THE TURKISH JOURNAL OF PEDIATRICS

A joint publication of the Turkish National Pediatric Society and the Turkish and International Children's Center

ADDRESS FOR SUBSCRIPTIONS AND CORRESPONDENCE

The Turkish Journal of Pediatrics
P.K. 66, 06240 Samanpazarı, Ankara, Turkey

Telephone : (4) 311 22 53 - 324 23 26 - 324 49 90
Telex : 42999 tcsm tr
Fax : (4) 311 22 53

This Journal is listed in *Current Contents, Index Medicus, Abstracts on Hygiene and Communicable Diseases, Tropical Diseases Bulletin,* the *CAB Abstracts* database and the *Excerpta Medica* database *EMBASE.*

VOLUME: 34 NUMBER: 2 APRIL - JUNE 1992

CONTESTS

Owner: Turkish and International Children's Center
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The Turkish Journal of Pediatrics 34: 63 - 69, 1992

EVALUATION OF GROWTH HORMONE SECRETION AND INSULIN – LIKE GROWTH FACTOR – 1 IN PREPUBERTAL CHILDREN WITH THALASSEMIA*

*Lebriz Sağlamer MD**, Lamia Ulukutlu MD***, Oya Ercan MD****
*Sezer Hatemi MD***, Hilmi Apak MD*****

Summary: Sağlamer L, Ulukutlu L, Ercan O, Hatemi S, Apak H. (Department of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey). Evaluation of growth hormone secretion and insulin-like growth factor-1 in prepubertal children with thalassemia. Turk J Pediatr 34: 63-69, 1992.

Growth retardation is a clinical feature of patients with thalassemia major, and endocrine studies have frequently revealed the presence of normal growth hormone (GH) secretion. The present study was undertaken in 14 prepubertal thalassemic children (9 males and 5 females), aged $2^{2/12}$ to $10^{3/12}$ years, with the aim of evaluating GH response to i.v. arginine, oral L-dopa stimulation and insulin-like growth factor-1 (IGF-1) levels. Eleven patients had peak serum GH levels <7 ng/ml and two patients had peak serum GH levels of 7-10 ng/ml with arginine. Similarly, 10 patients had peak levels <7 ng/ml and one patient had a peak level of 7-10 ng/ml with L-dopa. Thus, nine of the patients had GH deficiency and two had partial GH deficiency. Three patients had elevated basal GH values. The serum IGF-1 levels in the patients were not statistically different from the levels in the controls, but three patients had low IGF-1 values. These findings suggest a defect in the regulatory mechanisms of GH secretion. *Key words: thalassemia major, growth hormone, insulin-like growth factor-1.*

Growth retardation, once a constant feature of symptomatically transfused patients with thalassemia major, has been shown to be closely associated with the degree of chronic anemia[1-3]. Following hypertransfusion regimens keeping the hemoglobin (Hb) levels above 10 g/dl, near-normal growth has been reported up until the 8-10 year age range, with the pubertal growth spurt often lacking[4]. Transfusion therapy, although increasing the length and quality of life, causes lesions due to chronic iron overloading with subsequent organ damage[5]. Attention has thus been focused on endocrine abnormalities and chelation therapy[6-13].

Endocrine studies in thalassemic patients have frequently revealed the presence of normal growth hormone (GH) response to provocative stimuli[4,6-11]. Growth

* From the Department of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul.

** Assistant Professor of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine.

*** Professor of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine.

**** Pediatrician, İstanbul University Cerrahpaşa Faculty of Medicine.

***** Resident in Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine.

retardation has been attributed to a hepatic defect of insulin-like growth factor-1 (IGF-1) biosynthesis[14,15]. Series reported in the literature show differences in terms of age, transfusion and chelation characteristics of patients. The present study was undertaken to assess GH response to provocative stimuli and IGF-1 levels in our group of prepubertal thalassemic patients.

Material and Methods

Fourteen children with homozygous beta-thalassemia (9 males and 5 females), ranging in age from $2^{2/12}$ to $10^{3/12}$ years were studied. The clinical and laboratory findings of the patients are shown in Table I. All patients were given frequent transfusions beginning in the first two years of life except for patient 4 with thalassemia intermedia. As a general rule, blood was given at Hb levels of 9-10 g/dl. As a result of delays in transfusion, due to incompliance, the mean pretransfusion Hb concentration was 7.8 ± 1.0 g/dl. The total units of blood given ranged from 9 to 180 (mean 56 ± 53 units). The group was heterogenous in respect to chelation treatment with only seven having received s.c. desferrioxamine during the last 1-4 years. The mean age at the onset of chelation therapy was seven, due to problems of compliance and finance. Serum ferritin levels were high in all, the average value being 5035 ± 2465 ng/ml. Severe liver disease was excluded on the basis of normal protein levels, normal protein electrophoresis patterns, normal prothrombin activity in all and only modest elevations of the transaminases in some of the patients (mean SGOT 23 ± 13 mU/ml, mean SGPT 36 ± 28 mU/ml).

TABLE I: Clinical and Laboratory Findings of Patients

Case No.	Age (yrs)	Age at first transfusion (yrs)	Age at initiation of chelation (yrs)	Ferritin (ng/ml)	Pretransfusion Hb (g/dl)	Transfusion load (units)	SGOT (mU/ml)	SGPT (mU/ml)
1.	10 3/12	0 6/12	6	8474	7.5	180	32	64
2.	10 3/12	0 5/12	9	5314	6.0	140	12	19
3.	9 8/12	0 6/12	6	9803	7.0	142	43	68
4.	9 3/12	8 0/12	–	970	7.0	9	8	11
5.	9 0/12	0 6/12	5	4695	8.5	77	23	38
6.	7 11/12	0 7/12	–	5021	6.5	44	19	11
7.	7 10/12	0 10/12	5	5408	8.5	63	17	90
8.	6 4/12	1 10/12	–	3861	7.0	30	27	31
9.	4 8/12	0 6/12	2	5928	9.5	47	51	74
10.	3 8/12	0 5/12	2	1930	9.0	28	19	23
11.	2 6/12	0 4/12	–	3188	8.0	15	21	9
12.	2 5/12	1 4/12	–	1772	9.0	13	17	12
13	2 3/12	0 9/12	–	–	8.5	16	19	16
14	2 2/12	0 4/12	–	–	7.5	16	–	–
Mean	6 4/12	0 8/12*	7	5035*	7.8	56	23	36

* Case 4 excluded.

The patients were studied at least two weeks after the last transfusion. Height and weight were measured and bone age was determined according to the standards of Greulich and Pyle[16]. Arginine and L-dopa stimulation tests were performed on two different days to assess GH response. After collection of one baseline sample at 0 minutes in the fasting state, all patients received 0.5 g/kg arginine as a 10 % solution i.v. over a 30-minute period. Blood was collected at 30, 60, 90 and 120 minutes. Samples were drawn before and 30, 60, 90, 120, 180 and 240 minutes after oral L-dopa administration (patients over 22.5 kg, 500 mg; 11 to 22.5 kg, 250 mg; below 11 kg, 125 mg). The sera were stored at −20 °C, and plasma GH was measured by RIA (double antibody; Diagnostic Products Corporation, Los Angeles). Peak levels below 7 ng/ml on both tests were indicative of GH deficiency, while having at least one peak level of 7-10 ng/ml was accepted as partial GH deficiency. IGF-1 was measured by the immunoradiometric method with reagents from the Diagnostic Systems Laboratories. Control subjects were a group of healthy, prepubertal children (4 males and 5 females) aged 3 to $10^{7/12}$.

Results

The physical characteristics of the patients are shown in Table II. The bone age was significantly lower than the chronological age ($p < 0.01$) and the height age was also significantly lower than the chronological age ($p < 0.01$). Two of the

TABLE II: Physical Characteristics of Patients

Case No.	Sex	Age (yrs)	Bone Age (yrs)	Height age (yrs)	Height SDS
1.	M	10 3/12	8 0/12	9 9/12	−0.3
2.	M	10 3/12	7 0/12	9 9/12	−0.5
3.	M	9 8/12	7 0/12	8 3/12	−1.3
4.	M	9 3/12	6 0/12	6 6/12	−2.6
5.	F	9 0/12	9 0/12	8 6/12	−0.7
6.	F	7 11/12	6 10/12	7 2/12	−0.7
7.	M	7 10/12	7 0/12	6 9/12	−1.2
8.	M	6 4/12	5 0/12	6 6/12	+0.2
9.	M	4 8/12	4 0/12	5 0/12	+0.6
10.	F	3 8/12	3 0/12	3 6/12	+0.1
11.	F	2 6/12	–	1 6/12	−2.7
12.	M	2 5/12	1 9/12	1 9/12	−2.2
13.	F	2 3/12	2 4/12	2 3/12	−0.2
14.	F	2 2/12	2 0/12	1 9/12	−1.4
Mean		6 4/12	5 2/12	5 8/12	−0.92
± SD		3 3/12	2 5/12	3 0/12	1.03

patients were below the third percentile for height. The mean standard deviation score (SDS) for height was 0.92 ± 1.03.

The basal growth hormone level was above 7 ng/ml on three occasions. Peak levels were attained at 30 to 90 minutes with arginine and at 60 to 120 minutes with L-dopa. Although the mean peak levels were below 7 ng/ml, there was a statistically significant difference between the basal and peak values in both tests ($p < 0.05$). The basal and peak serum GH values with arginine and L-dopa stimulation and the IGF-I levels are shown in Table III. Arginine induced a normal GH response in one (> 10 ng/ml), subnormal responses in two, and responses characteristic of GH deficiency (< 7 ng/ml) in 11 subjects. L-dopa induced normal GH responses in three, a subnormal response in one and responses characteristic of GH deficiency in 10 subjects. When the results of the two provocative tests were evaluated together, nine patients had GH deficiency and two had partial GH deficiency.

TABLE III: Serum GH Response (ng/ml) to Arginine and L-dopa and IGF-I Levels (mU/ml) in Thalassemic Children

Case No.	Arginine		L-dopa		
	Basal	Peak	Basal	Peak	IGF-I
1.	0.28	8.80	0.21	13.50	1680
2.	1.10	4.80	1.40	2.20	348
3.	0.80	4.00	0.68	4.20	3000
4.	0.05	0.14	0.13	0.09	1800
5.	0.68	1.40	1.60	1.70	4320
6.	8.00	5.80	3.60	11.50	1680
7.	0.01	6.40	0.27	5.20	1440
8.	0.30	2.30	0.35	1.50	1440
9.	7.20	4.60	2.90	3.40	2280
10.	3.80	5.80	15.00	6.80	3000
11.	4.20	11.00	8.00	11.00	–
12.	2.10	0.18	0.01	1.30	360
13.	2.30	6.00	0.25	9.80	3000
14.	0.35	10.00	1.70	5.80	348
Mean	2.23	5.09	2.58	5.57	
± SD	2.64	3.37	4.16	4.40	

The mean serum IGF-1 level in nine boys was 2185.3 ± 1558.0 mU/ml and in four girls 2007.0 ± 1269 mU/ml. There was no statistically significant difference between the mean IGF-I levels of the patients and the controls ($p > 0.90$ and

$p > 0.30$ for boys and girls, respectively), but the IGF-1 levels of three patients were clearly low.

Discussion

Although the patients studied were given transfusions regularly, the pretransfusion Hb levels were suboptimal and some were receiving no or only short-term iron chelation therapy. In spite of this, the present data on growth agree with earlier observations, in that growth retardation in beta-thalassemia patients given frequent transfusions is not severe until the age of ten[4,6]. Impaired growth has been attributed to chronic anemia and iron overload. The height SDS values of our patients showed no statistically significant correlation with age, mean pretransfusion Hb, duration of chelation therapy, transfusion load or serum ferritin levels. A tendency to follow the height of parents, though slight, could be observed.

Chronic anemia, tissue damage by hypoxia and severe iron overloading could cause endocrine abnormalities in thalassemia. In previous studies, mostly normal GH responses to provocative stimuli have been reported[6-9,11]. Stimulation by insulin-induced hypoglycemia has usually been the method used and the lower normal limit has been variable, down to 5 ng/ml. In a few patients, subnormal GH responses to different stimuli have also been reported[3,10]. By contrast, growth hormone release after provocative stimuli was found to be deficient in most of our patients, which was similar to the findings of Pintor et al[17] who also showed a reduction of the pituitary response to growth hormone releasing hormone (GH-RH)[17]. No correlation was found between peak GH levels and bone or chronological age, serum ferritin levels or total blood received. A normal GH response to GH-RH has also been reported[18]. Endocrine abnormalities have usually been attributed to iron toxicity. On the other hand, growth retardation with defective GH responses to stimulation have been reported in thalassemic patients receiving little or no blood support therapy[19].

As the results of provocative tests have usually suggested normal pituitary secretion of GH, attention has been focused on IGF-I as the cause of growth retardation. IGF-1 levels have invariably been reported to be low in thalassemic patients[14,15,17]. We observed a decrease of IGF-1 in only three of our 14 patients, which can partly be explained by the young age of our group, since IGF-1 levels have been found to be low in only a small percentage of thalassemic children below 10 years of age[14]. In older children, the IGF-1 levels considered low for age could be normal for the pubertal status, since puberty is frequently delayed. Studies on the mechanism for deficiency of IGF-1 have implicated a specific defect at the hepatic GH receptor or post-receptor levels[20]. It has been considered unlikely that generalized hepatic damage is a major cause of this condition since no major hepatic disease was present and hepatic protein

synthesis was normal in these patients[14,20]. Serum IGF-1 values have been found to correlate with the degree of iron overload[14].

Contrary to a previous belief, that endocrine function in terms of GH secretion is normal in thalassemic patients, recent observations reveal various defects, especially in spontaneous GH secretion[21]. The discordance between low GH responses to provocative stimuli and normal IGF-1 values in our patients might be explained by the positive correlation shown between IGF-I levels and mean 24-h GH concentrations[22]. High basal GH levels in some of our patients also merit attention. Patients with elevated basal GH levels, indicating decreased somatostatin secretion, have been reported[22]. Alterations in the regulatory mechanisms of GH secretion are suggested, but further investigations such as endogenous GH secretion are necessary.

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The Turkish Journal of Pediatrics 34: 71 - 78, 1992

THE ROLE OF VIRUSES AND MYCOPLASMA PNEUMONIAE IN LOWER RESPIRATORY TRACT INFECTIONS IN CHILDHOOD*

*Ayhan Göçmen MD***, Feyzullah Çetinkaya MD****
*Şemsettin Ustaçelebi MD*****, Dürdal Us MD******

SUMMARY: Göçmen A, Çetinkaya F, Ustaçelebi Ş, Us D. (Departments of Pediatrics and Microbiology, Hacettepe University Faculty of Medicine, Ankara, Turkey). The role of viruses and mycoplasma pneumoniae in lower respiratory tract infections in childhood. Turk J Pediatr 34: 71-78, 1992.

Acute lower respiratory tract infections are one of the major causes of childhood morbidity and mortality in developing countries. This study was undertaken at Hacettepe University Children's Hospital to determine the role of viruses and *Mycoplasma pneumoniae* in respiratory tract infections in children. Eighty-three patients with lower respiratory tract infections were selected at random from among the children admitted to the hospital for evaluation of respiratory symptoms. Acute and convalescent serum samples were collected from all patients for the complement-fixation test and the following antigens were used: respiratory syncytial virus (RSV), adenovirus, parainfluenza virus Type 1, influenza viruses A and B, and *Mycoplasma pneumoniae*. The test was positive in 39 of 83 patients (47 %), and RSV was the most frequent agent detected serologically (15.7 %). *Key words: lower respiratory tract infection, respiratory syncytial virus, viral respiratory infection, Mycoplasma pneumoniae.*

Acute lower respiratory tract infections are an important cause of pediatric morbidity and mortality throughout the developing world[1]. There are about 4 million children in the world under the age of five who die each year from acute respiratory infections. This figure constitutes 27 percent of all childhood deaths in this age group[1].

Although bacteria can cause significant diseases of the respiratory tract, it has been possible to establish them as causative agents in only a small proportion of such illnesses. Respiratory syncytial virus (RSV), adenovirus, parainfluenza virus, influenza viruses and *Mycoplasma pneumoniae* are the most important pathogens causing lower respiratory tract infections in childhood[2].

The present study was undertaken at Hacettepe University Children's Hospital to determine the role of viruses and *Mycoplasma pneumoniae* in lower respiratory tract infections in children in Turkey.

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* From the Departments of Pediatrics and Microbiology, Hacettepe University Faculty of Medicine, Ankara.

** Professor of Pediatrics, Hacettepe University Faculty of Medicine.

*** Pediatrician, Adana.

**** Professor of Microbiology, Hacettepe University Faculty of Medicine.

***** Specialist in Microbiology, Hacettepe University Faculty of Medicine.

Material and Methods

The study population consisted of 77 out-patients and six hospitalized patients with lower respiratory tract infections who had been selected at random between November 1988 and January 1989 for inclusion in this study.

The illnesses were considered acute if the symptoms of the respiratory disease had been present for less than one month. Only patients with acute illnesses were included in the study.

Lower respiratory tract diseases were defined as infections of the epiglottis, larynx, trachea, and the lungs[2]. These diseases were characterized as croup, tracheobronchitis, bronchiolitis, or pneumonia. Croup was diagnosed by a hoarse barky cough and inspiratory stridor with or without drooling; tracheobronchitis by a cough and rhonchi; bronchiolitis by inspiratory and/or expiratory wheezing without radiographic evidence of consolidation, and pneumonia by rales and radiologic evidence of consolidation or patchy infiltrates[2].

At the first presentation, a history of symptoms related to lower respiratory tract disease, such as cough, fever, tachypnea, wheezing, dyspnea, and cyanosis, was noted. All patients were systematically examined by the same doctor.

The following tests were performed on each patient enrolled in the study: complete blood count, throat culture, chest X-ray and complement-fixation test of sera for serological detection of the agents involved.

Acute and convalescent sera samples were obtained from all patients and were used for complement-fixation tests performed at intervals of 12 and 21 days[3].

Paired sera from 83 patients were studied by means of a microtechnique in which two units of antigens and complement were used simultaneously. RSV, adenovirus, parainfluenza virus Type 1, influenza viruses A and B, and *Mycoplasma pneumoniae* antigens were used. The test was considered positive if there was at least a four-fold rise in antibody titers between the first and second serum specimens.

Results

There were 25 females and 58 males. The mean age was 33 months. Thirty percent of the patients were under one year and 68.5 percent under three years of age. There was a 2.7: 1 male predominance in all groups (Fig. 1).

Cough was the most frequent symptom while cyanosis was the least encountered symptom. Table I lists the physical findings of all patients.

The age-specific frequencies of the clinical pictures are shown in Table II. The positive results of the complement-fixation tests according to age group are

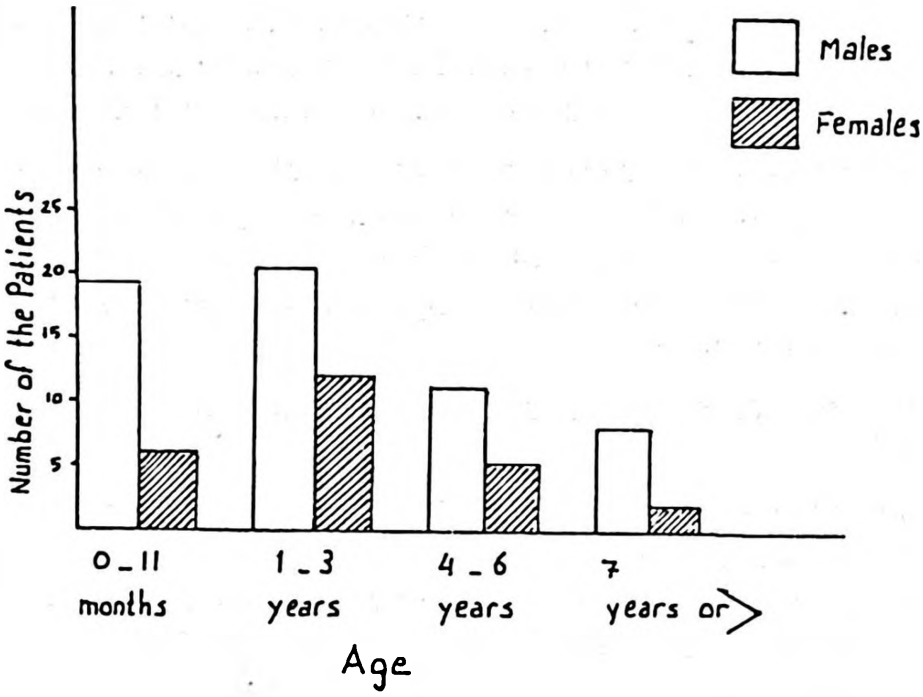

Fig. 1: Age and sex distribution of patients.

TABLE I: Clinical Findings of Patients

Clinical Findings	No. of Patients
Rales	73 (87 %)
Fever	59 (71 %)
Tachycardia	30 (36 %)
Rhonchi	29 (34 %)
Wheezing	20 (24 %)
Tachypnea	16 (19 %)
Cyanosis	4 (4.8 %)

TABLE II: Clinical Picture Distribution According to Age Group

Clinical Diagnosis	0-11 Mos	1-3 Years	4-6 Years	≥ 7 Years	Total
Pneumonia	7	19	12	9	47 (56.6 %)
Bronchiolitis	15	11	2	1	29 (35 %)
Croup	3	1			4 (4.8 %)
Tracheobronchitis		1	2		3 (3.6 %)
Total	25	32	16	10	83 (100 %)

shown in Table III. Thirty-nine of the 83 paired sera were positive and two had serological evidence of infection with more than one agent.

Seriologically, RSV was found to be the agent most frequently associated with lower respiratory tract infections followed by adenovirus, parainfluenza virus Type 1, *Mycoplasma pneumoniae,* and influenza viruses A and B (Table III).

The results in relation to the clinical presentation of the diseases are shown in Table IV. The complement-fixation test results were positive in 42 percent of pneumoniae patients, 58 percent of bronchiolitis patients and 66 percent of tracheobronchitis patients. No etiological agent was detected serologically in the sera of the patients with croup.

Group A *beta-hemolytic streptococcus* was isolated from the throat cultures of seven patients.

A white blood cell count of more than 10,000/mm^3 was noted in 18 of the 39 patients with positive complement-fixation tests. The differential count noted lymphocyte predominance in 20 of these 39 patients, however, polymorphonuclear leukocytes were predominant in all four of the patients infected with *Mycoplasma pneumoniae.*

TABLE III: Agents Serologically Detected in Relation to Age

Agent	0-11 Mos	1-3 Years	4-6 Years	≥ 7 Years	Total (%)
RSV	6	3	3	1	13-15.6
Adenovirus	1	5	1	2	9-10.8
Parainfluenza 1	3	3		2	8-9.6
M.pneumoniae		2	2		4-4.8
Influenza A		1		1	2-2.4
Influenza B	1				1-1.2
Influenza A plus		1			1-1.2
Parainfluenza 1 Influenza A plus				1	1-1.2
RSV					
Total	11	15	6	7	39-47

Most of the symptoms and signs of the patients resolved in a short period of time. A patient with an adenovirus infection was later diagnosed as having asthma. Another patient with influenza A and parainfluenza Type 1 virus infections concomitantly showed no improvement and died in three months.

TABLE IV: Agents in Relation to Clinical Condition

Diagnosis	No. of Patients tested	Number of Patients with Serological Evidence of Infection: RSV	Para 1	Adeno V.	MP	Inf A	Inf B	Inf A + Para 1	Inf A + RSV	Total
Pneumonia	47	6	4	4	3	1	–	1	1	20
Bronchiolitis	29	7	4	4	–	1	1	–	–	17
Croup	4	–	–	–	–	–	–	–	–	–
Tracheobronchitis	3	–	–	1	1	–	–	–	–	2
Total	83	13	8	9	4	2	1	1	1	39

RSV : Respiratory syncytial virus.
Para 1 : Parainfluenza type 1 virus.
Adenov: Adenoviruses.
MP : Mycoplasma pneumoniae.
Inf A : Influenza A virus.
Inf B : Influenza B virus.

Discussion

Mortality from acute lower respiratory disease is a serious problem in children under five years of age. Most children with respiratory tract illnesses are frequently seen in primary care clinics. Although minor upper respiratory tract infections are very common in childhood, they are not life-threatening for well-nourished children since death rates resulting from respiratory illnesses are closely linked to malnutrition. A study in Costa Rica showed that deaths resulting from bronchopneumonia in malnourished children are 12 times greater than in nourished children[1].

According to a study carried out in 1979 in Turkey, pneumonia was found to be the major cause of death in children in the 1-4 year age group[4]. In this age group, 31% of all deaths resulted from pneumonia[5]. The results of serologic studies in our study are consistent with the results of many other studies. In a WHO[6] study on respiratory ailments in children, 41 percent of patients developed a serological response to one or more of the agents we had investigated. Although this study was conducted in various countries, the results are similar to ours. Ong et al[7] reported that 32.6 percent of Malaysian children with respiratory infections exhibited serological evidence of infection with one or more of the same agents.

RSV is known as a major viral pathogen of respiratory tract diseases in children, especially in infections such as pneumonia and bronchiolitis in young infants. The disease may follow a fatal course in high risk infants such as those with congenital heart disease and bronchopulmonary dysplasia[8].

In our study serological evidence of RSV infection was found in 15.6 percent of patients. This agent was associated with 12.7 percent of pneumonia patients and 24 percent of bronchiolitis patients.

A study involving 662 children under three years of age hospitalized in Uganda showed that RSV was the pathogen which caused the lower respiratory tract infections in 17 percent of patients[9]. RSV infections were found to be present in 11 percent of 53 patients under two years of age, who had been hospitalized for bronchiolitis in Lagos, Nigeria[10].

RSV infections were associated with lower respiratory tract illnesses in about half of the patients we studied who were under one year of age.

The failure to detect an antibody rise to RSV in young infants using the complement-fixation technique has been previously reported[11], and, therefore, the exact value may actually be higher than what we found. Adenovirus infection was detected in 10.8 percent of the study group; five of these patients were in the 1-3 year age-group. Adenovirus infections account for 2-5 percent of all respiratory illnesses in children[12].

Parainfluenza virus Type 1 was detected in 9.6 percent of patients, and half of them were clinically diagnosed as having pneumonia and the rest as having bronchiolitis. Although parainfluenza virus Type 1 is the predominant agent associated with croup[13], it was not detected in our patients, probably because of the minimal number of patients with croup.

Influenza viruses were detected in five of our patients, two of whom also had other viruses. A patient infected with influenza A virus and parainfluenza virus Type 1 was clinically diagnosed as having pneumonia. This patient showed no improvement and died in three months.

Several aspects of respiratory disease due to *Mycoplasma pneumoniae* are different from those caused by viral pathogens[2]. *Mycoplasma pneumoniae* generally causes respiratory infections in children between the ages of 5-15[14]. Nagayama et al[15], demonstrated by using serological and isolation procedures that *Mycoplasma pneumoniae* infections occur frequently in young children. In this study, *Mycoplasma pneumoniae* was found to cause lower respiratory infections in 6.6 percent of children in the 0-2 year age-group and in 23.3 percent of children in the 3-6 year age-group.

We detected *Mycoplasma pneumoniae* infections in four patients, three of whom were diagnosed as having pneumonia and one as having tracheobronchitis. A WHO[7] study showed that 5 percent of pneumonias were caused by *Mycoplasma pneumoniae.*

Lower respiratory tract infections are common in young children, and decrease with age. Sixty-eight and a half percent of our patients were under three years of age and approximately 90 percent of these children who had bronchiolitis were under three years of age.

Pneumonia was seen in all age groups. Denny and Clyde[16] reported that the age - specific attack rate peak for pneumonia was reached between 3 - 5 years of age; tracheobronchitis occurred more frequently in the first two years of life, and the highest age - specific attack rates for bronchiolitis were seen in the second six months of life and for croup in the second year of life.

Wheezing, a common manifestation of acute respiratory illness in children was found in 24 percent of all patients and in 69 percent of patients with bronchiolitis. RSV has been reported to be the most common cause of wheezing associated with respiratory infections[17]. Wheezing was found in all seven RSV related-patients with bronchiolitis we studied.

Two agents were detected in the sera of two of our patients concomitantly. In respiratory diseases associated with significant rises in antibody titers to multiple agents, the increases may be due to simultaneous or sequential infections with two or more viruses occurring immediately before or during the period of time encompassed by the paired sera samples[18]. Simultaneous increases in titer may also be due to antigenic cross reactions among related agents, e.g., parainfluenza virus and mumps virus. In a study. Hilleman et al[19] showed 5 percent serologic responses to two agents.

Group A beta - hemolytic streptococci was isolated in the throat cultures of seven patients. In five of these patients, viruses were also detected. The association of viruses with group A streptococci has been reported in other studies[2].

An etiological agent could not be detected in 53 percent of our patients We believe that these patients were infected by other viruses or bacteria and that the agents under study in this paper could not be isolated because of the method used. Göçmen and Baki[20] have shown that some bacterial agents were the cause of pulmonary infections in 54.8 percent of 31 patients studied at Hacettepe University Children's Hospital.

Our patients will be followed for long - term complications of childhood lower respiratory tract infections because these infections, especially bronchiolitis, may lead to recurrent respiratory problems in subsequent years[21].

The results of this study suggest that various viruses are the major causes of lower respiratory tract infections in children in our region.

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The Turkish Journal of Pediatrics 34: 79 - 92, 1992

CARRIER DETECTION BY DNA ANALYSIS IN DUCHENNE MUSCULAR DYSTROPHY FAMILIES*

*Esra Battaloğlu MS**, Milhan Telatar BS**, Feza Deymeer MD***
*Piraye Serdaroğlu MD****, Coşkun Özdemir MD*****, Faik Kuseyri MD******
*Memnune Yüksel Apak MD*******, Aslıhan Tolun PhD********

SUMMARY: Battaloğlu E, Telatar M, Deymeer F, Serdaroğlu P, Özdemir C, Kuseyri F, Apak MY, Tolun A. (Department of Biology, Boğaziçi University, İstanbul, and the Department of Neurology, İstanbul University Faculty of Medicine, İstanbul, Turkey). Carrier detection by DNA analysis in Duchenne muscular dystrophy families. Turk J Pediatr 34: 79-92, 1992.

We applied DNA analysis techniques to Turkish families whose members were afflicted with Duchenne / Becker muscular dystrophy. The aim of this study was to establish a prenatal diagnosis of this anomaly and to determine the carrier state.

All of the techniques used in established diagnosis centers are now applied routinely in our laboratory. Both Southern analysis and polymerase chain reaction (PCR) methods were used for deletion detection in patients and restriction enzyme fragment length polymorphism (RFLP) determination for linkage analysis in women at risk. CA repeated sequence length polymorphism, the most recent technique for linkage analysis, was also applied.

About 250 individuals from seventy - nine families were investigated and thirty - six entire families were screened. Twenty - five women were found to be carriers while thirty seven were non - carriers. The carrier state could not be determined in three women. *Key words: Duchenne muscular dystrophy, polymerase chain reaction, restriction enzyme fragment length polymorphism, deletion, Southern analysis.* ·

Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disease, which affects about one in 3,400 males. Boys afflicted with this X - linked recessive disorder become nonambulatory at around age ten and die at about age twenty. Becker muscular dystrophy (BMD) is the milder and rarer form of the disorder in which nonambulation occurs much later in life. Both disorders are caused by mutations in the dystrophin gene[1,2].

In families with DMD, high levels of lactic dehydrogenase (LDH) and creatine phosphokinase (CPK) enzymes indicate the carrier state in women. However,

* From the Department of Biology, Boğaziçi University, İstanbul, and the Department of Neurology, İstanbul University Faculty of Medicine, İstanbul.

** PhD candidate in Biology, Boğaziçi University.

*** Associate Professor of Neurology, İstanbul University Faculty of Medicine, İstanbul.

**** Assistant Professor of Neurology, İstanbul University Faculty of Medicine.

***** Professor of Neurology, İstanbul University Faculty of Medicine.

****** Resident in Medical Genetics, İstanbul University Institute of Child Health, İstanbul.

******* Professor of Medical Genetics, İstanbul University Institute of Child Health.

******** Professor of Biology, Boğaziçi University.

This work was supported by the Boğaziçi University Research Fund and the French Myopathy Association (FMA).

about 30 percent of all carriers have normal levels of these enzymes. Therefore, DNA analysis should be carried out to determine the carrier state in all women at risk. It is then possible to perform prenatal diagnosis using the same techniques. In addition, the use of DNA analysis techniques allows for early diagnosis in about 60 percent of patients.

DNA analysis techniques that have been developed in the last five years are routine in our laboratory and are applied to DMD families. A variety of methods are employed in order to identify carriers related to both familial and sporadic cases. We are currently examining seventy - nine affected families. In this study, the strategies that have been developed for carrier detection and prenatal diagnosis are discussed and examples of interesting cases are presented.

A commonly accepted strategy for DNA analysis of this disorder circumvents problems arising from the unique features of the gene, such as its very large size and great variety of mutations. Dystrophin gene mutations are different in each DMD family and can be observed as large mutations (mostly deletions and some duplications) in 60 - 70 percent of patients via the commonly used DNA analysis techniques. Identification of the deletion reveals the site of the mutation, enabling linkage analysis to be performed more precisely. This is because in such a large gene, the probability of crossing - over in each meiosis is about 5 percent. Moreover, the DNA of a female carrier may be assayed for the presence of a particular deletion in one of her X - chromosomes. For these reasons, deletion detection simplifies carrier determination to some degree. This direct method assures a fast, simple prenatal diagnosis. However, in 30 - 40 percent of DMD families, the mutation is not detectable. Thus for carrier testing and prenatal diagnosis in such families, linkage analysis determines whether or not the DMD allele has been inherited.

The strategy for DNA analysis in DMD families may be summarized as follows:

A) Deletion Detection in Patients:

Either the Southern analysis or the polymerase chain reaction may be used. The first method detects deletions in about 60 - 70 percent of patients[3]. Ninety - eight percent of these deletions are detectable by the latter method which is fast and lower in cost[4,5].

1. Southern Analysis:

This technique detects specific DNA fragments (generated by restriction enzymes) using of complementary DNA (cDNA) probes. The probes themselves are part of the fragment to be detected. The method may be summarized as follows: a patient's DNA sample is cleaved by a restriction enzyme and subjected

to agarose gel electrophoresis to separate the fragments according to size. The fragments are then denatured, transferred onto a membrane and fixed. A radioactively labeled probe specific for the fragment is hybridized to the DNA samples on the membrane and the target fragment (s) is identified after autoradiography on X - ray films. A total of eight cDNA probes (specific to exons of the dystrophin gene) are used to screen the entire gene. Detection of the absence of a fragment in a patient is interpreted as a deletion. The first probe using Southern analysis takes at least eight days and four days are required for each additional probe.

2. Amplification by polymerase chain reaction (PCR):

The multiplex PCR technique was first applied in DMD in 1988 and was shown to detect about 50 percent of the deletions detectable by Southern analysis[4]. Recently, a second assay (multiplex II) has been developed[5]. Both assays together detect about 98 percent of all deletions and require only two days for results. PCR assays can be used in deletion detection in patients and in a fetus at risk because they are simple, fast, do not require radioisotopes, and need only 5 percent as much DNA as the Southern assay. However, all deletions detected by PCR should be confirmed by Southern analysis, which is completely reliable.

In each multiplex assay, nine pairs of primers, members of each pair being complementary to the opposite DNA strands, are used to amplify nine regions of differing lengths, each in a different exon commonly deleted in DMD patients. The fragments are amplified by 10^5 - 10^6 - fold in a single reaction and visualized after gel electrophoresis. Failure of amplification of one or more exons indicates a deletion.

B) Deletion Detection in Carriers:

Whenever a deletion is identified in a patient, DNA samples of women at risk in the family may be analyzed for the presence of a particular deletion by dosage analysis, even though the assay is complicated by the existence of two X - chromosomes. Alternatively, the mutation can be directly identified if a junction fragment is observable.

1. Dosage Analysis:

A female's DNA sample is subjected to Southern analysis using the same probe that detects the deletion in the related patient. A deletion in one of her X - chromosomes results in autoradiographic bands with half the intensity.

2. Junction Fragment Detection:

A junction fragment arises as a result of fusion of the two DNA fragments flanking the deletion. Such a fragment acquires an abnormal size as compared to a standard. It is this feature that renders analysis very simple: the mutation can be

identified by a single analysis in all females at risk in the family. Unfortunately, junction fragments are rarely detectable by cDNA probes.

C) Linkage Studies to Determine Inheritance of DMD Allele:

The most commonly applied technique comprises the use of restriction enzyme length polymorphisms (RFLP) which arise as a result of differences in DNA sequence in the noncoding regions of the gene. An RFLP does not change the sequence of the gene product (protein), but may be used to distinguish between the two alleles of the gene in the mother. Analysis in affected and unaffected boys in a family will reveal which allele is linked to DMD in that particular family. Intergenic RFLPs are detectable by some probes specific to introns and by some of the cDNA probes. In order to minimize the uncertainty in diagnosis due to the probability of crossing - over, the large size of the gene necessitates that at least three RFLPs be used: one intergenic and two which flank the gene. In cases where the site of deletion is known, linkage analysis using only two probes that flank the deletion is sufficient.

For prompt detection, the PCR technique can be used for three RFLP analysis: pERT87 - 8 (TaqI), pERT87 - 15 (BamHI) and pERT87 - 15 (XmnI)[6]. A region containing the restriction enzyme site is amplified, digested by the restriction enzyme and assayed via gel electrophoresis.

Recently the PCR technique has also been applied to another type of polymorphism: CA repeated - sequence length polymorphisms. Two such sequences are defined for the dystrophin gene: one is at the 3' end while the other is at the 5' end[7,8]. Because the lengths of the CA repeated sequences vary in different X - chromosomes, the alleles may be directly analyzed via gel electrophoresis after amplification. These polymorphisms are very useful since few RFLP assays exist in these regions.

Material and Methods

Seventy - nine unrelated patients diagnosed as having DMD or BMD in the Neurology Department of İstanbul University Faculty of Medicine, and 175 of their family members were enrolled in this study. Diagnosis was based on pedigree data, clinical examination, elevated serum creatine kinase values and electromyograms. In some patients, the muscle biopsy histopathology was also determined.

Plasmids containing the probe insert were grown in *E.coli* HB101 strain and isolated by the alkaline lysis method. The insert was cleaved out using the appropriate restriction enzyme and separated from the plasmid vector by agarose gel electrophoresis. The gel piece containing the probe fragment was then subjected to electrophoresis in closed dialysis tubing and the probe was recovered.

DNA was isolated from 10 ml peripheral blood samples. Cleavage of DNA by restriction enzymes and the Southern transfer were carried out essentially as previously described[9]. Radiolabeling was performed by the random - priming technique. 50 ng of probe DNA was labeled using 40 μCi ^{32}P - dCTP in 30 μl of reaction volume. The hexanucleotide primers were supplied by Dr. Linné; ^{32}P - dCTP was supplied by Amersham at a specific activity of 3.000 Ci / μg. The percentage of incorporation was determined after TCA precipitation. The whole reaction mix was used for hybridization without prior separation of unincorporated ^{32}P - dCTP typically in a volume of 20 - 40 ml. Autoradiography took 1 - 4 days at −70 °C with Kodak Xomat XAR film and Dupont lighting plus or Quanta III screens.

When the DNA samples on the membrane were to be reprobed, the previously hybridized probe was removed in 0.4 N NaOH and the membrane was neutralized in 0.2 x SSC and 0.1 % SDS. Labeled probes in hybridization buffer were occasionally reused after denaturation (100 °C for 10 minutes).

Multiplex assays were performed as described by Chamberlain et al[4], except that only two temperatures were employed in the amplification cycles: four minutes at 60 °C for annealing and elongation and one minute at 92 °C for denaturation. After 30 cycles, fragments were separated on a 2 % NuSieve, 1 % agarose gel. Primers were supplied by Dr. P. Ray and Dr. T. Friedman and Taq polymerase by Cetus and Boehringer.

PCR for RFLP analyses, CA repeat polymorphisms and the Y - chromosome sequence were performed in three steps: one minute at 52 °C for annealing, two minutes at 72 °C for elongation and one minute at 92 °C for denaturation. For RFLP analysis, 15 μl of the reaction mix was digested with the appropriate restriction enzyme in 20 μl of digestion buffer containing 10 units of the enzyme. pERT87 - 15 (XmnI) digestion products were assayed on 1 % agarose gel and the 3' CA repeat polymorphisms on 15 % acrylamide gels. All other assays were on 3 % NuSieve, 1 % agarose gel.

For isolation of DNA from the chorionic villi, the sample was first washed in TE and resuspended in 0.45 ml SE. SDS was added to 1 %, proteinase K to 1 mg/ml and the sample was incubated at 56 °C for two hours. DNA was precipitated by the addition of two volumes of ethanol after phenol - chloroform (1:1) extraction (twice) and chloroform extraction (twice). DNA was dissolved in 0.2 ml of TE. 20 - 30 mg villi yielded about 100 μg of DNA.

Results

In our studies with DMD families in Turkey, both deletion detection and RFLP analyses were carried out simultaneously. In cases where a deletion was detected, DNA samples of women at risk were analyzed for linkage in regions

flanking the deletion; some were also analysed for dosage. In cases where no deletion was detectable, RFLP linkage analysis was carried out: three polymorphisms (one intergenic, two flanking) were used for sporadic cases whereas two intergenic polymorphisms were sufficient for familial ones to establish an accurate diagnosis with all certainty. All of the above - mentioned DNA analysis techniques can now be routinely applied in our laboratory, where all the results described in this work were obtained.

A) Mutation Screening:

1. Southern Analysis:

Eight different cDNA probes were employed spanning only the first 9 kilobase pairs of the protein coding region of the gene, since no deletion had been reported at the outset in the remaining 5 kilobase pairs. A DNA sample of each patient was cleaved separately with two different restriction enzymes to obtain accurate results (Fig. 1). Of seventy - nine unrelated patients, forty - seven (59.5 %) were observed to have deletions.

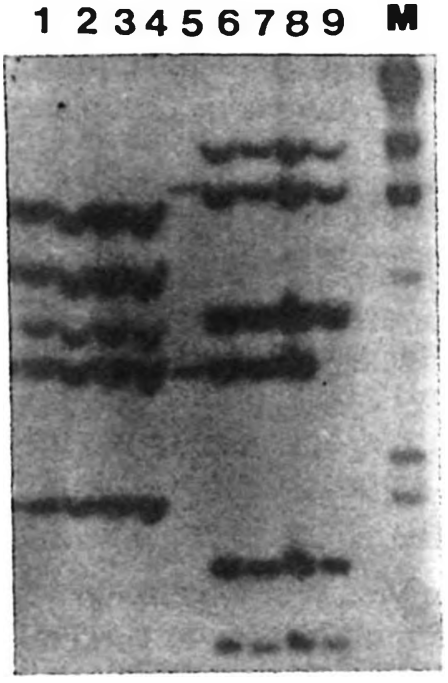

Fig. 1: Southern analysis of different patient DNA samples using the probe cDNA8. Samples 1-5 were digested with the restriction enzyme Taq^{I}, 6-9 with Hind III. There is a large deletion in sample 5, spanning fragment 1, 3, 5 and 6. The last lane is the lambda DNA/Hind III size marker.

Women at risk were screened for the deletion carried by the patient in the family. A decrease in dose in autoradiographic bands as a result of a deletion in one of the X - chromosomes could be detected upon careful observation (Fig. 2). Of the eighteen women included in dosage analysis, ten had a pattern similar to the standard; the others were carriers. In a few samples, band intensities were also determined by a density scanner.

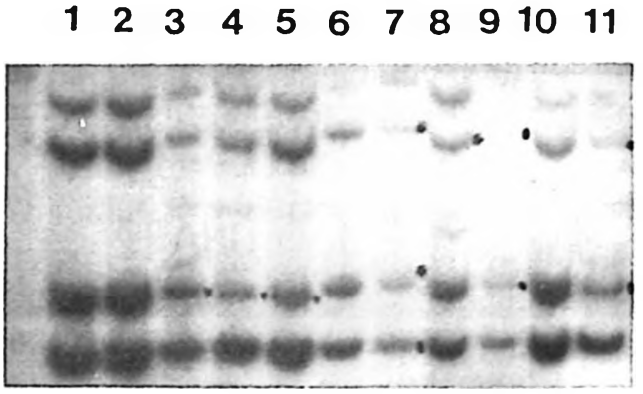

Fig. 2: Dosage analysis for DNA samples of women at risk, using the probe cDNA8 and Hind III. Samples 1 and 5 are standards. A decrease in band intensity relative to the control pattern was observed insamples 3, 4, 5, 7, 8, 9 and 11 as marked to the right of the bands.

2. Polymerase Chain Reaction Analysis:

DNA samples from seventy - nine patients were amplified using multiplex I and multiplex II primers. An example is shown in Fig. 3. Deletions were detected in forty-five patients (57 %) after agarose gel electrophoresis was performed.

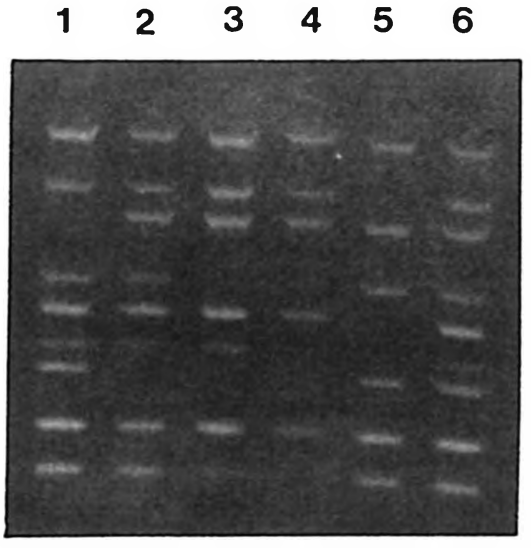

Fig. 3: Multiplex II amplification products of different samples. The last sample is an unaffected male, all others exhibit deletions corresponding to one or more regions in the gene.

B) Junction Fragment Detection:

In deletion screening of patient DNA samples with cDNA probes, some fragments, not of the expected size, were observed. These junction fragments were detected in six patients and were used to perform carrier analysis in women at risk. Out of nineteen women assayed, the junction fragments were present in six, indicating the carrier state; thirteen were non - carriers.

C) Linkage Analysis:

The mothers of DMD boys were screened for RFLP using five intergenic probes

(pERT87 - 1, pERT87 - 8, pERT87 - 15, XJ1.1 and XJ2.3), two flanking (754 and 99.6) and one cDNA probe (cDNA8). Probes pERT87 - 15 and cDNA8 were used to assay two RFLPs each. RFLP sites are shown in Fig. 4 and an example of RFLP detection in Fig. 5.

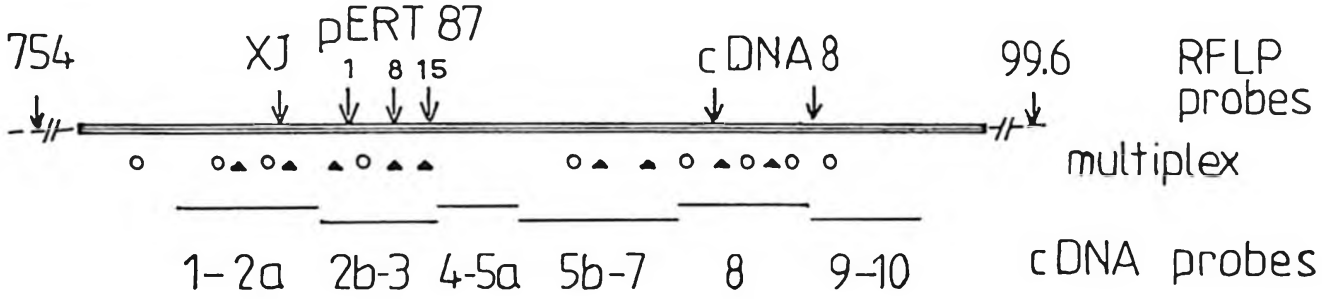

Fig. 4: Map of the dystrophin gene showing the locations of the probes detecting RFLP ( ), the regions spanned by the cDNA probes (—) and regions amplified by multiplex (▲) and multiplex II (o).

Fig. 5: An example of RFLP detection in 18 unrelated females using the probe 754 and the restriction enzyme PstI. Females exhibiting both bands are heterozygous for the RFLP.

Not all the mothers were assayed for all of the ten RFLPs, but the assay was terminated whenever:

1. Three RFLPs (one intergenic, two flanking the gene) were detected.
2. Two RFLPs flanking the mutation were detected.
3. One RFLP close to the mutation was detected in the familial cases.
4. A junction fragment was detected.

Among the RFLPs for which the mother was found to be heterozygous, the most suitable ones were chosen to be used in the analysis of all family members. The allele associated with DMD was determined and the carriers were identified. Interesting families are described individually to demonstrate the analyses.

Family I : The patient was reported to be a sporadic case, and no deletion could be detected either via multiplex PCR assays or by hybridization to cDNA probes. The mother was found to be heterozygous, having one intergenic and two flanking probes. Haplotypes were determined in five members of the family. The maternal grandfather's haplotype had to be deduced since his blood sample was not available (Fig. 6 a). The patient was shown to have inherited one of the grandmother's alleles, while the sister had inherited the grandfather's. Therefore, the sister was not a carrier. The data did not rule out inheritance of DMD from the grandmother.

Family II : The patient was reported to be a sporadic case, and no deletion was detected either via multiplex PCR assays or by hybridization to cDNA probes. The

father's blood sample was not available, thus the two sisters' common allele was assumed to have been inherited from the father (Fig. 6 b). Sister 2 had the same maternal allele as the patient, but sister 1 had the other, and was therefore definitely not a carrier. Sister 2 would be a carrier if the mutation was carried by the mother. Since the mutation was not defined, she should be considered a carrier.

Family III : The patient was reported to be a sporadic case and a deletion was detected with the probe cDNA 1 - 2 a. The mother was found to be heterozygous for two probes that closely flanked the deletion. Analysis of the family revealed that the sisters inherited a different maternal allele than the patient, thus they were definitely not carriers (Fig. 6 c).

Family IV : The patient was reported to be a sporadic case and a deletion was found in the cDNA8 region. Blood samples from only six of eleven members of the family were available. The mother was heterozygous with only the probe cDNA8 (PstI), which is very close to the mutation. The three sisters' common allele was assumed to be the paternal allele (Fig. 6 d). Sisters 2 and 5 inherited the same maternal allele as the healthy brother, so they were definitely not carriers.

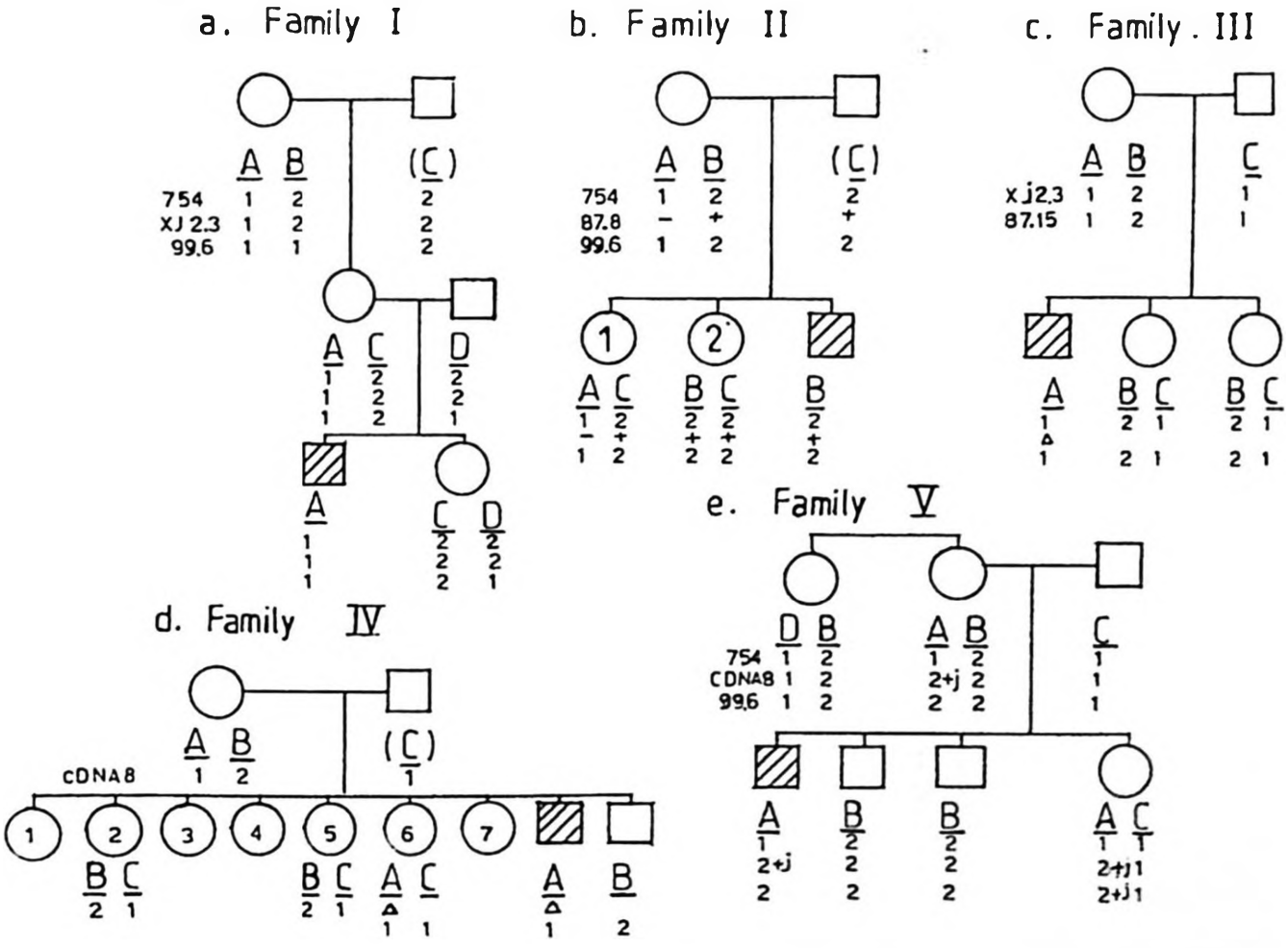

Fig. 6 a, b, c, d, e: Analysis in families I-V using various, probes. Δ indicates a deletion and J a junction fragment. Father's allele if deduced is indicated by parenthesis.

Sister 6 inherited the same maternal allele as the patient. Dosage analysis with the densitometer revealed that she carried the deletion, so did the mother, indicating that the mutation had occurred before the mother's. The densitometric analysis was the determining factor for this sister since the case was known to be sporadic.

Family V : Since the patient's maternal uncle also had DMD, both the sister and the maternal aunt sought carrier analysis. No deletion could be detected in this patient; moreover, he seemed to be heterozygous for the probe cDNA8. However, his DNA sample could be amplified using Y - chromosome specific primers via the PCR technique, indicating that the sample had not been mixed up with a female's Southern analysis after prolonged electrophoresis revealed that one polymorphic fragment was actually not the same size but smaller than the larger polymorphic allele, and so was identified as a duplication junction fragment. Both mother and sister were found to carry the junction fragment, and therefore were carriers, while the aunt did not have it, thus was not a carrier (Fig. 6 e). Probes cDNA8 and 99.6 also confirmed that she had inherited a different maternal allele.

Family VI : The patient was reported to be a sporadic case and the gene was totally deleted in the probe cDNA8 region. The mother was not heterozygous with either of the two RFLP assays in the same region: cDNA8 (PstI) and cDNA8 (Taq I). However, the maternal aunt was heterozygous with probe cDNA8 (PstI), indicating that she carried no deletion (Fig. 7 a). Thus she, and consequently her three daughters, were not carriers. Expectedly, the sister of the patient was "homozygous" for the probe: her alleles were either 3.4 / 3.4 or deletion / 3.4. The family was further analyzed with another probe (pERT87 - 15) for which the mother was heterozygous, and it was shown that the sister had inherited a different maternal allele than the patient's. She was considered to be a carrier with only 5 % probability (probability of crossing-over) since the mutation was rather distant from the pERT87 - 15 region.

Family VII : The two brothers were the only ones known to be afflicted in the family. No deletion could be detected in their DNA samples. The mother was heterozygous for only probes XJ1.1 and cDNA8P. The sisters were assayed and shown to have the same maternal allele with the first probe but different ones with the second, revealing a crossing - over event (Fig. 7 b). The first sister was not a carrier because she had inherited a maternal allele that was different from that of her brothers'. Whether the second sister was a carrier or not was not determined since the relative locations of the crossing - over and the mutation could not be determined.

Family VIII : This family was one of the most difficult to be completely screened. The two brothers were the only ones known to be afflicted in the family and no

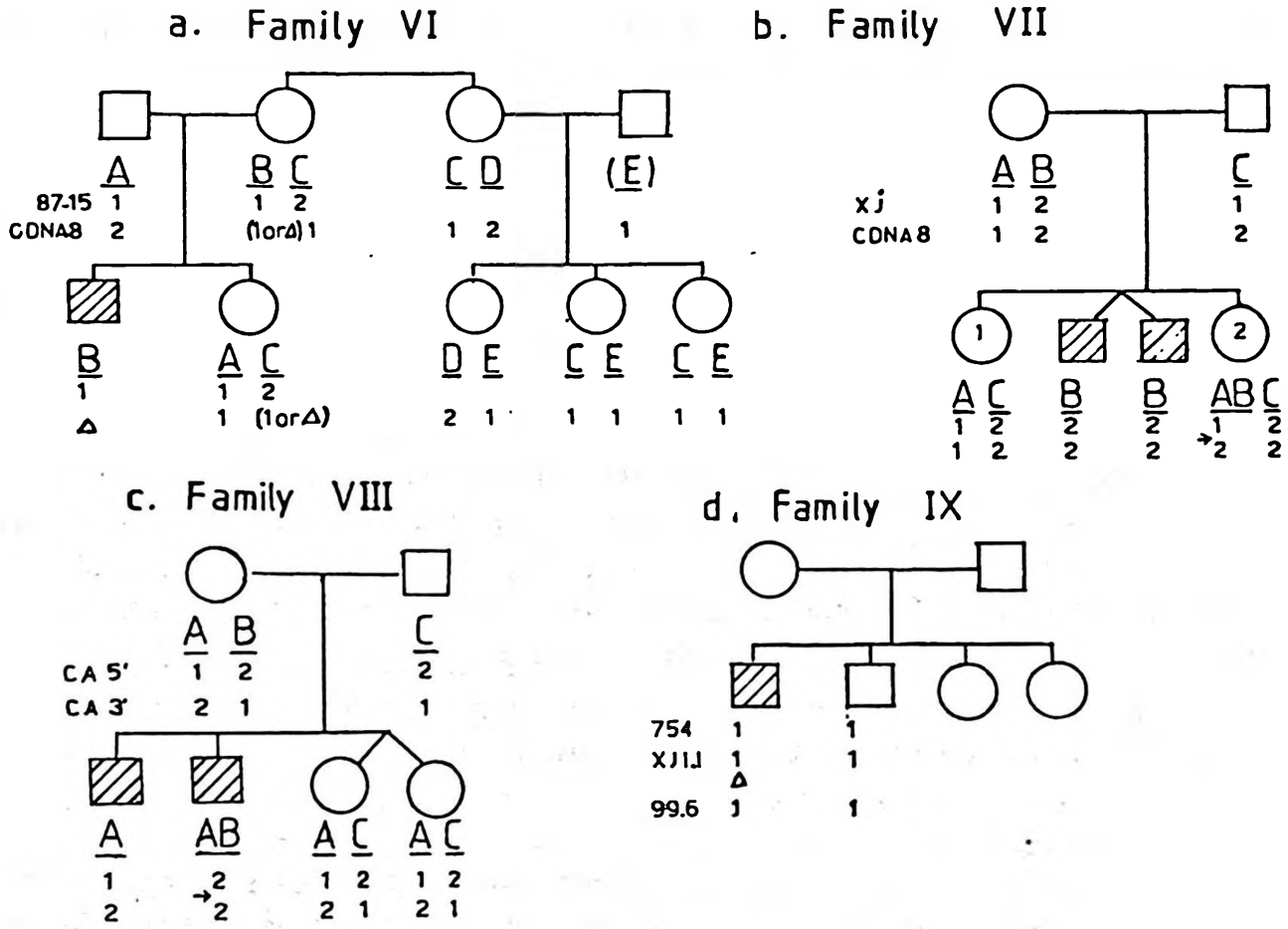

Fig. 7 a, b, c, d: Analysis in families VI-IX using various probes. Δ indicates a deletion. Father's allele if deduced is indicated by parenthesis.

deletion could be detected. The mother was homozygous for all RFLP probes studied. Later, when the CA repeat polymorphisms were applied, she was found to be heterozygous with both of them. Analysis showed that the sisters inherited a common maternal allele and that there was a crossing - over event in one of the patients (Fig. 7 c). Since the first brother had the same allele as the sisters, it was concluded that the crossing - over was in the second brother and that both of the sisters were carriers.

Family IX : The patient was reported to be a sporadic case and a deletion was found in the cDNA8 region. The unaffected brother had inherited the same haplotype, but not the deletion (Fig. 7 d).

Prenatal Diagnosis:

A woman with two affected brothers sought prenatal diagnosis at twelve weeks of pregnancy. The following assays were carried out within six days: blood samples from the family members were obtained and DNA was isolated. The mother of the pregnant woman was assayed for RFLP by PCR analysis and found to be heterozygous for pERT87 - 15 (BamHI). Family analysis revealed that the

pregnant woman had inherited the same maternal allele as the affected brothers and was therefore, a carrier. When the samples of the affected brothers were assayed via multiplex I and multiplex II, three fragments failed to amplify in each assay. There was not enough time to confirm the deletion by Southern analysis, but it was unlikely that failure of six consequent fragments to amplify was due to factors other than a deletion. DNA of the chorionic villi sample (CV DNA) was assayed by multiplex PCR to show that the fetus was not an affected male (all nine fragments amplified in both assays). Moreover, the following additional PCR analyses showed that the fetus was a female:

a) Absence of a Y - chromosome sequence: reaction mix contained 50, 100 or 250 ng of CV DNA with and without 100 ng of a male control DNA. Only the samples containing the male DNA showed a 112 bp product, indicating that the CV DNA contained no inhibitors for the reaction and that the sample itself did not contain the Y - chromosome sequence. Thus the fetus was female.

b) pERT87 - 8 (TaqI) RFLP for which the pregnant woman was a heterozygote: the assay showed that the fetus was a homozygote, indicating that the CV sample was not significantly, if at all, contaminated with the mother's.

Discussion

At present the only way to perform prenatal diagnosis for DMD is via application of DNA analysis techniques which are also used for carrier testing. These techniques will retain their importance in the future since a cure for the disease does not seem feasible at present due to the nature of the defect, and new affected families arise at high frequency unlike other genetic disorders (one third of all DMD cases are sporadic).

DNA analysis techniques that are widely used in genetic diseases were applied to Turkish DMD families in the Biology Department of Boğaziçi University. To our knowledge, this is the largest family study on a genetic disorder ever performed in Turkey. The study covered 254 individuals in 79 families. Thirty-six families were completely screened. In all the families, a total of twenty-five women were found to be carriers, while thirty-seven were non - carriers. In three women, it was impossible to determine the carrier state for various reasons. One subject had a crossing - over within the gene (family VII), while the other two belonged to different families and had inherited a maternal allele common to both the affected and healthy brothers. However, in a significant number of the families, analyses could not be completed due to unavailability of blood samples from some family members. Recently, families have become more interested and cooperative after being informed about the anomaly by the Turkish Myopathy Association.

The greatest difficulty in carrier and prenatal diagnosis of DMD is the strict requirement for haplotype analysis in all family members, unlike in certain other

genetic diseases. Such family studies are employed for several reasons in all established centers studying DMD. Some of these reasons are demonstrated in families described in this work. If a crossing - over has occurred in a patient, a single assay will mislead the analysis (different 5' - CA alleles were associated with the mutation in two brothers in family VIII). If a crossing - over in a sister has occurred, a single analysis will lead to an incorrect conclusion (probe XJ1.1 in family VII). Some cases are sporadic, so inheritance of the same haplotype does not necessarily indicate inheritance of DMD (two brothers in family IX).

Analysis is further complicated by germinal mosaicism in mothers, which is due to a mutation prior to proliferation of the germ cells. Thus the germ cells may contain three alleles, one of which harbors a mutation and is not present in the blood cells. Germinal mosaicism in DMD mothers is estimated to be above 10 percent, therefore, all sporadic cases should be suspected as being germinal mosaic if the mother desires more children.

Familial cases are much easier to analyze since they do not harbor the difficulties inherent in sporadic cases including germinal mosaicism. In general, only about one half of families are believed to be sporadic. However, in our families only 16 percent (16 / 79) were known to be familial for certain, which may simply be due to misinformation, and resulted in an otherwise avoidable extra burden in our analyses. To simplify the analysis, the carrier state of a mother may be determined via dosage analysis. However, the equipment used was not designed for this purpose, therefore, the analysis was very time consuming and cumbersome, although reliable.

This study also resulted in the early diagnosis of some suspected cases. Of fourteen boys who were too young for diagnosis, five had detectable deletions in the dystrophin gene. Considering the fact that deletions were detected in 47 of the total 79 diagnosed patients studied, 60 percent of the patients may be expected to be diagnosed with certainty via deletion detection.

We have shown that the RFLP probes employed in this work may all be used in Turkish families since they detect heterozygotes with reasonable frequencies. CA repeat length polymorphisms are also high enough to be conveniently employed. Application of the 3' - CA repeat polymorphism is more difficult than that of 5' - CA, not only because acrylamide gels are more difficult than NuSieve - agarose, but also because the interpretation is complicated by the hetero duplex band apparent only on acrylamide gels.

The greatest obstacle during this study was financial. Families were not charged a fee for the assays because the study was supported by a research grant. We should like to mention that costs were kept at a minimum by not using kits, and by optimizing the techniques applied.

Our aim is to decrease the number of new cases in families by prenatal diagnosis. Only one prenatal diagnosis established via PCR assays proved to be fast and efficient However, a lot of cases are expected to be more difficult. These cases include families with patients in whom no deletion is detected or in whom the deletion corresponds only to a single multiplex band. Southern analysis is essential in diagnosing these families: in the first group for RFLP analysis and in the latter group for confirmation of the deletion. Thus fresh radioactive material is needed, which has a half - life of fourteen days and is costly. Generally, radioactive material is purchased every four to six weeks.

Acknowledgments

We would like to express our gratitude to Dr. Richard R. Gatti and Dr. Peter Ray for their support. We also wish to thank M. Engin Sözen for dosage analysis and Dr. Kılıç Aydınlı and Dr. Seher Başaran for providing the CV sample.

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The Turkish Journal of Pediatrics 34: 93 - 98, 1992

THE SIGNIFICANCE OF EARLY PANENDOSCOPY IN CAUSTIC INGESTION IN CHILDREN*

*Wrzeslaw Romanczuk MD***, *Ryszard Korczowski MD****

SUMMARY: Romanczuk W, Korczowski R. (Pediatric Clinic, Institute of Clinical Medicine, Rzeszow, Poland). The significance of early panendoscopy in caustic ingestion in children. Turk J Pediatr 34: 93-98, 1992.

Fourteen cases of esophageal and gastric burns following the accidental ingestion of a caustic substance are presented. Early fiberoptic endoscopy was necessary, for it made possible a precise assessment of the severity and extent of injury and the application of proper conservative therapy. Signs and symptoms of caustic burns in children are an unreliable guide to injury, therefore, panendoscopy should be performed as soon as possible. A delay in the performance of a fiberoptic examination could result in the perforation of the affected organs. *Key words: caustic ingestion, esophageal perforation, early panendoscopy.*

The ingestion of caustic agents in children, though infrequent, is a serious therapeutic problem. It requires immediate and complex treatment and any delays may make the prognosis considerably worse[1-6].

Corrosive esophagitis and gastritis follow oropharyngeal burns in frequency. The effects of the intake of a strong base (NaOH, Ca $(OH)_2$), concentrated sulphuric or hydrochloric acid, salts of heavy metals, formalin, boiling water or hot food may permanently damage the organs. In order to prevent the possible consequences of such ingestions, i.e. perforation, stenosis, scar formation, ulceration and inflammation of mucosal membranes of the upper segment of the alimentary tract, prompt and intensive treatment must be initiated.

The peak incidence of caustic ingestions, mainly those occurring by accident, is seen in children under five years of age[2,3,5,6]. There is usually a lack of reliable information on the amount and kind of toxic product ingested. Very often we do not even know whether the intake of a caustic substance had actually occurred. Therefore, the necessity for early esophagogastroscopy, even in children only suspected of having consumed a corrosive compound, has been generally accepted[1,5-7].

In addition, in patients with no external signs of burns in the oral cavity or pharynx, flexible fiberoptic endoscopy is absolutely necessary to make a proper diagnosis[1-

* From the Pediatric Clinic, Institute of Clinical Medicine, Rzeszow, Poland.

** Pediatrician, Pediatric Clinic, Institute of Clinical Medicine.

*** Professor of Pediatrics, Pediatric Clinic, Institute of Clinical Medicine.

[8,9]. A delayed diagnosis will inevitably result in permanent post - burn changes in the upper segments of the alimentary tract (cicatrical stricture, scarring and stenosis) in which some improvement in the condition may be attained only through surgical intervention.

Material and Methods

Fourteen children aged 17 / 12 - 5 3 / 12 years (mean: 28 months) who were hospitalized in the Pediatric Clinic of the Institute of Clinical Medicine, Rzeszow between 1988 - 1989 because of the accidental ingestion of a caustic agent were enrolled in this study.

All patients underwent panendoscopy within the first 24 hours of ingestion. Type GIF - P 10 or GIF - XP 10 Olympus fiberscopes were used in the panendoscopies performed under general anesthesia of short duration (intravenous ketamine). Steroids, antibiotics, H_2 blockers (cimetidine) and drugs to protect the mucosa (antacids) were also given to the patients. For the first three to seven days, depending on the general condition and the endoscopic findings of the patient, only parenteral nutrition was used. Liquid, semi - solid or pap meals were gradually introduced within two to three weeks until oral feeding could be re - established.

Results

All the children were males and two - thirds of them were from the town in which the hospital was located. The agents most frequently ingested by seven patients were either hydrochloric acid or sulphuric acid in concentrated solutions. In the remaining seven cases, either formalin, potassium permanganate, liquid detergents, drain "decloggers" ("Kret and Swider") or the boiling contents from a juice extractor were ingested. With one exception (patient N.R.), all patients were brought to our clinic within the first 24 hours of ingestion.

Early endoscopy revealed traces of burns in the esophagus, cardia, stomach and pylorus in all patients. The intensity (congestion, edema, erosions, ulcerations and mucosal fragility) and extent (involving only the esophagus or the esophagus along with the stomach and pylorus) of the lesions varied from patient to patient. The most pronounced changes were noted at the level of the physiological narrowing of the esophagus and in the angulus of the stomach. Each endoscopy took three to ten minutes and produced no complication.

Early fiberoptic endoscopy was coupled with immediate medical treatment. Recovery was sustained in 13 patients, who are still being followed at our outpatient clinic of the Gastroenterology Department. No late complications were encountered in our series after an average 18-month follow - up. In one case,

however, N.R., a 27-month-old boy, the clinical course was very complicated and resulted in sequelae. For a demonstrative discussion, we present below the clinical course and outcome of the afore - mentioned case.

The patient was admitted to the intensive Care Unit of our hospital because he had accidentally ingested a large quantity of concentrated sulphuric acid. He was in critical condition, evidenced by tachypnea, cardiovascular collapse, unconsciousness and post - burn changes in the oral cavity, pharynx, and in the lungs. After nearly two weeks of intensive medical treatment, he was transferred to the Hospital for Infectious Diseases because of infectious diarrhea. After one week, he was readmitted to our clinic for further therapy. His general condition was poor. He had severe pneumonia and was fed by an orogastric tube. Panendoscopy revealed erosive changes throughout the esophagus and stomach, with ulceration of the angulus of the stomach (Fig. 1). The therapeutic regimen consisted of cimetidine and antacids. Oral feeding was gradually introduced. Ten

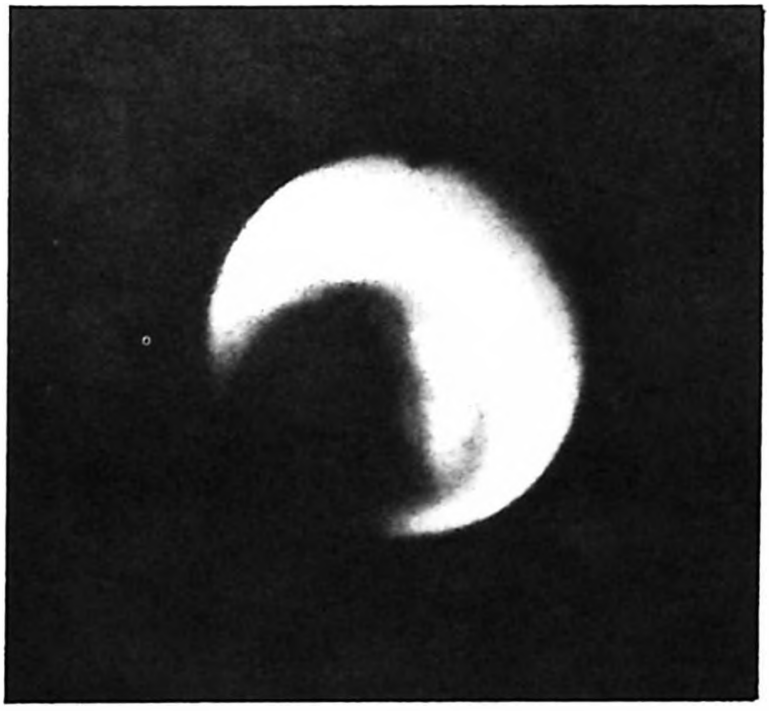

Fig. 1: Changes revealed by panendoscopy in the gastrointestinal tract.

days after initiation of oral feeding, he started to vomit following feedings and refused food. A repeat emergency endoscopic examination was performed. Narrowing of the lumen of the esophagus was detected 7 - 8 cm. down the incisors (Fig. 2). During the withdrawal of the endoscope, pneumothorax developed and the patient was immediately transferred to the Intensive Care Unit where pleural cavity drainage was performed. The chest X - ray showed a right-sided pneumothorax with a shift of the mediastinum to the left and

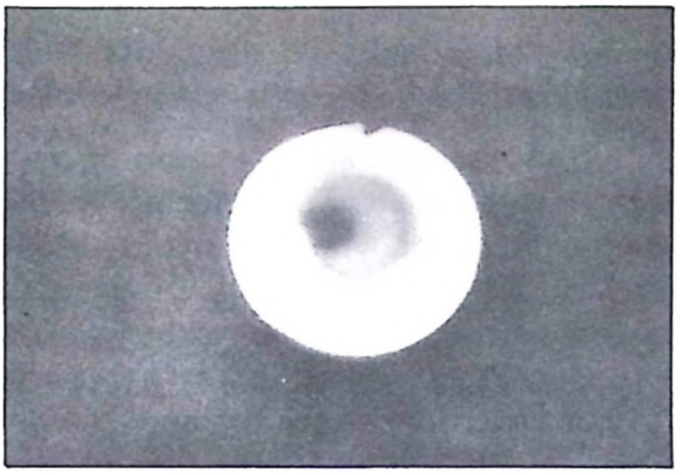

Fig. 2: Permanent narrowing of the lumen of the esophagus.

subcutaneous emphysema involving the right side of the thorax and neck (Figs. 3, 4). A barium follow-through revealed esophageal stenosis at the level of the fifth thoracal vertebra. The patient was treated for three days and was then transferred to the Children's Health Center in Warsaw for dilatation therapy.

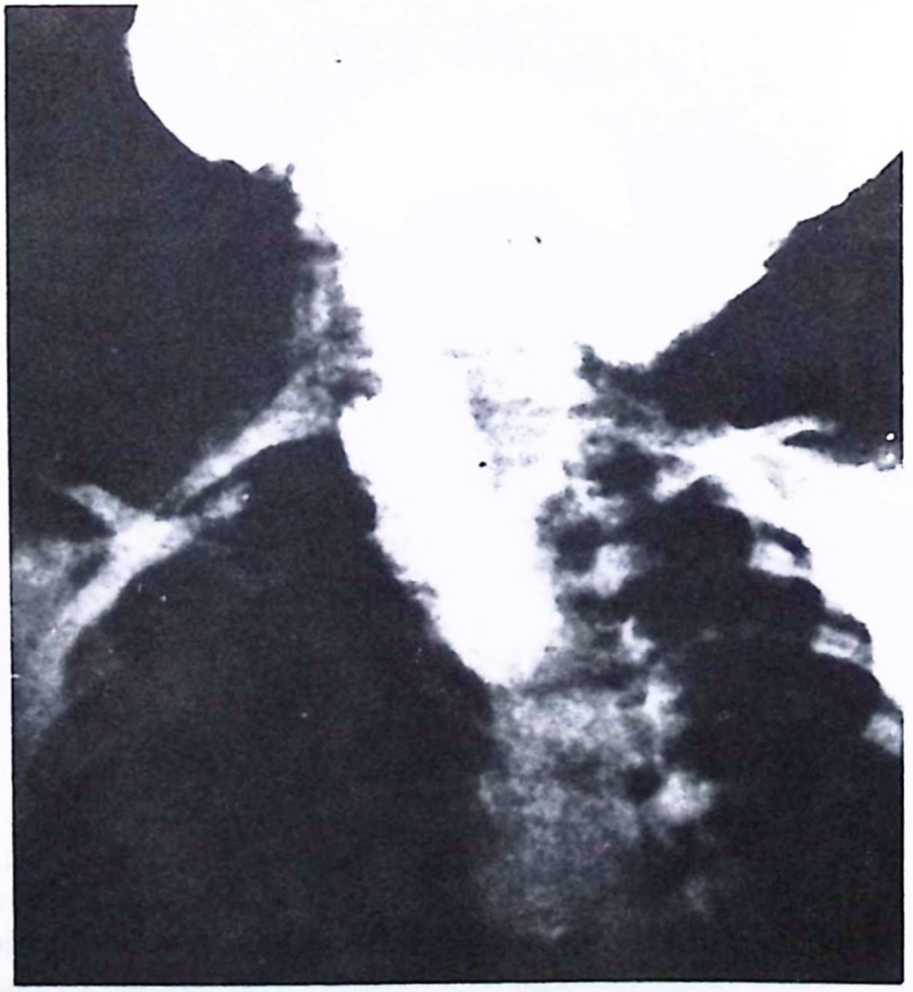

Fig. 3: Esophageal stenosis at the level of the fifth thoracal vertebra and a leakage of air into the mediastinum.

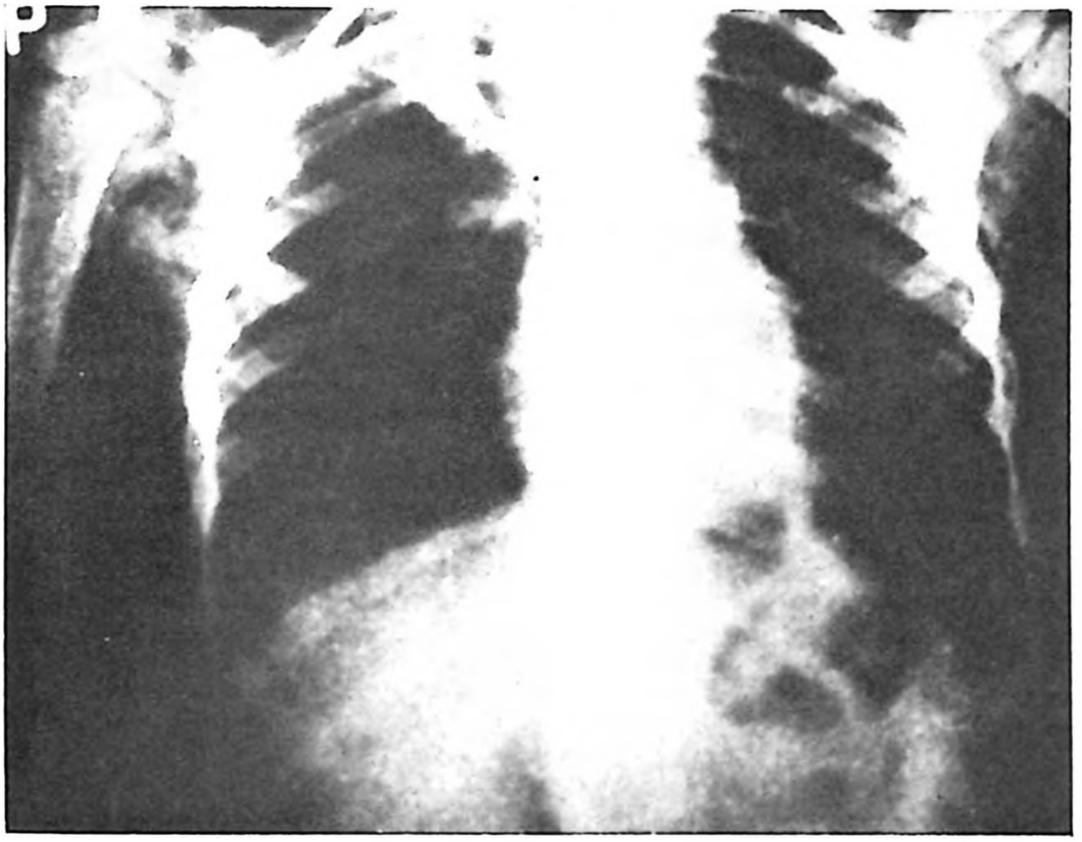

Fig. 4: Esophageal stenosis at the level of the fifth thoracal vertebra and a leakage of air into the mediastinum.

Discussion

It is generally assumed that in children who have ingested a caustic substance, early fiberoptic endoscopy is necessary. This also refers to cases with no external traces of burns in the oral cavity and / or throat[1,2,3,5,7]. It is also believed that endoscopy should be performed no later than the fourth day after the incident, since during this period only surface changes (edema and hyperemia of the esophageal mucosa with no violation of its continuity) occur and the esophageal wall shows a high resistance to mechanical injury, which may occur during the examination. This type of injury may be avoided by use of a flexible fiberoptic endoscope with axial optics along with a short - term general anesthesia.

Early endoscopy is decisive in selecting the type of therapy. It makes possible, when necessary, the immediate application of esophageal dilatation, thus preventing permanent stenosis or total obliteration[1,2,4,8-10].

Five days following ingestion, superficial lesions in the walls of the esophagus and stomach tend to be deeper and involve the muscular layer with derangement of mucosal continuity, which make these organs more vulnerable to mechanical injuries, iatrogenic perforations and permanent effects like esophagitis dissecans superficialis et profunda and eosophagitis corrosiva. American investigators refer to this state as the "ulcerative granulation phase"[3,6]. Proper treatment does not

necessarily ensure a satisfactory outcome and the general prognosis may be poor. These suggestions are in accord with our experience in treating patient N.R., in whom a repeat endoscopy was performed because of his life - threatening condition on the 32nd day after ingestion of strong sulphuric acid, resulting in perforation of the esophagus. In the other cases, early panendoscopy allowed for the introduction of pharmacological treatment and feeding, thus resulting in complete recovery. In half of the children, repeat endoscopies four to five months after the incident revealed normal findings. These children were developing normally and eating normal food.

Our experience with 14 cases indicates the usefulness of early endoscopic diagnosis in children suspected of having ingested a caustic substance. It is worth noting that panendoscopy performed in the seven other children suspected of having ingested a caustic substance enabled us to rule out this possibility and prevented unnecessary intensive treatment and longer hospitalization. After an observation period of one to two days, all these children were sent home.

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The Turkish Journal of Pediatrics 340 99 - 101, 1992

THE VALUE OF CSF ADENOSINE DEAMINASE LEVELS IN THE DIFFERENTIAL DIAGNOSIS OF CHILDHOOD MENINGITIS*

*Ali Baki MD***, Birsen Uçar MD***, Orhan Değer PhD*****

SUMMARY: Baki A, Uçar B, Değer O. (Department of Pediatrics, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey). The value of CSF adenosine deaminase levels in the differential diagnosis of childhood meningitis. Turk J Pediatr 34: 99-101, 1992.

In this study, adenosine deaminase (ADA) levels of serum and cerebrospinal fluid (CSF) in a total 28 children (13 with bacterial meningitis, 5 with mumps meningoencephalitis and 10 with febrile convulsions) were determined. The comparisons between the serum values were insignificant ($p > 0.05$) but the CSF levels of the children with bacterial meningitis were higher than in the others ($p < 0.05$). These findings suggest that serum ADA levels are not important in the diagnosis and differential diagnosis of these diseases. However, ADA levels of CSF may be useful in differentiating between bacterial and viral meningitis. *Key words: adenosine deaminase, cerebrospinal fluid (CSF), children, meningitis.*

Adenosine deaminase (ADA) specifically reacts with adenosine and several adenine nucleoside analogues. The enzyme is widely distributed in animal and human tissues and its activity is particularly high in lymphocytes and leukocytes[1]. Until now, the determination of its activity has been used in diagnosing pleural, peritoneal, pericardial and meningeal tuberculosis, and sarcoid meningitis[2-4].

Its prognostic importance in bacterial meningitis has been reported and these studies have been conducted primarily on adult patients[5]. After a review of the literature, we were unable to find any studies concerning the comparisons of serum and cerebrospinal fluid (CSF) levels in children with bacterial meningitis, viral meningoencephalitis (a central nervous system manifestation of mumps) and febrile convulsions. Therefore, we decided to investigate the significance of ADA in the diagnosis of meningitis and also in the differential diagnosis of bacterial and viral meningitis.

Material and Methods

The study population comprised a total 28 children hospitalized in Farabi Hospital between January 1990 and June 1991. ADA levels were determined by the

* From the Department of Pediatrics, Karadeniz Technical University Faculty of Medicine, Trabzon.

** Associate Professor of Pediatrics, Karadeniz Technical University Faculty of Medicine.

*** Resident in Pediatrics, Karadeniz Technical University Faculty of Medicine.

**** Associate Professor of Biochemistry, Karadeniz Technical University.

method of Giusti and Galanti[1] in 13 children with bacterial meningitis, aged between 6 months and 14 years (mean: 6.6 years), in 5 children with mumps meningoencephalitis (all having parotitis), aged between 2 and 11 years (mean: 7.2 years), and in 10 children with febrile convulsions, aged between 11 months and six years (mean: 2 years). Initial lumbar puncture fluids were used to determine the enzyme. The causative agents of the bacterial meningitis were *Streptococcus pneumoniae* in 5 patients and *Neisseria meningitis* in 8 patients. A viral study could not be carried out. The Mann - Whitney U and Sperman's tests were used for statistical analyses.

Results

Adenosine deaminase levels are shown in Table 1.

No correlation was found between serum and CSF adenosine deaminase activities (Sperman's test). There were statisically significant differences between the CSF levels of children with bacterial meningitis, mumps meningeoencephalitis and febrile convulsions ($p < 0.05$). However, the comparisons involving the serum values and the differences between the ADA levels in the children with mumps meningoencephalitis and febrile convulsions were insignificant ($p > 0.05$).

The mean number of cells in CSF was 4158 (97 % polymorphonuclear leukocytes and 3 % percent lymphocytes per ml) for the cases with bacterial meningitis and 693 for those with mumps meningoencephalitis (13 % polymorphonuclear leukocytes and 87 % lymphocytes). However, no cells were observed in the CSF of the patients with febrile convulsions.

TABLE I: Serum and CSF Adenosine Deaminase Levels of the Patients (mean and range values)

	Serum U / L		CSF U / L	
Bacterial meningitis (n = 13)	15.6 ± 6.8	(1 - 26)	7.9 ± 2.05	(5 - 18.0)
Mumps meningoencephalitis (n = 5)	20.5 ± 13.12	(2 - 44)	4.0 ± 1.1	(0.1 - 7.0)
Febrile Convulsions (n = 10)	18.1 ± 13.1	(3 - 44)	1.7 ± 0.3	(0.1 - 2.0)

Discussion

CSF adenosine deaminase activity has been found to be considerably high in cases of tuberculous meningitis (generally > 9 IU/L)[4,6,7]. It has been reported that very high ADA levels (> 15 U/ml) indicate a poor prognostic factor in purulent meningitis[5]. In this study, ADA levels in CSF were higher in the children with bacterial meningitis than in those with mumps meningoencephalitis or febrile convulsions. ADA values greater than 7 U/L in CSF may suggest acute bacterial meningitis while values below 5 U/L may signify viral meningitis. Very low ADA levels (< 2 U/L) in a child with fever and convulsions may be reason to rule out bacterial meningitis if this opinion is supported by other clinical and laboratory findings. In addition, no correlation was found between ADA levels and the number of cells in CSF ($p > 0.05$). This result has been corroborated by another study[5]. Our findings also demonstrate that serum ADA levels are of no value in making differential diagnoses.

Although, the number of cases in our study was limited, we nevertheless suggest that a test for CSF adenosine deaminase activity be carried out. This test is simple, rapid, inexpensive and available in most hospitals and may be useful in the differential diagnosis of the diseases mentioned. A more extensive study is needed to confirm our preliminary data.

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The Turkish Journal of Pediatrics 340 103 - 105, 1992

THE EFFECT OF NIFEDIPINE ON SECRETORY DIARRHEA IN MICE*

*Kadriye Yurdakök MD***, Şeyda Diker DVM****

SUMMARY: Yurdakök K, Diker Ş. (Department of Social Pediatrics, Hacettepe University Institute of Child Health, Ankara, Turkey). The effect of nifedipine on secretory diarrhea in mice. Turk J Pediatr 34: 103 - 105, 1992.

The aim of this study was to determine whether castor oil - induced secretory diarrhea in mice could be ameliorated by nifedipine, a calcium channel blocking agent in mice. Ten animals received nifedipine in a dose of 1 mg/ 30 g/ body weight and another ten animals received only distilled water via an orogastric feeding tube. Fifteen minutes after the administration of the drug or the distilled water, castor oil was given to all animals. The mean body weight loss was found to be higher in the control group than in the nifedipine treated group two hours after the administration of castor oil. These findings indicate that nifedipine may have a therapeutic role in castor oil - induced secretory diarrhea. *Key words: nifedipine, calcium blocking agent, secretory diarrhea, mice.*

Intracellular free calcium has an important role in active intestinal water and electrolyte transport and in intestinal motility[1, 2]. Raised intracellular free calcium is known to decrease intestinal sodium and chlorine absorption and stimulate active chlorine secretion while decreased levels of free calcium have an opposite effect[2]. The calcium influx across cell membranes also plays a major role in the contraction of intestinal smooth muscle[3]. The constipating effect of loperamide may be related to its calcium antagonist properties since verapamil and other calcium channel blockers have been reported to cause constipation[4-7]. Therefore, this study was designed to determine whether nifedipine, a calcium channel blocking agent, has any effect on castor oil - induced secretory diarrhea in mice.

Material and Methods

Twenty adult male albino mice each weighing 20 - 30g were used as study animals. The mice were randomly divided into two groups. Ten mice received a solution of 0.5 ml/ 30 g/ body weight in a single dose which contained 2 mg nifedipine (Nidilar^{R}) per 1 ml distilled water (1 mg nifedipine/ 30 g/ body weight) via an orogastric feeding tube. Ten animals, comprising the control group, received only 0.5 ml of distilled water /30 g/ body weight in the same manner as the study group. Fifteen minutes after the administration of nifedipine or distilled water, all the animals were given 0.6 ml of castor oil (Ricinor^{R}) through an orogastric feeding

* From the Department of Social Pediatrics, Hacettepe University Institute of Child Health, Ankara.
** Pediatrician, Hacettepe University Institute of Child Health.
*** Veterinary Internist, Gazi University Faculty of Medicine, Ankara.

tube[5]. All the mice were weighed prior to and two hours after the administration of the castor oil. The results were expressed as a mean ± standard deviation and statistical analysis was carried out using the Student's *t* test.

Results

During the study diarrhea was observed in most of the animals in the control group (9/10) while only three of ten mice had wet, loose stools in the study group. The mean body weights in each group prior to and after the administration of castor oil were not found to be statistically different (Table I). However, the mean body weight loss was found to be significantly higher in the control group than in the group treated with nifedipine (1.53 ± 0.97 and 0.46 ± 0.37, respectively; $p < 0.02$).

TABLE I: Body Weight Changes in Both Groups

	Mean Body Weights (g)		
	Initial	Two Hours Later	Mean Body Weight Loss
Nifedipine (n: 10)	27.20 ± 2.64	26.75 ± 2.65	0.46 ± 0.37*
Placebo (n: 10)	27.63 ± 4.85	26.10 ± 4.44	1.53 ± 0.97*

* $p < 0.02$

Discussion

Due to its antimotility effect on colonic smooth muscle, nifedipine has been used to inhibit gastrocolonic response to food in patients with irritable bowel syndrome[8]. In addition, nifedipine has been shown to increase ileal water, and sodium and chlorine absorption in animals[9]. Another study has demonstrated that nifedipine reduces the intestinal fluid response to *E.coli* heat - stable enterotoxin in the suckling mouse[10].

Our findings show that nifedipine also has a weight - reducing effect on the castor oil - induced model of secretory diarrhea in mice. Therefore, nifedipine may have a potential therapeutic role in secretory diarrhea. Further clinical studies are needed to show if this drug can be used safely and effectively in the treatment of secretory diarrhea in animals as well as in humans.

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The Turkish Journal of Pediatrics 340 107 - 109, 1992

NEUROBLASTOMA PRESENTING AS PROTEIN – LOSING ENTEROPATHY*

*Turgay Coşkun MD***, Hasan Özen MD***, Münevver Büyükpamukçu MD**** Gülsev Kale MD*****

SUMMARY: Coşkun T, Özen H, Büyükpamukçu M, Kale G. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Neuroblastoma presenting as protein - losing enteropathy. Turk J Pediatr 34: 107-109, 1992.

Protein - losing enteropathy is often reported to be associated with malignancies such as Hodgkin's disease, non - Hodgkin's lymphoma, and mesenteric mesenchymoma, but it seldom complicates neuroblastoma. In this report, we describe a case of neuroblastoma presenting as protein - losing enteropathy in which neurohumoral mechanisms were involved. *Key words: neuroblastoma, protein - losing enteropathy.*

Protein - losing enteropathy (PLE) is characterized by loss of plasma proteins from the gastrointestinal tract and the eventual development of hypoproteinemia[1]. Cardiac failure, nephrotic syndrome and various neoplasms may cause PLE as well as primary diseases of the digestive system which may involve any part of the gastrointestinal tract[2].

Neuroblastoma, which is one of the most common malignant tumors of childhood, seldom causes chronic diarrhea[3, 4]. In this report, we describe a case of neuroblastoma which appeared as PLE, and we wish to emphasize that malignant tumors should also be considered among the diseases causing protein - losing chronic diarrhea.

Case Report

A 2½ –month –old female infant was admitted to our hospital for evaluation of mild edema of the feet and watery diarrhea containing no blood and/or mucus which had developed one month prior to admission. She had been breast - fed until the onset of the diarrhea. She had no history of vomiting or fever and had lost 300 g within a period of one month.

Physical examination revealed an infant weighing 5,900 g with acidotic respiration. The temperature was 36 °C, pulse rate 120/min and respiratory rate 40/min.

* From the Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.
** Associate Professor of Pediatrics, Hacettepe University Faculty of Medicine.
*** Resident in Pediatrics, Hacettepe University Institute of Child Health.
**** Professor of Pediatrics and Pediatric Oncologist, Hacettepe University Institute of Oncology.
***** Professor of Pediatrics and Pediatric Pathologist, Hacettepe University Faculty of Medicine.

The liver was palpable 4 cm below the right costal margin and mild pretibial edema was detected.

Initial laboratory studies revealed hemoglobin 14.4 g/dl, white blood cells 10,700/µl with 55 % neutrophils, 37 % lymphocytes, 7 % monocytes and 1 % eosinophils, and platelet count 310,000/µl. Prothrombin time was 20 seconds and partial thromboplastin time 45 seconds. Other studies indicated serum sodium 128 mEq/L, potassium 1.8 mEq/L, chloride 98 mEq/L, CO_2 8.2 mEq/L, SGOT 117 IU/L, SGPT 56 IU/L, alkaline phosphatase 134 IU/L, total protein 2.5 g/dl, and albumin 1.4 g/dl. Routine urinalysis, chest x-ray and bone marrow examination were negative. Urine sodium, potassium and protein concentrations were within normal limits.

The patient had three episodes of tachycardia, in addition to diaphoresis, peripheral cyanosis and hypertension during follow - up. Generalized edema developed. On a repeat examination, a mass was detected upon deep palpation of the epigastric region. Contrast radiography revealed slight slurring of the large curvature of the stomach which implied external pressure. Ultrasound examination confirmed the presence of a 34 x 25 mm solitary mass in the right lobe of the liver. Scintigraphic studies of the liver using 90^m Tc - sulphur colloid showed a solitary nodule at the same site with irregular uptake of radioactivity. Vanyl mandelic acid was positive in a spot urine sample.

An exploratory laparotomy revealed a lobulated 3 x 4 x 3 cm mass alongside the large curvature of the stomach which involved the right adrenal gland and extended into the pancreas and duodenum. There were multiple metastases in the liver. The diagnosis of neuroblastoma was arrived at from biopsies of the mass and the liver. Chemotherapy was instituted. The diarrhea abated and the hypoproteinemia improved in three weeks after the onset of treatment.

Discussion

There are two para-neoplastic syndromes which may coexist with neuroblastoma in childhood. One is myoclonic encephalopathy with opsoclonus, myoclonus, and cerebellar ataxia, while the other is a clinical syndrome of chronic diarrhea, hypokalemia and achlorhydria or more often hypochlorhydria. The latter is caused by oversecretion of vasoactive intestinal polypeptides (VIP)[4]. This syndrome is observed not only in neuroblastoma, but also in other neoplasms of the sympathetic nervous system which secrete VIP[5]. VIP is a peptide of 28 - amino acids mainly synthesized in the duodenum. It acts as a neurotransmitter not only in the gastrointestinal tract, but also in neural tissue elsewhere and shows a wide range of biologic activity such as relaxation of smooth muscles, stimulation of intestinal water and electrolyte excretion, and an increase in the secretion of insulin, glucagon and various hormones of adenohypophysis. VIP is synthesized

from a precursor (pro - VIP) in neuroblastoma cells and the synthesis of pro - VIP is stimulated by Bt_2 cAMP[3].

Although PLE is reported to be more frequently associated with such malignancies as non - Hodgkin's lymphoma, Hodgkin's disease, and mesenteric mesenchymoma, it seldom complicates neuroblastoma[2]. Neoplasms cause PLE mainly by two mechanisms. The first is the direct protein loss from the ulcerated or inflamed mucosa like that seen in primary neoplasms of the gastrointestinal tract, while the second is secondary lymphangiectasia caused by lymphatic obstruction which in turn results in the loss of protein and lymphocytes[1]. Intestinal lymphangiectasia and PLE were reported by Gerdes et al[6] in an 8-month-old baby and by Schusseim[1] in two 3-year-old children with neuroblastoma.

In our case, there was no proteinuria evidenced. The hypoproteinemia was thought to be caused by losses from the gastrointestinal tract. Lymphopenia, which is expected in cases of lymphangiectasia, was not observed and neither was there any external pressure detected during surgery performed on the intestinal wall. Since there was no evidence of lymphangiectasia, intestinal protein loss was attributed to neurohumoral mechanisms. This view was supported by the amelioration of diarrhea and the normalization of the serum protein and albumin levels after the intiation of chemotherapy.

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The Turkish Journal of Pediatrics 34: 111 - 114, 1992

A CASE OF WAARDENBURG SYNDROME AND AGANGLIONOSIS*

*Ender Arıtürk MD***, Nadir Tosyalı MD***, Nurşen Arıtürk MD*****

SUMMARY: Arıtürk E, Tosyalı N, Arıtürk N. (Departments of Pediatric Surgery and Ophthalmology, Dicle University Faculty of Medicine, Diyarbakır, Turkey). A case of Waardenburg's syndrome and aganglionosis. Turk J Pediatr 34: 111-114, 1992.

Waardenburg's syndrome is characterized by a broad nasal root, pigmentation disturbance and congenital deafness while aganglionosis is described as the partial or complete lack of ganglion cells in the alimentary tract. This report describes a five-day-old male infant with Waardenburg's syndrome associated with total aganglionosis of the colon, ileum and distal jejunum and draws attention to the causal relationship between these two entities. *Key words: Waardenburg's syndrome, Hirschsprung's disease, neural crest.*

The principal characteristics of Waardenburg's syndrome are facial deformities exhibited by a broad nasal root, pigmentary disturbance, and congenital deafness. It is inherited as an autosomal dominant condition with variable penetrance. Aganglionosis is a congenital anomaly characterized by partial to complete colonic obstruction associated with the absence of intramural ganglion cells in the alimentary tract[1, 2]. This report presents both anomalies in one patient and stresses the etiological relationship.

Case Report

A five-day-old male infant was referred to the Pediatric Surgery Department of Dicle University Hospital for evaluation of intestinal obstruction. The baby was the first - born child of a first cousin marriage with the family history being otherwise unremarkable. The mother was 21 years old and the father 23. The patient's weight was 2800 g and length 49 cm. Physical examination revealed an extensive white forelock, broad nasal root, megalocornea, hypopigmentation of the irides and retina, and parietal deviation of the lacrimal puncta. He was totally unresponsive to noise. A cleft palate, systolic murmur along the mesocardiac region and bilateral hydrocele were striking features. Plain X - ray showed gas - fluid levels and an unused colon was found with contrast enema (Figs. 1 and 2). After correction of fluid and electrolyte imbalances and other preoperative

* From the Departments of Pediatric Surgery and Ophthalmology, Dicle University Faculty of Medicine, Diyarbakır.

** Associate Professor of Pediatric Surgery, Dicle University Faculty of Medicine.

*** Resident in Pediatric Surgery, Dicle University Faculty of Medicine.

**** Resident in Ophthalmology, Dicle University Faculty of Medicine.

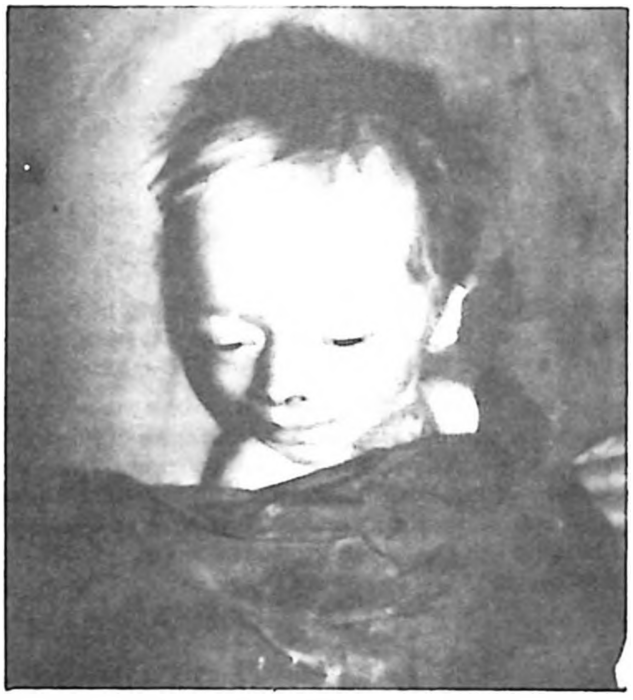

Fig. 1: Extensive white forelock and broad nasal root indicative of Waardenburg's syndrome.

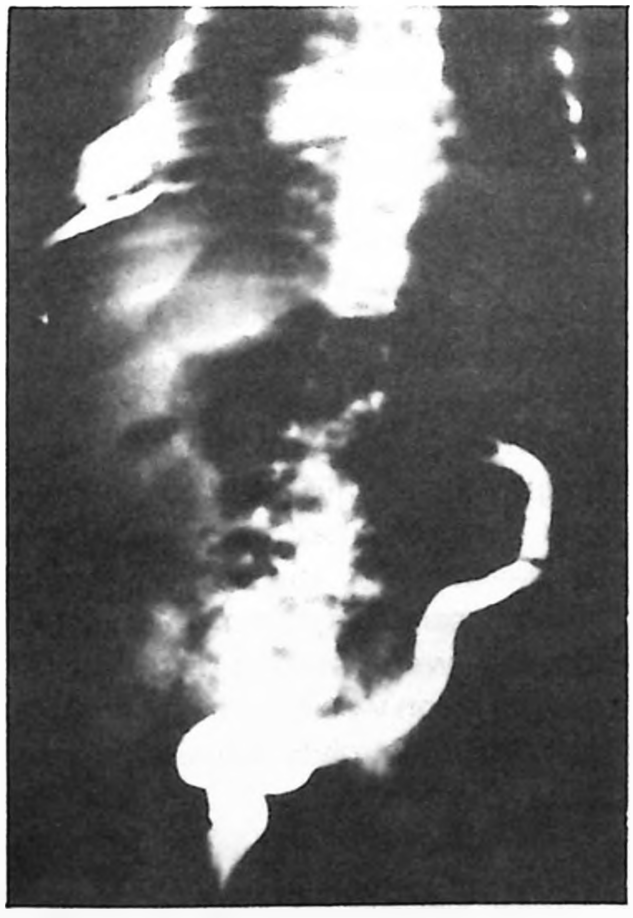

Fig. 2: Unused colon of patient with aganglionosis.

assessments, an exploratory laparotomy was performed. The whole colon and 25 cm of the terminal ileum were very narrow and believed to be aganglionic. An ileostomy was performed. Three biopsies were taken from above, below and at the site of the ileostomy. The ileostomy was not effective during the postoperative period. Parenteral nutrition was initiated. The infant died of sepsis on the 14th postoperative day. A postmortem examination revealed total aganglionosis extending to the upper jejunum. Hepatomegaly, edema, hyperemia of the other intraabdominal viscus and reactionary fluid were striking features of the intraabdominal examination. The pancreas and thyroid tissues were found to be normal. The retroperitoneal area was scrutinized for the sympathetic ganglion chain and surrenal glands. The surrenal glands were present at their normal location; both had normal findings. Intrathoracic examination revealed patent ductus arteriosus.

Discussion

As the neural folds fuse to form the neural tube, some neuroectodermal cells lying along the crest of each neural fold lose their epithelial affinities and attachments to neighboring cells. These are termed neural crest cells and are differentiated as melanocytes, Schwann cells, dorsal root ganglion cells (cochlea), sympathetic ganglion cells, chromaffin cells of the surrenal medulla, celiac ganglion cells and plexuses of the intestinal tractus[3]. If there are differentiation or migration problems of the neural crest cells, some of the pathological features of Waardenburg's syndrome and Hirschprung's disease can be seen together. Although these two conditions seldom occur together (2: 100,000 and 2: 10,000, respectively), numerous cases have been reported of Waardenburg's syndrome associated with Hirschprung's disease, indicating a possible etiological relationship. Moreover, after a review of the literature, we found that the length of the aganglionic intestinal segment is directly correlated with the variety of symptoms relevant to anomalies of neural crest origin[4-6]. This was also the case in our patient who had multiple anomalies and an aganglionic segment consisting of the entire colon, ileum and distal jejunum.

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The Turkish Journal of Pediatrics 34: 115 - 120, 1992

KAWASAKI SYNDROME*

*Nur Olgun MD**, Hasan Özkan MD***, Hale Ören MD***
*Nesrin Erdem MD**, Necla Çevik MD*****

SUMMARY: Olgun N, Özkan H, Ören H, Erdem N, Çevik N. (Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Kawasaki syndrome. Turk J Pediatr 34: 115-120, 1992.

Kawasaki syndrome, also known as mucocutaneous lymph node syndrome, is an acute vasculitis of infants and young children. We describe a four-year-old girl who presented with fever, a diffuse erythematous maculopapular rash, bilateral nonpurulent bulbar conjunctivitis, dry, red, fissured lips, a tongue with a strawberry "appearance", an erythematous pharynx, indurative erythema, and edema and desquamation of the face, hands and feet. She probably developed mitral valve prolapse during the course of the disease. The diagnosis of Kawasaki syndrome was arrived at by excluding other diseases and by the presence of all the clinical criteria for Kawasaki syndrome. Since this syndrome is rarely encountered in Turkey, this case is presented and the literature regarding the syndrome is reviewed. *Key words: Kawasaki syndrome, mucocutaneous lymph node syndrome.*

Kawasaki syndrome (KD), also known as mucocutaneous lymph node syndrome, is an acute vasculitis of infants and young children. Standard diagnostic criteria for Kawasaki syndrome include A) Fever persisting for five days or longer. B) The presence of four of the five following conditions: 1) bilateral conjunctival injection; 2) change(s) in the mucous membranes of the upper respiratory tract, such as infected pharynx, injected, dry, fissured lips and protuberance of tongue papillae ("strawberry tongue"); 3) change(s) in the extremities such as edema of the hands and feet, erythema of the palms and soles, desquamation of the skin including the periungal region; 4) Polymorphous exanthema of the body trunk without vesicles; 5) cervical lymphadenopathy; c) Illness cannot be explained by any other known disease process[1].

Approximately 20 percent of children with KD develop cardiovascular sequelae, the major cause of morbidity and mortality in this disease[2]. The etiology of KD remains unknown but the infrequent person-to-person transmission, the differences in racial incidence, and the clinical findings of multi - system vasculitis suggest that it may be caused by an infective agent(s) that leads to an immune - mediated syndrome in certain genetically predisposed individuals[1].

* From the Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir.
** Associate Professor of Pediatrics, Dokuz Eylül University Faculty of Medicine.
*** Research Assistant in Pediatrics, Dokuz Eylül University Faculty of Medicine.
**** Professor of Pediatrics, Dokuz Eylül University Faculty of Medicine.

Kawasaki disease occurs worldwide and affects children of all races, with Asians, particularly Japanese and Koreans, at highest risk, followed by blacks and then Caucasians who seem to be at lowest risk[1]. This syndrome, rarely encountered in Turkey, was reported in this country by Özsoylu et al[3] in 1976. We describe a four-year-old girl who fulfilled all the diagnostic criteria for KD and developed mitral valve prolapse.

Case Report

A four-year-old girl was admitted to the pediatric clinic of Dokuz Eylül University Hospital for evaluation of a 15 - day history of fever, rash, cervical adenopathy, congestion of the eyes, redness and fissuring of the lips, and swelling of the hands and feet.

Physical examination revealed a well developed child. Her weight was 18 kg and height 103 cm. She appeared irritable and acutely ill. The temperature was 40.5 °C rectally. She had a diffuse erythematous maculopapular rash covering her entire body, dry, red, fissured lips, an erythematous pharynx and a tongue with a "strawberry" appearance. She also had bilateral nonpurulent bulbar conjunctivitis and a lymph node measuring 1.5 cm in diameter in the right cervical region, Beau's lines on her fingernails, mucosal erythema of the vulva, indurative erythema, and edema and desquamation of the face, hands and feet. The remainder of the physical examination was within normal limits (Figs. 1 and 2).

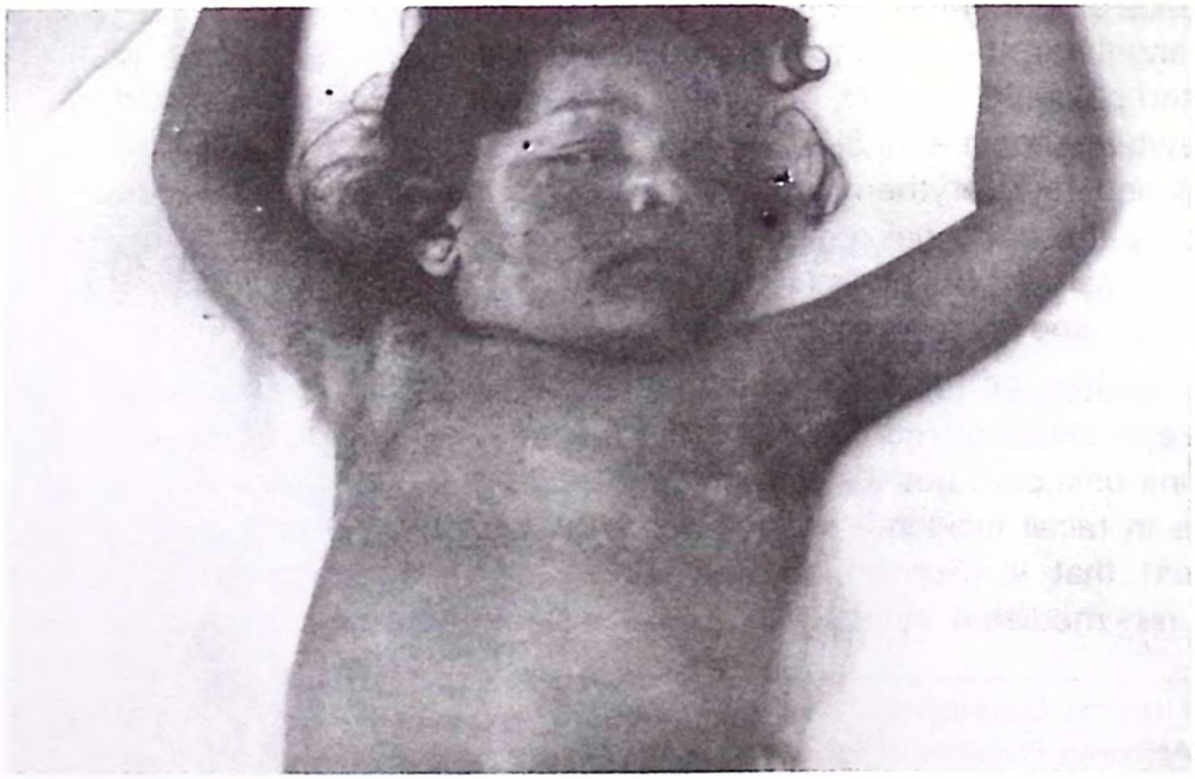

Fig 1: Diffuse erythematous maculopapular rash covering patient's entire body.

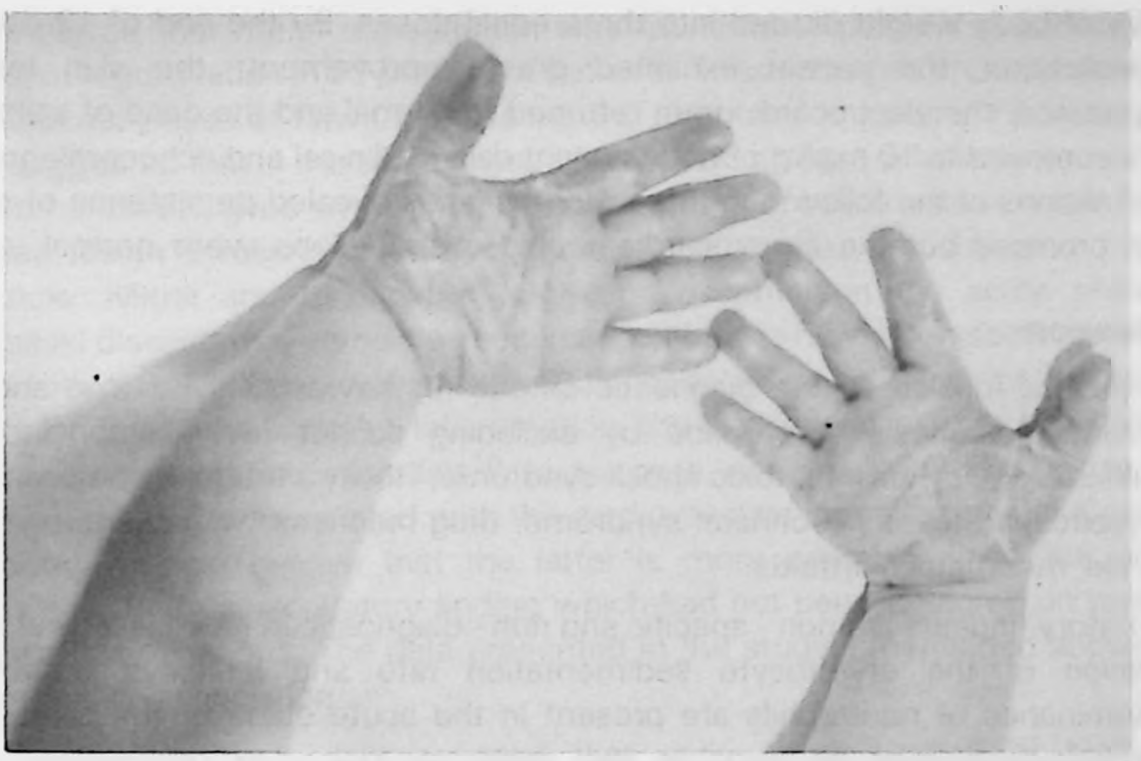

Fig. 2: Desquamation of the hands.

Initial laboratory studies revealed hemoglobin 14 g/dl and white blood cells 12,200/mm^3 with 54 % segmented neutrophils, 44 % lymphocytes and 2 % eosinophils. The platelet count was 350,000/mm^3. The erythrocyte sedimentation rate was 6 mm/hr. Urinalysis, total protein, serum electrolytes, glucose, urea, creatinine, X-ray examinations and the electrocardiogram were within normal limits. Liver function tests were normal except for a mild to moderate elevation of serum transaminases (SGOT = 81 IU/L; SGPT = 104 IU/L). The PPD test was negative. The following examinations were either normal or negative: blood, stool and nasopharynx cultures; viral serology (Epstein - Barr virus, cytomegalovirus, toxoplasma; hepatitis B and rubella); Venereal Disease Research Laboratory test for syphilis; antistreptolysin O; C-reactive protein; antinuclear antibodies; rheumatoid factor and parasitological examination of stool specimens. *E.coli* was isolated from the urine culture. The cutaneous biopsy specimen demonstrated perivascular mononuclear cell infiltration in the dermis.

During the first four days of hospitalization, the patient continued to have daily fevers of up to 40 °C, and the rash increased, despite erythromycin therapy. On the 5^{th} day, a 2/6 midsystolic murmur introduced by a click, was heard at the apex and along the left sternal border. An electrocardiogram showed sinus tachycardia and a first-degree atrioventricular block. The M - mode echocardiogram and two - dimensional studies showed posterior mitral valve prolapse. During the acute phase, salicylate therapy was administered orally in a daily dose of 100

mg/kg of body weight divided into three equal doses. By the end of 12 days of hospitalization, the patient exhibited great improvement; the skin lesions disappeared, the electrocardiogram returned to normal and the dose of salicylate was decreased to 10 mg/kg of body weight daily[7]. Clinical and echocardiographic examinations at the follow - up three months later revealed persistence of mitral valve prolapse but the electrocardiographic examinations were normal.

Discussion

Our patient fulfilled all the diagnostic criteria for Kawasaki syndrome and the differential diagnosis was made by excluding scarlet fever, staphylococcal "scalded skin" syndrome, toxic shock syndrome, Rocky Mountain spotted fever, leptospirosis, Stevens - Johnson syndrome, drug reactions, viral exanthems and juvenile rheumatoid arthritis.

Laboratory findings are non - specific and non - diagnostic in Kawasaki syndrome. Elevation of the erythrocyte sedimentation rate and leukocytosis with a predominance of neutrophils are present in the acute stage of the illness and thrombocytosis may be seen in the subacute stage. In our patient, mild leukocytosis with a predominance of neutrophils was present but the erythrocyte sedimentation rate and the thrombocyte count were normal probably because the patient had been admitted to our clinic in the third week of her illness.

Hepatic dysfunction with mild obstructive jaundice and mildly to moderately elevated levels of serum transaminases have been reported in 10 percent of children with Kawasaki syndrome[4]. Our patient also demonstrated hepatic involvement with elevated liver enzymes.

Echocardiographic and angiocardiographic data reveal that about 20 percent of patients with Kawasaki syndrome develop cardiovascular complications[1,2,5,6]. The earliest cardiac complications occur during the acute stage of the illness and include myocarditis, congestive heart failure, pericarditis with pericardial effusion, mitral and / or aortic insufficiency, arrhythmias and coronary artery aneurysm formation. Electrocardiographic changes are present in at least one - third of patients and include flattening and depression of the ST segment and T wave, decreased voltage and conduction disturbances. Over the next five weeks, these findings generally resolve, but acute coronary arteritis during the first two weeks of illness may lead to coronary artery aneurysm formation[1]. In our case, first - degree heart block and posterior mitral valve prolapse were detected at the end of the acute stage of the illness. The electrocardiogram repeated one week later showed a normal PR interval but echocardiographic examinations at the follow - up three months later revealed persistence of mitral valve prolapse which could have been attributed to papillary muscle dysfunction or valvulitis. Suzuki et

al[7], reported that mitral regurgitation was found in 47 percent of patients and tricuspid regurgitation in 53 percent of patients. These changes are often transient in the acute phase of Kawasaki disease and attributed to carditis. Fujiwara et al[8] also suggested that in the acute phase of Kawasaki disease valvular lesions due to inflammation of valves are very rare, and that most of these lesions result from a cardiac lesion involving the valvular roots, the papillary muscle and the left ventricle. Mitral and tricuspid regurgitation occurring in the acute phase of Kawasaki disease may remain as sequelae, and there have been some necropsy-based reports of severe valvulitis[9]. Additionally , despite a lack of clinical and laboratory evidence of active inflammation, the excised valves may demonstrate both acute and chronic vasculitis[10]. In our case, mitral valve prolapse may have been congenital or associated with the cardiovascular complications of Kawasaki disease. But, we believe that the latter is more probable since the patient presented a new auscultatory finding which had not been detected on previous physical examinations. The data presented in the studies mentioned above may support this association[7-10].

Recent studies have demonstrated that early administration of high-dose intravenous gammaglobulin (400 mg/kg/d for 4 days) plus aspirin (100 mg/kg/d for 14 days) will hasten the resolution of acute manifestations of the disease and reduce the prevalence of coronary artery aneurysms[1,2,11,12]. Low doses of aspirin (3-10 mg/kg/d) are then used as antiplatelet therapy until the aneurysms are resolved or the coronary arteries are demonstrated to be normal.

Our patient was admitted to our hospital in the third week of her illness and she was in the low risk group for development of coronary artery disease, therefore, high - dose aspirin therapy was initiated. Following defervescense, we reduced the dosage of the aspirin to 10 mg/kg/d and continued low - dose aspirin until the end of the convalescent period.

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ANNOUNCEMENTS

MEETINGS

ANKARA, 4 - 9 MAY 1992

THE INTERNATIONAL SOCIETY OF PAEDIATRIC ONCOLOGY (SIOP) AND THE EUROPEAN SCHOOL OF ONCOLOGY'S POSTGRADUATE COURSE IN PAEDIATRIC ONCOLOGY

Inquiries to: Prof. M. Büyükpamukçu
institute of Oncology
Hacettepe University
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JERUSALEM, 30 AUGUST - 4 SEPTEMBER 1992

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