ($p < 0.01$). Similarly, metabolic parameters (glycemia, HbA1c) and endogenous insulin secretion (fasting and stimulated C-peptide levels) improved during remission in both groups. The remission findings of our patients are proof of decelerated autoimmunnity at this phase.

A factor that, though unproven, may contribute to the maintenance of endogenous insulin secretion during remission is regeneration of beta cells[33]. The selected immunosuppressive agent in our study, MP, has a well-known regenerative effect on beta cells, as showed experimentally[17]. We previously revealed that long-term oral MP treatment during the remission period of IDDM prolonged this phase for at least 18 months in 60 percent of treated patients. Although we did not see severe side effects during the course of oral MP, these patients had to undergo long-term hospitalization. "MP-pulse therapy" is one of the therapeutic modalities applied for many autoimmune diseases. e.g. rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

At one-year of follow-up, about two-thirds of patients in the "MP-pulse" group reached remission, while the percentage of remitted patients among the control group was only 18 percent ($p < 0.01$). No patient under the age of 12 years was found among CR patients. Approximately 80 percent of ICR patients were over 10 years of age (Fig. 1).

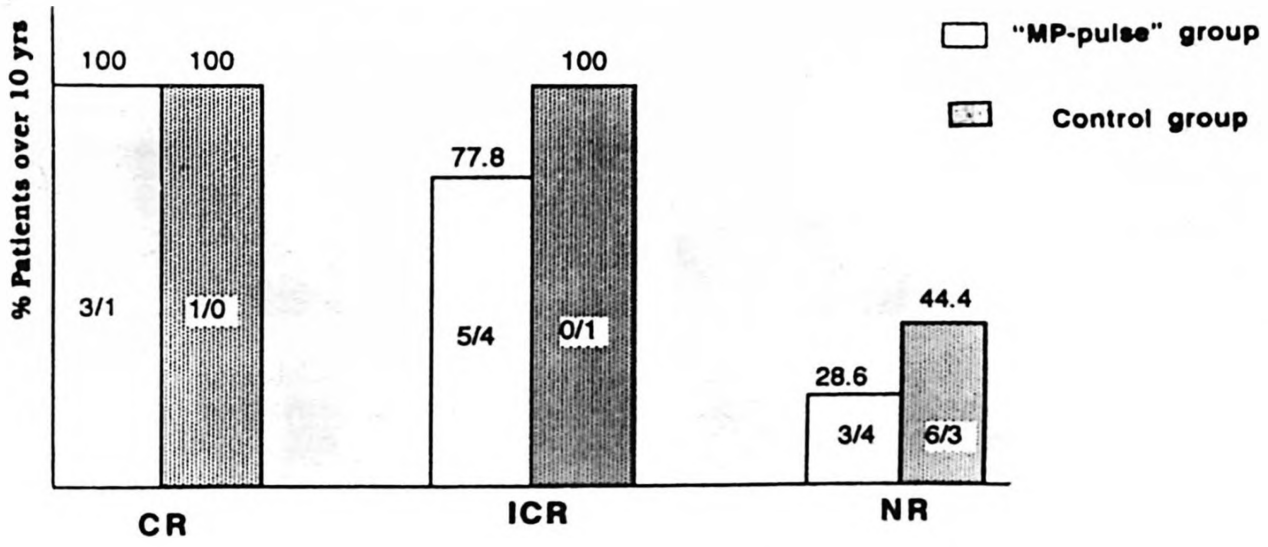

Fig. 1: Percentage of patients greater than 10 years of age among CR, ICR and NR patients (*figures inside each column indicate male/female ratio). CR: chronic remission. ICR: incomplete remission. NR: non-remitted cases.

In the first study with azothioprine, Harrison et al.[11] reported that seven out of 13 patients (mean age 25 years) were remitted completely. Later studies with the same drug in younger-onset patients (mean age 11 years) did not show such a high CR rate[6, 12]; thus the age of the patient at entry was considered as a possible predictor of outcome. The disease may progress more rapidly in younger than older children. In our study three out of four CR cases in the "MP-pulse" group and one patient who developed CR in the control group were males. Previous studies by our groups have revealed that male patients, especially in older-age-onset IDDM, may have better metabolic outcomes and a higher possibility of remission with IIT and immunotherapy[7, 32, 34]. This finding requires further confirmation.

CR patients had higher beta cell reserves than did ICR and NR patients at onset, as defined by percentage increase in serum C peptide after glucagon stimulation ($p < 0.05$ vs. NR, although CR vs. ICR was not significant). This reserve did not change significantly at the first year of follow-up; ICR patients had a lower capacity for beta-cell stimulation than did CR patients during MSII-induced remission ($p < 0.001$). However, this reserve was difficult to preserve later. NR patients had a limited beta-cell reserve at onset which could not be maintained during follow-up (Fig. 2).

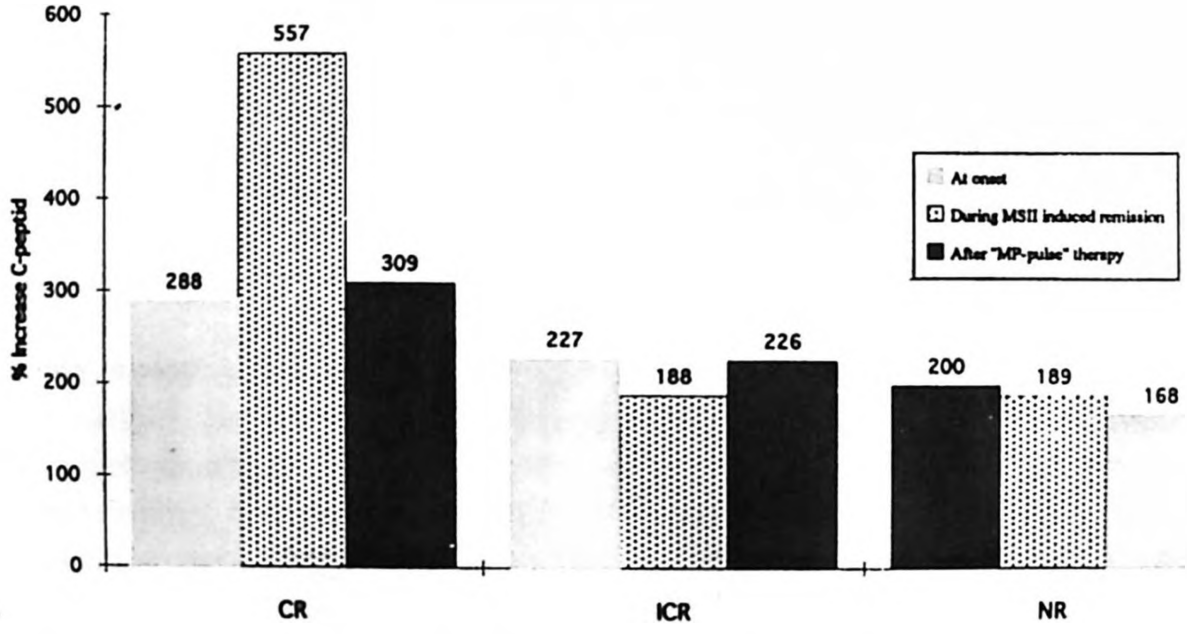

Fig. 2: Mean percentage increase in C-peptide level after glucagon stimulation in "MP-pulse" group. (*$p < 0.01$, **$p < 0.02$, ***$p < 0.05$, ****$p < 0.01$ vs. each onset).

The mean total duration of remission for consecutive CR and/or ICR in the "MP-pulse" group was found to be remarkably longer than in the control group ($p < 0.01$). Figure 3 depicts life-table analyses of "MP-pulse"-treated and control groups. The "MP-pulse" group had better metabolic control and significantly higher

beta-cell reserve than the control group at follow-up. Our results revealed that male gender, disease onset during late childhood and a higher beta cell reserve at clinical onset may point to a possible complete and/or long-term remission. This confirms the previous findings reported by our group, Schiffrin et al.[35, 36] and Karjalainen et al.[37] It seems that ICA positivity is not related to the likelihood of remission. ICA differences of our MP and control groups were thought to be due to limited numbers of patients enrolled in the study.

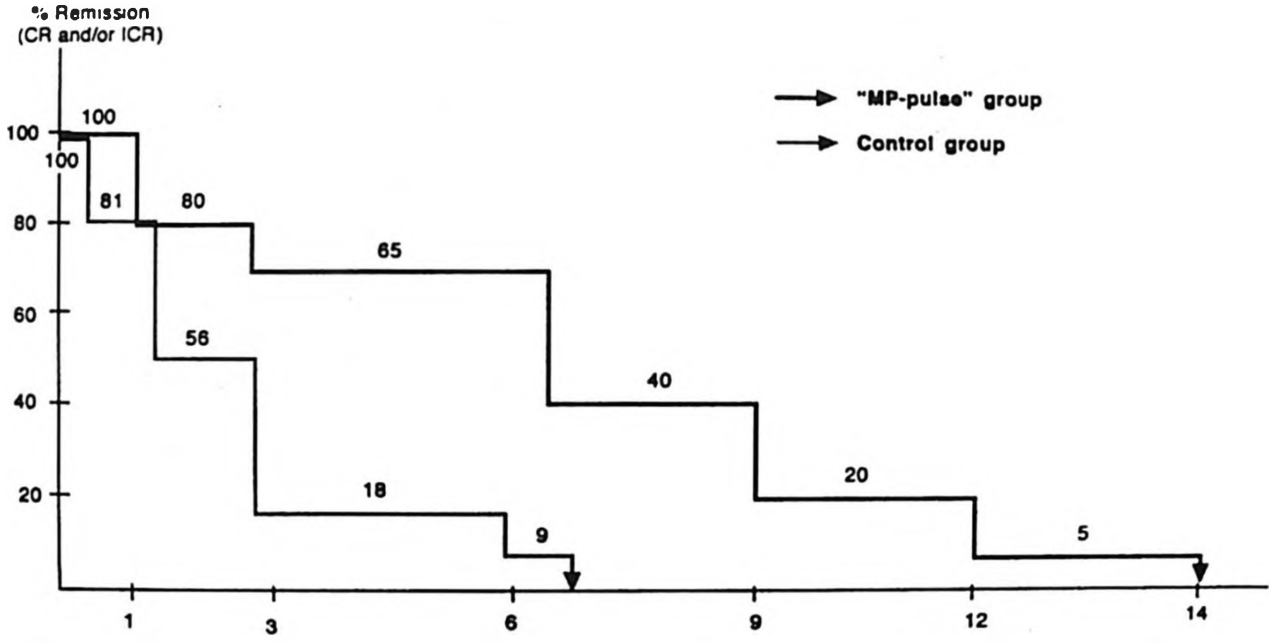

Fig. 3: Life table analyses for total remission in "MP-pulse" and control groups. Figures in parentheses indicate number of cases in remission in each time-period.

This work was conducted as part of a development plan relevant to immune-interventional trials in the remission period of IDDM. To our knowledge this study is the first to investigate the influence of "MP-pulse" therapy specifically on MSII-induced remission. The preliminary results suggest that "MP-pulse" therapy may have utility in prolonging the remission phase by helping to preserve beta-cell function from further deterioration during the MSII-induced remission phase of IDDM, particularly in patients diagnosed after early childhood ages.

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RELATION BETWEEN LIMITED JOINT MOBILITY AND PERIPHERAL NERVE FUNCTION IN DIABETIC CHILDREN*

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*Perihan Baslo MD******

SUMMARY: Fıçıcıoğlu C, Kızıltan M, Aydın A, Baslo P. (Metabolism-Nutrition Unit, Department of Pediatrics, and Department of Neurology, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey). Relation between limited joint mobility and peripheral nerve function in diabetic children. Turk J Pediatr 1996; 38: 431-437.

In order to assess the relation between limited joint mobility (LJM) and peripheral nerve function in diabetic children, peroneal nerve amplitude and motor conduction velocity as well as sural nerve amplitude and distal latency were measured with a Medelec MSG electromyograph. LJM was examined by observing the hands in the prayer position of 60 unselected diabetic children (average age 11.2 ± 3.1 years; mean duration of diabetes 4.1 ± 3 years) and 31 healthy controls. Of 60 patients, 38.3 percent had limited joint mobility. no influence of sex on expression of LJM was found. Its appearence was influenced by age, duration of diabetes and metabolic control (HbA1c) ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively). The mean amplitude, nerve conduction velocity and distal latency values in diabetic children with LJM were slower than those in the normal control group (peroneal amplitude $p < 0.01$, peroneal MCV $p < 0.001$, sural amplitude $p < 0.001$, sural distal latency $p < 0.001$). Of four patients with stage IV LJM, three had symptomatic neuropathy diagnosed using the criteria suggested by Dyck. Consequently, LJM identifies a population at risk for development of diabetic neuropathy. Thus, being easy and sensitive, LJM determination may provide both the physician and the patient himself with an important motivation to improve diabetic control in an effort to prevent dibetic neuropathy. *Key words: diabetes, neuropathy, limited joint mobility, childhood.*

Since first reported by Rosenbloom in 1974, limited joint mobility (LJM) has been increasingly recognized as a common manifestation of childhood diabetes, with reported prevalences varying between eight and 43 percent[1-6]. These contractures may involve the hands, feet and larger joints. Thickening of the skin may also be associated with LJM. LJM may precede the onset of clinical complications of IDDM by sufficient time to be utilized as a marker for these complications.

Diabetic neuropathy is a frequent complication of diabetes mellitus. The classical signs of neuropathy are rarely found in children with insulin-dependent diabetes.

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The frequency of electrophysiological abnormalities and peripheral neuropathy has varied widely from nine to 72 percent in different studies reported on diabetic children[7-15].

The aim of this study was to evaluate the relationship between LJM and peripheral neuropathy in diabetic children.

Material and Methods

The study group consisted of 60 insulin-dependent diabetic children from four to 16 years of age (mean ± SD: 11.1 ± 3.1) in whom the duration of diabetes ranged from one to 12 years (mean ± SD: 5.1 ± 3.1), and 31 nondiabetic children from four to 16 years of age (mean ± SD: 10.9 ± 2.8). The diabetic children were treated with two daily injections of human insulin, the dosage of which was adapted according to blood glucose monitoring with dextrostix and a glucometer. No children were treated with isoniazid, and none had a history of drug dependence or rheumatologic disease. None had a family history of hereditary neuropathic disorders.

Serial glycated hemoglobin (HbA1c) values every three months were measured by the colorimetric technique originally described by Fluckiger and Winterhalter[16] with normal concentrations of 6.3 to 7.2 percent.

During routine physical examinations, to in order demonstrate joint mobility, each patient was asked to approximate the palmar surfaces of the interphalangeal joints of both hands with the fingers fanned. Normal extension (approximation) at the interphalangeal joints is usually a minimum of 180°. For definitive diagnosis and staging of LJM, passive extension of the fingers, wrist, and elbow was done.

LJM was classified using Brink-Starkman[1] staging criteria as follows:

Stage 0; no stiffness, no joint abnormalities; Stage I: stiffness of skin only; Stage II: flexion contractures of opposing fifth fingers bilaterally; Stage III: bilateral contractures of more than the fifth fingers; Stage IV: wrist involvement bilaterally; Stage V: other joint involvement.

LJM was assessed by two pediatric diabetologists (each time the same physicians) together at more than one visit in order to confirm the presence of LJM and to stage it correctly. The absence of diabetes was known to the examiners who assessed LJM in the control group.

The amplitude and conduction velocity of the motor fibers of the peroneal nerve in both lower extremities, and the sensory amplitude and distal latency of the sural nerve were measured in the patients and healthy children by the same observer using a Medelec (MSG) electromyograph.

Complete neurologic examination and electromyographic measurements were performed on all children by a neurologist who was not aware of the presence or absence of LJM. Muscle strength was evaluated according to the principles

suggested by the Medical Research Council[17]. Tendon reflexes were evaluated in the biceps brachii, triceps brachii, brachioradialis, quadriceps and gastrocnemius soleus muscles. Sensory deficit was evaluated at three different points from distal to proximal on each extremity. Cotton wool was used to assess touch-pressure. Disposable pins and a 25 cm long, 28 Hz tuning fork were used for pain and vibratory sensation, respectively. Joint position was tested by manually displacing the great toe or finger.

The findings of the neurologic examination were scored as to neurological symptoms and neurological disability. The criteria for the diagnosis and staging of diabetic neuropathy were as follows[15,17]:

Stage 0-no neuropathy.

Stage Ia-early subclinical (asymptomatic) neuropathy: abnormality of amplitude or nerve conduction (more than 3 SD below the mean for normals) or distal latency (more than 3 SD above the mean for normals) of one or more attributes in two or more nerves and not due to a disease other than IDDM or to faulty electrode placement or low nerve temperature.

Stage Ib-subclinical (asymptomatic) neuropathy: abnormalities of both peripheral nerve test and neurologic examination (abnormality of one or more of muscle strength, tendon reflexes or sensation, judged to be due to diabetic neuropathy).

Stage II-symptomatic neuropathy: two or more abnormalities of nerve conduction, neurologic examination or neuropathic symptoms. Neuropathic symptoms present but of lesser severity than in Stage III.

Stage III- disabling neuropathy: two or more abnormalities of peripheral nerve test, neurologic examination or neuropathic symptoms. Disabling neuropathic symptoms present.

Statistical methods: Statistical analyses were performed using Chi-square test, Fisher's exact test, Kruskal-Wallis analysis of variance, Mann-Whitney U-test, and the stepwise regression procedure.

Results

Of 60 patients, 38.3 percent (n: 23) had limited joint mobility. Two subjects (8.6%) had stage I, 11 subjects (47.8%) had stage II, four subjects (17.3%) had stage III and six subjects (26%) had stage IV involvement of their interphalangeal joints. The presence of contractures was significantly related to mean longitudinal HbA1c (coefficient of variation for HbA1c value was 12.1%), duration of diabetes and age ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively). We did not find LJM in the nondiabetic control group (Table I). The peroneal motor conduction velocity (MCV) was the most significant correlate with LJM, followed by HbA1c, neuropathy

(symptomatic + asymptomatic) and duration of diabetes, which were next in importance as determined by step-wise regression analysis (Peroneal MCV: r^2: 0.77, F: 19.3, $p < 0.001$; HbA1c: r^2: 0.49, F: 11.7, $0.001 < p < 0.01$; neuropathy: r^2: 0.47, F: 11.5, $0.001 < p < 0.01$; duration of diabetes: r^2: 0.43, F: 11.1, $0.001 < p < 0.01$).

Table I: Duration of Diabetes, HbA1c Values and Age (Mean ± SD) in Diabetic Children with and without LJM and in Normal Controls

	Diabetic children with LJM (n: 23)	Diabetic children without LJM (n: 37)	Non diabetic children (n: 31)	Significance		
Duration of diabetes	7.3 ± 2.05	2.1 ± 1.6		$p < 0.001$*		
HbA1c	14.3 ± 1.6	9.5 ± 1.3	6.1 ± 0.4	$p < 0.001$*	$p < 0.001$**	$p < 0.001$***
Age	13.5 ± 2.3	9.9 ± 2.8	10.9 ± 2.8	$p < 0.001$*	$p < 0.001$**	$p > 0.05$***

* Significance between diabetic children with and without LJM.
** Significance between diabetic children with LJM and controls.
*** Significance between diabetic children without LJM and controls.

No influence of sex on expression for LJM was found (p: 0.97, chi-square test). Of 23 diabetic children with LJM, 13 (56.5%) had either clinical or subclinical neuropathy. Diabetic neuropathy was significantly more common in diabetic children with LJM (p: 0.0000149, Fisher's exact test). The mean amplitude, nerve conduction velocity and distal latency values in diabetic children with LJM were slower than in the normal control group (common peroneal amplitude $p < 0.01$, peroneal MCV $p < 0.001$, sural amplitude $p < 0.001$, sural distal latency $p < 0.001$) and in the diabetic group without LJM (peroneal amplitude $p > 0.05$, peroneal MCV $p < 0.001$, sural amplitude $p < 0.001$, sural distal latency $p < 0.001$) (Table II). Of six patients with stage IV LJM, three had symptomatic neuropathy diagnosed using the criteria suggested by Dyck[17]. Three other patients had both abnormal peripheral somatic electrophysiologic nerve test results in two or more nerves and pathologic findings in the neurologic examination (asymptomatic neuropathy). Three of the patients with stage IV LJM had retinopathy and one had nephropathy. The duration of diabetes and the values of HbA1c as the degree of metabolic control of patients with stage IV LJM were significantly greater than those of patients with less severe LJM ($p < 0.001$, $p < 0.001$, respectively). One of the four diabetic children with stage III LJM had asymptomatic neuropathy. Three of the 11 patients with stage II LJM had asymptomatic neuropathy. Three other patients with stage II LJM had only abnormal electrophysiological test results in one or more attributes of two or more nerves, and were diagnosed as having early subclinical neuropathy. Subclinical neuropathy was diagnosed in only two patients without LJM. The frequency of both clinical and subclinical neuropathy was 25 percent.

Table II: Electrophysiologic Measurements (Mean ± SD) in Diabetic Children with and without LJM and Control Group

	Diabetic children with LJM (n: 23)	Diabetic children without LJM (n: 37)	Non diabetic children (n: 31)	Significance		
Common peroneal CMAP* (milivolts)	2.9 ± 1.9	3.1 ± 1.6	4.5 ± 2.5	$p > 0.05^{**}$	$p < 0.001^{***}$	$p < 0.001^{****}$
Common peroneal MCV* (m/s)	39.7 ± 6.9	48.5 ± 9.2	49.4 ± 6.9	$p < 0.001^{**}$	$p < 0.001^{***}$	$p > 0.05^{****}$
Sural SNAP* (microvolts)	11 ± 6.3	17.4 ± 5.9	17.4 ± 6.9	$p < 0.001^{**}$	$p < 0.001^{***}$	$p > 0.05^{****}$
Sural distal latency (m/s)	4.8 ± 1.04	3.7 ± 0.9	3.4 ± 0.7	$p < 0.001^{**}$	$p < 0.001^{***}$	$p > 0.05^{****}$

*CMAP: Compound muscle action potential amplitude.
MCV : Motor conduction velocity.
SNAP : Sensory nerve action potential amplitude.

* Significance between diabetics with and without LJM.
** Significance between diabetics with LJM and controls.
*** Significance between diabetics without LJM and controls.

Discussion

LJM is a common complication of IDDM and is seen more frequently in diabetic children than in the normal population[1-6]. A simple screening test to find LJM is to ask the patient to press the palms of the hands together as if in prayer. Patients with LJM are unable to oppose the palmar surfaces together. The exact pathogenesis of LJM is not clear, but it is thought to be due to connective tissue glycosylation in children and adults with diabetes mellitus; these patients are more likely to develop complications associated with diabetes because their microvascular systems and intracellular structures undergo glycosylation in a similar fashion[18, 19]. It is often overlooked because it causes little disability and is therefore thought to be of little clinical importance.

Our data suggest an association of LJM with diabetic neuropathy in children with IDDM. LJM was noted in 23 (38.3%) of the 60 children with IDDM. The presence of LJM was significantly associated with longitudinal HbA1c values and with the duration of diabetes. Of 23 diabetic children with LJM, 13 (56.5%) had either clinical or subclinical neuropathy. Three of the patients with stage IV LJM had both clinical neuropathy and retinopathy, and one of them had nephropathy. Ten patients with stage I, II or III LJM had no other diabetic complications except subclinical neuropathy. Our results suggest that the more severe the LJM, the more likely that diabetic complications will be diagnosed.

The relationship between LJM, diabetic renal and retinal complications is well known[5, 18, 20, 21]. The relative risk of nephropathy and retinopathy if a patient has LJM is reported as 6:1 and 4:1, respectively[1]. Although the prevalence of microvascular complications has been reported in most studies on LJM, the prevalence of neuropathy has been stated in few of them[22, 23]. Brink et al.[1] reported the relative risk for diabetic neuropathy in the presence of LJM as 4:1. Jung et al.[23] described 23 adult diabetics with LJM who also had evidence of neuropathy. Starkmann et al.[21] reported a significant association of LJM and neuropathy in patients less than 40 years of age. We could not compare our results with those of others because there has been no similar study in diabetic children. LJM may be used as a marker to identify diabetic patients who are at risk for the development of diabetic neuropathy. Thus, LJM determination may provide the physician and the patient himself with an important motivation to improve diabetic control in an effort to prevent diabetic complications, including diabetic neuropathy. Further studies are necessary in order to delineate the links between the pathogenetic mechanisms of LJM and peripheral neuropathy in diabetes.

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RELATIONSHIP BETWEEN MATERNAL AND NEONATAL IRON STORES*

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SUMMARY: Tekinalp G, Oran O, Gürakan B, Saraçel M, Erdem G, Yurdakök M, Gürgey A. (Neonatology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Relationship between maternal and neonatal iron stores. Turk J Pediatr 1996; 38: 439-445.

In this study, hemoglobin (Hb), mean corpuscular volume (MCV) and serum ferritin (SF) levels were measured in 76 neonates and their mothers at delivery. Infants were grouped according to their gestational ages. Group I (< 34 weeks), group II (34-37 weeks) and group III (> 37 weeks) consisted of 15, 33 and 28 infants, respectively. Blood studies were repeated at two months of age in 50 neonates (26 from group II and 24 from group III). Among the three groups of infants, SF levels were lowest in group I, and among mothers, Group III had the lowest levels.

There were positive correlations between maternal and neonatal SF levels in groups II and III. There was no difference between the SF levels of neonates born to mothers with depleted or adequate iron stores. Anemia with a normal SF level was present in 14.4 percent, subclinic iron deficiency (normal Hb and low SF level) in 11 percent and iron deficiency anemia (low Hb and low SF levels) in 7.8 percent of the mothers. At two months of age 38.4 percent of preterm and 16.6 percent of term infants had Hb concentrations less than 10 g/dl. Only one of these infants had a low SF level. There was a negative correlation between maternal SF levels and the Hb concentration of term infants at two months of age. *Key words: serum, iron, ferritin, newborn infant.*

Iron deficiency continues to be one of the most prevalent nutritional deficiencies in developed as well as developing countries. The main source of iron for hemoglobin formation in the first few months of life derives from fetal iron stores at birth, originally from the mother. However, the status of iron stores in infants born to iron-depleted mothers remains controversial and has been inadequately investigated. While some observers have recorded a small but definite lowering of iron stores in the offspring of iron-deficient mothers, others have failed to corroborate these findings[1-4].

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The purpose of this study was to determine whether the amount of stored iron in the mother is related to the stored iron levels and other hematological indices of preterm and full-term newborns. This is the first study that has investigated the iron status of preterm neonates in our country, as far as we know.

Material and Methods

This study included 76 mothers and their neonates born at the Gynecology and Obstetrics Department of Hacettepe University Faculty of Medicine between March and December 1992.

Infants were grouped according to their gestational ages. Group I consisted of 15 small, preterm infants born before the 34th gestational week, group II consisted of 33 infants born in the 34th to 37th gestational weeks, and group III consisted of 28 term infants (> 37 weeks). The mean gestational ages were 31.5 + 2.6 weeks, 35.6 + 2.1 weeks, and 39.2 + 1.8 weeks, respectively.

Maternal and neonatal blood samples were drawn immediately after delivery from peripheral veins. Blood studies were reevaluated at two months of age in 50 infants (26 from group II and 24 from group III). Unfortunately we failed to follow most of the infants from group I.

During the first two months, no infant received iron supplements and all the infants were fed exclusively with breast milk. Hemoglobin and MCV values were measured by coulter counter model S plus VI (Coulter electronics INC, Hialeah, Florida USA). Serum ferritin levels were measured by using ferritin enzyme testing kits of Immuno Diagnostic Systems Ltd, with immunoradiometric assay.

Data were assessed by Student's t test, and linear regression analyses were done[5]. Reslts are expressed as mean ± standard deviation.

Results

Maternal and neonatal Hb, MCV and SF values are shown in Table I. Neonatal Hb, MCV and SF levels were significantly higher than maternal values ($p < 0.01$). Maternal Hb and MCV values were not significantly different among the three groups ($p > 0.05$). However, the mean maternal SF level of group III was lower than the others ($p < 0.01$). While the neonatal Hb level was found to be highest in group III ($p < 0.001$), the MCV level was higher and the SF level was lower than the others in group I ($p < 0.01$). Neonatal SF levels were higher than maternal values for all groups ($p < 0.001$). A positive correlation was found between maternal and neonatal SF levels in groups II and III (n: 33, r: 0.380, $p < 0.05$; and n: 28, r: 0.371, $p < 0.05$, respectively).

Table I: Maternal and Neonatal Hemoglobin, Mean Corpuscular Volume and Serum Ferritin Levels

		Group I (n: 15)	Group II (n: 33)	Group III (n: 28)	Total (n: 76)
Hb:	M	12.0 + 3.2 (8.5-14.6)	11.9 + 3.6 (7.7-14.6)	12.6 + 3.8 (7.4-15.9)	12.2 + 3.7 (7.4-15.9)
	N	17.7 + 4.2 (13.8-21.6)	17.2 + 4.4 (14.0-22.3)	19.6 + 4.0 (15.7-23.9)	18.2 + 4.8 (13.8-23.9)
MCV:	M	91.1 + 26.4 (75-120)	85.5 + 13.5 (67-99)	89.1 + 12.8 (78-105)	87.9 + 16.8 (67-120)
	N	114.7 + 14.8 (99-125)	106.4 + 14.8 (92-124)	106.8 + 10.0 (99-122)	108.2 + 14.6 (92-125)
SF:	M	46.4 + 62.8 (3-94)	51.4 + 70.0 (3-149)	26.7 + 37.0 (7-61)	41.36 + 62.0 (3-149)
	N	161.9 + 218.8 (34-427)	348.6 + 521.6 (49-1000)	303.8 + 608.2 (72-1500)	295.2 + 526.9 (34-1500)

M : Maternal values. N : Neonatal values. Hb: Hemoglobin (gr/dl).
MCV : Mean corpuscular volume (fl). SF : Serum ferritin (ng/ml).

Fifteen mothers had depleted iron stores (SF < 12 ng/ml)[3]. There was no significant difference between the SF levels of newborns born to mothers with depleted and adequate iron stores (Table II).

Table II: Comparison of Neonatal Serum Ferritin Levels of Infants Born to Mothers with Depleted and Adequate Iron Stores

	Neonatal ferritin (ng/ml)		
Maternal ferritin (ng/ml)	Groups I and II	Group III	All infants
< 12	209.5 + 292.4 (n: 7)	262.0 + 170.2 (n: 8)	237.5 + 232.4 (n: 15)
> 12	304.0 + 502.2 (n: 41)	320.6 + 714.8 (n: 20)	309.4 + 474.6 (n: 61)

p values for columns were 0.397, 0.242 and 0.943, respectively.

No correlation was found between the maternal and neonatal SF values when the maternal iron stores were depleted (n: 15, r: 0.164, $p > 0.05$); however, there was a positive correlation when the mothers' iron stores were adequate (n: 61, r: 0.333, $p < 0.01$).

The hemoglobin level was less than 10 g/dl in 11 of 76 mothers (14.4%), while their ferritin levels were normal. In contrast, nine mothers (11.8%) had low SF levels and normal Hb values. Only six mothers (7.8%) had low levels of both parameters.

At two months of age, no statistically significant difference was found between preterm and term levels of Hb, MCV and SF (Table III).

Table III: Hemoglobin, MCV and Serum Ferritin Values of Infants at Two Months of Age

	Group II (n: 26)	Group III (n: 24)
Hemoglobin (g/dl)	10.3 + 2.2 (8.6 – 12.4)	10.9 + 2.4 (7.7 – 13.4)
MCV (fl)	85.5 + 9.8 (75 – 93)	84.3 + 8.0 (78 – 90)
Ferritin (ng/ml)	165.3 + 309.2 (11 – 599)	160.2 + 310.8 (23 – 595)

Ten out of 26 preterm infants (38.46%), and four out of 24 term infants (16.6%) had low Hb levels (< 10 g/dl). Only one preterm infant had a low ferritin level. There was no correlation between the Hb values of mothers and infants at two months of age in the preterm or term groups. A negative correlation was found between maternal SF levels and the Hb values of term infants at two months of age (n: 24, r: -0.412, $p < 0.05$, Fig. 1); this was not true for preterm infants.

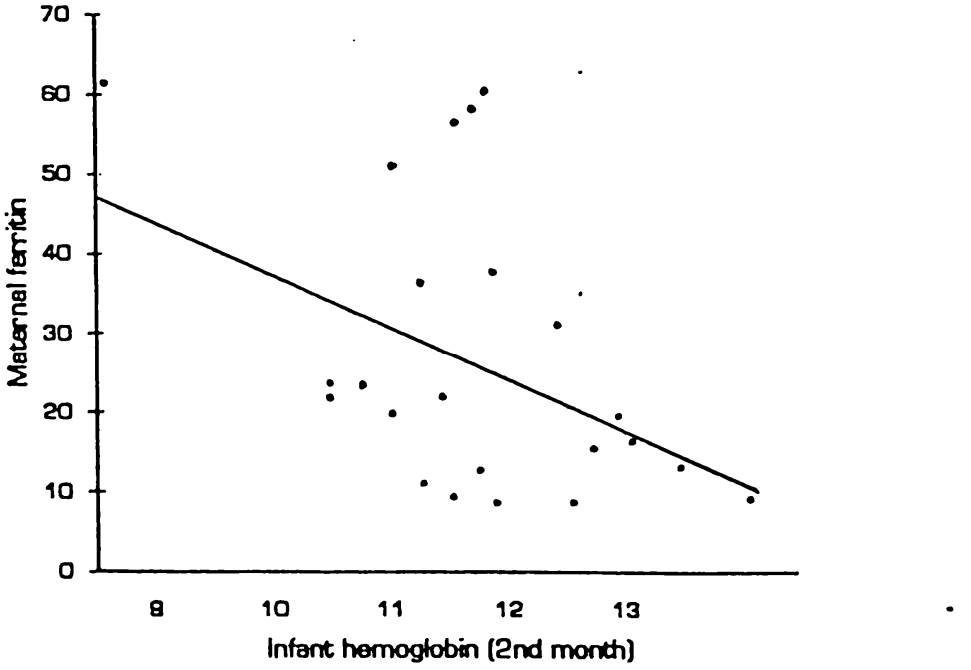

Fig. 1: Correlation between maternal ferritin levels at birth and hemoglobin concentrations of infants at two months of age.

Discussion

Iron is transported from mother to fetus against a concentration gradient; stored iron can be estimated from serum ferritin levels in infants as well as adults[6, 7].

Some studies have suggested that advancing gestation was associated with an increased SF concentration in neonates[8]. Others have found no correlation with gestational age or birth weight[9]. In the present study, the SF values of the mothers of term infants were significantly lower, and small premature newborns (group I) had the lowest SF levels. These results indicate that the accumulation of fetal iron stores and depletion of maternal iron stores continues during the last few weeks of pregnancy. A number of investigations have failed to demonstrate a relation between maternal and neonatal SF values[3, 4, 8]. On the contrary, Milman et al[1]. found a positive correlation. In our study, similar to Milman's report, there were positive correlations between maternal and neonatal ferritin levels in groups II and III. This indicates that moderately preterm and full-term infants' iron reserves are dependent on maternal iron stores.

Most of the studies have been inconclusive regarding the effect of maternal iron depletion on fetal ferritin levels. Kelly et al[9]. found significantly lower concentrations of ferritin in neonates when the maternal ferritin was less than 10 ng/ml. In other studies there were no significant differences between the SF levels of infants born to mothers with low and normal SF levels[10]. Serum ferritin levels less than 12 ng/ml are considered to represent iron-deficiency[3]. In the present study, we found no difference between the SF levels of neonates born to mothers with depleted or adequate iron stores (Table II). Thus, the presence of low maternal iron stores at the end of pregnancy does not necessarily signify reduced iron transfer to the fetus.

Jansson et al[8]. found no correlation between maternal and neonatal SF levels, whether the infants were born at or before term. In other studies a positive correlation has been found in term infants[1, 11]. In this study, the results of 15 mothers whose SF levels had been depleted did not correlate with those of their preterm or term babies. However, the SF levels of 61 mothers with adequate iron stores correlated positively with their term infants, except in preterm infants. The correlation of maternal and neonatal SF levels in term infants was similar to that cited in some previous reports[1, 11].

Iron deficiency and hemodilution are the most important factors causing anemia in pregnancy[1, 10, 12]. In our study group, low hemoglobin levels (< 10 g/dl) associated with normal SF levels were found in 14.4 percent of the mothers (11/76). The MCV value was greater than 95 fl in one of these mothers. This patient may have megaloblastic anemia while the remainder may have dilutional anemia.

The incidences of clinical (hemoglobin < 10 g/dl and SF < 12 ng/ml) and subclinical iron deficiencies (hemoglobin > 10 g/dl and SF < 12 ng/ml) have been reported as six to 12.9 percent and 15.4 to 30 percent, respectively[13, 14]. These parameters were found in 11.8 percent of (9/76) our cases respectively. Nine of 15 mothers with low SF levels had normal hemoglobin concentrations. This indicates that hemoglobin itself is not a reliable parameter for detecting iron-deficiency anemia in pregnant women, and the SF assay should be performed[13].

Preterm infants are particularly susceptible to anemia, owing to shortened survival of the fetal red cells, inadequate erythropoietic response and insufficient iron stores[15, 16]. The lowest values are found in infants of the shortest gestation[16]. In our study, however, there were no significant differences in hemoglobin, MCV and SF levels between preterm and term infants at two months of age. This may be due to the absence of small preterm infants in our group. In the present study, 38.4 percent of preterm (10/26) and 16.6 percent of term infants (4/24) had hemoglobin concentrations of less than 10 g/dl. However, only one of these infants had an SF level of less than 12 ng/ml. This result indicates that in the second month, iron deficiency is not an important cause of anemia.

No relationship has been found between the predelivery SF level the infant's SF level at six weeks of age[2]. On the other hand, hemoglobin concentrations have been found to be inversely correlated with SF values in term infants[2]. In our study there was a negative correlation between maternal SF levels and the hemoglobin concentrations of term infants at two months (Fig. 1). This correlation was not found in preterm infants. These results may be explained by the absorption of more iron from mothers by term infants, resulting in decreased SF levels of term infants' mothers at the end of pregnancy.

In conclusion, it appears that iron-deficiency anemia proven with SF levels at two months of age is not an important problem for term infants or for preterm infants born after 34 weeks of gestation.

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THE RELATIONSHIP BETWEEN APGAR SCORE AND UMBILICAL ARTERIAL BLOOD GAS VALUES IN NEWBORNS*

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SUMMARY: Energin M, Karakelleoğlu C, Orbak Z, Alp H, Selimoğlu MA, Ersoy M. (Department of Pediatrics, Atatürk University Faculty of Medicine, Erzurum, Turkey). The relationship between Apgar score and umbilical arterial blood gas values in newborns. Turk J Pediatr 1996; 38: 447-457.

The data for this report were derived from 61 newborns and their mothers. Of the newborns 45.9 percent had one-minute Apgar scores of less than seven, and 54.1 percent had Apgar scores of seven or greater. The five-minute Apgar score was less than 7 in 6.6 percent of cases.

While 23 percent of newborns had pH values of less than 7.20, 77 percent had pH of 7.20 or greater. Only 39.3 percent of the 28 newborns with one-minute Apgar score of less than 7 and 75 percent of the four newborns with five minute Apgar scores of less than 7 had pH values less than 7.20. Of 33 newborns, 9.1 percent who had Apgar scores of seven or more had pH of less than 7.20. We determined the sensitivity of the one minute Apgar score in acidemia to be 78 percent, the specifity to be 63 percent, the positive predictive value as 39 percent, and the negative predictive value as 90 percent. The one minute score is very poor for detecting acidosis when present but is rarely misleading when acidosis is absent.

There was a positive correlation between the Apgar score and pH ($p < 0.001$). The best correlation with umbilical artery pH values was observed with base excess (-BE) values ($p < 0.001$). Severe acidosis ($pH < 7.11$) was detected in eight cases. As ΔpH increases, pH, pO_2 and HCO_3 decrease and pCO_2 increases. Of 11 infants with $\Delta pH \geq 0.20$, 63.6 percent were sick infants and only one (9%) had normal Apgar scores. *Key words: Apgar score, umbilical cord blood gas, newborn, acidemia.*

The Apgar score was originally devised as a rapid and reproducible method for evaluation of an infant's condition at birth[1]. The Apgar score is not a specific indicator of birth asphyxia. In fact, newborn depression has many diverse causes, including maternal drug ingestion, trauma, neonatal manipulation, congenital anomaly, prenatal fetal insults, prematurity and, finally, birth asphyxia, which is a relatively minor contributor. Most authorities agree that it is extremely important to obtain umbilical cord blood pH samples in the presence of newborn depression since it will rule out birth asphyxia in approximately 80 percent of cases[2].

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In this study we aimed to demonstrate the relationship between Apgar scores and umbilical arterial blood gas values and to determine the importance of these values in fetal distress and asphyxia.

Material and Methods

The data for this report were derived from 61 newborns and their mothers in whom clinical characteristics were prospectively documented, and fetal and maternal blood gas and acid-base assessments were carried out at delivery.

The gestational ages of newborns ranged from 38 to 41 (39.3 ± 1.2) completed weeks with birth weights of 3022 ± 541 g. All newborns were appropriate for gestational age.

Blood gas and acid-base assessment of umbilical arterial blood was performed on blood drawn from a clamped segment of the umbilical cord obtained immediately after delivery. Maternal blood samples were taken from the antecubital vein.

All samples were obtained in the delivery room with pre-heparinized, 3-ml, plastic, sterile syringes with attached 1.5-inch, 22-gauge needles to obtain a consistent preparation and concentration of heparin. The analysis was performed within 15 minutes of delivery, using a blood gas analyzer (NOVA STAT PROFILE 1988) that measured pH, pCO_2, pO_2 and base deficit, hemoglobin (HB), hematocrit (Hct) and ΔpH calculated as maternal pH-umbilical arterial pH.

Apgar scores were determined by a pediatrician during the first and fifth minutes using a chronometer after vaginal delivery. In our study group, perinatal risk factors were as follows: meconium aspiration, cord prolapsus, preeclampsia-eclampsia, urinary tract infection, diabetes mellitus and smoking during pregnancy. The sick infants with meconium aspiration, two with cord prolapsus, and two with eclamptic and preeclamptic mothers. Only one newborn among the sick infants needed resuscitation, and the correlation between resuscitation and blood gasses could not been investigated.

Univariate analyses were performed with the student's t test. Data were analyzed with the unpaired student's test and linear regression analysis.

Results

Of 61 newborns, 28 had one-minute Apgar scores of less than seven, representing 45.9 percent of the births. Thirty-three newborns (54.1%) had Apgar scores of seven or greater. Umbilical arterial gas determinations of those groups are shown in Table I. There was no statistical difference between the gestational ages of these groups. The five-minute Apgar score was less than seven in four cases

(6.6%). While 14 (23%) of 61 newborns had pH values of less than 7.20, 47 (77%) had a pH of 7.20 or greater. The other parameters were also decreased, and the mean Apgar score was lower in the acidemic group than in the nonacidemic group. The values are displayed in Table II.

Table I: Umbilical Arterial Acid-Base Values and Mean Values of Hemoglobin, Hematocrit and ΔpH in Newborns with One-Minute Apgar Scores < 7 and > 7

Parameters	Apgar < 7 (n = 28)	Apgar ≥ 7 (n = 33)	p
pH	7.190 ± 0.132	7.287 ± 0.60	< 0.001
pCO_2	49.05 ± 7.64	45.14 ± 4.67	< 0.01
pO_2	15.86 ± 4.09	18.69 ± 3.34	< 0.001
HCO_3	17.44 ± 4.28	19.11 ± 3.10	< 0.05
BE	-9 ± 6.20	-5.32 ± 3.03	< 0.001
Hb	17.60 ± 1.58	16.60 ± 1.24	< 0.001
Hct	52.38 ± 5.45	49.49 ± 3.59	< 0.01
ΔpH	0.18 ± 0.09	0.13 ± 0.04	< 0.001

BE: Base excess. Hb: Hemoglobin. Hct: Hematocrit.

Table II: Mean Umbilical Arterial Acid-Base Values, Hemoglobin, Hematocrit and Apgar Scores in Newborns with Arterial pH ≥ 7.20 and < 7.20

Parameters	pH ≥ 7.20 (n = 47)	pH < 7.20 (n = 14)	p
pCO_2	44.72 ± 4.61	54.38 ± 6.35	< 0.001
pO_2	18.52 ± 3.41	13.62 ± 3.25	< 0.001
HCO_3	19.27 ± 2.98	15.25 ± 4.48	< 0.001
BE	-5.01 ± 2.29	-13.85 ± 5.97	< 0.001
Hb	16.75 ± 1.34	18.11 ± 1.49	< 0.001
Hct	49.72 ± 4.19	54.51 ± 4.67	< 0.001
ΔpH	0.12 ± 0.04	0.24 ± 0.10	< 0.001
One-minute Apgar score	6.7 ± 1.1	5.3 ± 2.1	< 0.01
Five-minute Apgar score	8.1 ± 1.6	7.8 ± 1.8	< 0.01

Only 39.3 percent (11 cases) of 28 newborns with one-minute Apgar scores of less than seven and 75 percent (3 cases) of four newborns with five-minute Apgar scores of less than seven had pH values of less than 7.20. Of 33 cases, 9.1 percent (3 cases) who had Apgar scores of seven or more had pHs of less than 7.20. There was a positive correlation between the Apgar score and pH ($p < 0.001$). This positive correlation with one-minute Apgar score of less than seven is shown in Figure 1, and the positive correlation in the group with umbilical arterial pH of less than 7.20 is shown in Figure 2.

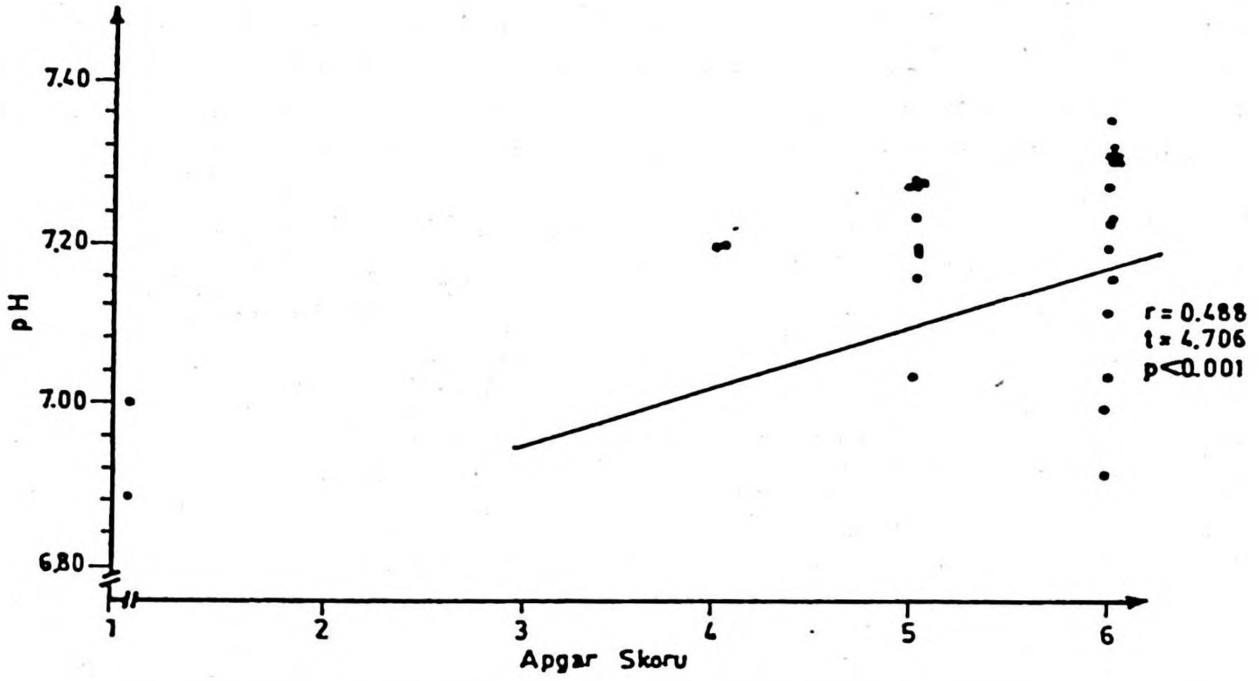

Fig. 1: The positive correlation between Apgar score and pH in newborns with one minute Apgar score < 7.

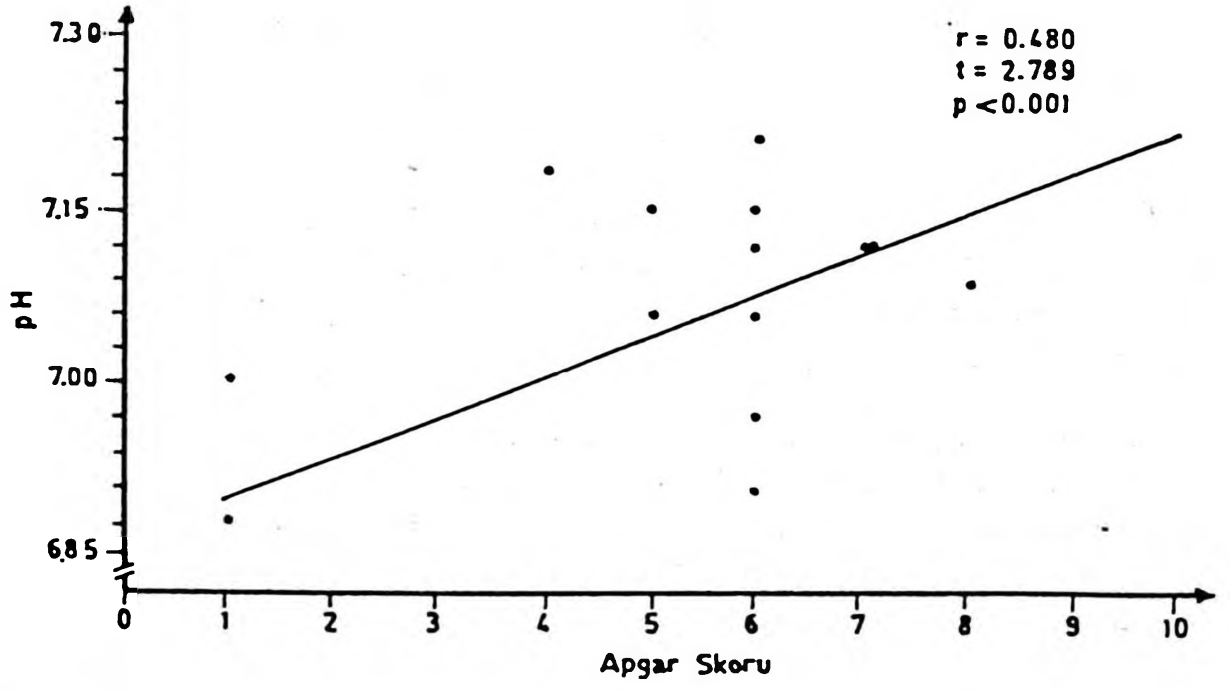

Fig. 2. The positive correlation between one minute Apgar score and pH in newborns with umbilical arterial pH < 7.20.

The best correlation with umbilical artery pH values was observed with base excess (-BE) values ($p < 0.001$). This correlation is shown in Figures 3 and 4.

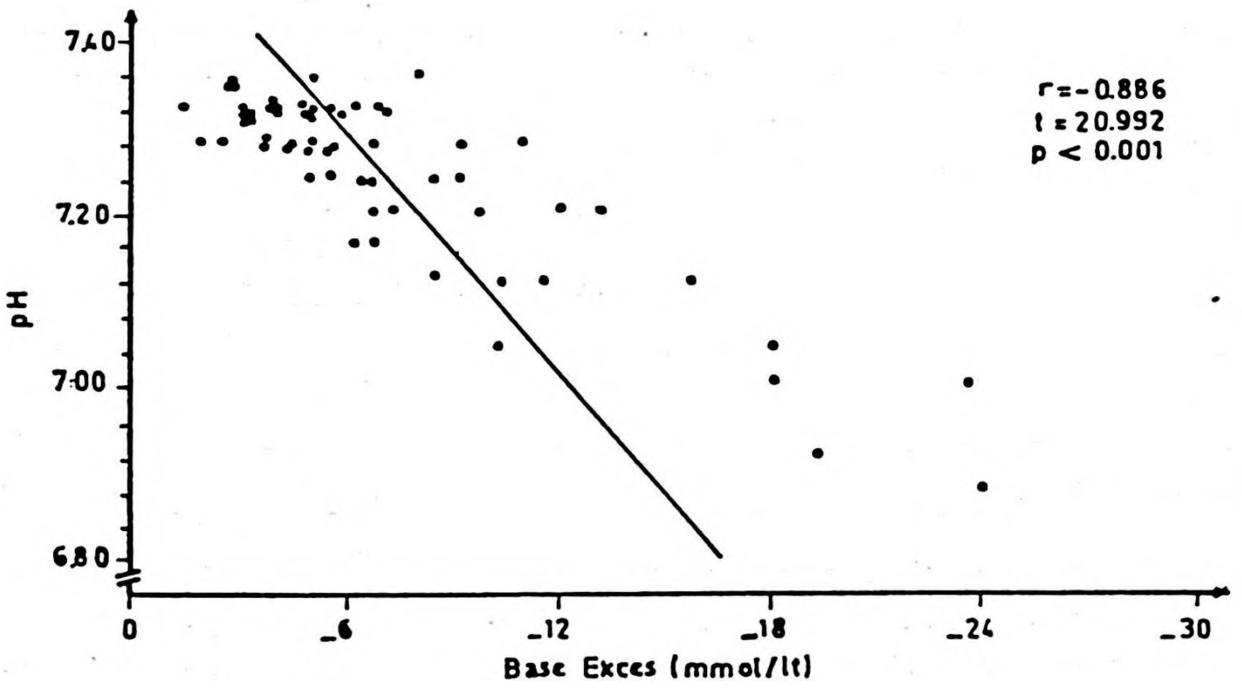

Fig. 3: The negative correlation between BE and pH in newborns with one minute Aapgar score < 7.

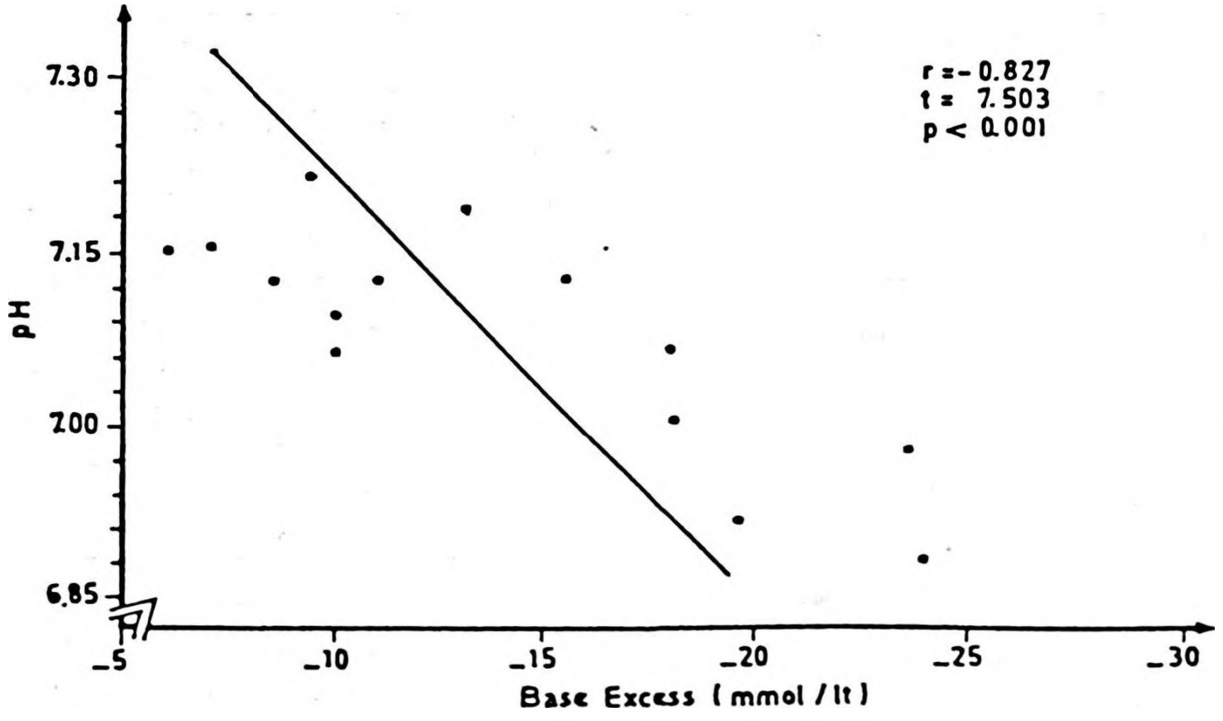

Fig. 4: The negative correlation between BE and pH in newborns with umbilical arterial pH < 7.20.

Severe acidosis (pH < 7.11) was detected in eight cases, as shown in Table III. The other parameters were observed to be correlated with the decreasing pH values.

Table III: Blood Gas Values, Mean ΔpH and Apgar Score of Newborns with Umbilical Arterial pH ≥ 7.11 and pH < 7.11

Parameters	pH ≥ 7.11 (n = 53)	pH < 7.11 (n = 8)	p
pCO_2	45.65 ± 5.46	55.42 ± 6.46	< 0.001
pO_2	17.88 ± 3.69	14.20 ± 4.26	< 0.01
HCO_3	19.25 ± 2.90	12.35 ± 3.29	< 0.001
BE	-5.48 ± 2.66	-17.36 ± 5.33	< 0.001
ΔpH	0.13 ± 0.04	0.27 ± 0.12	< 0.001
One-minute Apgar score	6.6 ± 1.2	5.0 ± 2.6	< 0.05
Five-minute Apgar score	7.9 ± 1.8	7.4 ± 2.2	< 0.05

According to ΔpH values, newborns were divided into three groups as less than 0.15, 0.15 to 0.19 and 0.20 or greater ΔpH. The arterial blood gas values and Apgar scores of these groups are shown in Table IV, and their statistical comparisons in Table V.

Table IV: Mean Values of Umbilical Arterial Acid-Base Values, Hemoglobin, Hematocrit and One-Minute Apgar Scores in Newborns with ΔpH < 0.15(A), ΔpH 0.15-0.19(B), ΔpH ≥ 0.20(C) and their Statistical Differences

Parameters	ΔpH < 0.15[a] (n = 40)	ΔpH: 0.15-0.19[b] (n = 10)	ΔpH ≥ 0.20[c] (n = 11)	a-b p	a-c p	b-c p
pH	7.29 ± 0.04	7.20 ± 0.09	7.08 ± 0.12	< 0.001	< 0.001	< 0.001
pCO_2	44.7 ± 5.44	49.22 ± 6.27	53.0 ± 5.86	< 0.01	< 0.001	> 0.05
pO_2	18.71 ± 3.54	16.20 ± 3.16	13.67 ± 3.41	< 0.01	< 0.001	< 0.05
HCO_3	19.12 ± 3.11	18.61 ± 4.03	15.26 ± 4.37	> 0.05	< 0.001	< 0.05
BE	-4.92 ± 2.11	-7.83 ± 5.20	-14.05 ± 6.36	< 0.05	< 0.001	< 0.05
HB	16.72 ± 1.43	17.45 ± 1.10	17.94 ± 1.62	< 0.05	< 0.01	> 0.05
Hct	49.64 ± 4.51	52.13 ± 3.37	53.93 ± 5.13	< 0.05	< 0.001	> 0.05
One minute Apgar score	6.8 ± 1.1	6.7 ± 0.8	4.6 ± 2.0	> 0.05	< 0.001	< 0.001
Five minute Apgar score	8.2 ± 1.3	7.6 ± 1.1	5.7 ± 1.9	< 0.05	< 0.001	< 0.001

As ΔpH increases, pH, pO_2 and HCO_3 decrease and pCO_2 increases. The negative correlation between ΔpH and umbilical artery pH ($p < 0.001$) and the negative correlation between one-minute Apgar scores and ΔpH are shown in Figures 5 and 6. Of the 11 infants (63.6%) with ΔpH ≥ 0.207, seven were sick infants and only one (9%) had normal Apgar scores.

Table V: Comparison of Acid-Base Values, Apgar Scores, ΔpH Values Between Groups with Perinatal Risk Factors and without Risk Factors

Parameters	With perinatal risk factor (n = 28)	Without perinatal risk factors (n = 33)	p
pH	7.201 ± 0.136	7.278 ± 0.067	< 0.001
pCO_2	48.13 ± 7.42	45.92 ± 5.45	> 0.05
PO_2	16.38 ± 4.33	18.25 ± 3.41	< 0.05
HCO_3	17.09 ± 4.35	19.41 ± 2.80	< 0.01
BE	-9.22 ± 6.43	-5.15 ± 2.40	< 0.001
ΔpH	0.18 ± 0.09	0.13 ± 0.04	< 0.001
One-minute Apgar score	5.9 ± 1.8	6.8 ± 1.0	< 0.01
Five-minute Apgar score	6.8 ± 1.7	8.2 ± 1.5	< 0.001

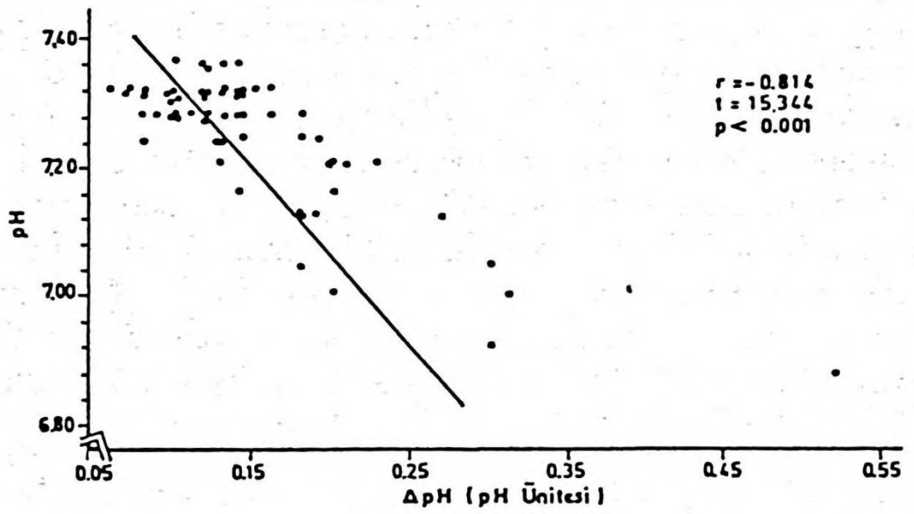

Fig. 5: The negative correlation between ΔpH and umbilical arterial pH.

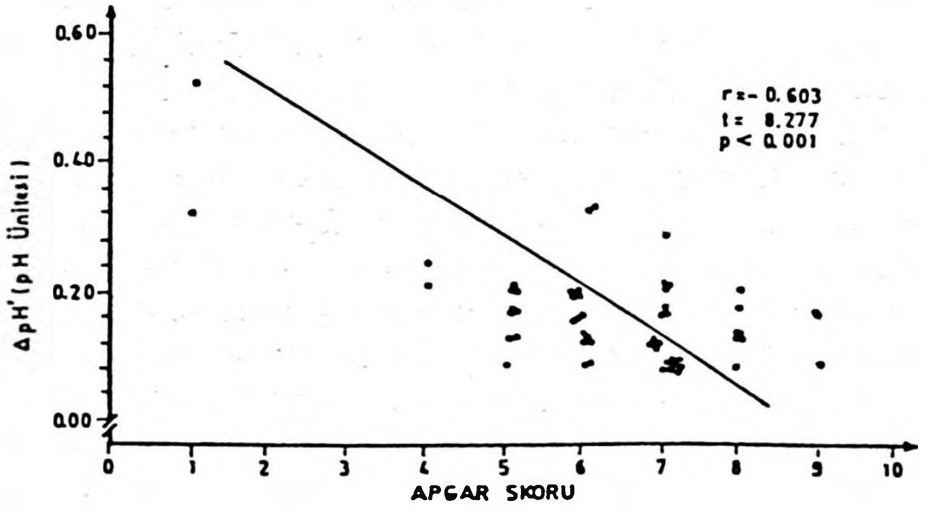

Fig. 6: The negative correlation between one minute Apgar score and ΔpH.

Discussion

The Apgar scores assigned at one and five minutes of age have long been used to define perinatal asphyxia, serve as a reference for other diagnostic tests, and attempt to prognosticate neonatal and later outcomes. Nevertheless, it has been well established that low Apgar scores are not synonymous with hypoxia, acidosis, or asphyxia[3]. Gestational age, maternal medications, infection, congenital disorders affecting the neuromuscular system, and neonatal cardiopulmonary condition at birth are but a few of the factors that can interfere with scoring. Improper scoring and inconsistency in scoring between observers must also be considered when interpreting Apgar scores[4].

Umbilical blood acid-base studies are objective measurements that can quantify the extent of fetal acidosis. The determination supplies information supplemental to the Apgar score in the evaluation of the neonate's condition[5]. Cord blood-gas measurements, obtained at the time of delivery in the case of a significant degree of metabolic acidosis, can indicate hypoxemia[5]. The absence of acidosis can exclude asphyxia in the presence of low Apgar scores and effectively shift the physician's attention to the identification of other possible causes of newborn depression. Preview data in the literature suggest that cord arterial pH is the most accurate reflection of fetal and newborn status when compared with all other cord arterial and venous blood-gas measurements[6,7]. The umbilical artery rather than vein has been used because the arterial values reflect tissue acid-base status while umbilical venous values reflect placental tissue acid-base status[8].

Many authors have demonstrated that cord arterial pH correlates best with Apgar scores[7,9-11]. We also detected a significant positive correlation between the first-minute Apgar scores and arterial pH values ($p < 0.001$) (Figs. 1, 2).

Sykes et al.[7] reviewed the relationship between Apgar score at one minute and cord arterial pH of less than or greater than 7.10. Only 27 percent of infants with a pH of less than 7.10 had one minute Apgar scores of less than seven (i.e., the Apgar score failed to identify severe acidosis in 73% of cases). On the other hand, only 21 percent of those with Apgar scores of less than seven at one minute had severe acidosis, and only 19 percent of infants with a five minute score of less than seven had severe acidosis. Boehm et al.[12] and Page et al.[10] reported the sensitivity of the one-minute Apgar score to be 78 and 46 percent, specificity 75 and 84 percent, positive predictive value 40 and 38 percent, and negative predictive value 94 and 88 percent, respectively. In our study, 28 newborns (45.9%) had one-minute Apgar scores of less than seven. pH values of less than 7.20 were detected in 14 (23%), and only 39.3 percent (11 cases) of 28 cases with Apgar scores of less than 7 had pH of less than 7.20 and in 9.1 percent (3 cases) of 33 cases who had Apgar scores greater had than seven

a pH of 7.20 or more. We determined the sensitivity of the Apgar score in acidemia to be 78 percent, the specificity to be 63 percent, the positive predictive value as 39 percent, and the negative predictive value as 90 percent. The one-minute score is very poor at detecting acidosis when present but is rarely misleading when acidosis is absent[13].

Cord, acid-base studies cannot distinguish whether fetal acidosis or alkalosis is the direct result of primary, fetal, pathologic conditions or the indirect result of maternal acid-base disorders. Maternal alkalosis secondary to hyperventilation can cause fetal hypocapnia and a rise in pH, which might obscure concurrent fetal metabolic acidosis[13].

The normal range of buffer base is 46-49 mEq/L. By definition, in nonpregnant women the normal base deficit or base excess equals 0 mEq/L. Actual calculations show the normal range of base excess to be -20 ± 1.0 mEq/L. During pregnancy, the mother hyperventilates and decreases her plasma bicarbonate but keeps her plasma pH constant. She also has a relative anemia and hypoproteinemia. In this setting, buffer base is depleted, creating a base deficit of 2-3 mEq/L. This can also be expressed as a base excess of -2 to -3 mEq/L[13].

In the presence of respiratory alkalosis, mild metabolic acidosis in an asphyxiated infant would not be detectable using cord pH measurements alone. If complete cord gas studies are obtained, metabolic acidosis would be apparent by the base deficit value. A less common acid-base disorder seen in laboring patients is metabolic acidosis resulting from the physical exertion of labor exacerbated by dehydration, infection or hypoglycemia. Fixed acids in the mother cross the placenta relatively slowly but can result in a harmless metabolic acidosis in the fetus in the absence of asphyxia. Fetal acidosis caused by the transfer of maternal fixed acids is usually characterized by minimal fetal base deficit[14]. A metabolic acidosis results in excess acid and a decreased buffer base. This is expressed as a negative base deficit or a base excess.

Gordon and Johnson[15] found the best correlation to pH with base excess. Goldenberg et al.[16] also stated that BE and HCO_3 are more important criteria for determining fetal acidemia than pH. Miller et al.[17] found a correlation between Apgar score, pH and BE. Silverman et al.[6] reported that a BE value of 10 mmol/L is more important than the Apgar score. Sykes et al.[7] determined severe acidemia (pH < 7.11) in newborns who had BE values greater than 12 mmol/L. We determined the best correlation to pH with -BE vlaues ($p < 0.001$), as shown in Figures 3, 4. Six of eight newborns who had BE of greater than 12 mmol/L (Table VI) had severe acidemia.

Another measure would be to determine the difference between maternal and fetal pH (usually measured on umbilical arterial blood). It has been reported that a difference in maternal-fetal pH of less than 0.15 units indicated maternal acidosis as the cause of fetal acidosis[18].

Table VI: Mean Acid-Base Values, Hemoglobin, Hematocrit ΔpH and One-Minute Apgar Scores in Sick and Healthy Newborns

Parameters	Sick newborns (n = 8)	Healthy newborns (n = 53)	p
pH	7.089 ± 0.025	7.266 ± 0.089	< 0.001
pCO_2	52.92 ± 5.73	46.03 ± 6.12	< 0.001
PO_2	13.37 ± 3.90	18.00 ± 3.60	< 0.001
HCO_3	14.312 ± 4.52	18.95 ± 3.25	< 0.001
BE	-14.93 ± 6.85	-5.85 ± 3.53	< 0.001
ΔpH	0.26 ± 0.11	0.14 ± 0.05	< 0.001
One-minute Apgar score	4.1 ± 2.2	6.7 ± 1.0	< 0.001
Five-minute Apgar score	5.4 ± 2.1	8.3 ± 1.4	< 0.001

Some authors have declared that ΔpH value is one of the most important criteria in the evaluation of the newborn at birth and estimating the neurological development in case maternal alkalosis is not present[18]. Rooth et al.[18] determined the mean ΔpH value in a group of newborns and their mothers without risk factors as 0.10 U, and as 0.15 U in the group with risk factors.

In our study the mean ΔpH was 0.15 ± 0.07 U. It was 0.18 ± 0.09 U in the group with risk factors and 0.13 ± 0.04 U in the normal group, and the statistical difference was significant ($p < 0.001$). The negative correlation between and umbilical artery pH ($p < 0.001$), and the negative correlation between the one-minute Apgar score and ΔpH are shown in Figures 5 and 6. The difference of 0.03 U in ΔpH between our study and the study of Rooth et al.[18] may be due to the applied anesthesia and analgesia to the mothers in their group to avoid hyperventilation during labor.

When we accept acidemia as ΔpH of greater than 0.20 (the same as Rooth et al.[18]), only one (9%) of 11 newborns with ΔpH values greater than 0.20 had one-minute Apgar scores of seven or greater. Seven of eight newborns in the sick group (87.5%) had ΔpH of 0.20 or greater while 62.5 percent of the eight sick newborns had pH of less than 7.20. Seven percent had one-minute Apgar scores of less than seven, and 87.5 percent had ΔpH values of 0.20 or greater. ΔpH values are more reliable than umbilical arterial pH and Apgar scores.

We conclude that in the evaluation of newborns and sick infants using Apgar scores and acid-base values in umbilical arterial blood (especially pH and base excess), evaluating the ΔpH by determining mothers' pH values is an appropriate method.

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The Turkish Journal of Pediatrics 1996; 38: 459-466

BLADE ATRIAL SEPTOSTOMY: EXPERIENCE WITH 18 PATIENTS*

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*Süheyla Özkutlu MD****, *Semra Özer MD***

SUMMARY: Çeliker A, Bilgiç A, Alehan D, Özkutlu S, Özer S. (Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Blade atrial septostomy: experience with 18 patients. Turk J Pediatr 1996; 38: 459-466.

Blade atrial septostomy was performed in 18 patients, including 14 with transposition of the great arteries, three with tricuspid atresia and one with severe mitral stenosis and ventricular septal defect. The patients' ages ranged from 25 to 210 days (mean: 108 ± 64 days), and weights ranged from 3.0 to 6.0 kg. Following blade septostomy, partial arterial pressure of oxygen (PaO_2) increased from an average of 22 ± 3.3 mmHg to 38.9 ± 7.6 mmHg ($p < 0.001$), and prompt clinical improvement was observed in the majority of patients. Significantly decreased atrial pressures (from 8.16 ± 6.1 to 3.93 ± 2.2 mmHg, $p < 0.01$) and significantly enlarged inter-atrial openings (from 1.6 ± 0.9 to 7.5 ± 1.8 mm, $p < 0.001$) were achieved with successful blade atrial septostomy. Blade atrial septostomy is an effective and life-saving procedure in patients who require nonrestrictive inter-atrial communication. *Key words: blade atrial septostomy, cardiac catheterization.*

An adequate inter-atrial opening is essential for survival in certain varieties of congenital heart diseases. Transposition of the great arteries, tricuspid atresia, mitral atresia, total anomalous pulmonary venous retum, pulmonary atresia with an intact interventricular septum, and double-outlet right ventricle with a restrictive ventricular septal defect are the main congenital heart diseases where inter-atrial mixing is obligatory or beneficial[1]. Atrial septostomy with a retractable knife blade was first performed by Parks et al.[2,3] to create an atrial septal defect in patients in whom balloon atrial septostomy was not possible or adequate. Later the clinical use and efficacy of blade atrial septostomy was established in various studies[4,5]. The aim of this retrospective study was to review the indications and results of blade atrial septostomy.

Material and Methods

The medical records of patients with blade septostomy performed between September 1989 and February 1994 were reviewed. The decision for blade septostomy was made according to echocardiographic findings (buldging of the inter-atrial septum, inadequate or no inter-atrial opening).

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The procedure was performed by cut-don technique in six patients, and by percutaneous technique through the femoral vein in 12 patients. The patients were sedated with premedication and given local anesthesia before cardiac catheterization. After catheterization and angiocardiography, a long sheath was introduced into the left atrium using a guide wire and a regular NIH catheter.

When the sheath and the regular catheter were in the left atrium, the catheter was removed, leaving the tip of the sheath in the left atrium. Then the blade septostomy catheter was passed through the sheath into the left atrium. The blade component was opened in the left atrium and the catheter was pulled into the right atrium. These procedures were repeated at various angulations to ensure an adequate inter-atrial opening. After blade septostomy, balloon atrial septostomy was performed as described previously, to enlarge the created inter-atrial opening[6]. After the procedure, a control echocardiographic study was performed to measure the dimensions of the created inter-atrial opening. Students' paired t test was used for statistical analysis.

Results

Blade atrial septostomy was performed in 18 patients (9 male and 9 female). The mean age of the patients was 108 ± 64 days, with a range of 25-210 days, and the mean weight was 4.4 ± 1.0 kg with a range of 3.0 to 6.0 kg.

Patients with transposition of the great arteries (TGA) comprised the largest group that underwent blade atrial septostomy (14 patients). Of the TGA patients, seven had no associated cardiac lesion while the other seven did. Among the latter, five patients had a small ventricular septal defect (VSD), one patient had a small atrial septal defect and one had pulmonary artery stenosis. Three cases had tricuspid atresia. Two of them had associated VSD, while the third had complex cardiac anomalies (dextrocardia, situs inversus totalis, large VSD, malposition of the great arteries, infundibular pulmonary stenosis, and absence of the right pulmonary artery). The last patient had parachute mitral valve, severe mitral stenosis and VSD (Table I).

Successful blade atrial septostomy and clinical improvement were achieved in 16 patients. Control echocardiographic study revealed a significantly enlarged inter-atrial opening. Before the procedure, seven patients had restrictive inter-atrial openings of two to three milimeters in diameter, while the remaining had patent foramen ovale. After the procedure, the inter-atrial openings were enlarged significantly to a diameter of 7.5 ± 1.8 mm ($p < 0.001$, Fig. 1). In Figure 2 an adequate inter-atrial opening achieved after blade atrial septostomy is demonstrated.

Table I: Some Clinical and Hemodynamic Parameters of the Patients

Patient	Cardiac Lesion	Age (Days)	MAP (mm Hg) LA (B)	MAP (mm Hg) LA (A)	IAO (mm) (B)	IAO (mm) (A)	PaO_2 (mm Hg) (B)	PaO_2 (mm Hg) (A)
1	TGA	210	6	4	1	6	24	47
2	TGA	30	18	5	3	4	29	42
3	TGA, VSD	75	14	10	3	8	19	28
4	TGA	38	NR	NR	2	8	24	42
5	TGA, VSD, PH	90	7	3	1	3	21	32
6	TGA, VSD	25	10	6	2	5	26	48
7	TGA	210	9	6	2	9	22	42
8	TGA, VSD, PS	75	4	4	1	7	22	40
9	TGA	60	1	1	1	NR	NR	NR
10	TGA	60	NR	NR	2	7	20	34
11	TGA	45	6	3	1	5	18	18
12	TGA, VSD, PH	120	4	2	1	8	18	24
13	TGA, ASD, PH	120	16	5	4	9	26	38
14	TGA, PS	120	2	2	2	7	20	42
15	Mitral stenosis, VSD	180	20	5	1	10	NR	NR
16	Tricuspid atresia, VSD	180	6*	2*	1	9	22	38
17	Tricuspid atresia, VSD	90	6*	3*	1	8	21	38
18	Tricuspid atresia, Complex cardiac anomaly	210	14*	6*	2	8	18	28

ASD: Atrial septal defect.
(B) : Before septostomy.
LA : Left atrium.
NR : Not recorded.
PDA: Patent ductus arteriosus.
PS : Pulmonary artery stenosis.
VSD: Ventricular septal defect.
(A) : After septostomy.
IAO : Inter-atrial opening.
MAP : Mean atrial pressure.
PaO_2: Partial pressure of arterial oxygen,
PH : Pulmonary hypertension.
TGA : Transposition of the great arteries.
* : Right atrial pressure.

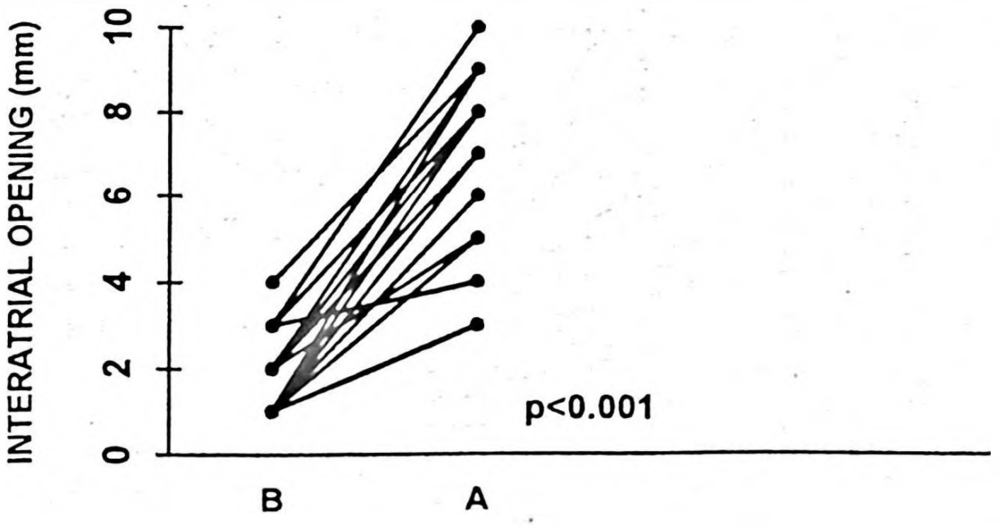
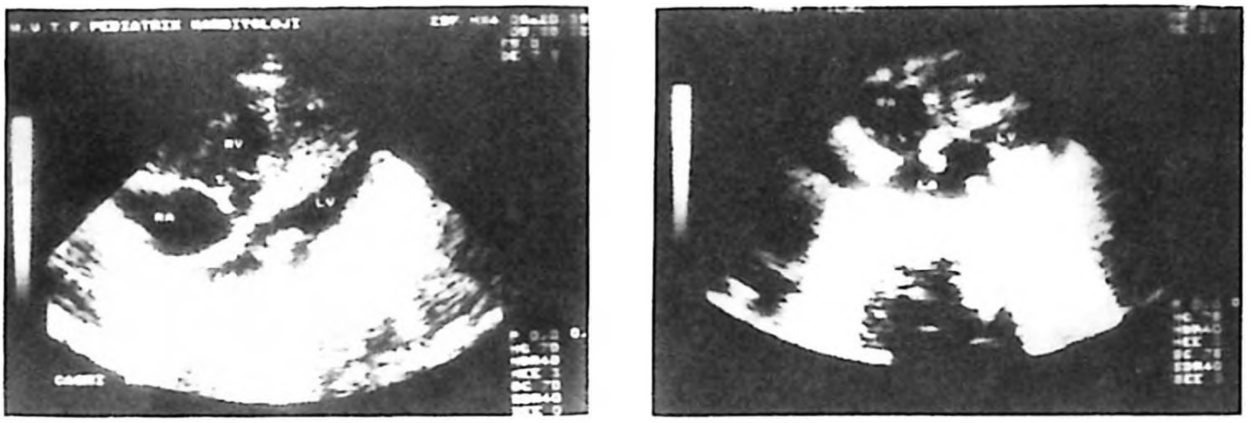


Fig. 1: Changes in the diameter of the inter-atrial opening before and after blade atrial septostomy. B: Before septostomy, A: After septostomy.



(A) (B)

Fig. 2: Two-dimensional echocardiogram demonstrating an intact inter-atrial septum (A). After the blade atrial septostomy procedure, an adequate inter-atrial opening was obtained (B). LA: left atrium, LV: left ventricle, M: mitral valve, RA: right atrium, RV: right ventricle, T: tricuspid valve, Arrow: interatrial opening.

Parallel to the echocardiographic findings, in the TGA and mitral stenosis group, the mean left atrial pressure decreased significantly from 9.0 ± 5 mmHg to 4.2 ± 2.2 mmHg ($p < 0.001$, Fig. 3). Similarly, in patients with tricuspid atresia, the right atrium pressure was reduced from 8.6 mmHg to 3.6 mmHg ($p < 0.05$).

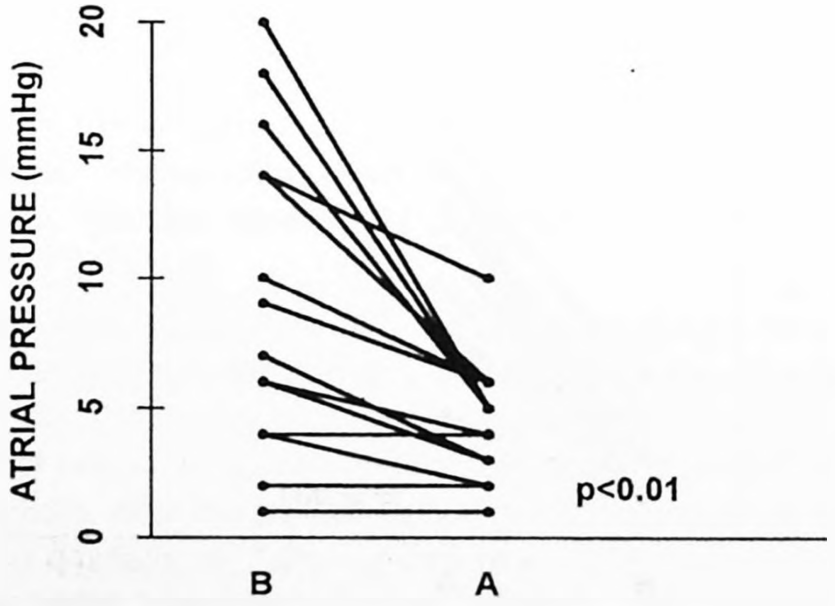


Fig. 3: Changes in the left atrial pressure before and after blade septostomy in patients with transposition of the great arteries or mitral stenosis. B: Before septostomy, A: After septostomy.

The partial arterial pressure of oxygen (PaO_2) is a valuable parameter that reflects the efficacy of blade atrial septostomy. In patients with TGA, a significant increase in PaO_2 was observed following septostomy owing to good interatrial mixing (from 22 ± 3.2 mmHg to 36.3 ± 8.3 mmHg, $p < 0.001$, Fig. 4).

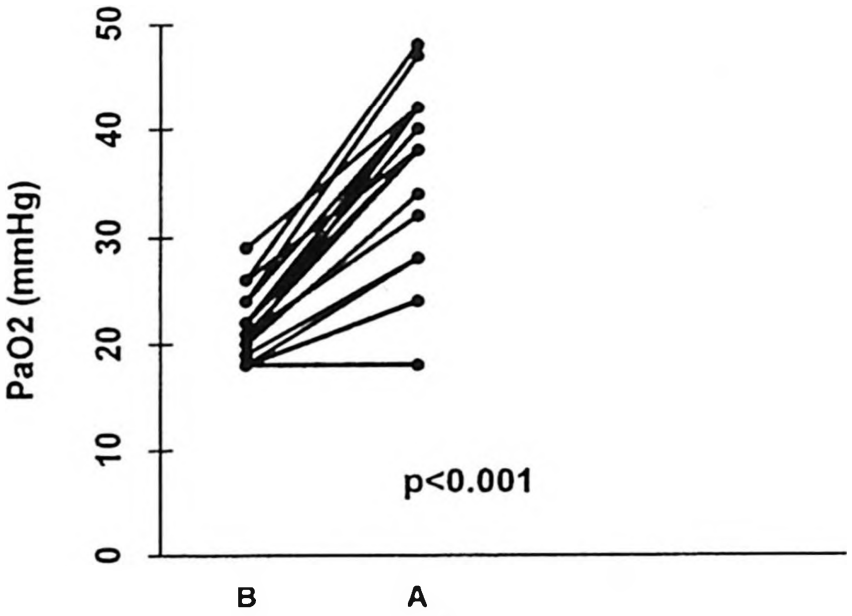


Fig. 4: Changes in the partial arterial pressure of oxygen (PaO_2) before and after blade atrial septostomy. B: Before septostomy, A: After septostomy.

Complications: There were two early deaths after the procedure. One was a seven-month-old male with TGA. During septostomy he became severely acidotic and bardycardic, and in spite of intensive care he died 24 hours after the procedure. The other patient had TGA, VSD and severe pulmonary hypertension, and the procedure was terminated because of severe bradycardia. Although he had bradycardia during the procedure, his heart rate increased to normal levels after septostomy, and subsequent echocardiography revealed an inter-atrial opening of three milimeters. He died seven days after the procedure before an operation could be performed. Therefore we believe that his death might not be directly related to blade septostomy but could be attributed to severe pulmonary hypertension.

Transient rhythm problems were the most frequently observed complications. Among them bradycardia was observed in three patients and ventricular fibrillation in one patient. One of the patients with bradycardia had an additional respiratory arrest for a brief period. One patient had rectal bleeding, which was presumed to be related to hypoxemia and necrotizing enterocolitis and was managed successfully.

Follow-up: Four of the patients lost contact with us. The remaining 12 patients were followed up for four to 48 months (mean: 13.9 ± 10 months). Among them four patients had a complete repair (Senning operation). One patient with tricuspid

atresia had palliative surgery (modified Blalock-Taussig shunt). Closure of VSD was performed in the patient who had parachute mitral valve, mitral stenosis and VSD. Pulmonary banding was performed in one patient who had pulmonary hypertension in addition to TGA and VSD. One patient with TGA died seven months after septostomy due to pulmonary infection and respiratory failure. Another patient with TGA died one month after septostomy. Three patients have not yet had surgery.

Discussion

During recent years, improved noninvasive diagnostic techniques have lessened the indications for diagnostic cardiac catheterization, while therapeutic catheter procedures are being used increasingly. Nowadays, the interventional workload is about 15 percent of total catheter procedures in larger pediatric cardiology centers, compared with only one to two percent only five years ago[7-9]. Balloon atrial septostomy, described in 1966 by Rashking and Miller[10], was the first clinically useful catheter-directed therapy. It has improved the survival rate of patients with TGA from 20 to more than 85 percent. Blade atrial septostomy was developed by Park et al.[2] in 1975 to supplement balloon septostomy in patients in whom the septum was too thick to be torn by balloon septostomy alone. Blade septostomy is generally considered to be appropriate for patients who are more than four to six weeks old and who require nonrestrictive inter-atrial communication.

Blade atrial septostomy has been shown to be effective in various reports[4, 5, 11, 12]. Equalization or reduction of the pressure gradient between the two atria, enlargement of the inter-atrial opening and an increase in systemic oxygen saturation are the most important indicators of success in the septostomy procedure[13]. In our study, all three of the criteria were met. Significant clinical improvement was noted in all of our patients. Patients with TGA and mitral stenosis showed a significant increase in PaO_2 and a significant decrease in left atrial pressure in response to blade atrial septostomy. Post-septostomy echocardiographic studies revealed a significantly enlarged inter-atrial opening. Although an inter-atrial opening larger than 10 mm was regarded as a criterion for improvement, we obtained favorable results in our patients with an inter-atrial opening of 7.5 milimeters. Our results show that while evaluating a patient and the success of the procedure, all the parameters (PaO_2, atrial pressures, clinical findings, size of the inter-atrial opening) should be taken into consideration, and that an opening of less than 10 mm may also be effective.

Balloon atrial septostomy is unlikely to provide long-term palliation, however blade septostomy may be very effective in providing adequate long-term palliation. In the collaborative study of Park et al.[13] excellent long-term results were obtained in 84 percent of patients with blade septostomy. Ali-Khan et al.[14] also reported

repeat septostomy procedure in only four of the 45 patients. Similarly, in our patients, long-term follow-up results were very good, and none of our patients underwent a repeat septostomy. However, it should be kept in mind that during the follow-up period these patients may show clinical deterioration and a repeat procedure may be required.

Our study has shown that blade atrial septostomy is a relatively safe procedure. Rhythm problems were the most frequently encountered problems that have not been stressed before. Most of the complications observed in our patients were transient and improved without any permanent sequelae. However, patients who undergo blade septostomy should be observed carefully, since most of them are clinically unstable and potentially prone to serious complications, as reported in various reports.

Recently, in a preliminary report a new approach was presented for creating inter-atrial communication in older infants by using a stationary fixed-diameter angioplasty balloon[15]. However, for the moment, blade atrial septostomy is the treatment of choice for creating an inter-atrial opening in older infants, since the safety and effectiveness of this new approach have yet to be established.

In conclusion, blade atrial septostomy is a relatively safe, effective and life-saving procedure in patients who require nonrestrictive inter-atrial communication.

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RADIOFREQUENCY CATHETER ABLATION OF ACCESSORY PATHWAYS AND MODIFICATION OF ATRIOVENTRICULAR NODE IN CHILDREN AND ADOLESCENTS*

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SUMMARY: Çeliker A, Brugada P. (Arrhythmia Unit, O.L.V. Hospital Cardiovascular Center, Aalst, Belgium). Radiofrequency catheter ablation of accessory pathways and modification of atrioventricular node in children and adolescents. Turk J Pediatr 1996; 38: 467-475.

Catheter ablation of an accessory pathway and atrioventricular node modification using 550 kHz radiofrequency was attempted in 23 children and adolescents between five and 19 years of age (mean = 15.7 years). Fifteen children had accessory-pathway-mediated tachycardia and eight had atrioventricular node reentrant tachycardia.

Accessory pathways were present in ten children. Two patients had associated congenital heart disease. Symptoms included disabling palpitations and episodes of syncope. Ablation was attempted from the right and left sides of the heart. Single-catheter technique was used in seven patients. Eleven of the 15 patients with accessory pathways were treated completely. Two patients had recurrences, and one of them died after the arrhythmia surgery. There were two failures. Two patients with incomplete interruption of the accessory pathways were followed up clinically. There were three cases with temporary systemic embolization and one with severe pain related to radiofrequency energy application.

Atrioventricular node modification was done by fast-pathway ablation in six, and by slow-pathway ablation in two patients. Two patients with fast-pathway ablation had recurrences of clinical arrhythmia, and one of them underwent a successful second session. There were no complications associated with the procedure in this group of patients.

Radiofrequency catheter application was initially successful in 21 (84%) out of 25 procedures, and ultimately curative in 16 (69%) out of 23 patients. There were some serious complications which resolved in the immediate post-procedure period. Radiofrequency catheter ablation appears to be a safe and successful method for the management of supraventricular tachycardia secondary to accessory pathways or atrioventricular node reentrant tachycardia in children and adolescents. *Key words: radiofrequency catheter ablation, supraventricular tachycardia, children.*

Supraventricular tachycardia is the most common sustained arrhythmia in children, and is usually associated with accessory-pathway-mediated tachycardia or atrioventricular (AV) node reentrant tachycardia[1]. These tachycardias are associated with significant symptoms, and medical therapy may be ineffective in some cases. Catheter ablation with high-voltage, direct-current shock has been used for ablation of accessory pathways and modification of the AV node in

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children[2-5]. However, direct-current shock ablation results in explosive gas formation and generation of a shock wave. Since 1987, transcatheter application of radiofrequency energy has been used in adults to manage these arrhythmias[6,7]. The absence of hazardous effects of direct-current shock makes the radiofrequency catheter ablation technique particularly encouraging for use in children. There have been a few reports of the use of this technique in children[8-12]. This report describes our initial experience with the use of radiofrequency catheter ablation in the management of children and adolescents with sustained symptomatic accessory-pathway-mediated tachyçardia and AV node reentrant tachycardia.

Material and Methods

Patient Selection : All patients were referred to our center (O.L.V. Hospital Cardiovascular Center, Arrhythmia Unit) for evaluation and management of frequent supraventricular tachycardia episodes, After an initial evaluation that included history, physical examination, electrocardiogram (ECG) and in some cases 24-hour ambulatory ECG, exercise testing, and echocardiography, eight patients underwent a standard electrophysiologic study; the mechanism of tachycardia was confirmed in these cases before the ablation procedure. Two patients had Ebstein's anomaly of the tricuspid valve, and one of them had been operated on for a previous right-lateral accessory pathway.

Patients who would have otherwise been incurable by antiarrhythmia drugs became candidates for radiofrequency catheter ablation. The criteria were as follows:

1. An accessory pathway or AV node reentrant tachycardia was present in a patient with frequent symptomatic episodes of supraventricular tachycardia which occurred despite treatment with several antiarrhythmic drugs.
2. Antiarrhythmic drugs partially or completely controlled the supraventricular tachycardia but were associated with side effects.
3. The patient or parents did not want to use antiarryhythmic drugs because of the possibility of long-term drug dependency. Twenty-three patients met these criteria and underwent the catheter ablation procedure. The patients or their parents gave written consent for the catheter ablation intervention.

RF Catheter Ablation Technique

Radiofrequency energy was supplied by a radiofrequency energy generator system (Hat 200, Dr. Osypka GMBH, Germany) to obtain a continuous unmodulated current at 550 kHz. Energy was applied between the distal pole of a 7F steerable quadripolar electrode catheter with a 4 mm distal electrode (Mansfield Webster, USA), and a large surface area skin electrode was placed over the left scapula. All patients received anticoagulant therapy using 100 U/kg (maximum 5000 U) of intravenous heparin.

Accessory Pathway Ablation : The accessory pathway location was classified according to the electrophysiologic study, the pattern of the delta wave in sinus rhythm and retrograde atrial activation polarity during a supraventricular tachycardia attack. In patients with left-sided accessory pathways, the ablation catheter was introduced from the femoral artery retrograde to the left ventricle and positioned against the AV ring. When this approach failed, the left atrial approach was used. In patients with a posteroseptal accessory pathway, the ablation catheter was advanced from the right femoral vein and positioned around the coronary sinus ostium. In patients with a right lateral accessory pathway, the ablation catheter was inserted from the right femoral vein and positioned in the right atrium.

The ablation catheter was positioned to record both atrial and ventricular electrograms and the shortest AV interval during the sinus rhythm in patients with manifest Wolff-Parkinson-White syndrome (WPW), and the shortest ventriculoatrial interval during orthodromic supraventricular tachycardia or during ventricular pacing in patients with a concealed accessory pathway. We tried to obtain accessory pathway potentials and continuous electrical activity before the ablation. Radiofrequency energy was applied at a power output of 30-40 W for 30 seconds. After the ablation, ventricular stimulation was then performed to evaluate the absence of ventriculo atrial conduction. Also, atrial stimulation was carried out in order to check whether tachycardia was still inducible.

AV Nodal Modification : A 7F steerable quadripolar catheter was advanced to record bipolar electrograms in the vicinity of the AV node prior to radiofrequency application. First the catheter was placed across the tricuspid annulus to record the His bundle potential (HBP) and was slowly withdrawn so that a larger atrial deflection was recorded. The target sites have an atrial/ventricular ratio greater than one and a small (< 100 mV) or absent HBP. Radiofrequency energy was used until the atrium-to-His interval was prolonged by approximately 50 percent when a non-conducting P-wave was seen. Ablation of the slow AV nodal pathway occurred when the RFA catheter was placed superiorly to the site of recording of a HBP, while ablation of the fast AV nodal pathway was seen when locating the catheter inferior to the His.

Results

Twenty-five procedures were performed in 23 patients between five and 19 years of age (mean = 15.7, median = 16 years, Tables I, II). Fifteen patients had an accessory pathway, and eight had AV node reentrant tachycardia. Radiofrequency catheter ablation was initially successful in 21 (84%) of the 25 procedures and ultimately curative in 16 (69%) of 23 patients.

Table I: Clinical, Electrophysiologic and Radiofrequency Ablation Data of Patients with Accessory Pathway-Mediated Tachycardia

						RFA		Shock			Follow-up			
Patient	Sex	Age (Years)	Symptoms (Years)	Drug	Diagnosis	RET (Min.)	PD (Min.)	N	Power (Watts)	Complications	EPS	Drug	Months	Results
1.	F	15	P (2)	S	LPS, AS, LL Manifest	134	360	–	–	None	–	S	8	Sæ
2.	M	16	P, Pre (2)	V, S	Para His# Manifest	–	–	24	–	None	–	–	1	S
3.	M	5	P (5)	D, P	LL*@ Pro	33 Concealed	70	6	–	None	+	–	–	R
4.	M	16	P (7)	D, S, A	LL* Concealed	67	240	38	–	Embolization	+	–	4	S
5.*	F	18	P, Pre (14)	A, Pr Pro	LL Manifest	19.4	45	2	40	Embolization	–	–	–	S
6.*	M	17	Pro P, Pre	Pro	ParaHis Manifest	14	45	1	25	None	–	–	–	S
7.	M	15	P (1)	S	LL Concealed	65.6	160	30	30	None	–	–	1	Sæ
8.*	M	15	P (2)	S	LL Manifest	89.2	210	60	23	None	–	S	1	F
9.*	M	15	P (2)	None	LL Manifest	24.4	55	10	32	Embolization	–	–	–	S
10.	F	15	P (1)	Pro	LPS, LP Concealed	26.5	105	6	30	None	–	–	1	S
11.	F	15	P (1)	None	LL Manifest	2.7	30	2	35	None	–	–	2	S
12.	F	19	P (6)	Pr, Pro A	RL Manifest	6.4	15	2	35	None	–	–	1	R
13.*	M	16	P* (3)	None	AS Manifest	86	175	–	–	Pain	–	S	–	F
14.*	M	18	P, Pre (2)	None	LL Manifest	9.6	30	6	35	None	–	–	–	S
15.	M	15	P (2)	S	LL Concealed	18.1	40	6	35	None	–	–	–	S

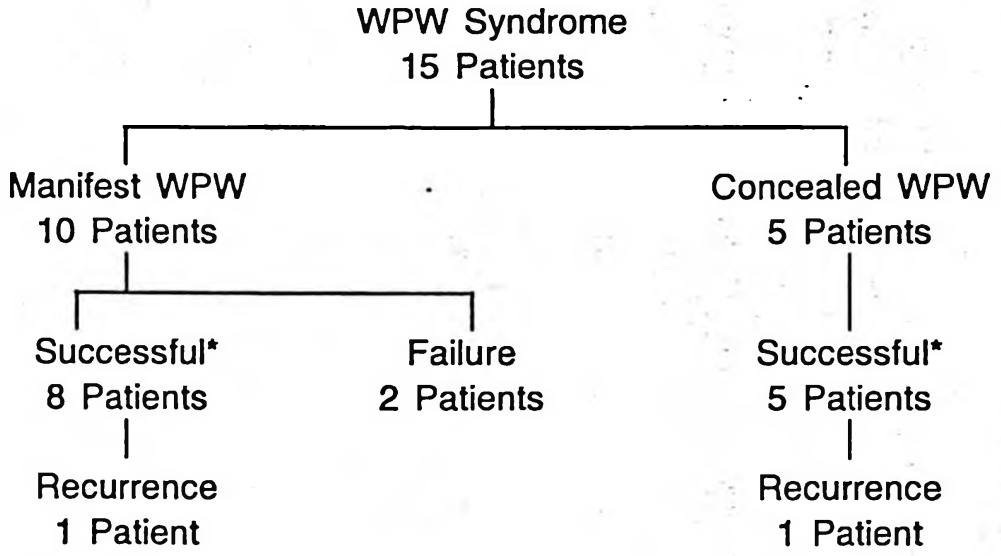
RFA: radiofrequency application, RET: radiation exposure time, PD: procedure duration, Min: minutes, N: number of shocks, EPS: electrophysiologic study, F: female, M: male, P: palpitation, Pre: presyncope, S: sotalol, V: verapamil, A: amiodarone, Pr: propranolol, Pro: propafenone, D: digoxin, LPS: left posteroseptal, AS: anteroseptal, LL: left-lateral, #: associated with AV node reentrant tachycardia, *: Ebstein's anomaly of tricuspid valve, @: second ablation session, LP: left posterior, RL: right-lateral, S: successful, æ: partially successful, F: failure, R: recurrence.

Table II: Clinical, Electrophysiologic and Radiofrequency Ablation Data of Patients with AV-Node Reentrant Tachycardia

						RFA		Shock			Follow-up				
Patient	Sex	Age (Years)	Symptoms (Years)	Previous Intervention	Drug	RET (Min.)	PD (Min.)	N	Power (watts)	Ablation Site	Complications	EPS	Drug	Months	Results
1.	F	18	P, Pre (6)	EPS	Pr	20.6	75	3	–	F PW	None	–	–	7	No arrhythmia
2.	M	13	Syn (3)	–	Pr	6.8	45	2	30	S PW	None	–	–	6	No arrhythmia
3.	F	15	P (4)	EPS	D, S	–	–	1	32	S PW	None	–	–	7	No arrhythmia
4.	F	18	P, Pre (2)	–	S	20	75	3	–	F PW	None	–	–	3	No arrhythmia
5.	F	17	P (3)	EPS	D, Pr	–	–	3	30	F PW	None	–	–	5	Recurrence
6.	M	18	P (5)	–	–	–	45	3	31	F PW	None	–	–	2	No arrhythmin
7.	F	17	P	EPS	S, Pr	16.3	65	9	30	F PW	None	–	–	3	Recurrence
8.	F	18	P (1)	–	S	32	90	26	32	F PW	None	–	–	2	No arrhythmin

Syn: syncope, F PW: fast-pathway, S PW: slow-pathway, #: second ablation session, RFA: radiofrequency application, RET: radiation exposure time, PD: procedure duration, N: number of shocks, EPS: electrophysiologic study, F: female, M: male, P: palpitation, Pre: pesyncope, Pr: propranolol, D: digoxin, S: sotalol.

Accessory pathway ablation (patients 1-15, Table I). Fifteen children and adolescents (11 boys, 4 girls) between five and 19 years of age (mean 15 years) underwent radiofrequency catheter ablation of accessory pathways. Wolff-Parkinson-White (WPW) syndrome was present in 10 children, while the other five were found to have a concealed accessory pathway capable of only retrograde conduction. All procedures required a median of 10 applications of radiofrequency energy (range 1 to 38) at an application output power of 25 to 40 W (mean = 33 W). The mean duration of procedures was 100 min (range 15 to 360 min) and the mean radiation exposure time 35 min (range 2.7 to 134 min). One patient had two, and one patient had three accessory pathways. One patient also had AV-node reentrant tachycardia. The single catheter technique was used in five patients with left lateral AP, and one patient with parahisian AP with manifest WPW. Thirteen of the 15 procedures were successful (Fig. 1). In seven successful procedures in patients with manifest preexcitation, the delta wave disappeared. Immediate restudy showed the absence of preexcitation with atrial stimulation and the absence of ventriculoatrial conduction.



*: one case partially successful.

Fig. 1: RF ablation results in patients with WPW syndrome.

Eleven patients had absent ventriculoatrial conduction, and supraventricular tachycardia was no longer inducible. There were two partially successful procedures and two failures. The single catheter technique failed in these patients. Three patients developed signs of systemic embolization and one patient experienced severe pain during the radiofrequency catheter ablation. The patients with systemic embolization recovered completely in the immediate post-ablation period. During follow-up, two patients had recurrences and one of them died

immediately following the successful surgical division of the left lateral accessory pathway. Follow-up electrophysiologic studies were performed in two patients who had successful procedures, and confirmed a recurrence in one of them. Ten patients were asymptomatic without medication after a follow-up interval of one to eight months. Six of the ten successfully treated patients who had preexcitation before the procedure had no preexcitation on follow-up ECG.

AV node modification (patients 16-23, Table II). Eight children and adolescents (two boys, six girls) between 13 and 18 years of age (mean 16 years) underwent radiofrequency catheter modification of the AV node. Four of the eight patients had electrophysiologic studies done before catheter modification of the AV node, showing anterograde dual AV node conduction curves in all of them. Six patients had fast-pathway and two slow-pathway ablation. These procedures required a median of three applications of radiofrequency energy (range 1-26) at an application power output of 30 to 32 W (mean = 30 W). The mean duration of procedures was 65 min (range 45-90 min) and the mean radiation exposure time was 19 min (range 6.8-32 min). There were no complications. Immediate testing after the procedure showed the absence of ventriculoatrial conduction, and this arrhythmia was no longer inducible. PR and AH intervals were prolonged in all patients who had fast-pathway ablation. During follow-up of these patients, two of them had recurrences, and the PR interval was shortened in these patients. One of them was managed with a second ablation session. Seven patients were asymptomatic without medication after a follow-up period of two to seven months (mean = 4.4 months).

Discussion

After initial procedures of catheter ablation of the AV junction in adults using direct-current shock, the same technique has been extended for use in patients with all types of supraventricular tachycardia. There is now wide experience with direct-current catheter ablation of the posteroseptal accessory pathway and more recently of accessory pathways in other locations. Direct-current shock has also been used to modify the AV node in patients with AV-node reentrant tachycardia. However, experience with direct-current shock ablation in children is limited[2-5]. Direct-current shocks of 50 to 250 J were used by Gillette et al.[5] for ablation of ectopic atrial tachycardia foci in four children, with success in two. Smith et al.[4] used shocks of 25 to 300 J delivered to the mouth of the coronary sinus in four children with a permanent form of junctional reciprocating tachycardia, achieving permanent ablation of the retrograde accessory pathway in only one. Bromberg et al.[3] reported on six children, eight to 18 years in age, with an accessory pathway who had undergone direct-current catheter ablation procedures. Only two patients were without signs of accessory pathway function

on follow-up, and three patients underwent surgical division of the accessory pathway. AV node modification using direct-current shock catheter ablation has not been reported in children. Also, the direct-current approach has many disadvantages, such as apparent lack of energy titration and the need for general anesthesia.

The application of radiofrequency energy is not associated with barotrauma. Its physiological efficacy results from resistive heating of cardiac tissue, and the lesions are found to be rather circumscript and small. Radiofrequency energy application can be controlled on the anatomical substrate and requires no general anesthesia[6,7]. Radiofrequency energy has now been applied successfully and safely in adults and children for AV-node modification of AV-node, reentrant tachycardia, and ablation accessory pathways located in various positions. Saul et al.[12] described the initial successful results in children with accessory-pathway-mediated tachycardias. Dick et al.[13] reported the success rate of radiofrequency catheter ablation of accessory pathways as 74 percent, and this rate was even higher in the left-sided accessory pathway in 10 of 13 children after a follow-up interval 1.25 to seven months. They also described successful modification of the AV node in children with AV-node reentrant tachycardia. Another study, performed by Schluter and Kuck[9], showed the effectiveness of the single-catheter technique in three children with left-sided accessory pathways. They also reported complete ablation of accessory pathways in various positions in 10 of 11 children in the same study.

In our study, complete ablation was achieved in nine of 15 children with accessory pathways. Two patients with partially successful results were followed without symptoms. Totally symptom-free lives were obtained by eleven patients.

Although AV-nodal reentry tachycardia is not as common in children as adults, it may cause severe problems in childhood. Slow-pathway ablation is the treatment of choice because of the low risk of permanent AV block. However, the technique for this approach is not easy, and the duration of the procedure and the number of radiofrequency applications needed is higher than in fast-pathway ablation[13]. Also, titration of radiofrequency energy and increased experience prevent inadvertent AV block. Van Hare et al.[8] reported high success and low complication rates with fast-pathway ablation. In one collaborative study the results were similar in success and complication rates to those with adults[11]. In our study group we observed two recurrences in the fast-pathway ablation group without AV conduction block. These results were consistent with previous reports. Fast-pathway ablation may be done by experienced centers with no complications and a reasonably low recurrence rate.

In conclusion, radiofrequency catheter ablation is becoming an acceptable and preferred method for the treatment of supraventricular tachycardia in children. The low risk of complications and high success rate compared to arrhythmia surgery and direct-current ablation demonstrate its safety and efficacy.

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PERIPHERAL AND NASAL EOSINOPHILIA AND SERUM TOTAL IMMUNOGLOBULIN E LEVELS IN CHILDREN WITH ASCARIASIS*

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SUMMARY: Yazıcıoğlu M, Öneş Ü, Yalçın I. (Allergy, Clinical Immunology and Infectious Disease Unit, Department of Pediatrics, İstanbul University Faculty of Medicine, Çapa-İstanbul, Turkey). Peripheral and nasal eosinophilia and serum total immunoglobulin E levels in children with ascariasis. Turk J Pediatr 1996; 38: 477-484.

In order to evaluate peripheral and nasal eosinophilia and serum total IgE levels in ascariasis, 30 children between two and 12 years of age whose fecal samples were found to contain Ascaris lumbricoides, and 30 children between 1.5 and 12 years of age who did not have ascaris in their fecal samples, were enrolled in the study. Peripheral eosinophil counts and serum total IgE levels were significantly higher in the study group, but there was no significant difference between the nasal eosinophil counts of the two groups. Since total serum IgE levels differ according to age, the patients and the control groups were also evaluated within three age-groups. Peripheral eosinophil counts and serum total IgE levels of patients with ascariasis in all age ranges were significantly higher, but nasal eosinophil values did not differ significantly. These results show that in ascariasis the presence of peripheral eosinophilia and high total serum IgE levels are similar to those in atopic diseases, and some allergic symptoms such as nasal itching can be caused by parasites. However, nasal itching does not correlate with nasal eosinophilia in ascariasis. This is an important feature in the differential diagnosis. *Key words: ascariasis, peripheral eosinophil counts, nasal eosinophil counts, total serum IgE levels, children.*

In diseases mediated by type 1 hypersensitivity reactions, the increase in serum total IgE levels and peripheral eosinophilia are well-known findings[1].

Parasitic infections are also associated with high IgE level and peripheral eosinophilia in children[1,2]. Thus parasitic infections are important to consider in the differential diagnosis of allergic diseases with eosinophilia[3]. In Turkey, ascariasis in particular is frequently seen[4]. Furthermore, ascaris infestations may also cause asthma symptoms. Allergic rhinitis symptoms usually accompany asthma. Nasal itching and eosinophilia help to diagnose allergic rhinitis. In children with ascariasis, itching of the nose is frequently seen[5-7]. Therefore, our study aimed to reveal the value of nasal eosinophilia in the differential diagnosis of ascariasis by investigating serum total IgE levels and peripheral and nasal eosinophil counts.

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Material and Methods

Thirty children who were seen at the Department of Pediatrics at İstanbul University Faculty of Medicine and whose fecal samples were found to contain Ascaris lumbricoides were enrolled in the study. There were 16 female and 14 male patients ranging in age from one to 12 years. Thirty children within the same age-groups but without ascaris in their fecal samples were accepted as the control group. The children and the families in the patient and control groups had no histories of allergic disease or infections.

The history, symptoms and physical findings of each patient were recorded, laboratory tests leukocyte counts and peripheral blood smears were done and eosinophil counts were calculated. In order to examine nasal cytology, a specimen of nasal secretions was transferred to a glass slide and stained with Hansel's stain. Eosinophil ratios were calculated by counting 100 cells under oil immersion. Total serum IgE concentrations were measured by Enzymoimmunoassay (Bio Merieux Total IgE kit, France).

Statistical analyses were done by using the chi-square test, Student's t-test and Mann-Whitney test.

Results

The data on age and sex and the results of peripheral and nasal eosinophil values, total serum IgE determinations and nasal symptoms of the patient and control gorups are displayed in Tables I and II. The mean (± SD) ages of the patient and control groups were 6.48 ± 3.3 and 5.4 ± 3.2 years, respectively. There was no significant difference in either age or sex distribution between the two groups (Table III).

Since total serum IgE levels differ according to age, the patient and control groups were evaluated within three different age-groups consisting of one to three, four to five, and six to 12-year-old children. In the study group, peripheral eosinophil counts and total serum IgE levels were found to increase with age, but only the total serum IgE levels of children between six and 12 years of age were found to be significantly higher.

Peripheral eosinophil numbers and serum total IgE levels of patients in all age ranges were higher than controls. The differences were significant for peripheral eosinophil counts for those between one and three ($0.001 < p < 0.01$), four to five ($p < 0.001$), and six to 12 years of age ($0.001 < p < 0.01$). When the serum total IgE levels of the study and control groups were compared by the Mann-Whitney test (one-tailed), significant differences were found in one to three, ($p = 0.0029$), four to five ($p = 0.04$) and six to 12-year-olds ($p = 0.0014$).

Table I: Characteristics of the Patient Group

Case no.	Age (years)	Sex	Peripheral eosinophilia (/mm^3)	Nasal eosinophilia (%)	Total serum IgE
1	10	M	704	0	235
2	3	M	162	0	34
3	4	F	364	0	54
4	9	M	420	1	250
5	9	M	434	0	143
6	11	M	395	6	120
7	9	M	534	0	128
8	3	M	432	0	57
9	2	F	384	0	74
10	4	F	660	2	260
11	10	M	528	0	151
12	2	F	496	2	136
13	5.5	F	426	0	74
14	3	F	584	1	175
15	5.5	F	572	2	98
16	2	F	288	0	162.5
17	2.5	F	364	0	129.5
18	7	F	700	1	725
19	4	M	336	0	48
20	10	M	250	7	53
21	7	F	320	0	125
22	11	F	576	0	116
23	11	F	308	0	30
24	10	F	352	0	172.5
25	9	M	376	0	160
26	12	M	270	0	117
27	5	M	248	2	121
28	3	M	68	0	33
29	5.5	F	240	5	95
30	5.5	F	460	0	52.5

Table II: Characteristics of the Control Group

Case no.	Age (years)	Sex	Peripheral eosinophilia (/mm^3)	Nasal eosinophilia (%)	Total serum IgE (IU/ml)
1	2.5	M	208	1	69
2	5	F	280	0	115
3	5	M	210	0	25
4	4	F	160	4	33
5	2.5	F	140	0	29.5
6	5	M	80	2	17
7	2.5	F	184	0	16
8	2	F	94	0	3
9	5	F	40	3	29.5
10	2	F	62	0	26
11	10	F	124	2	17
12	10	M	184	0	58
13	5.5	M	204	0	48
14	12	M	330	2	98
15	4	M	114	0	44
16	2	F	108	0	34
17	10	M	318	0	138
18	6	M	78	0	10
19	7	M	268	0	106
20	9	F	150	0	45
21	8	M	208	0	43
22	5.5	F	172	1	90
23	4	F	264	0	104
24	3	M	130	0	33
25	1	F	166	1	25
26	4	F	188	2	90
27	1.5	F	224	0	93
28	3	M	168	0	25
29	11	M	252	5	84
30	10	M	156	2	85

Table III: Comparison of Clinical and Laboratory Data in Patient and Control Groups

	Age (years) Mean ± SD (Range)	Sex F/M	Peripheral eosinophilia (/mm^3) Mean ± SD (Range)	Nasal eosinophilia (%) Mean ± SD (Range)	Total serum IgE (IU/ml) Mean ± SD (Range)	Nasal itching Number (%)
Patient group (n = 30)	6.48 ± 3.3 (2-12)	16/14	408.37 ± 153.69 (68-704)	0.97 ± 1.87 (0-7)	137.63 ± 127.11 (30-725)	11/30 (36.6%)
Control group (n = 30)	5.4 ± 3.2 (1.5-12)	15/15	175.47 ± 73.43 (40-330)	0.83 ± 1.34 (0-5)	54.4 ± 36.61 (3-138)	(–)
Statistical evaluation	t = 1.287 p = 0.203 NS	X^2 = 0.000 p = 1 NS	t = 7.489 p < 0.001	t = 0.33 p = 0.742 NS	u = 748.5 z = 4.413 p < 0.05	

Nasal eosinophil values were comparable in the two groups, and no significant difference was found when compared using Student's t-test ($p > 0.05$).

Nasal eosinophilia was investigated in cases with the symptom of nasal itching; the percentages of eosinophils were found to be five, six and seven percent in three of the cases. In these cases severe nasal itching was reported.

Discussion

Ascariasis has an important place in the differential diagnosis of allergic diseases presenting with eosinophilia and elevations of total serum IgE levels. Although many studies that investigated the relation between allergic diseases and ascariasis have been reported, many problems remain inconclusive[8-11].

Tullis[8] reported that 99 percent of this patients with bronchial asthma had one of the three intestinal parasites Ascaris lumbricoides, Strongyoides stercoralis or Necator americanus, and concluded that the worms were an important cause of asthma; others have not confirmed his findings, however[9].

It has been suggested that helminthic infections can suppress allergic reactions to environmental allergens, Turton[10] has proved this hypothesis by describing his self-infection with Necator americanus, which cured his seasonal hay fever. In another study, the skin-test reactivity to common inhalant allergens was found to be low in populations heavily parasitised with Ascaris lumbricoides; it was concluded, however, that other influences, such as racial, cultural, nutritional and environmental factors, also affect the expression of the atopic state in the tropical environment[11].

In a recent study, Lynch et al.[12] reported that helminthic infection does increase IgE synthesis and that this increase, when moderate, can enhance allergic reactivity; however, when it is excessive, it can actually be suppressive through both saturation of mast-cell FCE receptors and inhibition of specific IgE antibody synthesis.

Ascaris lumbricoides can cause diseases such as asthma, angioneurotic edema, wheezing and anaphylactic shock syndrome. Urticarial reactions, which are most prominent during pulmonary migration, have also been reported[13-15].

A symptom which is found in ascariasis and has similarities with allergic diseases is nasal itching. Although it is reported as a frequent symptom in ascariasis, no studies on this subject have yet been reported.

Since there are similarities between ascariasis and allergic diseases, it is important to establish the differences between these two diseases. In this study peripheral eosinophil counts were observed to be higher in the study than the control group. The serum total IgE levels in cases infected with Ascaris were found to be higher than the controls, but these levels did not exceed 260 IU/ml except in one case (Table I, Case no: 18).

Nasal itching was experienced by 36.6 percent of the patient gorup. When the cases with nasal itching were evaluated for nasal eosinophilia, only three cases (Table I, Cases no: 6, 20, 29) had values greater than normal. However, there were no significant statistical differences between the patient and control groups with respect to nasal eosinophilia. This result shows that nasal eosinophilia is not a prominent finding in children with ascariasis. In children with allergic rhinitis, nasal eosinophilia is more common. In our study nasal itching was not associated with nasal eosinophilia in children with ascariasis. On the other hand, nasal itching is seen in children with allergic rihinitis and correlates with nasal eosinophilia.

Allergic reactions associated with Ascaris infestations are reported to be mediated by reaginic antibody[16]. Aerosolization of the Ascaris antigen stimulates local IgE antibody production in the respiratory system, and this event is followed by the release of mediators and clinical symptoms. Ascaris-induced pneumonic reactions are the result of contact of the parasite and host tissues, which is a local effect induced during larval migration. Urticarial reactions are thought to be due to generalized hypersensitivty reactions which are associated with high IgE levels[13]. Ascaris-induced anaphylactic shock syndrome has been recognised and the finding of degranulated mast cells throughout the body tissues were ascribed to the release of a mast cell degranulator by the worms or a reagin-ascaris allergen interaction at the mast cell surface[14].

Nasal itching seen in ascariasis can be explained by the distant effects of parasites and immediate hypersensitivity reactions, but is not associated with nasal eosinophilia. Eosinophilic chemotactic factor A (ECF-A) released by the parasite when larvae migrate to the nose can induce eosinophil accumulation, but larval migration to the nose is well known to be rare. The reason we could not detect eosinophils in nasal secretions in ascariasis may be due to the fact that we did not examine the cases while the allergic symptoms were more significant, during the migration of the larvae, or we could not detect the brief period of tissue eosinophilia.

In conclusion, the presence of peripheral eosinophilia and high serum IgE levels in ascariasis are similar to that in atopic diseases, and some allergic symptoms such as nasal itching could be an effect of parasites. However, nasal itching is not correlated with nasal eosinophilia in ascariasis. This is an important feature in the differential diagnosis of ascariasis and allergic rhinitis.

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N – ACETYL – β – D – GLUCOSAMINIDASE EXCRETION IN PATIENTS WITH UNILATERAL RENAL AGENESIS OR NEPHRECTOMY IN CHILDHOOD*

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SUMMARY: Tekin N, Kural N, Uslu S. (Department of Pediatrics, Osmangazi University Faculty of Medicine, Eskişehir, Turkey). N-acetyl-β-D-glucosaminidase excretion in patients with unilateral agenesis or nephrectomy in childhood. Turk J Pediatr 1996; 38: 485-490.

Renal functional impairment due to the damaging effect of hyperfiltration of the remnant kidney was evaluated in patients who had been uninephrectomized in childhood or had unilateral agenesis. Sixteen patients (8 male, 8 female) a mean of 7.1 years (1-26 years) postnephrectomy were evaluated. Blood pressure values, clearances of creatinine (Ccr) and phosphorus (Cp), urine osmolality, total protein excretion and NAG (N-acetyl-beta-d-glucosaminidase) excretion were determined. Ccr values were slightly but not significantly lower (90.390 ± 11.223 ml/min) than those of control subjects (110.818 ± 7.755 ml/min). Daily urinary protein excretion adjusted for body surface was significantly higher in the study group ($p < 0.05$). Urinary NAG excretion measured with a spectrophotometric method and described as the Cr ratio was significantly higher in the patients than the control subjects ($p < 0.05$). It was concluded that the damaging effects of hyperperfusion not only involve the glomerulus but also the proximal and distal tubulus. *Key words: nephrectomy, proteinuria, NAG excretion.*

A person can have a solitary kidney congenitally or as a result of nephrectomy for renal disease. On the other hand, some people are renal donors for live transplantation and live with a single kidney for a long time. It has been shown in experimental studies that hyperperfusion of the remnant glomeruli leads eventually to the development of glomerulosclerosis in these patients. Most studies have focused on glomerular functions[1-4]; tubular functions of single-kidney patients have rarely been studied.

In this study, in order to assess the effect of uninephrectomy on both glomerular and tubular functions, we evaluated patients who had been nephrectomized in childhood or had unilateral renal agenesis. Besides clearance tests, the proximal tubulus was also evaluated with N-acetyl-β-D-glucosaminidase (NAG), which is a proteolytic enzyme located in the lysosomal part of the proximal tubulus epithelial cells; raised levels indicate renal tubular damage[5-8].

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Material and Methods

We reviewed the charts of patients who had undergone unilateral nephrectomy during childhood (before 18 years of age) and those with unilateral agenesis. At the time of the study, patients were between two and 31 years of age (median age 15.5 years). The follow-up period with a single kidney was 7.1 years (range 1-26 years). none of the nephrectomized patients had any sign, symptom or laboratory finding of renal functional impairment, hypertension or any systemic disorder causing deterioration in renal function at the time of nephrectomy. Sixteen patients fullfilled those criteria. Fourteen of them were nephrectomized and two had renal agenesis. Table I shows the indications for nephrectomy. At the time of this study, all of the subjects were in good health. Twenty-one control subjects (thirteen female, eight male) with no history of renal disease were also studied. The mean age of the control group was 12.8 years (range 7-18 years).

Table I: Indication for Nephrectomy

No.	Age	Sex	Years post-uninephrectomy	Reasons for nephrectomy
1	10	F	1.5	Left hypoplastic kidney
2	10	M	4	Right hydronephrosis
3	16	M	9	Right xantogranulomatous PN
4	9	M	5	Right kidney aplasia
5	11	M	7	Left xantogranulomatous PN
6	18	F	1	Chronic PN, Right hydronephrosis
7	8	M	8	Left hydronephrosis
8	17	F	7	Chronic PN, right atrophic kidney
9	23	M	12	Left xantogranulomatous PN
10	26	M	8	Chronic PN, right nephrolithiasis
11	2	F	2	Left total multicystic displasia
12	24	F	6	Traumatic hemorrhage
13	20	F	20	Agenesis of the left kidney
14	19	F	1	Multiple nephrolithiasis
15	5	F	5	Agenesis of the left kidney
16	31	M	26	Right hydronephrosis

The subjects greater than 18 years old were considered hypertensive if they had an average systolic blood pressure greater than 140 and/or diastolic greater than 90 mmHg. the prevalence of hypertension in the uninephrectomized group and control group among patients less than 18 years old was compared with the prevalence expected in the general population matched for age and sex.

Laboratory studies: A 24-hour urine sample was collected for the determination of clearances of creatinine and phosphorus (Ccr, P), urine osmolality, total protein

excretion and NAG excretion. Ccr and P were calculated and corrected for body surface area and expressed per 1.73 sqm. Fractional excretion of sodium (FENa), tubular reabsorption of phosphorus (TRP), osmolar clearance and free water clearance were also calculated.

Urinary NAG activity was determined spectrophotometrically[9]. The enzyme activity in each urine sample was expressed as the NAG U/mmol creatinine. Creatinine was measured by the Jaffe reaction[10]. Quantitative urine protein excretion was determined by precipitation with sulfosalicylic acid after clearing the urine of phosphate with acetic acid. The turbidity produced was measured against a reference standard on a spectrophotometer.

The statistical comparison between groups was made using the chi-square test.

Results

Blood pressure values were within normal limits in six patients older than 18 years and in 10 patients younger than 18 years of age. The data of the clearance studies are presented in Table II. The endogenous creatinine clearance values of the patients were slightly but not significantly lower (90.390 ± 11.223 ml/min) than those of control subjects (110.818 ± 7.755 ml/min). The FENa results were not significantly different between the patients with a single kidney and the control subjects ($p > 0.05$).

Table II: The Statistical Comparison of Laboratory Values of Nephrectomized Patients and the Control Group

	Single kidney		Control group			
	Mean	Standard deviation	Mean	Standard deviation	t	p
Ccr (ml/min)	90.390	11.223	110.818	7.755	-1.56	$p > 0.05$
FEna (% mg)	1.292	0.168	1.168	0.135	0.58	$p > 0.05$
TRP (% mg)	84.725	2.357	95.476	0.600	-5.77	$p < 0.001$
Cosm (ml/min)	2.268	0.232	3.188	0.261	-2.54	$p < 0.05$
Prot/Cr (urine)	0.601	0.115	0.285	0.042	3.10	$p < 0.01$
NAG/Cr (U/mmol)	238.433	45.125	133.157	21.537	2.40	$p < 0.05$

The mean phosphorus clearance value in patients with a single kidney was 11.150 ± 1.413, which was significantly higher that of the healthy control subjects (4.774 ± 0.646) (t: 4.67, $p < 0.001$).

Tubular reabsorption of phosphorus was also significantly different from control values (95.476 ± 0.600) in the patients with a single kidney (84.725 ± 2.357) (t: -5.77, $p < 0.001$). The mean osmolar clearance (Cosm) of the patients with one kidney was significantly lower than that of control subjects ($p < 0.05$). The free water clearance of the patients was -1.313 ± 0.208, which was significantly different from that of the control group ($p < 0.001$).

There was also a significant difference in daily protein excretion adjusted for body surface area between the two groups ($p < 0.05$). The ratio of urinary protein to urinary creatinine excretion also differed significantly between the two groups ($p < 0.01$). The mean value of NAG/Cr was 238.433 ± 133.157 in the study group, which was significantly higher than that of the control subjects ($p < 0.05$, Table II).

Discussion

Nephrectomy is performed under various conditions, even in childhood. Urologists and nephrologists routinely reassure patients that a single kidney is sufficient to meet the metabolic demands of the body without development of proteinuria, hypertension and renal insufficiency. However, clinical reports have documented development of proteinuria and hypertension as a result of focal glomerulosclerosis in renal transplant donors[3,4,11].

Terry Watnick et al.[1] found a higher prevalence of hypertension in donors (62%) compared with the expected prevalence adjusted for age, sex and race (42%). The prevalence of hypertension was reported to be higher in male donors as compared to pre-uninephrectomy values and to age-and sex-matched inpatient and outpatient control subjects. Renotropins, which were circulating factors detected in the nephrectomized patients, were suggested to be responsible for the development of hypertension[11].

Ingrid Wikstad et al.[12] studied renal functions and did not find hypertension in their group of patients who had been nephrectomized in childhood. Similar to that study, blood pressure measurements were within normal limits in all of our patients. Perhaps the high prevalence of hypertension among donor nephrectomy patients was due to their ages, as it is known that the incidence of hypertension increases with age. If the follow-up period of our group is longer [7.1 years (1-26)] the incidence of hypertension could be higher.

In most studies the creatinine clearance of the nephrectomized paitents has been within normal limits. In our patient group, the mean creatinine clearance was 81 percent of the control group, but the difference was not statistically significant as it was stated in the literature[13-16].

Following nephrectomy all parts of the nephron enlarge in compensatory hypertrophy; thus, changes involve not only the glomerulus but also the tubulus.

Reabsorbing and secreting salts and water, and concentrating and diluting the urine to maintain homeostasis of the body are the functions of the tubulus. Sodium, bicarbonate, phosphate, amino acids and glucose are reabsorbed primarily by the proximal tubule. In our patient group, while FENa values were normal, Cp and TRP, which show early pathologic changes in the proximal tubulus, were deteriorated; when compared with the control group, the differences were statistically significant.

Proteinuria, which is an early sign of the increase in glomerular permeability, has been detected in nephrectomized patients, and the rate of proteinuria correlates well with the duration of the postnephrectomy period[11, 14]. It has been noted that protein excretion in urine is not albumin, but the source of proteinuria is the impaired absorption of the tubular surface[12, 17]. To prove this, Tapson et al.[8] studied four enzymes [alanine aminopeptidase (AAP), alkaline phosphatase (Akp), NAG and LDH] with high molecular weights that would not permit urine to pass by way of glomerular filtration. If the high concentrations of these enzymes were detected in urine, increased metabolism of the proximal tubulus cells and the defective function of tubular reabsorption could be responsible for the protein excretion.

For this reason we determined NAG in urine, as it has a high molecular weight that cannot be filtrated through the glomerulus. Increased urinary NAG activities indicate renal tubular damage and reflect the activity of the disease[8, 19, 20]. In chronic renal disease, urinary tract obstruction, nephrotic syndrome, glomerulonephritis, rejection of the transplanted kidney, and renal tubular disorders due to the use of aminoglycosides, increased NAG activity in urine has been detected. In our study, statistically significantly high levels of NAG adjusted for Cr values pointed out the increased metabolism of proximal tubulus epithelial cells.

The mean value of NAG/Cr in the study group was significantly higher than that of control subjects. Given the similar results documented in the literature, we concluded that this can be accepted as an indicator of renal tubular damage, as previously suggested by Kunin et al.[5] the presence of normal Ccr values, significant proteinuria and high NAG/Cr values could be interpreted as deterioration in proximal tubular functions.

In summary, we evaluated 16 patients an average of 7.1 years following unilateral nephrectomy or with unilateral agenesis, we concluded that the expected deterioration in renal function, which we detected earlier than reported in the literature, suggests that the defect is mainly at the tubular level, and NAG could be used as an indicator of proximal tubular damage.

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SERUM ERYTHROPOIETIN AND GRANULOCYTE - MACROPHAGE - COLONY STIMULATING FACTOR LEVELS WITH MEGADOSE METHYLPREDNISOLONE TREATMENT*

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SUMMARY: Şaylı TR, Özsoylu Ş. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Serum erythropoietin and granulocyte-macrophage-colony stimulating factor levels with megadose methylprednisolone treatment. Turk J Pediatr 1996; 38: 491-495.

Serum erythropoietin (EPO) and granulocyte-macrophage-colony stimulating factor (GM-CSF) levels were determined in 12 children with acute and seven with chronic idiopathic thrombocytopenic purpura (ITP) prior to and during megadose methylprednisolone (MDMP) treatment by using enzyme-linked immunosorbant assay (ELISA) kits. It was shown that elevation of EPO and GM-CSF levels was correlated with an increase in hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), leukocyte (Leu), polymorphonuclear leukocyte (PMN) and monocyte (Mo) counts during MDMP treatment. *Key words: erythropoietin, granulocyte-macrophage-colony stimulating factor, megadose methylprednisolone.*

During the treatment of childhood acute and chronic idiopathic thrombocytopenic purpura (ITP) cases, we observed that megadose methylprednisolone (MDMP), besides correcting thrombocytopenia, elevated hemoglobin (Hb), packed cell volume (PCV), leukocyte (Leu), polymorphonuclear leukocyte (PMN) and monocyte (MO) counts[1-3]. Therefore, erythropoietin (EPO) and granulocyte-macrophage-colony stimulating factor (GM-CSF) levels were measured prior to and during MDMP treatment, and the corresponding Hb, PCV, red blood cell (RBC), PMN, Mo and lymphocyte (Lym) counts were determined in patients with ITP.

Material and Methods

Hemoglobin, PCV, Leu, PMN, Mo and Lym counts were obtained in 19 patients with ITP one and two weeks after MDMP treatment. Twelve of these children (10 girls 2 boys) had acute and seven had chronic (2 girls, 5 boys) ITP with age ranges of five to 13 ($\bar{X} \pm$ SD: 9.25 ± 2.73) and six to 14 (10.5 ± 2.99) years, respectively.

The diagnosis of ITP was based on platelet counts of less than 50×10^9/L, with excessive or normal numbers of megakaryocytes in the bone marrow. Platelets were enumerated by phase contrast microscopy[4]. Hepatosplenomegaly and lymphadenopathy were not detected, and there was no recent history of drug ingestion, including aspirin; no patients had received platelet or blood transfusions prior to the study. Underlying diseases were excluded as far as possible by lupus erythematosus (LE X 3) preparations, throat culture and antiglobulin test. Chronic ITP was considered when thrombocytopenia persisted longer than six months.

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Blood was obtained before 9:00 am. and prior to administration of MDMP. Serum EPO and GM-CSF levels were determined by enzyme-linked immunosorbant assay (ELISA) using Clinigen (Code No: ADC 060960) and Janssen Biochimica (Code No: 28.010.74) prior to the 3rd, 7th and 14th days of MDMP treatment (daily 30 mg/kg for three days, then 20 mg/kg for four days; 30 mg/kg and 20 mg/kg for one week each, administered before 9:00 am. orally, for acute and chronic ITP cases, respectively). The EPO and GM-CSF levels were calculated from the linear region of corresponding standard curves. Hb, PCV, RBC and Leu counts were obtained by using a coulter counter (Model S-plus VI); absolute values for polymorphonuclear leukocytes, monocytes and lymphocytes were calculated from total counts and simultaneous 100-cell differential counts on the 3rd, 7th and 14th days of the treatment and weekly for two more weeks after the treatment. For statistical evaluation, the Mann-Whitney U-test, Wlcoxon Rank test and regression correlation analysis were used.

Results

Erythropoietin (Fig. 1) and GM-CSF increased significantly ($p < 0.05$) after three days of MDMP administration. However, a hematologic response was first observed on the seventh day of treatment without being related to the platelet response. Elevation of GM-CSF was more marked than EPO on the third day of the treatment, which coincided with a significant elevation in Leu, PMN and Mo counts. GM-CSF elevation was not parallel to the platelet response in each case. PMN count changes were well correlated with leukocyte counts ($p < 0.05$). Lym counts did not change with Leu counts, and no decrease was observed with MDMP administ (Table I).

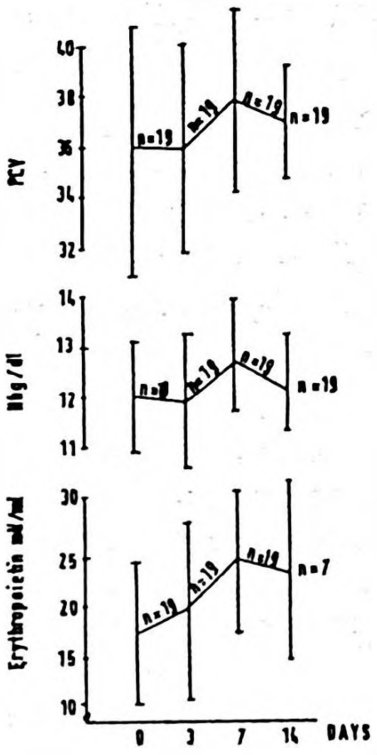

Fig. 1: Hb, PCV and serum EPO levels (mean ± SD) in patients prior to and during MDMP treatment.

Table I: EPO, Hb, PCV, RBC, GM-CSF, Leu, PMN, Mo, Lym Values Prior to, During and After MDMP Treatment in 19 Patients with ITP

	EPO (mU/mL)	Hb (g/dL)	PVC (%)	RBC (x 10^{12}/L)	GM-CSF (pg/mL)	Leu (x 10^9/L)	PMN (x 10^9/L)	Mo (x 10^9/L)	Lym (x 10^9/L)
Initial	17.21 ± 7.1	12.02 ± 1.07	36.07 ± 4.06	4.68 ± 0.5	7.62 ± 5.6***	6.98 ± 2.17	4.54 ± 2.13	0.18 ± 0.14	2.26 ± 0.84
	(8.8-30.4)	(10.3-13.62)	(28-44)	(4.02-5.58)	(5-25)	(5.00-14.30)	(1.70-10.30)	(0-0.57)	(1.05-4.86)
3rd day	19.59 ± 8.63	11.87 ± 1.44	36.02 ± 3.97	4.57 ± 0.58	13.25 ± 6.5***	11.17 ± 3.52	8.94 ± 3.18	0.32 ± 0.16	(1.91 ± 0.61)
	(12.2-36.4)	(9.8-14.2)	(31.4-43)	(3.36 ± 5.56)	(5.3-28)	(6.30-20.60)	(5.00-17.7)	(0.1-0.,82)	(1.0-3.36)
7th day	24.45 ± 7.27	12.77 ± 1.41	38.33 ± 3.44	4.91 ± 0.54	18.26 ± 6.9***	16.05 ± 4.33	12.96 ± 4.3	0.45 ± 0.20	2.64 ± 1.13
	(13.7-35.7)	(10.7-14.62)	(32-45)	(4.0-5.94)	(11-36)	(8.00-25.40)	(5.9-22.3)	(0.16-0.96)	(1.26-5.28)
14th day	23.53 ± 8.98***	12.21 ± 1.01	37.06 ± 3.29	4.90 ± 0.64**	19.1 ± 5.6*	14.61 ± 7.66	11.89 ± 6.95	(0.34 ± 0.24)	2.38 ± 1.37
	(10.2-36.0)	(11.0-13.48)	(32.8-44)	(4.02-5.67)	(12.6-26)	(5.20-29.0)	(2.7-26.1)	(0-0.10)	(1.1-6.8)
21st day	–	11.87 ± 1.32	35.79 ± 3.91	–	–	7.27 ± 2.02	5.13 ± 1.78	0.20 ± 0.11	1.94 ± 0.75
		(9.94-14.62)	(28-43)			(5.10-11.7)	(2.9-8.7)	(0-0.46)	(0.8-3.55)
28th day	–	12.32 ± 1.23	36.86 ± 3.8	–	–	7.05 ± 3.39	4.48 ± 3.2	0.16 ± 0.14	2.41 ± 0.68
		(10.9-14.9)	(32-45)			(5.00-19.3)	(2.1-17.1)	(0-0.48)	(1.4-4.28)

$\bar{X}$ ± SD ranges in parentheses.

* Only in 6.
** in 7.
*** in 12 patients.

EPO : Erythropoietin.
Hb : Hemoglobin.
Leu : Leukocyte.
MDMP: Megadose methylprednisolone.
PCV : Packed cell volume.
RBC : Red blood cell.
GM-CSF : Granulocyte-macrophage-colony stimulating factor.
ITP : Idiopathic thrombocytopenic purpura.
Lym : Lymphocyte.
Mo : Monocyte.
PMN : Polymorphonuclear leukocyte.

Discussion

Elevation of Hb and PCV during MDMP administration was observed[3]. The increase in the EPO level in this study could be an explanation for these observations, which strongly suggest bone marrow activation. Although chronic, large doses of EPO have been shown to cause thrombocytopenia in mice[5], this was not observed in this study at the detected EPO levels.

The in-vitro effects of GM-CSF on megakaryocyte progenitors have been well documented[6, 7]. Augmentation of GM-CSF levels in this study may be responsible for the elevation of platelet counts, but did not coincide in each patient. Prolongation of survival of human PMN by GM-CSF has been shown[8]. The observed increase in PMN and Leu counts may also be related to elevation of GM-CSF in our study, in addition to the well-known steroid effects[9, 10].

High-dose methylprednisolone has been shown to increase GM-CSF in children with leukemia[11]. In this study, oral MDMP administration in children with ITP resulted in elevation of EPO and GM-CSF. However, other cytokines could not be determined. A large body of data has indicated that platelet production is regulated by several humoral factors[12-16], and a megakaryocyte-colony-stimulating factor has been detected in the plasma and urine of severely thrombocytopenic individuals[13, 15, 16].

Elevation of EPO and GM-CSF levels during MDMP treatment may help to explain increases in Hb, PVC, RBC, Leu, PMN and Mo. Although other cytokines should also be evaluated to explain the increase in platelet counts, we believe that the increase of GM-CSF may also be a factor for improvement of thrombocytopenia with this treatment.

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RFLP DISCORDANCE IN A PKU FAMILY DUE TO A DELETION IN THE PAH GENE*

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SUMMARY: Bosco P, Ceratto N, Cali F, Goltsov AA, Eisensmith RC, Novelli G, Piccola BD, Romano V. (Istituto per la Ricerca sul Ritardo Mentale e l'Involuzione Cerebrale (IRCCS), Troina, and the Università degli studi di Roma Tor Vergata, Rome, and the Casa Sollievo della Sofferenza, S. Giovanni Rotando, Italy, and the Department of Cellular Biology, Baylor College of Medicine, Houston, Texas, USA). RFLP discordance in a PKU family due to a deletion in the PAH gene. Turk J Pediatr 1996; 38: 497-504.

We have previously reported preliminary data on a PKU family showing a discordant segregation of Pvu II (b) alleles at the PAH locus. A combination of several restriction enzymes and probe C2.6 (intron 2) as well as STR typing were used to dissect the molecular structure of the PAH gene around exon 3. In this family, the results of this analysis and a re-examination of the physical map of the 5'-end of the gene provided strong evidence for the occurrence of a deletion removing exon 3. The "Sicilian" (approximately 2.5 kb) and "Yemenite Jew" (6.7 kb) deletions, the latter one also deleting exon 3, are different in terms of both the 5'-end breakpoint and apparent length. This study, besides adding a new member to the long and increasing list of nearly 200 PAH gene mutations, also proposes to undertake a careful evaluation of RFLP discordances incidentally detected at the PAH locus. *Key words: classical PKU, PAH gene mutation, RFLP discordance.*

In Sicily the study of the mutant alleles causing phenylalanine hydroxylase (PAH) deficiency has recently led to the identification of 40 mutations altering the structure and function of the PAH enzyme[1]. These mutations account for approximately 98 percent of all mutant alleles in this population, and 22 of these defects are individually represented at a low frequency (0.9%). This observation is consistent with the presence of many founding populations for phenylketonuria (PKU) in Sicily, as is also documented by historical and archeological records. According to a consultation of the PKU Consortium Database[2], 24 deletions of the PAH gene have been reported to date. Large deletions are rare. One of the largest deletions reported so far has been well characterized at the molecular

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level and removes 6.7 kb of genomic DNA containing exon 3 and intronic flanking regions. It is the only mutation responsible for classical PKU in Yemenite Jews, probably because of founder and genetic drift effects[3]. We have previously reported preliminary data on the identification of a new mutation, also deleting exon 3 of the PAH gene, found in a single mutant chromosome borne by a Sicilian, classical PKU patient[4]. In this study we extend the molecular analysis of this mutation and provide strong evidence that the deletion removes approximately 2.5 kb of genomic DNA. This molecular lesion is responsible for the discordant segregation of alleles of the Pvu II (b) restriction fragment length polymorphism (RFLP) in the affected family. Although this mutation partially overlaps the deletion described by Avigad et al.,[3] in Yemenite Jews it could be demonstrated that the two deletions have different 5' breakpoints.

Material and Methods

Patients and Diagnosis

The index case in family F2 is a 22-year-old, mentally retarded boy who was sent to the OASI Institute (Troina) for diagnosis. Determination of his blood phenylalanine level showed that the had classical PKU, untreated since birth. His younger sister (16 years old) was also affected by classical PKU but had been enrolled in the regional neonatal screening program for PKU and had been successfully treated since birth. The occurrence of defects involving homeostasis or synthesis of the phenylalanine hydroxylase cofactor (BH4) was ruled out by urinary pterins testing.

Polymorphism and Mutation Analyses

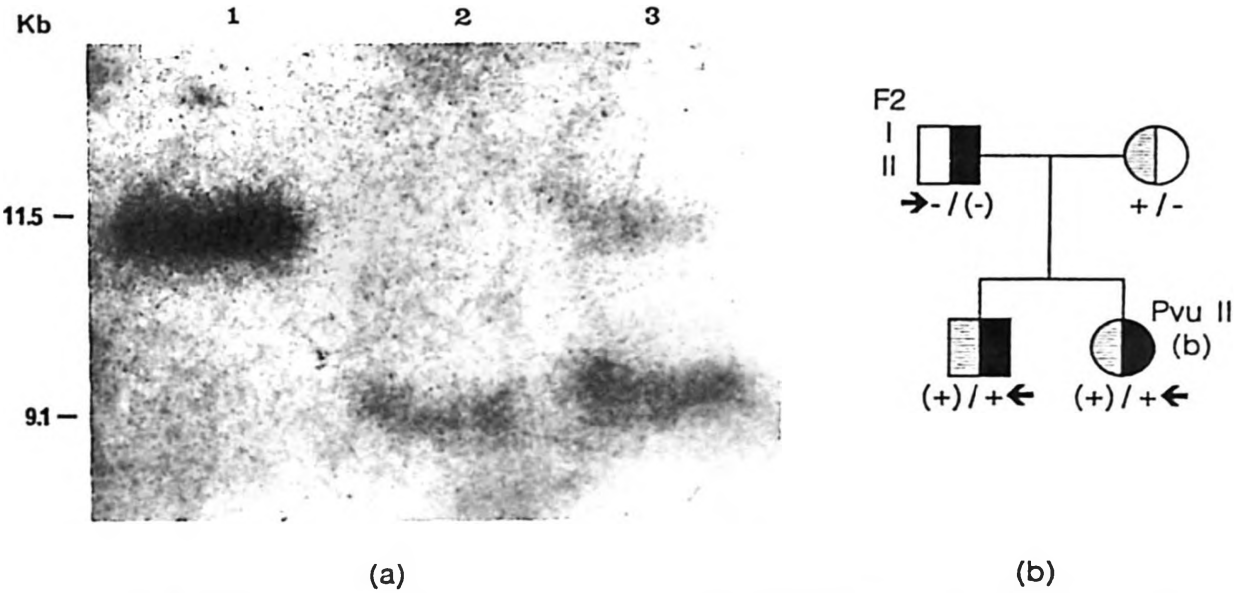
RFLP analysis at the PAH locus was performed as described by Lidsky et al.[5]. The haplotypes identified in this family (F2) have been previously reported[4]. A previous mutation analysis performed in this family had shown that the two affected siblings and the mother were heterozygous for the IVS X nt 546 mutation[1-4]. Probe C2.6 (kindly provided by Dr. Shiloh, Tel Aviv, Israel) was hybridized to genomic DNAs digested with one of the following enzymes: Eco RI, Pvu II or Hind III (biolabs, USA). Probe C2.6 is a genomic fragment, 2.6 kb long, derived from intron 2 of the PAH gene. It was generated by digesting with Hind III and Eco RI probe C, which is 4 kb long. The 3' end of probe C2.6 maps in intron 2, 0.7 kb away from exon 3. Further details about probes C2.6 and C have been reported by Avigad et al.[3]. Analysis of the intragenic short tandem repeats (STR) polymorphism was performed as described by Goltsov et al.[6] STR allele sizes determined according to the latter authors were two base pairs shorter than the true size[7]. DNA fingerprinting analysis was carried out by typing D1S80[8], ApoB[9] and HLA DQ[10] polymorphic loci. Mendelian inheritance was demonstrated for each allele of the latter three polymorphisms in family F2. Statistical analysis was performed according to Balazs et al.[11] and Wong et al.[12].

Illegitimate Transcription

This analysis was performed according to Abadie et al.[13]. Two oligonucleotides were used for primer extension on RNA (primer 2) and PCR amplification (primers 1 and 2). Primer 1: 5'CTT TGG ACA GGA AAC AAG CT 3'; primer 2: 5' GAT CTT TAA AAC GAG GGT GGT CA 3'. Both primers were synthetized according to the published sequence of the PAH cDNA[14] with an automated oligo-synthetizer (Applied Biosystem, USA). They span the junctions between exons 1 and 2 (primer 1) and exons 4 and 5 (primer 2), respectively.

Results

Fig. 1a shows the results of Pvu II (b) analysis performed in family F2. The father (lane 1) carries a single 11.5 kb band, the mother (lane 3) carries both the 11.5 and 9.0 kb bands, and the affected boy (lane 2) bears only the maternal 9.0 kb allele. Identical results were obtained for the affected sister (II-2 in Fig. 1b). Apparently the paternal 11.5 kb allele was not transmitted to the two affected sibs. All other RFLPs showed concordant segregation[4]. DNA fingerprinting analysis was undertaken to exclude the occurrence of false parenthood. In particular, three mini-satellites corresponding to the highly polymorphic variable number of tandem repeats ApoB, D1S80 and HLA DQ loci were used to fingerprint this family. Mendelian segregation analysis of these three polymorphisms showed identical haplotypes between parents and the putative siblings (data not shown). Haplo-identity could be ascertained with a probability of 99.99 percent.



(a) (b)

Fig. 1: Autoradiograph (a) and pedigree (b) of a Sicilian PKU family showing discordant segregation of Pvu II (b) RFLP alleles. The Pvu II (b) 11.5 allele was not transmitted by the father to the affected boy. The mother transmitted the Pvu II (b) 9.0 kb allele. 1, 2, and 3 refer to the father, the affected son and the mother, respectively.

To further analyze the region surrounding exon 3, a combination of probe C2.6 with several enzymatic digestions (Eco RI, Pvu II and Hind III) of genomic DNAs was used. Hind III digestion showed the presence of a normal 3.3 kb fragment in the father and in the two affected siblings (Fig. 2, lane 1 ⇨ 3, lanes 4 ⇨ 7 refer to normal individuals). Eco RI analysis (Fig. 3) allowed the identification of a new 6 kb fragment in the father[1-2], in the two affected siblings (II-3 and II-4) and in the two sisters fathered by 1-2 in a previous marriage (II-1 and II-2). The abnormal Eco RI 6 kb fragment showed concordant Mendelian segregation only with the mutated chromosome, thus suggesting that II-1 and II-2 were PKU carriers. Also, the father's sister[1-4] was known to be a PKU carrier because of the presence of the Eco RI 6 kb fragment. Pvu II-digests of genomic DNAs also probed with C2.6 in this family uncovered a new 8.8 kb fragment that was only found in all individuals who also bore the new Eco RI 6 kb band (data not shown). Consistent with Eco RI results, segregation studies also showed, as expected, that the new Pvu II fragment (8.8 kb) was transmitted by the father to the affected daughter (Fig. 4). STR analysis showed that the father (224/240), the mother (244/252), the affected son (224/252) and the affected daughter (224/252) were all heterozygous for this intragenic microsatellite polymorphism. STR allele no. 224 is associated with the paternal mutant PAH gene and has not been previously described. A tentative map of exon 3 and flanking intronic regions based on the results of this study and on published[15-3] and unpublished (Eisensmith and Goltsov: personal communication) data is displayed in Fig. 5. Based on Hind III, EcoRI and Pvu II data, the map shows a deletion of approximately 2.5 kb which removes exon 3. Finally, illegitimate transcription analysis carried out in RNA isolated from peripheral blood lymphocytes of the affected boy led to the identification of a normal (375 bp) cDNA fragment.

Fig. 2: Autoradiograph obtained by hybridization of probe C2.6 to Hind III digested genomic DNA from the father and the two affected siblings (asterisks) or from other normal individuals. Only the normal 3.3 kb fragment is seen. The Hind III 4.2 kb fragment which is diagnostic for the deletion observed in Yemenite Jewish patients is missing.

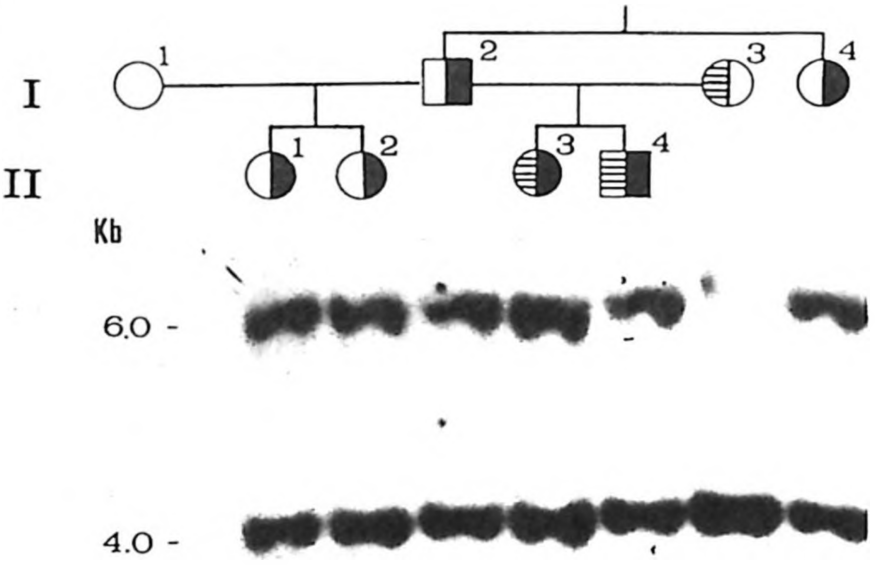


Fig. 3: Segregation analysis of the abnormal Eco RI 6 kb fragment detected with probe C2.6 in family F2. Individuals indicated with half-filled squares or circles bear the EcoRI 6 kb fragment diagnostic for the deletion of exon 3 in the PAH gene. Half-striped squares or circles indicate that the mother and the two affected siblings are heterozygous for the IVS X nt546 mutation. Only the mother (I-3) did not carry the abnormal fragment.

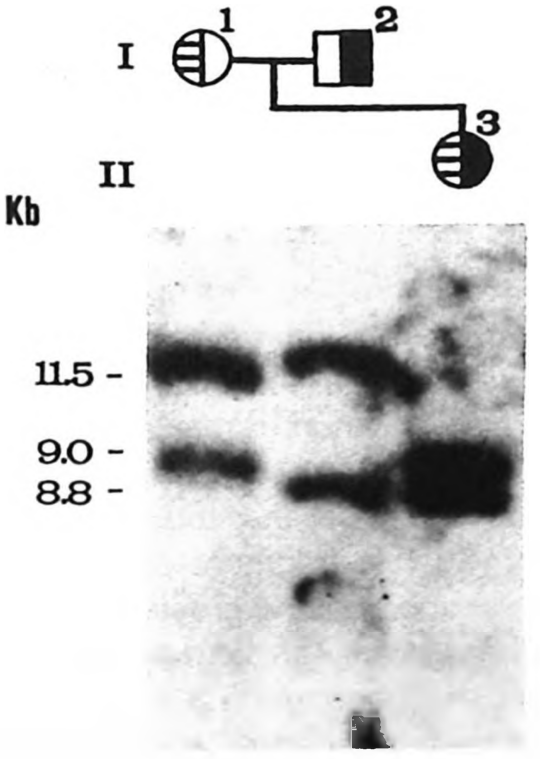


Fig. 4: Segregation analysis of the abnormal Pvu II 8.8 kb fragment hybridizing to probe C2.6. This fragment was transmitted from the father to the daughter, who was affected by classical PKU. Other PKU carriers and the affected brother also bear this fragment (data not shown).

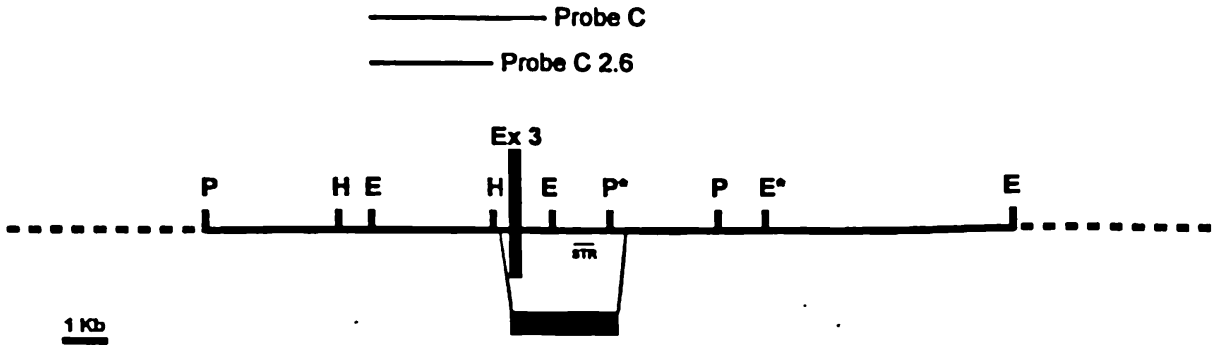


Fig. 5: Restriction map of the PAH gene in the exon 3 region, showing the deletion of approximately 2.5 kb (filled rectangle below the restriction map) removing exon 3 in PKU family F2. The position of Hind III (H) and EcoRI (E) sites were located according to Avigad et al.,[3] Di Lella et al.,[15] and Eisensmith and Goltsov (personal communication), respectively. P* indicates the Pvu II (b), and polymorphic site E* refers to an EcoRI site not reported in the map published by Di Lella et al.[15] The STR locus map 0.7 kb downstream to exon 3[6], however its relative position to the polymorphic Pvu II site is tentative.

Discussion

The results of DNA fingerprinting analysis make unlikely the possibility that the Pvu II (b) RFLP discordance was due to false parenthood. An alternative explanation would assume that the father is hemizygous for the Pvu II (b) 11.5 kb allele, and the mother transmits to the two affected siblings the IVS X nt 546 mutation. Such a hypothesis implies that exon 3 in the PAH gene inherited from the father is deleted, since this is the only coding region contained in the Pvu II (b) polymorphic fragments recognized by the phPAH247 probe[15]. The deletion is probably masked by the presence of the other normal 11.5 kb allele in the father and by the presence of the maternal PKU chromosome Pvu II (b) 9.0 kb allele in the affected son and daughter. The new fragments seen with Eco RI and Pvu II using probe C2.6 are likely to reflect the occurrence of a gene rearrangement around exon 3, rather than the presence of restriction fragment-length polymorphisms. For instance, the occurrence of a new 6 kb fragment could be due to a new Eco RI polymorphism. However, this possibility is unlikely because, according to the map published by Di Lella et al.,[15], neither the appearance of a new Eco RI site nor the abolition of a preexisting one, would lead to a 6 kb fragment. Such a fragment could only be generated by the contemporary (and unlikely) abolition and creation of two Eco RI restriction sites. The Eco RI data are rather consistent with the deletion of a DNA fragment including exon 3 and the Eco RI site neighboring the 3' end of exon 3. Consequently, the Eco RI 6 kb band could be generated as a junction fragment linking the two deletion breakpoints. In our preliminary report on this family[4], a deletion of approximately 9-10 kb was suspected, based on the restriction map published by Di Lella et al.[15] However, a recent reexamination of the Eco RI restriction map

of the original cPAH 10 lambda clone (Eisensmith and Goltsov: personal communication) allowed the identification of one additional Eco RI site (see E* in Fig. 5) not reported in the map of the PAH gene published by Di Lella et al.[15] Accordingly, the length of DNA removed by the deletion would be approximately 2.5 kb long. The latter estimate fits well now with the reduction of the Pvu II (b) fragment size from 11.5 kb to 8.8 kb (Fig. 4). On the other hand, by taking into account the STR data only, the deletion could be shorter (i.e., less than 1.4 kb). The discrepancy between STR and restriction enzyme analysis clearly prevents a precise estimate of the deletion width, and it is likely to be due to the still incomplete characterization of the physical map in this specific region of the PAH gene. Furthermore, no other mutations altering the coding sequence were detected by DGGE and sequence analyses carried out on the paternally inherited, mutant PAH gene[1]. On the other hand, our attempts to identify a shorter transcript due to the deletion of exon 3 by illegitimate transcription analysis were unsuccessful. This result suggests that the deletion probably impairs the correct splicing of exons 2 and 4. Since probe C2.6 identifies in these patients a normal Hind III 3.3 kb fragment (Fig. 2) 3, the deletion must have its 5' breakpoint further downstream compared to the Yemenite Jews' mutation (the closest Hind III site upstream to exon 3 is conserved). It is possible that intronic regions flanking exon 3 contain hot spots for recombination. According to previous reports[1-4], the frequency of this mutation in Sicily is approximately one percent. The results of this study also emphasize the importance of undertaking a detailed molecular analysis of RFLP discordances incidentally detected at the PAH locus, since they may underlie the presence of PKU-causing mutations.

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FEVER AND ALTERED CONSCIOUSNESS ARE NOT ALWAYS EQUAL TO CENTRAL NERVOUS SYSTEM INFECTION: NEUROLEPTIC MALIGNANT SYNDROME IN A 15 - YEAR - OLD GIRL*

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SUMMARY: Ünal F, Hızlı Ş, Özyürek H, Sonuvar B. (Departments of Child Psychiatry and Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Fever and altered consciousness are not always equal to central nervous system infection: neuroleptic malignant syndrome in a 15-year-old girl. Turk J Pediatr 1996; 38: 505-510.

Neuroleptic malignant syndrome, the most serious and potentially fatal side effect of neuroleptics, is characterized by altered consciousness, extrapyramidal symptoms, hyperthermia, elevated plasma creatine phosphokinase and leukocytosis. In the child and adolescent population, the syndrome may be underrecognized or underreported. We describe a 15-year-old girl who developed neuroleptic malignant syndrome after a single injection of two different neuroleptics. We find the case interesting because the patient's course was unusual, and she was treated successfully with the combination of diazepam and biperiden. Our aim is to discuss several aspects of the syndrome through this case, particularly the problem of recognition. *Key words: neuroleptic malignant syndrome, adolescent, diazepam, biperiden, haloperidol, chlorpromazine.*

Neuroleptic drugs (antipsychotics) produce numerous side effects, including serious extrapyramidal symptoms consisting of akathisia, dystonia, tremor, akinesia, rigidity, tardive dyskinesia and neuroleptic malignant syndrome (NMS). Among these side effects, the most serious and potentially fatal complication is NMS[1]. The syndrome is characterized by altered consciousness; extrapyramidal symptoms such as muscle rigidity, dystonia, akathisia and tremor; symptoms of autonomic dysfunction such as hyperthermia, tachycardia, hypertension or hypotension; diaphoresis; elevated plasma creatine phosphokinase (CPK); and leukocytosis[2, 3].

NMS typically develops within days of a recently initiated course of neuroleptic drug treatment. Potential risk factors for NMS may include dehydration, agitation, affective disorder, intramuscular drug application and a higher drug dosage[4, 5]. In the last decade, numerous reports have provided sufficient data to estimate the frequency of NMS in patients treated with neuroleptic drugs[6-10]. Risk estimates

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for NMS on exposure to neuroleptic drugs vary between 0.07 percent and 2.44 percent. The mean estimated frequency from pooled data is reported to be 0.67 percent. In the child and adolescent population, NMS may be underdiagnosed or underreported, perhaps due to the infrequent use of neuroleptic drugs in this population as well as difficulties in recognizing the condition. In Addonizio et al.'s[12] review covering all of the 115 cases reported in the English literature, 15 patients were in the range of 10 to 19 years old. Other investigators have reported single cases or small series of children and adolescents who developed NMS[13-20].

The case we describe here is a 15-year-old girl who developed NMS after a single injection of two different neuroleptics. We find the case interesting because the patient's course was not typical, she was treated successfully with the combination of diazepam and biperiden. Our aim is to discuss several aspects of NMS through this case, particularly the problem of recognition.

Case Report

The patient was a 15-year-old female with no history of medical or psychiatric illness. Her father was reported to have the diagnosis of schizophrenia, paranoid type. There was no history of other psychiatric illness in the family.

One week prior to her admission to our hospital she developed an acutely progressing clinical picture consisting of insomnia, agitation and paranoid delusions. This clinical picture was considered to be a psychotic episode by a local hospital, and a single intramuscular injection of the combination of haloperidol 5 mg and chlorpromazine 25 mg was administered. Approximately eight hours after this injection, a gradually progressing clinical picture consisting of fever, fluctuating consciousness, weakness and muscle rigidity developed. She was admitted to the local hospital with the preliminary diagnosis of encephalitis because of her high fever, confusion and neck stiffness. All of the laboratory findings including urine, blood. cerebrospinal fluid (CSF) analysis and EEG were reported to be normal. It was remarkable that plasma creatine phosphokinase (CPK) was not evaluated in the local hospital. Dexamethasone 4 mg g.i.d., mannitol 120 cc t.i.d. and nifedipine 15 mg t.i.d. were administered for the additional symptom of hypertension, but she was referred to the university hospital because a clinical change was not observed within five days with that treatment.

On admission to our hospital, the patient's physical, neurological and psychiatric examinations were remarkable for hyperthermia (38 °C), confusion, agitation, possible auditory and visual hallucinations, "cogwheel" muscular rigidity, labile blood pressure and diaphoresis. Abnormal laboratory findings included a WBC of 12,300/mm^3, and a CPK of 832 IU/L. Routine blood, urine, CSF analysis, blood and urine cultures, B_{12}, serum protein electrophoresis, anti-DNA, complement

C_3/C_4, EEG, ECG, abdominal ultrasonography and cranial computed tomography (CT) studies were normal. The patient fulfilled the criteria given by Caroff et al.[2] and Levenson[21] for the diagnosis of NMS, and diazepam 20 mg and biperiden 10 mg were administered daily. Five days later, her agitation disappeared and plasma CPK returned to normal levels. Although her confusion, hallucinations, muscular rigidity and autonomic dysfunction symptoms diminished gradually, the patient had recovered completely on the 20th day of that treatment. In the drug-free follow-up period of six months, she had neither psychiatric nor medical symptoms.

Discussion

With the increasing use of psychotropic drugs in the child and adolescent population, NMS has become an important diagnostic consideration for the pediatrician. Many cases of NMS may go unrecognized, particularly in the early stages of development of the syndrome[6]. We believe that this fact is particularly relevant in children and adolescents, as it was the case in our patient. Although she had almost all of the symptoms of the syndrome, NMS remained unrecognized in the local hospital. This may be due to insufficient knowledge about NMS or a lack in the information sources. Her parents helped a great deal by explaining that the fever and the confusional state had begun immediately after the injection. We believe that assumptions of the family about the etiology of the disorder should be taken into account.

NMS patients come to pediatricians mostly with altered consciousness and fever. Medical conditions to be considered in the differential diagnosis of this clinical picture include systemic and central nervous system (CNS) infections, septicemia, CNS mass lesions, metabolic conditions and autoimmune diseases. It is noteworthy that these conditions can be associated with NMS or superimposed. NMS is sometimes confused with intoxication with strychnine or CO, serotonin syndrome, malignant hyperthermia, heat stroke and lethal (psychogenic) catatonia[2,5].

Clinical pictures of intoxication, serotonin syndrome, malignant hyperthermia, heat stroke or lethal catatonia are infrequent but often considered, because of their specific etiology, prodromal and morbid features. CNS mass lesions, metabolic conditions and autoimmune diseases are also easy to detect with cranial CT or specific laboratory tests.

CNS infections are more frequently seen and may be a perplexing problem in the differential diagnosis, thus should be discussed in detail. In meningitis cases, fever is the cardinal sign, while headache, lethargy and irritability are also seen. In CSF findings, increased WBC and protein as well as decreased glucose are expected. Encephalitis is much more difficult to differentiate. Fever, headache, tremor, ataxia, cranial nerve VI palsy, irritability and lethargic appearance are

findings of encephalitis. CSF findings include increased WBC and protein, normal glucose and negative cultures. In the presented case, fever, altered consciousness, irritability and tremor were present, but the CSF findings were not consistent with meningitis or encephalitis. Although CSF findings may be normal in viral encephalitis at the beginning of the disease, a second CSF analysis performed later in our case was also found to be normal.

Although the exact pathophysiology of NMS is not known, several neuro-mediators including dopamine, serotonin, norepinephrine, gamma-aminobutyric acid (GABA), excitatory amino acid (EAA) and N-methyl-D-aspartate (NMDA) are claimed to play a role in the pathogenesis of the syndrome[5, 22]. Current concepts regarding the pathophysiology of NMS are based on a functional dopamine deficient state triggered by neuroleptic drug-mediated, post-synaptic dopamine receptor blockade and ensuing hyperactivity of excitatory amino acid neurotransmission in the basal ganglia and hypothalamus[5].

Recognition of NMS is the most important therapeutic action for a good outcome, because discontinuation of the causative drug is the first step of the treatment. Other therapeutic measures consist of reducing fever, adequate monitoring, and support of cardiovascular, respiratory, renal and other vital functions. Currently, dopamine agonists such as bromocriptine, amantadine and carbidopa/levodopa and dantrolene are usually administered for the treatment of NMS[7, 23]. Anticholinergics, benzodiazepines and electroconvulsive therapy are often considered to be controversial therapeutic alternatives[7]. However, bromocriptine may exacerbate psychotic symptoms, and dantrolene should be considered as a potentially hepatotoxic drug[23]. Benzodiazepines have been administered to more than 20 patients with transient relief of symptoms in about 40 percent of cases[12]. There are also reports of a beneficial response[23].

We think that our case fulfills the criteria for Stage 3 as discussed by Woodbury and Woodbury[20], and benzodiazepines are among the recommended treatments for this stage[12, 20, 23]. In our case diazepam was also the treatment of choice because of the patient's severe agitation. We observed a rapid recovery of that life-threatening symptom. The remaining features improved relatively slowly. Studies concerning the mode of action of benzodiazepines on NMS argue that this agent may have an indirect dopaminergic effect mediated by GABA-ergic feedback loops within nigrostriatal and mesolimbic centers[24]. Biperiden was also administered in our case for the extrapyramidal symptoms. Although there are various studies suggesting that anticholinergic agents have no effect on NMS, Leikin et al.[25] reported successful results with diphenhydramine. It is known that central dopaminergic blockade in the nigrostriatal pathway causes an excitatory effect on the cholinergic system, which may be responsible for the symptomatology seen in NMS[26]. The effectiveness of biperiden in NMS may be due to its blockade of the cholinergic component of the nigrostriatal pathway.

Another interesting point in our case was the administration of nifedipine to reduce the patient's blood pressure in the local hospital. This agent is reported to be effective in NMS by blocking the neural and muscular calcium channels, which might be involved in the pathophysiology of the syndrome[27]. It can be hypothesized that this agent also had a therapeutic role in our patient, who remained in Stage 3. To avoid unfortunate therapeutic outcomes, we suggest that pediatricians should be sensitive to the possible role of NMS among pediatric patients with fever of unknown origin, striking extrapyramidal symptoms and a recent history of psychopharmacological intervention.

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HEPATIC MUCORMYCOSIS IN A CHILD WITH FULMINANT HEPATIC FAILURE*

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SUMMARY: Erdem G, Çağlar M, Ceyhan M, Akçören Z, Kanra G. (Infectious Disease and Pathology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Hepatic mucormycosis in a child with fulminant hepatic failure. Turk J Pediatr 1996; 38: 511-514.

Mucormycosis is an uncommon, opportunistic infection in children. We present a case of fulminant hepatic failure with hepatic mucormycosis. Although the suggested defects and pathogenic mechanisms in infections related to hepatic failure and mucormycosis are similar, few cases with both mucormycosis and liver failure have been reported.
Key words: mucormycosis, liver, hepatic failure.

We report a postmortem-diagnosed case of mucormycosis with fulminant hepatic failure (FHF) due to hepatitis A infection. Mucormycosis appears in tissue as irregularly shaped, broad hyphae with right-angle branching in the midst of acute neutrophilic infiltrate and vascular invasion. Vascular invasion and tissue necrosis are the hallmarks of the disease[1]. Antemortem diagnosis has been made infrequently in mucormycosis, and tissue cultures give variable results[1,2].

Case Report

A five-year-old girl was admitted with a two-week history of jaundice. On admission she was conscious, icteric, and her liver was palpable eight cm from the right costal margin. Routine laboratory investigations were all normal except for mild bilirubinuria. Her transaminase levels were increased; SGOT was 1065 IU/L and SGPT was 632 IU/L; total bilirubin was 23.7 mg/dl with a direct fraction of 13.4 mg/dl. The alkaline phosphatase level was 310 IU/L, total protein was 9.5 g/dl and albumin was 3.8 g/dl. On admission she had a positive anti-HAV IgM.

During the follow-up period of four days, the patient became irritable and lethargic. Although inpatient treatment for hepatic encephalopathy was begun immediately, she lost consciousness completely within two days and developed coma with anisocoria. Her transaminase levels continued to decline during the hospitalization

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period, with a decrease in liver size and extreme increase in bilirubin levels (total bilirubin: 43.8 mg/dl; direct fraction: 17.4 mg/dl). Her blood ammonia and prothrombin times were within normal limits. On the eighth day of hospitalization she had a sudden cardiopulmonary arrest and did not respond to resuscitative efforts.

On postmortem examination the incisional tissue specimen from the liver was firm with a brownish-green color. On cut surface, nodular lesions of 0.2-0.3 cm in diameter were observed. Histopathological examination revealed necrosis of the hepatocytes around the central veins and dilation of the sinusoids. Intracellular cholestasis was evident. In areas of diffuse necrosis, the vessels of portal triads were filled with necrotic debris infiltrated by numerous broad, aseptate hyphae (Fig. 1). Hyphae with nearly 45-degree-angle branching were less condensed in the surrounding parenchyma and were stained positively with methenamine silver stain (Fig. 2). Cultures for mucormycosis were negative. Tissue specimens were obtained from the brain, kidney, and lungs, and no hyphae was seen in these organs.

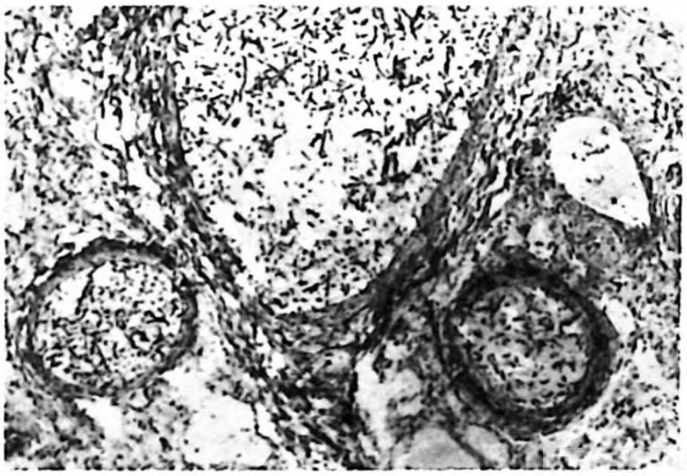

Fig. 1: The portal area and hyphae in the postmortem liver tissue sample (Methenamine silver stain 4 x 13).

Fig. 2: Broad nonseptate hyphae in liver tissue; branching is shown by an arrow (Methenamine silver stain 20 x 66).

Discussion

Viral hepatitis is the most common cause of FHF, which is complicated by infections in up to 40 percent of cases[3, 4]. Besides bacterial and viral infections, opportunistic fungal infections occur in most of these patients[3]. In a clinical study of 35 patients with FHF, fungi, especially the candida species, were cultured from 14 patients[5]. The increased risk of bacterial and fungal infections in these patients is thought to be due to a reversible immune suppression, including impaired neutrophil and Kupffer cell functions and deficiency of opsonins such as complement components and fibronectin[6-8].

The mucormycoses are an important class of opportunistic fungal pathogens in mostly immunocompromised hosts, but the underlying immunologic defects responsible for the development of mucormycoses are not well understood[9, 10].

Delayed chemotaxis of neutrophils seen in infected rabbits, defects in alternate complement pathway activation, generation of oxidative metabolites, defensins and cationic proteins may all contribute to the pathogenesis of mucormycosis[2, 11].

Although factors contributing to both mucormycosis and infections in FHF are still unclear, most of the accused defects in neutrophil and macrophage functions are similar. Immunologic studies were unfortunately not available in our case due to postmortem diagnosis. It is difficult to explain why mucormycosis is so rare in patients with hepatic failure. It may be due to the fact that most patients die from other complications of FHF before the development of fungal infections. The infrequency of reported cases of mucormycosis does not indicate low frequency of disease but rather decreased reportability of the condition[12]. Of the 34 reports of mucormycosis in children reported since 1954, only one patient with viral hepatitis developed mucormycosis with a perirenal abscess and total renal infarct without gastrointestinal tract involvement[12, 13]. In an adult patient with oliguric renal failure and renal mucormycosis, FHF was found to be the preceding disease[14].

Gastrointestinal mucormycosis is rare, and intrinsic abnormalities of the gastrointestinal tract, such as amebic colitis, thyphoid, pellegra and kwashiorkor generally have preceded the infection[9]. Those abnormalities were not present in our case. The organ most frequently involved is the stomach, with the large bowel next in frequency. The liver is involved secondary to a widespread dissemination from a primary site[10, 11]. It is interesting that in our case there were no hypae in the lungs, kidney or brain. Hyphae were only demonstrated in the liver tissue specimen.

To our knowledge, this is the first child with hepatic mucormycosis in FHF. The definitions of the underlying defects in both mucormycosis and in infections of patients with FHF may shed light on the rarity of mucormycosis in patients with hepatic failure.

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BALLOON DILATATION ANGIOPLASTY OF A STENOTIC BLALOCK - TAUSSIG ANASTOMOSIS*

A Case Report

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SUMMARY: Saltık İL, Güler-Eroğlu A, Sarıoğlu A, Batmaz G. (Pediatric Cardiology Unit, İstanbul University Institute of Cardiology, İstanbul, Turkey). Balloon dilatation angioplasty of a stenotic Blalock-Taussig anastomosis. Turk J Pediatr 1996; 38: 515-519.

A 12-year-old boy with tetralogy of Fallot was evaluated for increasing cyanosis and exercise intolerance. The patient had a right Blalock-Taussig shunt operation 11 years previously and a left modified Blalock-Taussig shunt operation one year previously. After demonstration of hypoplastic pulmonary arteries, the stenosed right Blalock-Taussig shunt and the totally occluded, left modified Blalock-Taussig shunt were successfully dilatated with a 7-mm-diameter angioplasty balloon resulting in a 5-mm-diameter patent shunt. We believe that, when possible, balloon dilatation angioplasty of stenosed Blalock-Taussig shunts is a reasonable and safe alternative to surgery.
Key words: balloon dilatation, Blalock-Taussig shunt.

Blalock-Taussig shunts are standard palliative treatment for various cyanotic, congenital heart defects associated with pulmonary blood flow. Early or late occlusion of Blalock-Taussig shunts occurs in approximately 10 percent of cases[1]. Recently, balloon dilatation angioplasty of stenotic Blalock-Taussig shunts has been done successfully in pediatric patients who were not amenable to total surgical correction in order to avoid the mortality and morbidity of a new systemic-pulmonary anastomosis[2-8]. We report the successful dilatation of a severely stenosed Blalock-Taussig shunt in a child with tetralogy of Fallot.

Case Report

A 12-year-old boy with tetralogy of Fallot was evaluated for his increasing cyanosis and exercise intolerance. The patient had a classical right Blalock-Taussig shunt operation 11 years previously and a left modified Blalock-Taussig shunt operation one year previously at another center. On admission he had severe cyanosis, clubbing and normal pluses in all extremities with the exception of an absent

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right brachial pulse. There was a grade 1/6° systolic murmur at the left sternal border, but a continuous murmur was not heard. Chest x-ray showed decreased pulmonary vascular marking. Right axis and right ventricular hypertrophy was noted on electrocardiogram. His hemoglobin was 17 g/dl with a hematocrit of 54 percent. Echocardiographic examination revealed tetralogy of Fallot, severe obstruction of right ventricular outflow, hypoplastic pulmonary arteries and aorto-pulmonary collaterals. Color doppler echocardiography demonstrated minimal turbulent flow in the right pulmonary artery, and there was no shunt flow in the left pulmonary artery.

Right heart catheterization was performed to determine the diagnosis and demonstrate pulmonary blood sources under general anesthesia. After percutaneous entry via the right femoral vein, 50 units/kg of intravenous heparin was administered A 7 French NH catheter was advanced into the aorta by the femoral venous route via the right ventricle and ventricular septal defect. After pressure and saturation measurements, a right innominate artery angiogram was performed. There was severe stenosis at the anastomotic site of the right Blalock-Taussig shunt with a tinny "jet" of contrast medium passing through the shunt into the right pulmonary artery (Fig. 1a). There was no evidence of a left modified Blalock-Taussig shunt in repeated angiograms. After demonstrating hypoplastic pulmonary arteries, the decision was made to perform balloon dilatation angioplasty of the stenosed Blalock-Taussig shunt. A Cordis Judkins 5 French right coronary artery catheter with a 4 cm curve was inserted in to the right femoral vein and advanced with floppy guide-wire manipulation across the Blalock-Taussig shunt into the right pulmonary artery by the transvenous route. A 260 cm 0.032 inch exchange guide-wire was advanced through the catheter and positioned distally within the left pulmonary artery. The catheter was removed and a 7 French balloon dilatation (Meditech) catheter with a balloon diameter of 5 mm was then inserted over the exchange wire and advanced into the right pulmonary artery through the Blalock-Taussig shunt so that the midsection of the balloon was at the point of stenosis. The balloon was dilatated to a maximum of 10 atmospheres pressure for three to four minutes (Fig. 1b). Because of inefficient dilatation, the procedure was repeated with a balloon diameter of 7 mm until the balloon waist disappeared. The balloon catheter was then removed and repeated angiography performed. The angiogram documented improved pulmonary flow to pulmonary arteries through the shunt, the diameter of the anastomotic site increased to 5 mm (Fig. 1c), and systemic arterial oxygen saturation increased from 72 percent to 80 percent. Immediately after the procedure a loud continuous murmur was easily heard. The child was discharged on the following day.

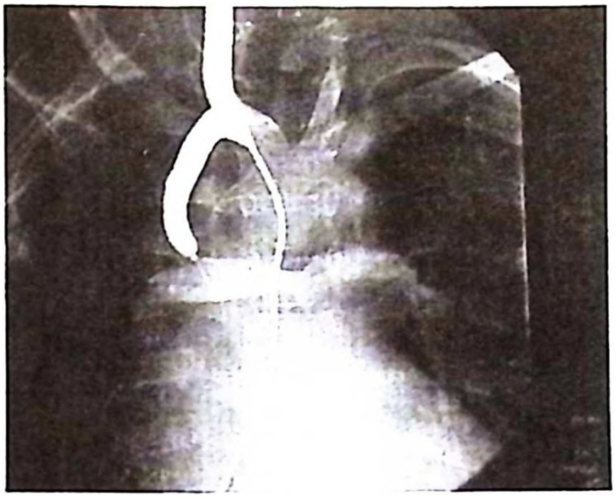

Fig. 1a: Angiographic injection into the right innominate artery before balloon dilatation demonstrates marked narrowing of the anastomotic site of the Blalock-Taussig Shunt.

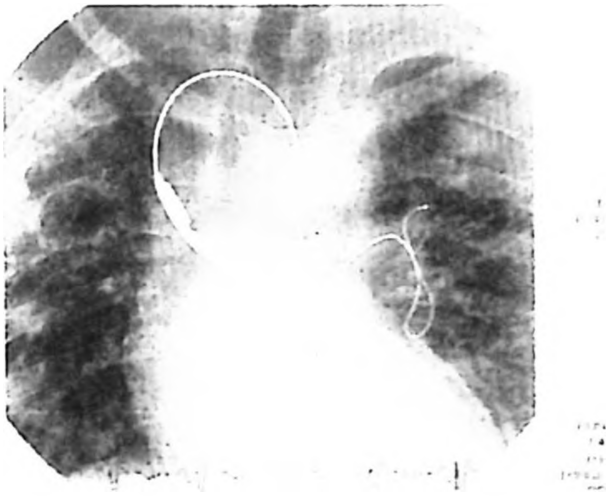

Fig. 1b: Position of 5 mm balloon dilatation catheter during inflation. Note waist of balloon.

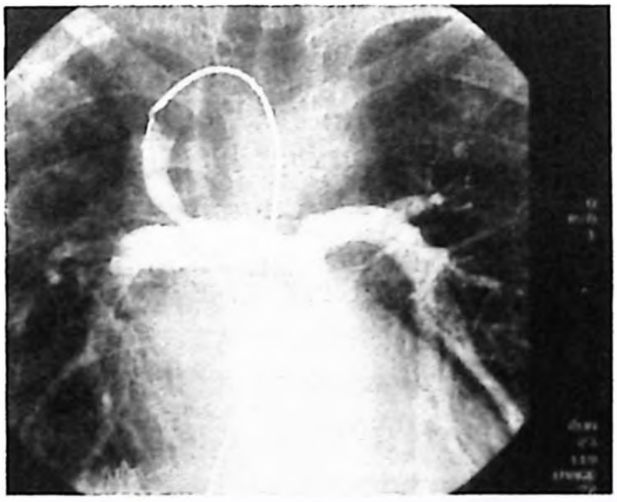

Fig. 1c: Angiographic injection into right Blalock-Taussig shunt after balloon dilatation shows relief of stenosis at the anastomotic site and improvement in pulmonary flow.

Discussion

When a Blalock-Taussig shunt becomes stenosed in the case of a cyanotic congenital heart defect with reduced pulmonary blood flow, therapeutic options include the creation of another systemic-pulmonary shunt or, if suitable, correction of the underlying cardiac malformation earlier than planned. Transcatheter balloon dilatation of stenosed shunts has been proposed as an alternative to a new systemic-pulmonary anastomosis[2-7]. Our patient had hypoplastic pulmonary arteries and was not amenable to total surgical correction. He also had a right Blalock-Taussig and totally occluded left modified Blalock-Taussig shunt; thus,

creating a new systemic to pulmonary shunt was too difficult. Therefore, balloon dilatation angioplasty of the stenotic Blalock-Taussig shunt was the ideal treatment for him.

Transcatheter balloon dilatation of a stenotic Blalock-Taussig shunt has achieved adequate palliation, but the results have depended on the size of the balloon in relation to the size of the shunt[3, 4, 9]. Oversized balloons, nonetheless, may cause increased pulmonary blood flow leading to congestive heart failure in the short term and pulmonary hypertension in the long term[9]. In order to avoid complications, we completed the procedure when the diameter of the anastomotic site increased to 5 mm.

A major limitation in the application of this technique for occluded systemic to pulmonary artery shunts in children is that patients in whom the shunt was the major (or singular) source of pulmonary blood flow often have severe hypoxia and metabolic acidosis at presentation. When other sources of pulmonary blood flow in the form of aorta-pulmonary collateral arteries or a stenosed pulmonary valve are present, however, the patient is often in a stable hemodynamic state despite shunt occlusion[1, 3, 5, 9]. Our patient had the advantage of having aorta-pulmonary collateral arteries, and hemodynamic disturbance was prevented during the procedure.

In most published reports, transcatheter balloon dilatation of stenosed shunts has been accomplished by the arterial route (retrograde). We used the transvenous route (antegrade) in this patient with tetralogy of Fallot. When possible, use of the transvenous route prevents femoral artery injury and permits safe advancement of larger balloons. However, there is a potential risk of arrhythmia with this technique, because the catheter passes through the heart. Our patient did not have any arrhythmia.

We believe that balloon dilatation angioplasty of stenosed Blalock-Taussig shunts is a reasonable and safe alternative to reoperation in patients who are unsuitable for surgical correction and in those for whom the correction needs to be delayed. Fundamental questions remain regarding the magnitude of dilatation, determination of which shunts will respond to dilatation and evaluation of both short-and long-term results.

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ACUTE ALTERNATING HEMIPLEGIA*

A Case Report

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SUMMARY: Tekgül H, Tütüncüoğlu S, Muhan A, Duman Y. (Neurology Unit, Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Acute alternating hemiplegia: a case report. Turk J Pediatr 1996; 38: 521-526.

Acute alternating hemiplegia in childhood is a rare disorder characterized by onset before 18 months of age and frequent attacks of alternating paralysis. In this case report, a 20-month-old boy having the diagnosis of acute alternating hemiplegia is presented. The diagnosis was based on clinical features. The frequency and severity of the hemiplegic attacks decreased following flunarizine therapy. In this case, cerebral perfusion was investigated during ictal and interictal periods. Tc-99m HMPAO-Brain single photon emission computed tomography (SPECT) revealed normal cerebral perfusion in ictal periods and hypoperfusion in interictal periods. *Key words: acute alternating hemiplegia, cerebral perfusion, flunarizine.*

Alternating hemiplegia in childhood is a rare disorder characterized by frequent transient attacks of hemiplegia lasting seconds or days. Verret and Steele[1] first described this disorder in 1971. Since then, fifty cases have been reported[1-5]. Krageloh and Aicardi[2] proposed the diagnostic criteria for this disorder: (1) Onset of disease before 18 months of age; (2) Repeated attacks of hemiplegia involving both sides of the body; (3) Oculomotor abnormalities during the attacks; (4) Autonomic disturbances associated with hemiplegia or occurring independently; (5) Mental and neurological abnormalities (Table I).

Table I: Diagnostic Criteria in Alternating Hemiplegia (Krageloh and Aicardi[2]), modified by Sakuragawa[6]

1. Onset of disease beore 18 months of age.
2. Repeated attacks of hemiplegia involving both sides of the body.
3. Oculomotor abnormalities during the attacks.
4. Autonomic disturbances associated with hemiplegia or occurring independently.
5. Mental and neurological abnormalities.
6. Ruling out related disorders such as metabolic or vascular disorders.

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Although the etiology and pathogenesis of alternating hemiplegia are unknown, it is considered a variant of migraine. The presence of acute onset and transient attacks of hemiplegia suggest a vascular mechanism. These is some evidence both to support and refute this theory[5,6]. Anticonvulsants and conventional antimigraine drugs are ineffective for hemiplegic attacks. A potent antivaso-constrictor, flunarizine, has been reported to be effective in decreasing the duration and severity of hemiplegia attacks[6-10].

In this report, a 20-month-old boy with acute alternating hemiplegia is presented with the findings of ictal and interictal 99m Tc-HMPAO brain single photon emission computed tomography (SPECT).

Case Report

A 20-month-old boy had been born at term with perinatal asphyxia. There was no family history of any neurological disease. At one year of age, attacks of hemiparesis/hemiplegia were noted. These attacks were considered to be postictal paralyses, and several anticonvulsant drugs including phenytoin, carbamazepine and vigabatrin were administered with no benefical effects.

The patient had recurrent attacks of hemiparesis/hemiplegia during the previous eight months. The frequency varied form three to four attacks per month to two to three attacks per day. The duration of the attacks ranged from 10 minutes to 96 hours. Oculomotor abnormalities such as nystagmus, gaze deviation and strabismus were noticed during some episodes. Various autonomic disturbances (tachycardia, dilated pupils, sweating, alterations in skin color) were associated with hemiplegia. He was miserable and very irritable during the attacks, and cried a lot. Sometimes he had bilateral involvement. Dysphagia and respiratory difficulty was observed during the bilateral attacks. Although he never lost consciousness completely during the attacks, he had a decreased response to external stimuli. Symptoms generally disappeared with sleep. He had moderate developmental delay. His neurological examination revealed brisk deep tendon reflexes, but he had no muscle weakness between attacks.

The following investigations were found to be normal: complete blood count; erythrocyte sedimentation rate; renal, liver, and thyroid function tests; and serum levels of potassium, magnesium, glucose, ammonia, creatine kinase, lactate, and pyruvate. Tests for antinuclear factor, anti-DNA and anticardiolipin antibodies were negative. Urine analysis for amino acids as well as metabolic screening were negative. The serum levels of protein C and S, antithrombin III, C_3 and apoprotein were normal. Electrocardiography, telecardiography and hemoglobin electrophoresis were normal. The nerve conduction study and electromyography were also normal.

EEG tracings were obtained during ictal and interictal periods. No epileptogenic discharge was recorded during the attacks. Subcortical paroxysmal activity was noted in the interictal EEG recording. Cranial computed tomography (CT) and magnetic resonance imaging (MR) showed cortical atrophy (Fig. 1). MR angiography was normal (Fig. 2).

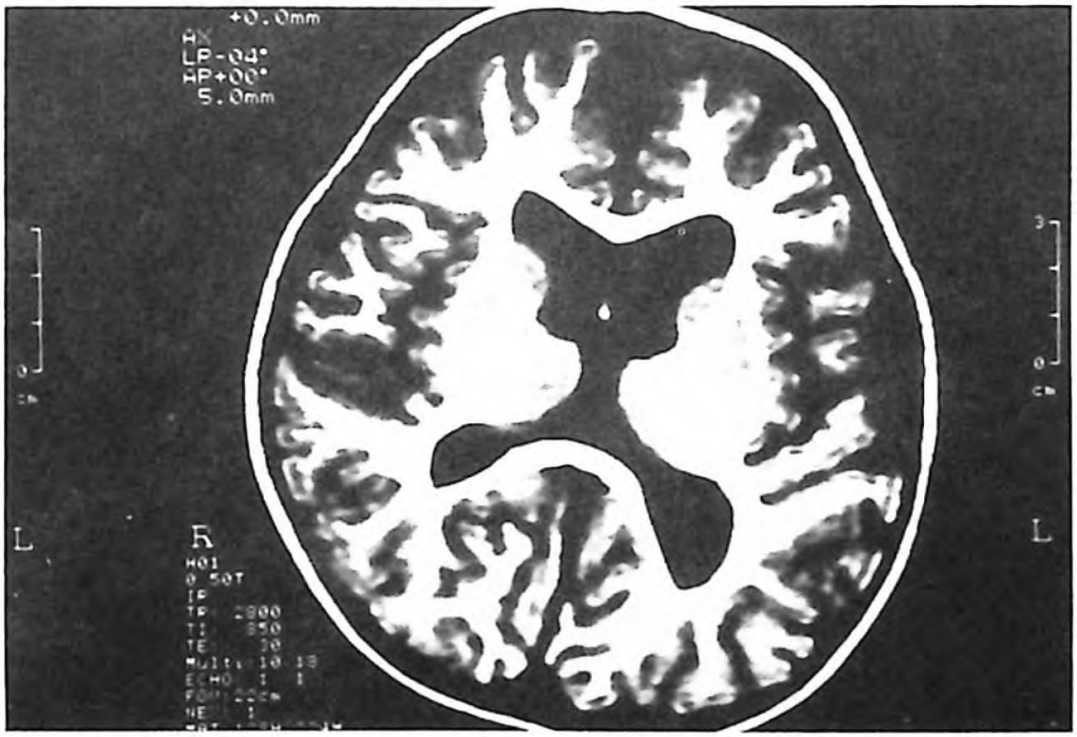

Fig. 1. Axial MR (T2W1): Cortical atrophy.

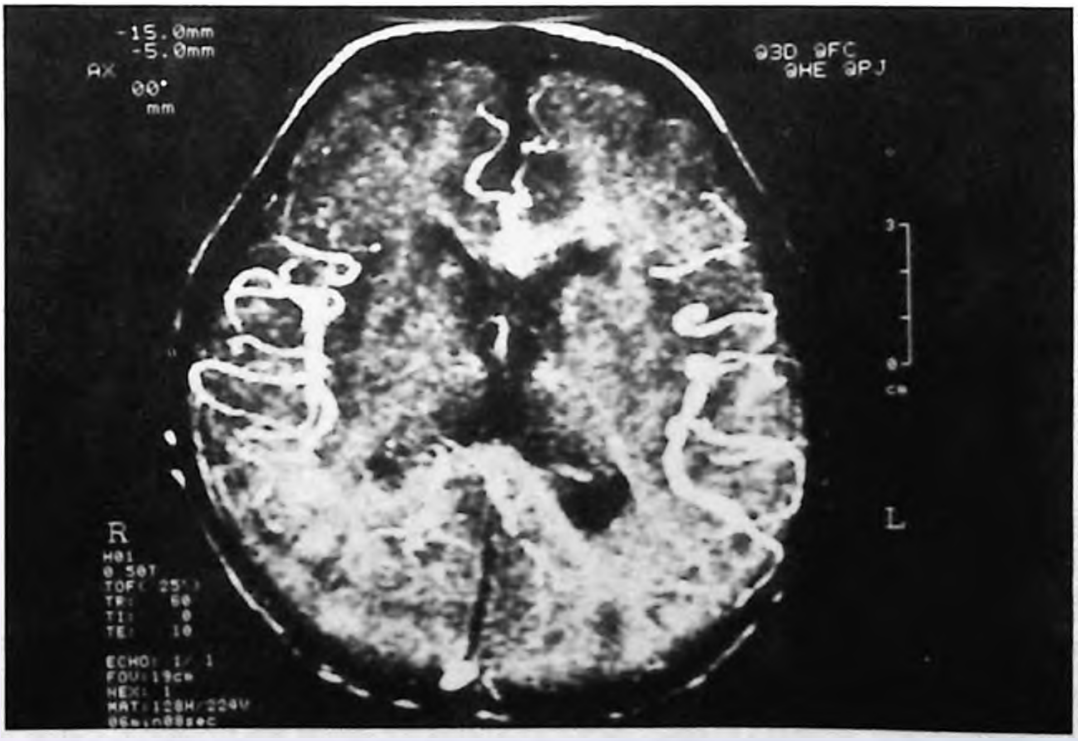

Fig. 2. MR angiography: Normal.

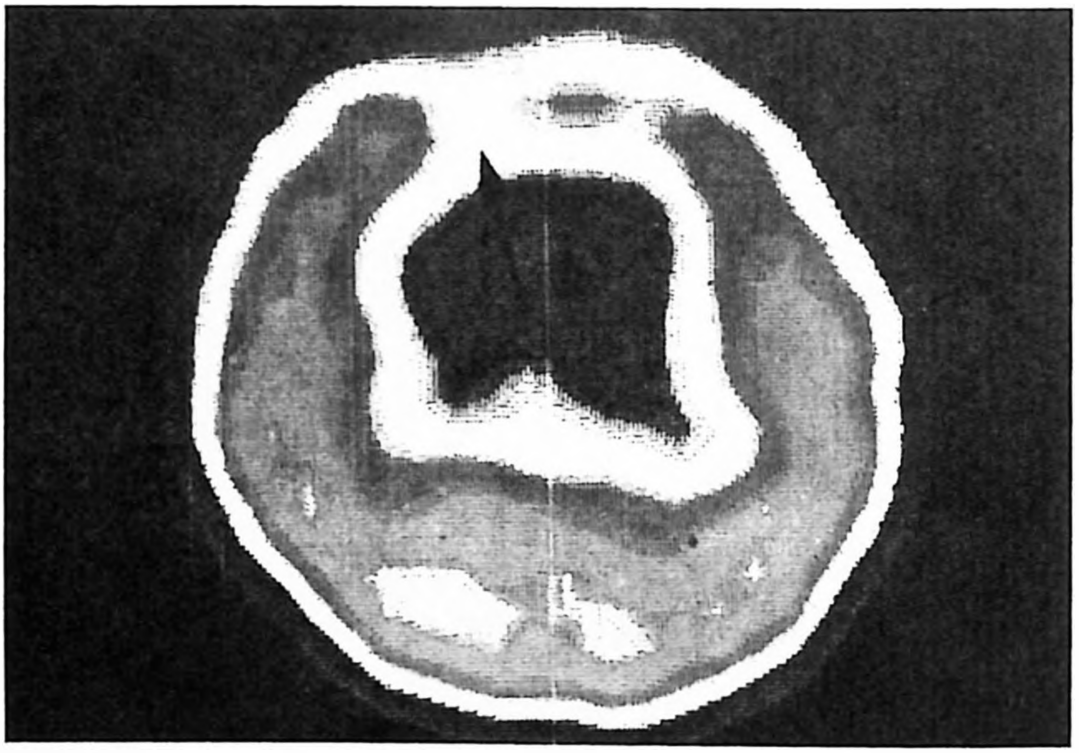

Fig. 3: Interictal Tc99m-HMPAO-brain SPECT: Bilateral hypoperfusion of frontal areas.

Technetium 99m hexamethyl propylamine oxime (HMPAO)-brain SPECT images were taken during the ictal and interictal periods. Although ictal SPECT examination revealed normal cerebral perfusion, interictal SPECT showed hypoperfusion of the bilateral frontal lobes (Fig. 3).

Flunarizine (5 mg/day), a calcium-channel blocker, was initiated to prevent the acute hemiplegic attacks. After five months of therapy, the duration and severity of attacks decreased significantly (1-2 attacks per month). No adverse reaction was observed.

Discussion

Acute alternating hemiplegia is a rare disease characterized by paroxysmal, alternating hemiplegia and/or tetraplagia beginning in infancy. The diagnosis of this disorder is based on clinical findings. Criteria for diagnosis were defined by Krageloh and Aicardi[2] and developed by Sakuragawa[6]. Epileptic seizure, mitochondrial encphalopathy and complicated migraine should be taken into consideration in the differential diagnosis of acute alternating hemiplegia. It may be difficult to determine whether the attacks are true epileptic, because ictal EEG abnormalities may be seen in both disorders; however, the hemiplegic attacks are unresponsive to anticonvulsant therapy. EEG abnormalities have been detected between hemiplegic attacks in two-thirds of patients. EEG slowing during the attacks has also been observed on the contralateral side in half of patients[11]. We could

not find any abnormality in the EEG recordings of our patient during the attacks, but subcortical paroxysmal discharges were noted in his interictal EEG recording. The patient had been diagnosed with epilepsy based on his interictal subcortical epileptic activity at a university hospital. Several anticonvulsant drugs had been prescribed by various physicians. During his stay in our hospital, a generalized tonic-clonic seizure was observed, and phenytoin therapy was continued while the other anticonvulsant drugs were discontinued.

MELAS syndrome (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) may manifest a somewhat similar clinical picture. The presence of normal muscle biopsy findings and normal lactate and pyruvate levels are indicative in distinguishing alternating hemiplegia and MELAS. Although the pathophysiologic mechanism of acute alternating hemiplegia remains obscure, this disorder may be a migraine variant. While a prominent family history of migraine and epilepsy among the first-degree relatives of patients is often found, the parents of our patient had no history of migraine or epilepsy.

A vascular mechanism has been proposed for alternating hemiplegia, but there is no clear evidence to support this theory[3-8]. Cranial CT/MR and angiographic studies do not reveal any evidence of cerebrovascular disease. In advanced cases, cortical atrophy and mild atrophy of the cerebellar vermis may be revealed by CT/MR. Our patient had cortical atrophy and secondary dilatation of ventricles on his cranial MR (Fig. 1). We considered these findings to be the consequence of severe perinatal asphyxia. MR angiography also revealed no abnormal finding (Fig. 2).

Investigations of cerebral perfusion during attacks and between attacks with the use of Brain-SPECT have revealed contradictory results. Ictal SPECT studies have showed hyperperfusion, hypoperfusion or normal findings on the contralateral hemisphere[6, 12, 13]. Zupanc et al.[12] reported hypoperfusion on the left cerebral hemisphere during an acute episode on the right by using 123 I-iodoamphetamine SPECT. They postulated vasospasm as the probable cause of the transient, reversible hypoperfusion of the contralateral hemisphere. In contrast, Kanazawa et al.[13] reported a case with hyperperfusion on the temporal and parietal regions contralateral to the hemiplegic side by 99m-Tc HMPA-SPECT examination. The authors postulated that hyperperfusion may have been due to an underlying seizure phenomenon. Their case was a 12-month-old boy having focal spike discharges on his interictal EEG.

Interictal studies measuring blood perfusion by SPECT and cerebral metabolic rate by PET have showed a decrease in cerebral blood perfusion and metabolism. Sakuragawa[6] reviewed 23 Japanese cases with alternating hemiplegia in childhood. He reported that six of the eight patients showed a slight decrease in regional cerebral blood flow between hemiplegic attacks, usually in the region of a middle cerebral artery by using positron emission tomography (PET) or 133 Xe inhalation. In our patient, 99mTC HMPAO-SPECT showed normal cerebral perfusion during the attack and bilateral hypoperfusion of the frontal areas between hemiplegic attacks (Fig. 3). Based on these ictal and interictal SPECT

findings, it may be postulated that transient vasospasm is the underlying mechanism for recurrent hemiplegic attacks due to brain perfusion changes. Contradictory SPECT results may be secondary to different scanning times after the onset of the hemiplegic attacks.

Acute hemiplegic attacks do not respond to any anticonvulsant drugs. A potent calcium-channel blocker, flunarizine, has been reported to be effective in decreasing the duration and severity of hemiplegic attacks[6-10]. In an international collaborative study, Casaer[10] reported that flunarizine was the first truly promising drug for alternating hemiplegia. The mode of action of flunarizine is unknown, but it may exert an effect depending on its antihypoxic, antivasoconstrictive and anticonvulsant properties. Our case also responded well to 5 mg/day aflunarizine therapy. After five months of therapy, the duration and severity of attacks decreased significantly.

Acute alternating hemiplegia should be considered in patients with recurrent hemiplegic attacks. Early diagnosis and treatment may change the course of the disorder.

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INFANTILE MYOFIBROMATOSIS: A CASE WITH UNUSUAL FEATURES AND REVIEW OF THE LITERATURE*

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SUMMARY: Kotiloğlu E, Göğüş S, Ruacan Ş, Akyüz C, Büyükpamukçu M, Sarıalioğlu F. (Pathology and Oncology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Infantile myofibromatosis: a case with unusual features and review of the literature. Turk J Pediatr 1996; 38: 527-532.

A three-month-old boy was admitted for a slowly progressing nodule in his right chin, first recognized at birth and recently accompanied by facial paralysis. It was found to be a soft tissue mass arising in subcutaneous tissue and extending deep into the temporal muscle, causing temporal bone erosion without infiltrating the dura. Initially interpreted as mesenchymal chondrosarcoma, combined chemotherapy (PULSE-VAC) was given. Eighteen months later an additional nodule developed in the paraspinal skin. Evaluation of both lesions showed vimentin and α-smooth-muscle-action positivity. As the final diagnosis was multicentric infantile myofibromatosis, the child was followed up without therapy. Thirty months later, multiple osteolytic lesions appeared on the skull and long bones of the extremities. Some lesions remained stable and some regressed during two years of follow-up. *Key words: myofibromatosis, mesenchymal hamartomas, immunohistochemistry, childhood tumors.*

Childhood mesenchymal tumors of fibroblastic and/or myofibroblastic deviation are an adverse group of tumors with pathological features occasionally causing uncertainty about clinical behavior, treatment and prognosis. Infantile myofibromatosis (IM) is the most frequent tumor type among these, but it is not widely known and has been frequently misdiagnosed[1,2]. Here we report a case with an unusual course and review the relevant literature to familiarize both physicians and pathologists with the unique features of this tumor.

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Case Report

A three-month-old male was admitted in April 1990 for evaluation of a mass in his right chin, first noticed at birth. Its growth had been slow and progressive. One month prior to admission, signs of facial paralysis were noticed by his parents while he was crying. He was born at term as the first child of healthy parents with no consaguinity. There was no history of similar lesions in the family.

On physical examination, the tumor was palpated as an immobile, non-tender and firm nodule about two centimeters in diameter. A computerized tomography (CT) scan revealed temporal bone erosion without involvement of the temporal lobe. Other physical and laboratory findings were normal.

The tumor was totally excised along with the temporal muscle that it invaded. Both the fascia and the dura were intact. Microscopically the tumor was composed of spindle-shaped cells arranged interlacing fascicles at the periphery with myxoid and chondroid areas, especially in the center (Fig. 1). Mitotic figures were frequent (2-3/high-power field). The initial diagnosis was mesenchymal chondrosarcoma. It was accepted as stage II disease and combined chemotherapy (PULSE-VAC) was initiated[3]. Because of the patient's age, low doses were administered (Vincristin: 1 mg/m^2, Actinomycin-D: 10 μ/kg, Cyclophosphamide: 10 mg/kg); radiotherapy was not given. This protocol was carried out for one year with no problem. Several CT scans and pulmonary x-rays taken during this period were normal.

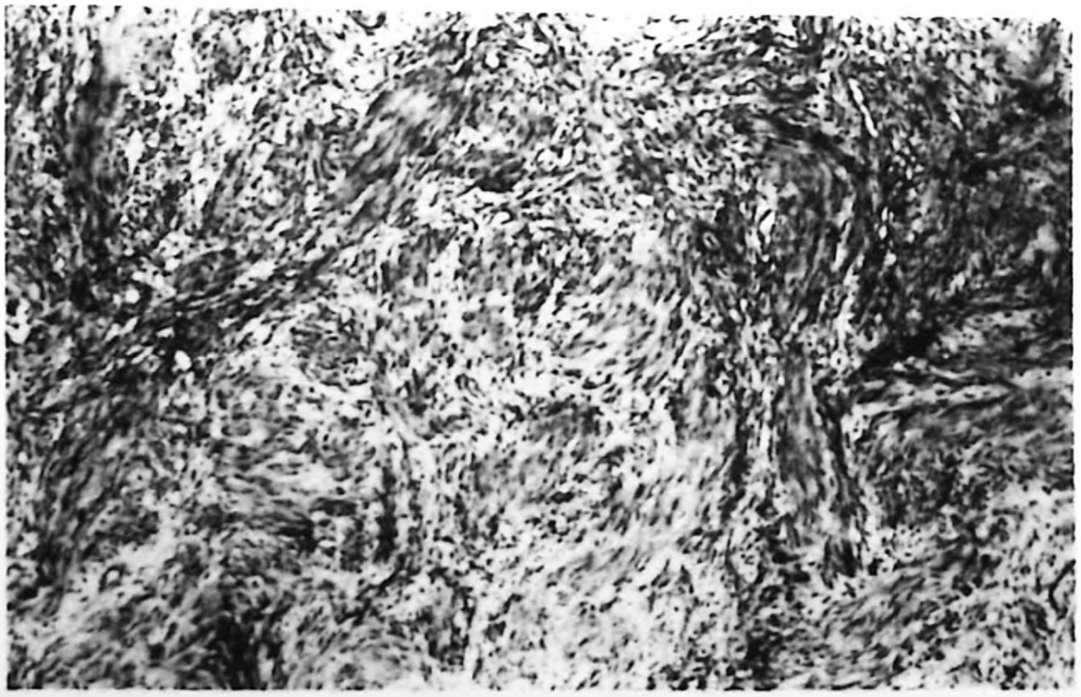


Fig. 1: Interlacing fascicles of spindle cells at the periphery of the tumor. (Trichrome stain, x 40).

In November 1991, a firm, paraspinal, subcutaneous 1.5 x 1.5 cm nodule was palpable in the left thoracolumbar area. X-ray and CT scan of this area were normal. Histologic evaluation of the excisional biopsy revealed hyalinized to hypercellular interdigitating bundles and nodules of spindle cells (Fig. 2). Mitotic figures were sparse. There were neither ossification and cartilage formations necrosis nor hemagiopericytoma-like areas. These findings were consistent with IM. The first tumor was also re-evaluated, and both tumors were found to be vimentin and a-smooth muscle actin-positive (Avidin-biotin peroxidase method with BioGenex Lab. antibodies). The diagnosis was revised as the multicentric form of IM. Chemotherapy was dicontinued. Skeletal radiograms, cranial CT and magnetic resonance imaging revealed no additional lesions. However, in October 1992, a skeletal survey revealed several osteolytic lesions on the skull and long bones of the extremities (Figs. 3, 4). Some of these lesions remained stable and some even regressed during the two-year follow-up period.

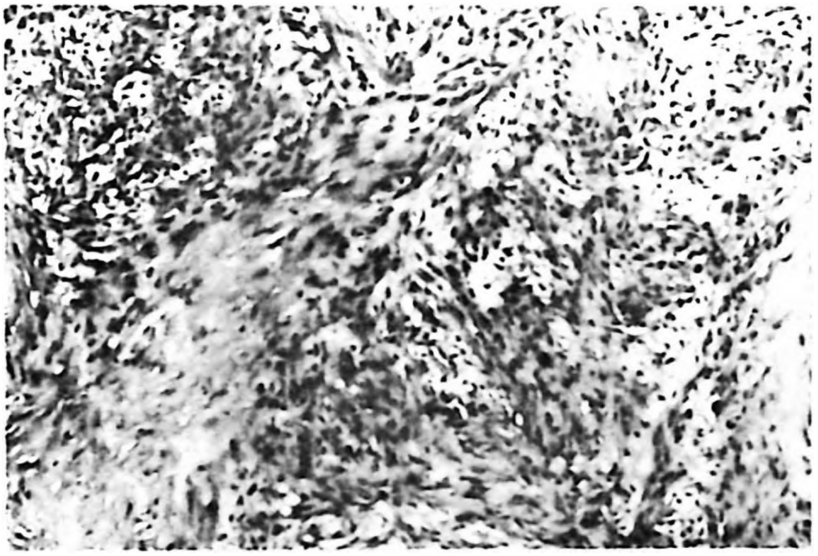
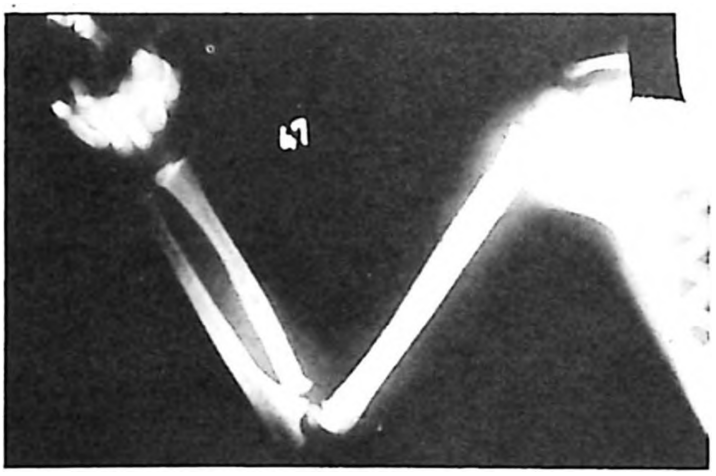
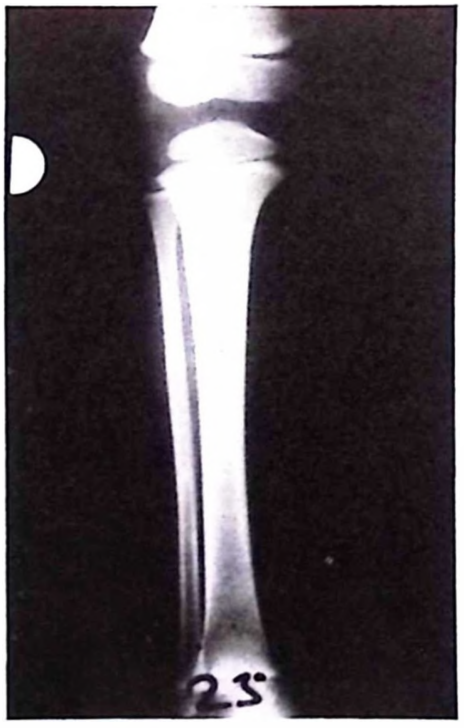


Fig. 2: Hypercellular, partially hyalinized interdigitating bundles of spindle cells. (H + E, x 100).

Fig. 3. Several osteolytic lesions of the skull



(a)



(b)

Fig. 4: Well-defined radioluscencies in the metadiaphyseal regions of the (a), radius and (b) tibia.

Discussion

Infantile myofibromatosis has formerly been described as multiple congenital mesenchymal hamartomas; fibromatoses; congenital diffuse, generalized hamartomatosis; congenital generalized fibromatosis and multiple vascular leiomyomas[1,2,4]. The diagnosis is established either at birth or during the first year of life[1,5,6]. A solitary, adult counterpart of this entity has also been described[7]. There is a male predominance of 1.6: 1[8,9].

The lesions cause few symptoms except for occasional pain caused by compression of nerves[8, 9]. In our case, facial paralysis was observed.

Although the cause of IM is unknown, its relation to estrogen hormones has been proven experimentally[8]. Both an autosomal-dominant[5] and an autosomal-recessive[10] pattern of inheritance have been described. Major malformations such as esophageal atresia and hypoplastic right kidney have been reported in an infant with IM[11].

There is still considerable debate as to the nature and histogenesis of this condition. It is probably of hamartomatous origin. The prominent cell type is the myofibroblast, which may exhibit a range of phenotypes including vimentin, α-smooth muscle actin, desmin and collagen type IV[1, 2, 12]. IM seems to display more advanced smooth-muscle differentiation than conventional fibromatoses[12, 13]. This differentiation was featured by α-smooth-muscle-actin positivity in our case.

IM occurs in either a solitary or multicentric form[1, 2, 4]. Chung and Enzinger[14] found solitary lesions to be at least twice as common as multiple lesions. Solitary lesions arise in subcutaneous tissue, muscle or bone. In the multicentric form, there are multiple soft-tissue, bone and visceral lesions[2]. Visceral lesions are not always associated with the multicentric form[14, 15]. If visceral lesions are present, the prognosis is poor[11, 16, 17]; otherwise the tumor follows a benign course, often with spontaneous regression[1, 9, 10]. Thus, conservative surgical treatment with local excision is recommended, but local recurrence has been documented in some cases[1, 6].

In the multicentric form, there may be a single nodule at the time of initial diagnosis, and formation of new nodules may be observed during the first few months of life[2, 8, 9, 18, 19]. New nodules are usually reported to appear within eight weeks, but in familial IM new nodules developed as late as four age[20]. Although there was no family history of similiar lesions in our case, a second subcutaneous nodule and multiple osteolytic lesions appeared 18 and 30 months after the initial nodule, respectively.

Bone involvement is represented as well-defined radioluscencies in the medulla of the craniofacial skeleton and metadiaphyseal regions of long bones[1, 2, 6, 7]. Therefore the radiological differential diagnosis of bone lesions includes Langerhans' cell histiocytosis, fibrous dysplasia, metaphyseal fibrous defect, aneurysmal bone cyst, chondromyxoid fibroma, familial multiple non-osteogenic fibromata, neurofibromatosis, metastatic neuroblastoma and hemangiomatosis[5, 6]. The histologic differential diagnosis presents greater difficulties, especially if the tumor is solitary. It must be differentiated from several soft tissue neoplasms, including fibrosarcoma, leiomyoma, leiomyosarcoma, hemangiopericytoma, neurofibroma and juvenile hyaline fibromatosis[5, 16]. Because of these difficulties, some cases have been misdiagnosed as sarcomas and given chemotherapy[11, 19].

Furthermore, chemotherapy has been initiated to prevent the rapid course of a multicentric form with visceral involvement[10] and based on the decision of the clinician in another case[20], but did not alter the clinical course in either.

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HYPOTHALAMIC HAMARTOMA WITH GELASTIC EPILEPSY, PRECOCIOUS PUBERTY AND POLYDACTYLY*

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SUMMARY: Turanlı G, Aynacı M, Yalnızoğlu D, Renda Y. (Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Hypothalamic hamartoma with gelastic epilepsy, precocious puberty and polydactyly. Turk J Pediatr 1996; 38: 533-536.

An entity including gelastic epilepsy, precocious puberty, polydactyly and a hypothalamic hamartoma type IIa is described in a 16-year-old female patient. Polydactyly was detected at birth, she developed precocious puberty at four years of age, and gelastic epilepsy was diagnosed at age seven. The precocious puberty was successfully treated medically and her treatment was discontinued at the age of 10 years, but the gelastic seizures were difficult to control. When the patient was 11 years old, MRI revealed a hypothalamic hamartoma. The combination of these four features is very rare in the literature. *Key words: gelastic epilepsy, hypothalamic hamartoma, precocious puberty, polydactyly.*

Primary tumors of the hypothalamus are less common than other primary, intracranial tumors in childhood[1]. Hamartomas are non-neoplastic malformations and constitute most hypothalamic tumors[2]. Although various associations between hypothalamic hamartoma and gelastic epilepsy have been reported in the literature, the association of hypothalamic hamartoma, gelastic epilepsy, precocious puberty and skeletal abnormalities is very rare[1, 3, 4]. The first case in which all four features have been described was reported in 1994[1]. Here too we present a girl with hypothalamic hamartoma, gelastic epilepsy, precocious puberty and polydactyly.

Case Report

The patient was first admitted to Hacettepe University's Department of Pediatric Endocrinology with symptoms of vaginal bleeding and breast development at four years of age. She was diagnosed with isosexual precocious puberty after laboratory investigations were completed, and was given cyprateron acetate therapy. Treatment was discontinued at the age of 10 years after ensuring that puberty was progressing normally.

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At 12 years of age she was admitted to Hacettepe University's Department of Pediatric Neurology with gelastic seizures at a frequency of three to four episodes per day. Since the age of seven she had been experiencing uncontrollable laughing attacks lasting one to two minutes followed by urinary incontinence every two to three weeks. She also had generalized tonic-clonic seizures lastings one to two minutes which had started two years before admission. Aside from mild mental retardation and polydactyly, her physical and neurological examinations were normal on admission. EEG revealed normal background rhythm with left centro-temporo-occipital spike and slow-wave discharges and secondary generalization. Cranial tomography was normal. She was put on carbamazepine therapy, but after three months of treatment no improvement was observed in her seizures. Her magnetic resonance imaging (MRI) revealed a mass in the tuber cinereum. T_1-weighted MRIs demonstrated a hypo-intense hypothalamic mass, 2 cm in diameter, bulging into the floor of the third ventricle, with contact with the mamillary bodies. This lesion was seen observed to be hyperintense on T2-weighted images. There was no abnormality in her cerebral angiography.

Surgery for cranial mass resection was recommended but refused by her family. After a four-year interval with anti-epileptic treatment, she returned to our clinic. At present she is 16 years old and has been receiving carbamazepine therapy for four years, but she experiences one to three laughter episodes every two weeks.

Discussion

Hypothalamic hamartomas are defined as heterotopic and hyperplastic tissue resembling gray matter in various sizes[5]. They usually originate from the tuber cinereum and mamillary bodies[6,7]. They are often associated with cerebral and extracerebral congenital abnormalities such as microgyria, cysts, callosal defects, polydactyly, facial abnormalities and heart defects[8,9]. Nurbhai et al[10] reported an infant with skeletal abnormalities including syndactyly which seems to be the most common skeletal defect occurring with hypothalamic hamartomas. Clinically, hypothalamic hamartomas may be accompanied with precocious puberty, gelastic epilepsy and other type of seizures[7]. MRI is the most sensitive method for detecting hypothalamic hamartomas[11]. In our case, the lesion did not appear on cranial tomography but was detected on MRI.

Abnormal neuronal connections or independent endocrine activity of hypothalamic hamartomas may result in precocious puberty[12-14]. The pathogenesis of gelastic epilepsy has been described as mechanical compression the mamillary bodies and/or pathological neuronal connections to the hypothalamus and limbic system[2].

Uncontrollable pathological laughter may result from several causes, such as bilateral cortico-bulbar lesions, multiple sclerosis, amyotrophic lateral sclerosis, bilateral frontal lobe disease, hysterical laughter and hebephrenic schizophrenia.

The criteria for gelastic epilepsy were described by Gascon and Lombroso[15] in 1971. Thesė criteria are: stereotypical recurrence, the absence of external precipitating factors, concomitance of other epileptic manifestations, the presence of ictal and interictal EEG abnormalities, and the absence of other causes of pathological laughter. All of these criteria were also present in our patient.

Valdueza et al.[2] classified hypothalamic hamartomas into two groups and types Ia, Ib and IIa, IIb according to their localizations, type of attachment, extent of hypothalamic displacement and size. Type Ia and Ib lesions are characteristically medium in size, either asymptomatic or associated with precocious puberty. There is no hypothalamic displacement. Type Ia originates from the tuber cinereum, and Ib from the mamillary body.

Type IIa hamartomas are usually larger than 1.5 cm, their attachment is sessile, and they are localized on the tuber cinereum or mamillary body. They result in slight hypothalamic displacement. Patients usually suffer from mixed seizures (gelastic, other seizures and memory disturbances). Our patient was classified as type IIa with the presence of generalized tonic-clonic and gelastic seizures and the absence of hypothalamic displacement on MRI. In type IIb hypothalamic displacement is marked. The prognosis is poor and control of seizures is difficult in these patients. Precocious puberty can be managed medically with a good effect[1]. According to Valdueza et al.[2], the surgical approach is a realistic alternative in certain cases, including large and broad-based type IIb hamartomas associated with gelastic epilepsy and behavioral disorders. Medical therapy controlled precocious puberty in our patient but the treatment was unable to control her gelastic seizures.

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The Turkish Journal of Pediatrics 1996; 38: 537-542

TRICHO - RHINO - PHALANGEAL SYNDROME TYPE I*

*Yasemen Eroğlu MD***, *Derya Erçal MD****

SUMMARY: Eroğlu Y, Erçal D. (Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Tricho-rhino-phalangeal syndrome type I. Turk J Pediatr 1996; 38: 537-542.

Tricho-rhino-phalangeal syndrome type I (TRPS I) is characterized by a bulbous nose, sparse hair and epiphyseal coning. Autosomal dominant and recessive transmission are suggested. The presence of cone-shaped epiphyses, the major complaint of patients due to swelling over the phalangeal joints, requires differential diagnosis among various syndromes. This paper, describing a ten-year-old girl with TRPS I, aims to bring the features of the syndrome to medical attention. *Key words: tricho-rhino-phalangeal syndrome, cone-shaped epiphysis.*

Tricho-rhino-phalangeal syndrome type I (TRPS I) is characterized by sparse, fragile scalp hair, bulbous nose and radiological abnormalities of the hands, mainly epiphyseal coning[1]. Unusual facies and hair may be detectable from birth, but usually children are first brought to medical attention because of swelling over the phalangeal joints. This report presents a 10-year-old girl who had the characteristic features of TRPS I.

Case Report

A 10-year-old girl was admitted to our clinic because of a one-year history of painless swelling of her fingers. Her history was otherwise unremarkable, except that her parents had first-degree consanguinity. There were no similar symptoms among relatives or siblings. Her height was 131 cm (10^{th}-25^{th} percentile). She had sparse and fine hair, bulbous nose, prominent ears, a long philtrum and micrognathia (Figs. 1a, b). A thin upper lip covering the lower, a high-arched palate and malpositioned teeth were noted. Widely positioned nipples were also detected (Fig. 2), along with broadening and slight flexion of the proximal interphalangeal joints and deviation of the phalangeal axis, with no limitation of motion (Fig. 3). She had fragile and brittle toenails, hypoplasia of the fourth and fifth toes and a short first toe as well as cutaneous syndactyly between the second and third toes (Fig. 4). her mental ability was not impaired.

X-ray examination of the hands revealed cone-shaped epiphyses in the proximal and middle phalanges of the second, third and fifth digits, in the proximal and distal phalanges of the first digit and in the middle phalanx of the fourth digit (Fig. 5).

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*** Associate Professor of Pediatrics, Dokuz Eylül University Faculty of Medicine.

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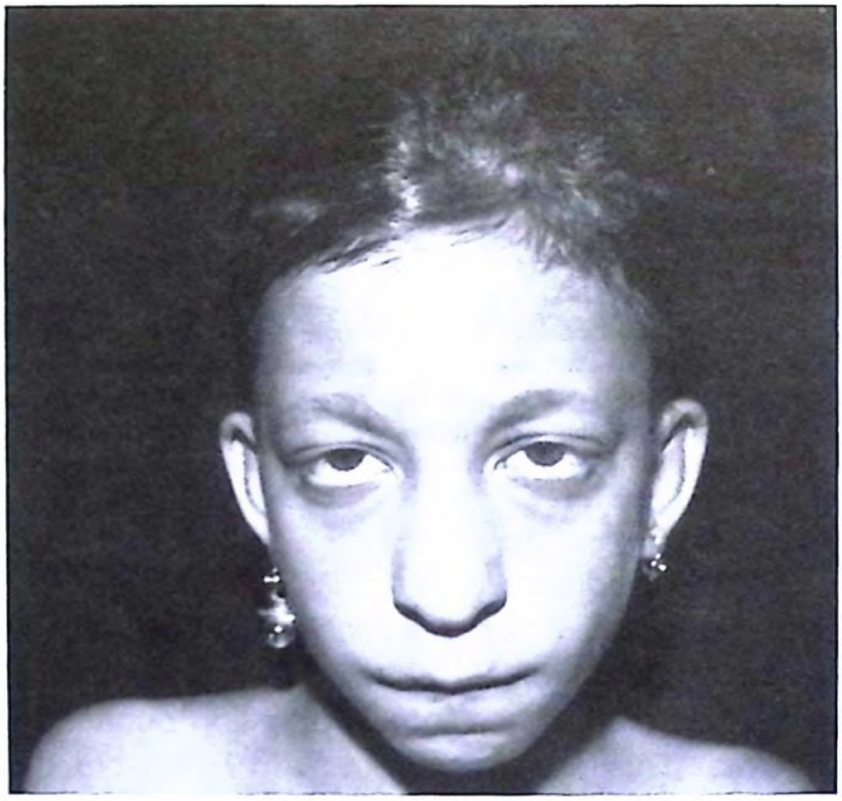
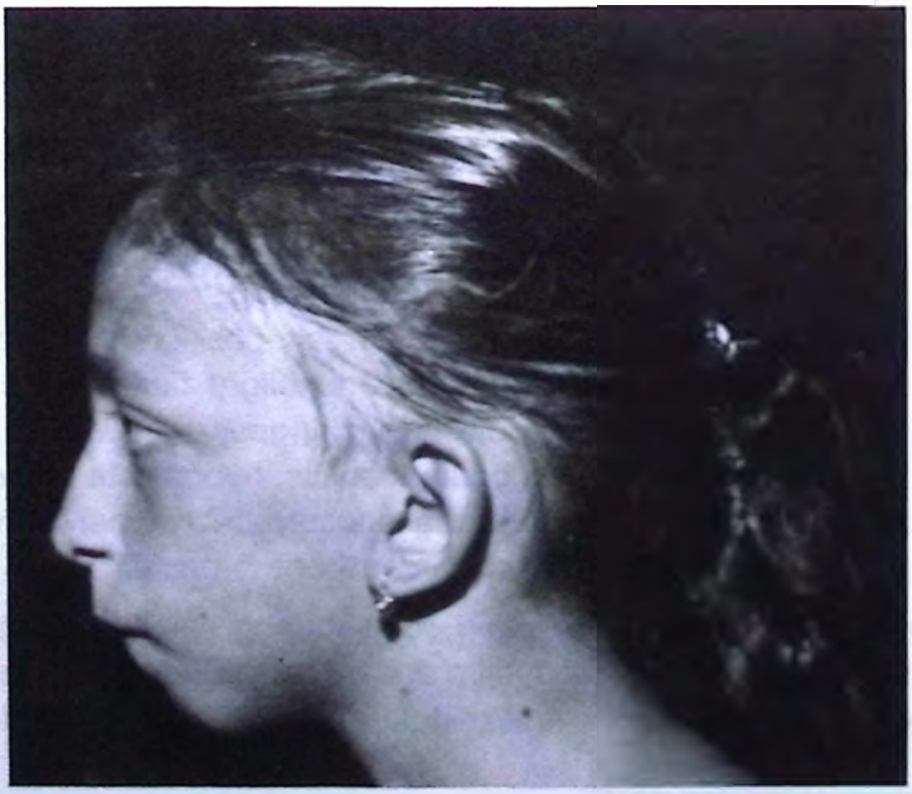


(a)



(b)

Figs. 1a, b: Sparse hair, bulbous nose, prominent and low-set ears, long philtrum, micrognathia and thin upper lip covering the lower were the prominent features.

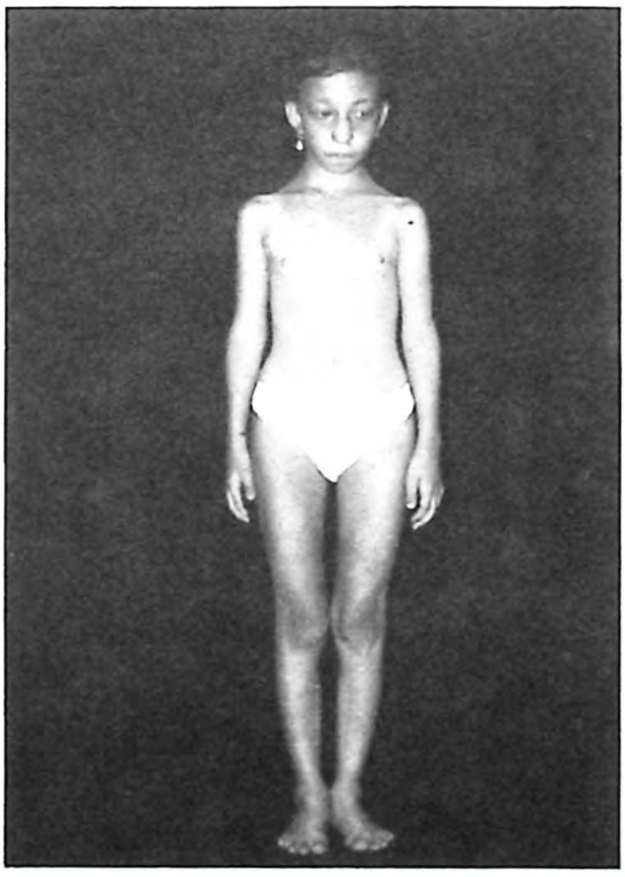


Fig. 2: Widely positioned nipples and coxa vara deformity.

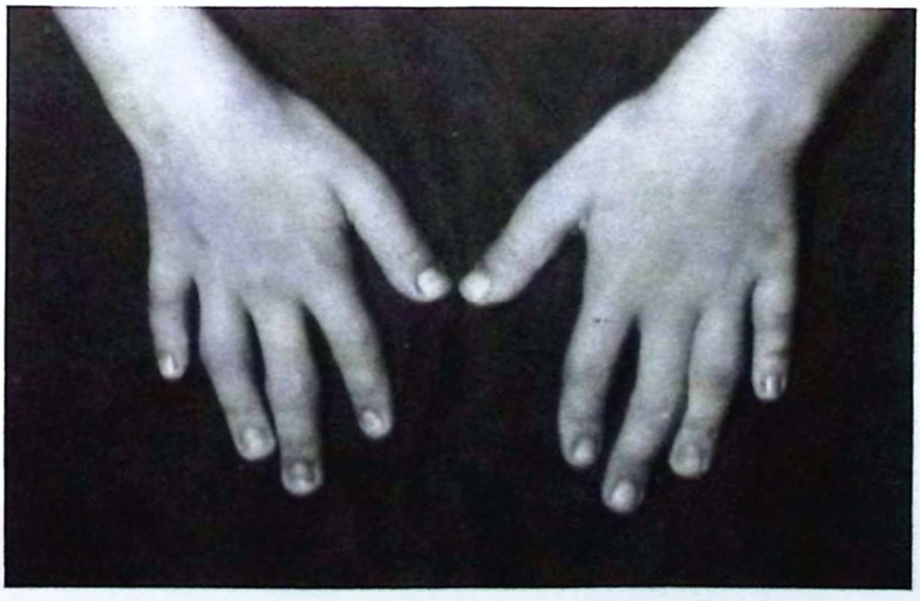


Fig. 3: Broadening and slight flexion of the proximal interphalangeal joints plus deviation of the phalangeal axis can be seen.

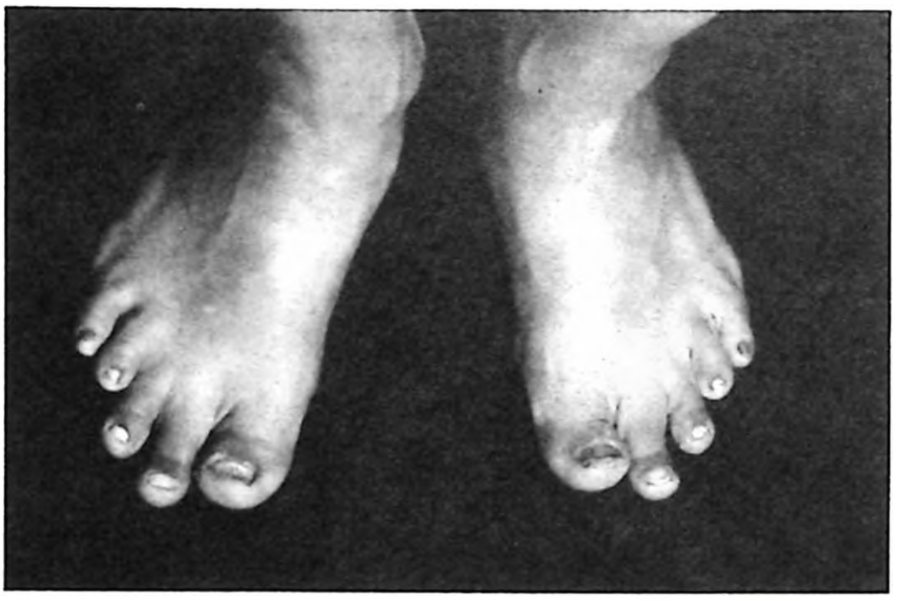


Fig. 4: Fragile and brittle toenails, hypoplasia of the first, fourth and fifth toes and cutanous syndactyly between the second and third toes.

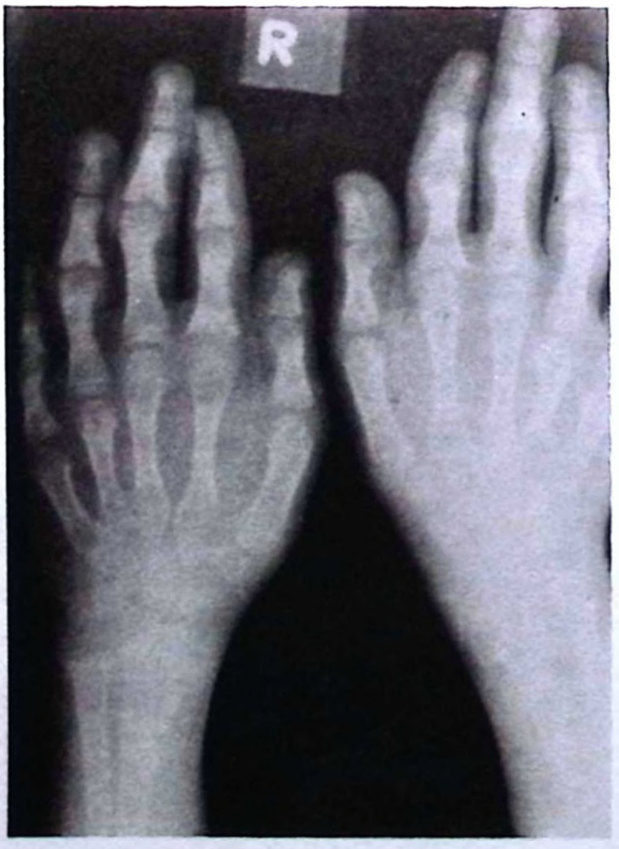


Fig. 5: Cone-shaped epiphysis in the proximal and middle phalanges of the second, third and fifth digits, in the proximal and distal phalanges of the first digit and in the middle phalanx of the fourth digit.

Discussion

The main features of TRPS I are facial dysmorphism; sparse, brittle and usually light-colored scalp hair; and short hands and feet with finger deformities. Facial dysmorphism includes a bulbous or pear-shaped nose, large and prominent ears, a long, broad philtrum, a thin upper lip and poorly developed eyebrows laterally. Skeletal changes include numerous, phalangeal, cone-shaped epiphyses of the hands, often associated with brachymetacarpism and brachymetatarsism. Clinically, cone-shaped epiphyses appear as swelling of the interphalangeal joints, mainly the proximal ones, and sometimes lead to deviation of the phalangeal axis[2]. There is little or no limitation of motion. Flattening of the capital femoral epiphyses, partial syndactyly, scoliosis, kyphosis, winged scapula, thoracic deformity, dental malocclusion, short stature and mental deficiency may sometimes accompany the main features. The nails are usually thin and brittle[1,2].

Our patient demonstrated the characteristic presentation of the syndrome. She did not have growth failure or mental deficiency. She was the only person with syndromic features in the family. Considering the parents' consanguinity, this patient represents the autosomal-recessive type of the syndrome. In TRPS I, autosomal-dominant transmission is usually suggested, but an autosomal-recessive pattern apparently occurs in affected children of normal parents, as in our case[3,4]. The chromosomal basis of the syndrome has been studied with prometaphase chromosomes; lately, small deletions in chromosome 8 (8q23, 8q24.12) have also been demonstrated and this region (8q24.12) provides the TRPS I gene[5-9].

The differential diagnosis of TRPS I should include TRPS II (Langer-Giedion syndrome) and TRPS III (Sugio-Kajii syndrome) as well as the disorders associated with cone-shaped epiphyses. In TRPS II, multiple cartilaginous exostoses of the long tubular bones, microcephalic mental retardation and short stature are the additional characteristic findings. TRPS III is characterized by the clinical features of TRPS I plus a severe form of generalized shortness of all phalanges, metacarpals and metatarsals and sometimes limitation of joint movements, pectus carinatum and thoracic scoliosis. Our patient had none of these features. Apart from normal variants, cone-shaped epiphyses can be seen in various disorders (Table I)[1-3,11]. Associated features of these syndromes are important in the differential diagnosis of TRPS I.

TRPS I is a syndrome with a normal life expectancy. Osseous changes may develop in early childhood and worsen until adolescent growth is complete. Progressive osteoarticular changes and degenerative hip disease may necessitate orthopedic care. Psychological guidance for dysmorphic features and genetic counseling may be considered.

Table I: Disorders Associated with Cone Epiphyses

Normal Variant	
Congenital Syndromes	
Achondroplasia	Dyggve-Melchior-Clausen
Acrodysostosis	Kaschin-Beck
Acromesomelic dysplasia	Orofaciodigital
Asphyxiating thoracic dysplasia	Osteoglophonic
Beckwith-Wiedemann	Otopalatodigital
Bilginturan	Peripheral dysostosis
Chondrodysplasia punctata	Pseudoachondroplastic dysplasia
Chondroectodermal dysplasia	Saldino-Mainzer
Christian	Seckel
Cleidocranial dysostosis	Trichorhinophalangeal I
Cockayne	trichorhinophalangeal II
Acquired	
Frostbite	
Infection (meningococcemia, viruses)	
Infarction (sickle cell)	
Trauma	

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ANNOUNCEMENTS

BOOK REVIEW

Böhles H, Hofstetter R, Sewell AC (Editors). *Metabolic Cardiomyopathy.* Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH, 1995. 176 pp. DM 72 ISBN 3-8047-1429-3.

There is mounting evidence that a considerable number of cardiomyopathies are genetically determined and are due to various biochemical defects. Correct diagnosis of such disorders may sometimes enable clinicians to treat the conditions successfully, as in carnitine replacement therapy in cardiomyopathy due to carnitine deficiency, and may make it possible for them to help affected families by way of prenatal diagnosis.

In addition to three editors, 11 scientists contributed to this book, which consists of 14 chapters with 52 figures and 34 tables. The first chapter of the book is devoted to cardiac energy metabolism, and serves as a basis for the following chapters. Covering almost all aspects—symptomatology, clinical and laboratory diagnosis, including endomyocardial biopsy and treatment—of metabolic diseases with cardiac involvement, this book is highly recommended for all who are interested in metabolic disorders or cardiology, as well as for general pediatricians.

Turgay Coşkun, MD

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References to books:

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

References to chapters in books:

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

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