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CONTENTS

Newborn PKU screening in Turkey: at present and organization for future

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SUMMARY: Özalp İ, Coşkun T, Tokatlı A, Kalkanoğlu HS, Dursun A, Tokol S, Köksal G, Özgüç M, Köse R. Newborn PKU screening in Turkey: at present and organization for future. Turk J Pediatr 2001; 43: 97-101.

At present, pkenylketonuria screening is a national child health program in Turkey which is carried out collaboratively by the Ministry of Health and three University Children's Hospitals in Ankara, İstanbul and İzmir. Since 1986 the number of cities included in the screening program has gradually increased, now and it covers all the metropolises the country. A total of 383 babies were found with persistent hyperphenylalaninemia (1:4172) among 1,605,582 babies screened by the Guthrie test at the Hacettepe Screening Center in Ankara. By taking into account pretreatment phenylalanine levels and phenlyalanine tolerances at five years of age, the numbers of classical and mild-moderate phenylketonuria and mild hyperphenylalaninemia cases were 216, 102 and 58, respectively.

The major problems encountered in the screening program and in management of the detected cases were unsatisfactory sample collection, early discharge from maternity hospitals, difficulties in reaching some detected cases, and noncompliance with dietary therapy due to illiterate parents or to lack of social insurance. To screen and treat all newborns for phenylketonuria and to include at least hypothyroidism in the screening program, there is a need for a more disciplinary intersectoral approach than exists at present.

Key words: phenylketonuria, national screening, screening problems.

Due to improvements in health services, and in environmental and socioeconomic conditions, the pattern of morbidity has changed significantly in Turkey. It can be clearly observed that preventable diseases such as infectious and nutritional disorders became minor problems, while other disorders such as inborn errors of metabolic diseases, congenital malformations and malignancies are listed among the most common diseases[1]. Thus it becomes very important to assess the magnitude of problems related to the above-mentioned disorders, to take preventive measures to decrease their incidence, and to screen for the ones that can be treated when diagnosed early.

Based on our clinical observations and then a pilot study we carried out in Ankara, the incidence of phenylketonuria (PKU) is quite high and screening for this particular disease needs to be considered a desirable part of preventive medicine[2,3]. Therefore we began in 1986 to screen newborn babies for PKU on a nation wide basis in collaboration with the Ministry of Health. We will report here the screening organization we have reached at present, some of our results, problems encountered thus far, and our proposed organization for a more effective mass-newborn screening in future.

PKU Screening Program at Present

The screening program at first covered newborns born in maternity hospitals in the metropolitan areas of 27 cities in Turkey. The number of provinces included in the program was increased gradually, and the program now covers all the metropolises in Turkey. At present

we have three PKU screening centers. In addition to Ankara, newborns born in 77 out of 80 cities in Turkey are currently being screened at the oldest and largest screening center in Ankara at Hacettepe University Children's Hospital. Infants born in İstanbul and in İzmir can be screened locally in the other two centers at the Department of Pediatrics, İstanbul University, İstanbul Faculty of Medicine and at the Department of Pediatrics, Dokuz Eylül University Faculty of Medicine. It is a national child health program which is being carried out collaboratively by the Ministry of Health and the above-mentioned universities. Collection of blood samples from the newborns is organized by the Ministry of Health. Laboratory costs of the screening in the beginning were supported financially by The Turkish Society for PKU and Allied Disorders. At present costs are partly supported by the Ministry of Health as well. There is a recently established foundation METVAK to cover the costs of therapy and management of the detected cases with PKU who do not have any insurance.

PKU Screening Results

Table I shows the newborn screening results of 1,605,582 infants from 78 cities using the Guthrie bacteriological inhibition assay. High pressure liquid chromatography (HPLC) is used for the quantitative phenylalanine (phe) determinations. For the classification of PKU pretreatment phe levels of detected cases and their phe tolerance around five years of age are taken into account[4]. Since we could not assess phe tolerance in detected cases too young to be evaluated, results of newborns screened after June 1998 are not included in this Table. A total of 383 babies were found to have persistent hyperphenylalaninemia. (HPA) (1:4172). Three hundred and eighteen out of these cases were diagnosed as having PKU (1:5049). Two hundred and sixteen out of 318 cases with PKU were classified as having classical PKU and 102 cases as having mild-moderate PKU. Only 58 cases had mild HPA and did not need any diet therapy. Interestingly, initial serum phe levels were around 10 mg/dl in a few patients who were fed solely by breast milk. Blood phe levels of these patients, however, increased to levels compatible with classical PKU. These cases point to the fact that there might be similar cases in countries like Turkey were sole breast-feeding is a common practice in the first months of life. In fact, Berlin et al.[5] also reported similar cases with PKU.

Table I. Incidence of Hyperphenylalaninemias (HPA) in Turkey (n: 1,605,582)*

Type of HPA	No of detected cases	Incidence
PKU	318	1/5049
Classical	216	
Mild-Moderate	102	
Mild HPA	58	
Defect in BH4 Metabolism	7	
Total	383	1/4192

* January 1986-June 1998
False positivity: 0.4%

We have rather recently begun to routinely screen detected cases for BH_4 deficiency. The frequency of this type of HPA seems to be at least two percent, which is comparable with the incidence of BH_4 deficiency in some other eastern countries. In earlier reports it was also indicated that BH_4 deficiency is not rare in Turkey[6,7].

False positivity was 0.4 percent. Since neither false positive nor false negative results were observed in the unknown samples periodically sent from Germany for interlaboratory quality insurance, it was thought that false positivity was due to the contamination of the cards during sampling.

Turkey seems to have the highest PKU incidence, next to Ireland, when the results of the present survey were compared with those obtained in other countries[8-12]. The rate of consanguineous marriages among the parents of the persistent HPA patients was 45 percent, almost twice the current figure given for Turkey[13]. Presence of no consanguinity in more than half of the families, however, may reflect the high frequency of mutations in the phenylalanine hydroxylase (PAH) gene in Turkey. Carrier testing of 209 individuals supports the high mutated gene frequency hypothesis in Turkey[14].

Problems Encountered in the Screening Program

We have faced a great number of difficulties which prevented our reaching in total the objectives of the screening program (Table II). First, some health personnel do not yet consider neonatal screening as a part of preventive medicine, a fact that results in poor blood collection. Second screening specimens are not

being collected from about 40 percent of the 1.3 million deliveries yearly which do not take place in hospitals. In fact, most newborns are discharged within 24 hours, some even a few hours after delivery. Since not all babies have the opportunity of a second test, some cases may be missed. In recent years, however, some actions. Have been taken to motivate parents and to stimulate health personnel. At present, coverage has reached 54 percent of all the deliveries, and the percentage of babies having a second test has increased.

Table II. Major Problems Encountered in the PKU Screening Program

1. Poor collection
   - low percentage of coverage
   - inadequate sampling
   - late arrival of the blood samples at screening laboratories
2. Reaching the positive case late
   - ignorance of health presonnel and of the parents
   - incorrect or incomplete address
   - difficulties in the transport of the samples to screening laboratories
   - lack of intersectoral approach
3. Problems related to the follow-up of the affected cases
   - illiteracy and ignorance of the parents
   - limited number of experienced centers for the treatment and follow-up of the cases with PKU
   - difficulties in the supply of low-protein and low-phenylalanine products

Incomplete, incorrect or even non-existent addresses and lack of information among families about the importance of screening for abnormalities resulted in difficulty in finding some of the patients with positive screening results and in a relatively long interval between initial sampling and the onset of therapy. In fact, in around 15 percent of detected cases, therapy could not be initiated until after the first months of life.

Illiteracy as well as ignorance of the parents decrease the rate of compliance with the rigid and long-term dietary treatment[15,16]. Difficulty in obtaining special formulas and low-protein products locally and the low income of the families who are not insured have been the other problems we encountered in follow-up of the detected cases. Furthermore, general practitioners, even most pediatricians, lack experience in the management of PKU, and dietitians are not present in every hospital. At present we have only a few experienced centers for PKU cases. These are located in central and western regions of the country, and some of the patients cannot attend these centers as frequently as necessary. Consequently, owing to all the above-mentioned factors, it has been difficult, sometimes impossible, to keep the phenylalanine levels of detected cases within normal limits. The percentage of patients with good metabolic control was 92 percent in the first year, but dropped to 62 percent at the end of the second year. Only 51 percent of the cases who completed six years of therapy were found to have good metabolic control (Table III).

Tablo III. Quality of Dietary Control in PKU Patients (n: 123)

Duration of good metabolic control*	No of patients	%
For the first year	113	92
For the first two years	76	62
Through treatment period	55	45

* Average plasma phenylalanine level is ≤ 6 mg/dl.

Proposed Mass-Newborn Screening Organization for the Future

In the beginning we decided that in countries like Turkey with socioeconomic limitations and complicated demographic and geographic conditions, it was more important to start and develop a neonatal screening program without waiting until all of the conditions required for a very comprehensive program were met. It has been so, and since, 1986 the number of cities included in the screening program has gradually increased to at least cover newborns born in maternity hospitals in metropolitan districts in all cities in Turkey. In spite of all the problems encountered, we believe that implementation of the PKU screening program in Turkey has been accomplished. However, to screen all newborns for PKU, and to include at least hypothyroidism into the screening as well, there is an urgent need for a more disciplinary intersectoral approach. To achieve this, we propose organization as outlined in Figure 1, taking into account the problems we encountered thus far, health care structure and the socioeconomic conditions of the country.

Because the newborn population to be secreened is large, there should be regional screening laboratories, at least one in each geographical region of the country. To provide frequent

follow-up of identified cases there is an urgent need for specialized pediatric departments, at least one in each geographical district of Turkey. Since Turkey has financial limitations, screening laboratories could be placed in pediatric departments. Personnel involved in the screening program can be trained by pediatricians, dietitians and laboratory personnel in centers which already exist.

infants born at home. They can take part in the management of detected cases locally as well. Sample collection for second testing could also be made by midwives who work at mother and child health (MCH) centers in the cities.

At present prenatal diagnosis is performed only in the pregnant mothers with a previous PKU child and with informative molecular findings and on condition that the costs of the procedure

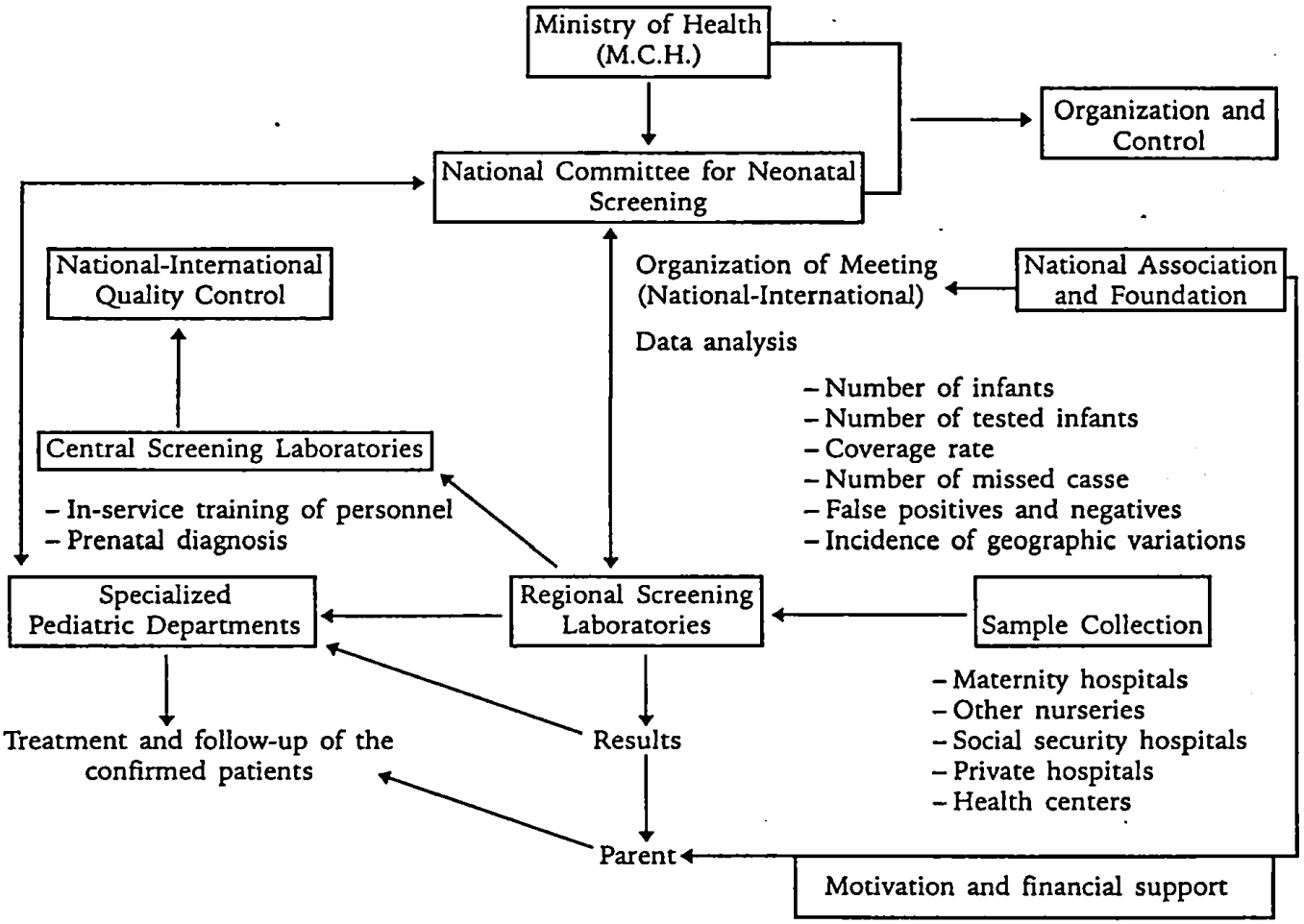

Fig. 1. Proposed neonatal screening organization for Turkey.

One of the essential components of the neonatal screening program should be national and/or international interlaboratory quality control. National interlaboratory quality control can be carried out in the screening center at Hacettepe University Faculty of Medicine that has already been under the control of the international quality assurance program in Germany[17].

In rural areas of Turkey, community-based midwives are the health personnel in close contact with the mothers. The screening program could be organized so that these midwives collect blood samples from the all

are met. Since the dietary formulas are very expensive and since at present one fourth of the mothers are not educated enough to follow rigid and long-term dietary therapy, prenatal diagnosis of PKU should be integrated into the screening program on a large scale. Molecular studies for prenatal diagnosis could be made in reference screening centers.

Pilot studies that have been carried out for hypothyroidism, biotinidase deficiency and galactosemia indicate that their incidence rates are also high[18,19]. We do not yet have any figure about the incidence of medium chain acyl-CoA

dehydrogenase deficiency, glutaric aciduria, maple syrup urine disease, or homocystinuria, which may also benefit from early diagnosis[20]. In fact, the organization illustrated in Figure 1 can be modified to allow additional screening for the above-mentioned diseases. Except for hypothyroidism, however, before conducting such a large-scale screening program for the other metabolic disorders, cost-benefit analyses should be made taking into account Turkey's present socioeconomic conditions.

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Fluoride content of infant formulas and market milk in Turkey

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SUMMARY: Ataç A, Altay N, Ölmez S. Fluoride content of infant formulas and market milk in Turkey. Turk J Pediatr 2001; 43: 102-104.

The aim of the present study was to test the fluoride contents of infant formulas and market milk in Turkey. Fifteen formulas and nine market milks were prepared according to manufacturers' instructions with deionized water. Fluoride contents were analyzed with a spectrophotometer (CADAS 50S) and results were obtained as ppm (μgF/ml). Mean fluoride content of the formulas was 0.101 ppm F and of the milks was 0.08 ppm F. Formulas reconstituted with water containing < 0.3 ppm F do not provide a daily fluoride intake at any age. To decrease dental caries among children in Turkey, fluoride supplements could be prescribed at minimal dosages according to the ADA fluoride schedule.

Key words: fluoride, fluoride supplements, infants formulas, milk.

The goal of the dental profession is to help individuals achieve and maintain maximum oral health throughout their lives. Success in attaining this objective is highlighted by the decline of caries throughout the western world[1,2]. This progress has been mainly due to the use of water fluoridation and fluoride products and acceptance of primary preventive dentistry procedures. Due to technical and/or financial considerations it has not been possible to fluoridate water supplies in Turkey. But in this case, it is still possible and even easy to receive the systemic or topical benefits of fluorides, for example by installing small fluoridation units in school water supplies or by taking dietary supplements like fluoride tablets or drops. Fluoride applied directly to the teeth through fluoridated dentifrice gels or mouth rinses is referred to as topical applications and requires education and/or professional help.

Although the prevalence of dental caries has declined markedly over the past 20 years in most countries, the disease is still a major problem for both adults and children, especially in developing and underdeveloped countries. The change in the mean number of decayed, missing and filled surfaces in permanent teeth (DMFS) is well documented from four surveys carried out in the United States[3-6]. The earlier reductions in dental caries of 40-70 percent resulted from the fluoridation of public water supplies and use of fluoride supplements; the most recent resulted from use of topical fluorides[7].

The oral health status of the Turkish population was well documented in 1988 and reported as dft (decayed and filled primary teeth-total) scores in five year olds as 5, and DMFT (decayed, missing and filled permanent teeth-total) scores in 12-year-olds as 3.16, which is quite high in comparison to developed countries[8]. To reduce dental caries in children, fluoride supplements like fluoride tablets are recommended starting at six months to 16 years of age[9].

To avoid excessive fluoride intake by an infant or child, the fluoride level of drinking water, infant formulas, milk (breast and cow milk) and foods must be determined before hand. For an undesirable degree of dental fluorosis to be avoided, the total daily fluoride intake should not exceed 0.05-0.07 mgF/kg of body weight[10]. The fluoride levels of drinking water available at markets and from tap water sources in Turkey have been determined before[11,12].

This study was designed to evaluate the fluoride content of 15 infant formulas and nine market milks to prepare recommendations for fluoride tablets or drops for children in Turkey.

Material and Methods

The 15 infant formulas and nine market milks selected for analysis are listed in Table I.

Infant formulas were prepared with deionized water according to manufacturers' instructions. Milk and freshly prepared formulas were diluted

with deionized water adding 1/10 ratios. To analyze the fluoride contents, 5 ml from each sample were converted into Photometric (Reagent) Test Kits and placed in a CADAS 50S spectrophotometer, which is automatically calibrated after each measurement. Results were obtained directly as ppm units (μgF/ml). Five measurements were made from each sample and mean values are shown in Table I.

topical effects of the fluoride supplement in addition to the systemic effects.

To prescribe such supplements for children, pediatric dentists and pediatricians should be aware of the daily dietary fluoride intake of infants. As mentioned before, the fluoride level of water sources and drinking water in Turkey is not at optimum levels (0.7-1.2 ppm F)[11,12].

Table I. Fluoride Content of Infant Formulas and Market Milk

Infant formulas	ppm F mean	Market milk	ppm F mean
Humana 1 (Mamsel İlaç Sanayi)	0.03	Mis	0.13
Humana 2 (Mamsel İlaç Sanayi)	0.07	Mis Kalsiyum	0.16
Humana HN (Mamsel İlaç Sanayi)	0.15	Kay Yağlı	0.13
Aptamil 1 (Milupa)	0.09	Nestle Anne	0.04
Almiron (Nutricia)	0.19	Migros	0.01
Nutrilon forte (Nutricia)	0.18	SEK	0.02
Nutrilon soya (Nutricia)	0.19	AOÇ	0.10
Nutrilon 1 (Nutricia)	0.01	Pınar Light	0.14
Protifar (Nutricia)	0.11	Pınar	0.04
Milupa (Milk and Fruit) (Milupa)	0.09		
Milupa (Milk and Rice) (Milupa)	0.11		
Milupa (Milk and Banana) (Milupa)	0.08		
SMA-S-26 (Wyeth)	0.16		
Starmil (Wyeth)	0.05		
Nutrilon 2 (Nutricia)	0.01		
MEAN AMOUNT	0.101		0.08

Results

The fluoride levels of infant formulas and market milks are seen in Table I. The fluoride levels observed in this study in infant formulas ranged from 0.01-0.19 ppm F, and in milk samples from 0.01-0.16 ppm F. Almiron and Nutrilon-Soya (Nutricia, Holland) showed the highest fluoride levels in infant formulas (0.19 ppm F). Mis Kalsiyum showed the highest level of fluoride among the milk samples (0.16 ppm F).

Discussion

Recommendations regarding fluoride supplementation are not readily available around the world. Banting[13] reported 21 countries' current recommendations for fluoride supplementation by tablets, salt and milk. Fourteen countries had specific recommendations for fluoride tablets. In Turkey, there is not a specific recommendation for fluoride tablets, although the oral health status of children is very poor.

Fluoride supplements in chewable form are effective in reducing dental decay rates. In this way, all teeth benefit from the cumulative

The fluoride content of the market milk tested in this study ranged from 0.01-0.16 ppm F with a mean value of 0.08 ppm F. Dabeka and McKenzie[14] reported cow milk fluoride contents as 0.41 ppm F in Canada, which is five times higher than those in Turkey.

The fluoride content of the infant formulas tested in this study ranged from 0.01-0.19 ppm F with a mean value of 0.101 ppm F. Dabeka and Mckenzie[14] reported the mean fluoride concentration of powdered infant formulas available in Canada as 1.13 ppm F, which is higher than that found in the formulas available in Turkey. The difference is probably due to the fluoride content of the water used in preparation of the powdered formulas.

Silva and Reynolds[15] reported that when infant formulas available in Australia are reconstituted with water not containing fluoride, the range is 0.240 ppm F. In another study, Johnson and Bawden[16] evaluated infant formulas purchased in seven cities across the USA, and reported fluoride contents as 0.03-0.38 ppm F. These authors showed that when the infant formulas

were reconstituted in water containing 0.1 ppm F, their estimated contribution to an infant's daily fluoride intake at six months of age ranged from 0.018-0.076 mg/kg/body mass. In fact, even if the water content is 1 ppm F, it ranges from 0.124-0.184 mg/kg/body mass[15].

In this study, the mean value of fluoride content of formulas was 0.101 ppm F, and they were reconstituted with deionized water containing less than 0.1 ppm F. However, none of the formulas, when reconstituted with water containing up to 0.3 ppm F should provide a daily fluoride intake, at any age, above the suggested threshold for fluorosis of 0.05-0.07 mgF/kg/body mass/day.

If water fluoridated at 1.0 ppm F was used to reconstitute the infant formulas, all would provide a daily fluoride intake above the suggested threshold for fluorosis[15].

According to Ataç et al.[11], almost all market drinking waters contain fluoride levels less than 0.3 ppm F. Usmen et al.[12], reported tap water fluoride concentrations around Turkey and found fluoride levels very low. Thus, it is safe to assume that infant formulas reconstituted with these drinking or tap waters could not cause fluorosis in infants' teeth.

Heilman et al.[17], reported that fluoride concentrations found in infant foods ranged from 0.01-8.38 ppm F, and that the highest values were obtained from chicken-based infant foods. On the other hand, Van Winkle et al.[18] analyzed infant formulas and reported that 17 soy-based formulas showed the highest level of fluoride at 0.26 ppm F. In our study, Nutrilon-Soya (Nutricia, Holland), a soy-based formula, also showed the highest level of fluoride.

Chowdhury et al.[19] emphasized that in non-fluoridated areas infants' intake of dietary fluoride is five to seven times less than in optimal fluoridated areas.

In conclusion, consumption alone of the infant formulas and/or market milk tested in this study is unlikely to be a risk factor for dental fluorosis in non-fluoridated communities in Turkey.

The dental status of children in Turkey is very poor when compared to that in Western countries. Prescription of fluoride supplements at minimal dosage[9] together with application of topical fluorides can decrease dental caries among children in Turkey.

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Monotherapy with meropenem versus combination therapy with piperacillin plus amikacin as empiric therapy for neutropenic fever in children with lymphoma and solid tumors

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The purpose of this study was to compare meropenem monotherapy with combination therapy for empirical treatment of neutropenic fever in children with lymphoma and solid tumors. Ninety episodes of neutropenic fever in children (0.7-16.0; mean age 7.7 years) with solid tumors in a single center were randomized to receive either meropenem (50 mg/kg/dose-maximum 1 g, every 8 hours) or piperacillin (200 mg/kg/dose, every 6 hours) plus amikacin (15 mg/kg daily). Failure was defined as treatment modification. Non-Hodgkin's lymphoma (NHL) accounted for 62.2 percent of all episodes, and solid tumors (37.8%) for the rest. Blood cultures were positive in 23 percent of all episodes. Sixty-seven percent of all isolated microorganisms stained Gram-positive. Overall success was 70.0 percent (63/90). The success with meropenem was comparable to that seen with piperacillin plus amikacin: 76.6 versus 64.6 percent ($p = 0.25$). The failure rate was 33 percent with Gram-positive culture and 78 percent with Gram-negative or mixed cultures. The solid tumor group had significantly less bacteremia (4/34 versus 17/56; $p < 0.05$) and treatment failure (3/34 versus 24/56; $p < 0.001$) than the NHL group. No serious drug-related adverse event was noticed. Meropenem monotherapy was as effective as piperacillin plus amikacin combination in the empirical treatment of neutropenic fever in children with lymphoma and solid tumors.

Key words: neutropenic fever, children, meropenem, monotherapy.

Empirical broad-spectrum antibiotic therapy has reduced the risk of death in patients with neutropenic fever. During the past two decades combination therapy with betalactams and aminoglycosides has been standard therapy[1-4]. The invention of extended spectrum cephalosporins and carbapenems offered single-agent therapy. Although studies conducted by De Pauw et al.[5] and Pizzo et al.[6] showed that ceftazidime, the most widely used cephalosporin monotherapy, was as effective as combination therapy, its lack of activity against some Gram-positive organisms and increasing Gram-negative resistance against ceftazidime may limit its use[5,6]. Carbapenems, with their microbiological activity against both Gram-positive and Gram-negative bacteria, were promising candidates for empirical therapy in neutropenic fever. The Meropenem Study Group revealed that meropenem monotherapy was as effective as ceftazidime monotherapy[7]. The European Organization for Research and Treatment of Cancer Study showed that monotherapy with meropenem was equivalent to combination therapy with ceftazidime plus amikacin[8].

The present study was a single center, prospective, randomized trial to compare meropenem monotherapy versus combination therapy for empirical treatment of neutropenic fever in children with lymphoma and solid tumors.

Material and Methods

Patient Eligibility: This trial was conducted in a single center, Pediatric Oncology and Infectious Diseases Units of Hacettepe University, İhsan Doğramacı Children's Hospital, Ankara, Turkey. Patients (≤ 16 years of age) with lymphoma and solid tumors were eligible for randomization if they had fever (defined as a single oral temperature above 38.3 °C or a temperature of 38.0 °C or greater taken on two occasions at least 1 hour apart), neutropenia (absolute neutrophil count, ANC ≤ 500/mm³) and were without an apparent infection. Patients with leukemia were not included in this study since they were treated in another department. Patients were excluded if they had received antibiotics within the last four days, had blood or blood product infusion or had been given colony stimulating factor within the last six hours. None of our patients had a known allergy to any of the antibiotics prescribed in the protocol.

Initial Evaluation: A complete history was taken from each patient, and a detailed physical examination was performed. Complete blood count, peripheral blood smear, CRP, erythrocyte sedimentation rate, routine chest X-rays, urinalysis, urine culture and blood cultures (two sets, from different venipunctures at 30-minute intervals; two additional sets from central venous line if the patient had any), and other cultures (when indicated) were performed.

Study Design and Procedure: A total of 90 consecutive neutropenic fever episodes were evaluated. Episodes were randomized to receive either meropenem (50 mg/kg/dose-maximum 1 g, every 8 hours, infused over 20-30 minutes) or piperacillin (200 mg/kg/dose, every 6 hours) plus amikacin (15 mg/kg daily) in this one-side blinded study. Treatment modification was done after 72-96 hours (depending on the clinical condition) if the patient still had fever and neutropenia. The minimum duration of treatment was seven days, four of which were consecutive without fever. Failure was defined as treatment modification.

Statistical Analysis: All data were entered into a computerized data base and analyzed using SPSS programs. Chi-square (Fisher's exact tests for small samples) and Mann-Whitney U tests were used for comparison.

Results

The mean age was 7.7 years for the whole group (0.7-16.0; ± 4.46). The characteristics of the 90 episodes are given in Table I. Non-Hodgkin's lymphoma (NHL) (56 episodes) made up 62.2 percent of all episodes, and solid tumors the remainder (37.8%): rhabd-myosarcoma (8), Ewing's sarcoma (6), neuroblastoma[6], medulloblastoma (3), nasopharynx carcinoma (2), germ cell tumor (2), PPNET (2), and Hodgkin's disease, mesothelioma, Wilms's tumor, retinoblastoma, hepatoblastoma (1 episode each). Patients had central venous line in only 10 episodes (11%). The two treatment groups were comparable according to the parameters given in Table I.

Table I. Characteristics of 90 Episodes

Characteristics	Treatment groups	
	Meropenem	Piperacillin + Amikacin
Episodes (n)	45	45
Age [mean (range)]	7.0 (1.0-15.0)	8.4 (0.7-16.0)
Male/Female	27/18	21/24
Lymphoma (n)	27	29
Solid tumor (n)	18	16
Central line (yes/no)	5/40	5/40
Radiotherapy (yes/no)	13/32	12/33
Antimicrobial prophylaxis	None	None
Status of the disease		
Progression and relapse	10	14
Other status	35	31
Interval with the last chemotherapy (days) mean (range)	10 (2-16)	10 (3-19)
Hemoglobin (gr/dl) [mean (range)]	7.9 (4.0-13.0)	8.2 (5.2-12.1)
WBC (/mm³) [mean (range)]	805 (300-1600)	882 (100-2100)
Platelet (/mm³) [mean (range)]	68000 (9000-321000)	72000 (5000-444500)
ESR (mm/hour) [mean (range)]	82 (4-145)	92 (5-150)
CRP p(mg/dl [mean (range)]	6.9 (0.1-22.0)	8.7 (0.2-25.3)
ANC		
mean (range) (/mm³)	148 (0-500)	155 (0-450)
episodes with ≤ 100/mm³ at entry	19	23
Duration of ANC (median days)		
≤ 100/mm³	1	1
≤ 500/mm³	3	3
≤ 1500/mm³ (/mm³)	5	4
Success rate (%)	76.6	64.6

Duration of Neutropenia and Fever: The mean duration of neutropenia was 4.1 days (1-20); the difference between groups was not statistically significant. Neutropenia duration in episodes with progression and relapse was longer than in ones without active disease: four days (1-13) versus three days (1-21); $p < 0.05$). The mean duration of fever was 3.8 days (1-20). Although fever subsided earlier in the meropenem group, the difference between groups was not statistically significant (meropenem 3.4 days; piperacillin plus amikacin 4.3 days; $p = 0.27$).

Bacteremia: Blood cultures were positive in 23 percent of all episodes (21/90, 6 of them were polymicrobial: 1 double Gram-positive, 5 mixed). The distribution of 27 microorganisms isolated in 21 episodes is shown in Table II [single Gram-positive 44% (12/27), single Gram-negative 15% (4/27), mixed 41% (11/27)]. Gram-positives constituted 67 percent of all isolated microorganisms. One-third (4/12) of staphylococci were methicillin-resistant (MRSA). The stage of disease seemed to increase the risk of bacteremia: the risk was higher in cases of progression and relapse (38% versus 18%, $p = 0.055$). If the patient still had fever after 96 hours, the risk of bacteremia increased to 42 percent (vs 17%, $p < 0.05$).

Table II. Gram Staining of Blood-Isolated Microorganisms

Gram staining	Single (n)	Polymicrobial (n)	n (%)
Gram-positive	11	7	18 (66.7)
S. coagulase (-)	7	3	10 (37.0)
S. aureus	1	1	2 (7.4)
α-hemolytic Streptococcus	3	2	5 (18.5)
S. pneumoniae	–	1	1 (3.7)
Gram-negative	4	5	9 (33.3)
E.coli	1	3	4 (14.8)
P. aeruginosa	1	–	1 (3.7)
S. maltophilia	1	1	2 (7.4)
Klebsiella	–	1	1 (3.7)
Acinetobacter	1	–	1 (3.7)
Total	15	12	27 (100.0)

Mortality: The proportion of episodes ending in death was 2.2 percent (2/90). The first patient was a 13.5-year-old boy, stage III, abdominal NHL, with partial response to LMB-B chemotherapy. E. coli and MRSA had been isolated from blood culture. He had been given amphotericin B starting on the 7th day, and died on the 21st day when his ANC was still < 100/mm^3 and platelet count was < 50,000/mm^3. The second one was a 13.8-year-old girl, stage IV NHL, with relapsed disease. S. maltophilia and MRSA had been isolated from blood culture. She had been given amphotericin B starting on the 7th day, and died on the 9th day when her ANC was still < 100/mm^3 and platelet count was < 50,000/mm^3. The underlying disease was in terminal stage in this patient.

Non-Hodgkin's Lymphoma Versus Solid Tumors: The differences between the non-Hodgkin's lymphoma and solid tumor groups was striking. The solid tumor group had significantly less bacteremia (4/34 versus 17/56; $p < 0.05$) and treatment failure (3/34 versus 24/56; $p < 0.001$) than the NHL group.

CRP, ESR: Neither the CRP nor ESR level was associated with the risk of bacteremia, type of microorganism isolated, treatment failure, or amphotericin B use. No serious drug-related adverse event was noticed.

Discussion

With intensive chemotherapy, the five year disease-free survival rate in childhood cancer is 75 percent[1]. Neutropenic fever is one of the leading causes of morbidity and mortality either directly or indirectly by causing a delay or dose reduction in the chemotherapy regimen. Continuous monitoring of parameters (characteristics of patients, isolated microorganisms, causes of treatment failure, etc.) is crucial for determining a policy for treating neutropenic fever in an oncology center. Monotherapy leads to fewer adverse effects; morover, it reduces the amount of time and supplies needed for administering the antibiotics.

The median duration of neutropenia was three days in our study (1-21) compared to 11 days, 10 days (1-68) and nine days in studies conducted by EORTC and GIMEMA[8], Lucas et al.[3] and Petrilli et al.[4], respectively. The absence of leukemia and bone marrow transplantation patients in our trial very likely resulted in this difference.

Bacteria were isolated from blood cultures in 23 percent of all episodes, which was consistent with recent trials (24%, EORTC and GIMEMA; 24.4%, Lucas et al.)[8,3]. The significant difference between NHL and solid tumor groups (30.4% vs 11.8%) that we detected was also noticed by Lucas et al.[3] (24.4% in leukemia/lymphoma vs 3.1% in solid tumors)[3]. This fact probably contributed to less antibiotic modification in episodes with solid tumors in our study (3/34

vs 24/56; $p < 0.01$). With their shorter duration of neutropenia, and lesser culture positivity and requirement of antibiotic modification, neutropenic fever patients with solid tumors are promising candidates for early discharge, outpatient management and oral treatment.

Gram-positives constituted 67 percent of isolated microorganisms, as is the case in most centers[7-11]. In many centers 80 to 90 percent of patients have intravenous catheters. Lindblad[9] reported that 25 percent of patients with a central venous line had bacteremia (69% Gram-positive) compared to 14 percent of patients without a central venous line (40% Gram-positive)[9]. Although central venous lines were present in only 10 episodes (11%), Gram-positives still constituted two-thirds (67%) of isolated microorganisms (63% even if episodes with central venous line are excluded). Some factors other than increased use of intravenous device (such as hand washing and resistance to β-lactam antibiotics) may explain Gram-positive predominance.

Success against Gram-positive bacteremia without antibiotic modification was much higher in our center [67% vs 29% (Behre et al.[11]) and 28% (EORTC GIMEMA)[8]]. On the other hand, we had a much lower success rate against Gram-negative and mixed bacteremia [22% vs 47% (EORTC GIMEMA)[8]] irrespective of the antibiotic group.

Because there has been a considerable reduction in Gram-negative bacteremic episodes over the last 10 years, some authors believe that the need for aminoglycoside-containing regimens should be reassessed. Meropenem, which is highly stable to inactivation by renal dehydropeptidase, does not require cilastatin; moreover, its spectrum covers most Gram-positive, Gram-negative and anaerobic bacteria. It is successfully used in intraabdominal infections, meningitis, urinary tract infections and lower respiratory tract infections as an alternative to some combination therapy[12]. It does not increase the risk of seizure and has a lower incidence of nausea and vomiting than does imipenem/cilastatin.

Previous clinical studies with meropenem monotherapy in neutropenic fever were conducted in adult patients[7-11]. Only the EORTC GIMEMA trial included both children and adult patients[8]. Success rates varied between 45-60 percent. It is well known that neutropenia is shorter in duration in children than in adult patients[13]. The absence of leukemia and bone marrow transplantation patients and shorter duration of neutropenia very likely contributed to a higher success rate in our study. We also strictly avoided early modification before 96 hours (compared to 72 hours in other studies) unless the patient deteriorated clinically.

There are limited studies concerning meropenem monotherapy for neutropenic fever in children. Our study showed that meropenem monotherapy was well tolerated and was as effective as piperacillin plus amikacin combination therapy. The success rates of both groups were comparable with other empirical regimens. We conclude that meropenem monotherapy can be used in the empirical treatment of neutropenic fever in children with lymphoma and solid tumors.

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Molecular genetic anaylses of cystinuria type 1 in 24 Turkish patients

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M467T mutation (exon 8) in rBAT gene is found to be the most common mutation in cystinuria type I patients. In our series consisting of 24 patients, the allele frequency of the M467T mutation was 8.3 percent (4/48). The second most frequent mutation at the same nucleotide position was M467K, with an allele frequency of 4.2 percent (2/48). The polymorphism which is found in linkage disequilibrium with the M467T is 231T/A (exon 1). We also found that 231T/A was associated with the M467T mutation in our series.

Key words: cystinuria type I, molecular genetics, mutation, polymorphism.

Cystinuria is an autosomal recessive disease characterized by the development of stones in the uniray tract. It is caused by the defective transport of cystine and dibasic amino acids (lysine, arginine, ornithine) through the renal tubular cells and intestinal mucosa[1]. Three different types of cystinuria (I, II and III) have been described. Type I heterozygotes excrete normal amounts of cystine in their urine, whereas types II and III heterozygotes excrete high and moderate amounts of cystine, respectively[2]. The disease has a prevalence of 1/7,000 worldwide[3]. In a screening of metabolic disorders, included 6,050 high-risk infants, the prevalence of cystinuria in Turkey was reported as 1/1,000[4]. The prevalence was also reported by Gültekin et al.[5] and Durmuş-Aydoğdu et al.[6] as 1/2,155 and 1/2,065, respectively, in a screening of normal children for cystinuria.

Cystinuria disease gene rBAT (SLC3A1), which is localized to 2p16.3-21, has been shown to be responsible for type I but not for type II and type III cystinuria[7-9]. Linkage analysis demonstrated the presence of genetic heterogeneity and mapped type III cystinuria locus to 19q13.1[10]. It was also suggested that cystinuria type II might be due to a defect at this locus[11]. Description of this new locus will allow the identification of cystinuria type II and III genes. This will be useful in understanding the biochemistry of cystine reabsorption.

The rBAT gene spans about 45 kb of genomic DNA and is composed of 10 exons[12,13]. The 2.2 kb mRNA is expressed in the kidney cortex and intestinal epithelium and encodes for a 685 amino acid, 79 kDa transport protein, for cystine and dibasic amino acids[14].

Thirty-eight mutations[15] and nine polymorphisms[14] have been described at the rBAT locus so far. M467T, which is the most common mutation, is a T→C substitution at nucleotide position 1400 (exon 8). It destroys a N1aIII restriction site in the 60 bp PCR product.

Another mutation at the same nucleotide position is M467K, which is a T→A substitution. It destroys a N1aIII restriction site and creates an AluI restriction site, yielding two fragments of 37 and 23 bp[16].

The polymorphism which is found in linkage disequilibrium with the M467T mutation is 231T/A in exon 1 in the rBAT gene. It creates a DdeI restriction site yielding two fragments of 263 and 102 bp[17].

In this study, 24 Turkish cystinuria type I patients were analyzed for the M467T and M467K mutations and 231T/A polymorphism.

Material and Methods

The cyanide-nitroprusside test was routinely applied to urine samples of all patients evaluated at the Metabolism Unit's laboratory as a component of a selective screening procedure. Patients having a positive cyanide-nitroprusside test were further analyzed by urine and blood amino acid chromatography. All patients with an increase in cystine and dibasic amino acids in their urine samples were diagnosed with cystinuria. Twenty-four patients with high urinary excretion of cystine whose parents had a normal urinary amino acid profile were diagnosed with cystinuria type I.

DNA was isolated from white blood cells according to standard protocols[18]. M467T and M467K mutations were analyzed by amplifying genomic DNA (F: 5' GCG TTT GGG GAA TCA GTA TG 3', R: 5' -GTT CCA GGG AGT GTG AAA AG 3'). For the mutations and the polymorphism, a total of 35 cycles of amplification were performed (2 min. at 94 °C, 1 min. at 55 °C and 1 min. at 72 °C). For the detection of mutations, PCR products were digested with N1aIII and AluI according to the manufacturer's instructions and were electrophoresed on a 15% polyacrylamide gel at 300V for 45 minutes[16].

231T/A polymorphism was analyzed by amplifying DNA (F: 5' AGA GAG GGC AAT GAT GGC TA 3', R: 5' GAA GGC ACT CCG AAG ACA TAA 3'). PCR products were digested with DdeI and electrophoresed on a 3% agarose gel[7]. In addition to 24 cystinuria patients, 20 healthy control individuals were analyzed for 231T/A polymorphism. Gels were stained with EtBr and viewed under UV fluorescence.

Results

Among 24 cystinuria type I patients, 75 percent had stones in their urinary tract. Consanguinity was present in 37.5 percent of the families. The characteristics of the patients and the results of DNA analysis are shown in Table I.

Table I. Characteristics of the Patients and Results of DNA Analysis

No.	Age	Sex	Consanguinity	Urinary stone	M467T	M467K	231T/A
1	3 y	M	(+)	(+)	-/-	-/-	-/-
2	8 m	M	(-)	(+)	-/-	+/-	-/-
3	12 y	M	(-)	(+)	+/-	-/-	+/-
4	3 y	M	(-)	(+)	-/-	-/-	-/-
5	6 y	M	(+)	(+)	-/-	-/-	-/-
6	2 y	M	(+)	(+)	-/-	-/-	-/-
7	7 y	M	(+)	(+)	-/-	-/-	-/-
8	2 m	F	(-)	(+)	-/-	-/-	-/-
9	11 y	M	(-)	(-)	+/-	-/-	+/-
10	9 y	F	(-)	(-)	-/-	-/-	-/-
11	6 y	F	(+)	(+)	-/-	-/-	-/-
12	5 y	M	(+)	(-)	-/-	-/-	-/-
13	25 y	M	(-)	(-)	-/-	-/-	-/-
14	6 y	M	(+)	(+)	-/-	+/-	-/-
15	8 y	M	(-)	(-)	-/-	-/-	-/-
16	?	M	(-)	(+)	-/-	-/-	-/-
17	1.5 y	M	(+)	(+)	-/-	-/-	-/-
18	2 y	M	(+)	(+)	+/+	-/-	+/+
19	3 y	M	(-)	(+)	-/-	-/-	-/-
20	?	F	(-)	(+)	-/-	-/-	-/-
21	18 y	M	(-)	(+)	-/-	-/-	-/-
22	14 y	M	(-)	(+)	-/-	-/-	-/-
23	10 y	F	(-)	(+)	-/-	-/-	-/-
24	3 y	M	(-)	(-)	-/-	-/-	-/-

Among the 24 patients, two were heterozygous and one was homozygous for the M467T mutation. The allele frequency was 8.3 percent. Another two patients were heterozygous for M467K, which is the second most frequent mutation at the same position, and the allele frequency was 4.2 percent. It has been shown previously that 231T/A polymorphism was found in linkage disequilibrium with the M467 mutation[17]. We also analyzed 231T/A in our group of patients T→A transversion was found in three patients that had the M467T mutation (8.3%). This polymorphism was not found in the rest of our patient group or in the 20 healthy control individuals.

Discussion

At present, 38 cystinuria type I mutations in the rBAT gene have been described[15] in populations in Italy, Spain, the Middle East, Eastern Europe (mainly people of Jewish origin) and Japan. Of the mutations there were missense, deletion, insertion, nonsense, splice site and frameshift mutations[19].

The most frequent mutation was M467T in Italian (8.7%) and Spanish (40%) populations[16,20]. In our patients the allele frequency of the M467T mutation (8.3%) was similar to the Italian results. M467K was analyzed in Spanish patients, and the frequency was 1.8 percent[21], which is lower than the frequency in the Turkish mutant alleles (4.2%).

In the rBAT gene, besides 38 mutations, nine polymorphisms were identified. In the Mediterranean cystinuria type I patients, the 231T/A polymorphism in exon 1 was found in linkage disequilibrium with the M467T mutation (11.8%). 231T/A polymorphism was only found in affected chromosomes, not in normal ones. This observation was confirmed by our results and the allele frequency of the 231T/A polymorphism (8.3%) was found similar to that in Mediterranean populations.

In our study, 12.5 percent of the cystinuria type I mutant alleles could be identified. This leaves a large proportion of alleles still to be analyzed. Heterogeneity of mutations in recessive disorders is observed in the Turkish population[22]. This creates a difficulty in population screening or carrier identification by DNA analysis. Further characterization of unknown mutations is underway.

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A practical GnRH analogue (triptorelin) stimulation test to distinguish constitutional delay of puberty from hypogonadotropic hypogonadism in prepubertal boys

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At present, no established practical and reliable endocrine test exists for differentiating between constitutional delay of puberty (CDP) and hypogonadotropic hypogonadism (HH). The most discriminating results have been reported by measuring LH and testosterone (T) responses to gonadotropin-relasing hormone (GnRH) analogues. In this study, 23 prepubertal boys aged 14 to 16.5 years underwent a modified triptorelin (a GnRH analogue) stimulation test, and they were followed clinically for up 24 to months. Sixteen subjects developed spontaneous puberty during the follow-up period and thus were diagnosed with CDP, and the remaining seven were diagnosed with HH. Retrospective evaluation of their LH, FSH and T responses revealed significant differences without any overlaps in serum LH levels at 4 h (CDP: 33.2 ± 9.3 vs. HH: 3.3 ± 2.6 mIU/ml, $p < 0.0002$) and in serum T levels at 24 h (CDP: 369.3 ± 128.1 vs. HH: 61.4 ± 22.6 ng/dl $p < 0.0002$). We conclude that CDP can be clearly differentiated from HH by the LH response at 4 h and/or T response at 24 h after a single-dose triptorelin administration.

Key words: constitutional delay of puberty, hypogonadotropic hypogonadism, triptorelin.

Differentiation of teen-age boys with constitutional delay of puberty (CDP) from those with hypogonadotropic hypogonadism (HH) remains difficult because of the similarity in their clinical and laboratory characteristics. At present, no established practical and reliable endocrine test exists for differentiating between these two entities[1]. Thus, the diagnosis of HH is often delayed until the exclusion of CDP by spontaneous appearance of pubertal signs. Recent literature focused on gonadotropin and testosterone (T) responses to gonadotropin-releasing hormone analogues (GnRH analogue) to differentiate between these two conditions. A nafarelin stimulation test has been used the most and reported as a useful tool to distinguish the two conditions by LH and/or T response in the great majority of the cases[2-4]. Leuprolide was reported to have been useful in one study, although it failed to produce distinctive results in another[5, 6]. The most encouraging results were reported with another GnRH agonist, triptorelin[7]. Triptorelin, administered at 4 am, was reported to have differentiated these two conditions in all subjects by their LH but not by their T response four hours later, at 8 am. Our primary aim was to develop a practical triptorelin stimulation test. To this end, we modified the triptorelin stimulation method in that we administered triptorelin at 8 am instead

of 4 am. Our second modification was the extension of the test to 24 h to see whether the T response could be another diagnostic parameter as in the other GnRH agonist stimulation tests. Here we report similarly differentiating results obtained after using a modified triptorelin stimulation test.

Material and Methods

Twenty-three prepubertal boys, aged 14-16.5 years, were studied at three different pediatric endocrinology clinics. Location of the cases and the time period of the study were as follows: 10 cases at Hacettepe University, İhsan Doğramacı Children's Hospital between June 1997 and February 1998; 10 cases at Atatürk University between July 1998 and November 1999, and three cases at Mersin University between March 1999 and December 1999. The following inclusion criteria were used: 1. no evidence of systemic diseases to cause delayed puberty, 2. a weight for height within normal standards for Turkish children [8], 3. a bone age equal to or greater than 11.5 years according to Greulich-Pyle method [9], and 4. a testicular volume less than 4 ml.

All subjects underwent a triptorelin stimulation test at the time of enrollment after the nature of the study had been explained and informed consents were obtained from the subjects or their families. Blood specimens were collected for baseline LH, FSH, and T levels at 8 am. Then, triptorelin (D-trp^6 -luteinizing hormone-releasing hormone) was administered subcutaneously at a dose of 0.1 mg/m^2 (Decapeptyl^R, Ipsen-Biotech, Paris, France). Following the triptorelin injection, blood samples for LH, FSH and T were obtained at 4, 8 and 24 h. Serum LH, FSH and T concentrations were determined by chemiluminescent enzyme immunometric assay (Immulite, Automated Analyzer, Diagnostic Product Corporation, Los Angeles, CA, USA) in duplicate on the same day. The subjects were regularly followed up until determination of the physical evidence of pubertal advancement for up to 24 months. No subject received short-term T treatment. Sixteen subjects who had spontaneous pubertal development were diagnosed with CDP. In this group, evidence of pubertal advancement was determined in 9 ± 5.2 (5-24) months at the age of 15.6±0.83 (14.6-17) years. Their mean testicular volume was 4.5 ± 1.8 (4-8) ml at the time of the last examination. The remaining seven subjects who showed no pubertal signs during 17.4 ± 5.0 (12-24) months follow-up period were diagnosed with HH. The mean chronological age of the subjects was 16.8 ± 0.7 (16-18) years at the time of the last examination. Their mean testicular volume was 2.7 ± 0.5 (2-3) ml at the end of the follow-up period. Of the subjects with HH, four had anosmia, micropenis and history of bilateral orchidopexy (Kallmann's syndrome), two had panhypopituitarism and had undergone surgical removal of the craniopharyngioma. Except for these two patients with panhypopituitarism, none of the subjects in the entire study population showed clinical or laboratory features of growth hormone deficiency. Clinical and hormonal variables between the groups were compared with Mann-Whitney U test. Z-scores for 4 h LH and 24 h T responses in the CDP group were calculated using SPSS software package.

Results

The initial clinical characteristics of the patients are summarized in Table I. There were no statistically significant differences between the groups in height, chronological age, bone age, or testicular volume (Table I). Mean basal values of LH, FSH, and T also did not differ significantly between the groups. The patterns of responses to triptorelin in both groups are shown in Table II. After triptorelin administration, the difference in mean serum LH levels between the groups was most pronounced at 4 h (33.2 ± 9.3 vs. 3.3 ± 2.6 mIU/ml, for CDP and HH respectively, $p < 0.0002$) without any overlaps (min: 14.1, max: 44.4 for CDP and min: 0.7, max: 8.1 for HH groups). Mean incremental LH response, Δ LH (LH at 4 h minus LH at time 0) was significantly higher the in CDP group (32.3 ± 9.2 mIU/ml vs. 2.6 ± 2.5 mIU/ml, $p < 0.0002$). Δ LH at 4 h ranged from 13.4 to 43 mIU/ml for the CDP group, and from 0.0 to 7.7 in the HH group. Although the mean LH responses to triptorelin at 8 and 24 h in subjects with CDP were significantly higher, the responses of the two groups were overlapping. No significant differences in mean FSH response was observed at any timepoints. Mean T response to triptorelin in the CDP group was significantly higher than in the HH group (369.3 ± 128.1 ng/dl vs. 61.4 ± 22.6 ng/dl respectively, $p < 0.0002$) at 24 h without

overlapping (range 110 to 571 ng/dl for CDP, and 21 to 90 ng/dl for HH group). Δ T at 24 h (T value at 24 h minus T value at time 0) was 334.3 ± 124.0 ng/dl vs. 12.8 ± 34.0 ng/dl for CDP and HH groups, respectively (p: 0.0002). Δ T at 24 h ranged from 90 to 551 ng/dl for the CDP group, and from -40 to 51 ng/dl in the HH group. Although the mean T responses to triptorelin at 4 and 8 h in subjects with CDP were significantly higher, the responses of the two groups were overlapping.

Table I. Initial Clinical Characteristics of the Subjects with Constitutional Delay of Puberty (CDP) and Hypogonadotropic Hypogonadism (HH)

	Age (year)	Height (cm)	Height SDS*	Testicular volume (ml)	Bone age (year)
CDP	14.8 ± 0.8 (14-16.5)	147.6 ± 3.2 (142-154)	-2.4 ± 0.8 (-4.0-1.5)	2.6 ± 0.4 (2-3)	12.0 ± 0.6 (11.5-13)
HH	15.8 ± 1.2 (14-16.5)	153.5 ± 8.8 (144-163)	-2.0 ± 1.1 (-4.0-(-0.99)	2.4 ± 0.5 (2-3)	12.6 ± 0.9 (11.5-14)

Data are means ± SD (Range), *SDS: Standard deviation score.

Table II. LH, FSH and Testosterone (T) Responses to Triptorelin in Constitutional Delay of Puberty (CDP) and Hypogonadotropic Hypogonadism (HH)

	Hour	HH (n:7)	CDP (n:16)	p
LH (mIU/ml)	0.	0.6 ± 0.2 (0.4-1.0)	0.8 ± 0.2 (0.4-1.4)	NS*
	4	3.3 ± 2.6 (0.7-8.1)	33.2 ± 9.3 (14.1-44.4)	0.0002
	8.	2.8 ± 1.6 (0.7-5.0)	12.8 ± 5.9 (4.4-26.5)	0.0003
	24.	1.1 ± 0.5 (0.7-2.1)	6.5 ± 4.8 (0.7-15.6)	0.003
FSH (mIU/ml)	0.	0.6 ± 0.2 (0.1-0.9)	0.8 ± 0.3 (0.6-1.7)	NS
	4.	5.1 ± 2.3 (2.0-7.2)	4.4 ± 2.3 (2.7-9.5)	NS
	8.	3.3 ± 2.1 (1.5 ± 6.1)	3.9 ± 2.8 (2.2-9.6)	NS
	24.	1.6 ± 1.2 (0.2-3.5)	1.8 ± 1.1 (1.0-4.9)	NS
T (ng/dl)	0.	22.8 ± 13.8 (11-54)	29.3 ± 18.7 (10.72)	NS
	4.	35.7 ± 17.1 (22-70)	61.8 ± 24.8 (20-103)	0.02
	8.	42.8 ± 21.3 (21-82)	120.6 ± 35.1 (50-194)	0.0003
	24.	61.4 ± 22.6 (21-90)	369.3 ± 128.1 (110-571)	0.0002

Data are means ± SD (Range), * NS: non-significant.

Discussion

Distinguishing CDP from HH in boys with delayed puberty remains a diagnostic challenge. Synthetic GnRH analogues have been used extensively to distinguish CDP from HH. However, a simple and reliable diagnostic test is still lacking [1]. We found that when triptorelin is given at 8 am, the increase in serum LH at 4 h or T at 24 h is sufficient to distinguish those two clinical entities. In a pilot study (HH n: 8, CDP n: 3), Ehrmann et al. [2] reported that LH responses to nafarelin in subjects with CDP at the 0.5 -4 h did not overlap with those in HH patients. However, one subject with HH had a T response similar to those of CDP at 8-24 h. Ghai et al. [3] reported that LH levels in subjects with CDP were significantly higher than those in subjects with HH at 4 h. One patient with HH, however, was misclassified in the CDP group by the test. Only 55 percent (n: 11) of the patients with CDP displayed a significant T response 8-24 h after nafarelin injection. In a smaller study (CDP n: 6, HH n: 5), Kletter et al.[4] found that LH, FSH and T responses in subjects with CDP were higher than in those with HH without overlapping after nafarelin administration. Recently, Zamboni et al.[7] reported that triptorelin administration at 4 am clearly distinguished CDP (n: 18) from HH (n: 10) by the LH responses at 4 h without overlapping. However, starting a test at 4 am on an outpatient basis would be almost impossible in practice, as the authors suggested. Therefore, we modified their method and administered triptorelin at 8 am instead of 4 am and extended the test period to 24 h to see whether the T response could be another

diagnostic parameter. In this study, we were able to reproduce the 4h LH results of the original study in a comparable subject population. This reproduction might not have been possible because of the wellknown nocturnal increase in the number and magnitude of LH pulses in early puberty, which suggests enhanced sensitivity to GnRH during the night [10]. Moreover, we found that the 24h T response is also completely discriminatory. Zamboni et al.[7] looked for T responses only at 4 h; however, based on the results obtained in the other GnRH analogue stimulation studies, larger differences in T response between the two conditions should be expected at 16-24 h[2-4]. For that reason, we extended the study period to 24 h, and we found significantly different, non-overlapping results at 24 h. Our data indicate that an LH level below 14.1 mIU/ml (-2 SD) 4 h or a T level below 110 ng/dl (-2SD) 24 h after triptorelin administration could be useful as a cut-off point to discriminate CDP from HH. We conclude that CDP can be clearly differentiated from HH by the LH response at the 4 h and/or T response at 24 h after triptorelin administration. This test, we think, is superior to the others in that: i) Both 4h LH and 24 h T responses are significantly different between the groups without any overlaps ii) It is practical since it is started at 8 am. The patient can return either at noon on the same day or at 8 am on the following day. The latter option can be particularly useful in that absence from school for the test is unnecessary. iii) Measuring T response at 24 h enables assessment of the integrity of not only pituitary but also gonadal function.

In conclusion, patients with CDP, with bone age equal to or greater than 11.5 years, display a distinct increase in LH at 4 h and in T concentrations at 24 h after 8 am triptorelin administration, thus enabling clear differentiation of subjects with CDP from those with HH.

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Breast milk β-glucuronidase levels in hyperbilirubinemia

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Breast milk β-glucuronidase was thought to be one of the etiological factors in the pathogenesis of late-onset breast-milk jaundice, but results of these studies are conflicting. In this study breast milk β-glucuronidase levels were determined in groups with physiologic jaundice, early breast-feeding jaundice and late breast-milk jaundice. No difference in β-glucuronidase levels of these three groups was found in samples taken on the 4th and 15th days of life. β-glucuronidase activity in breast milk declined from the 4th to 15th day in all groups.

These results imply that factors other than breast milk β-glucuronidase activity should be investigated to reveal the pathogenesis of late-onset breast-milk jaundice.

Key words: β-glucuronidase, breast-milk jaundice, hyperbilirubinemia.

Neonatal jaundice associated with breast-feeding is a problem frequently encountered in neonatology[1]. Early breast-feeding jaundice occurs in the first days of life in breast-fed newborns, as effective breast-feeding is not established[2]. Late breast-milk jaundice is defined as jaundice occurring after the first three to five days of life when there is no hemolysis, and weight gain and intestinal function are normal[1,3]. Of infants who develop bilirubin levels high enough to require phototherapy and who do not have evidence of isoimmunization or other obvious hemolytic disease, 80 to 90 percent are fully or partially breast-fed[4]. Several etiological hypotheses have been postulated to explain the pathophysiological mechanisms of late breast-feeding jaundice. These hypothesis are related to substances such as pregnanediol and non-esterified fatty acids which are thought to inhibit glucuronyl transferase, and there are additional mechanisms-proposed responsible such as increased enterohepatic circulation of bilirubin and increased β-glucuronidase activity[5-15]. There are conflicting reports on β-glucuronidase activity in early and late breast-feeding jaundice[5,7,9-11,13-15]. Our previous report showed no effect of β-glucuronidase activity in early breast-feeding jaundice[7]. In this study we intended to compare maternal milk β-glucuronidase levels in babies with early and late breast-milk jaundice.

Material and Methods

The study group consisted of 41 healthy jaundiced newborn babies, which were exclusively breast-fed. The control group consisted of 18 healthy breast-fed newborn babies without visible jaundice. The study group was screened to exclude those with blood group incompatibility, hypothyroidism, cholestatic disorders, diabetic mothers, asphyxia, septicemia, cephalhematoma, bruising and glucose-6-phosphate dehydrogenase deficiency. Information on the perinatal period, birth weight, maternal drug use, first feeding time, feeding frequency, Apgar scores and postnatal weight loss was obtained for each infant. After informed consent was taken, two samples of breast milk were collected with a manual pump from the mothers. The first sample was collected between the 3rd-5th days; the second sample was collected on the 15th day. Bilirubin levels of the babies were determined at the same time. The samples were kept at -20 °C until analyzed.

The β-glucuronidase assay was performed using phenolphthalein mono β-glucuronic acid as substrate (Sigma kit No.325), and the results

were expressed as modified Sigma Units/ml. Statistical lanalysis was performed using Mann-Whitney U and chi-square tests.

Results

Around the 4th day of life 27 (65.8%) of the 41 infants in the study group had bilirubin peak levels between 7-12 mg/dl (physiologic jaundice) while the other 14 (34.2%) babies had bilirubin peak levels higher than 16 mg/dl (significant hyperbilirubinemia, breast-feeding jaundice)[1,16]. The infants with significant hyperbilirubinemia were treated with phototherapy according to our hospital practice. There was no difference between groups in first feeding time, feeding frequency, and weight loss within the first week of life. On the 15th day of life 22 (53.6%) of the 41 infants had bilirubin levels higher than 7 mg/dl (prolonged jaundice). Nine of these 22 infants were in the physiologic jaundice group and 13 were in the early breast-feeding jaundice group around the 4th day of life. These nine infants with prolonged jaundice were excluded from the physiologic jaundice group. Table I shows the characteristics of the three groups: those with physiologic jaundice, breast-feeding jaundice and the control group around 4th day of life. There was no significant difference in β-glucuronidase levels between the three groups around the 4th day of life. Furthermore, no correlation was found between bilirubin and β-glucuronidase levels in those with physiologic jaundice, or in the breast-feeding jaundice group ($r = -0.33$, $r = 0.42$). On the 15th day of life no significant difference was found in β-glucuronidase levels between the prolonged jaundice group and the infants with bilirubin levels below 7 mg/dl. There was also no sifnificant difference in β-glucuronidase levels between the prolonged jaundice group and the control group on the 15th day (Table II). In these groups no correlation was found between bilirubin and β-glucuronidase levels on day 15. In all groups there was a decrease in β-glucuronidase levels between the 3rd-5th and 15th days of life.

Table I. β-glucuronidase Levels Around the 4th Day of Life and Characteristics of Groups

	Physiologic jaundice group (n=18)	Breast-feeding jaundice group (n=14)	Control group (n=18)	P
Birth weight (g)	3330 ± 319 (2640-3900)	3315 ± 420 (2600-3900)	3341 ± 215 (3000-3750)	> 0.05
Gestational age (week)	38.8 ± 0.9 (37-40)	38.4 ± 1.0 (37-40)	39.2 ± 1 (37-41)	> 0.05
Maternal age (year)	29.1 ± 5.7 (22-40)	28.5 ± 6.3 (21-38)	28.6 ± 4.9 (20-36)	> 0.05
Serum bilirubin levels (mg/dl)	10.2 ± 1.7 (7.9-13.1)	18.2 ± 1.8 (16.2-21.8)	-	0.00
β-glucuronidase levels (U/ml)	385.6 ± 364.6 (144-1350)	259.9 ± 281.3 (60-1179)	276.0 ± 125.9 (84-621)	> 0.05

Table II. β-glucuronidase Levels on the 15th Day of Life and Characteristics of Groups

	Babies with bilirubin levels < 7 mg/dl (n=19)	Babies with bilirubin levels > 7 mg/dl (n=22)	Control group (n=18)	P
Birth weight (g)	3339 ± 312 (2640-3900)	3252 ± 396 (2600-3900)	3341 ± 215 (3000-3750)	> 0.05
Gestational age (week)	38.7 ± 0.8 (38-40)	38.6 ± 1.0 (37-41)	39.2 ± 1.1 (37-41)	> 0.05
Maternal age (year)	28.8 ± 5.7 (22-40)	29.4 ± 5.8 (21-39)	28.6 ± 4.9 (20-36)	> 0.05
Serum bilirubin levels (mg/dl) (day15)	3.6 ± 1.9 (0.7-7.0)	9.4 ± 1.7 (7.1-13.1)	-	0.000
β-glucuronidase levels (U/ml) (day 15)	104.2 ± 47.7 (45-222)	134.6 ± 78.3 (33-336)	128.1 ± 72.5 (42-294)	> 0.05

Discussion

Breast-feeding is a common cause of jaundice in normal newborns in the first week of life and beyond[1,16]. The cause of early-onset breast-feeding jaundice has been related to the frequency of feedings and to inadequate caloric intake[3]. Late-onset breast-milk jaundice is thought to be associated with an abnormality in the composition of breast milk[16]. Breast milk β-glucuronidase was thought to be an etiological factor in the pathogenesis of late-onset breast-milk jaundice, and studies were performed to clarify the effect of β-glucuronidase activity in human milk[5,7,9-11,13-15]. The results of these studies are conflicting. Gourley et al.[11] found that bilirubin levels were related to concentrations of β-glucuronidase in breast milk. However, other studies showed no correlation[5,7,9,13,15]. As breast-milk jaundice may show a broad clinical spectrum ranging from physiologic jaundice to significant hyperbilirubinemia[1], this study was undertaken to investigate the β-glucuronidase levels in breast milk in two groups of hyperbilirubinemic babies with physiologic jaundice and significant hyperbilirubinemia. In this study, similar to other studies[5,7,9,13,15], we did not find any correlation between bilirubin and β-glucuronidase levels in the prolonged jaundice group. Furthermore, the changes in β-glucuronidase concentration in breast milk were found to decrease from the 4th to the 15th day.

Babies with physiologic jaundice in this study were also investigated for breast milk beta-glucuronidase levels. The onset and time of peak bilirubin levels of physiologic jaundice and early-onset breast-milk jaundice are similar, but in early breast-feeding jaundice, peak levels exceed the normal range of physiologic jaundice on the 3rd or 4th day of life. Although no significant statistical difference was shown in β-glucuronidase concentrations between any of the groups (Table I), it is surprising to observe the highest mean breast milk β-glucuronidase level in the physiologic jaundice group.

In the prolonged hyperbilirubinemia group, 13 of 22 infants had been grouped as early breast-feeding jaundice around the 4th day of life. Although there is a classification as early-onset and late-onset breast-feeding jaundice[1,3], our results indicate that nearly all (except one) babies with early-onset breast-feeding jaundice had prolonged hyperbilirubinemia on day 15. These results show that distinction of early and late type breast-feeding jaundices may not be clear, as some cases may overlap.

These results confirm that a simple hypothesis, involving the single action of β-glucuronidase, is insufficient to explain the pathogenesis of breast-feeding jaundice.

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Serum ferritin, iron levels and iron binding capacity in asymmetric SGA babies

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SUMMARY: Karaduman D, Ergin H, Kılıç İ. Serum ferritin, iron levels and iron binding capacity in asymmetric SGA babies. Turk J Pediatr 2001; 43: 121-124.

The concentration of serum ferritin reflects the extent of iron stores in premature infants. We aimed to determine serum ferritin levels and iron status in asymmetric small for gestational age (SGA) babies.

This study was performed on 21 SGA babies and 19 appropriate for gestational age (AGA) babies. Hemoglobin, iron, iron binding capacity and ferritin levels were inevestigated in the first six hours after the birth. Hemoglobin levels in the SGA and control groups were 20.9 ± 1.3 (19.4-23.4 g/dl) and 19.6 ± 0.8 (18.5-21.5 g/dl), respectively ($p = 0.001$). Serum ferritin levels in the SGA and AGA groups were 58.36 ± 20.1 ng/ml and 90.46 ± 30.5 ng/ml, respectively. Ferritin levels were found lower in the SGA group ($p < 0.001$). In the SGA group, decreased serum iron and increased iron binding capacity were found but the difference was not significant ($p > 0.05$).

Decreased ferritin levels may result from either impaired iron transport associated with uteroplacental vascular insufficiency or increased iron utilization during enhanced erythropoiesis in conditions characterized by chronic fetal hypoxia. Our results stress the significance of iron supplementation and careful anemia follow-up in term SGA babies. Because anemia progress early, beginning iron therapy as soon as possible is a necessity in SGA babies as in prematures.

Key words: small for gestational age, SGA, serum ferritin, iron stores.

Fetal growth is affected by fetal growth potential in the first half of the pregnancy and by maternal environment and uteroplacental functions in the second half[1]. Various criterions are used in defining babies as small for gestational age (SGA). SGA may be defined as birth weight below the 10th percentile for gestational age or as more than 2 standard deviations below the mean for gestational age[2]. The ponderal index can be used to identify infants whose soft tissue mass is below normal for the stage of skeletal development. Thus, a pondreal index below the 10th percentile may be used to identify SGA infants. Ponderal index is the most commonly used parameter[3,4]. Since it is not affected by gestational age, race or sex, it is preferred to define SGA.

The concentration of serum ferritin reflects the extent of iron stores in infants[5,6]. For this reason serum ferritin level is useful in the evaluation of iron status.

In this study, iron, iron binding capacity and ferritin levels were searched in the sera of term SGA babies that are thought to be the result of intrauterine chronic hypoxia.

Material and Methods

All infants were examined during the first day after birth; gestational age was determined by Dubowitz score[7]. Forty term babies were between 38-42 weeks' gestational age according to the Dubowitz score. Twenty-one of 40 babies were SGA and 19 were appropriate for gestational age (AGA). In the determination of the babies with SGA, the ponderal index (PI) (body weight/length^3 x 100) was used. Babies having PI under 10 percent were considered as asymmetric SGA[3,4].

Reasons for growth retardation included severe preeclampsia (n = 4), chronic hypertension (n = 2), maternal smoking (n = 2) and chronic

heart disease (n = 1). No underlying disease which could have caused SGA was found in the other patients. All of these infants were asymmetrically growth retarded. Non was retarded on the basis of intrauterine infection, as determined by titers for congenital infectious agents. Infants whose mothers had hemoglobin concentrations < 9 g/dl were excluded as were mothers and infants with fever, bacteremia, or leukocytosis.

The sera of the venous blood samples were taken in the first six hours after birth, and hemoglobin, iron, iron binding capacity and ferritin levels were investgiated. Hemoglobin was determined on the Abbott Coulter Counter (Cell-Dyn 3500). The serum was separated by centrifugation and was frozen at -40 °C until analysis for ferritin. Serum ferritin levels were determined by immunoenzymatic assay (ELISA) in the Exim-Abbott automatic analyzer using Boehringer Mannheim system (Abbott Labarotories, USA, 1997). Iron and iron binding capacity were determined by deproteinization method in the Hitachi 704 automatic analyzer with Boehringer Mannheim system in heparinized plasma (Boehringer Mannheim Gmblt. Mannheim, Germany, 1994).

Values are mean ± SD. Student's t test and linear correlation analysis were used for statistical analysis. A $p < 0.05$ value was considered to represent a significant difference between the compared values (SPSS 5-0, 1992).

The study was approved by the Medical Ethics Committee of Pamukkale University Hospital.

Results

Hemoglobin, iron, iron binding capacity and ferritin levels were investigated in the first six hours after birth in the 21 SGA and 19 AGA babies. SGA infants included 11 females (52.4%) and 10 males (47.6%) and AGA infants included 10 females (52.6%) and 9 males (47.4%). The mean birth weights were 2370 ± 211 grams (1800-2600) in the SGA group and 3228 ± 295 grams (288-3700) in the control group. There was a significant difference between the birth weight of the two groups ($p < 0.001$). Hemoglobin levels in the SGA and control groups were 20.9 ± 1.3 (19.4-23.4 g/dl) and 19.6 ± 0.8 (18.5-21.5 g/dl), respectively. There was a significant difference between the hemoglobin levels of the two groups ($p = 0.001$).

Serum ferritin levels in the SGA and AGA groups were 58.4 ± 20.1 ng/ml and 90.5 ± 30.5 ng/ml, respectively. Ferritin levels were found lower in the SGA group ($p < 0.001$). Serum iron levels in the SGA and AGA groups were 10.1 ± 3.4 µmol/L and 10.6 ± 5.0 µmol/L, respectively. Serum iron binding capacities were 74.5 ± 11.8 µmol/L and 70.4 ± 10.0 µmol/L, respectively. In the SGA group, decreased serum iron and increased iron binding capacity were found, but the difference was not significant ($p > 0.05$) (Table I).

Table I. Perinatal Data of SGA and Control Groups (mean ± SD)

	SGA	Control	P
Birth weight	2370 ± 211	3228 ± 295	< 0.001
Gestational age (weeks)	39.7 ± 0.9	40.0 ± 0.5	> 0.05
Hemoglobin	20.9 ± 1.3	19.6 ± 0.8	= 0.001
Iron (µmol/L)	10.07 ± 3.4	10.6 ± 5.0	> 0.05
Iron binding capacity (µmol/L)	74.5 ± 11.8	70.4 ± 10.0	> 0.05
Ferritin (ng/ml)	58.4 ± 20.1	90.5 ± 30.5	< 0.001

SGA: small for gestational age.

Twelve (57.1%) of the infants in the study group had abnormally low serum ferritin levels, whereas only three (15%) of the control infants had such low values. An abnormally low serum ferritin level was accepted as lower than 60 ng/ml[8].

We found clear correlation between serum ferritin and birth weight (r: 0.50, $p < 0.001$).

Discussion

Since the fetus gains 85 percent of its body weight in the second half of pregnancy, any impaired uteroplacental blood flow in this period will definitely disturb food and oxygen transfer to the baby[9]. Length and head circumference are protected; however, because of inadequate weight gain, these babies have a lower ponderal index and are referred to as asymmetric SGA.

In 50 percent of asymmetric SGA babies, intrauterine chronic hypoxia risk is increased because of their impaired uteroplacental blood flow[9]. These babies, when compared with AGA babies, presented higher cord hemoglobin levels[10] and an increased incidence of neonatal polycythemia[11]. Usually hematocrit levels are ≥ 60 percent. Neonatal polycythemia is associated with impaired fetoplacental blood flow during fetal hypoxia episodes or with chronic fetal tissue hypoxia and a compensatory increase in erythropoietin levels[12].

Levels of serum ferritin reflecting the extent of iron stores in children have been reported in recent studies[5,6]. Serum ferritin levels were searched in premature babies[13-15]. Correlations were detected between maternal and fetal serum ferritin levels and gestational age[8,16,17]. However, sufficient studies in SGA babies have not been done.

Cord ferritin levels were found significantly lower in preterm babies in a study based on 22 term and 32 preterm infants[15]. Tekinalp et al.[18] determined serum ferritin levels in 76 neonates and showed the lowest levels in less than 34 weeks of gestational age prematures. In a similar study, Jansson and Holmberg[13] showed significantly lower serum ferritin levels in less than 34 weeks of gestational age prematures. There was a positive correlation between serum ferritin levels and birth weights. In this study, we found clear correlation between serum ferritin levels and birth weights.

The results of the limited number of studies on SGA babies are as follows: Olivares et al.[19] showed that serum ferritin levels were lower in term SGA babies than in preterm AGA babies. On the other hand, serum ferritin levels were higher in term SGA babies than in preterm SGA babies.

Abbas and Snijders[20] in 1994 found a small decrease in ferritin levels in SGA babies and an important increase in maternal serum ferritin concentration. They demonstrated that the decreased feto-maternal ferritin ratio could be the consequence of impaired placental perfusion.

Chockalingam et al.[21] studied 50 high-risk infants consisting of 16 SGA, 21 from preeclamptic mothers and 13 from diabetic mothers. They showed a significant inverse correlation between increasing serum transferrin and decreasing ferritin levels compared to the control group. The reason for the increased transferrin levels was unknown, but may reflect an increase in iron binding capacity compensating for low fetal iron stores.

In this study, serum transferrin and maternal ferritin levels were not measured. However, in the SGA group, decreased serum iron and increased iron binding capacity were found, but difference was not significant. There results could be evidence of increased iron requirement in SGA babies. Serum ferritin levels were determined to be significantly decreased in SGA babies. Decreased ferritin levels may result from either impaired iron transport associated with uteroplacental vascular insufficiency[22] or increased iron utilization during enhanced erythropoiesis in conditions characterized by chronic fetal hypoxia.

Chockalingam et al.[21] determined abnormally low ferritin levels (< 60 ng/ml) in 64 percent of the infants. Similarly, we found abnormally low ferritin levels in 57.1 percent of the study group.

This study demonstrated that newborn infants at risk for impaired uteroplacental blood flow or chronic fetal hypoxia had depressed ferritin levels and body iron stores. Decreased iron stores may result from either impaired iron transport associated with uteroplacental vascular insufficiency or increased iron utilization during enhanced erytrhopoiesis in conditions characterized by chronic fetal hypoxia.

Further studies are needed in order to determine the time to administer iron supplement in the event of iron deficiency anemia in SGA babies.

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Seroprevalence of helicobacter pylori in a pediatric population

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SUMMARY: Altuğlu İ, Sayıner AA, Özacar T, Egemen A, Bilgiç A. Seroprevalence of Helicobacter pylori in a pediatric population. Turk J Pediatr 2001; 43: 125-127.

Helicobacter pylori (H. pylori) is one of the common bacterial infections in humans. In this study, seroprevalence of H. pylori infection in a pediatric population in İzmir and its relationship with different variables were investigated. Two hundred twenty-six children (115 boys, 111 girls, age range: 1-18) were tested for anti-H. pylori IgG. Socioeconomic conditions, living area (urban or rural), and number of people living in the same house were noted for each subject. H. pylori antibodies were determined by an enzyme immunoassay. Overall, 120 (53%) subjects were seropositive for H. pylori. The seroprevalence of H. pylori increased significantly with age and poor socioeconomic conditions. Seroprevalence did not differ according to sex, number of people living in the same house or living area.

Key words: Helicobacter pylori, seroprevalence, children, age, sex, socioeconomic conditions.

Helicobacter pylori (H. pylori) is now thought to be one of the most important factors in the etiology of chronic gastritis, peptic ulcers, noncardia gastric cancer and gastric mucosa associated lymphoid tissue lymphoma[1].

H. pylori infection is probably the most common chronic bacterial infection in humans. The prevalence of the infection varies from 50% of adults in developed countries to nearly 90% in developing countries[2].

Overcrowding and poor socioeconomic conditions during childhood appear to be risk factors for H. pylori infection. The data available is consistent with the suggestion that the infection may be acquired in early life and that person to person contact plays an important role in its transmission[3,4].

The aim of this study was to investigate the seroprevalence of H. pylori infection in different parts of İzmir and to determine the relationship of seropositivity with age, sex, number of people living in the same house, socioeconomic conditions and living area (urban or rural).

Material and Methods

The study was planned as part of the rubella seroprevalence study. In the mentioned study, a cluster sample design was carried out for the selection of the study population[5]. Among those, children aged 1-18 were selected for the presented study. The study population consisted of 226 asymptomatic children (115 boys, 111 girls, age range 1-18). The children were distributed into four groups by age: group A, 1-5 years; group B, 6-9 years; group C, 10-13 years; group D, 14-18 years.

The children were selected from different regions of Izmir and examined in two different groups, urban and rural. Socioeconomic conditions and number of people living in the same house were noted. Socioeconomic conditions were classified into three main categories (low, middle and high) according to the annual household income. Children were examined in two groups according to the people living in the number of same house (group I: 1-4 people, group II: ≥ 5 people).

Serum samples obtained from the children were stored at -20 °C until tested.

IgG antibodies to H. pylori were determined qualitatively by a commercial enzyme linked immunosorbent assay (Zeus Scientific Inc, USA).

The seroprevalence of H. pylori infection was analyzed in relation to all considered variables (age, sex, number of people living in the same house, socioeconomic conditions and living area). Chi-square test was used to examine the relation of H. pylori infection and the variables.

Results

Seropositivity for H. pylori was found in 120 of the 226 subjects (53%). Characteristics of the study population and H. pylori status are shown in Table I.

Table I. Characteristics of the Study Population

Groups		H. pylori IgG (+) Number (%)	H. pylori IgG (-) Number (%)	p value
Sex	Boys	60 (52.2)	55 (47.8)	NS
	Girls	60 (54.1)	51 (45.9)	
No of people living in the same house	1-4	64 (50.8)	62 (49.2)	NS
	≥ 5	56 (56)	44 (44)	
Socioeconomic conditions	Low	45 (65.2)	24 (34.8)	p = 0.048
	Middle	65 (48.5)	69 (51.5)	
	High	10 (43.5)	13 (56.5)	
Age	1-5	16 (33.3)	32 (66.7)	p = 0.003
	6-9	28 (50)	28 (50)	
	10-13	34 (55.7)	27 (44.3)	
	14-18	42 (68.9)	19 (31.1)	
Living area	Urban	90 (52.6)	81 (58.4)	NS
	Rural	30 (54.5)	25 (45.5)	

NS: Non-significant.

The seroprevalence of H. pylori increased significantly with age and poor socioeconomic conditions. There was only one seropositive child (1/11) among the one-year-old group. The number of children infected reached 46.8 percent (57/122) by the age of 10 years. Seroprevalance did not differ according to sex, number of people living in the same house or living area.

Discussion

In this study, the prevalence of antibodies to H. pylori and associated risk factors were determined in asymptomatic children living in different parts of Izmir.

Published studies on the epidemiology of H. pylori infection show considerable differences in the prevalence of antibodies to H. pylori in the different populations studied. In a study from India, the frequency of H. pylori infection increased with age and was > 80 percent by age 20[6]. In a study from Italy, seropositivity was 29 percent in the same age group, increased significantly with age and did not differ according to sex[7]. In France, seropositivity was 16 percent in a group aged between 10-19[9].

In a Japanese study, of 1,043 healthy adults, 416 (40%) were seropositive for H. pylori. The seropositivity rates for H. pylori significantly increased with age[8]. In another study from Japan[10] the data strongly suggested that the highest infection rates for H. pylori occur among infants and children.

Dominici et al.[11] reported that children in families where both parents were infected had a higher prevalence of H. pylori infection (44%) than children from families with only one (30%) or no parents (21%) infected. In the mentioned study, it was concluded that social status may be a risk factor for H. pylori infection.

In a study from Estonia, of the 421 schoolchildren selected from urban and rural areas, H. pylori prevalence rate was 65 percent in rural areas and 49 percent in towns[12]. In our study, no significant difference was found in the seropositivity between the groups from different living areas.

In a Turkish study in the Çukurova region, 25.9 percent of 54 asymptomatic children were found to be anti-H. pylori positive[13]. In adults anti H. pylori IgG seropositivity was determined in two different study groups in Izmir. The results were 62.5 percent in 40 asymptomatic infants and 73.8 percent in 244 adults, respectively[14,15]. Seropositivity was related to socioeconomic conditions in both studies. In another study from Turkey, 59 children with dyspeptic complaints and 48 asymptomatic children (age range 5-17) were tested for H. pylori antibodies, and 52.5 percent and 41.7 percent, respectively,

were found to be positive. The difference was not statistically significant. Seropositivity increased with age in both groups[16].

There are common patterns in most of the studies. The acquisition of H. pylori infection is early in life. The high prevalence of H. pylori infection in developing countries is a reflection of the lower socioeconomic level of those areas[6].

A number of studies indicate that crowding and number of siblings were associated with H. pylori seropositivity[7,15]. In the presented study, H. pylori seropositivity increased from 50.8 percent and 56 percent when the number of people living in the same house was ≥ 5, but the difference was statistically nonsignificant.

In this study, high seroprevalence of H. pylori and a relationship with age and socioeconomic conditions were determined. Our findings also support the fact that H. pylori is usually acquired early in life. Children in poor socioeconomic conditions are at a greater risk of infection. However, in this study, no correlation was found between seropositivity and number of people living in the same house.

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Critical learning period for speech acquisition and screening techniques in early detection of hearing impairment

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SUMMARY: Şerbetçioğlu MB. Critical learning period for speech acquisition and screening techniques in early detection of hearing impairment. Turk J Pediatr 2001; 43: 128-132.

In order to develop verbal communication skills, an infant nervous system needs sound stimuli, especially human speech, in the early and critical learning period of life. The maturation of hearing and language learning abilites of an infant is considered to be complete within the first two to three years of life. Therefore, detection of hearing impairment before the end of the critical language learning period is considered crucial if a child is expected to acquire his/her native language properly. Parents or caregivers may suspect the presence of a delay in language acquisition; however, relying only on parental awareness could cause a delay in detection of the hearing impairment. Even mild hearing loss may interfere with normal development of speech and language in infants, therefore, universal screening of infants for hearing loss as early as possible should be the goal.

The purpose of this paper is to discuss the concept of the critical language learning period and its relevance to language acquisition. Furthermore, it is the aim of the paper to evaluate the available techniques for early identification of hearing impairment in general and the feasibility of the various techniques in Turkey.

Key words: newborn, congenital, sensorineural, hearing loss, deafness, early identification, language, newborn hearing screening.

Hearing impairment seems to be one of the most common diseases in the world. According to statistics, this is even true in a highly developed country such as the United States of America, where hearing impairment in general is considered second only to arthritis as the most prevalent chronic disease. In fact, the incidence of children born with mild to profound degrees of permanent hearing loss in the United States is at least 1 in 750[1].

Although there are several types of disabling conditions, among these, hearing loss in the early period of life is considered to be a unique problem. Parents can recognize the problems of a blind infant at once but it is not easy to recognize a congenital hearing loss. Late identification of infant hearing impairment is considered a major public health problem. The purpose of this paper is to review the reasons for early identification of hearing impairment in general and the feasibility of the techniques for detecting hearing impairment in Turkey.

1. Hearing Impairment and Language Disability

There is common agreement that congenital hearing loss inevitably leads to some type of language learning disability. According to Bamford and Saunders[2], poor language performance related with congenital sensorineural hearing loss comes about not only because of lack of sensory input (e.g. speech sounds) -the primary cause- but also because of associated secondary effects. The secondary effects have been described as "contextual aspects of language acquisition". Again, according to the same authors, contextual aspects of language acquisition can be divided into two main processes.

The first process is the internal contextual aspects of language development, which are concerned with cognitive processes and strategies. Cognitive processes are involved with the intelligence and may be defined as an ability to recognize relationships and solve problems. In the past, it was thought that intelligence was mainly determined by heredity. However, it is

now felt that intelligence (at least cognitive processes) is partly determined by the sensory inputs a child receives.

The second type of contextual process involves the outside world of the infant, such as adult-child interactions and the communicative abilities of the infant. These abilities are mainly needed for adaptation of the infant to the social environment. The main cause of several psychological disorders of the child, indeed, may be related to insufficient adaptation of the child. In fact, it is reasonably easy to discover the relationship between hearing loss and psychodynamic factors in the childhood period. Thus, although the hearing loss seems to be limited to his/her ears, the child's total life is affected by this condition.

2. The Critical Learning Period

The maturation of hearing and language learning abilities of the infant is essentially completed within the first two to three years of life. In fact, these skills, which are highly integrated, start to develop as soon as the child is born. Thus, it is almost a consensus that in order to develop communication skills, an infant nervous system needs sound stimuli, essentially the human speech, in the early and critical learning period of life. In a study of adopted children, Dennis[3] identified the first two years of life as the critical period for exposure to a good language environment.

Actually, the critical period of speech acquisition is a maturational concept and does not help to illustrate the deficient part of the nervous system. On an anatomical basis, Rubin[4] argued for the early plasticity of the brain. The brain requires stimulation in the first three years of life, if auditory perception and language acquisition are to occur:

".... Available information would indicate that the maturation of the auditory system is centripetal, proceeding from cochlea to the auditory cortex. Environmental sounds would appear to have its greatest effect in shaping auditory ability from the time the cochlea and eighth nerve first become functional (i.e. just after the birth) to the time when maturation of the central nervous system is achieved."

There are controlled studies on the hearing-impaired population that seem to support the concept of early plasticity of the brain. For instance, in an earlier study, the Lexington School for the Deaf[8] compared infants who had been admitted to the audiological habilitation program before 16 months of age with those admitted between 16 and 24 months. After the habilitation program had been carried out, the results showed that mother-infant communication was far more superior in the earlier admitted group. In addition, the language learning abilities of these children were higher than those of the late admitted group. The lower age limit for the language acquisition period could be as early as six months[6]. According to recent studies which compared early intervention versus intervention after six months, the hearing-impaired infants who received early recognition as well as early habilitation were far more superior in terms of receptive as well as expressive language abilities[7].

3. Parental Awareness

One of the main targets could be to develop parental awareness for hearing impairment all over the country. This effort may not be sufficient to detect hearing impairment within the first 12 months of life; however, it may lower the overall age of detection of hearing impairment and help to improve the services supplied to these children. Parents could recognize the hearing impairment earlier than anyone else, if they are properly informed. Since it is difficult to inform parents individually, mass media could be used to make parents conscious and possibly suspicious about their child's hearing and language learning problems.

Generally, only parents can be sure of their child's hearing loss at a time when there is an obvious delay in speech production. This is probably related to the observation that parents as well as medical professionals are mainly concerned with only the delay in speech production. Tucker and Nolan[8] also reported that parental detection of hearing loss is 70 percent successful. Unfortunately, this detection is often dependent upon concern at the lack of language development[8] which, by definition, occurs too late for remedial action to be taken (see the section above). On the other hand, hearing loss can be detected early if the simple reactions of an infant to a ordinary speech signal are considered as the criteria of normal hearing, instead of speech acquisition.

According to a study of deafness in the European Community[9], only 22 percent of affected children were suspected by their parents

of being hearing-impaired in the first year of life and only about two-thirds by the time they reached their third birthday. This is an another example which indicates that it is not an easy task to detect hearing impairment before children reach the end of the critical language learning period, if we relied only on parental awareness.

4. Screening Methods for Hearing Impairment

A traditional definition of screening is "a process of applying to large numbers of individuals certain rapid, simple measures that will identify those individuals with high probability of disorders in the function tested"[10].

If a very young child has suffered some sort of hearing loss or any kind of mental retardation or cannot participate in a familial language environment, the central nervous and auditory systems will not develop correctly. Of these adverse conditions which might lead to some sort of deviation in the normal development of language acquisition, congenital hearing loss can be detected within the first three days of life. Thus, in terms of screening criteria, the screening of congenital hearing loss in infants is considered as acceptable and feasible. In the search of a technique for screening hearing in the infant population, the main difficulty is that there is no established rapid, simple and perfect measure to employ all over Turkey. There are some types of subjective and objective hearing tests which can be used. However, in the newborn population, only objective measures can essentially provide a rapid, simple and perfect detection of hearing loss[12].

Recent technological developments have produced objective screening methods that are rapid, reliable, sensitive, and easily administered. These advances offer an opportunity, for the first time, to initiate universal screening for hearing impairment in early infancy. Objective screening tools are becoming more and more popular and are almost acknowledged as routine hearing screening tests[8]. Two objective measuring techniques, auditory brainstem response (ABR) and transiently evoked oto-acoustic emission (TEOAE), are currently available and used for infant hearing screening. ABR is the most reliable measure currently available with which to assess the functional integrity of a child's peripheral auditory system. while ABR can estimate the degree of hearing loss if peresent, it is time consuming and requires experienced personnel. In order to decrease the time required as well as the qualification of the personnel, an automated measurement of ABR has been developed[11]. In the latter study, 41,000 newborns screened using automated ABR equipment as well as a TEOAE device improved the screening results to near 100 percent sensitivity and an as low as 2 percent false-positive rate. However, such successful results need to be replicated by other researchers in order to be convincing. The TEOAE technique, on the other hand, has been proposed as an objective, rapid and noninvasive screening test for auditory dysfunction in neonates. This technique is sensitive to cochlear hearing losses and provides "pass or refer" results. The Joint Committee on Infant Hearing stated on June 21, 1994 that even mild hearing loss may interfere with normal development of speech and language, and recommended the goal of universal detection of infants with hearing loss as early as possible. This committee endorsed the goal of identification of hearing impairment using two-stage screening (i.e. initially applying TEOAE testing and then utilizing ABR technique for those infants who failed the first stage) before discharging from the newborn nursery[12]. Both of these screening techniques satisfy all the necessary criteria required for screening the infant population. In a paper to be published soon, the results of a two stage screening program applied by the author and colleagues in 100 neonates with high-risk in a Neonatal Intensive Care Unit in İzmir revealed a hearing loss rate in the high-risk group in Turkey in the range of 13 percent which was higher than expected.

5. Screening High-Risk Infants

High-risk infants can be described as a small group of children whose history and physical conditions identify them as possessing a high chance of having the handicap searched for. Since the incidence of moderate to profound hearing loss in this high-risk group is fairly high (i.e. 2.5 to 5%), full audiological testing of this group is required, once this group has been identified (see Appendix I). In the past, as screening the entire newborn population (universal screening) is expensive (in terms of expenditure and human resources), screening

only risk newborns was considered sufficient[13]. However, screening only the infants with high-risk criteria identifies only half of the infants with significant hearing loss, and the other half of the infants with hearing loss (which are not within the high-risk population group), would be detected as having hearing loss later than the age of two years. Thus, it is envisaged that screening high-risk infants would not achieve the goal of detecting hearing loss before the end of the critical language learning period.

6. Universal Screening

In the United States of America, which is one of the leading countries in terms of health and technology, there is a tendency to apply universal screening in the first months of life. In the United Kingdom, the Health Visitor Screening Program has been developed. This type of universal hearing screening test is routinely carried out on nine-month-old babies in some regions of the U.K., by specially trained Health Visitors employing behavioral distraction tests[8]. This kind of screening test, while adventageous in that the whole hearing pathway is tested, is not considered an appropriate technique for Turkey, since it relies heavily on highly trained and experienced staff.

In 1993, The National Institutes of Health in the U.S.A. issued a statement on Early Identification of Hearing Impairment, and concluded that "significantly reduced auditory input also adversely affects the developing auditory nervous system and can have harmful effects on social, emotional, cognitive, and academic development, as well as on a person's vocational and economic potential[14]. 'This panel endorsed the recommendations of the Joint Committee on Infant Hearing that all hearing-impaired infants should be identified and treatment initiated by six months of age. In order to achieve this objective, the latter panel recommended universal screening for hearing impairment prior to three months of age. Because of the unique accessibility of almost all infants in the newborn nursery, the consensus panel recommended screening of all newborns, both high and low-risk, for hearing impairment prior to hospital discharge. Clearly, universal screening will increase the number of infants identified with hearing impairment. This will necessitate adequate diagnostic follow-up and treatment facilities. Comprehensive intervention and management programs (i.e. audiological rehabilitation and special education centers) must be an integral part of a universal screening program. Thus, universal newborn screening is only the initial step if early detection and intervention are considered the target of the public health policy in this country.

Because 20-30 percent of hearing-impaired infants will acquire their hearing loss during early childhood, universal neonatal screening is not a replacement for ongoing surveillance throughout infancy and early childhood. Even though universal screening is an essential step in the early detection of hearing impairment, education of primary caregivers and primary health care providers on early signs of hearing impairment remains an important goal.

Conclusion

The infant nervous system is extremely sensitive to human communication sounds, especially at the beginning of life. If the infant cannot participate in a familial language environment or suffers from some type of hearing impairment during the first three years of life, then the speech and language learning abilities of the child will be inevitably affected. As the mental and psychological development of the infant is closely dependent on communication the infant is generally affected by these conditions. However, if the main reason for interruption in communication is analyzed, and remedial action can be taken before it is too late, the development of the child will be minimally affected. In other words, "the earlier, the better".

In the past, detection of hearing impairment was often dependent upon concern at the lack of language development. According to current concepts, it is not a sufficient way to detect hearing impairment, especially if the mental and psychological development of the child, have been affected.

To deal with congenitally hearing-impaired children, team work is necessary. Various groups of people should coordinate in order to organize better screening programs: parents of hearing-impaired infants, audiologists, physicians, teachers, psychologists and so on.

In an ideal large-screening program, it is assumed that all infants will be checked for hearing impairment, i.e. universal hearing

screening. If the universal hearing screening is not considered feasible, then audiological evaluation of high-risk infants should be done.

Consequently, there is widespread agreement about the need for very early identification of a child suffering from congenital hearing impairment. It is difficult, to cure congenital sensorineural hearing impairment. However, if it can be recognized earlier, children suffering from hearing impairment can be habilitated and specially educated, only then minimizing the damaging effects.

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Apoptosis in renal disease: a brief review of the literature and report of preliminary findings in childhood lupus nephritis

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Apoptosis, a programmed form of cell death, is an important mechanism that maintains cellular homeostasis. The cellular content of tissues is regulated by a balance between cell proliferation and cell loss. Apoptosis is important not only in physiological conditions but in pathological processes as well.

Apoptosis has been implicated in the pathogenesis of certain renal diseases. In human models, systemic lupus erythematosus (SLE) and IgA nephropathy have been the main interests. These studies have mainly shown that apoptosis is important in the control of mesangial cell population.

We have attempted to define the role of apoptosis in a cohort of childhood lupus nephritis. We have analyzed apoptosis by the terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP-biotin nick end-labeling (TUNEL) method in eight SLE pediatric patients, two of whom had hereditary deficiencies of complement components. Although the sample size was small because of the rarity of hereditary complement deficiencies, we have shown that apoptotic activity was the greatest among these pediatric patients. It has been previously suggested that in lupus, autoimmunity develops as a result of inadequate clearance of apoptotic blebs containing nuclear elements; complement deficiencies are the most important hereditary factors predisposing to the inadequate clearance of apoptotic particles. This is the first time this hypothesis has been evaluated in the tissue samples of hereditary complement deficiency-related proliferative lupus nephritis. On the other hand, apoptosis was not different from the other mesangial proliferative glomerulopathies in the lupus nephritis samples. Further studies are needed to confirm our preliminary findings.

Apoptosis has been implicated in other renal diseases as well, such as autosomal polycystic kidney disease, and in experimental models. A short review of the relevant literature is presented highlighting the role of apoptosis in the pathogenesis and prognosis of certain renal diseases.

Key words: apoptosis, renal disease, systemic lupus erythematosus, hereditary complement deficiency, childhood.

Extensive studies during the past 30 years in histopathology, genetics and molecular biology have shown that virtually all animal cells are armed with the genetic machinery to commit suicide. Under normal conditions damaged or senescent cells sacrifice themselves through a non-inflammatory, energy-dependent form of cell death, termed apoptosis, for body cell homeostasis[1-3]. This type of cell death was first named by Kerr et al[3]. as apoptosis, taken from a Latin word describing the process of leaves falling from the tree or petals from a flower. Apoptosis is a programmed form of cell death. It is required for elimination of unwanted cells in processes as diverse as embryological remodeling, development of the immune repertoire, and resolution of inflammation. In turn, the mechanisms of apoptosis and removal of some cells are required to protect the organism from inflammation and autoreactivity[1].

A Worm's Tale: A Guide for Understanding the Molecular Aspects of Apoptosis

A number of detailed research as in the early 1930's in the nematode worm, Caenorhabditis elegans have shed light on the genetic and

molecular mechanisms of apoptosis[3]. In this worm, specific genes are activated to kill precisely 131 cells, leaving 959 in the adult worm[4,5]. Studies of this worm revealed that apoptosis has four consecutive episodes: 1- Extracellular or intracellular signaling to trigger committed cell death, 2- Cell killing by activation of intracellular proteases, 3- Removal of the apoptotic cell by other cells, and 4- Degradation of the cell corpse within the lysosomes[5]. These stages are controlled by CED (for cell death abnormal) genes in C. elegans which are highly conserved throughout animal evolution from worm to human. The products of CED-3 and CED-4 (homologous to human caspase) are required for execution of apoptosis while CED-9 (homologous to human Bcl-2) hinders apoptosis by inhibiting CED-3 and 4. CED-3 is a cysteine aspartyl protease (caspase) which activates a variety of cellular proteins such as DNA repair enzymes, components of nuclear membranes and endonucleases responsible for cleaving the DNA in the apoptotic cell. CED-3 is homologous to caspases in mammalian cells, while CED-4 is to apoptotic protease activating factor-1 (Apaf-1) and CED-9 is to the Bcl-2 family which protects apoptosis[4].

A number of switches are operative in the control of apoptosis. The main death signals are Fas, Fas-ligand, TNF, c-myc, and the main death promoters are Bad, Bax (Table I, Fig. 1). On the other hand, Bcl-2 is an intracellular, apoptosis inhibitory protein that inhibits Fas/APO-1 -induced apoptosis.

Table I. Genes Controlling Apoptosis in Mammals

Signals leading to apoptotic death	c-myc (myelocytoma oncogene) Fas (APO-1, CD-95) Tumor necrosis factor (TNF, Fas-ligand, etc.) Hid p53 E1A/E1B
Death promoters	Bad Bax Bak (Bcl-2 homologous) Bcl-x_s
Cysteine proteases (caspases)	Ced-3, ced-4 (Caenorhabditis elegans genes)
Survival promoters (anti-apoptotic)	Bcl-2 (B-cell follicular lymphoma, ced-9) Bcl- x_L

The apoptotic process begins with the shrinkage of the cell characterized by condensation of the cell cytoplasm and pyknosis of the nucleus. In the next stage, the nucleus becomes fragmented[4-6]. At the final stage the cell is broken up into several membrane-bound

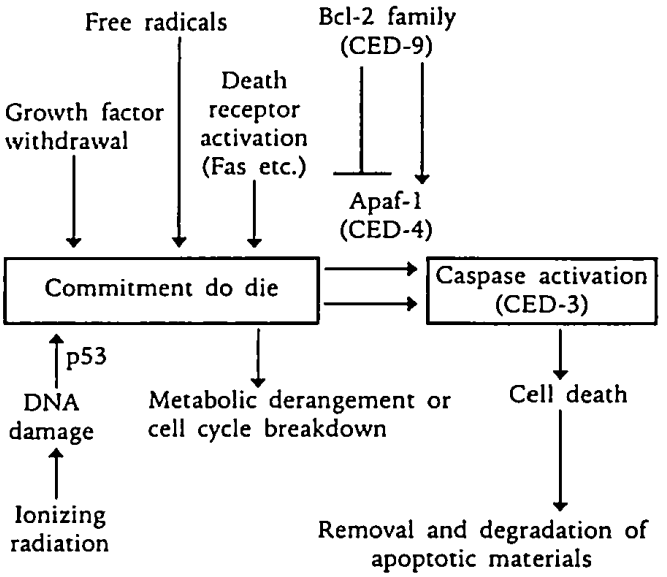

Fig. 1. A simplified scheme of the apoptotic program in mammalian cells[5].

vesicles, which include several intact organelles and nuclear fragments. These are called apoptotic bodies which are then internalized by adjacent cells or macrophages without inciting inflammatory events which might be caused by the uncontrolled release of noxious contents of the cell. Nuclear disintegration is exclusively achieved by a specific endonuclease (CAD-caspase–activated deoxyribonuclease). These enzymes act on multiple intracellular targets causing the formation of DNA laddering (endonuclease-mediated internucleosomal chromatin cleavage) and alterations of plasma membrane, including the exposure of phospholipid phosphotidylserine. Once initiated, the process quickly proceeds. Since the apoptotic cells are rapidly removed by neighboring cells and macrophages within 1-6 hours, only a few examples are visible in tissues. These cells are seen as shrunken cells with a dark pyknotic nucleus usually surrounded by a clear halo visible on light microscopy (Fig. 2). This is an energy–requiring process, differentiating apoptosis from necrosis, which requires no energy. The intracellular levels of adenosine triphosphate (ATP) also affect the form of the cell death[5]. For instance, in experimental studies, it has been demonstrated that apoptosis is the preferable mode of cell death in ATP-supplying conditions, whereas necrosis occurs in ATP-depleted situations. Based on the experimental data, it was concluded that ATP supply by glycolysis and mitochondrial respiration is needed for the final stage of apoptosis leading to nuclear condensation and DNA degradation.

ATP is also an important factor in the activation of executioner caspases that are triggered in the late stages of apoptosis[3-6].

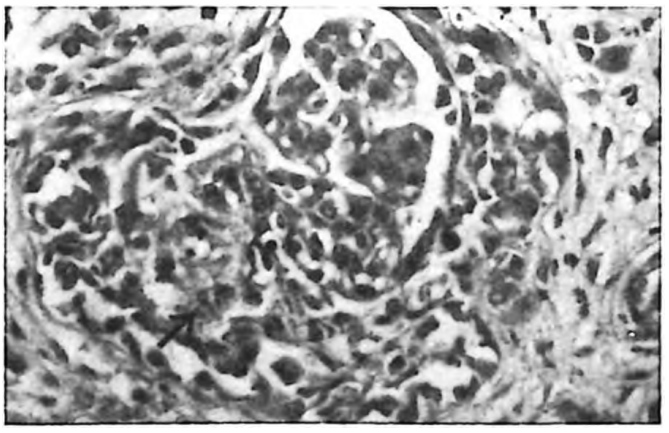

Fig. 2. The glomerulus with endocapillary proliferation with marked variation of its intensity among the segments of glomerular luft, focal karyorrhexis and complete epithelial crescent with partially obliterated Bowman's capsular space where pseudotubular formation is present in a case with C_{1q} deficiency (HE x 400).

Necrosis is different from apoptosis in many aspects (Table II). For example, cellular membranes remain intact during the apoptotic process, whereas cellular swelling, caused by loss of permeability and integrity of cell membrane, is one of the most prominent alterations during necrosis[4]. As a consequence, the disturbance of cellular membranes results in secondary damage of intracellular organelles, such as mitochondria, endoplasmic reticulum and polysomes. In the later stages of necrosis, rupture of plasma membrane and mitochondrial swelling are followed by a spilling out of the cytoplasmic organelles and release of lysosomes. The ruptured lysosomes release hydrolases leading to accelerated cellular disintegration. During the later stages of necrosis, nuclear DNA is degraded in nonspecific fragments.

Apoptotic cell fraction can be easily identified and quantified by Terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP-biotin nick end-labeling (TUNEL) method, as described by Gavrieli et al[7]. The reaction is so specific that only apoptotic nuclei are stained. The method is based on the specific binding of Tdt to 3'-OH ends of DNA following synthesis of a polydeoxynucleotide polymer. Nuclear DNA on renal sections is first exposed to proteolytic treatment, and then Tdt is used to incorporate biotinylated deoxyuridine at sites of DNA breaks. The signals were amplified by avidin-peroxidase system enabling conventional histochemical identification by light microscopy[7,8] (Figs. 3 and 4).

Table II. Comparison of Cardinal Features of Apoptosis and Necrosis

Cardinal features	Apoptosis	Necrosis
Triggers leading to	Physiologic or pathologic conditions without ATP depletion	Severe hypoxia, toxins, conditions of ATP depletion
Biochemistry		
ATP requirement	Yes	No
DNA fragmentation	Laddering of DNA internucleosomal cleavage as 185 base pairs	Randomly
Protein	Caspase activation	Nonspecific degradation
Substrates	Specific substrates	Nonspecific hydrolysis
Histology		
Cell	Chromatin condensation, single deaths, apoptotic bodies	Cellular swelling, death of group of cells, disintegrated nucleus
Organelles	Intact	Damaged
Mitochondria	Swelling, cytochrome-C release	ATP-depleted, swollen, ruptured
Plasma membrane	Intact, blebbed	Disrupted, enhanced permeability
Removal of death cells	Adjacent cells	Immigrant phagocytes
Tissue reaction	Little or none	Inflammation

Fig. 3: Numerous apoptotic bodies with preserved cellular membrane in the glomerular tuft (stained in black) detected by the TUNEL method in the case with C1q deficiency. Note the silhouette appearance of the crescentic glomerulus in the background (x 640).

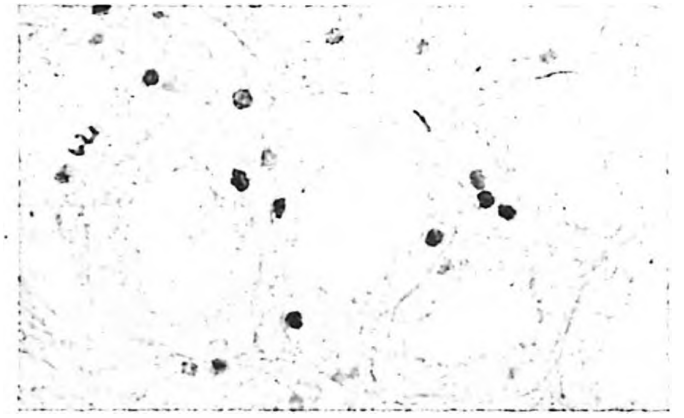

Fig. 4: Same case. Extensive apoptosis in tubuli detected by the TUNEL method (x 400).

Recently, the role of apoptosis in the pathogenesis of renal diseases has attracted much attention[1,2]. Apoptosis has contradictory effects in kidney diseases[2] . In certain experimental models of proliferative glomerulonephritis, apoptosis has been suggested to be an essential process in the regulation of endothelial or mesangial cells, in the repair stage[9-11]. Apoptosis has been assigned a beneficial role in proliferative glomerulopathy induced by anti-Thy-1.1 antibody administration. In this model apoptosis has been suggested to be necessary for regulating the number of intrinsic endothelial cells and for removing the unwanted cells of inflammation[10,11]. On the other hand, apoptosis has been implicated in the sclerotic process within the kidney: apoptotic bodies have been demonstrated in the sclerotic areas of experimental and human renal diseases[10,11]. Makino et al.[12] observed TUNEL-positive cells mainly in the sclerotic lesions. Again, the role of apoptosis in sclerosis was evident in the remnant kidney model in rats[12]. Thus although apoptosis may initially be a regulatory mechanism, 'uncontrolled' apoptosis seems to be involved in the sclerotic process. In the present review, summarize the findings of the various studies on apoptosis in certain renal diseases, and present our data in lupus nephritis in children.

Apoptosis and Glomerulonephritis

The experimental model of Thy-1 glomerulonephritis is a reversible model of glomerulonephritis. In this model it has been demonstrated that apoptosis is the major mechanism mediating the resolution of glomerular hypercellularity. In a study of human IgA nephropathy, there was no correlation between glomerular cell proliferation and Fas antigen expression[11]. In the same study there was remarkable Fas Ag expression in glomeruli of proliferative lupus nephritis.

Apoptosis and Glomerulosclerosis

In the experimental model of anti-GBM glomerulonephritis, induced in WKY rats, apoptosis was associated with sclerosis. In human IgA nephropathy, TUNEL (+) cells were mainly observed in the sclerotic lesions. Makino et al.[12] concluded that in these models, apoptosis in glomerulosclerosis is "uncontrolled apoptosis".

Apoptosis and Systemic Lupus Erythematosus (SLE)

Exciting findings in the study of apoptosis have shed light on the pathogenesis of SLE[13,14]. Increased apoptosis has been detected in the peripheral blood of SLE patients. We and others have shown that lymphocyte apoptosis was significantly increased in SLE patients as compared to controls. In our study, lymphocyte apoptosis was significantly increased, and annexin stainings for lymphocytes in SLE patients (n:6) and healthy controls (n: 12) were 11.3 ± 3.3 and 1.2 ± 0.9, respectively. Lymphocyte apoptosis was significantly increased as compared to controls ($p = 0.008$, CI: 1.9 - 18.2).

Along with this increased apoptosis in SLE, there is impaired removal of apoptotic cells. This is because phagocytosis of apoptotic cells is defective in SLE. Complement system is also required in the processing and disposal of these apoptotic particles. Thus it has become clear that the main reason for the association of complement deficiencies with SLE is the inefficient removal of the apoptotic products.

When endonucleases cleave the chromatin, the resultant product is a unit called nucleosome, with a stretch of DNA covered by histones. Exposure to abnormal normally sequestered amounts of nuclear Ag and nucleosomes elicits the autoimmune response[1]. In fact, it has now become clear that the autoantibodies in SLE nephritis are mainly directed against nucleosomes and not DNA. The positively charged histones may also explain the trapping of these immune complexes at the negatively charged glomerular basement membrane[1].

Subsequently these apoptotically derived self-antigens, particularly nucleosomes, drive a T-cell dependent autoimmune response to formation of autoantibodies. There is persistence of inflammation with these unremoved self-antigens[14].

In SLE, peripheral tolerance to self-antigens is also impaired, resulting in more autoantibody production; recent evidence suggests breakdown of peripheral deletional mechanisms and a lack in control of B cells. This results in aberrant polyclonal activation of B cells favoring autoantibody formation. At this stage, environmental factors such as infections and genetic factors such as MHC repertoire may also be important[14].

The immune complexes comprised of nucleosomes and autoantibodies are the mainstay of the pathogenesis in lupus nephritis. A number of studies in proliferative lupus nephritis point to a decreased apoptosis. In tissue samples of Class IV lupus nephritis, an increased transcription of the Bcl-2 gene, an inhibitor of apoptosis, has been demonstrated[1]. Thus, glomerular trapment of autoimmune complexes is followed by depressed autoinflammatory mechanisms with impaired clearance of infiltrating cells. The persistence of these inflammatory cells provokes secondary molecules of inflammation. When proliferating cell nuclear antigen (PCNA) staining in used for the proliferative index and TUNEL staining for the detection of apoptosis, the imbalance between PCNA and TUNEL+ cells may define the severity of the disease[1].

The importance of regulating of apoptosis has also been confirmed in an experimental model. Bcl-2 is an apoptosis inhibitor. At about one year of age, Bcl-2 transgenic mice develop high titers of antinuclear antibodies and usually die of immune complex glomerulonephritis. Thus, the increased apoptosis with inefficient clearing mechanisms seems to be the main drive for lupus nephritis[5,13,14].

We have attempted to study apoptosis in proliferating lupus nephritis in childhood SLE cases. Of these, two cases had C1q and C3 deficiencies[15,16]. TUNEL staining of proliferative lupus nephritis patients (n: 7) were compared to staining in cases with mesangial proliferative glomerulonephritis (n: 4). There was no significant difference between these two groups. However, the glomerular TUNEL staining in two SLE patients with complement deficiencies was very high. The glomerular TUNEL staining was detected in 42.9 percent of all glomeruli of these two patients, whereas it was 20 percent in the control group. Although the numbers were not sufficient for statistical analysis, we aim to pursue these findings. This is the first tissue representation of increased apoptosis in complement deficient states.

Apoptosis and Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Recent studies have shown that increased apoptosis is a contributing factor for the development of cysts in this disease. Increased Bcl-2 mRNA staining accompanies the increased apoptotic index in the cystic lesions of ADPKD[1]. In conclusion, larger studies in human subjects will enlighten the role of apoptosis in the pathogenesis and/or development of the aforementioned kidney diseases.

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Lupus anticoagulant and protein S deficiency in a child who developed disseminated intravascular coagulation in association with varicella

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Varicella is not always a benign disease it may cause serious complications. We report a two-year-old boy with disseminated intravascular coagulation in association with varicella. The patient had the lupus anticoagulant, the antiphospholipid antibody, acquired free protein S deficiency, and increased concentrations of the prothrombin F 1+2 fragment. Intravenous immunoglobulin was administered due to its potential antibody-blocking activity, and the patient responded well. We recommend that children with varicella and disseminated intravascular coagulation should be examined for the lupus anticoagulant, the free protein S antigen, the prothrombin fragment F 1+2 and the other coagulation parameters. Intravenous immunoglobulin administration could be useful in such conditions because of its antibody-blocking activity.

Key words: protein S, lupus anticoagulant, varicella, disseminated intravascular coagulation.

Varicella is generally benign in healthy children and the complications of disease are not common[1]. However, recent epidemiological studies suggest that varicella complications may be more frequent than previously reported[2-5]. Life-threatening thrombotic complications such as purpura fulminans, disseminated intravascular coagulation, and thrombosis have been rarely reported in children with varicella[6,7]. Investigators have described children with thromboembolism and acquired protein S deficiency in association with varicella[8-11].

We report the occurrence of disseminated intravascular coagulation in a case with varicella. The patient also had a lupus anticoagulant (LA) and acquired free protein S deficiency.

Case Report

A two-year old boy was admitted to our hospital with the complaints of fever, cough and dyspnea for four days. He had been diagnosed with varicella 10 days previously. He was a healthy child before varicella infection. There was no family history of thrombosis.

On admission, physical examination revealed tachypnea, intercostal, subcostal and suprasternal retractions, and rales at both lung fields. The patient was febrile (temperature 38.2 °C). The hemoglobin level, and the white cell and platelet count were normal. The erythrocyte sedimentation rate and the concentrations of C-reactive protein were slightly elevated. Activated partial thromboplastin time (APTT) and prothrombin time (PT) were normal. Tests of liver and kidney function were normal. The serum immunoglobulin levels and lymphocyte subgroups were within the normal range. No pathogenic microorganism was detected in cultures taken from the throat, urine, blood or stool. Anti-varicella IgG and IgM antibodies were positive. Chest x-ray showed bilateral nodular infiltrates throughout both lung fields.

Therapy was started with intravenously administered ceftriaxone 100 mg/kg/day and acyclovir 1500 mg/m^2/day, but the patient did not respond. Three days after his admission he developed hematemesis, generalized tonic-clonic convulsions and subconjunctival hemorrhage in the temporal chamber of the right eye.

Funduscopic examination and cranial computed tomography were normal. Cerebrospinal fluid examination revealed a slightly elevated protein concentration with no pleocytosis. There was no growth in the cerebrospinal fluid culture. Thrombin time (TT) and APTT were prolonged (Table I). Platelet count and fibrinogen were decreased. D-dimer level was extremely elevated (7.24 μg/ml). Protein C and antithrombin activity were determined with Sta-Compact Analyzer Coagulometry using Diagnostica Stago (Asniéres, France) kits. The thrombin antithrombin (TAT) complex and the prothrombin fragment F 1+2 (PF 1+2) were measured with an ELISA (Enzygnost, Dade-Behring/Germany). Free protein S antigen, tissue plasminogen activator (tPA), plasminogen activator inhibitor 1 (PAI-1) and antiphospholipid antibody (APA) were assessed by ELISA (Asserachrom, D.Stago/France). Activated protein C-resistance (APC-resistance) was measured using Dignostica Stago kits. Lupus anticoagulant was determined by Staclot-PNP (D.Stago/France). The results of the tests were compared with the results of healthy children reported by Andrew et al[12]. Protein C activity was normal (Table I). Free protein S concentration was markedly reduced (0.21 U/ml). The concentrations of PF 1+2, TAT complex, tPA and PAI-1 were elevated. Lupus anticoagulant was positive, and APA was elevated.

A diagnosis of disseminated intravascular coagulation was considered. Infusion of fresh frozen plasma and intravenous immunoglobulin (400 mg/kg/day, for 5 days) were given. Clinical symptoms improved rapidly, and disseminated intravascular coagulation was controlled with the normalization of PT, APTT, fibrinogen and platelet count. However, free protein S remained below the normal range (0.31 U/ml) and LA was positive. The concentration of PF 1+2 was found to be slightly elevated (1.36 nmol/L). The concentrations of tPA, PAI-1, TAT were increased (Table I).

One month after admission, all coagulation parameters including free protein S antigen and PF 1+2 were normal. Lupus anticoagulant also disappeared (Table I).

Family investigation was normal in terms of hemostasis. Protein S was normal in both parents and sister.

Discussion

Varicella may be associated with severe purpura fulminans and disseminated intravascular coagulation [7,8]. In the patient presented here, the disseminated intravascular coagulation was diagnosed by the determination of prolonged APTT and TT, elevated D-dimer levels and a decreased platelet count.

Table I. Laboratory Findings of the Patient on the 3rd, 7th and 30th Days of Admission

	On the 3rd day	On the 7th day	On the 30th day
PT (s)	14	13	12
APTT (s)	42	30	28
Platelet count (/mm³)	101000	207000	260000
Fibrinogen (g/dl)	1.60	2.60	3.66
D-dimer (μg/ml)	7.24	2.2	0.42
Thrombin time (s)	23	22	19.1
Protein C (U/ml)	0.49	0.49	0.92
Free protein S (U/ml)	0.21	0.31	0.72
PF 1+2 (nmol/L)	1.66	1.36	0.67
TAT (μg/L)	18.32	6.5	3.61
tPA (ng/ml)	23.6	20.3	11.94
PAI-1 (ng/ml)	75	75	40
APC-resistance	150	155	145
Lupus anticoagulant	+	+	-
APA (UPL/ml)	41.46	27.8	14.2

PT ..... : prothrombin time.
APTT ..... : activated partial thromboplastin time.
PF 1+2 ..... : prothrombin fragment F 1+2.
TAT ..... : thrombin antithrombin complex.
tPA ..... : tissue plasminogen activator.
PAI-1 ..... : plasminogen activator inhibitor.
APC-resistance ..... : activated protein C-resistance.
APA ..... : antiphospholid antibody.
The reference ranges [12] for PT: 12-16 s.
APTT ..... : 25-33 s, thrombin time: 15-22 s.
fibrinogen ..... : 1.7-4 g/dl.
D-dimer ..... : <0.5 μcg/ml.
protein C ..... : 0.40-0.92 U/ml.
free protein S antigen : 0.71-0.97 U/ml.
PF 1+2 ..... : 0.4-1.1 nmol/L.
TAT ..... : 1.0-4.1 μg/L.
tPA ..... : 1-12 ng/ml.
PAI-1 ..... : 4-43 ng/ml.
APC-resistance ..... : > 75%.
APA ..... : < 15 UPL/ml.

Protein S is a plasma protein involved in the regulation of the protein C anticoagulant pathway. Free protein S is the active plasma form of protein S with anticoagulant activity. Several cases of varicella associated with severe thromboembolism and isolated acquired free protein S deficiency have been reported[8,9]. Recently, the presence of the LA has been described in children with varicella who acquired protein S deficiencies[10]. We also found the LA in association with acquired free protein S deficiency. In addition, our patient had increased concentrations of PF 1+2, tPA and PAI-1. The prothrombin fragment F 1+2 is generated when prothrombin is converted to thrombin, and is a current marker of excessive thrombin generation. In our patient, increased concentration of the PF 1+2, in our opinion, suggested the presence of excessive thrombin and hypercoagulability. The prothrombin fragment F 1+2 returned to normal levels when LA and protein S deficiency had resolved. This observation suggests that reduction in free protein S and LA may exacerbate the disseminated intravascular coagulative response. However, it is not clear whether the acquired protein S deficiency and the LA are the causes or the consequences of disseminated intravascular coagulation.

In our patient, the elevated D-dimer level and prolonged TT were considered due to the thrombosis in the microvascular plexus, and increased tPA, PAI-1 and D-dimer levels probably reflected that the fibrinolytic system was activated secondary to extensive microvascular thrombosis.

Currently, protein S concentrates are not available. Therefore, replacement with fresh frozen plasma might be the best alternative strategy in patients with protein S deficiency in association with varicella. However, because fresh frozen plasma contains only a small amount of protein S and has potential risks, the use of fresh frozen plasma has not been recommended by some authors [13,14]. Our patient was given fresh frozen plasma as a supportive therapy for disseminated intravascular coagulation. We could not use heparin due to hematemesis.

Recently, autoantibodies against protein S were shown in patients with protein S deficiency in association with varicella, and it has been reported that protein S antibodies may result in acquired free protein S deficiency [8,10]. The nature of the protein S antibodies is unclear; it has been postulated that the protein S antibodies may be the LA[10]. Because of the presence of the LA in our patient, we decided to administer intravenous immunoglobulin due to its potential antibody-blocking activity. Our patient responded well to its administration.

In conclusion, varicella is not always a benign disease of childhood. It may cause serious complications such as disseminated intravascular coagulation and thrombosis. Clinicians should be aware of thromboembolic complications. We recommend that children who develop disseminated intravascular coagulation in association with varicella should be examined for the LA, the free protein S antigen, the PF 1+2 and other coagulation parameters. Intravenous immunoglobulin administration could be useful in such conditions because of its antibody blocking activity.

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Dermatomyositis with membranous nephropathy

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Dermatomyositis is the connective tissue disease with the least renal involvement. Although some renal findings like proteinuria, hematuria, pyuria, progressive renal insufficiency, and glomerular and tubular calcium deposits with arteriolar fibrosis have been described, glomerulonephritides have rarely been associated with dermatomyositis, especially in childhood cases. We describe a 10-year old boy with the clinical picture of dermatomyositis who underwent renal biopsy due to microscopic hematuria demonstrating membranous glomerulonephritis with C1q deposition. Children with "full-house" membranous glomerulonephritis with deposition of C1q and the other immunoglobulins have been reported to present in the future with the clinical findings of systemic lupus erythematosus. However, laboratory evaluation of our patient for systemic lupus erythematosus was negative at the present time. Thus, we think this case should be followed up closely with special attention to the possible clinical and laboratory findings of systemic lupus erythematosus.

Key words: dermatomyositis, membranous nephropathy, systemic lupus erythematosus, childhood.

Dermatomyositis (DM) is the connective tissue disease with the least renal involvement. Although DM is considered a necrotizing vasculitis, there is very little evidence to suggest that endothelial injury occurs in the kidney. However, some renal findings like proteinuria, hematuria, pyuria, progressive renal insufficiency, and glomerular and tubular calcium deposits with arteriolar fibrosis have been described[1]. Glomerulonephritis [membranous glomerulonephritis (MGN) specifically] has been associated with DM in only a few cases[1-3]. Full-house MGN has been suggested as a preceding marker of systemic lupus erythematosus (SLE) in childhood[4].

We describe a 10-year old boy with DM who developed microhematuria two months after the diagnosis of DM. Persistence of hematuria led to a percutaneous renal biopsy demonstrating C1q-positive MGN.

Case Report

A 10-year-old boy presented with a two-month history of weakness and pain in his arms and legs and difficulty in walking. There were in addition erythema of his eyelids, nasal speech and difficulty in eating solid food. Personal and family histories were unremarkable. Physical examination revealed heliotropic rash (Fig. 1), weakness and tenderness in the proximal muscles of the extremities, and decreased deep tendon reflexes in the lower extremities. Laboratory analysis demonstrated Hb 11.9 g/dl, Hct 36%, WBC 16,900/mm^3, platelets 251,000/mm^3, MCV 81 fl; ESR 13 mm/h, ASO 85 IU/ml, CRP 5 mg/dl, RF (–); BUN 17 mg/dl, creatinine 0.4 mg/dl, SGOT 108 IU/ml, SGPT 26 IU/ml, and CPK 955 IU/ml. Pulmonary function tests showed moderate restrictive disease. Urinalysis revealed specific gravity 1030, pH 5.0, protein (–), blood (–), and 1-2 WBC/hpf. Electromyogram demonstrated diffuse muscle fiber involvement in the proximal muscles with intense spontaneous fibrillation indicating acquired inflammation. Muscle biopsy showed mononuclear cells infiltrating vessel walls within muscle and fat tissue and atrophic changes in muscle cells in some areas.

Treatment was started with prednisolone, 2 mg/kg/day in four divided doses, and the dose was decreased gradually after two months along with institution of physiotherapy, upon improvement of the active symptoms and signs of the disease. However, microscopic hematuria without proteinuria was noted in routine urinalysis

three months after the diagnosis. His blood pressure was normal. Urine culture was sterile and urinary system ultrasonography was normal. Complete blood count, erythrocyte sedimentation rate, C-reactive protein, C3, C4, and urinary calcium excretion were normal. ANA was negative. Persistence of microscopic hematuria led us to perform a renal biopsy six months after the diagnosis of DM. Histopathologic evaluation of the renal tissue revealed basal membrane thickening without mesangial proliferation, minimal interstitial fibrosis and tubular atrophy in light microscopy (Fig. 2). Direct immunofluorescent microscopy demonstrated 3+ membranous, fine granular, linear IgG, IgA and IgM deposition with minimal C3, C1q, fibrinogen and albumin deposition (Fig. 3). Thus, the diagnosis of MGN was made histopathologically.

Fig. 1. Heliotropic rash on the face of the patient.

Fig. 2. Slight basal membrane thickening in a glomerulus (periodic acid-Schiff PAS, x 200).

Discussion

Glomerulonephritis has been described in only a few cases of DM[1-3,5]. Review of the literature revealed only one pediatric patient with MGN developing DM during follow-up. That child had developed proteinuria with nephrotic syndrome before the diagnosis of MGN. Our case is a 10-year old boy diagnosed as DM before he developed microscopic hematuria.

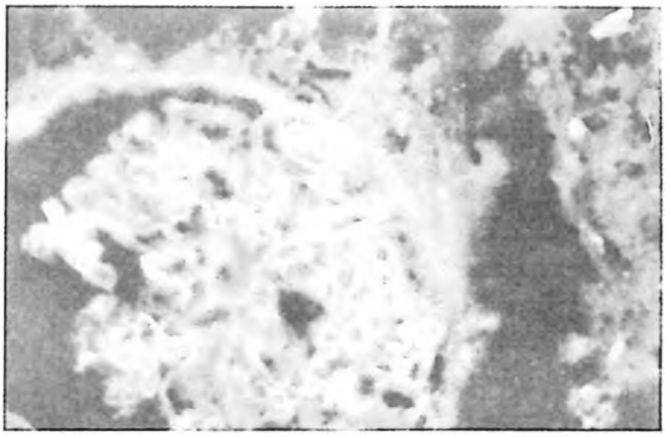

Fig. 3. Fine granular 3+ IgG depositon in the basal membrane (DIF, IgG, x 400).

Since renal involvement is so rare in DM, we reevaluated the patient to exclude other collagen vascular diseases such as SLE, but no clinical or laboratory evidence of SLE was detected. In addition, hypercalciuria could have been suspected as a cause of hematuria, since the patient developed hematuria during treatment with corticosteroid therapy. However, hematuria was detected during the dose reduction period of steroid therapy, and renal calcium excretion was determined to be normal in serial measurements. Seraches for the other causes of hematuria were also negative.

Persistence of hematuria without any known cause led us to perform a renal biopsy that demonstrated MGN. Lack of marked mesangial proliferation resembled idiopathic MGN histopathologically, not the membranous nephropathy of SLE. However, accumulation of C1q, a marker of renal tissue involvement in SLE, was noted. Presence of C1q-positive MGN in renal histopathology with the clinical findings of DM in this child suggested that our patient represented a case of seronegative SLE with renal involvement. It has been reported that children with "full-house" MGN with deposition of C1q and the other immunoglobulins could present in the future with the clinical findings of SLE, especially when cytoplasmic tubuloreticular inclusions are detected in electron microscopy[4,6]. Unfortunately, we could not evaluate the renal biopsy specimen by electron microscopy.

However, in view of the data mentioned above[4,6], although the clinical picture of our case is compatible with DM at the present time, other clinical and biological symptoms of SLE may appear in this patient in the future.

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Central nervous system involvement of polyarteritis nodosa: a case report

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SUMMARY: Altınok D, Yıldız YT, Ruşen E, Eryılmaz M, Tacal T. Central nervous system involvement of polyarteritis nodosa: a case report. Turk J Pediatr 2001; 43: 146-150.

Polyarteritis nodosa (PAN) is a necrotizing vasculitis involving small and medium-sized arteries and it affects multiple organ systems in the body Central nervous system (CNS) involvement appears less frequently, and usually develops after the disease is established. Although aneurysms are common in visceral arteries in PAN, intracranial aneurysms are uncommon and have been documented rarely. This case is reported to raise awareness among radiologists as it has characteristic and rare, if not specific, imaging findings of CNS involvement of PAN.

Key words: polyarteritis nodosa, aneurysms, hematoma, central nervous system.

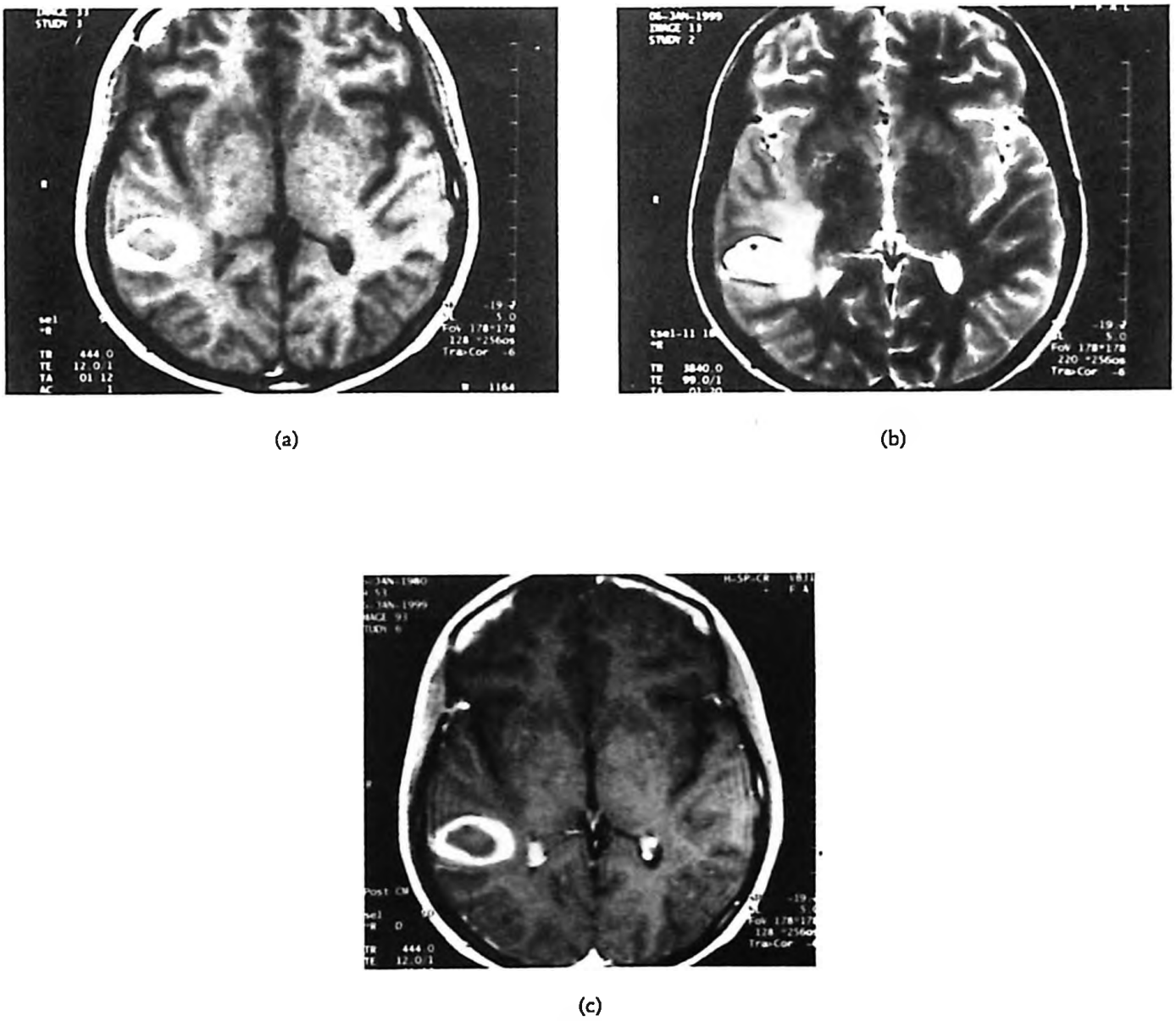
Polyarteritis nodosa (PAN) is a necrotizing vasculitis involving small and medium-sized arteries, and it affects multiple organ systems in the body, except for the lungs and spleen[1-4]. Central nervous system (CNS) involvement appears less frequently, and usually develops after the disease is established. The most common neuroradiological findings in PAN are nonspecific focal ischemic areas followed by intracranial hemorrhage. Although aneurysms are common in visceral arteries in PAN, intracranial aneurysms are uncommon and have been documented rarely[4-6]. In this paper, the central nervous system involvement of PAN in a young male patient with intracranial aneurysms and intracerebral hemorrhage is reported.

Case Report

An 18-year-old male patient presented with a three-week history of abdominal pain, headache, high blood pressure of 200/120 mm Hg, weakness in legs and weight loss of 5 kg. He also had fever, disseminated skin rash on arms in the form of livedo reticularis and cyanosis of the hands. He had suffered from abdominal pain for 10 years for which he had an operation for suspected perforation. A renal angiogram performed previously because of his recurrent hypertension was normal.

Laboratory examination showed a high erythrocyte sedimentation rate and C-reactive protein. ANA, anti-DNA, and RF antibodies were negative. Thyroid hormones were in normal range. Abdominal ultrasound and computed tomography scans were normal. He had signs of pericarditis on echocardiography. Although medication was started with antihypertensive drugs and steroids, the patient had left hemiparesis, left hemihypoesthesia and nystagmus during his stay at the hospital. Magnetic resonance imaging (MRI) revealed a subacute hematoma localized in grey and subcortical white matter of the right temporoparietal region. The lesion was hypointense and had a hyperintense rim in T1 W images (Fig. 1a), and was hyperintense in T2 W scans with moderate peripheral edema (Fig. 1b). After gadolinium injection, there was no contrast enhancement (Fig. 1c). As a ruptured aneurysm was suggested in differential diagnosis, a cerebral angiogram was performed. In his cerebral angiography, there were multiple aneursmal dilatations in peripheral segments of the left middle cerebral artery (Fig. 2a). There were several aneurysms in occipital, internal maxillary (Fig. 2b) and superficial temporal branches of the right external carotid artery (Fig. 2c). The diagnosis of PAN was made on

the basis of the American College of Rheumatology criteria for the classification of polyarteritis nodosa[2]. The patient was treated with cytotoxic agents, but 10 days later he developed spinal shock. In his thoracic spinal MRI study (scans unavailable), the spinal cord intensity was diffusely increased in T2 W images suggesting occlusion of Adamkiewicz's artery (anterior spinal artery). The patient died four days later.



(a) (b)

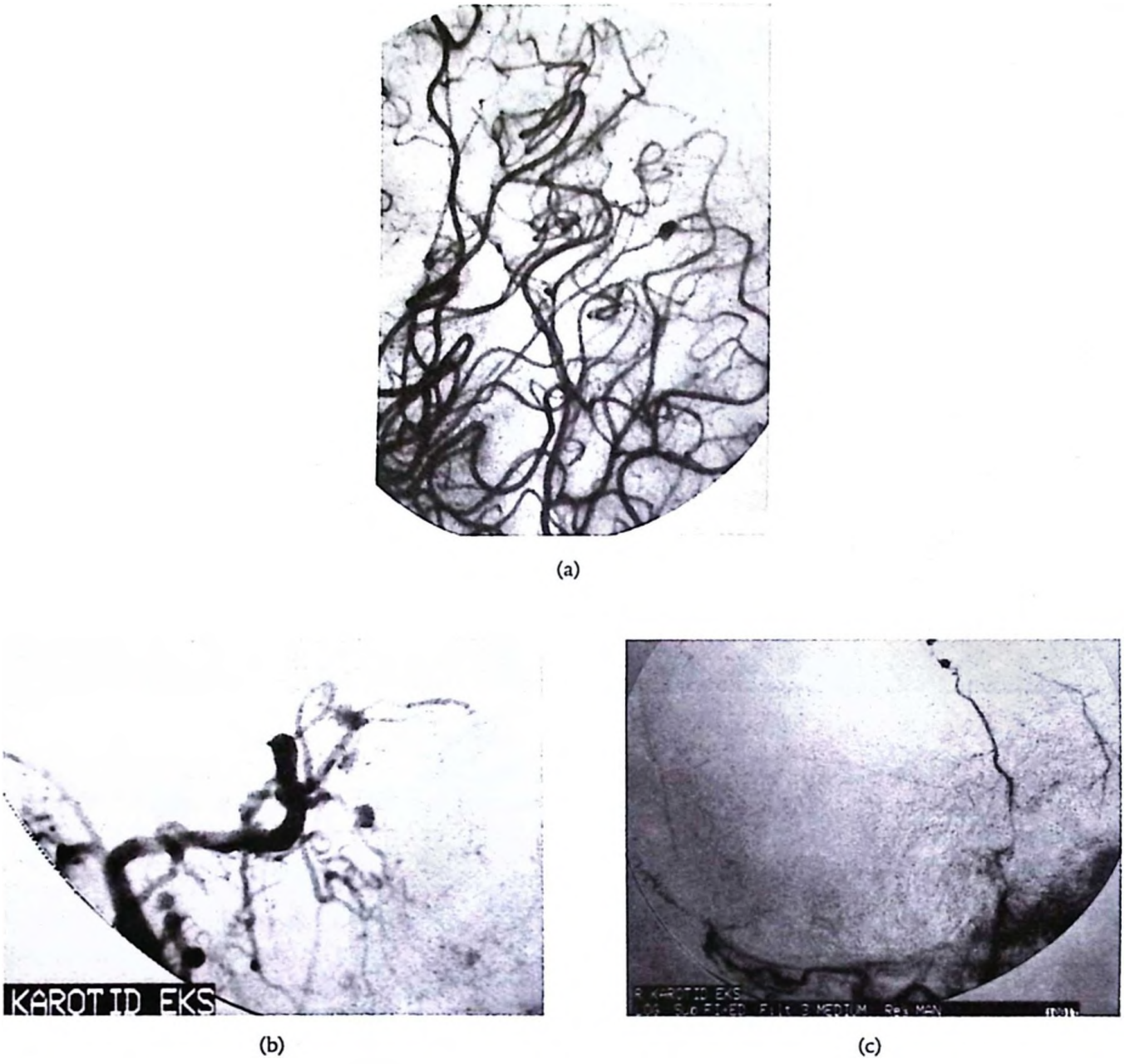
(c)

Fig. 1. a, b, c: Magnetic resonance imaging (MRI) findings of an 18-year-old-man with central nervous system (CNS) involvement of polyarteritis nodosa (PAN).

a) Transverse T1 W scan reveals a isointense lesion that has a peripheral hyperintense rim consistent with subacute hematoma localized in grey and subcortical white matter of the right temporoparietal region.

b) The lesion is hyperintense in T2 W scan and there is peripheral edema.

c) There is no contrast enhancement after gadolinium injection.



(a)

(b)

(c)

Fig. 2. a, b, c: Cerebral angiogram of the same patient.

a) Multiple aneurysmal dilatations in peripheral segments of left middle cerebral artery in left common carotid artery injection.

b) More aneurysms in occipital, internal maxillary.

c) Superficial temporal branches of the right external carotid artery.

Discussion

Polyarteritis nodosa is a systemic necrotizing vasculitis involving small and medium-sized arteries, believed to be initiated by immune complex deposition[6]. Multiple organ systems are involved including skin, joints, heart, kidneys, gastrointestinal system (GIS), and peripheral and central nervous system. The kidneys are involved in up to 80 percent of cases in the form of renal failure and hypertension, and GIS vasculitis is present in up to 50 percent of the patients, in the form of abdominal pain due to visceral infarction. Cardiac manifestations are congestive heart failure, myocardial infarction, hypertension related changes and, as seen in our patient, pericarditis. Fever and weight loss accompany the symptoms[2].

In PAN, neurological involvement is the leading cause of morbidity in 65 percent of the patients[2]. It is usually manifested as mononeuropathy multiplex, paresthesia, pain, weakness and sensory loss. The central nervous system is

affected in 20-40 percent of the patients in the form of hypertensive encephalopathy and hemorrhagic and ischemic infarcts in the brain and spinal cord[4]. There are reports that suggest that CNS involvement is likely to develop in patients who have renal and mesenteric involvement[7], but recent publications report that CNS findings may well be early disease manifestations[6].

The symptoms and findings in PAN are not specific, nor are there especific serologic tests for diagnosis[2]. Usually, laboratory findings vary with the degree of inflammation and vascular damage in specific organs. Angiographic studies may be extremely helpful in suggesting the diagnosis by demonstrating stenosis, occlusion or aneurysmal dilatations in the vasculitic vessels, but none of these abnormalities is pathognomonic[6]. Although aneurysms are common in renal and splanchnic circulation, they are rarely encountered in intracranial circulation in patients with PAN[4-6]. Definitive diagnosis is made by arterial wall biopsy and by confirming the presence of granulocytes and mononuclear leukocytes in the arterial wall[2].

Our patient had six out of 10 classification criteria of PAN defined by the American College of Rheumatology (Table I): weight loss, livedo reticularis, myalgia, mononeuropathy multiplex, hypertension and aneurysmal dilatations in cerebral angiography. It is stated that the presence of three or more of these 10 criteria is associated with a sensitivity of 82.2 percent and specificity of 86.6 percent[2].

In the differential diagnosis of PAN, it is advised to exclude systemic lupus erythematosus, rheumatoid arthritis and Wegener's granulomatosis prior to submitting a patient to the tests required by the PAN criteria. It is stated that the diagnosis of PAN should not be excluded until angiograms and relevant vessel biopsies are performed[2].

The MRI findings in our case were nonspecific, and a set of differential diagnoses such as hypertensive hemorrhage and rupture of aneurysm had to be considered. On the basis of imaging findings alone, with hemorrhagic lesions on MRI and aneurysms in cranial angiography, primary angiitis of the CNS would be the first on a differential diagnosis list. Primary angiitis of the CNS presents with ischemic lesions or hemorrhage caused by stenosis and vascular wall fragility[3]. Hemorrhage from ruptured aneurysms resulting from angiitis has ben reported in previous cases. The patients present with headaches and multifocal neurological deficits. On cerebral angiography, several areas of segmental arterial narrowing are demonstrated. A leptomeningeal or parenchymal biopsy specimen should demonstrate granulomatous changes with chronic polynuclear lymphocytes and multinucleated giant cells with focal fibroid necrosis. This well-defined characteristic distinguishes it as a different clinicopathological entity from other vasculitides affecting the CNS, such as collagen disease, Takayasu's disease, and rheumatoid arteritis. As our patient had symptoms related to other organ systems,

Table I. 1990 Criteria for the Classification of Polyarteritis Nodosa (Traditional Format)

Criterion	Definition
1. Weight loss > 4 kg	Loss of 4 kg or more of body weight since illness began, not due to dieting or other factors
2. Livedo reticularis	Mottledd reticular pattern over the skin of portions of the extremities or torso
3. Testicular pain or tenderness	Pain or tenderness of the testicles not due to infection, trauma or other sources
4. Myalgias, weakness or leg tenderness	Diffuse myalgias (excluding shoulder and hip girdle) or weakness of muscles or leg tenderness
5. Mononeuropathy or polyneuropathy	Development of mononeuropathy, multiple mononeuropathies or polyneuropathy
6. Diastolic BP > 90 mm Hg	Development of hypertension with the diastolic blood pressure higher than 90 mm Hg
7. Elevated BUN or creatinine	Elevation of BUN > 40 mg/dl or creatinine > 1.5 mg/dl, not due to dehydration or obstruction
8. Hepatitis B virus	Presence of hepatitis B antigen or antibody in serum
9. Arteriographic abnormality	Arteriogram showing aneurysms or occlusions of the visceral arteries, not due to arteriosclerosis, fibromuscular dysplasia, or other noninflammatory causes
10. Biopsy of small or medium-sized artery containing PMN	Histological changes showing the presence of granulocytes or granulocytes and mononuclear leukocytes in the artery wall

(*) For classification purposes, a patient shall be said to have polyarteritis nodosa if at least three of these 10 criteria are present. The peresence of any three or more criteria yields a sensitivity of 82.2 percent and a specificity of 86.6 percent.

BP: blood pressure; BUN: blood urea nitrogen; PMN: polymorphonuclear neutrophils.

primary angiitis of the CNS was excluded from the differential diagnosis list. Many of the patients misclassified as PAN actually have syndromes such as giant cell (temporal) arteritis, Wegener's granulomatosis and Churg-Strauss syndrome. The facts that the patient lacked classical findings of granulomatous histopathology (giant cell arteritis) and necrotizing upper airways disease (Wegener's granulomatosis) and had no long history of atopia and eosinophilia (Churg-Strauss) helped us to make the definitive diagnosis of PAN.

Since the vasculitides comprise a diverse group of diseases and there is a great variability among individual cases in the same type of vasculitis, it is imperative to use a universally accepted classification system[8]. Our patient' signs and symptoms fullfilled seven out of 10 minor diagnostic criteria for PAN proposed by Özen et al[9]. Renal involvement and musculoskeletal findings, which were defined as major criterion, were absent in our patient. The presence of five criteria, including at least one major finding, correlated with the histopathologic diagnosis of PAN in at least 97 percent of their patients[9].

In conclusion, among several vasculitis syndromes, PAN remains one of the most difficult to classify[2]. The definitive diagnosis requires angiograms or vessel biopsies. This case is reported to raise awareness among radiologists as it has characteristic and rare, if not specific, imaging findings of CNS involvement of PAN. Contrary to common belief, CNS involvement may present early in the course of the disease, as in our patient.

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Rapidly progressive bronchiectasis complicating ulcerative colitis in a child

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SUMMARY: Türktaş I, Bostancı I, Altuntaş B. Rapidly progressive bronchiectasis complicating ulcerative colitis in a child. Turk J Pediatr 2001; 43: 151-154.

Patients with ulcerative colitis may have a presentation dominated by extraintestinal manifestations. These manifestations, particularly bronchiectasis, are very rarely seen in pediatric patients. A 13-year-old boy with ulcerative colitis who was diagnosed by colonic mucosa biopsy is presented. He developed unexplained productive cough after the appearance of colonic disease. He was treated and followed up at his primary care hospital with the sole diagnosis of ulcerative colitis, with little attention given to the chest symptoms. The relation of the bronchial involvement to the ulcerative colitis was not established until two years after the onset of disease. Thoracal computed tomography (CT) examination after this period showed evidence of bronchiectasis and pulmonary involvement. Despite prophylactic inhaled corticosteroid treatment, no clinical or radiographic improvement was observed and widespread bronchial destruction developed very rapidly. More effective treatment with oral steroids was probably necessary in this patient, if the early chest symptoms were related to the ulcerative colitis.

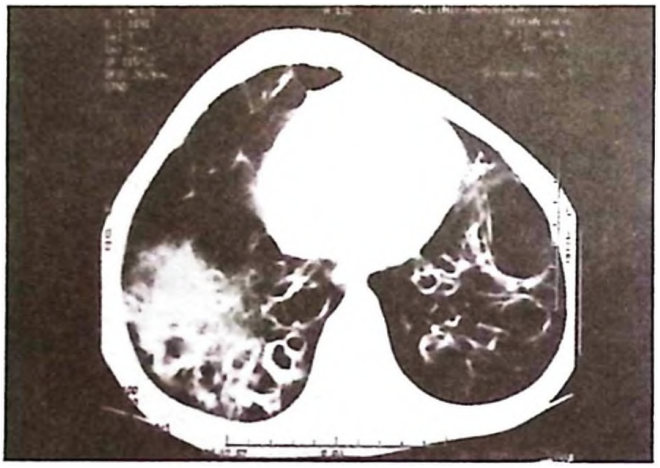
Key words: bronchiectasis, inhaled corticosteroid, ulcerative colitis.

Over the course of the past 20 years, varied patterns of pulmonary involvement in ulcerative colitis have been identified in case reports[1-4]. These manifestations, particularly bronchiectasis which is the characteristic pulmonary lesion of adult ulcerative colitis, are very rarely seen in pediatric patients. We present a 13-year-old boy with ulcerative colitis who developed a rapidly progressive bronchiectasis after the appearance of colonic disease despite inhaled corticosteroid treatment.

Case Report

A 13-year-old boy was admitted to the emergency room in December because of a productive cough, cyanosis and dyspnea at rest. Colonic mucosa biopsy proven ulcerative colitis had been diagnosed in 1993 with a one-year history of persistent mucous diarrhea, abdominal pain and intermittent rectal bleeding, and sulfasalazine treatment was started. Within three months of his medication he had an unexplained productive cough. There was no history of significant respiratory illness (including no sinusitis, wheezy bronchitis or asthma, or gastroesophageal reflux), nor family history of pulmonary disease. A switch from sulfasalazine to mesalazine produced no change in respiratory symptoms. In 1995, he experienced hepatic dysfunction and transient, multiple joint swelling and tenderness which involved the large joint and was asymmetric in distribution without exacerbation of the colitis. Investigations included hepatic biopsy which confirmed the chronic active hepatitis diagnosis. During follow-up, there were two minor exacerbations of his chronic hepatitis, but arthritis did not reappear. He experienced three to four episodes of gastrointestinal disturbance each year which responded to intermittent oral corticosteroid therapy. However, increase in his productive cough and dyspnea continued with progressive changes on the chest X-ray. In June 1995 thoracal computed tomography (CT) scan showed newly developing cystic bronchiectasis (Fig. 1). Subsequently, prophylactic treatment was started with inlaled budesonide, 800 μg/day. Between 1995-1998 he was hospitalized several

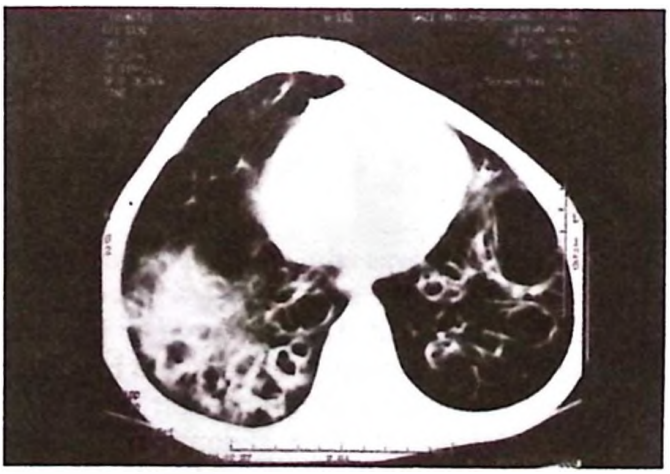
times for pulmonary disease, and had received prednisone in doses ranging from 20 mg to 30 mg daily for treatment periods of seven to 10 days, with dramatic improvement. The other intermittent medications included mesalazine, antibiotics and bronchodilators. Despite prophylactic inhaled corticosteroid treatment, productive cough and dyspnea increased and home oxygen treatment was started, seven months prior to our evaluation.



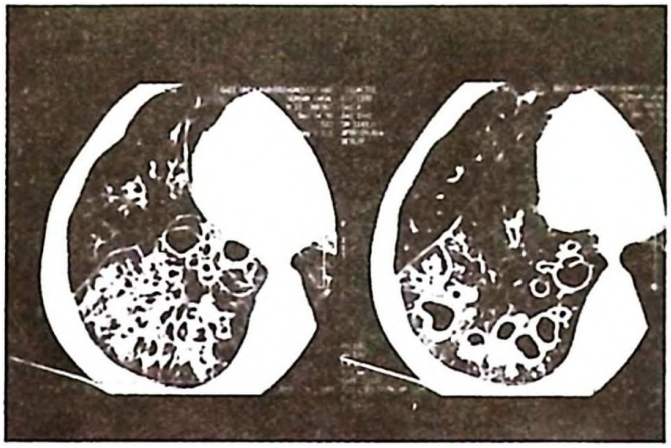
Fg. 1. Thoracal computed tomography (CT) scan in 1995 demonstrated newly developing cystic bronchiectasis affecting both the lower lobes and the middle lobe. The bronchiectasis was more severe in the left paracardiac region (arrow).

At the time of admission, his parents reported the above history. On his examination, the patient's general condition was poor. He was unable to walk and could not do his normal activities. He was cyanotic and orthopneic with intercostal retractions. Physical examination showed a thin and weak boy, with a blood pressure of 110/79 mmHg and a heart rate of 88 beat/min. Examination of the chest revealed widespread wheezing and crackles at both lungs. There was considerable finger clubbing and pectus carinatum. The liver was palpable 2 cm below the costal margin; the spleen was not palpable.

The arterial blood gas values on room air showed an arterial oxygen tension (PaO_2) of 65 mmHg, arterial carbon dioxide tension ($PaCO_2$) of 40 mmHg and pH of 7.4. Hemoglobin was 10 g/dl. White blood cell count, serum electrolytes, liver function tests, autoantibody screen (ANA, SMA, LKM-1, cANCA, pANCA) and sweat sodium levels were negative. Hepatitis B surface antigen and anti-hepatitis C antibody were negative. HLA B27 was negative. Erythrocyte sedimentation rate was 60 mm in the first hour, and C-reactive protein was 38 mg/dl. His serum IgE was 20 IU/L; other quantitative immunoglobulins, including IgG subclasses, were normal. There was no serological evidence for bronchopulmonary aspergillosis at the time of evaluation. In addition, sputum cultures did not reveal Aspergillus organisms or other fungi, and dermal hypersensitivity to Aspergillus antigen was absent. Skin-prick tests for the other common allergens were negative. Repeated bacteriologic studies of sputum, including cultures for mycobacteria, were negative. The cell type was neutrophils. A tuberculin skin test (ppd, 5 TU) was negative. Repeated colonic mucosal biopsies showed ulcerative colitis in remission. Abdominal ultrasonography showed multiple gallstones in gallbladder, of which the greatest was 1x1 cm. Consecutive CT scans of the thorax showed progression to widespread, severe cystic bronchiectasis (Figs. 2a, 2b). Treatment with parenteral fluid, oxygen, cefuroxime, nebulized salbutamol and budesonide (200 µg/day) and intravenous methylprednisolone (40 mg/day) was started. Three weeks later, the patient's clinical condition was somewhat improved and he was able to perform the pulmonary function test: forced expiratory volume in ones (FEV_1) was 0.50 L (31% of predicted), forced vital capacity (FVC) was 1.10 L (62%) of predicted), FEV_1/FVC: 45% and 25-75% of forced expiratory flow (FEF_{25-75}) was 0.25 L/sec (12% of predicted). FEV_1 increased to 0.57 L after inhaling 5 mg nebulized salbutamol (an increase of 15%). Intravenous methylprednisolone was changed to oral methylprednisolone without dose change, and



Fg. 2a. Repeated thoracal CT scan at the same level through the lower lobes three years after diagnosis showed progression to widespread severe cystic bronchiectasis.



Fg. 2b. A thoracal high resolution CT scan shows multiple rounded lucencies with discrete walls representing severe cystic brnochiectasis with prominent bronchial wall thickening almost entirely replacing the right lower lobe.

then was gradually tapered off while he continued inhaled budesonide (2000 µg/day). He was discharged with inhaled budesonide, salbutamol, continuous oxygen, and oral methylprednisolone (30 mg/day).

Discussion

Occasionally, patients with ulcerative colitis may have a presentation dominated by extraintestinal manifestations. This accounts for less than five percent of pediatric disease[5]. Camus et al.[1] recently described the largest series of patients with inflammatory bowel disease-associated large airway disease, which included 28 patients with ulcerative colitis. In that series, only six patients developed bronchiectasis. A study in 1998, the second largest case series of such patients, reported by Spira et al.[2], included a series of six ulcerative colitis adult patients who had new, persistent and unexplained symptoms of respiratory disease, particularly chronic productive cough. In four patients, a subsequent diagnosis of ulcerative colitis-associated bronchiectasis was made using CT scan. The pattern of ventilatory impairment was predominantly obstructive in patients with ulcerative colitis[3,4]. Generally, the course and severity of the extraintestinal involvement do not correlate with the colonic disease[1,2]. On long-term follow-up, our patient had experienced three to four colonic attaks each year, whereas supurative pulmonary disease progressed very rapidly. Concurrent exacerbations of bowel, hepatic and airway disease never occurred. Only pulmonary disease was severe enough to require hospital treatment.

The mechanisms of bronchiectasis in patients with ulcerative colitis has not been clearly defined yet, but it may simply represent inflammation at two different sites of common embryological roots[1]. There is accumulating evidence for a cell-mediated pattern of immune response with infiltration of activated CD4+ or CD8+ T lymphocytes within the bronchial mucosa in bronchiectasis[6]. These cells have been noted in a number of other systemic diseases including ulcerative colitis[6]. The similarities of the mucosal immune system of the lung (bronchial-associated lymphoid tissue) and intestine (gut-associated lymphoid tissue) suggest that the immune cells may migrate from the gut to the lung, possibly leading to inflammatory damage at both the bronchial and colonic mucosa[7]. Gaga et al.[8] clearly demonstrated that in patients with bronchiectasis treated with inhaled corticosteroids, the T cell infiltrate (CD4+ T cells) was significantly lower than in untreated patients. This finding supports a role for cell-mediated immune mechanisms in the pathogenesis of ongoing airway damage in bronchiectasis.

Our patient had no prior viral or bacterial infections of the lungs, including no mycobacterial infections, which could predispose to development of recurrent infections and bronchiectasis. The absence of alternative factors to account for his bronchiectasis; failure to isolate bacterial pathogens on repeated sputum culture; presence of bowel disease preceding the onset of pulmonary symptoms; and an impressive response to oral steroids, particularly during the onset of disease, all support that the pathogenesis of bronchiectasis in ulcerative colitis may be primarily autoimmune. It is possible that his bronchiectasis may have represented, the end stage of an active inflammatory process seen in all of the bronchia. Cholelithiasis and chronic active hepatitis were associated with ulcerative colitis in our patient. The relationship between chronic active hepatitis and inflammatory bowel disease remains unclear. Although there was no autoantibody positivity in our patient, the type of chronic active hepatitis found to be associated with inflammatory bowel disease has usually been the so-called autoimmune type[9].

In our patient, the relation of the bronchial involvement to the ulcerative colitis was not established until two years after the onset of disease. Thoracal CT examination after this period showed evidence of bronchiectasis and pulmonary

involvement, leading to bronchial destruction after only three years. Inflammatory bowel disease-associated lung disease is generally responsive to inhaled[2] and oral steroids[1-3]. Our patient received the minimal dose of systemic steroid medication for periods too short in duration. He was treated with inhaled corticosteroids for a period of about three years, but no clinical or radiographic improvement was observed and widespread bronchial destruction developed very rapidly. Probably more effective treatment with oral steroids was necessary in this patient.

Some of the pulmonary involvements make a substantial contribution to the considerable fatality[4,10]. It is impotant to recognize this association in patients with ulcerative colitis who have had unexplained persistent pulmonary symptoms. To our knowledge, in inflammatory bowel diseases, so destructive and rapid a pulmonary complication as seen in our case has not been reported previously. Because of its extremely rapid progression, proper anti-inflammatory treatment should be started immediately in the early stage in such patients.

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Unilateral late-onset tibia vara associated with bilateral proximal femoral growth disturbance in monozygotic twins: case report

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SUMMARY: Yazıcı M, Atilla B. Unilateral late-onset tibia vara associated with bilateral proximal femoral growth disturbance in monozygotic twins case report. Turk J Pediatr 2001; 43: 155-158.

Clinical and histopathological similarities and rare association of Blount's disease with various proximal femoral physeal affections (i.e. adolescent coxa vara and slipped capital femoral epiphysis) are well known. Association of tibia vara with another epiphyseal disease of the proximal femur has not been reported previously.

In this paper, a monozygotic set of twins with concordant bilateral epiphyseal growth disturbance of the proximal femur and unilateral late-onset tibia vara is presented. Radiological characteristics of the affected knees revealed a wedging in the proximal tibial epiphysis, depression of the medial joint surface and varus deformity of the tibia. Proximal femurs of both cases showed aspheric congruity, coxa magna, shortness of the femoral neck, and subchondral cystic changes.

The presented cases support the genetic etiology of tibia vara, and association of the two conditions is unique.

Key words: adolescent Blount's disease, tibia vara, proximal femoral epiphysis.

In tibia vara (Blount's disease), enchondral ossification is disturbed in the medial aspect of the proximal tibia (i.e. epiphysis, physis, and metaphysis). Many authors differentiate two main groups of the disease depending upon age at clinical onset, namely, early-onset (or infantile) and late-onset tibia wara[1-4]. Histopathological studies of affected individuals indicate a similar pathologic process in developmental coxa vara and in slipped capital femoral epiphysis (SCFE)[3-5]. Previous manuscripts reported the interesting association of these two proximal epiphyseal affections in the same patient without determining the exact relation of the diseases[6].

A monozygotic set of twins with unilateral late-onset tibia vara associated with proximal femoral growth disturbance is presented in this paper.

Case Reports

The patients were 16-year-old monozygotic twin males. HLA screening indicated they were identical for all locations.

Their chief complaint was progressive bow-leg deformity. At approximately 14 years of age, angulation of their left knees initially appeared. They had mild left knee pain which increased with standing, walking and vigorous activities over the last few months prior to presentation. The patients presented with no other musculoskeletal problems.

The father was heroin addict. The mother had taken some unknown pills to terminate her pregnancy. Prenatal, birth, and early childhood histories were not remarkable. It was reported that both siblings walked when they were 14 months old and did not have any hip pain or difficulties in walking during their childhood.

Review of the systems was normal in both patients. Biochemical and hematological screening revealed no abnormalities. The range of flexion-extension of the hip joints was within normal limits in both patients. There was minimal restriction in rotational movements. No deformities were observed. Minimal waddling was seen when they were waddling.

In the first patient (Patient A), the left knee range of motion was normal. Grade I medial collateral instability was detected when the involved knee was examined in 20° flexion. There were no differences of length in the lower extremities.

In the second patient (Patient B), an apparent lateral thrust of the left knee during ambulation was observed. The left knee demonstrated full extension, with flexion being limited (approximately 20°). When the left knee was examined in 20° flexion, grade II medial collateral instability was detected. An inequality of 1.5 cm in length of the two lower extremities was found (Fig. 1).

Fig. 1. Picture demonstrating clinical features of Patients A and B (at the right side).

On anteroposterior (AP) view of the pelvis (Patient A), growth disturbance at the lateral part of the proximal, shortening of the femoral neck and enlargement of the head with mild increase of the neck-shaft angle was apparent in both hips. There was aspheric congruity and cystic changes at right hip (Fig. 2a). A weight-bearing AP X-ray of the left knee showed a wedging in the proximal tibial epiphysis, depression of the medial joint surface (metaphyseal-diaphyseal angel [MDA] of 25°), and varus angulation of the tibia (Fig. 2b).

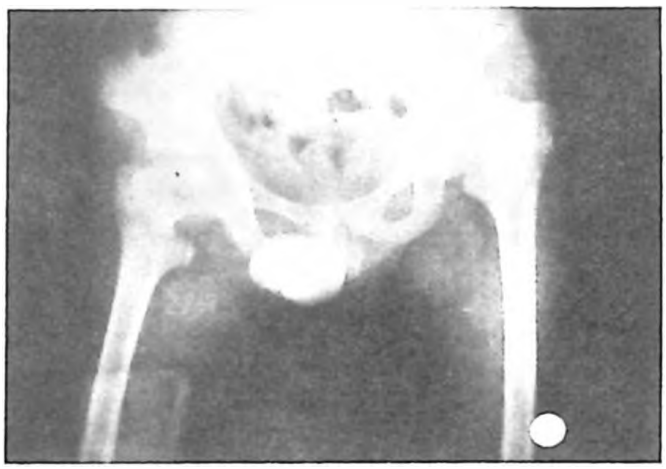

Fig. 2a. Anteroposterior (AP) view of the pelvis (Patient A).

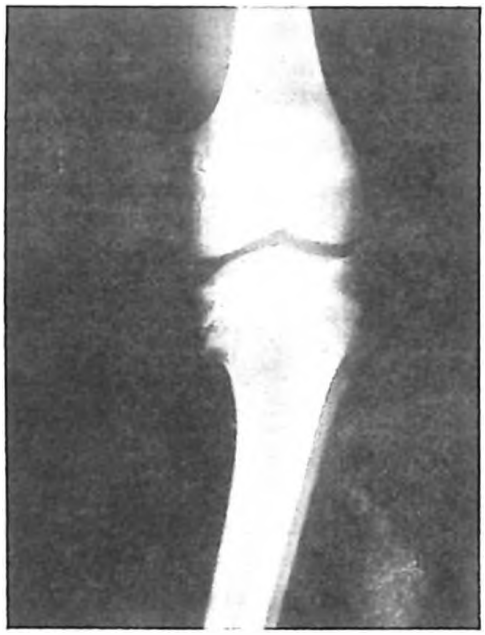

Fig. 2b. Weight-bearing anteroposterior (AP) X-ray of the left knee (Patient A).

Pelvis AP view of Patient B showed bilateral coxa magna with deformation of the femoral heads with short femoral necks, very similar to his brother. On AP weight-bearing X-ray of the left knee, we observed severe wedging of the medial tibial epiphysis and metaphysis, severe depression of joint surface (MDA of 30°), and adaptive overgrowth in the medial femoral condyle (Fig. 3).

The X-ray examinations of the knees and hips of the other members of the family (except one distant relative with tibia vara) disclosed no deformities of the tibia or proximal part of the femur. This observation suggested that this was a new mutation.

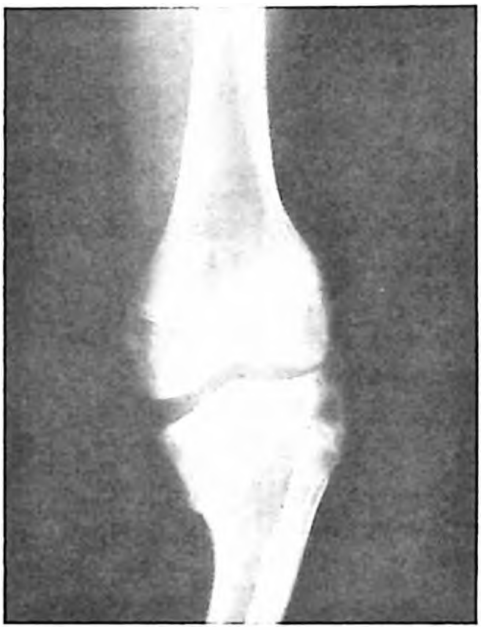

Fig. 3. Anteroposterior (AP) weight-bearing X-ray of the left knee (Patient B).

Discussion

The similarity between the clinical courses of tibia vara and developmental coxa vara was first reported by Lagenskiöld[1,2]. Later research showed that this similarity also existed at a microscopic level[3]. Although Say et al.[7] reported a family with congenital coxa vara and late adaptive degeneration of the proximal tibia, coexistence of adolescent tibia vara and coxa vara has not been previously published. Lovejoy and Lovell[8] described two cases in which adolescent tibia vara presented with SCFE. Wenger et al.[6] also reported one similar case.

Although trauma, infection, and nutritional and genetic factors have been implicated as causative factors, the etiology of tibia vara still remains to be elucidated.

Proximal physis of the femur consists of three parts: longitudinal growth plate, trochanteric growth plate and femoral neck isthmus. Separate or combined growth arrest of these plates eventually leads to different types of deformities. In line with such deformities, the acetabulum shows adaptive changes.

Growth disturbance of the proximal femur may occur due to a multitude of factors such as Legg-Perthes-Calvé disease, trauma, infection, and congenital and developmental conditions. Each condition (except the genetic and insidious developmental diseases) presents its clinical symptoms with varying radiological features in relation to the etiology and the severity of the epiphyseal damage[9]. In the presented cases, broader femoral neck, relative overgrowth of the trochanter major and close-to-normal appearance of the medial side of the neck suggested growth arrest at the lateral side of the longitudinal growth plate.

Certain studies investigating tibia vara draw attention to the high probability of genetic etiology, though currently the mode of inheritance has not been determined[10-13]. Concurrence of the same disease in monozygotic twins with the absence of any defined predisposing factors like marked obesity, black race, or early walking supports the genetic etiology. In this report the association of the two distinct conditions in twins also supports the genetic etiology and genetic relation of these conditions. While there is no clear evidence for the common etiology of these two conditions, it seems that there is a variable coexistence for the affections of the proximal epiphysis of the femur and tibia.

Tibia vara coexisting with SCFE has been reported previously in three cases. This is the first report mentioning coexistence of a different epiphyseal growth problem of the proximal femur. We suggest that the proximal femur should be examined for possible epiphyseal problems in patients presenting with adolescent tibia vara.

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Cutis marmorata telangiectatica congenita: an unusual cause of lower extremity hypoplasia

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SUMMARY: Avcı S, Çalıkoğlu E, Şaylı U. Cutis marmorata telangiectatica congenita: an unusual cause of lower extremity hypoplasia. Turk J Pediatr 2001; 43: 159-161.

Cutis marmorata telangiectatica congenita is a relatively benign, rare cutaneous disorder with various manifestations. A seven-year-old girl, who presented with extremity hypoplasia, had the characteristic reticular, patchy, blue-pink cutaneous lesions, which became more prominent with exposure to cold temperatures. She had 4.8 cm shortening of her right lower extremity, which was also thinner than on the left side. The patient did not have skin atrophy, ulcers, glaucoma or macrocephaly. She is being followed for a future extremity lengthening procedure.

Key words: telangiectasis, cutis marmorata, skin abnormalities, leg length inequality.

Hypoplasia of the extremities may be classified as congenital or acquired, and there are many causative factors. Cutis marmorata telangiectatica congenita (CMTC) is a rare cutaneous syndrome, and limb asymmetry may be one of its manifestations. Here, we present a case of CMTC with limb hypoplasia and a review of the literature.

Case Report

A seven-year-old girl, second child of a nonconsanguineous marriage, was brought with the complaint of limping. She was born with red stains on most parts of her body that became more prominent when the child was exposed to cold. Soon, the family noticed that her right leg was shorter and thinner than the left, and length discrepancy became more apparent as the child grew older. Skin lesions remained unchanged during this period. Her landmarks of development were normal and she was attending primary school as a successful student. None of the family members had similar cutaneous lesions.

On examination, there was port wine stain on the scalp without any skin lesions on the face. The diffuse port wine stain on the right side of the trunk ended at the midline both posteriorly and anteriorly. All over the right lower extremity there were pale, pink-blue, reticular, macular lesions (cutis marmorata), which became more prominent when the child was exposed to cold (Fig. 1). Similar lesions were found on the anterior part of her left cruris. Skin was not atrophic and there were no ulcers.

Her right lower extremity was 4.5 cm shorter than on the left side by measurement from the superior iliac spine to the medial malleolus (Fig. 2). The right thigh circumference, measured 10 cm proximal to the superior pole of the patella was 5.5 cm thinner than on the left side. The right cruris, measured from the midpoint, was 3 cm atrophic compared to the left side. There were no other deformities and muscle testing was normal.

Blood pressure was measured 95/55 mmHg from both arms. A skeletal survey revealed no abnormalities of the spine or extremities. Leg length discrepancy, measured by computerized tomography (CT) scanogram, was 4.8 cm in total, 2.9 cm from the femur and 1.9 cm from the tibia. Bone age determined from the left wrist was 6 years 10 months. Urinalysis and complete blood count were normal. The genetic karyotype was also reported to be normal. Punch biopsy specimen, obtained from the right arm skin, showed dilated venules in the superficial dermis.

The patient was diagnosed as cutis marmorata telangiectatica congenita. She was prescribed a 3 cm shoe-lift for the right side and is being followed for a future extremity-lengthening procedure.

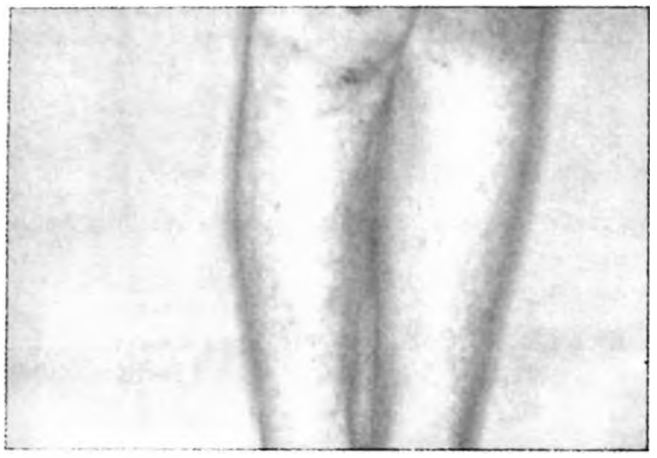

Fig. 1. Closer view of the reticular skin lesions, especially on the right leg.

Fig. 2. Atrophy of the right lower extremity is apparent.

Discussion

Physiological cutis marmorata is frequently observed in newborn babies of Caucasian origin and is accentuated by crying and cold temperatures. Van Lohuizen[1] first described CMTC in 1922, as a localized or generalized, reticulated, blue-violet network, usually present at birth. Various synonyms, such as congenital generalized phlebectasia, nevus vascularis reticularis, and congenital livedo reticularis have also been used in the literature.

Heredity and environmental agents have been proposed in the etiology, but the theory of Happle[4], which suggested a lethal gene surviving by mosaicism for the Klippel-Trenaunay and Sturge-Weber syndromes, better explains the patchy distribution of the lesions and sporadic occurrence of the disease[2-4]. It has also been suggested that Klippel-Trenaunay and Sturge-Weber syndromes and CMTC should be classified as a group of vascular diseases associated with other developmental defects of the mesodermal system during embryonic life[5].

Frequently associated conditions are skin atrophy and ulcerations, nevus flammeus, capillary and cavernous hemangioma, hypertrophy or atrophy of the affected limb, macrocephaly and glaucoma[6,7]. Skin lesions are always patchy and never involve the whole body. When located on the trunk they tend to end at the midline. Skin lesions usually improve, especially during the first two years of life, and this has been attributed to the maturation of the skin, with an increase in the dermis and keratin layers[5,7]. In the current case, the skin lesions were not as dark as observed in photographs of cases presented in the literature, but the family had not noticed any improvement since birth either.

In a review of 35 cases by Devillers et al.[7], 40 percent of the patients had limb asymmetry and in half of these, asymmetry was both in length and circumference. The affected extremity may be atrophic or hypertrophic. In contrast to our patient, who had 4.8 cm shortening, maximum leg length discrepancy in that series was 4 cm. We will follow the patient to observe the changes in discrepancy, and a leg-lengthening procedure will be planned according to the obtained data.

Glaucoma has also been reported, but all of these patients had a skin lesion around the affected eye; routine ophthalmologic evaluation is not recommended[8]. In our case, lesions on the head were restricted to the hairy scalp, and physical examination of the eyes was normal.

To our knowledge, this is the third case of CMTC published from Turkey. One of the previously published cases did not have any anomalies other than the skin lesions[9]. The other case was a newborn with the typical skin lesions and atrial septal defect, but there was no extremity asymmetry[10].

In conclusion, CMTC is a rare and relatively benign condition that should be considered in the differential diagnosis of extremity asymmetry, and associated conditions should be searched.

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Chronic idiopathic neutropenia associated with gingival enlargement

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SUMMARY: Olcay L, Çelenligil-Nazlıel H, Korkmaz A, Yetgin S. Chronic idiopathic neutropenia associated with gingival enlargement. Turk J Pediatr 2001; 43: 162-165.

A girl with chronic idiopathic neutropenia who developed gingival enlargement at seven years of age is presented. Intraoral examination revealed generalized gingival inflammation with a tendency to bleeding and inflammatory gingival enlargement localized to the anterior region. A considerable amount of bacterial plaque was noted on the teeth. There were also 4-5 mm pocket depths around the first molars. Radiographic examination also indicated the presence of incipient bone loss around the first molars in both jaws. The patient, who was diagnosed as localized prepubertal periodontitis with generalized gingival inflammation and anterior gingival enlargement, accentuates the importance of evaluation of periodontal status in patients with chronic idiopathic neutropenia, to avoid the destruction of supporting structures of the dentition.

Key words: neutropenia, gingival enlargement, prepubertal periodontitis.

The most frequent hematological disease associated with gingival enlargement is accepted as leukemia. However, in patients with immunodeficiency, as in neutropenia, neutrophil dysfunction and aplastic anemia[1-8], periodontal manifestations ranging from-marginal gingivitis to rapidly progressive periodontitis may be prominent[5-8]. Sometimes stomatitis with ulceration is the only major clinical manifestation of neutropenia[7]. This presented case describes localized prepubertal periodontitis with generalized gingival inflammation and anterior gingival enlargement in a seven-year-old girl with chronic idiopathic neutropenia.

Case Report

A 4 ½ -year-old girl was referred to the Hematology Unit because of recurrent upper respiratory tract infections, tonsillitis, pneumonia, sinusitis, and sinopulmonary infections since one year of age, and was treated for pulmonary tuberculosis. Her white blood cell count (WBC) ranged between 3.5-6.5 x 10^9/L and absolute neutrophil count between 0.18-0.58 x 10^9/L. The bone marrow aspiration revealed arrest in promyelocyte maturation. The immunoglobulin levels and lymphocyte subsets were normal. There was fourth degree consanguinity between the parents; however, neither the parents nor her two siblings had neutropenia. Her mother complained of recurrent aphthous lesions. She was treated with granulocyte colony stimulating factor (G-CSF) for 10-20 days during infections, which recurred every 15-45 days with the diagnosis of chronic idiopathic neutropenia. When she was seven years of age, gingival enlargement was observed and she was referred to the Periodontology Department of the Faculty of Dentistry for further evaluation.

Intraoral examination revealed abnormal teeth relations and generalized gingival inflammation with a tendency to bleeding and inflammatory gingival enlargement localized to the anterior region (Figs. 1, 2). A considerable amount of bacterial plaque was noted on the teeth. There were also 4-5 mm pocket depths around the first molars. Accordingly, radiographic examination indicated the presence of incipient bone loss around the first molars in both jaws (Fig. 3). On the basis of clinical and radiographic findings, the patient was diagnosed as localized prepubertal periodontitis with generalized gingival inflammation and anterior gingival enlargement.

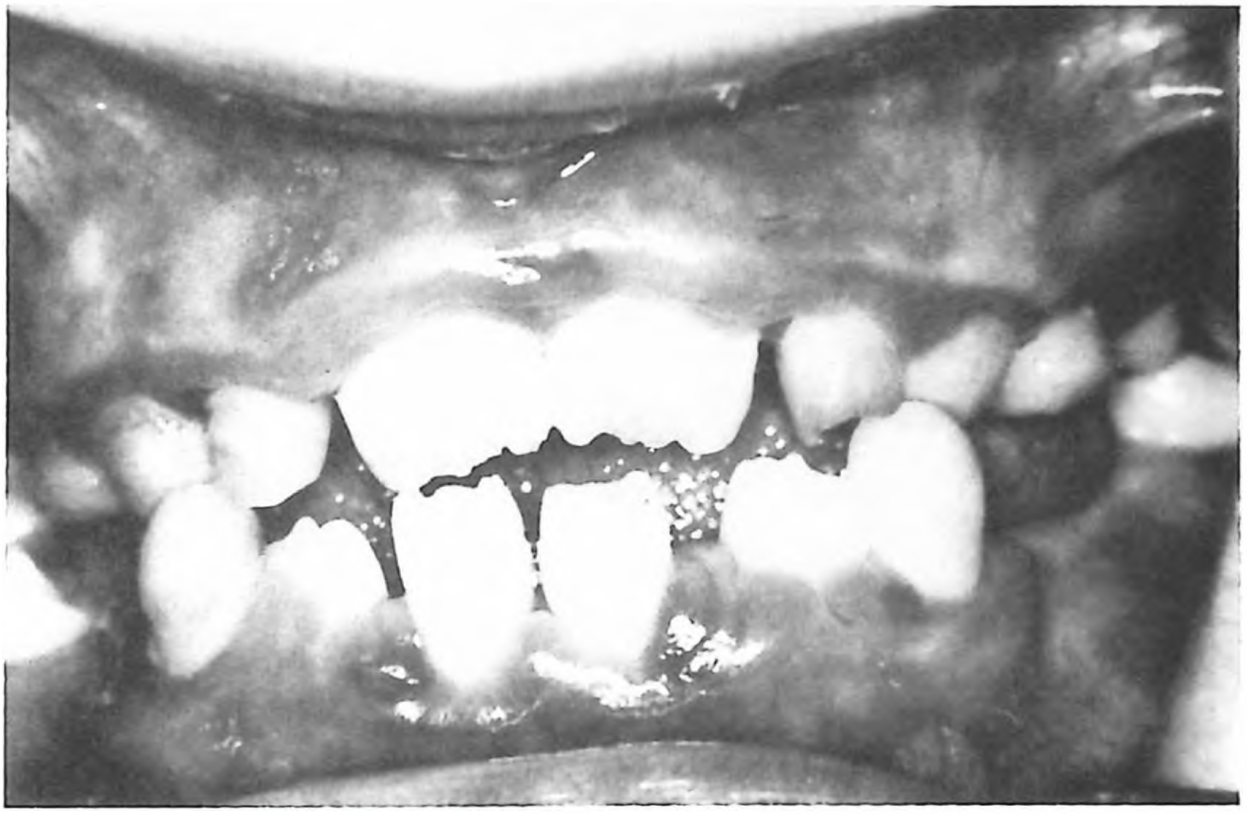

Fig. 1. Chronic inflammatory gingival enlargement localized to the anterior region.

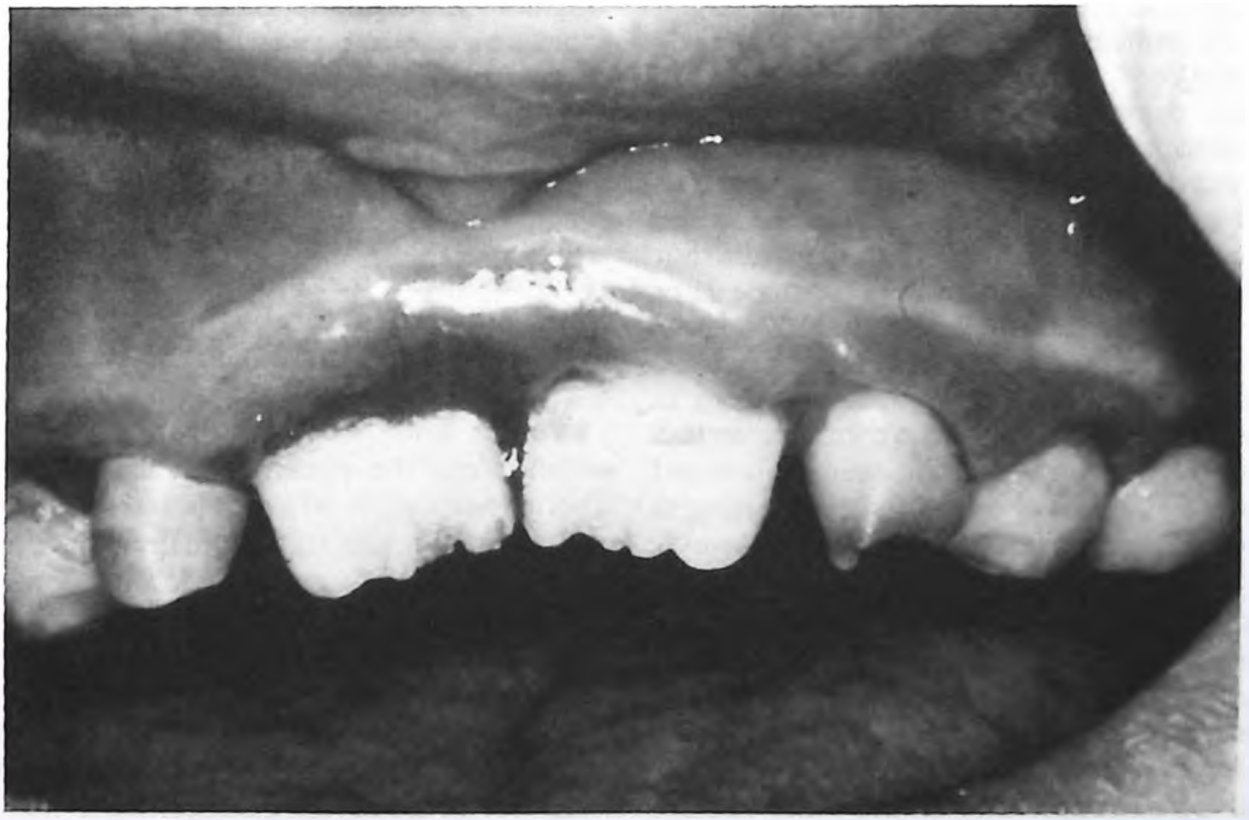

Fig. 2. Bleeding upon gentle manipulation due to the severe inflammation of the gingiva.

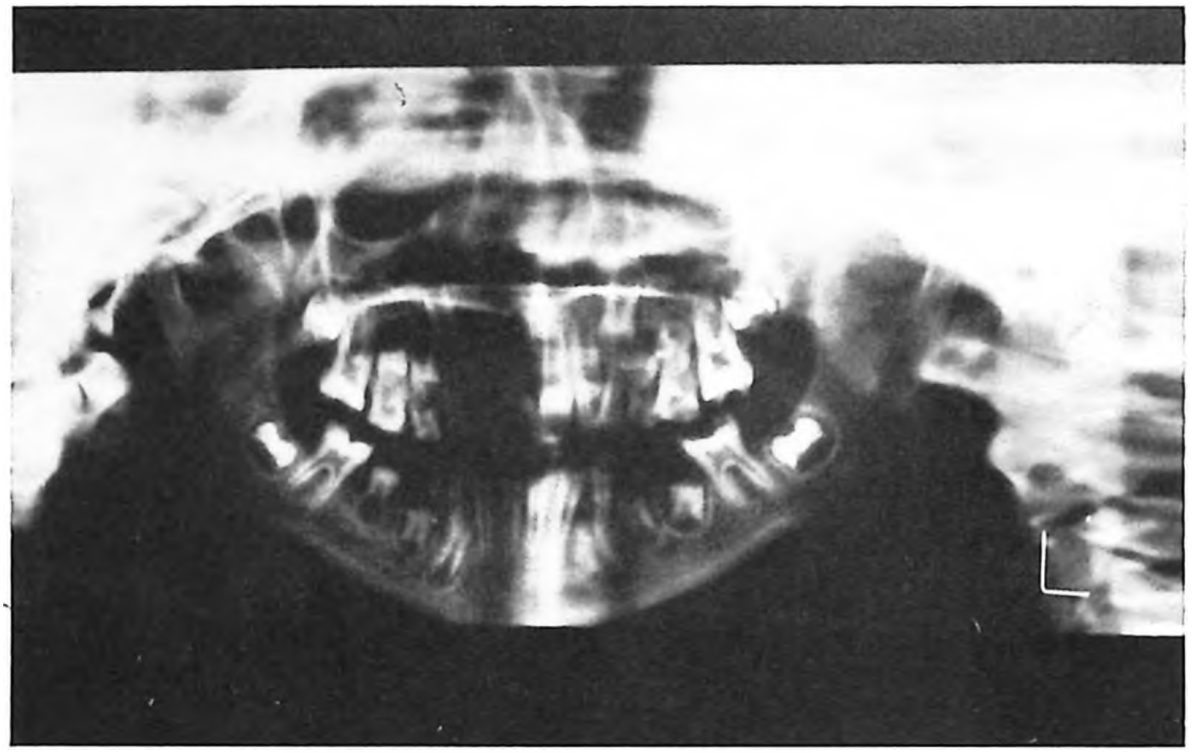

Fig. 3. Alveolar bone loss around the first molars.

Discussion

Chronic neutropenia of childhood is a group of disorders in which production of neutrophils is decreased or ineffective. The underlying mechanism has not been well identified yet. Autosomal dominant pattern of transmission has been reported. On the other hand, there are several reports indicating the absence of genetic transmission[9]. In the present case, Fanconi's anemia, dyskeratosis congenita, cartilage-hair hypoplasia, and Schwachman-Diamond syndrome were ruled out based on normal phenotype. The age at onset and the course of the disease as well as normaley of neutrophil counts were not consistent with congenital severe neutropenia or cyclic neutropenia. Therefore, she was diagnosed as chronic idiopathic neutropenia. In general, depressed resistance against microorganisms in chronic neutropenia and neutrophil dysfunction may disturb the interactions between the host and the microorganisms of bacterial plaque, leading to increased susceptibility to preiodontal disease[1-8].

Both gingivitis and chronic inflammatory gingival enlargement are caused by prolonged exposure to dental plaque[10]. Factors that favor plaque accumulation and retention include poor oral hygiene, abnormal relationships of adjacent teeth and opposing teeth, improper dental restorations, nasal obstruction and habits like mouth breathing[10]. Our patient had the habit of mouth breathing due to the nasal obstruction caused by recurrent upper respiratory tract infections and tonsillary hypertrophy. In addition, since she was in the mixed dentition period, abnormal teeth relations existed. Furthermore, her father noted that she resisted attempts to brush her teeth because of painful aphthous lesions. We think that the factors causing bacterial plaque accumulation may contribute to the clinical picture of generalized gingival inflammation and inflammatory gingival enlargement.

There are a few reports[2,3] describing periodontal findings in chronic idiopathic neutropenia, and they document that this type of neutropenia is characterized by oral symptoms of severe persistent gingivitis, recurrent occurrence of aphthous-type lesions and/or rapidly advancing alveolar bone destruction. As previously mentioned, the patient also had prepubertal periodontitis[11,12] which has its onset before 11 years of age in the primary or mixed dentition. Prepubertal periodontitis appears in Papillon-Lefèvre syndrome. Down syndrome. Chédiak-Higashi syndrome, hypophosphatasia, acute and subacute leukemia, leukocyte adhesion deficiency and neutropenias[11,12]. In our case, alveolar bone destruction, a characteristic sign of prepubertal periodontitis, was very limited at this stage, but she was included into

a periodontal follow-up program in order to prevent further alveolar bone loss which may result in exfoliation of the teeth.

In view of the previous reports[1-8,11,12] and our findings, we suggest that periodontal evaluation of patients exhibiting neutropenia is of utmost importance to avoid the destruction of supporting structures of the dentition. We also want to point out that gingival enlargement is not a hallmark of leukemia only.

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A case with some clinical findings overlapping to Rubinstein-Taybi, Rubinstein-Taybi-like syndrome or multiple pterygium syndrome: coincidental findings or a new entity?

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SUMMARY: Kargı AE, Balcı S, Aköz T, Kargı Ş, Erdoğan B. A case with some clinical findings overlapping to Rubinstein-Taybi, Rubinstein-Taybi-like syndrome or multiple pterygium syndrome: coincidental findings or a new entity? Turk J Pediatr 2001; 43: 166-171.

We report a case with broad, deviated thumb and big, duplicated, deviated toes resembling Rubinstein-Taybi syndrome. But the patient did not have severe mental retardation as in Rubinstein-Taybi syndrome and had no microdeletions on chromosome 16 by FISH-based assay. This patient had mild webbing as seen in multiple pterygium syndrome, but broad-deviated thumb has not been reported in this syndrome. We discuss whether these are coincidental or overlapping findings or whether this is a possible new clinical entity.

Key words: Rubinstein-Taybi syndrome, Rubinstein-Taybi-like syndrome, multiple pterygium syndrome, FISH-based assay for Rubinstein-Taybi syndrome.

In 1963, Rubinstein and Taybi[1] described a new syndrome characterized by typical facial appearance, broad thumbs and halluces, and angulation deformities of thumbs and halluces, rarely hexadactyly of the feet and partial cutaneous syndactyly of the toes[1]. Since then, these cases have been called Rubinstein-Taybi syndrome (RTS)[2-6]. A submicroscopic deletion in chromosome 16p13.3 has recently been found in RTS patients. But it is only detected in one-fourth of these patients[7].

Multiple pterygium syndrome is characterized by webbing of the knee, elbow, axillary region, and neck, and flexion contractures of the joints[8,9].

In this report we present a new case with some classical findings of RTS such as broad and deviated thumb and some typical findings of multiple pterygium syndrome or Rubinstein-Taybi-like syndrome. Interestingly, we could not demonstrate a microdeletion of chromosome 16 in our patient by FISH-based assay.

Case Report

A nine-year-old male child was referred to the Plastic and Reconstructive Surgery Department because of malformations involving his face, hands and feet. He was the second child of nonconsanguineous parents with no family history of similar conditions, and he had two healthy brothers. He was born normally at term after an uneventful pregnancy. At birth, he weighed 3100 g (25^{th} - 50^{th} percentile) with a length of 45 cm ($<5^{th}$ percentile) and a head circumference of 35 cm (50^{th} - 75^{th} percentile). He grew regularly at about -3SD for height and weight until nine years of age. He attended school at seven years of age with average achievement.

He had severe webbing neck and cup-shaped ears. His cup ear malformation was operated under general anesthesia at eight years. He was also operated for webbing neck at eight years.

On admission, physical examination showed short stature with height 120 cm (3^{rd} percentile), and span 112 cm. His weight was 24 kg (10^{th} - 25^{th} percentile) and occipitofrontal circumference (OFC) was 52 cm. He had frontal bossing, hypertelorism (intermedial canthal distance 37 mm), wide palpebral fissures, upper eyelid ptosis, loss of hair on the lateral parts of eyebrows, depressed and enlarged nasal bridge, short and upturned nose and prominent columella, long philtrum, large and triangular mouth with

downturned corners, low-set ears with hypoplasia of scafal and helical configurations and a cup ear like malformation of the 1/3 upper part of both ears, small and recidive chin, gingival hyperplasia, dental abnormalities, bifid uvula, and short frenulum (Fig. 1). Operation scar for the webbed neck can be seen on the lateral view (Fig. 2). He had a short, bilaterally webbed neck and a low posterior hairline was noted (Fig. 3).

Distal phalanges of both thumbs were broad and radially deviated. There was incomplete syndactyly involving the fourth web of both hands. There was fifth finger clinodactyly on the right side (Figs. 4, 5). His feet were deviated laterally and he had big toes and preaxial duplication on the right side (Fig. 6). His joints were hyperextensible and hypermobile with range of motions wider than normal. The external genitalia were hypoplastic with bilateral retractile testes and inguinal hernia on the right side (Fig. 7).

He had pectus excavatum, bilateral inverted nipples and hypoplastic umbilical region. Another web involving apparently his right anterior axillary region and mildly on the left side was noted (Fig. 8).

Other systems were normal, and particularly no cardiac, renal or vertebral anomalies were noted. Routine laboratory tests, karyotyping, and ultrasonography of the abdomen and pelvis revealed normal findings. By FISH based assay and by using five cosmids covering the entire CBP gene on 16p; all cosmids, RT 100, RT 191, RT 102, RT 203, and RT 166 were present on both copies of 16p in our patient, excluding a microdeletion [Personal communication; Breuning MH, 1998].

Radiological studies showed delayed bone age. Hand radiographs showed split distal phalanx and abnormal angulation of the thumbs, clinodactyly of the right fifth finger, and hypoplasia of the proximal and middle phalanges. Feet radiographs showed preaxial duplication of the toe on the right side and abnormal angulation of the halluces (Figs. 9, 10).

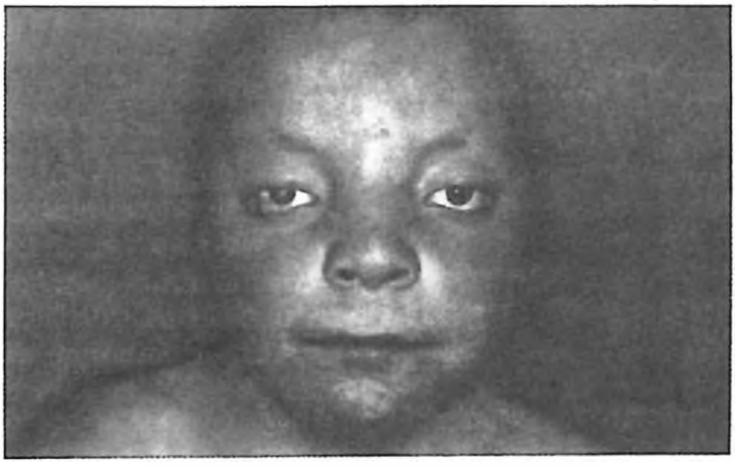

Fig. 1: Facial appearance (anterior view).

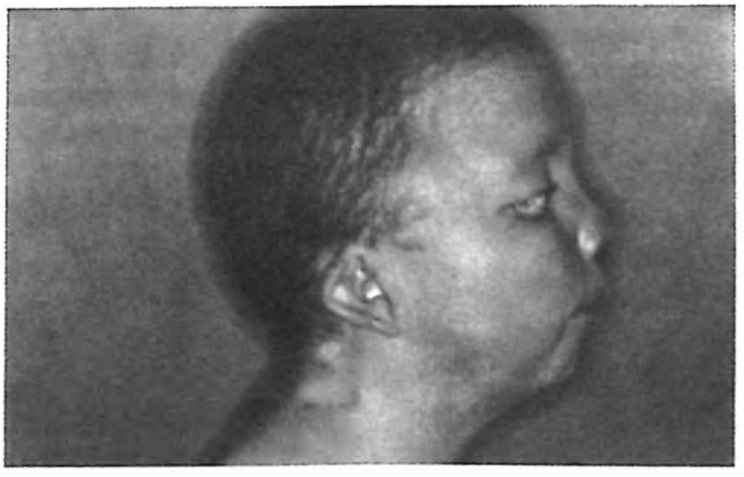

Fig. 2. Facial appearance (lateral view).

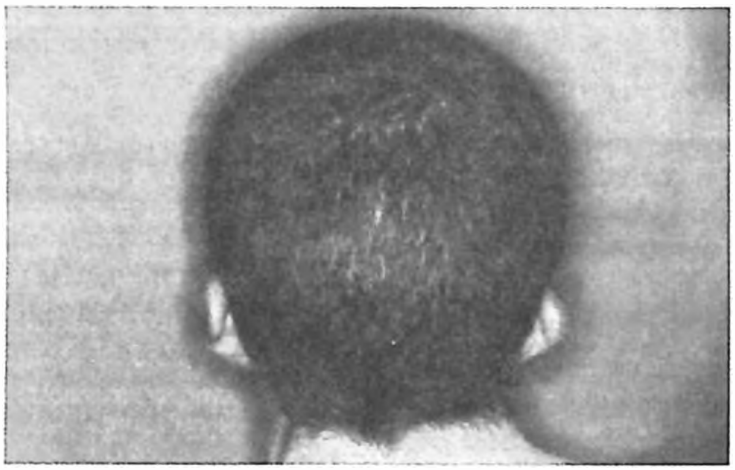

Fig. 3. Posterior view of the head.

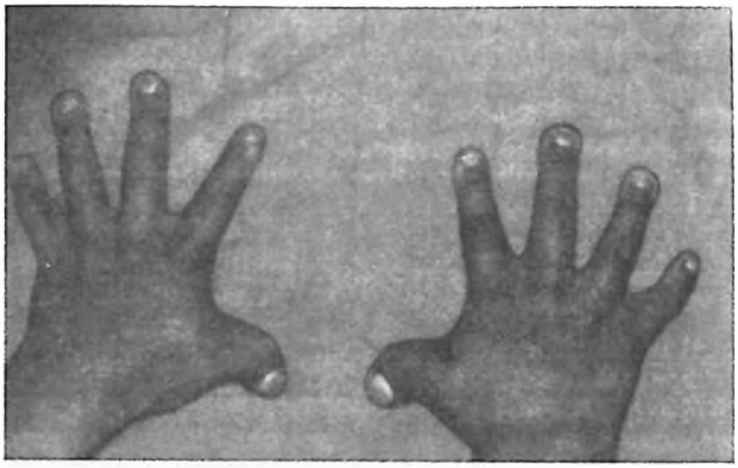

Fig. 4. Hand deformities (dorsal view).

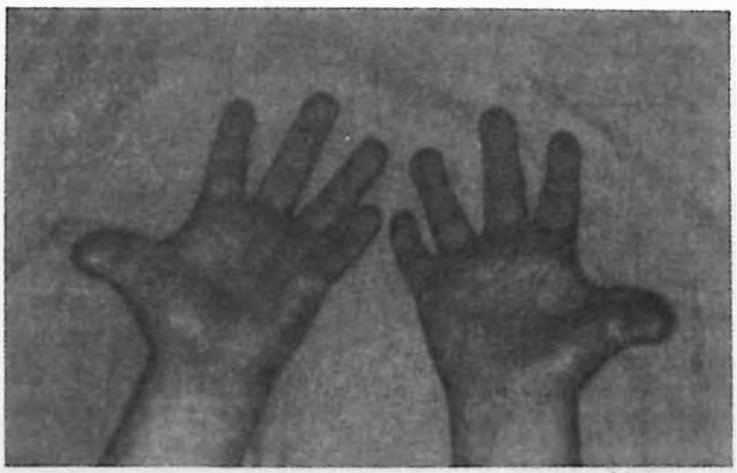

Fig. 5. Hand deformities (palmar view).

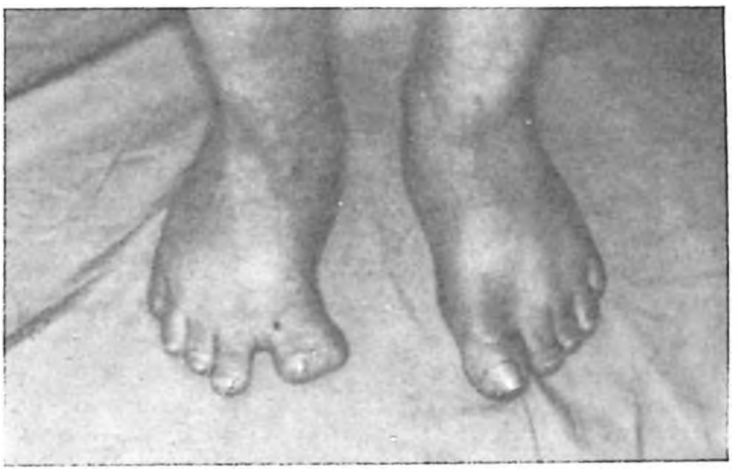

Fig. 6. Patient's feet.

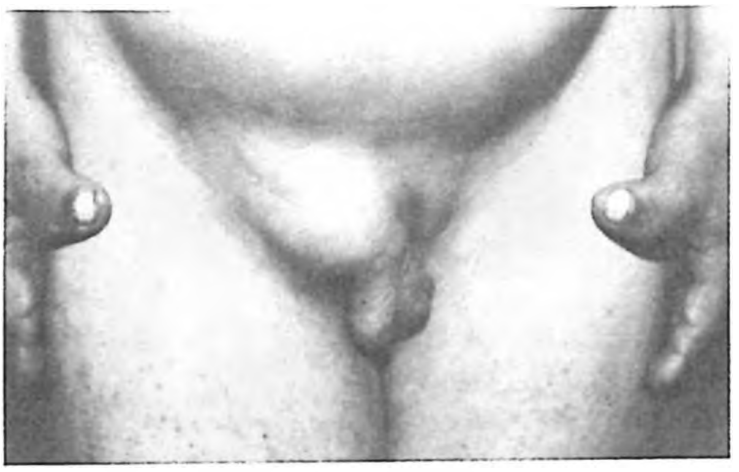

Fig. 7. Patient's genital region.

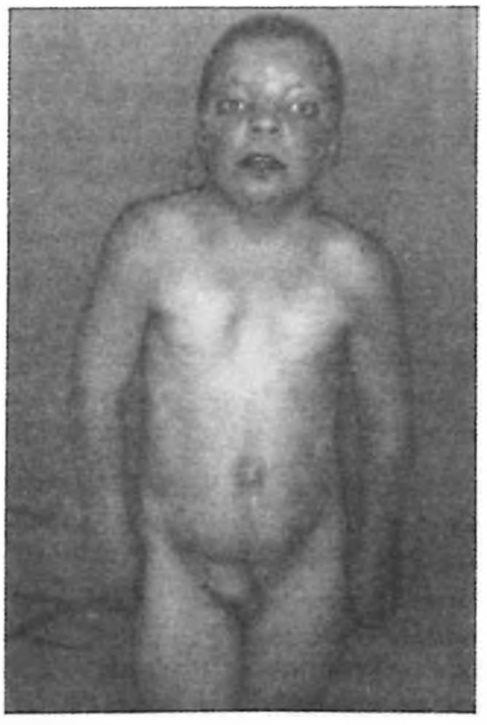

Fig. 8. Anterior view of the patient's body.

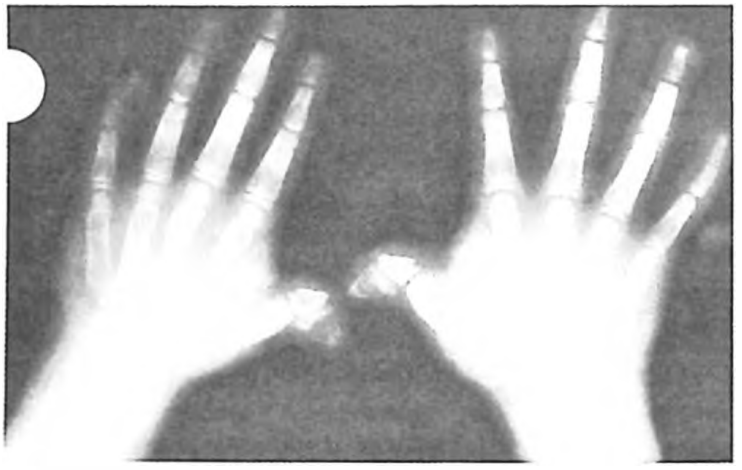

Fig. 9. Radiographs of the hands showing bilaterally abnormal angulation of the thumbs and the right split distal phalanx.

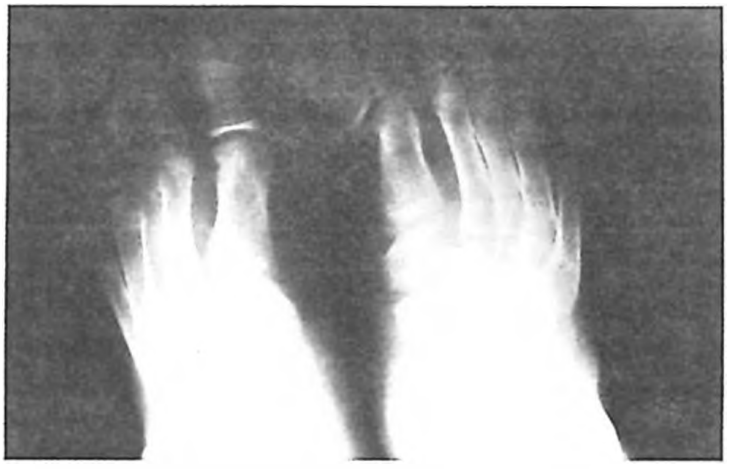

Fig. 10. Radiographs of the feet demonstrating preaxial duplication of the toe on the right side and angulation of the halluces.

Discussion

We present here a new case with some characteristic findings of RTS, some of multiple pterygium syndrome and some of Rubinstein-Taybi-like syndrome, and discuss whether findings of our case are coincidental or whether this is possibly a new entity.

Rubinstein-Taybi syndrome is characterized by specific facial appearance, psychomotor retardation, small stature and anomalies of the hands and feet with broad distal phalanges, especially of the thumbs and halluces[3,10,11].

Our patient's hand and feet abnormalities were very similar to that seen in RTS. He had mild mesomelic shortening of extremities. Both thumbs were broad with radial angulation. There was also syndactyly between the fourth and fifth fingers and clinodactyly on both hands. His feet were deviated laterally and he had big toes with preaxial duplication on the right side. These hand malformations are characteristics of RTS[3,10,11]. Our patient also had delayed bone age and we detected split distal phalanx of the thumbs, and hypoplasia of the proximal and middle phalanges of the hands by radiological studies, as is common in RTS. Other locomotor system abnormalities in RTS are laxity of joints and joint dislocations[6]. Our patient also had mildly hyperextensible joints.

While the hand and feet abnormalities in this patient are highly suggestive of RTS, facial findings not consistent with RTS suggested another syndrome. Again, our patient's intelligence was not severely affected. Recently, a more definite diagnosis of RTS has been made possible with FISH technique to determine the presence of microdeletion on chromosome 16p13.3. Though detected in one-fourth of RTS patients, further cytogenetic studies did not confirm this diagnosis in our case[7].

Though our patient's facial appearance suggested Robinow's syndrome, this patient did not have any vertebral malformations or mesomelic dysplasia, which are seen in that syndrome[12,13]. In 1993, Balcı et al.[14] noted a new hand malformation in Robinow's syndrome, such as split hand, and they also noted syndactyly between the fourth and fifth fingers and toes, such as in our case.

Multiple pterygium syndrome is another clinical entity characterized by webbing of the knee, elbow, axillary region, and neck, and flexion contractures of the joint. Webbing has never been reported in Robinow syndrome or in RTS. Neck and right axillary region webs of our patient were suggestive of multiple pterygium syndrome. Minor facial anomalies of this syndrome are ptosis, antimongoloid slant, low-set ears, and epicanthus, and some of these were present in our case. Other characteristics of multiple pterygium syndrome are vertebral anomalies, syndactyly, camptodactyly, and short stature[8,9,15]. However, these hand and feet abnormalities have never been reported in multiple pterygium syndrome in medical literature. In our patient, inverted hypoplastic nipple seen in multiple pterygium syndrome and pectus excavatum seen in RTS are remarkable[8-10].

This case should also be differentiated from Rubinstein-Taybi-like syndrome. In the syndrome reported by Cotsirilos and Pavone[16,17], facial features were not like RTS, while hand and feet abnormalities were similar to that seen in RTS. Reported facial features of this syndrome as also seen in our patient, were low-set ears, hypertelorism, ptosis of eyelids and palpebral fissures downward slanted down[16,17].

In conclusion, we report a possible new entity which had some clinical findings of RTS, Rubinstein-Taybi-like syndrome and multiple pterygium syndrome. RTS could not be definitely excluded cytogenetically. The question to be answered is whether these findings are overlapping or just coincidental or whether this is a new entity. Further similar studies are needed to resolve the question.

Acknowledgments

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Bleomycin-induced hyperpigmentation and hypersensitivity reactions to etoposide and vinblastine in a child with endodermal sinus tumor

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SUMMARY: Mutafoğlu-Uysal K, Sarıalioğlu F, Olgun N. Belomycin-induced hyperpigmentation and hypersensitivity reactions to etoposide and vinblastine in a child with endodermal sinus tumor. Turk J Pediatr 2001; 43: 172-174.

We report a pediatric case who developed bleomycin-induced hyperpigmentation and hypersensitivity reactions to both etoposide and vinblastine while receiving chemotherapy for germ cell tumor. Skin hyperpigmentation related to chemotherapeutic agents has been reported only rarely in pediatric patients. This patient developed a characteristic skin hyperpigmentation which was "flagellate" in appearance. Two features of the hyperpigmentation were noteworthy: development at a low cumulative dose of bleomycin and persistence after cessation of chemotherapy. Additive effect of cisplatinum-induced hyperpigmentation was suggested. Although hypersensitivity reactions to etoposide have been previously reported, hypersensitivity reactions to vinblastine are almost unknown. To our knowledge, this is the first report of hypersensitivity reaction to vinblastine in a child in English literature.

Key words: hyperpigmentation, hypersensitivity reactions, bleomycin, etoposide, vinblastine.

Unlike some serious and life-threatening side effects of anticancer drugs, hyperpigmentation has been reported only rarely in pediatric patients because it is usually accepted as a problem of simple cosmetic concern. Hypersensitivity reaction (HSR) is another toxic side effect of anticancer therapy which may necessitate alterations in the chemotherapy regimen and may even be life-threatening. Certain drugs like L-asparaginase cause HSRs in a significant number of patients, while HSRs to some other chemotherapeutic agents like Vinca alkaloids are almost unknown[1]. An increasing number of HSRs have appeared in pediatric oncology literature, as in the case of epipodophyllotoxins, but have been noted only recently anecdotally[2].

We herein present a pediatric patient who demonstrated bleomycin-induced skin pigmentation and HSRs to both etoposide and vinblastine (VBL).

Case Report

In December 1996, a previously healthy 14-year-old girl presented with an abdominal mass, and she was diagnosed as stage IV endodermal sinus tumor originating from the left ovary with hepatic metastasis. After excision of the primary tumor, chemotherapy was started with combined PEB protocol (P: Cisplatinum 120 mg/m^2, 5 hour parenteral infusion, on day 1; E: Etoposide 100 mg/m^2 parenteral infusion over 1 hour on days 1-3; B: Bleomycin 15 mg/m^2 IV, on day 2).

During the first course of chemotherapy, a HSR developed with urticaria, flushing, bronhcospasm, cyanosis and dyspnea at the 15th minute of the first etoposide dose. The infusion was discontinued and the reaction was controlled with parenteral antihistaminic and corticosteroid. Her past history revealed no record of asthma, atopy, drug allergy or adverse reactions to radiological contrast media. Since VBL is another active agent against germ cell tumors, etoposide was replaced with VBL (6 mg/m^2 IV days 2 and 3). She tolerated the next two courses of chemotherapy well without any significant toxicity.

Three weeks after the third course, when she had already received a total of 60 mg bleomycin and 576 mg CDDP, she was noted to have areas of linear, dark brown hyperpigmentation over

the neck, inner thighs and anterior chest wall (Fig. 1). The skin lesions were not pruritic. On the fourth course, during intravenous administration of VBL, a generalized cutaneous rash developed at the 3rd minute of injection in the form of erythematous macules and urticarial plaques which were pruritic. These lesions showed a rapid resolution process after intravenous diphenhydramine and prednisolone. Two more chemotherapy courses including CDDP, VBL and bleomycin were administered with premedication (dexamethasone and diphenhydramine) in an attempt to prevent HSRs. No further HSR was observed.

Fig. 1. Close-up of linear dark brown hyperpigmentation on the inner thigh.

Renal and hepatic functions, which were monitored before each course, remained intact. Dark brown linear hyperpigmented skin lesions were mostly prominent just after the chemotherapy courses. Their color changed to light brown after the first week of chemotherapy, but never disappeared. Six courses of chemotherapy were completed in May 1997 and she has been in complete remission without any late toxic side effects since July 1999. The linear hyperpigmentation of the skin did not disappear, but got lighter during the follow-up.

Discussion

The major toxicity of bleomycin is dose-related pulmonary toxicity, which has been well established, but skin hyperpigmentation, another dose-related toxicity, has come to attention only infrequently because it causes only cosmetic trouble. Our patient developed skin hyperpigmentation in the form of fine, linear streaking over the neck, chest wall and inner thighs. This type of skin hyperpigmentation, which is "flagellate" in appearance and scattered mainly over the chest wall, is unique to bleomycin[3-5]. So for this case, hyperpigmentation was thought to be mainly related to the skin toxicity of bleomycin. Bleomycin-related skin toxicity is dose related and usually develops after a cumulative dose of 90-285 mg[3]. We observed this side effect at a lower dose (60 mg) level. Concomitantly administered CDDP may play an additive role in the early development of hyperpigmentation. Localized hyperpigmentation induced by pressure related to CDDP has been reported more recently[6]. There was no pressure effect to induce hyperpigmentation for this case, so CDDP probably played only an additional role. The exact pathogenetic mechanism for bleomycin and CDDP-induced hyperpigmentation is still not known Bleomycin-related hyperpigmentation is a reversible toxicity after the drug is discontinued, but CDDP-induced cases seems to be permanent[3,6]. The permanent hyperpigmentation in this patient may also suggest an additional role of CDDP.

Hypersensitivity reactions to two different agents developed in this patient whose past and family histories were unremarkable for allergic reactions. A severe HSR was observed at the 15th minute of the first etoposide dose, so non-immunologic mechanism was suspected. At the time, she was receiving one-hour infusion of etoposide in saline, with an etoposide concentration of less than 0.5 mg/ml; therefore, a reaction related to rapid infection was unlikely. Although the exact mechanism of HSR to etoposide is not known, it is believed to be of non-immunogenic origin since many of these reactions occur during the first exposure[7]. However, some cases suggested an immunologic base for HSRs to etoposide[8]. Although previous isolated reports seemed to establish etoposide HSRs as an uncommon event, HSRs were observed in 33 percent of pediatric patients with acute lymphoblastic leukemia in a recent study, However, only 14 percent of the reactions were grade 3[2]. The parenteral formulation of etoposide used for this case contained polysorbate 80 and benzyl alcohol, but no studies have been performed to determine whether these substances are to blame in some HSRs.

The deicision to stop or continue with the same agent after development of a HSR depends on the nature and severity of the reaction, the type of cancer that is treated and the availability of a suitable analog or another drug in the same

chemical class. Because of a grade 3 reaction, we replaced etoposide with VBL.

Most antitumor agents have been recognized to cause HSRs in at least a few cases, but HSRs to Vinca alkaloids are almost unknown[1]. Young et al.[10] reported a patient who developed HSR to VBL during a phase I study. To our knowledge, this is the first pediatric patient who developed HSR to VBL.

More data on uncommon toxic side effects of chemotherapy should appear in pediatric oncology literature to help clinicians be aware of the potential side effects and to prevent unnecessary interventions. This may also stimulate studies searching for the pathogenesis of some uncommonly occurring toxic side effects. Future prospective studies on bleomycin-induced hyperpigmentation, especially at lower cumulative doses and during the acute stages of toxicity, may help to clarify the pathogenetic mechanism for this unique toxicity.

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Subadventitial hematoma of a patent ductus arteriosus and thrombus formation within the aorta secondary to cardiac catheterization

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SUMMARY: Doğan R, Bilgiç A. Subadventitial hematoma of a patent ductus arteriosus and thrombus formation within the aorta secondary to cardiac catheterization. Turk J Pediatr 2001; 43: 175-176.

An 18-month-old boy with patent ductus arteriosus (PDA) underwent surgical closure of PDA. Cardiac catheterization determined the PDA was not suitable for transcatheter closure. After the chest was opened, subadventitial hematoma was seen on the aortic end of the PDA. Incision of the aorta revealed a thrombus secondary to intimal laceration. The thrombus was extracted and the PDA was closed using division technique. Because no similar report was found in English-language literature, the technique and the surgical strategy are discussed.

Key words: patent ductus arteriosus, cardiac catheterization, complication.

Persistent patent ductus arteriosus (PDA) is a common congenital cardiac abnormality, occurring in approximately 0.01 to 0.08 percent of live births[1]. Congestive heart failure is an urgent indication for PDA closure, if antifailure treatment is not successful. In children without congestive heart failure, PDA closure can be planned electively. All PDAs should be closed because of a small but definite risk of endarteritis (estimated incidence of 0.45/year)[2].

The first successful PDA operation was performed in 1939 by Gross[3], and since that time, closure of PDAs by ligation or division has been done throughout the world[4-5]. In addition to the surgical closure techniques, transcatheter closure of PDAs using different devices has been widely used over the last 20 years.[6-8] This technique is accepted as an alternative to surgery in all except pre-terms. Inadvertent embolization, residual shunts, hemolysis and vascular trauma are the main complications of transcatheter ductus closure[7-9]. Although vascular trauma is a rare occurrence, it is a possible risk of catheterization procedures.

Herein we report a case of an 18-month-old boy who underwent an elective transcatheter closure of PDA.

Case Report

In October 1998, an 18-month-old boy with PDA was admitted to Hacettepe Medical Center for elective transcatheter closure of the PDA using a controlled-release coil. Transthoracic echocardiography demonstrated a moderately large PDA. Cardiac catheterization was performed according to the protocol of the Pediatric Cardiology Department. However, the PDA was found to have a larger diameter than 5 mm at its narrowest point, and the patient was determined not suitable for such a procedure (Fig.1). The patient was then scheduled for elective surgical closure of his PDA.

The operation was performed according to our standard technique, including posterolateral thoracotomy made through the fourth intercostal space on the left side, two months after the catheterization. The lung was gently retracted medially, and a hematoma around the aortic end of the ductus was unexpectedly found. After the posterior pleura was opened, dissection was continued above and below the level of the ductus. The dissection was then slightly more extensive, and the two teflon slings were passed around the aorta above and

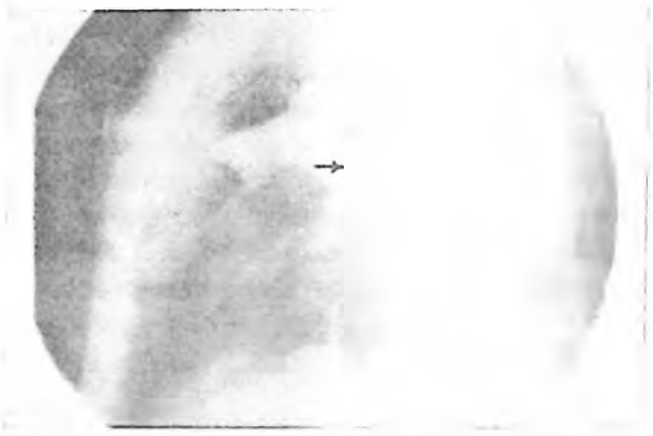

Fig. 1. Lateral aortography showing large patent ductus arteriosus.

below the ductus. A curved dissector was then used, and it was well seen that a subadventitial hematoma, was clearly seen on the posterionferior side of the aortic end of the ductus. Two vascular clamps were applied on the aorta and another one was placed on the pulmonary end. The aortic end of the ductus was opened on its transverse plane, revealing an unsuspected partially organized thrombus. The thrombus were extracted by releasing the vascular clamps and washing out the clots. The residual clot was easily dissected with a coronary dissector. An intimal laceration of 2-3 mm was than noted seen on the posteroinferior portion. The aortic end and then the pulmonary end were oversewn with a double row of continuous sutures. A pledget of Surgicell® was left between the divided ends of the PDA to keep the suture lines from rubbing each other.

The postoperative course was uneventful and the patient was discharged home five days after operation.

Discussion

Surgical closure of PDAs by ligation or division is effective, safe and economical treatment[5,7,8]. Hospital mortality in recent years has in fact approached zero.

Within the past 20 years, of transcatheter and thoracoscopic closure of PDAs have rapidly gained popularity[6-9,10] as an alternative to surgical correction, and they avoid thoracotomy, incision scar, convalescence and the psychological impact of surgery on the child.

The major complications of the invasive procedures are hemorrhage, dissection, false aneurysm on the catheter side, embolization, diminished extremity pulse, hemolysis and infections[7,8,9]. However, to the best of our knowledge, there is no report of subadventitial hematoma and thrombus formation within the aorta after a catheterization made for a PDA.

In some circumstances in which the PDA is short and wide or calcified, division should be indicated, because ligation of such a PDA may cause rupture of a friable duct. It is well known that division is performed by applying fine vascular clamps or, alternatively, a partial occlusion clamp is used at the aortic end to obtain more length for division.

In the case of PDAs, in which a subadventitial hematoma develops after the catheterization or an invasive procedure, it should be kept in mind that a thrombus may cover the ductal orifice and intrude into the aorta. The ductus should not be clamped before the aorta is cross-clamped and opened due to the risk of embolism of thrombotic material. To our knowledge this is the first report of subadventitial hematoma and thrombus formation within the aorta at the aortic end of a PDA due to intimal laceration.

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Global fibrinolytic capacity in healthy newborn infants

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The fibrinolytic system removes the fibrin clot once it has been activated. Fibrinolysis uses elements from plasma, platelets, tissue, and other blood cells to regulate the degradation of fibrin. This is brought about by the conversion of plasminogen to plasmin. The process of plasminogen activation can occur through two distinct pathways including the extrinsic activator system [tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA)] that exists in tissues throughout the body and the intrinsic (plasma) activator system (kallikrein, factor IXa and factor XIIa). The pathway employed in vivo appears to be the extrinsic pathway, however, both the intrinsic as well as exogenous-associated systems could play an important role in the activation of plasminogen. A wide variety of natural inhibitors of fibrinolysis exist in plasma, blood cells, tissues, and other extracellular matrices. These natural inhibitors can act to either inhibit plasmin directly (α_2-antiplasmin, α_2-macroglobulin, antithrombin-III and C1 esterase inhibitor), or block the conversion of plasminogen to plasmin (plasminogen activator inhibitors). At least four plasminogen activator inhibitors (PAI) have been identified. These include PAI-1, PAI-2, PAI-3, and protease nexin-1. The PAI-1 is the main plasminogen activator inhibitor that exists in plasma and serves an important role in the regulation of fibrinolysis[1].

Evaluation of the body's fibrinolytic potential is difficult in contrast to blood clotting in vitro because fibrinolysis is only activated after coagulation. Fibrinolysis is generally initiated by the local release of t-PA in the vascular micro-environment. In this micro-environment, the concentrations of t-PA and of PAI-1 are considerably higher than in the blood circulation. When a fibrin clot is present, t-PA binds to it and becomes protected from inhibition by PAI-1 and other inhibitors. By contrast, free t-PA in plasma is rapidly inactivated by PAI-1. In addition, t-PA and PAI-1 have short half-lives in plasma. For this reason, determinations of plasma levels of plasminogen, its activators and inhibitors do not reflect fibrinolytic activity properly.

This fundamental principle has limited the ability of investigators to define the nature of plasminogen activators and the regulation of fibrinolysis for years. To resolve this problem Amiral et al.[2] developed a standardized assay to measure global fibrinolytic capacity (GFC) in plasma. This assay is a sensitive and reliable parameter to evaluate the fibrinolytic potency of plasma in vitro. GFC assay also allows us to evaluate the sum of the known and unknown factors that regulate plasminogen activators and inhibitors.

The fibrinolytic mechanism of the normal newborn infant is poorly understood. Numerous investigators have reported conflicting results such as reduced plasminogen activity and antigen, normal t-PA antigen, normal to increased t-PA activity, normal to increased PAI-1 antigen, normal to decreased PAI-1 activity, and normal to reduced α_2-antiplasmin[3-5]. All these indirect findings of the fibrinolytic system suggest that there is reduced fibrinolysis in the first days of life. However, abnormally short whole blood clotting times, short euglobulin lysis times, and increased plasma concentrations of the Bβ 15-42 fibrin-related peptides all suggest that the fibrinolytic system is activated at birth[6]. At the same time, the capacity of the fetal fibrinolytic system to generate plasmin in response to stimulation with a thrombolytic agent is decreased compared with adults[7]. We therefore studied GFC in the first six hours of life in newborn infants. As far as we know, this is the first report on GFC of newborn infants in the medical literature.

Twelve preterm and 13 full-term healthy infants, who were consecutively admitted to the Neonatal Care Unit of Hacettepe University Children's Hospital, Ankara, Turkey, were studied. The mothers of the infants showed no amnionitis, prolonged rupture of membranes (24 h before delivery), toxicosis, the syndrome of hemolysis, elevated liver enzymes, low platelet count (HELLP) nor any other diseases that could influence the activation of clotting and fibrinolysis in their infants. Dexamethasone,

which may inhibit the fibrinolytic system[8], was given to the mothers of four infants in the preterm group more than two days before delivery. No other specific drug that could influence the activation of clotting and fibrinolysis was given to the mothers. For ethical reasons, all neonates received vitamin K1 2 mg intramuscularly upon delivery.

In all infants, blood samples for GFC testing were taken within six hours after birth before any medical teratment. In preterm infants, blood samples were obtained from an indwelling umbilical arterial catheter. Peripheral arterial blood samples were used in full-term infants, for ethical reasons. None of the infants had severe acidosis (umbilical arterial blood pH < 7.10) at the time of blood sampling, and no clinical abnormalities were observed during the first month of life. All samples were collected in soft plastic tubes containing an appropriate anticoagulant to prevent activation of blood clotting before analysis. At each sampling 1 ml of blood was anticoagulated with citrate (0.3%), immediately centrifuged (1500 x g, 20 min) and the supernatant stored at -20 °C until determination of GFC.

Global fibrinolytic capacity was determined by the method of Amiral et al.[2] Briefly, a freeze-dried fibrin clot (obtained with fibrinogen, depleted from all the lysine binding proteins, then clotted with thrombin in the presence of silica, calcium and factor XIII-A), is used for preparing standardized fibrin tablets. These latter are introduced in 200 µl test plasma, supplemented with a constant and limited amount of tPA, and the mixture is incubated for 1 hour at 37 °C. Fibrinolysis is then stopped with 50 µl of an aprotinin solution. Generated D-dimer is measured and its concentration is directly related to GFC.

Data are presented as mean ± SD. Gestational age, birth weight and D-dimer concentrations were compared using Mann-Whitney U tests. Correlations were determined by calculating Spearman's rank correlation coefficient. Statistical significance was assumed when the p value was less than 0.05.

Global fibrinolytic capacity, which is expressed as generated D-dimer concentrations, was significantly higher in preterm infants compared to full-term infants ($q < 0.001$, Table I). The D-dimer concentration was negatively correlated with the gestational age ($r = -0.38$; $p < 0.05$), but was not correlated with the birth weight ($r = -0.22$; $p > 0.05$).

Table I. Global Fibrinolytic Capacity in the First Six Hours of Life in Healthy Full-term and Preterm Infants

	Full-term infants (n = 13)	Preterm infants (n = 12)
Gestational age (weeks)	39.2 ± 1.1	32.5 ± 2.5
Birth weight (g)	3332 ± 440	1683 ± 713
Generated D-dimer (µg/ml)	3.8 ± 2.6*	9.9 ± 8.1*

* $p < 0.001$ by Mann-Whitney U test.

Our findings suggest that GFC is increased in preterm infants when compared to full-term infants. Normally plasma t-PA plasminogen concentrations of preterm infants are lower than in term infants[6]. Increased fibrinolytic activity in preterm infants in our study seems to contrast with low plasminogen levels in these infants. However, GFC assay allows evaluation of not only plasminogen levels, but also the sum of the known and unknown factors that may regulate plasminogen activators and inhibitors. Although indirect parameters of fibrinolysis (e.g. plasma plasminogen, t-PA, PAI-1, etc) could not be determined in this study, simultaneous determination of these parameters may not clarify the cause(s) of increased GFC. In addition, increased GFC can be responsible for the low levels of plasma plasminogen due to consumption in preterm infants. On the other hand, increased thrombin-antithrombin III complex and plasmin-antiplasmin complex have been reported in healthy preterm infants without clinical evidence of thrombosis[9]. We may hypothesize that the increased thrombotic activity may be balanced by increased GFC activity. The cause(s) of the increased GFC in preterm infants remains to be determined. Further studies are needed to assess the increased GFC in preterm infants before making a reliable conclusion.

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Neutrophil elastase gene mutations in cyclic neutropenia

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To The Editor

A case of cyclic neutropenia associated with renal AA amyloidosis was reported by Dr. Metin et al. in the Journal (200; 42: 61-64). It was a surprise to me that reference #13, which belongs to some of the authors, was given as "in press", despite it being two years later.

But the main point of my letter is to point out that mutations in the gene encoding neutrophil elastase have been shown in this disorder as well as congenital neutropenia[1,2].

If everyone takes time to indicate the newest developments related to the published papers in the Journal, I believe readers would be easily exposed to these achievements in a short period of time.

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ANNOUNCEMENTS

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SAMSUN, 25 - 28 JUNE 2001

XI[th] NEONATOLOGY CONGRESS AND RECENT ADVANCES IN NEONATOLOGY COURSE

Inquiries to : Alişan Yıldıran, MD
Congress Secretariat
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PRAGUE, 4 - 7 SEPTEMBER 2001

IXXXX[th] ANNUAL SYMPOSIUM SOCIETY FOR THE STUDY OF INBORN ERRORS OF METABOLISM (SSIEM)

Inquiries to : Ms. Martina Souckova
Congress Secretariat
Opletalova 22
110 00 Praha 1
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Phone : +420-2-8400 1444
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web site : www.ssiem2001.cz

BEIJING, 9 - 14 SEPTEMBER 2001

THE XXIII[rd] INTERNATIONAL CONGRESS OF PEDIATRICS (The IPA World Congress of Pediatrics)
THE II[nd] INTERNATIONAL CONGRESS ON PEDIATRIC NURSING

Inquiries to : Secretariat
XXIII[rd] ICP
International Convention Services
Chinese medical Association
42 Dongsi Xidajie
Beijing 100710
CHINA
Phone : +86 10 6225 0394
Fax : +86 10 6512 3754
web site : http://www.chinamed.com.cn/pediatrics

ANKARA, 17 - 20 OCTOBER 2001

III[rd] NATIONAL HEMATOLOGY CONGRESS

Inquiries to : Fatma Gümrük, MD
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06100 Hacettepe, Ankara
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Fax : +90-312-324 16 81
e-mail : fgumruk@gen.hun.edu.tr
mcetin@gen.hun.edu.tr

ADANA, 26-30 OCTOBER 2001

II[nd] NATIONAL CHILDHOOD INFECTIOUS DISEASES CONGRESS

Inquiries to : Emre Alhan, MD
Secretariat
II[nd] National Childhood Infectious Diseases Congress
P.O. Box 1318
01122 Cemalpaşa, Adana
TURKEY
Phone : +90-322-338 60 60 Ext. 31 55
Fax : +90-322-338 70 82
e-mail : cocukenf@mail.cu.edu.tr
ealhan@mail.cu.edu.tr
web site : ww.cu.edu.tr/ucehk
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LJUBLJANA, 13 - 15 JUNE 2002

XIII[th] ANNUAL CONGRESS OF THE EUROPEAN SOCIETY OF PEDIATRIC AND NEONATAL INTENSIVE CARE (ESPNIC)

ESPNIC NURSING SYMPOSIUM

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

ALICANTE, 19 - 21 SEPTEMBER 2002

XVII[th] UNION OF MIDDLE EASTERN AND MEDITERRANEAN PEDIATRIC SOCIETIES (UMEMPS) CONGRESS

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

ANKARA, 26 - 30 SEPTEMBER 2002

VI[th] INTERNATIONAL CONGRESS OF TROPICAL PEDIATRICS

Inquiries to : Congress Secreteriat
International Children's Center
Bilkent, Ankara
TURKEY
Phone: +90-312-290 23 66
Fax : +90-312-266 46 78
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