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CHILDHOOD TUBERCULOSIS: A REPORT OF 2,205 CASES*

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SUMMARY: Göçmen A, Cengizlier R, Özçelik U, Kiper N, Şenuyar R. (Chest Diseases Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Childhood tuberculosis: a report of 2,205 cases. Turk J Pediatr 1997; 39: 149-158.

A total of 2,205 children with the diagnosis of tuberculosis followed at Hacettepe University İhsan Doğramacı Children's Hospital between the years 1972 and 1992 were investigated in this retrospective study. Sixty-two percent of the patients were less than six years old. There was no gender preponderance among the cases. Thirty-four percent of the patients had predisposing diseases for tuberculosis, such as malnutrition, measles and pertussis. Thirty-five percent of the patients had a history of contact with a tuberculous patient. The most common type of tuberculosis was pulmonary (39%), but extrapulmonary cases outnumbered pulmonary tuberculosis patients. Thirty-three percent of the patients had been vaccinated with BCG. The Mantoux test was found positive in 62 percent of patients. The tuberculous culture was found to be positive in the sputum of 28 percent of pulmonary tuberculosis cases. Patients were treated with daily (59%) or intermittent (41%) antituberculous therapy regimens. The response to treatment was excellent. Relapse was observed in five cases. Mortality from tuberculosis was eight percent, 23 percent of which were from tuberculous meningitis. Althouh there was a decrease in the number of children with tuberculosis seen at our hospital between 1972 and 1992, the prevention and control of tuberculosis are still significant health problems for our country. *Key words: tuberculosis, children.*

Because of its high morbidity and mortality rates, tuberculosis (TB) remains a major public health concern for the world. In children less than 15 years of age in developing countries, 1.3 million cases and 450,000 related deaths occur annually[1]. Despite the numerous efforts to control TB in developed countries, the disease is still considered to be a major health problem-especially in urban areas[2].

Significant progress in the control of TB has been made in Turkey, but the disease is still one of the most significant public health problems. The aim of this study was to evaluate the clinical and laboratory features of TB in 2205 children at Hacettepe University İhsan Doğramacı Children's Hospital between the years 1972 and 1992.

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Material and Methods

The records of 2205 patients with the diagnosis of TB between 1972 and 1992 at Hacettepe University İhsan Doğramacı Children's Hospital, which is a tertiery care hospital in the capital of Turkey, were reviewed retrospectively. The diagnosis criteria for TB were: clinical and radiological evidence, a history of direct contact with an adult with TB, positive Mantoux test reaction, positive bacteriology and histopathology for tuberculosis, and characteristic findings on cerebrospinal fluid, pleural fluid and ascites fluid examination.

At this institution, 5 TU ppd solution stabilized with tween 20 was used for the Mantoux testing, and induration was measured after 48-72 hours. A Mantoux response greater than 10 mm and 15 mm were considered as positive for children without BCG and with BCG, respectively.

Radiologic examination included chest roentgenogram (postero-anterior and lateral) for all children, bone and joint roentgenogram for bone and joint tuberculosis, abdominal ultrasonography for some of the abdominal tuberculosis cases, and computed tomographic scans for some of the meningeal tuberculosis cases.

Sputum, gastric aspirate, pleural fluid, pericardial fluid, urine, cerebrospinal fluid (CSF) and biopsy specimens were inoculated in Löwenstein Jensen medium. Sputum or gastric aspirate materials were collected from 712 of the patients.

Before 1978, patients were treated with a daily antituberculous chemotherapy regimen consisting of isoniazid plus rifampin plus streptomycin daily for one month, and isoniazid plus rifampin daily for 11 months. Since 1978, uncomplicated patients have received an intermittent, antituberculous chemotherapy regimen. This regimen comprised isoniazid plus rifampin plus streptomycin daily for 15 days, then isoniazid plus rifampin twice weekly for a total of nine months until the year 1986. The regimen was then changed to isoniazid plus rifampin daily for 15 days, and then the same drugs twice weekly for a total of nine months. Patients with severe tuberculous meningitis or miliary tuberculosis received daily antituberculosis therapy for nine to 12 months, with the recent addition of pyrazinamide. Corticosteroids were used in all patients with meningitis.

Results

The number of cases per year peaked in 1977 at 168. In the following years there was a gradual decrease in cases (Fig. 1). The children's ages ranged from three months to 17 years. Sixty-two percent of the cases were less than six years of age (Table I). There was no gender preponderance among the cases.

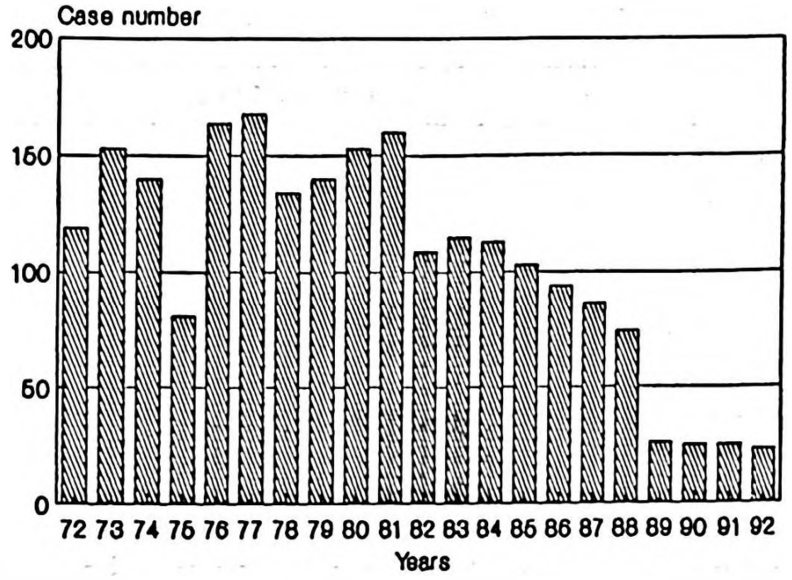

Fig. 1: Number of tuberculous cases according to year.

Table I: Distribution of Cases According to Age-groups

Age	Number of Cases	%
0-23 months	568	26
24 months-6 years	801	36
7-12 years	483	22
13 years and over	353	16
Total	2205	100

As for the predisposing diseases, 22 percent of cases had malnutrition, 11 percent had a history of measles and one percent had pertussis in the recent past.

The most common type of tuberculosis was pulmonary, followed by meningeal, miliary, pleural, lymph node, bone and joint, abdominal, pericardial, skin and genitourinary tuberculosis (Table II).

The criteria for the diagnosis of tuberculosis are listed in Table III. Thirty-five percent of the cases had a history of contact with a tuberculous patient, and 17 percent of them were living in the same household. The symptoms and physical examination findings, and the radiological findings in pulmonary TB are shown in Tables IV and V, respectively. In pulmonary TB cases, 28 percent had positive TB cultures in sputum. In extrapulmonary TB, positive cultures were obtained in 18 percent of various specimens. The Mantoux test was found to be positive in 62 percent of cases 70 percent in pulmonary and 54 percent in extrapulmonary tuberculous cases. Thirty-three percent of the patients had been vaccinated with BCG; pulmonary and extrapulmonary cases comprised 17 and 16 percent of these cases, respectively.

Table II: Sites of Tuberculosis

Site	Number of Cases	%
Pulmonary	937	39
Meningeal	537	22
Miliary	237	10
Pleurisy	186	8
Lymph node	174	7
Skeletal	173	7
Intraabdominal	93	4
Pericardial	45	2
Skin	18	1
Genito-urinary	13	0.5
Total	2413*	

* In some patients tuberculosis were detected at more than one site.

Table III: Criteria for the Diagnosis of Tuberculosis

	Pulmonary tuberculosis	Meningeal tuberculosis	Miliary tuberculosis	Pleural tuberculosis	Lymph nodes tuberculosis	Bone and joint tuberculosis	Abdominal tuberculosis	Pericardial tuberculosis	Skin tuberculosis	Genito-urinary tuberculosis
Criteria	n: 937 (%)	n: 537 (%)	n: 237 (%)	n: 186 (%)	n: 174 (%)	n: 173 (%)	n: 93 (%)	n: 45 (%)	n: 18 (%)	n: 13 (%)
Clinical	937 (100)	537 (100)	237 (100)	186 (100)	174 (100)	173 (100)	93 (100)	45 (100)	18 (100)	13 (100)
Radiologic	937 (100)	141 (26)	237 (100)	186 (100)	18 (10)	173 (100)	46 (50)	45 (100)	1 (5)	7 (53)
PPD +	656 (70)	209 (39)	19 (8)	148 (80)	131 (75)	149 (86)	76 (82)	40 (89)	12 (67)	13 (100)
Contact	347 (37)	302 (56)	116 (49)	41 (22)	9 (5)	11 (6)	3 (3)	16 (14)	2 (11)	4 (30)
Bacteriologic	262 (28)	126 (23)	42 (18)	14 (8)	9 (5)	43 (25)	12 (13)	15 (33)	2 (11)	2 (15)
Pathologic	4 (0.4)	–	2 (1)	–	60 (34)	35 (20)	8 (7)	7 (16)	2 (11)	4 (30)

Table IV: The Symptoms and Physical Examination Findings in Pulmonary Tuberculosis

Finding	Number of Cases	%
Fever	750	80
Cough	637	68
Abnormal breath sounds	440	47
Sweating	363	39
Anorexia and weight loss	365	39
Weakness	281	30
Peripheral lymphadenopathy	234	25
Normal findings	197	21
Hepatosplenomegaly	169	18
Dyspnea/respiratory distress	112	12
Erythema nodosum	19	2

Total number: 937

Table V: Radiological Findings of Pulmonary Tuberculous Patients

Finding	Number of Cases	%
Hilar lymphadenopathy	402	43
Infiltration	356	38
Pleural effusion	122	13
Miliary spread	84	9
Atelectasis	28	3
Calcification	28	3
Cavern	19	2

Total number: 937

The cases were treated with daily or intermittent antituberculous therapy regimens (Table VI). Patients who completed the therapy numbered 1,742 (79%), and the clinicoradiologic response to treatment was observed to be excellent. Relapse was noted in five patients: one with pulmonary tuberculosis and Hodgkin's disease, one with pulmonary tuberculosis and cyclic neutropenia, one pulmonary tuberculosis case, and two lymph-node tuberculosis patients. Two cases of relapse were in the intermittent therapy group. These patients were successfully retreated with intermittent anti-tuberculous chemotherapy. Serious side effects of the drugs were not observed in any patient. A transient increase in liver enzymes was observed in 67 patients, 12 of them in the intermittent therapy group. These high levels returned to normal with the cessation of therapy or by decreasing the drug levels for a while. Changing the drug regimen or stopping the antituberculous therapy for a long time was not necessary in any case.

Table VI: Treatment of Patients

Treatment	Site of Tuberculosis	
	Pulmonary (%)	Extrapulmonary (%)
Daily*	254 (27)	1166 (79)
Intermittent a**	646 (69)	280 (19)
b***	37 (4)	30 (2)
Total	937 (100)	1476 (100)

*: Isoniazid plus rifampin plus streptomycin or pyrazinamide daily for one month, and isoniazid plus rifampin daily for 8 to 11 months.

**: Isoniazid plus rifampin plus streptomycin daily for 15 days, then isoniazid plus rifampin twice weekly for a total of 9 months.

***: Isoniazid plus rifampin daily for 15 days, then the same drugs twice weekly for a total of 9 months.

According to the obtainable data from the patients who were followed up, the mean mortality rate was eight percent for all tuberculosis patients, the highest found in tuberculous meningitis (23%) (Table VII). Of the patients who died, two pulmonary tuberculosis patients had congenital heart disease and malnutrition, one pulmonary tuberculosis patient had chronic renal failure, one bone and joint tuberculosis patient had chronic renal failure, and two bone and joint tuberculosis patients had malnutrition.

Table VII: Distribution and Ratio of Tuberculosis Mortality

Site of Tuberculosis	Number of Patients	Number of Deaths	% of Deaths
Meningeal	537	124	23
Miliary	237	25	11
Abdominal	93	7	8
Skeletal	173	7	4
Pericardial	45	6	13
Pulmonary	937	5	0.5
Pleural	186	–	–
Lymph node	174	–	–
Skin	18	–	–
Genito-urinary	13	–	–
Total	2413	174	8

Discussion

The number of TB cases that were admitted to our hospital was 119 in 1972. This number increased to 168 in 1977 and decreased to 23 in 1992. During this period the total number of patients admitted to our hospital each year did not vary significantly, the mean being 135,000 patients. This decrease in TB cases over the years may be due to the diagnosis and treatment of the disease by peripheral health units. The overall incidence of TB in Turkey has also decreased in recent years. According to data from the Turkish Ministry of Health, the number dropped to 51/100,000 in 1988 from 108 in 1971[3-5]. The other change was in the pulmonary-extrapulmonary TB distribution. Between the years 1972 and 1981, the prevalences of pulmonary and extrapulmonary TB were 54 and 46 percent, respectively. Since 1982, there has been a decrease in pulmonary TB cases from 54 to 38 percent, while there has been an increase in extrapulmonary TB cases from 46 to 62 percent. Similar changes have been reported from other countries: while there is an increase in extrapulmonary TB, the incidence of pulmonary TB is decreasing[6-8].

Tuberculosis is seen relatively more frequently in early childhood and the elderly[8-10]. Sixty-two percent of our patients were less than six years of age. This number was also compatible with that observed in other countries[11-14].

Resistance to tuberculosis may be lowered by several factors, such as malnutrition, certain infections (measles, varicella, pertussis, HIV and certain other viral illnesses), corticosteroids and other immunosuppressive therapy, stress and hormonal changes. Twenty-two of our patients had malnutrition, 11 percent had a history of measles, and one percent had pertussis in the recent past. Bhakoo and colleagues[11] decribed a 22 percent incidence of measles, 18 percent pertussis and five percent incidence of varicella in 149 tuberculosis patients. In Hernandez and colleagues' study[15], the incidence of malnutrition was 82 percent in 277 meningeal tuberculous patients.

An important factor in the spread of the disease is the presence of undiagnosed cases. A history of contact with some one with TB was present in 35 percent of cases. In a previous study done in Turkey, a history of contact was found in 42 percent of patients[16]. Reports from other countries reported this figure at 80 percent[12], 60 percent[17] and 44 percent[18], and contact with TB has been stressed as an important factor in the spread of the disease. In India, where the incidence of tuberculosis is high, a history of contact was found to be 10-15 percent[11] and 30 percent[19]. The reason for this low rate of contact was thought to be due to concealment of the history of contact because of feelings of guilt expressed at the occurrence of disease in the family or because the presence of the disease was unknown[11]. It is important, therefore, that patients be persistently questioned about TB contact and that family members be examined for TB.

Tuberculosis may present with a variety of symptoms and physical examination findings. Symptoms and physical findings seen among our pulmonary tuberculous patients were similar to those of other reports[20-23]. Infants and young children with pulmonary tuberculosis were more likely to have symptoms such as cough, fever, weight loss, and wheezing or decreased breath sounds[14]. In our study, clinical findings, including signs and symptoms, played an important role in the diagnosis of tuberculosis.

The predominant radiological findings in pulmonary tuberculosis patients were lymphadenopathy (43%) and parenchymal infiltration (38%). In different studies, lymphadenopathy was by far the most common abnormality in pulmonary tuberculosis patients' x-ray findings (100% in the study by Abernathy and colleagues[23] and 93.5% in Pineda and colleagues'[24] study). In the study of Biddulph and colleagues[26] this finding was similar to that in our study at 53 percent of patients.

A positive culture was detected in 28 percent of pulmonary tuberculosis cases.

A positive culture, generally associated with low ratios in childhood tuberculosis, was reportedly detected in seven to 35 percent cases in previous studies[13, 26]. The positive culture rate was very low in extrapulmonary tuberculosis because of difficulties in obtaining samples.

The Mantoux test was positive in 62 percent of cases. In tuberculous meningitis 39 percent and in miliary TB only eight percent had positive Mantoux test reactions. Similar results have been reported in the medical literature: ppd was found positive in only 16 percent of patients with TB meningitis[9], and 41 percent in miliary TB[8].

Thirty-three percent of our patients had been vaccinated with BCG. In the literature, the protective efficacy of BCG vaccination is controversial and ranges from zero to 80 percent[27]. However, it has been stressed that in childhood the vaccination protects against serious complications tuberculous meningitis[3, 18, 27, 28].

We used intermittent antituberculous chemotherapy for a large patient group and found it to be as effective as daily treatment. The results of intermittent antituberculous chemotherapy in children have been reported as successful in a number of studies[23, 25, 29]. Relapse was observed in five patients in the late period, and two of them were immunocompromised. It is well known that poor compliance with therapy and immunosuppression play important roles in the response to antituberculous therapy and relapse. Serious side effects were not observed in any patient. The explanation for the rarity of side effects could be the use of drugs at low dosages and young age-groups.

The most serious complication that led to a fatal outcome was TB meningitis, with an incidence of 23 percent. The mortality associated with TB meningitis has been reported to be 12 to 72 percent[6, 23, 30]. The overall mortality rate in our series was eight percent. According to data released by the Turkish Ministry of Health, mortality due to TB is 3.68 per 100,000. While in the 1950s TB was the leading cause of death in Turkey, in 1987 it had dropped to 18th place[3, 28]. The reason for our high mortality rate may be the fact that complicated cases are referred to our hospital. Consequently, complications such as TB meningitis, are seen more frequently.

There has been a decrease in TB cases in our hospital over the last 21 years. The ratio between pulmonary and extrapulmonary involvement has shifted slightly towards extrapulmonary disease. Predisposing factors such as infectious diseases, malnutrition and contact with another person with TB play important roles in the occurrence and spread of the disease. With a mortality rate of eight percent, the prevention and control of TB are still important health concerns.

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HYPOGLYCEMIA IN SMALL – FOR – GESTATIONAL – AGE NEONATES*

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SUMMARY: Karahasanoğlu Ö, Karatekin G, Köse R, Nuhoğlu A. (Pediatric Clinics, Şişli Etfal Hospital, İstanbul, Turkey). Hypoglycemia in small-for-gestational-age neonates. Turk J Pediatr 1997; 39: 159-164.

In this prospective study, we investigated the frequency of hypoglycemia and the proper intervals for screening in small-for-gestational-age (SGA) neonates. We determined test-strip blood glucose values at two, three, six, 12, 24 and 48 hours of age in 25 SGA and 16 appropriate-for-gestational-age (AGA) infants who were born after 37 completed gestational weeks at the Obstetrics Clinics of Şişli Etfal Hospital. Serum glucose determination was immediately done if a test-strip value was less than 40 mg/dl. The frequency of hypoglycemia in SGA neonates was significantly higher (p: 0097) than in AGA neonates. The first three hours, the sixth hour and the 48th hour postnatally were the most common hours for encountering hypoglycemia. Clinical signs were not true indicators of hypoglycemia. These data suggest that screening for hypoglycemia in SGA neonates should continue for 48 hours. *Key words: hypoglycemia, small-for-gestational-age infants.*

Regardless of the presence or absence of abnormal clinical signs, neonatal hypoglycemia can cause damage to both neuronal and glial cells, resulting in severe handicap or death[1,2]. Small-for-gestational-age (SGA) neonates are at risk of developing hypoglycemia for reasons such as increased utilization of glucose, decreased gluconeogenesis, decreased stores of fat and glycogen, and inefficiency in converting fatty acids to ketone bodies[3-11].

Though screening for hypoglycemia in SGA infants is recommended using a recent definition of hypoglycemia, the frequency of hypoglycemia and the proper duration of screening have only been documented in one work[12]. The purpose of this study was to determine the frequency of hypoglycemia and the age at which it occurred in SGA infants, using Srinivasan et al's[13] definition of hypoglycemia.

Material and Methods

The study group consisted of 25 proportional SGA neonates, and the control group consisted of 16 appropriate-for-gestational age (AGA) neonates who were born at term between March 1, 1993 and July 15, 1994, at Şişli Etfal Hospital.

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Reasons not to include other term SGA neonates who were born during this time period are listed in Table I. Infants were followed in the neonatal unit or in mothers' rooms in the Gynecology Department at Şişli Etfal Hospital.

Table I: Criteria for Not Enrolling in the Study

Maternal Reasons	Reasons Concerning Infant
– Toxemia	– Meconium staining of amniotic fluid
– Diabetes	– Apgar scores < 7 at 5 minutes
– Anemia	– Polycythemia
– Hypertension	– Respiratory distress syndrome
– Prenatal infection	– Congenital anomalies
– Fetal distress	– Neonate treated with intravenous dextrose solution for reasons other than hypoglycemia
– Medication ingestion during the last four weeks of pregnancy	

Only infants who were 38 to 42 weeks gestational age were included. Assessment of gestational age was done using the Ballard Score[14]. Neonates below the 10th percentile for birth weight according to Denver Intrauterine Growth Charts[15] were accepted as SGA, and between the 10th and 90th percentiles were accepted as AGA.

Birth weight, birth length, head circumference, sex, gestational age, ponderal index, presence of a twin pregnancy and type of delivery were documented in each case.

Serial blood samples were drawn from each infant in the study and control groups at two, three, six, 12, 24 and 48 hours of age using a capillary-glucose-test-apparatus, Glucocard (GT-1610, Kyoto Daiichi Kagaku Co.). Serum glucose determination was immediately done if a test strip reading was less than 40 mg/dl[12]. Hypoglycemia was defined as a serum glucose less than 35 mg/dl at less than three hours of age, less than 40 mg/dl at three to 24 hours of age, and less than 45 mg/dl at greater than 24 hours of age[13].

Only infants who were followed in the neonatal unit for some minor problems were fed 66 kcal/100 ml infant formula; all other infants were breastfed. All infants were nursed or fed immediately after the first blood glucose measurement was performed in the second hour.

All infants were fed or nursed every three hours. Neonates were observed for the symptoms of hypoglycemia prior to the collection of samples. Lethargy, apnea, cyanosis, poor feeding, irritability, tremors, weak or high-pitched cry and seizures were accepted as symptoms of hypoglycemia. Neonates with low test-strip values were fed 10-15 milliliters of formula after venous blood samples were drawn. If there were symptoms or if hypoglycemia persisted after feeding,

treatment was started immediately with an intravenous infusion of 200 mg/kg dextrose (2 ml/kg dextrose water, 10% concentrated) given over one minute and followed by a continuous infusion of 8 mg/kg/min.

Statistical analyses were done using Fisher's test, chi-square analysis, Student's-t test and Mann-Whitney U test, and p values greater than 0.05 were accepted as insignificant.

Results

While 11 infants in the study group were found to be hypoglycemic (44%), only one infant in the control group was hypoglycemic (6.2%) ($p < 0.05$). No statistical difference could be found between the study and control groups in terms of sex, multiple gestations and type of delivery (Table II).

Table II: Comparison of Study and Control Groups*

	SGA Neonates (n: 25)	AGA Neonates (n: 16)	P Value
Hypoglycemia	11	1	0.0097
Twins	6	2	0.31
Sex	14 males 11 females	8 males 8 females	0.84
Type of delivery			
Cesarean section	5	1	
Vaginal delivery	19	15	0.32
Other	1		

*: Fisher test and chi-square analysis were used.

There was no significant difference between hypoglycemic SGA infants and euglycemic SGA infants in terms of sex and with seven females and four males in the former group, and five females and nine males in the latter group ($p > 0.05$). Seven of the hypoglycemic SGA infants and 13 of the euglycemic SGA infants were delivered vaginally ($p > 0.05$).

As shown in Table III, there was no significant difference between hypoglycemic and euglycemic SGA infants in terms of birth weight, birth length, head circumference, ponderal index or presence of twin pregnancy.

Blood glucose values of hypoglycemic SGA neonates at two, three, six, 12, 24 and 48 hours of age are shown in Table IV. The first six postnatal hours and the 48th hour were the most commonly encountered ages for hypoglycemia in SGA neonates. Five infants in the study group had symptoms of hypoglycemia. These symptoms included irritability (n: 4), tachypnea (n: 2), poor feeding (n: 2), cyanosis (n: 2), high-pitched cry (n: 2) and tremors (n: 1).

Table III: Comparison of Hypoglycemic and Euglycemic SGA Infants in Terms of Birthweight, Birth Length, Head Circumference, Ponderal Index*

	Euglycemic Infants (n: 14)	Hypoglycemic Infants (n: 11)	P Value
Birthweight (g)	2085,71 ± 358.1	2027,27 ± 216.06	> 0.05
Birth length (cm)	46.57 ± 2.24	45.54 ± 2.21	> 0.05
Head circumference (cm)	32.10 ± 1.53	31.68 ± 1.66	> 0.05
Ponderal index	2.13 ± 0.18	2.12 ± 0.46	> 0.05

* Mean ± SD, Student's-t test was used.

Table IV: Blood Glucose Values of Hypoglycemic SGA Neonates in the First 48 Hours of Life

Postnatal Age (hours)	n	Blood Glucose (mg/dl)
2	5	25, 30, 33, 34, 35
2	3	25, 34, 36
6	4	30, 35, 35, 38
12	2	35, 39
24	1	44
48	3	35, 38, 40

Discussion

In the present study, we found the incidence of SGA neonates born after 37 completed weeks to be 6.4 percent. Similar results have been reported by others[8, 17, 20].

The definition of hypoglycemia is controversial. There is a wide variation in the definitions given, ranging from a glucose concentration of < 18 mg/dl to < 72 mg/dl[1, 5, 13, 20-22]. The definition of Srinivasan and colleagues[13] was accepted in our study. The reported incidence of hypoglycemia in SGA infants, a high-risk group for hypoglycemia, ranges between 12.7 and 29 percent[12, 16, 23]. In our study the incidence of hypoglycemia in SGA infants was found to be 44 percent. This difference may be related to the fact that the other studies, except for one[12], did not screen or test for glucose as often as we did. In the above-mentioned exceptional study, the incidence of hypoglycemia in SGA neonates was found to be 14.7 percent using Srinivasan et al's[13] definition. This difference between the two works may be related to the difference between birthweights of SGA neonates. In Holtrop's[12] study, the mean birthweight was 2,498 ± 363 grams in hypoglycemic SGA neonates, and 2,512 ± 336 in euglycemic SGA neonates. In our study, the mean birthweight was 2,027 ± 216 grams in nypoglycemic SGA

neonates and 2,085 ± 358 grams in euglycemic SGA neonates. Lower birth weights of SGA babies lead to more decreased stores of fat and glycogen, resulting in more severe and frequent episodes of hypoglycemia.

In the present study, 54 percent of hypoglycemic SGA infants had no clinical symptoms. This confirms the results of previous reports[1, 2, 12, 24]. In the present study, hypoglycemia was reported in 65 percent of hypoglycemic SGA infants at six hours after birth. Holtrop[12] reported that the mean age at which hypoglycemia occurred was 6.1 hours in SGA neonates. Sexson[25] reported that hypoglycemia occurs more frequently between 0.5 to 12 hours after birth. In the present study, three infants were found to be hypoglycemic at 48 hours of age, and in two of these, blood glucose values were normal for the first 48 hours. Similar results were reported by Holtrop[12].

On the basis of our findings, we recommend that SGA neonates be carefully screened for hypoglycemia for at least 48 hours, and the first six hours of life should be accepted as the most critical hours. Clinical signs alone should not be accepted as indicators of hypoglycemia.

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STANDARDIZATION OF METHACHOLINE INHALATION CHALLENGE*

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SUMMARY: Şekerel BE, Saraçlar Y, Tuncer A, Adalıoğlu G, Çetinkaya F. (Allergy Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Standardization of methacholine inhalation challenge. Turk J Pediatr 1997; 39: 165-172.

Methacholine inhalation challenge has been shown to be an extremely useful diagnostic test. The purpose of this study was to document the reproducibility of methacholine inhalation challenge used in our clinic. In addition, we also examined the output of the delivery system, the stability of four-month-old methacholine solution, and the cumulative effect of methacholine.

To document reproducibility, two identical challenges were performed in each of 19 asthmatic children. The influence of the previous doses of methacholine on bronchial response was examined by performing a third challenge with a single dose of methacholine in ten of these children. The remaining nine children were also tested for the third time with four-month-old methacholine solution to examine the stability of that solution. The output of the delivery system was assessed by measuring the change in weight of the nebulizer. Responses to methacholine were highly reproducible within one doubling dose interval. There was a small but significant cumulative effect of methacholine. Comparable results were obtained with newly prepared methacholine solution and four-month-old solution. The variability of the output of the same nebuliser was less than that of different nebulisers of the same model.

The major clinical implication of our results is that our methacholine inhalation challenge procedure is standardized. This encourages more widespread use of this important diagnostic test for demonstration of airway hyper-responsiveness. *Key words: methacholine inhalation challenge, children, airway responsiveness, bronchial asthma.*

The narrowing of airways in response to various stimuli, such as methacholine, histamine, adenosine, exercise and cold, dry air, is called airway responsiveness (AR). In various respiratory problems, an individual's response to these agents may be increased; these individuals are said to have airway hyper-responsiveness (AHR)[1].

A quantitative assessment of the presence and degree of AHR can be made by measuring the response to standardized stimuli. Most tests are performed by

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administration of an agonist in the form of an aerosol followed by measurement of lung function. The most commonly used nonspecific agents are methacholine (Mch) and histamine[2].

Patients with asthma have AHR. Measurement of AR is widely used to confirm the presence of asthma, quantify the severity of the disease and assess the response to anti-inflammatory therapy[3].

This study was designed to define the short-term reproducibility of the methacholine inhalation challenge test used in our clinic. We also examined the cumulative effect of Mch and the stability of methacholine solution after four months of storage and described nebuliser output variability.

Material and Methods

Study Population

Nineteen asthmatic children currently being followed at the Pediatric Allergy Unit of Hacettepe University İhsan Doğramacı Children's Hospital participated in the study. All had episodic dyspnea, wheezing and reversible airway obstruction following bronchodilator therapy. At the time of study their symptoms were well controlled, and their forced expiratory volumes in one second (FEV1) were greater than 70 percent of their predicted values. FEV values did not vary by more than five percent on each study day. There was no history of asthma exacerbation or respiratory tract infection in the four weeks preceding the first study. Aerosol bronchodilators were withheld for 12 hours and oral bronchodilators for 24 hours before each study day.

Test Procedure

Our methacholine inhalation challenges were carried out by a method similar to that described by Cockcroft et al[4]. In each test an aerosol of saline was inhaled first, followed five-minute intervals by inhalation of methacholine in two-fold increasing concentrations, from 0.03 to 8 mg/ml. Salbutamol aerosol was administered to obtain recovery following completion of each challenge.

The aerosol was produced by a DeVilbiss 646 (Somerset, PA, USA) nebuliser which was operated by DeVilbiss air compressor (Pulmo-Aid, Somerset, PA, USA). On each occasion 4 ml of diluent or normal saline was placed into the nebuliser. The patient, wearing a nose clip, inhaled the aerosol through a mouthpiece for two minutes during tidal breathing. The same nebuliser was used for each challenge. The response, measured as the change in FEV_1 with the child in a standing position, was assessed using a dry rolling seal spirometer (SensorMedics 2130 Spirometer, California, USA). Initially, the forced expiratory maneuvers were repeated until three reproducible FEV_1 values were obtained.

After each inhalation, FEV_1 was measured at 0.5 and 1.5 minutes, and if necessary at three minutes to obtain the lowest post-inhalation FEV_1. The FEV_1 was measured only once on these occasions unless the measurement was technically unsatisfactory. Inhalations were continued until the post saline FEV_1 had fallen by 20 percent or more, or until a concentration of 8 mg/ml had been administered.

The responses were expressed in terms of the provocative concentration of methacholine required to produce a 20 percent fall in FEV_1 (PC20). This was obtained from the log dose response curve by linear interpolation of the last two points.

Solutions of methacholine were made from methacholine chloride powder and normal saline, as reported previously[5]. The prepared solution of 32 mg/ml methacholine was filtered using a 0.22 µm filter. Four ml of the solution was then placed in a sterile, single-use, black-covered vial and stored at 4 °C until the day of the challenge. To assess the stability of four-month-old Mch solution, ten of these vials were used in further challenges after four months. Thirty minutes before each challenge, the solution was diluted with normal saline and then placed in a sterile, single-use vial marked with a different color.

Study Design

The short-term reproducibility of the test was investigated in a single-blind manner by two identical challenges in each of the 19 patients. The challenges were performed at the same time on two of three days.

Nine patients, each with a PC20 greater than 0.25 mg/ml, underwent a third test to determine the cumulative dose effects of Mch. Initial saline inhalation was followed by inhalation of the final concentration of that individual's first Mch challenge. The PC20 obtained from a single-dose challenge of Mch [PC20(s)] was calculated by the following formula:

$$PC20(s) = \frac{\text{Concentration of Mch X 20}}{\%\ \text{fall in } FEV_1}$$

In the remaining ten patients, the original challenge was repeated for a third time (PC20(4m)) with four-month-old Mch solution to assess its stability following storage. PC20(4m) was then compared with the first challenge (PC20(1)).

The output of the three nebulisers (A, B and C) was determined as fallows: 4 ml of normal saline was placed in each nebuliser which was then operated with a DeVilbiss compressor (Pulmo-Aid, Somerset, PA, USA) for two minutes. Output was determined by measuring the resultant change in weight of the nebulises. The mean of trials was calculated to determine the individual variability of each nebuliser and the variability between nebulisers of the same model.

Statistical Analysis

The results of the methacholine inhalation challenges were analyzed after logarithmic transformation of each PC20 value. Spearmann rank correlation, Wilcoxon test and coefficient of variation were used for statistical analysis. A significance level of $p = 0.05$ was used.

Results

The age of the children ranged from seven to 16 years with a mean age of 11.37 ± 0.7 years. Each child underwent three challenges on three out of seven days. The first two were identical challenges to estimate the reproducibility of the test. A third challenge was performed in 10 children with four-month-old Mch solution stored at 4 °C, and in the remaining nine children with signle-dose Mch. The results of the challenges are shown in Table I.

Table I: Results of Methacholine Inhalation Challenges

Case No.	Age	Sex	PC20(1)	Pc20(2)	Pc20(s)	Pc20(4M)
1	16	F	0.11	0.18	0.22	
2	13	M	0.35	0.22	0.5	
3	13	M	3.1	3.07	5	
4	10	F	0.33	0.45	0.55	
5	10	M	3.4	2.3	3.63	
6	14	F	3.24	4.01	5.7	
7	11	F	1.2	1.75	2.9	
8	12	M	1.9	1.6	2	
9	14	F	0.59	0.7	1	
10	12	F	0.1	0.07		0.11
11	7	M	0.1	0.08		0.13
12	8	F	0.17	0.13		0.3
13	9	M	0.14	0.21		0.18
14	8	M	0.61	0.46		0.81
15	14	M	5.66	6.4		4.4
16	8	M	0.26	0.3		0.13
17	6	F	0.66	0.91		1.1
18	16	F	0.17	0.16		0.21
19	15	F	0.91	0.75		1.23

There was a high degree of reproducibility in response to methacholine [PC20(1) and PC20(2)] with a coefficient of determination (r^2) of 0.98. The measurements were within the tow-log-dose response, as shown in Figure 1. Inhalation of at least four doubling concentrations of methacholine at intervals of five minutes

produced a cumulative effect in every subject in that all the PC20 values obtained by the single-dose challenge [PC20(s)] were lower than the first individual PC20 values [PC20(1)], as shown in Figure 2. For the group the difference was significant (Wilcoxon test, $p < 0.05$).

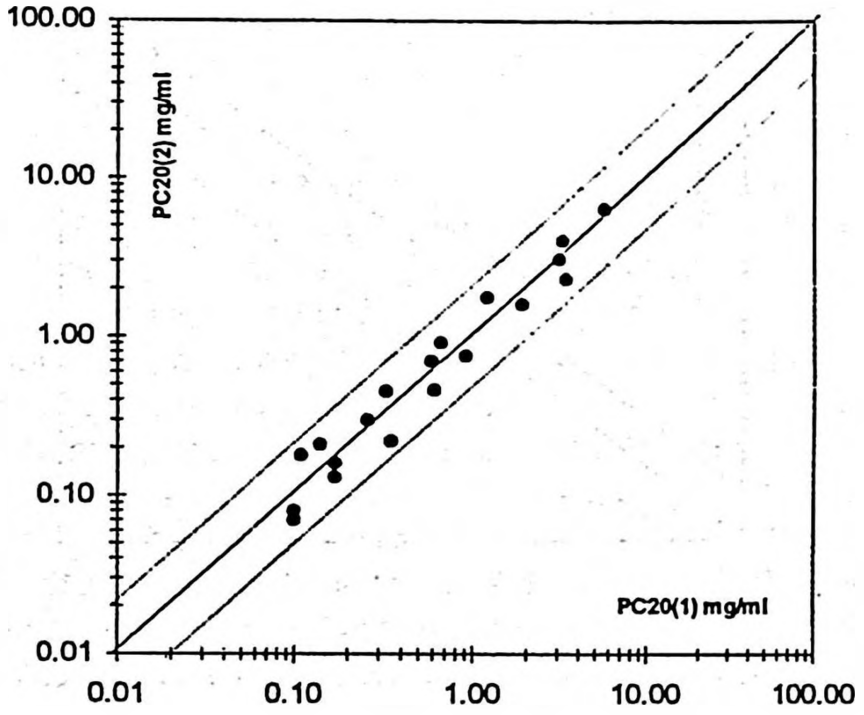

Fig. 1: Relation between the PC20 of the first and second tests. The solid line is the line of identity, and dotted lines indicate one doubling dose interval from the line of identity.

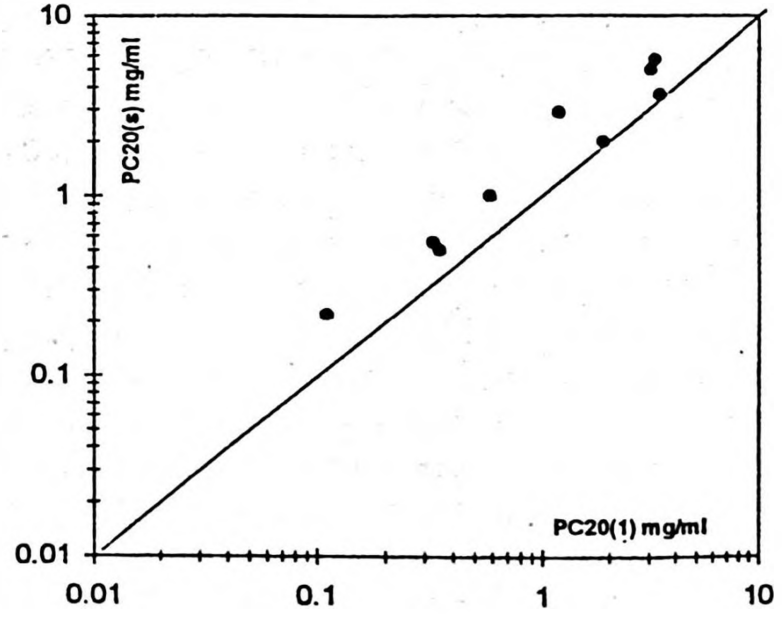

Fig. 2: Relation between the PC20 of the first test and the single-dose test. The solid line is the line of identity.

There was a significant coefficient of determination ($r^2 = 0.99$) between PC20 values obtained with fresh prepared [PC20(1)] and four-month-old [PC20(4m)] methacholine challenges. The two challenges are displayed in Figure 3. For the group the difference was not significant (Wilcoxon test, $p > 0.05$).

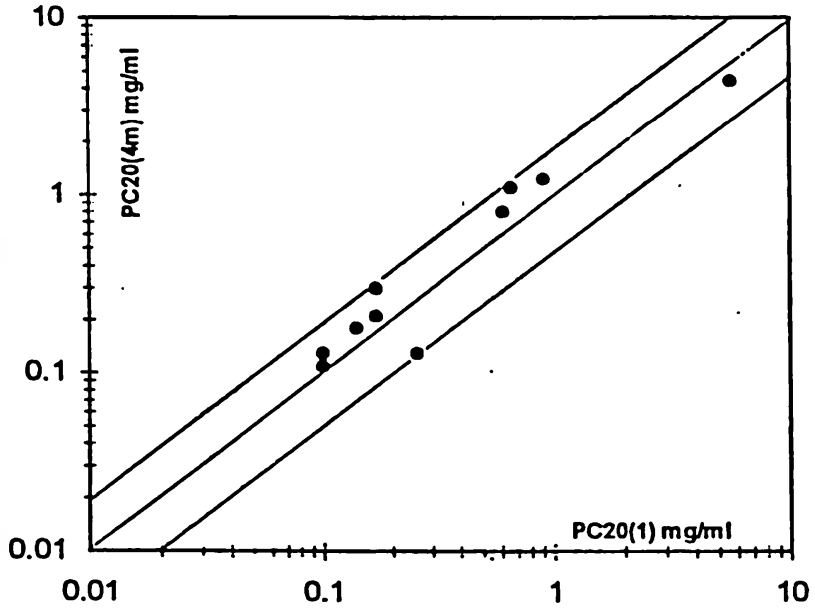

Fig. 3: Relation between the PC20 of the first and third tests with four-month-old Mch. The solid line is the line of identity, and the dotted lines indicate one doubling dose interval.

The aerosol outputs of DeVilbis 646 nebulisers, which were operated with a consistent compressor, are shown in Table II. The variabilities (coefficient of

Table II: Nebuliser Output

Nebuliser	Nebuliser Output (ml/min)	Mean ± SD (ml/min)
A	0.240 0.234 0.242 0.228 0.242	0.234 ± 0.009
B*	0.264 0.268 0.260 0.260 0.258	0.262 ± 0.008
C	0.272 0.266 0.270 0.270 0.272	0.270 ± 0.008

*Nebuliser used in all challenge tests.

variation) of different nebulisers from the same model were 3.75, 3.1 and 3.13 percent for nebulisers A, B, and C, respectively. The variation increased to 6.9 percent when all three nebulisers were taken into account.

The majority of children had no difficulty in complying with the test. There was only one child with technically unsatisfactory data because of a communication problem (data not included).

Discussion

For valid interpretation of test results (in this case the concentration of Mch producing a 20 percent decrease in FEV_1), it is essential to know the degree of variability within the involved test. To this end, we carried out a study on 19 asthmatic children by performing two identical Mch challenges to document the reproducibility of this test. Highly reproducible results at a one-doubling-dose interval were obtained, suggesting our procedure is technically standardized. The results of previous reports studying the variability of this method did not differ from ours[4, 6]. Aside from technical variables, individual variables also affect the results. Since subjects with mild to moderately severe asthma were included in our study, the variability might also be affected by individual variation, which suggests that our results may be applicable to clinical practice.

The dosimeter technique is the other method for measurement of AR. In this method, which was established by Chai et al[7], a dosimeter is used to deliver a consistent amount of agonist during the first 0.6 seconds of each of five inspiratory capacity breaths. It has been concluded that both tidal breathing and the dosimeter method are suitable for measurement of AR in children and adults[6, 8]. We prefer the tidal breathing method, since less cooperation and equipment (dosimeter) are required. The main disadvantages of our method are the following: first, the test takes more time than the dosimeter method, and second, it is not possible to be sure of the actual dose delivered to the patient. The results of the nebuliser output study demonstrate that the variation of output in each individual nebuliser was approximately four percent, increasing to seven percent when different nebulisers of the same model are used. As reported previously[9], nebuliser output must be known and kept constant to avoid becoming a variable in the inhalation test. Using different nebulisers of the same model may increase the variability of the test, so the results may not be interpreted as accurately. The output of nebulisers was also shown to be determined by the operating flow rates[9]. To eliminate this technical factor, we prefer to use an air compressor rather than a central air source. In the latter situation, the respiratory technician would adjust the air flow, and differences in this adjustment would increase the variation of the challenge tests.

Two other conclusions that merit comment emerged from our results. First, Mch has a cumulative effect. Therefore, shorter test protocols may increase the variability of the challenge when compared to a full protocol. Second, Mch solution of 32 mg/ml is stable after four months when refrigerated at 4 °C, which indicates that Mch solution needs to be made no more frequently than every four months. This finding is also in agreement with Pratter et al[10].

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INTERFERON – ALPHA THERAPY FOR REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDREN*

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SUMMARY: Yeşilipek MA, Yeğin O. (Hematology Unit, Department of Pediatrics, Akdeniz University Faculty of Medicine, Antalya, Turkey). Interferon-alpha therapy for refractory idiopathic thrombocytopenic purpura in children. Turk J Pediatr 1997; 39: 173-176.

Interferon (IFN)-alpha therapy was presented as a possible treatment for refractory immune thrombocytopenic purpura (ITP). We used 3 x 10^6 U recombinant IFN alpha, subcutaneously three times a week, every other day, for a total of 12 doses, in five children with refractory ITP. Three patients showed no response and were classified with Type III. One patient gave a partial response, and the other one had been in remission for 71 weeks as of this writing. This is the longest remission period reported for IFN therapy. The patients were classified Types IIb and I, respectively. The therapy was well tolerated. We conclude that further studies are to needed evaluate IFN-alpha therapy in childhood ITP. *Key words: interferon alpha, immune thrombocytopenic purpura, childhood.*

Acute idiopathic thrombocytopenic purpura (ITP) represents the most frequent hemorrhagic diathesis in childhood. ITP is an acquired thrombocytopenia accompanied by reduced platelet survival which is caused by premature degradation of the platelets by the reticuloendothelial cell system of the spleen or liver. The incidence of ITP is approximately one per 10,000 children. When the condition persists (about 10% of cases), with or without therapy, for more than six months, traditionally it is referred to as chronic ITP. It is well known that the risk of intracranial hemorrhage (ICH) is higher in chronic patients. The incidence of ICH increases with the passage of time, starting at 0.3 percent immediately after diagnosis, and increasing to two percent at 24 months[1].

Sometimes the management of chronic or refractory patients may fail with conventional regimens (steroids, immuneglobulins, splenectomy, ascorbic acid, mega-dose methylprednisolone, Anti-D, etc.) and may cause a clinical problem.

We studied the effect of IFN-alpha on children with refractory ITP who had failed to improve with conventional therapies.

Material and Methods

We used IFN-alpha in five children who had no response to previous ITP treatment modalities. The cases are summarized in Table I. All of the patients had the symptoms of petechiae, ecchymoses and epistaxis. Case 5 also had

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menorrhagia. Thrombocytopenia associated with increased immature megakaryocytes and absence of malign cells in the bone marrow were accepted as the criteria for the diagnosis of ITP. None of the patients had clinical or laboratory evidence of cytomegalovirus, Ebstein Barr virus, Hepatitis B virus infection or systemic lupus erythematosis. The treatment course consisted of 12 injections of 3 x 10^6 U recombinant IFN-alpha (IFN-alpha 2a for Cases 1, 4 and IFN alpha 2b for Cases 2, 3, 5) given subcutaneously three times a week, every other day. The responses are shown in Table II. We classified the responses according to a scale that has previously been described[2]. Type I indicates a complete response, with a return of the platelet count to greater than 200 x 10^9/L for longer than three months. Type IIa indicates a partial response with a platelet count of 30 to 200 x 10^9/L for longer than six weeks; Type IIb represents a partial response with platelets of 30 to 200 x 10^9/L for less than six weeks; Type III means no response with a static platelet count; and Type IV indicates worsening of the platelet count, with an increased clinical bleeding tendency.

Table I: Clinical Characteristics of the Patients

Patient	Sex	Age (yrs)	Time from Diagnosis to IFN Therapy (Months)	Previous Treatments (*)
1	M	13	30	P, D, G, M, S
2	M	2	8	P, G, M, Vit C
3	F	3.5	13	P, G, M
4	F	7	31	P, G, M, Vit C
5	F	15	22	P, G, M

(*) P: Prednisolone, D: Danazol, G: Gammaglobulin,
M: High-dose methylprednisolone, S: Splenectomy.

Table II: Response to IFN Treatment

Patient	Maximum PLT* Count (x10^9/L)			Time to Max Response (days)	Duration of Response (weeks)	Last PLT* Count (x10^9/L)	Response Type
	Pre IFN	During	Post				
1	8	64	500	21	71	250	I
2	9	150	57	–	–	57	IIb
3	19	29	28	–	–	25	III
4	46	64	60	–	–	60	III
5	6	16	19	–	–	15	III

* PLT: Platelet.

Results

The therapies were well tolerated. No response was detected during treatment in the first patient, except a rise on the fourth day of treatment for three days; however, after 21 days the patient's platelet count increased to 380 x 10^9/L, and he had been in remission for 71 weeks as of this writing. Case 2, whose response was classified as IIb, showed an increase in the platelet count after the third dose of IFN (9 to 150 x 10^9/L), but it decreased three days later. The Cases 3 and 4 showed minimal and Case 5 no response; thus the last three patients were classified as type III.

Discussion

Conventional therapies may fail in some children with immune thrombocytopenic purpura, (ITP). Because of the life-threatening risk of bleeding, new therapeutic agents are being investigated. Some authors have reported that (IFN-alpha) therapy may lead to remission in some, but not all, of these chronic or refractory patients[3-6].

Proctor et al.[6] demonstrated that short-course, low-dose IFN-alpha was useful in some cases of severe steroid-non-responsive ITP in adult patients. However, Hurtado et al.[5] reported that neither platelet count nor bleeding manifestations improved with IFN. A few studies have been reported in childhood, but there is still controversy over the use of IFN-alpha therapy in ITP.

Our first patient experienced an increase in the platelet count after therapy was completed. This finding is in contrast with previous reports in children, as platelet counts usually rise during the treatment[4]. The patient was still in remission after 71 weeks, as of this writing, which is the longest reported period in the literature for IFN-alpha therapy in chronic ITP. Spontaneous remission is another possibility that must be kept in mind in these patients.

The mechanism of action of IFN in ITP is unclear. There is evidence as to inhibitory effect of IFN on B-cell immunglobulin production[4]. The correlation between the low serum levels of IgG and PAIgG and increased platelet counts confirms this suggestion[7]. Hurtado et al.[5] found that IFN stimulates natural killer activity. Because these cells are responsible for antibody-dependent cell-mediated cytotoxicity, they suggested that it is probably the cause of lack of response.

Ishii et al.[8] administered six million units of IFN-alpha and compared the improved response rate to previous studies using three million units. No difference was found, and the authors concluded that a dose of six million units may be too large to treat some ITP patients.

In conclusion, IFN therapy may be one treatment choice for patients with refractory ITP because of its having fewer side effects and being safe for viral transmission. However, this therapy is still controversial, and the results vary

from center to center. Large collaborative trials in refractory patients are needed in order to clarify the treatment protocols and the possible value of IFN-alpha therapy in these special patients.

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TUMOR NECROSIS FACTOR – α AND INTERLEUKIN – 1β LEVELS IN CHILDREN WITH BACTERIAL, TUBERCULOUS AND ASEPTIC MENINGITIS*

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SUMMARY: Ceyhan M, Kanra G, Ecevit Z, Seçmeer G, Erdem G, Akan Ö, Müftüoğlu O. (Infectious Diseases Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Tumor necrosis factor-α and interleukin-1β levels in children with bacterial, tuberculous and aseptic meningitis. Turk J Pediatr 1997; 39: 177-184.

Cerebrospinal fluid levels of tumor necrosis factor-α and interleukin-1β in 78 children with nonbacterial, bacterial and tuberculous meningitis, and in 34 control subjects were analyzed in order to evaluate the involvement of these cytokines in the pathogenesis of acute bacterial meningitis and their discriminative value between different etiologies of meningitis. Tumor necrosis factor-α and interleukin-1β levels were significantly higher in bacterial and tuberculous meningitis than in aseptic meningitis and in control subjects ($p < 0.0001$). There was no difference in the levels of tumor necrosis factor-α and interleukin-1β between nonbacterial meningitis and control groups. The finding that both tumor necrosis factor-α and interleukin-1β are increased in the cerebrospinal fluid of patients with bacterial and tuberculous meningitis whereas normal levels of these two cytokines have been found in patients with nonbacterial meningitis signifies that these cytokines may be used to differentiate between bacterial and nonbacterial meningitis. *Key words: TNF-α, IL-1β, bacterial meningitis, aseptic meningitis.*

One of the most important advances in immunology in recent years is the understanding of the effects of some cytokines on inflammatory responses to infectious agents. Tumor necrosis factor alpha (TNF-α), which is produced mainly by the macrophages as a response to microbial or endotoxic stimuli, has been implicated in the pathogenesis of both local and systemic inflammatory reactions[1].

The importance of TNF-α has been established especially in fatal endotoxemia[2]. In experimental models, common physiological and pathological changes of gram-negative septicemia were observed after TNF-α administration[1,3]. TNF-α

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concentrations are also found to be increased in meningococcemia and meningococcal meningitis[4,5]. The mortality rate and neurologic sequelae have also been found to be higher in cases with increased concentrations of TNF-α[6].

TNF-α has been found to be responsible for at least some of the inflammatory events in bacterial meningitis in controlled studies, and dexamethasone administered before endotoxin was demonstrated to inhibit TNF-α secretion[7]. The important role of TNF-α in the pathogenesis of childhood meningitis has also been shown in clinical studies. TNF-α activity was demonstrated in 93 percent of 42 newborns with gram-negative meningitis in a study done by McCracken et al[3]. In another study, TNF-α levels were found to be increased in 75 percent of infants with bacterial meningitis[8]. Differences in these studies may originate from differences in the sampling time of cerebrospinal fluid (CSF) after the onset of symptoms and differences in the hosts' responses. There was no increase in TNF-α levels in nonbacterial meningitis cases in any of these studies. This finding may help to differentiate between bacterial and nonbacterial cases of meningitis in the presence of high TNF-α levels.

Another important mediator emphasized in the pathophysiology of bacterial meningitis is interleukin-1-beta (IL-1β). Like TNF-α, it is mainly produced by macrophages, but epidermal cells, synovial fibroblasts, lymphocytes, vascular endothelial cells, astrocytes and microglia can all secrete IL-1β[9]. IL-1β secretion can be stimulated by many factors, such as endotoxin in gram-negative bacterial cell walls, the teichoic acid component of the gram-positive bacterial cell wall, immune complexes, C5a and TNF[10].

In clinical studies, IL-1β levels have been detected in the CSF of 95 percent of cases with bacterial meningitis[9]. Although the degree of elevated CSF concentrations of IL-1β was much lower than that of bacterial meningitis, IL-1β concentrations were also found to be elevated in 88 percent of nonbacterial meningitis cases[11]. IL-1β activity was not detected in control subjects without meningeal inflammation. Dexamethasone also inhibits IL-1β secretion, and some of its anti-inflammatory effect is attributed to this inhibition[12,13]. Inexpensive and rapid assays for these two cytokines may help to distinguish between bacterial and nonbacterial causes of meningitis[14].

The etiologies of both bacterial and nonbacterial meningitis are different in Turkey than in developed countries; pneumococcus pneumonia is the major pathogen in bacterial meningitis, and most nonbacterial meningitis cases are due to mumps[15]. Mycobacterium tuberculosis poses a great problem in distinguishing tuberculous meningitis from other causes of bacterial meningitis. TNF-α and IL-1β are thought to be involved in the immune response to mycobacterial infection because they exhibit antimycobacterial effects in vitro; moreover, the mycobacterium tuberculosis cell wall component lipoarabinomannan,

mycobacterial heat shock protein, and M.tuberculosis culture filtrate devoid of contaminating lipopolysaccharide are reported to stimulate the production of TNF-α and IL-1β[16, 17]. TNF-α levels were found to be moderately increased in tuberculous meningitis in only one clinical study[14]. Still, the importance of these cytokines in tuberculous meningitis must be evaluated.

Material and Methods

Patients: Patients between the ages of one month and 15 years were evaluated in four study groups:

Group 1: Bacterial meningitis cases: Symptoms indicative of meningitis and fulfilling at least one of the following two criteria[18]:

1. Bacterial isolation and/or latex agglutination.
2. At least one of the following, if no pathogen were isolated:
   a) White blood cell (WBC) greater than 2,000 or polymorphonuclear leukocyte (PMNL) count greater than 1,180 per cubic milimeter of CSF.
   b) CSF protein greater than 220 mg/dl,
   c) CSF to blood ratio of glucose less than 0.25.

Group 2: Tuberculous meningitis cases: Cases with symptoms indicative of meningitis and routine CSF laboratory tests suggesting bacterial meningitis and detection of acid-resistant bacilli on Ziehl-Neelsen stain and/or culture-positive cases; or, cases with a positive family history for tuberculosis who were non-responsive to conventional antimicrobial therapy who had a clinical cure after anti-tuberculous therapy and who had lymphoycte-predominant CSF pleocytosis, hypoglycorrhachia and blockage of CSF circulation demonstrated by cisternography or other imaging techniques.

Group 3: Nonbacterial meningitis cases: Cytochemical values of CSF suggesting meningitis with normal CSF glucose and negative bacteriological tests (culture, gram stain, conventional antigen testing) and cases that had not received antimicrobial therapy during the previous two weeks.

Group 4: Control subjects: Cases with normal CSF findings. These cases were the patients whose clinical findings indicated the need for lumbar puncture to exclude the diagnosis of meningitis in the Emergency Clinic.

Laboratory Studies: Leukocyte counts, protein and glucose levels in CSF, CSF to blood ratio of glucose, gram-stained smears of CSF and routine cultures were done in all cases. Latex agglutination tests for pneumococcus, Haemophilus influenzae type b and meningococcus in the CSF were done in all patients. CSF samples were stored at -40 °C until analysis.

Detection of TNF-α and IL-1b: The levels of TNF-α and IL-1β in the CSF samples obtained during the diagnosis were evaluated. TNF-α concentrations were measured by an ELISA kit (Cistron TNF-α kit) with a sensitivity of ≤ 20 pg/ml. First, the wells were coated with 100 µL standard, control and test sera and incubated for two hours at 37 °C; the wells were then washed three times with washing buffer. 100 µL TNF-α antisera was then added to each well, incubated for two hours at 37 °C, and the washing was repeated three times. 100 µL anti-Ig G-HRP conjugate was added to each well, incubated for 30 minutes at room temperature and washed again. 100 µL substrate was then added to each well and incubated at room temperature without closing the tips of the wells. 50 µL of 4N sulfuric acid was added to each well, and the OD was read at 490 nm. The optic density values were averaged to obtain the mean values and their standard errors. Titers were expressed as mean concentration ± standard error for each group.

IL-1β levels were detected by an ELISA kit (Cistron TNF-α kit) with the same sensitivity. The same procedure was repeated, but instead of TNF-α antisera, IL-1β antisera were used.

Statistical Analysis: Student's t-test and Mann-Whitney U tests were used to compare the groups with respect to TNF-α and IL-1β levels.

Results

A total of 112 patients were analyzed; 38 of the patients had bacterial and 36 had nonbacterial meningitis. Since the incidence was low, we found only four cases with tuberculous meningitis. The remaining 34 patients were the control subjects. The ages of the patients ranged from two months to 13 years in the control group (mean 3.42 years), one month to 14 years in the nonbacterial meningitis group (mean 4.14 years), three months to 15 years in the bacterial meningitis group (mean 3.65 years), and three to 11 years in the tuberculous meningitis group (mean 6 years).

The CSF findings of the patients are sumarized in Table I. S. pneumoniae was isolated in seven patients, N. meningitidis in five patients and H. influenzae in two patients. A diagnosis of bacterial meningitis was suggested by CSF cytochemistry in 24 patients.

Table I: Cytochemical Findings of the CSF of Patients

	Groups			
	Control	Nonbacterial m.*	Bacterial m.	Tuberculous m.
Cell count (/mm^3)	0.82 ± 0.61	82.71 ± 72.20	175.60 ± 233.12	429.00 ± 148.94
PMNL (/mm^3)	0	31.08 ± 52.17	141.11 ± 158.44	71.50 ± 82.56
Lymphocyte (/mm^3)	0.82 ± 0.61	51.63 ± 48.36	34.49 ± 27.63	357.50 ± 163.28
Protein (mg/dl)	32.28 ± 17.41	79.95 ± 43.18	103.75 ± 43.07	169.25 ± 104.16
Glucose (CSF/blood)	0.81 ± 0.23	0.69 ± 0.17	0.36 ± 0.23	0.17 ± 0.08
Culture positivity	–	–	14/38**	–

* m.: meningitis.

** P.: pneumonia was isolated in 7 patients, N. meningitidis in 5 patients and H. influenzae in 2 patients.

TNF-α levels are summarized in Table II. The high standard deviation values were due to the significant differences between the cases. In the bacterial and tuberculous meningitis groups, the concentrations were significantly higher than in the control and nonbacterial meningitis groups. The TNF-α levels of the control and nonbacterial meningitis groups did not differ significantly ($p = 0.39$). The TNF-α levels were significantly higher in the bacterial and tuberculous meningitis groups than in the control and nonbacterial meningitis groups ($p < 0.0001$). There was no statistically significant difference in TNF-α levels between the bacterial and tuberculous meningitis groups ($p = 0.39$).

Table II: TNF-α Levels in the CSF

Groups	n	TNF-α (pg/ml) Mean ± Standard Deviation (Range)	p		
Control	34	0.57 ± 1.08 (0-4.36)	0.39	< 0.0001	< 0.0001
Nonbacterial meningitis	36	0.79 ± 1.09 (0-4.60)			
Bacterial meningitis	38	76.41 ± 58.10 (12.01-246.30)			0.39
Tuberculous meningitis	4	47.13 ± 16.78 (36.4-71.60)			

IL-1β levels were correlated with TNF-α levels in all groups (Table III). IL-1β levels were not significantly different between the control and nonbacterial meningitis groups ($p = 0.39$), but were significantly higher in the bacterial and tuberculous meningitis groups than in the control and nonbacterial meningitis groups ($p < 0.0001$). There was no statistically significant difference in IL-1β levels between the bacterial and tuberculous meningitis groups ($p = 0.73$).

Table III: IL-β Levels in the CSF

Groups	n	IL-β (pg/ml) Mean ± Standard Deviation (Range)	p		
Control	34	0.42 ± 0.58 (0-1.90)	0.39	< 0.0001	< 0.0001
Nonbacterial meningitis	36	1.04 ± 3.02 (0-17.90)			
Bacterial meningitis	38	54.91 ± 27.98 (18.20-148.20)			0.73
Tuberculous meningitis	4	61.22 ± 40.43 (28.91-118.12)			

The lowest levels of both TNF-α and IL-1β in the bacterial and tuberculous meningitis groups were significantly higher than the highest levels of both the control and nonbacterial meningitis groups. The highest TNF-α and IL-1β concentrations of the control and nonbacterial meningitis groups were 4.60 and 17.90 pg/ml, respectively, and the lowest values of the other two groups were 12.01 and 18.20 pg/ml, respectively.

Discussion

The role of TNF-α and IL-1β in the pathogenesis of meningitis has been demonstrated by many studies in the recent years. This progress has led to an understanding of the pathogenesis of bacterial meningitis, the usage of these cytokines in the diagnosis of meningitis and new modes of therapy aiming to inhibit the synthesis and secretion of these cytokines.

TNF-α and IL-1β concentrations are found to be increased in the CSF sampled just after the onset of meningitis symptoms[3, 6, 8, 19, 20]. We also observed that both TNF-α and IL-1β levels were increased in the bacterial meningitis group, whereas they were normal in children with nonbacterial meningitis and in control subjects. The increase in these two cytokines is an intermediate step in the CSF inflammation triggered by the teichoic acid in the gram-positive bacterial cell wall and the lipooligosaccharide in gram-negative bacteria. These components of bacteria first activate the natural killer cells, especially the macrophages, and then the Kuppfer cells, CNS astrocytes and glial cells; these cells synthesize TNF-α in response. TNF-α in return increases the synthesis of IL-1β by stimulating macrophages, endothelial cells, astrocytes and glial cells[21, 22]. TNF-α and IL-1β are in part responsible for the cytochemical findings of bacterial meningitis and some of its complications, such as hearing loss, by their direct effects and by increasing the synthesis of prostaglandins, platelet-activating factor and other inflammatory mediators[23].

Despite some studies demonstrating an increase in the secretion of TNF-α by blood mononuclear cells during viral infections, no increase in TNF-α levels were found in clinical studies of nonbacterial meningitis[20, 24-26]. In our study there was no significant difference between the CSF TNF-α levels of nonbacterial meningitis and control subjects.

We found that the lowest values of both mediators in the bacterial meningitis group were higher than the highest of the nonbacterial meningitis and control cases, indicating 100 percent specificity in distinguishing bacterial and nonbacterial cases. This specificity is higher than in the other reported studies[3, 8, 20].

Helminen et al.[27] showed that IL-1β levels were increased in bacterial meningitis but not in nonbacterial meningitis cases. Viruses also have an inhibitory effect on IL-1β synthesis[28, 29]. IL-1β levels were significantly higher in our bacterial meningitis group, confirming the former studies. The finding of high IL-1β levels in two patients with nonbacterial meningitis in a former study can be explained by ythe late sampling of CSF in these patients[20].

The exact role of inflammatory mediators in tuberculous meningitis is still unclear. One of the aims of this study was to determine the roles of TNF-α and IL-1β in tuberculous meningitis. The levels of both TNF-α and IL-1β were found to be

elevated in these cases, as in cases of bacterial meningitis. It was demonstrated that the level of TNF-α produced by monocytes from patients with pulmonary tuberculosis was related to the disease state of pulmonary tuberculosis; monocytes from patients with chronic refractory tuberculosis released significantly lower amounts of TNF-α[30]. The moderate increase in TNF-α in tuberculous meningitis cases in the present and former studies may be due to this fact. The number of cases is too low to draw a conclusion, but the findings suggest that tuberculous meningitis must also be considered in cases with increased CSF levels of TNF-α and IL-1β.

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NEONATAL SEPTICEMIA IN A NEONATAL INTENSIVE CARE UNIT*
Results of Four Years

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SUMMARY: Samancı N, Ovalı F, Akdoğan Z, Dağoğlu T. (Department of Gynecology and Obstetrics and the Neonatology Unit, İstanbul University Faculty of Medicine, Çapa-İstanbul, Turkey). Neonatal septicemia in a neonatal intensive care unit. Results of four years. Turk J Pediatr 1997; 39: 185-193.

The outcome of congenital and nosocomial septicemia has been documented in infants who were admitted to a neonatal intensive care unit over a four-year period. The overall incidence of neonatal septicemia in the neonatal intensive care unit was 5.4 percent. Common causes of neonatal septicemia were gram-negative bacilli and staphylococci. Gram-positive microorganisms were the major causative agents for early-onset septicemia. Since the most common pathogen in cases of nosocomial sepsis was gram-negative bacillus, higher mortality rates were observed in nosocomial sepsis. The overall mortality rate in neonatal sepsis was 44.2 percent. The mortality rate in infants in whom nosocomial septicemia developed was significantly higher than in the infants in whom early-onset septicemia developed. However, as gram-negative colonization of the nursery recently changed to gram-positive microorganisms, the mortality rate is hoped to decrease. *Key words: neonatal septicemia, nosocomial infection, early-onset septicemia.*

The incidence of neonatal sepsis is between one and 10 cases per 1000 live births, and almost four-fold that in cases of preterm infants; the incidence is higher (1-5%) for infants born to mothers with chorioamnionitis[1,2]. Despite the increasing use of newer antibiotics, the mortality rate in neonatal sepsis remains 20 to 75 percent[1].

The two principal sources of newborn infection are the mother and the nursery environment. Infection is acquired from the mother transplacentally at the time of delivery or in the postnatal period[3,4]. The infections that develop in the first 48 hours of life (early-onset) are not acquired in the nursery. Such infections are defined as congenital infection or early-onset sepsis. Infections occurring after this time are defined as nosocomial (nursery-acquired)[2,3].

The reported incidence of nursery-acquired infections varies considerably and is affected by multiple factors such as nursery personnel, respiratory equipment, sinks and incubators. The most important risk factor for neonatal sepsis is low

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birth weight; others include prolonged hospitalization, invasive procedures, placement of indwelling devices and overcrowded nurseries[2-4].

The overall incidence of nursery-acquired infection is reportedly 1.4 percent, but is considerably higher in neonatal intensive care units, ranging from five to 30 percent[4-6]. This incidence varies from nursery to nursery and depends on conditions predisposing to infection.

In the present report, we evaluated the frequency of congenital and nosocomial infections in our neonatal intesive care unit (NICU) during a four-year period, the influence of birth weight on infection frequency, and the association of nosocomial infection with infant mortality.

Material and Methods

Between January 1, 1991 and December 31, 1994, 3120 newborns were admitted to the neonatal intensive care unit. All of the infants were born at the Department of Gynecology and Obstetrics of İstanbul University Faculty of Medicine. In the neonatal intensive care unit, three rooms were designated as level I, level II and level III. Indications for admission to the neonatal intensive care unit included low birth weight, prematurity, respiratory distress syndrome, aspiration of meconium, resuscitation in the labor ward, the presence of congenital anomalies, hyperbilirubinemia, perinatal asphyxia, infants of diabetic mothers, prolonged rupture of membranes and maternal peripartum infection. During this period, 2.824 preterm infants were admitted to the NICU.

For each infant enrolled in this study, the following information was recorded: maternal obstetric history, evidence of maternal infection, history of prolonged rupture of membranes (longer than 24 hours), mode of delivery, APGAR score, gestational age, birth weight, isolated organism, the onset of sepsis and its outcome. The incidence of infection, isolated microorganisms, complications and mortality rates were evaluated. Gestational age was assessed by the method of Dubowitz et al.[7] or by maternal dates. Neonates were defined as premature if the gestational age was less than 37 completed weeks. Perinatal asphyxia was defined as a five-minute Apgar score of less than seven. Each infant was examined by a neonatal fellow.

Premature rupture of membranes or maternal peripartum infection as well as resuscitation in the labor ward were accepted as risk factors, and blood cultures were obtained from these infants after delivery.

When sepsis was suspected clinically, initial tests performed included complete blood cell counts (CBC), differential leukocyte counts, C-reactive protein (CRP) and peripheral blood cultures. Other investigations were done when clinically indicated, included gastric aspirate, urine and spinal fluid microscopy and culture,

tracheal aspirate culture, stool culture, and chest or abdominal radiographs. On completion of the inital investigation for infection, antibiotics were administered intravenously. Ampicillin and an aminoglycoside were combined to treat suspected early-onset septicemia or meningitis before identification of the pathogen. The diagnosis of sepsis was made when there were positive findings on peripheral blood culture. Most infants with septicemia present with nonspecific signs and symptoms. The presence of one or more clinical features, such as lethargy, temperature instability, increased gastric aspirate, glucose intolerance or hepatosplenomegaly are diagnostic signs and symptoms. A ratio of immature to total neutrophils of greater than 0.2, white cell count of either less than 5 x 10^9/L or greater than 30 x 10^9/L, platelet count of less than 50 x 10^9/L and CRP levels greater than 1 mg/dl are positive laboratory results[8] for diagnosing neonatal septicemia in infants with clinical features.

Generally antibiotic therapy was started in infants with risk factors for infection. Also, antibiotics were discontinued within three days if the neonatal fellow considered infection to be unlikely or blood cultures were sterile. If sepsis was diagnosed, the most susceptible antibiotics were selected. Patients responding to antibiotic therapy were treated for 2-3 weeks or at least 10 days from the time of the first negative blood culture.

If sepsis was suspected clinically, lumbar puncture was performed immediately. Interpretation of biochemical values of cerebrospinal fluid (CSF) in newborn infants was difficult. Any one or a combination of the following CSF values was considered positive for meningitis: more than 32 leukocytes/mm^3, of which more than 60 percent were polymorphonuclear cells; CSF glucose less than 50 to 75 percent of a simultaneous serum glucose; protein greater than 150 mg/dl; and microorganisms on gram-stained smears of CSF[9]. The diagnosis of meningitis was made when there were positive findings in biochemical values and CSF culture. If meningitis was diagnosed, cranial ultrasonography was performed to detect ventriculitis. Ampicillin and cefotaxime were combined for initial empiric therapy of neonatal meningitis. After the pathogen was identified and its antimicrobial susceptibilities were known, the most appropriate drug or drugs were selected. Therapy for meningitis was continued for a minimum of two weeks after sterilization of CSF cultures.

Since the incubation periods of infections occurring in the neonate are not well defined, it was sometimes difficult to determine whether an infection was perinatally or nosocomially acquired. Most infections that develop in the first 48 hours of life (i.e., early-onset)ore not nosocomial, and those developing after this interval are increasingly likely to be nosocomial.

Values are expressed as mean ± SD. Statistical analyses were performed using chi-square and the Fisher exact test. The results of statistical analysis were considered significant at $p < 0.05$.

Results

During four years of retrospective study, neonatal sepsis was diagnosed in 167 infants. Sepsis was confirmed by blood cultures in 141 cases (84.6%). In 26 cases (15.4%), sepsis was diagnosed by laboratory results. The mean gestational age was 32.4 ± 2.3 weeks (25-43 weeks), and birthweights were 1,762 ± 581 g (730-4,250 g) in infants with neonatal sepsis. Forty percent (67 infants) were female and sixty percent (100 infants) were male. A total of 27 infants (16%) had neonatal asphyxia. The type of delivery in cases with sepsis was vaginal in 78.2 percent and cesarean section in 21.8 percent. The overall incidence of prolonged rupture of membranes was 2.7 percent. One hundred and fifty-three infants were premature (91.6%) and 14 infants (8.4%) were mature. The overall incidence of sepsis was 5.4 percent in the neonatal intensive care unit. The incidence of neonatal sepsis in premature infants was 5.4 percent. The incidence of neonatal sepsis according to birth weight is shown in Table I. That total infection rate in the infants with birthweights of less than 1500 g was significantly higher than that of the larger infants ($p < 0.05$) (Table I).

Table I: Neonatal Sepsis in the Unit According to Birth Weight

Birth Weight	No. of Infants	No. of Infants with Infection	Incidence (%)
< 100 g	178	19	10.6
1000-1499	530	83	15.7
1500-1999	786	41	5.2
2000-2499	1192	18	1.5
> 2500	434	6	1.4
Total	3120	167	5.4

Forty-three infants had early-onset sepsis. The remaining 124 infants were defined to have nosocomial sepsis. The nosocomial sepsis rate in the neonatal intensive care unit was four percent. Meningitis was diagnosed in 16 cases. The frequency of pathogenic organisms in infants with neonatal sepsis and meningitis is shown in Table II. The most common etiologic agents isolated from the cases of septicemia were gram-negative enteric bacilli and Staphylococcus aureus. Gram-negative microorganisms were common causes of sepsis in ventilated neonates; five cases were diagnosed in ventilated neonates.

The risk factors for early-onset sepsis, such as prolonged rupture of membranes (PROM), maternal chorioamnionitis and meconium aspiration syndrome, were evaluated. Thirty-two cases had PROM, 11 cases had maternal amnionitis and 12 cases had meconium aspiration (12 of the 43 infants with early-onset sepsis had > 1 risk factor).

A total of 16 patients had sepsis plus meningitis. Ventriculitis and hydrocephalus developed in four cases (25%) with meningitis. The isolated microorganisms are shown in Table II. The most common etiologic agents were gram-negative bacilli.

Table II: Frequency of Pathogenic Organisms in Neonatal Sepsis and Meningitis

Organism	Early-Onset Sepsis	Nosocomial Infection	Cases of Meningitis
Klebsiella pneumoniae	7	39	9
Staphylococcus aureus (MRSA)	14	16	2
Coagulase (-) staphylococci	8	17	2
E.Coli	2	2	2
K.Oxytoca	2	8	
Enterobacter	3	9	1
Group B streptococcus	4	–	–
α-hemolitic streptococcus	3	5	–
Acineto bacter	–	2	–
Total	43	98	16

A total of 43 cases of early-onset sepsis were diagnosed, with gram-positive microorganisms being the most common pathogens isolated from cultures. The most common etiologic agents isolated from the cases of nosocomial sepsis were gram-negative bacilli.

In cases of septicemia, 74 infants (44.3%) died and 93 infants (55.7%) survived. The overall mortality rate in neonatal sepsis was 44.2 percent. The mortality rates in neonatal sepsis and meningitis are shown in Table III. The mean gestational age, birth weight and duration of hospitalization of the mortality and survival groups are shown in Table IV. Smaller infants (birth weight less than 1500 g) had increased infection and mortality rates (Table V). The total nosocomial infection rate in the infants with birth weights of less than 1500 g was significantly higher than that in nthe infants greater than 1500 g at birth ($p < 0.05$). The mortality rate in infants in whom neonatal septicemia of any type developed was significantly higher than that of the infants in whom such infections did not develop (Table V). The overall mortality rate in the neonatal intensive care unit was 15.1 percent.

Table III: Mortality Rates in Neonatal Septicemia and Meningitis

	No. of Infants	No. with Mortality
Early-onset sepsis	43	11 (25%)
Nosocomial infection (late-sepsis)	124	63 (50.8%)
Meningitis	16	9 (56%)

Table IV: Epidemiologic Characteristics in Neonatal Sepsis

	Survival Group	Mortality Group
Mean gestational age (weeks)	32.8 ± 2.4	29.8 ± 2.1
Mean birth weight (g)	1874 ± 595	1382 ± 208

Table V: Mortality Rates According to Birth Weight

	With Neonatall Sepsis		Without Neonatal Sepsis	
Birth Weight (g)	No. of Infections	Mortality (%)	No. of Infants	Mortality (%)
< 1000	19	79	159	71
1000-1499	83	54	447	38
1500-1999	41	24	745	17.2
2000-2499	18	17	1174	5.4
> 2500	6	17	418	0.6

The mortality rate (50.8%) in infants in whom nosocomial septicemia of any type developed was significantly higher than the rate (25%) in cases where early-onset sepsis developed ($p < 0.05$).

The average duration of hospitalization for the total infants admitted to the neonatal intensive care unit was 13.2 days. In the infants who developed nosocomial infection, the average duration of hospitalization was 22.1 days. We were unable to determine the precise role of nosocomial infection in prolonging the duration of hospitalization because of the presence of other risk factors in the infants with long hospital stays (e.g. lower birth weight, respiratory distress syndrome, perinatal asphyxia).

Discussion

Infections are significant causes of mortality in neonates, especially for premature infants of very low birth weight. The two principal sources of newborn infection are the mother and the nursery environment. Prematurity is a common risk factor for neonatal sepsis in both early-onset and nosocomial diseases[1, 10-12]. This increased susceptibility to infection correlates with the immaturity of the premature infant's immune system, including diminished humoral and cellular responses and decreased transplacentally acquired IgG. Environmental factors in the nursery affect the risk of acquiring a nosocomial infection. Prolonged hospitalization in neonatal intensive care units predisposes infants to colonization with the potentially pathogenic gram-negative bacterial flora of the nursery and possible subsequent infection[13]. Invasive procedures and indwelling foreign bodies increase the risk of nosocomial infection in newborns[3].

In infants weighing less than 1000 g at birth, the reported incidence of sepsis is 50 percent or higher, and in infants weighing 1000-1500 g at birth the reported incidence of sepsis is 12-14 percent[2-4, 6]. We observed similar results except in infants weighing less than 1000 g at birth. We suggested that high numbers of extremely low birth weight babies had been lost in the first few days because of asphyxia, intraventricular hemorrhage and respiratory distress syndrome.

In NICUs, common causes of neonatal septicemia include gram-negative bacilli, staphylococcus aureus and coagulase-negative staphylococci. A common characteristic of nosocomial, gram-negative, enteric pathogens in newborn nurseries is antibiotic resistance. In addition, colonization and infection with methicillin-resistant S.aureus in the NICU have occurred (Table II). In recent years, a leading cause of nosocomial infections has been coagulase-negative staphylococci. Other nosocomial pathogens in low-birth weight infants in NICUs have been fungi, a finding not observed in the present study.

We observed that the gram-negative, antibiotic-resistant bacilli (particularly K. pneumoniae) were still playing the greatest role in nosocomial infections, followed by staphylococci. The main source of gram-negative infection is the nursery environment. We observed a total of four cases of group B β-hemolytic streptococcus, all infants with early-onset septicemia. We found that gram-positive microorganisms (S. aureus and coagulase-negative staphylococci) were the major causative agents (68%) of early-onset septicemia.

Factors that appear to contribute to the high rate of nosocomial infection in the unit may be considered under two categories: host factors and environmental factors. The most important host factor for nosocomial infection is low birth weight[2-4]. The nosocomial infection rate in infants with birth weights of less than 1500 g was approximately 5.5 times that in larger infants. The mortality rate in early-onset septicemia was lower than in nosocomial sepsis. Since the most common pathogens in cases of nosocomial sepsis were gram-negative bacilli, higher mortality rates were observed in nosocomial sepsis[4, 11, 14, 15]. We estimated the incidence of septicemia in the NICU to be 5.4 percent, and the nosocomial infection rate was for percent. The infection rates were lower than those in other published works[4-6, 14, 16].

The complications of neonatal sepsis include focal infection and meningitis. The mortality rate was high in infants with sepsis plus meningitis.

Amnionitis was a significant problem. Eleven of the neonates with early-onset sepsis had maternal amnionitis. The statistically significant relationship between maternal amnionitis and sepsis was previously demonstrated in our hospital[17].

The outcome of neonatal infections can be improved if illness is recognised early and appropriate antimicrobial agents are promptly administered[4]. The mortality

rate in neonatal sepsis has been reported as 12 to 30 percent[4, 11]. In our study, the overall mortality rate was 44.2 percent. Since gram-negative, antibiotic-resistant, enteric bacilli were the predominant organism in cases of neonatal sepsis, the mortality rate was high. In cases of early-onset septicemia, the mortality rate was 25 percent. Since gram-positive organisms were predominant in this type of neonatal infection, the mortality rate was lower than in nosocomial sepsis[4, 11].

Our data emphasize the clinical importance of nosocomial infections in a patient population in a NICU. Although we have continually stressed the need for good handwashing technique, appropriate use of isolation procedures and the minimal handling method, we observe nosocomial infection frequently. However, the nosocomial infection rate in our unit was not high compared to that in the literature. The main problem was gram-negative infections two years ago. The colonization of the nursery changed to gram-positive microorganisms recently, Which is hoped to result in a decrease in mortality rate.

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COMPARISON OF GROWTH, SERUM PREALBUMIN, TRANSFERRIN, IgG AND AMINO ACIDS OF TERM INFANTS FED BREAST MILK OR FORMULA*

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SUMMARY: Kurugöl Z, Çoker M, Çoker C, Egemen A, Ersöz B. (Department of Pediatrics and the Research Laboratory, Ege University Faculty of Medicine, İzmir, Turkey). Comparison of growth, serum prealbumin, transferrin, IgG and amino acids of term infants fed breast milk or formula. Turk J Pediatr 1997; 39: 195-202.

This study was designed to compare growth parameters, biochemical indices of protein metabolism and serum amino acid patterns in newborn infants fed either human milk (n: 22) or formula with protein and amino acid concentrations similar to human milk (n: 19) during the first two months of life. Growth parameters were normal and similar in all infants. Serum levels of transferrin and IgG were not significantly different, whereas prealbumin concentrations were found to be significantly higher in infants fed formula compared to those fed human milk. In the formula group, glutamic acid and cystine levels were significantly lower while phenylalanine and proline were significantly higher compared to the breastfed group.

It was observed that feeding a formula with an amino acid pattern similar to that of human milk does not necessarily give rise to identical patterns of amino acids or indices of protein metabolism. *Key words: breastfed, formula, amino acids, growth.*

Human milk is accepted to be the most appropriate food for infants and is usually recommended during the first four to six months of life without any additional food. However, in some situations it may be necessary to use formulas for infant feeding. Formulas are being modified to be similar to human milk with respect to the composition and concentration of macro and micronutrients. Since optimal growth and development is achieved with mother's milk, the adequacy of formula is usually ascertained by comparing the growth of infants fed such products with that of breastfed infants. Along with other specifications, the protein content of formula is also modified to obtain a whey: casein ratio of 60:40, which is similar to that of human milk (63:37). It is suggested that the use of such formula

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with a protein concentration and amino acid pattern similar to human milk for infant feeding would result in indices of protein metabolism and amino acid patterns like those in breastfed infants[1-6].

Material and Methods

This study included 41 (21 male, 20 female) term, healthy infants who were being followed by the Social Pediatrics Section of the Department of Pediatrics at Ege University Medical School. These infants were registered for the study before birth, and they had no problems during the prenatal, natal or postnatal periods. Twenty-two were bearstfed and 19 received a formula in current use in Turkey, containing 15 g/L protein with a whey: casein ratio of 60/40 and 670 kcal/L. The claimed amino acid composition of the formula as well as that of human milk is shown in Table I. Mothers of the infants fed with formula were trained to prepare it, and the first serving was fed together with a nurse in the hospital. The daily food intake of the infants was regulated and followed on a feeding form completed by the mothers. Neither the formula-fed nor the breastfed infants received any additional nutrients throughout the study.

Table I: Amino Acid Composition of Human Milk[8] and Formula Used in This Study (mg/g of Protein)

Amino Acid	Human Milk	Formula
Threonine	54	40
Valine	59	66
Methionine	14	26
Isoleucine	59	46
Leucine	112	93
Phenylalanine	54	46
Lysine	82	73
Tryptophan	27	13
Cystine	17	6
Tyrosine	58	40
Arginine	44	33
Aspartate	109	73
Serine	58	53
Glutamate	194	193
Prolin	102	113
Glycine	31	20

Infants in both groups were followed up at regular intervals until the end of the second month of life. Measurements of weight, length and head circumference were made at birth and at the end of the first and second months. A digital

scale accurate to ± 5 g was used to measure weight, and an infant-measuring board with a millimeter ruler was used to record length. All measurements were made by the same examiner, using the same tools.

Samples of preprandial (2.5-3 hours after the previous meal), peripheral venous blood were obtained at the end of the second month between 9.00 and 10.30 a.m. Serum proteins, prealbumin, transferrin and IgG were measured by immunonephelometry (Beckmann Protein Array). Amino acids in serum were measured by gas chromatography with a flame ionization detector as their n-acetyl-n-propyl ester derivatives, the chromatographic separation being accomplished on a capillary column[7].

The parents' consent to the study and the Ethics Committee Agreement of the Medical Faculty of Ege University were obtained.

Statistical analysis was performed using Student's t and Mann-Whitney U test.

Results

Growth parameters at birth and at the end of the first and second months are summarized in Table II. No significant differences were observed with regard to gains in weight height or head circumference. The recorded data was evaluated at the end of the second month, revealing that the daily intake of formula, protein and energy in the formula group was 130-150 ml/kg/day, 1.95-2.25 g/kg/day and 87-100 kcal/day, respectively.

Table II: Anthropometric Measurements

	Birth	End of First Month	End of Second Month
Weight (g)			
Human milk (n = 22)	3334 ± 499	4457 ± 614	5450 ± 730
Formula (n = 19)	3161 ± 571	4380 ± 348	5341 ± 619
Length (cm)			
Human milk (n = 22)	50.1 ± 1.7	54.9 ± 2.1	58.2 ± 2.4
Formula (n = 19)	49.9 ± 1.7	54.6 ± 2.8	57.8 ± 2.4
Head circumference (cm)			
Human milk (n = 22)	35.1 ± 0.9	37.6 ± 1.0	39.3 ± 1.0
Formula (n = 19)	35.0 ± 0.7	36.9 ± 1.3	39.0 ± 1.5

Values are means ± SD. There were significant differences among groups with regard to gains in weight, height, and head circumference.

Table III shows serum prealbumin, transferrin and IgG values at the end of the second month. Serum levels of transferrin and IgG were similar in the two groups, while serum levels of prealbumin were significantly higher in the group fed formula ($p < 0.01$).

Table III: Serum Biochemical Assessment of Protein Nutriture

Biochemical indices (g/L)	Human Milk (n = 22)	Formula (n = 19)
Transferrin	2.8 ± 0.5	2.8 ± 0.4
Prealbumin	0.13 ± 0.04*	0.17 ± 0.04*
IgG	4.6 ± 1.6	4.6 ± 1.0

Values are mean ± SD. Prealbumin concentrations were significantly higher in infants fed formula than in infants fed human milk ($p < 0.01$).

The amino acid concentrations at the end of the second month are presented in Table IV. No significant difference in serum total and essential amino acid concentrations or in the ratio of essential to total amino acids (0.24 in the breastfed and 0.28 in the formula group) was present between the two groups. However, glutamic acid and cystine were significantly lower while phenylalanine and proline were significantly higher in the formula group compared to the breastfed group. The mean values for the valine to glycine ratio were estimated to be 1.8 in the formula and 1.6 in the breastfed group, with no significant difference between the two groups.

Table IV: Serum Amino Acid Concentrations of Infants fed Human Milk or Formula at the End of the Second Month (μmol/L)

Amino Acid	Human Milk (n = 22)	Formula (n = 19)
Phenylalanine	56.6 ± 8.6*	116.5 ± 22.0*
Methionine	104.1 ± 12.8	104.7 ± 17.1
Aspargine + aspartic acid	33.0 ± 5.7	45.1 ± 8.9
Glycine	96.0 ± 10.9	96.3 ± 13.1
Threonine	79.6 ± 15.0	99.9 ± 22.1
Proline	35.4 ± 5.9*	78.4 ± 13.5*
Arginine	63.0 ± 13.2	64.5 ± 13.1
Isoleucine	19.6 ± 2.4	24.3 ± 2.8
Leucine	50.6 ± 6.1	45.2 ± 6.1
Alanine	117.4 ± 15.1	130.8 ± 29.4
Tyrosine	92.4 ± 19.6	72.9 ± 9.9
Lysine	155.7 ± 20.1	184.2 ± 26.7
Hydroxyproline	133.1 ± 17.8	115.2 ± 22.3
Serine	87.6 ± 11.5	60.7 ± 8.2
Valine	167.1 ± 30.2	141.7 ± 27.8
Glutamine + glutamic acid	863.0 ± 110.0*	505.4 ± 58.2*
Cystine	211.7 ± 64.3*	46.3 ± 9.3*

Values represent means ± SEM.

* In the formula group, glutamic acid and cystine levels were significantly lower while phenylalanine and proline were significantly higher than in the breastfed group ($p < 0.05$).

Discussion

Human milk, the only physiological nutrient for the newborn, is also ideal for growth and development. However, today many commercial formulas are being used either together with or as an alternative to breast feeding. Formulas are modified to be similar to human milk by adding some amino acids and/or by lowering the total protein and amino acid concentrations. It is suggested that the growth and development as well as the indices of protein metabolism and plasma amino acid profiles of the infants fed such formulas are similar to those of breastfed infants[1, 4, 5]. In formula feeding, over-feeding with a bottle may lead to excessive weight gain. On the other hand, improper preparation of the formula may result in inadequate weight gain. Nonetheless, it has been shown in multiple studies that the growth parameters of breast- and formula-fed, term babies are similar[6, 8, 9]. In this study, no significant differences were detected between weight gain, increments of length and head circumference of infants fed human milk or formula, all values being in the normal ranges.

The serum albumin level has long been used as an index for evaluating protein energy metabolism[9]. However, the long half-life of albumin (20 days in adults), although somewhat shorter in infants, limits its usefulness in assessing nutritional status. Recently, biochemical parameters with shorter half-lives, such as prealbumin and transferrin, have been gaining interest[4, 6, 8, 10]. Studies on children with kwashiorkor have put forward that prealbumin is a sensitive indicator of protein energy malnutrition[11, 12]. It is also considered to be useful for the early diagnosis of malnutrition, reflecting short-term changes in nutritional status[13]. Furthermore, data have been presented that changes in prealbumin levels occur at an earlier stage than changes in anthropometric measurements at different protein intake levels in premature infants[14].

Polberger et al.[2] have shown that prealbumin, RBP and transferrin are the most valuable plasma proteins for assessing protein metabolism in low birth weight infants, particularly stressing prealbumin, which is strongly correlated with protein intake. The formula in this study contained 15 g/L protein, and no difference was observed in the infants' growth. In fact, it is possible to obtain similar growth in formula-fed babies merely by increasing protein intake, thus leading to higher levels of prealbumin in serum.

Transferrin is accepted as an index of nutrition by some authors, while others claim that it is not valuable[2, 15]. In accordance with Rigo et al.[6] and Picone et al.[8], no significant difference was observed in serum transferrin concentrations in infants fed either human milk or formula.

There is a controversy between various studies regarding the total amino acid concentration in the serum of infants fed formula with 15 g/L protein and a whey:

casein ratio of 60:40[5, 8, 16, 17]. No difference was observed in the total amino acid concentrations of the two groups of infants in this study.

However, compared to the breastfed group, glutamic acid and cystine levels proved to be significantly lower while phenylalanine and proline significantly higher in the formula group. In the neonatal period, due to a deficiency in cystathionase activity, methionine is not converted to cystine. Thus, cystine may be regarded as an essential amino acid for the neonate. Human milk supplies sufficient cystine. Picone et al.[8] have detected significantly higher levels of plasma total cystine in breastfed compared to formula-fed infants. Also, in this study serum cystine levels were found to be higher in infants fed human milk, which can be explained by the lower cystine content of the formula used.

Glutamine plays a regulatory role in protein synthesis. It has been shown that supplementation of the diet with glutamine speeds up the reparation of intestinal mucosa in infants[18].

The higher levels of glutamic acid in breastfed infants is considered to be a result of the rich glutamic acid content of human milk[19]. An enteresting finding of this study was that, although the glutamic acid content of the formula was equivalent to that of human milk, the glutamic acid levels were higher in breastfed infants.

In accordance with other studies, the phenylalanine concentration was found to be higher in formula-fed infants, even though the phenylalanine content of the formula was decreased in consideration of the fact that phenylalanine metabolism is inadequate in the newborn[3, 8]. However, it was also observed that phenylalanine values did not reach toxic levels in any of the infants.

Similar to phenylalanine, proline levels were determined to be significantly higher in the formula group. Parallel to this result, some previous investigators have found increased proline values in formula-fed infants[3, 8], while others had contrary findings[6]. The reason for the discrepancy in proline levels remains unclear.

Valine is a branched-chain amino acid which has an important role in insulin secretion[20]. The high valine/glycine ratio in formula-fed babies accounts for the hyperinsulinemia that leads eventually to insulin resistance[21]. Recent epidemiological studies have shown that the incidence of insulin-dependent diabetes is higher in children who have not received human milk[22]. Contrary to this finding, in this study the valine levels in serum and the valine/glycine ratio were higher in breast-fed infants, however the difference was not statistically significant.

In spite of continuous efforts to modify formula to be biologically equivalent to human milk, there are differences in the serum levels of micronutrients even though the macronutrients are similar in breast- and formula-fed infants. Also, in this study, the group fed formula had higher levels of prealbumin, phenylalanine and proline and lower levels of glutamic acid and cystine compared to the group fed human milk.

In conclusion, this study provides evidence that formulas with an amino acid profile similar to that of human milk do not necessarily produce plasma patterns similar to those of breast fed infants. Besides the protein and amino acid content of the nutrient, other factors such as the difference in the bioavailability of amino acids in human milk or formula, variable concentration and type of amino acids over the course of lactation, and hormonal response to feeding may also influence amino acid concentrations.

The clinical impacts and the intrinsic results of these discrepancies in the infant remain to be elucidated. It is not known whether the higher concentrations of some amino acids cause metabolic intolerance or whether the lower concentrations of others cause deficiency symptoms in formula-fed infants.

Further modifications in the protein content of infant formulas will depend on more expanded studies which evaluate macro-and micronutrients together to identify and determine the significance of the variables which affect the protein nutritional status.

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SERUM GRANULOCYTE – MACROPHAGE COLONY – STIMULATING FACTOR AND INTERLEUKIN – 1 LEVELS IN PATIENTS WITH REPEATED AND NON – REPEATED PULMONARY INFECTIONS*

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SUMMARY: Erduran E, Gedik Y, Aslan Y, Değer O, Ören A. (Departments of Pediatrics and Biochemistry, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey). Serum granulocyte-macrophage colony-stimulating factor and interleukin-1 levels in patients with repeated and non-repeated pulmonary infections. Turk J Pediatr 1997; 39: 203-211.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) has a stimulating effect on erythroid, megakaryocytic and granulocyte-macrophage progenitors. Activated T lymphocytes, monocytes, fibroblasts and endothelial cells release GM-CSF after stimulation by endotoxin and cytokines such as interleukin-1 (IL-1) and tumor necrosis factor. IL-1 is also released in response to bacterial infections and inflammation by phagocytic mononuclear cells. GM-CSF and IL-1 levels were examined in 10 patients with recurrent pulmonary infection (repeaters) and in 10 patients with acute pulmonary infection (non-repeaters) in the acute and recovery periods of infection.

The mean serum GM-CSF and IL-1 levels of non-repeaters were significantly higher than those of repeaters in the acute period of infection ($p < 0.002$), but the same parameters of both groups were not different in the recovery period ($p > 0.05$). In addition, both the serum GM-CSF and IL-1 levels of repeaters and non-repeaters in the acute period of infection were higher than those in the recovery period ($p < 0.05$).

There was a positive correlation between serum IL-1 and GM-CSF levels in non-repeaters ($r = 0.746$, $p = 0.013$), but no significant correlation between the same parameters in repeaters ($r = 0.395$, $p = 0.259$).

In this study, we could not explain why the serum GM-CSF and IL-1 levels in repeaters did not increase as they did in non-repeaters; moreover, there was no significant correlation between serum IL-1 and GM-CSF levels in repeaters during the acute period of infection. These findings may be due to microenvironmental factors in bone marrow and/or other factors. *Key words: granulocyte-macrophage colony-stimulating factor, interleukin-1, recurrent pulmonary infection.*

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is responsible for burst-promoting activity as well as stimulating activity on granulocyte-macrophage progenitors[1,2]. Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), two

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cytokines produced by activated monocytes, further stimulate fibroblasts and endothelial cells to produce GM-CSF[3,4]. Monocytes also release GM-CSF after stimulation with endotoxin[3].

Phagocytic mononuclear cells synthesize and release IL-1 in response to bacterial infections, inflammation, tissue damage, and antibody-antigen complexes[4,5]. The primary sources of IL-1 are blood monocytes, phagocytic lining cells of the liver and spleen, and other tissue macrophages; specialized cells such as keratinocytes, gingival and corneal epithelial cells, renal mesangial cells and brain astrocytes also produce IL-1-like molecules. IL-1 causes fever by inducing an abrupt increase in the synthesis of prostaglandins, most notably prostaglandin E_2, in the anterior hypothalamus[6]. Cytokines, including the colony-stimulating factors (CSFs), TNF and many interleukins, mediate the complex series of responses to infection[7,8]. Early studies identified colony-stimulating activity (CSA) in the serum of experimental animals exposed to pathogens[9,10]. These studies use bioassay, which was not always specific because of synergistic interactions or the effects of accessory cells[11-13]. Nonetheless, on the basis of marrow colony formation in-vitro, GM-CSF or a combination of granulocyte colony-stimulating factor (G-CSF) in the serum appeared to be part of the response to infection[10].

Epithelial cells lining respiratory airways can participate in inflammation in a number of ways. Airway epithelial cells also can act as "effector" cells, synthesizing and releasing cytokines, lipid mediators, and reactive oxygen species in response to a number of pathologically relevant stimuli, thereby contributing to inflammation[14]. In addition, alveolar macrophages also produce various inflammatory and immunomodulatory cytokines when stimulated with lipopolysaccharides (LPS) and play a role in local immunoregulation[15,16].

The aim of this study was to define as possible abnormalities IL-1 and GM-CSF production and to investigate the interactions of these two cytokines in patients with repeated and non-repeated pulmonary infections. We measured the serum GM-CSF and IL-1 levels in these groups of patients.

Material and Methods

Patients with recurrent pulmonary infections (repeaters), five boys and five girls between the ages of eight months and eight years (3.7 ± 2.4 years), and 10 patients with acute pulmonary infections (non-repeaters), six boys and four girls between ages 10 months and 10 years (4.1 ± 2.9 years), were included in this study. Four patients with recurrent pulmonary infections had immunodeficiency (three hypogammaglobulinemias, one hyperimmunoglobulin M syndrome), one patient had Down syndrome, one had pulmonary alveolar proteinosis and one had pulmonary hemosiderosis. The cause of recurrent

pulmonary infection in three patients could not be determined. No patient had malignant diseae, a hematological disorder or surgical problems.

Recurrent pulmonary infection is defined as more than three attacks of pulmonary infection within a year. Patchy or diffuse alveolar filling or consolidation with air bronchograms in chest x-ray suggested the diagnosis of non-repeated pulmonary infection; primarily interstitial changes and bronchial wall thickening on chest x-ray suggested the diagnosis of recurrent pulmonary infection.

GM-CSF, IL-1 and Other Measurements

Serum samples were drawn into heparinized tubes as soon as possible after the clinical onset of pulmonary infection (before administration of antibiotics) and after recovering from pulmonary infection (after discontinuing antibiotics). All samples were allowed to clot at room temperature. Immediately after clotting, they were centrifuged at 1000 x g for 20 minutes. Collected serum samples were kept at -70 °C until analyzed. Serum GM-CSF and IL-1 levels were determined by ELISA tests (Quantikine, cat. no: DGM 00, and Cistron Biotechnology, cat. no: 03-HS96, respectively). We also examined white blood cells (WBC) and absolute granulocyte count (AGC), C-reactive protein (CRP), serum total protein (TP) and albumin (Alb) levels in all patients during the acute and recovery periods.

Microbiological analyses could not be performed in either group (repeaters and non-repeaters) during the acute period, but we surmised that the cause of pulmonary infection in all patients was a bacterial agent because the patients had high fevers, WBC and AGC CRP positivity.

Statistical analyses were done using Mann-Whitney U and Wilcoxon matched-pairs signed-ranks tests, as well as linear regression analyses.

Results

The clinical and laboratory findings of repeaters and non-repeaters in the acute and recovery periods are shown in Table I. Fever, serum TP and Alb levels, and CRP positivity of both groups were similar in both the acute and recovery periods ($p > 0.05$). Although the total WBC and AGC of non-repeaters in the acute period of infection were significantly higher than those of repeaters (< 0.05), the total WBC and AGC of both groups were not different in the recovery period of infection ($p > 0.05$). In addition, the total WBC and AGC of both groups in the acute period were higher than those in the recovery period ($p < 0.002$).

The serum GM-CSF and IL-1 levels of both groups are plotted in Figures 1 and 2, respectively. In the acute period of infection, the mean serum GM-CSF and IL-1 levels of non-repeaters were significantly higher than those of repeaters ($p < 0.002$). However, in the recovery period of infection, the serum GM-CSF

and IL-1 levels were not different between repeaters and non-repeaters ($p > 0.05$). Both the serum GM-CSF and IL-1 levels of repeaters and non-repeaters were higher in the acute period of infection than in the recovery period ($p < 0.05$).

Table I: Clinical and Laboratory Findings of Repeaters and Non-Repeaters in Acute and Recovery Periods

Clinical and Laboratory Findings	Repeaters (n = 10)		Non-Repeaters (n = 10)	
	Acute Period	Recovery Period	Acute Period	Recovery Period
Age (years)	3.7 ± 2.4	–	4.1 ± 2.9	–
TP (g/dl)	7.2 ± 0.8	7.1 ± 0.9	7.4 ± 0.9	7.5 ± 0.7
Alb (g/dl)	4.2 ± 0.6	4.0 ± 0.5	4.5 ± 0.7	4.6 ± 0.6
WBC (count/µl)*	17.872 ± 3.645	8.670 ± 1.120	20.164 ± 4.215**	8.846 ± 1.214
AGC (count/µl)*	12.492 ± 2.955	5.329 ± 874	16.629 ± 3.471**	5.562 ± 912
CRP positivity	+ 2.9	–	+ 3.2	–
Fever (°C)*	38.2 ± 0.7	36.9 ± 0.3	38.9 ± 0.8	36.7 ± 0.3

Abbreviations: TP: total protein, Alb: albumin, WBC: white blood cell, AGC: absolute granulocyte count, CRP: C-reactive protein.

* The values represent statistically significant differences between the acute and recovery periods in the same group ($p < 0.001$).

** The values are significantly higher than those of the other group in the acute period ($p < 0.05$).

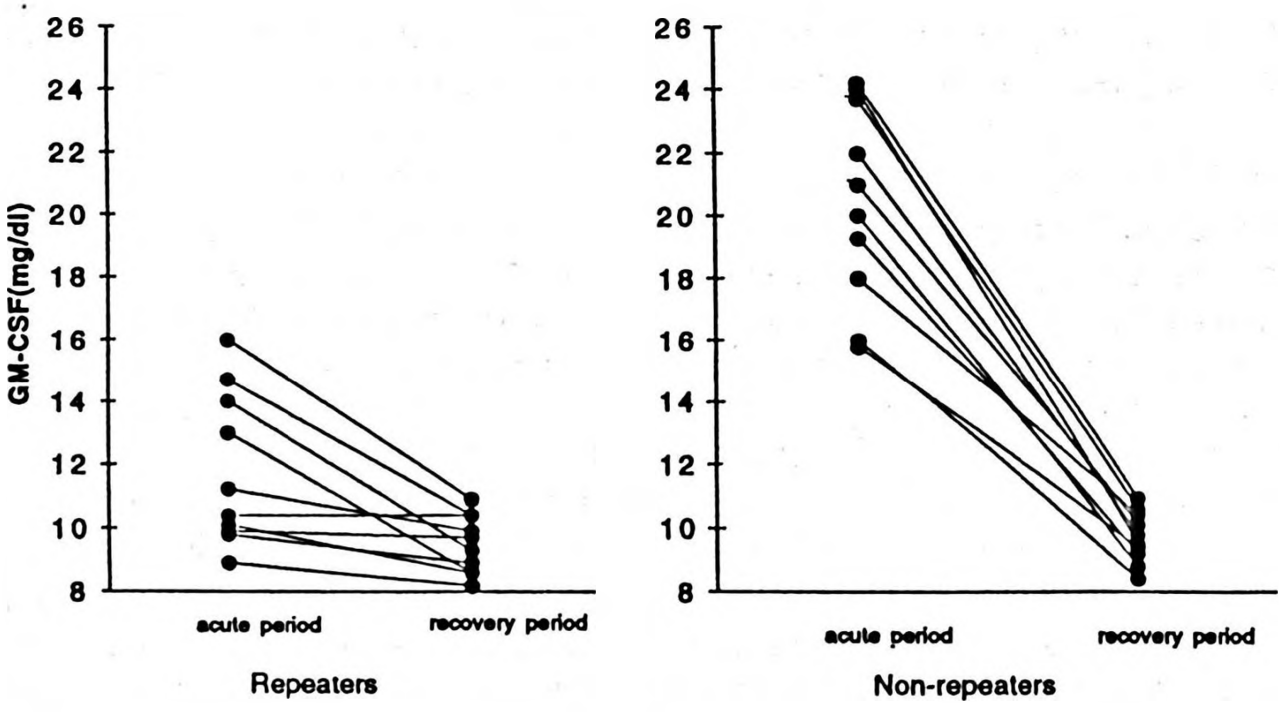

Fig. 1: Serum GM-CSF levels of patients.

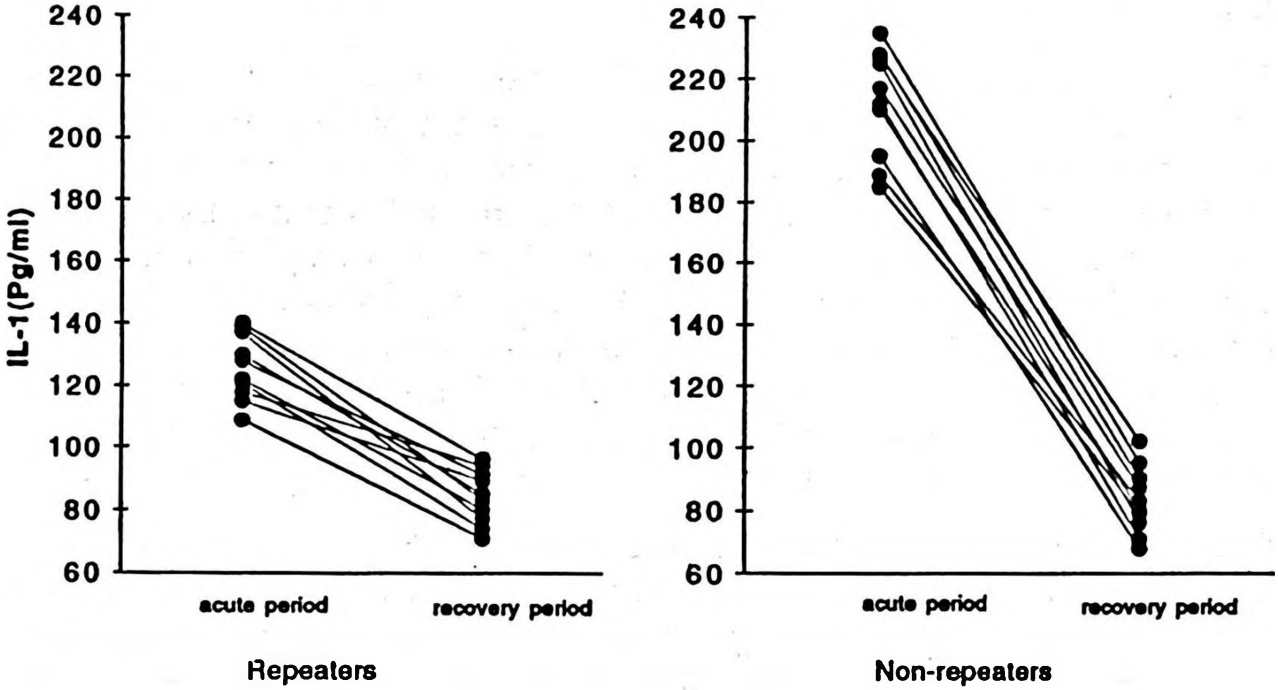

Fig. 2: Serum IL-1 levels of patients.

The mean serum GM-CSF level of the patients with immunodeficiency among the repeaters group in the acute and recovery periods was 13.2 ± 1.5 (11.2-14.7) pg/ml and 9.6 ± 0.8 (8.7-10.4) pg/ml, and the serum IL-1 level 121 ± 7 (115-130) pg/ml and 84 ± 9 (71-96) pg/ml, respectively. Both the serum GM-CSF and IL-1 levels of the patients with immunodeficiency in the acute period of infection were similar to those in the recovery period ($p > 0.05$).

In the acute period, there was a positive correlation between serum IL-1 and GM-CSF levels in non-repeaters ($r = 0.746$, $p = 0.013$), but no significant correlation between the same parameters in repeaters ($r = 0.395$, $p = 0.259$, Fig. 3).

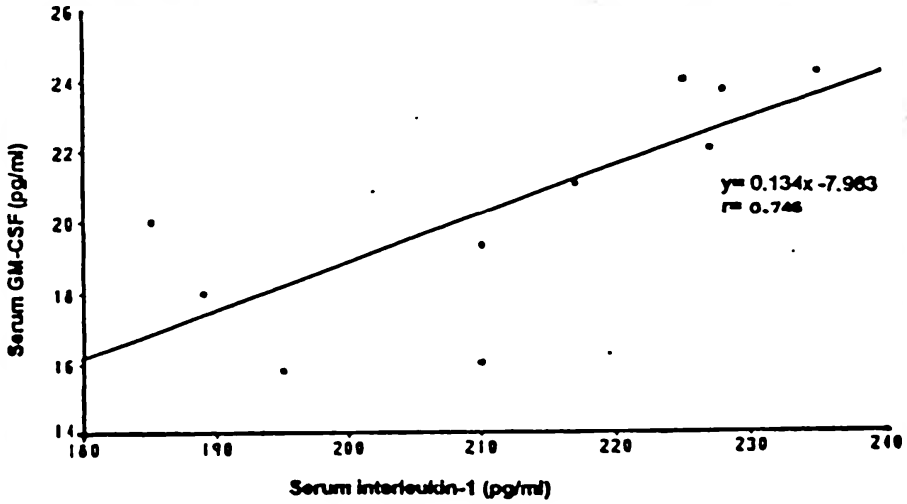

Fig. 3: Correlation-graph showing a positive correlation between serum IL-1 and CM-CSF levels of non-repeaters in the acute period ($r = 0.746$, $p = 0.013$).

Discussion

This study revealed higher IL-1 and GM-CSF levels in patients with non-repeated pulmonary infections compared to those with repeated pulmonary infections with an acute infectious status. We were not able to explain the cause of this condition. Hoffman-Goetz et al.[17] demonstrated that the production of IL-1 by peripheral blood monocytes was impaired in hospitalized patients with protein deficiency. We also found decreasing serum IL-1 levels in protein-depleted patients (unpublished data). However, it was demonstrated that there were no significant differences in serum TP and Alb levels between repeaters and non-repeaters, because it was thought that the difference in IL-1 levels between the two groups was not related to serum Alb levels.

One of the most sensitive responses to serum IL-1 levels is an increase in number and immaturity of circulating neutrophils. The release of neutrophils is apparently due to the direct action of IL-1 on bone marrow[13]. We think that in this study, the cause of higher WBC and AGC levels in non-repeaters compared to repeaters in the acute period may be due to higher serum IL-1 levels in non-repeaters.

Kanusova et al.[18] suggested that there was a significant increase in IL-1 production in children with respiratory bacterial infections, but IL-1 production decreased in acute respiratory viral infections. Significantly higher levels of IL-1 had been found in the bronchoalveolar lavage fluid of patients with bacterial pulmonary infection than in patients without such infection. Therefore, it was suggested that alveolar macrophages in inflammatory lung disease had an increased capacity to secrete IL-1 on stimulation with LPS[19, 20]. Nearly all infections, immunologic reactions and inflammatory processes stimulate mononuclear cells and phagocytes to synthesize and release IL-1. At the onset of the infection, blood monocytes and tissue macrophages become activated by exposure to either its products or toxins. Either process results in the synthesis and release of IL-1[21]. In our study, significantly higher serum IL-1 levels were determined in repeaters and non-repeaters with an acute infectious status compared to those in the recovery period. This finding may be due to an increased capacity of alveolar epithelial cells and macrophages to secrete IL-1 on stimulation with LPS or bacterial products.

A minor elevation in GM-CSF was observed in a few critically ill patients with gram-negative bacteremia, consistent with the observation that LPS induces GM-CSF production by monocytes[17]. IL-1 may indirectly induce GM-CSF release from the endothelium[3, 22]. The low levels of GM-CSF observed in some patients may represent "spill-over" into the circulation. Alternatively, GM-CSF may only be produced in small amounts sufficient to synergize with other factors or to activate mature cells without playing a major role as an independent circulating

mediator of leukocytosis[22]. As far as we know, no research related to serum GM-CSF levels in patients with acute pulmonary infections has been reported in the literature. Serum GM-CSF levels for repeaters and non-repeaters in the acute infectious state were found to be significantly higher than those in the recovery period.

We were not able to determine the etiologic agent in our patients in the acute infectious period. However, we believe that the cause of pulmonary infection in all patients was a bacterial agent because the patients had high fevers, WBC and AGC levels, and CRP positivity. These findings are observed more frequently in patients with bacterial infections than viral infections.

Helsinen et al.[23] suggested that monocytes from children with severe bacterial infections showed a high level of spontaneous production of IL-1. In viral respiratory infections, there was usually little or no spontaneous IL-1 production from monocytes, and the values did not differ from those of children without infections. Chen et al.[24] suggested that low serum G-CSF levels were significantly associated with fatal outcomes, and that GM-CSF played no obvious role as a systemic effector molecule in acute bacterial infections.

Neither the serum GM-CSF or IL-1 levels of the patients with immunodeficiency in the acute period of infection differed from those in the recovery period. Therefore, we think that humoral immunodeficiency may cause diminished IL-1 and GM-CSF responses in the acute infectious state.

The present study revealed that the serum GM-CSF levels of non-repeaters were higher than those of repeaters in the acute infectious state. This condition can be explained by higher serum IL-1 levels in non-repeaters compared to repeaters. However, we could not explain why serum IL-1 levels in repeaters did not increase as they did in non-repeaters; moreover, there was no significant correlation between serum GM-CSF and IL-1 levels in repeaters in the acute infectious state. We think that the cause may be microenvironmental factors in the bone marrow, and/or other factors. Further studies are required to clarify this point.

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TUBULAR MARKERS IN CHILDREN WITH INSULIN – DEPENDENT DIABETES MELLITUS*

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SUMMARY: Çalışkan S, Fıçıcıoğlu C, Hacıbekiroğlu M, Mıkla Ş, Kasapçopur Ö, Sever L, Aydın A, Arısoy N. (Department of Pediatrics and Central Research Laboratory, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey). Tubular markers in children with insulin-dependent diabetes mellitus. Turk J Pediatr 1997; 39: 213-218.

The aim of the present study was to investigate the prevalence of tubular dysfunction and to assess the clinical significance of low-molecular-weight proteinuria and enzymuria in children with insulin-dependent diabetes mellitus (IDDM). N.acetyl-beta-D-glucosaminidase (NAG) and beta-microglobulin (β_2M) excretion was determined in 52 children with insulin-dependent diabetes mellitus and 28 controls. Patients were grouped according to the duration of diabetes: group 1 (n = 7): less than one year; group 2 (n = 27): one to five years; groups 3 (n = 18): greater than five years. Both parameters were significantly increased in groups 2 and 3 compared to controls. Urinary β_2M levels correlated significantly with albuminuria and HbA_{1C}, while urinary NAG levels correlated only with HbA_{1C}.

Two to four samples were obtained from 35 of 52 diabetic patients in the study group at one-month intervals. Of these, 23 patients had elevated NAG levels, and 22 patients increased β_2M excretion. However, only six patients displayed persistent enzymuria, and nine low-molecular-weight proteinuria. The mean (SD) of coefficients of variation of each patient was 50.45 (± 28.24) for NAG and 68.25 (± 42.57) for β_2M excretion.

We concluded that early tubular dysfunction and/or damage occurs in IDDM but is not established in the majority of children. *Key words: beta$_2$-microglobulin, insulin-dependent diabetes mellitus, n-acetyl-beta-D-glucosaminidase.*

Nephropathy is a serious microvascular complication of insulin-dependent diabetes mellitus (IDDM). Numerous studies have been carried out in order to predict the occurrence of diabetic nephropathy. It is generally accepted that microalbuminuria precedes macroalbuminuria[1] and has a predictive value in IDDM, but a long-term study failed to demonstrate this[2].

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It has been suggested, however, that peritubular vasculopathy may be the cause of renal failure in diabetic nephropathy[3]. In experimental diabetes mellitus, peritubular capillaries[4] and the tubular epithelium[5] are involved. In a recent study concerning macroalbuminuric patients, a greater increase in low-molecular-weight (LMW) proteinuria was observed in patients with IDDM compared to those with primary glomerulonephritis[6]. On the other hand, tubular dysfunction has been reported early in IDDM[7-12]. The clinical significance and predictive value of early tubular involvement in diabetic children remain to be clarified.

The aim of the present study was to investigate the prevalence of tubular dysfunction and to assess the clinical significance of low-molecular-weight proteinuria (beta$_2$-microglobulin, β_2M) and enzymuria (n-acetyl-beta-D-glucosaminidase, NAG) early in IDDM.

Material and Methods

Fifty-two patients with IDDM and 28 healthy children were admitted to the study. Diabetic patients were divided into three groups according to the duration of diabetes: group I: less than one year; group 2: one to five years; group 3: greater than five years, Demographic and clinical data of the patients and controls are presented in Table I. None of the patients had an infection or ketoacidosis.

Table I: Clinical Data in Diabetic Children

	Group 1 < 1 Year (n = 7)	Group 2 1-5 Years (n = 27)	Group 3 > 5 Years (n = 18)	All Groups (n = 54)
Sex	4M/3F	16M/11F	8M/10F	28M/24F
Age (years)	6.4 (1.7-8.8)	11.3 (4.2-17)	14 (7-20.2)	11.3 (1.7-20.2)
High systolic blood pressure[a]	–	2 (7%)	2 (11%)	4 (7%)
High diastolic blood pressure[a]	1 (14%)	5 (19%)	4 (22%)	10 (19%)
Retinopathy	–	1 (4%)	3 (17%)	4 (7%)
HbA_{1c} (%)	9.4 (7.6-15.1)	10 (8-15.8)	10.5 (7.6-12.8)	10.2 (7.6-15.8)
Albuminuria (µg/mmol creatinine)	0.6 (0.2-2.4)	1.3 (0.1-11)	1.5 (0.3-19)	1.3 (0.1-19.0)

Figures represent median (ranges) or number (%).
[a] > 2 SD of normal population[15].

A total of 124 samples were collected from the diabetic children. From each of 17 patients only one urine sample was available, whereas two to four samples were obtained from the remaining 35 children at one-month intervals (follow-up group). Ten β_2M values tested in acidic urine were excluded from the study.

Urine samples were collected early in the morning. Each sample was divided into two parts, which were stored separately at + 4 °C (to test albumin) and -20 °C (to test other parameters). Urinary creatinine and NAG were determined photometrically by a Hitachi 717 automatic analyzer. Urinary creatinine was

measured according to the Jaffe method. Crezolesulfonftalein-glucosaminide was used as a substrate in measuring NAG (Boehringer-Mannheim)[13]. Urinary β_2M was determined by radioimmunoassay (DPC). Microalbumin was measured by double-antibody radioimmunoassay (DPC). Inter-assay coefficients of variation were 8.4 percent for NAG, 10.6 percent for β_2M, and 9.5 percent for albumin. Urinary NAG, β_2M and albumin were evaluated by determining the NAG: creatinine, β_2M: creatinine and albumin: creatinine ratios. The upper limit of normal was defined as the mean + 2 SD of controls for NAG (0.92 U/mmol creatinine) and β_2M (18.0 µg/mmol creatinine) and higher than 3.5 mg/mmol creatinine for albumin, as previously reported[14].

All patients underwent a fundoscopic examination every six months. Microaneurysm confirmed by angiography was accepted as retinopathy. Measurement of the glycosylated hemoglobin (HbA_{1C}) level was done every three months. The one-year average value was utilized in statistical calculations. The blood pressure of all patients was measured. The standard deviation score was calculated for each patient according to values obtained from healthy Turkish children[15]. Parental consent was obtained in every case.

Non-parametric statistical tests were utilized because the data were not normally distributed even after logarithmic transformation (Mann-Whitney U test for comparing two groups, Sperman's Rho for correlation). Group 1 (n = 7) was not included in the statistical analysis. Within-individual variability was compared by coefficients of variation (CV). The CV of each parameter was calculated in each subject. Mean and SD were determined among subjects for each parameter. For statistical tests other than CV, the median value of all urine samples was utilized. Statistical tests were performed using the SPSS PC program.

Results

Age and sex distribution were not significantly different between the diabetic and control groups. Urinary excretion of NAG and β_2M was significantly higher in groups 2 and 3 compared to controls (Table II). There was a positive correlation between NAG and β_2M excretion in groups 2 and 3. Both parameters correlated with HbA_{1C} levels in group 3. β_2M excretion also correlated with albuminuria in group 3 (Table III).

Table II: Excretion of Urinary NAG and β_2M in Normal and Diabetic Children

	Group 1[a] < 1 Year (n = 7)	Group 2 1-5 Years (n = 27)	Group 3 > 5 Years (n = 18)	Controls (n = 28)
NAG (U/mg creatinine)	6.5 (1.2-8.7)	8.1* (0.1-31.6)	10.2** (2.8-20.5)	3.5 (2-9)
β_2M (µg/mg creatinine)	329 (24-4164)	142* (1-2202)	184* (16-1539)	85 (16-157)

* $p < 0.05$, ** $p < 0.001$ vs controls.
[a] Not included in the statistical analysis.
Figures represent median and ranges.

Table III: Correlation of Urinary NAG and β_2M Levels with Albumin Excretion, HbA_{1C}, Systolic and Diastolic Blood Pressure in Diabetic Children

	NAG		β_2M	
	Group 2 1-5 Year	Group 3 > 5 Years	Group 2 > 1-5 Years	Group 3 > 5 years
Albumin	NS	NS	NS	$r_s = 0.6$ ($p = 0.009$)
HbA_{1C}	NS	$r_s = 0.72$ ($p = 0.001$)	NS	$r_s = 0.75$ ($p < 0.001$)
Systolic blood pressure[a]	NS	NS	NS	NS
Diastolic blood pressure[a]	NS	NS	NS	NS
β_2M	rs = 0.40 (p = 0.024)	rs = 0.52 (p = 0.027)		

Figures represent median (ranges) or number (%).
[a] > 2 SD of normal population[15].

Of four retinopathic children, one had only microalbuminuria, one had microalbuminuria with enzymuria and LMW proteinuria, another had enzymuria and LMW proteinuria without microalbuminuria, and the last one had neither microalbuminuria nor LMW proteinuria and enzymuria.

The frequencies of increased excretions of each parameter in the follow-up group are shown in Table IV. The CVs of NAG, β_2M and albumin excretions are reflected in Table V. The CV of NAG was lower than those of albumin and β_2M, but the difference was insignificant.

Tablo IV: Frequencies of Increased Levels of NAG, β_2M and Albumin Excretions in the Follow-up Group.

	NAG n = 35 (%)	β_2M n = 34 (%)	Albumin n = 33 (%)
Elevated level in at least one sample	23 (66)	22 (65)	12 (39)
Elevated levels in at least two samples	15 (43)	13 (38)	4 (12)
Elevated levels in all samples	6 (17)	9 (26)	4 (12)

Table V: Variation of NAG, β_2M and Albumin Excretion in the Follow-up Group

	CVs (Mean ± SD)
NAG	50.5 ± 28.2
β_2M	68.2 ± 42.6
Albumin	61.5 ± 34.6

CVs: Coefficients of variation.

Discussion

Our results confirm that early tubular dysfunction and/or damage occurs in IDDM. Large CVs and the fluctuation of tubular marker levels observed in the follow-up group demonstrated that tubular involvement was not established in the majority of patients. This finding is similar to Ginevri et al.'s[12], who observed that all increased urinary levels of brush border antigen and LMW protein returned to normal limits in children with IDDM. One possible explanation for this reversible tubular involvement is the toxicity of albuminuria. It is known that enzymuria and LMW proteinuria occur in proteinuric states[16, 17]. However, microalbuminuria did not seem to play a major role in our study group: firstly, not all microalbuminuric patients had LMW proteinuria and enzymuria or vice versa; secondly, there was no correlation between albuminuria and NAG. Another possible explanation is that metabolic changes may cause tubular toxicity. Glycosylated hemoglobin is a good parameter for judging metabolic status, as found in other studies[10-12, 18], HbA1c was correlated with NAG excretion LMW proteinuria in group 3. It is well known that HbA1c is a good predictor of microvascular disease. One can speculate that peritubular vascular changes may contribute to early tubular involvement in IDDM. Martin et al.[9] observed higher urinary NAG levels in adult type I diabetic patients with retinopathy and postulated that "once microvascular disease is established, the excretion rates of NAG and LMW proteins tend to be increased and unresponsive to metabolic control". However, in our study ony two of four patients with retinopathy had eleated urinary NAG and β_2M levels. Considering this finding and the "reversible" elevation of tubular markers, it seems unlikely that peritubular vascular changes are the major cause of early tubular involvement in IDDM. NAG is a lysosomal enzyme located in the proximal tubular epithelial cell. A number of factors causing proximal tubular perturbation produce urinary NAG elevation[20]. If the causative factor is eliminated, urinary NAG activity returns to normal limits. Hyperglycemia may alter tubular cellular metabolism, resulting in an increased loss of the enzyme into urine. Watanable et al.[21] have shown that altered blood sugar regulation causes elevation of urinary NAG levels.

The mechanism and the clinical significance of reversible tubular damage and/or dysfunction in early IDDM is not yet clear. Long-term follow-up studies are required to assess whether early reversible tubular involvement has a predictive value or is an only an epiphenomenon.

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SHORT – AND INTERMEDIATE – TERM EFFICACY OF AMIODARONE IN INFANTS AND CHILDREN WITH CARDIAC ARRHYTHMIA*

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SUMMARY: Çeliker A, Koçak G, Lenk MK, Alehan D, Özme Ş. (Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Short-and intermediate-term efficacy of amiodarone in infants and children. Turk J Pediatr 1997; 39: 219-225.

The antiarrhythmic effect of amiodarone was examined in this retrospective study in a group of 20 patients with a mean age of 8.5 ± 6.7 years (range 42 days to 20 years, median 9 years). Five patients with atrial flutter, one patient with atrial fibrillation, two patients with an intermediate rhythm between atrial flutter and atriala fibrillation, four patients with chaotic atrial tachycardia, three patients with atrioventricular reentry tachycardia, two patients with junctional ectopic tachycardia, and three patients with ventricular arrhythmias were treated with amiodarone. The mean duration of therapy was 9.1 ± 12.3 months (range 1 month to 4 years). Before amiodarone treatment, 18 patients had been unresponsive to various antiarrhythmic drugs (range 1-8, median 2). Two patients received amiodarone as an initial therapy. It was administered orally at a dose of 10 mg/kg once per day for 10 days and then decreased to 5 mg/kg once per day. Amiodarone was effective in 16 patients (80%).

Side effects occurred in three patients, including thyroid dysfunction, elevation of liver enzymes, and keratopathy. All side effects disappeared upon cessation of the therapy. We recommend amiodarone for the treatment of childhood arrhythmias, especially for the refractory types. *Key words: amiodarone, childhood, arrhythmia.*

Amiodarone is known to be effective for the control of various cardiac arrhythmias in adults[1-3]. Recently, successful use of the drug in the treatment of childhood arrhythmias refractory to conventional agents has been reported[2, 4, 5]. Side effects of amiodarone that occur during long-term administration have limited use of the drug in childhood. The purpose of this report was to investigate the efficacy and side effects of amiodarone in different types of arrhythmias in children.

Material and Methods

The study population consisted of 20 patients (7 female and 13 male, mean age 8.5 ± 6.7 years, range 42 days to 20 years, median 9 years). Seventeen patients (85%) had different types of supraventricular tachycardia (SVT), while three patients (15%) had ventricular arrhythmias (Table I). Rhythm was assessed by 12-lead electrocardiogram (ECG) and 24-hour continuous ambulatory electrocardiogram (cardioscan) in all.

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Table I: Clinical Characteristics of Patients

Case No.	Age	Sex	Arrhythmia	Heart Disease	Cardiac Surgery	Previous Drugs
1	11	M	AFL	TGA, PS	+	Dig, Quin, Propa
2	14	F	AFL	Restrictive CMP	–	Dig
3	14	M	AFL	Dilated CMP	–	Dig, V
4	18 months	M	AFL	–	–	Dig, Dilt
5	16	M	AFL	TGA	+	Dig, Propa
6	20	F	AFIB	MS, MI, TI	+	Quin
7	18	F	AFL + AFIB	ASD	+	Dig, Prop, Fle, Propa, V
8	15	M	AFL + AFIB	Single ventricle, PS	+	–
9	5 months	M	CAT	–	–	Dig, Propa, S
10	6 months	F	CAT	ASD	–	Dig, S
11	42 days	M	CAT	–	–	Dig, S
12	3 months	F	CAT	–	–	–
13	7	M	AVRT	Dilated CMP	–	Dig, Fle, Propa
14	5	F	AVRT	–	–	Dig, V
15	3.5 months	M	AVRT	Single ventricle	–	Dig, S, Propa
16	4	M	JET	Dilated CMP	–	Dig, Prop, Pind, Propa, Fle, Proc, Quin, V
17	6	M	JET	AS, AI	+	Dig, Propa
18	12	M	PVC	MI, MVP	–	Propa, S
19	11	M	PVC	TOF	+	Dig, S, Propa, Mex
20	13	F	VT	Dilated CMP	–	Dig, Propa, Epd

No: number, M: male, F: female, AFL: atrial flutter, AFIB: atrial fibrilation, CAT: chaotic atrial tachycardia, AVRT: atrioventricular reentry tachycardia, JET: junctional ectopic tachycardia, PVC: premature ventricular contractions, VT: ventricular tachycardia, TGA: transposition of great arteries, PS: pulmonary stenosis, CMP: cardiomyopathy, MS: mitral stenosis, MI: mitral insufficiency, TI: tricuspid insufficiency, ASD: atrial septal defect, AS: aortic stenosis, AI: aortic insufficiency, MVP: mitral valve prolapse, TOF: tetralogy of Fallot, +: surgery performed, –: surgery not performed, Dig: digoxin, Quin: quinidine, Propa: propafenone, V: verapamil, Dilt: diltiazem, Prop: propranolol, Fle: flecainide, S: sotalol, Pind: pindolol, Proc: procainamide, Mex: mexiletine, Epd:epdantoin.

Ten patients (50%) had congenital structural heart disease, and seven of them had undergone palliative or corrective heart surgery. Five patients (25%) had acquired heart disease, with restrictive cardiomyopathy (CMP) in one patient and dilated CMP in four patients. Three patients developed dilated CMP secondary to incessant tachyarrhythmias. Before amiodarone treatment, 18 patients had been unresponsive to various antiarrhythmic drugs (range 1-8, median 2) and seven patients had previously required one or more synchronized direct-current electrocardioversions. Two patients received amiodarone as an initial therapy.

Amiodarone was given orally at a loading dose of 10 mg/kg/day for 10 days, followed by a maintennace dose of 5 mg/kg/day. Digitalis was used in 11 patients concomitantly. Twelve-lead ECG, 24-hour cardioscan and echocardiography were performed in order to demonstrate the efficacy of amiodarone therapy. Ophthalmologic examination was performed, and serum levels of triiodothyronine (T_3), thyroxine (T_4), thyroid-stimulating hormone (TSH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were obtained one, three, six and 12 months after initiation of therapy and were repeated every six months thereafter. The drug was discontinued if it was not effective.

Results

Results were considered to be effective when normal sinus rhythm was reestablished and the clinical state improved in patients treated with amiodarone. In patients whose tachycardia rate was reduced but whose arrhythmia was not effectively suppressed, the results were considered to be partially effective. If there was no change in the clinical and electrocardiographic states, the treatment was considered ineffective.

The mean duration of therapy was 9.1 ± 12.3 months (range 1 month to 4 years). Amiodarone was effective in 13 patients (65%), partially effective in three patients (15%), and ineffective in four patients (20%) (Table II). In one patient in whom amiodarone was effective (Case 7), it was discontinued after four years and there was no subsequeut recurrence. The other patients without drug-related side-effects were still on amiodarone therapy as of this writing. Arrhythmia did not recur in any patients for whom amiodarone had been effective.

Table II: Efficacy and Side Effects of Amiodarone

Case Number	Outcome	Follow-up (months)	Side Effects
Atrial tachycardia			
1	+	6	(–)
2	+	1	(+)*
3	–	10	(–)
4	+	3	(–)
5	+	6	(+)**
6	–	2	(–)
7	+	48	(–)
8	–	5	(–)
9	+	8	(–)
10	+	3	(–)
11	+	1	(–)
12	+	1	(–)
AVRT			
13	–	5	(–)
14	+	8	(–)
15	+	13	(–)
JET			
16	+ (P)	36	(–)
17	+	1	(–)
PVC and VT			
18	+ (P)	4	(–)
19	+ (P)	2	(–)
20	+	19	(+)***

AVRT: atrioventricular reentry tachycardia, JET: junctional ectopic tachycardia, PVC: premature ventricular contractions, VT: ventricular tachycardia, +: success, –: failure, (P): partial, (–): side effects did not occur, (+): side effects occurred, *: hepatic dysfunction, **: keratopathy, ***: thyroid dysfunction.

Atrial Tachycardia

The twelve patients with some form of atrial tachycardia were treated with amiodarone for a mean of 9.8 months. The treatment was effective in nine patients (75%). Four of the five patients with atrial flutter responded well to amiodarone, but one patient required discontinuation of the drug on the seventh day of therapy because of side effects. One patient with atrial fibrillation did not respond to amiodarone. Only one of the two patients with an intermediate rhythm between atrial flutter and atrial fibrillation responded to treatment. Therapy with amiodarone was successful in all four children (100%) with chaotic atrial tachycardia (CAT).

Atrioventricular Reentry Tachycardia (AVRT)

In two of the three patients with AVRT the treatment was effective (67%), whereas in one it was ineffective. The patient who was unresponsive to therapy with a permanent form of junctional reciprocating tachycardia died suddenly due to congestive heart failure while he was still on amiodarone therapy (Case 13).

Junctional Ectopic Tachycardia (JET)

The two patients in this group were treated with amiodarone for a mean of 18.5 months. It was considered to be effective in these patients, one of them being partially responsive. He had dilated CMP and was given eight other antiarrhythmic drugs before the initiation of therapy with amiodarone. He had normal cardiac function assessed by echocardiographic study.

Premature Ventricular Contractions (PVC) and Ventricular Tachycardia (VT)

Two patients with PVC were considered to be partially responsive to amiodarone therapy. Twenty-for-hour cardioscan revealed that ectopic beats were not completely suppressed but were reduced in number. Amiodarone was effective in one patient with VT, but it had to be withdrawn because of side effects (Case 20).

Side Effects

During amiodarone therapy, side effects occurred in three (15%) of 20 patients (Table II). A 13-year-old female patient with VT who had dilated CMP developed hyperthyroidism in the 19^{th} month of therapy (Case 20). In a 14-year-old female patient with restrictive CMP and atrial flutter, amiodarone treatment was stopped because of elevated liver enzymes, nausea, vomiting and abdominal pain (Case 2). Amiodarone keratopathy occurred within four months in a 16-year-old male patient with atrial flutter (Case 5). The drug was discontinued due to side effects in three patients in whom it had been effective, and all side effects were reversible after discontinuation.

Discussion

Amiodarone hydrochloride is an iodinated benzofuran derivative which is structurally similar to thyroxine. It was first used in 1967 as an antianginal agent due to its ability to increase coronary blood flow and to decrease peripheral vascular resistance, but was later found to have antiarrhythmic properties[3]. It has class-III antiarrhythmic actions according to the Vaughan Williams classification[1,2]. Amiodarone prolongs the action potential duration and refractory period, but it also has calcium antagonist and β-receptor blocking effects on cardiac tissues. Consequently, the surface ECG may show sinus bradycardia, prolongation of the QT interval and changes in the T and U waves. Approximately 50 percent of the drug is absorbed after an oral dose, and it has a large volume of distribution. Oral loading is necessary to obtain an antiarrhythmic effect. It is generally used in conjunction with digoxin because of its inotropic properties[6].

The side effects of antiarrhythmics and drug resistance are challenging problems in the treatment of arrhythmias in childhood. After the surgical repair of most congenital heart diseases, patients are at risk for sudden death from various arrhythmias. Supraventricular tachycardia is generally considered benign, but in some patients it is incessant and these patients are at risk for the development of cardiomyopathy. Many children with such life-threatening arrhythmias respond to conventional antiarrhythmic drugs, but some do not. Recent data suggest that amiodarone is highly effective in controlling most of the supraventricular as well as ventricular tachyarrhythmias that have failed to respond to various conventional drugs[7]. Several reports have suggested that amiodarone has an efficacy of between 60 and 100 percent on different arrhythmias[2,4,5,8]. The efficacy of amiodarone in our study, including partial effectiveness, was 80 percent, which is similar to the findings of other researchers[8]. We observed complete control in 65 percent of patients, partial control in 15 percent, and failure in 20 percent. In our experience the efficacy of amiodarone in particular arrhythmias was 62.5 percent in atrial flutter and fibrillation, 100 percent in CAT, 66.6 percent in AVRT, and 100 percent in JET and ventricular ectopy. Guccione et al.[8] observed complete control in 80 percent of their 95 patients, partial control in four percent, and failure in 16 percent. In ventricular tachycardia they had an efficacy of 68 percent, in atrial flutter 97 percent, in SVT 75 percent and in JET 50 percent. Many reports[7-9] show a high efficacy of amiodarone in children with Wolff-Parkinson-White syndrome and JET. Therefore, use of amiodarone before surgical therapy for these arrhythmias is recommended.

Amiodarone has various side effects such as cutaneous disorders, photosensitivity, corneal deposits and keratopathy, hyperthyroidism or hypothyroidism because of the presence of iodine compound, elevation in liver enzymes and proarrhythmia[4,5,8,10,11]. The incidence of side effects associated

with amiodarone therapy ranges from 12.5 to 93 percent, and the side effects are usually reversible after drug discontinuation[5, 8, 11]. Clinical thyroid dysfunction and hepatic dysfunction are rarely seen in children. Intravenous amiodarone may cause acute bradycardia, hypotension and cardiovascular collapse[1, 12, 13]. We had a 15 percent incidence of side effects overall after seven days to 19 months of therapy, and we observed no increase in the incidence of side effects with a longer duration of treatment. It is suggested that amiodarone is more rapidly metabolized in young children, because side effects are less frequent in young patients than in adults[2, 4, 8]. In our study, all patients who had side effects were over greater than 10 years in age.

Pharmacological therapy for tachyarrhythmias is often effective but generally palliative and has side effects in long-term use. Transcatheter ablation using radiofrequency energy of arrhythmia foci or accessory pathways has been used, especially for life-threatening or drug-refractory arrhythmias[15, 16]. This treatment method is being used successfully in patients with atrial flutter, ectopic atrial tachycardia, JET, atrioventricular node reentrant tachycardia, and SVT due to accessory pathways, but side effects such as systemic embolization, myocardial damage or perforation, and rhythm disorders are serious limitations[15, 16]. It is also difficult to perform catheter ablation in infants with tachyarrhythmias.

It is known that amiodarone can be used in newborns and also during intrauterine life. The smallest patient that has been given amiodarone was a 23-week-gestation fetus who had hydrops fetalis secondary to tachycardia[5]. The smallest patient in our series was a 42-day-old infant with CAT.

Because of amiodarone's complex pharmacokinetics, the therapeutic and toxic range of serum levels has not yet been defined[2]. There is no known correlation between amiodarone dosage and serum level, or between serum level and toxicity[2, 14]. Therefore, monitoring amiodarone dosage and serum levels is not useful for evaluating amiodarone toxicity. For this reason, it is crucial to follow up the side effects of the drug. Serious side effects of amiodarone are relatively infrequent in children, but follow-up of patients, especially young ones who have rapid brain development and somatic growth, should be done carefully.

In conclusion, amiodarone is a very effective agent for infants and adolescents who have drug-refractory tachycardia, especially in postoperative patients. It should be used with caution because of its side effects.

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The Turkish Journal of Pediatrics 1997; 39: 227-238

IMPROVEMENTS IN MOTHER – CHILD HEALTH INDICATORS IN TURKEY*

*Ayşe Akın MD***, *Arzu Köseli MD****

SUMMARY: Akın A, Köseli A. (Directorate of Mother and Child Health and Family Planning, Ministry of Health, Ankara, Turkey). Improvements in mother-child health indicators in Turkey. Turk J Pediatr 1997; 39: 227-238.

Turkey has a young population as a result of high fertility and growth rates in the recent past. Thirty-five percent of the population is less than 15 years of age, and 25 percent of the population comprises reproductive-age woman. The latest estimate of the population growth rate was 19 per thousand for the 1990-1995 period. In recent decades dramatic declines in fertility rates have been noted. In the early 1970s, the overall fertility rate was approximately five children per woman, declining to 2.7 in 1993. The crude birth rate is currently estimated to be approximately 23 per thousand. The crude death rate has also declined from approximately 30 per thousand in the 1940s to 6.5 per thousand in the 1990s. Life expectancy at birth in Turkey is 65.9 for males and 70.5 for females. The infant mortality rate in the 1960s was approximately 200 per thousand, declining to 67 per thousand during the 1985-1990 period, and 53 per thousand for the period 1988-1993. It was 48 per thousand in 1995 and 42.2 per thousand in 1996, according to the State Planning Organization. The infant mortality rate has declined by 35 percent in the last ten years. The mortality rate for children under five years of age was 113.5 per thousand between 1978-1983 and 60.9 per thousand in 1993; it is currently 50 per thousand. The maternal mortality rate was greater than 200/100,000 in 1995. During the last five years the proportion of women receiving antenatal care has increased from 43 to 63 percent. The proportion of safe deliveries was 76 percent. Thirty-nine percent of all deliveries occur at home. The important point here is that the proportion of unsafe deliveries assisted by traditional birth attendants is 24 percent.

It is very obvious that during the last 15 years, maternal and child health (MCH), especially child health, has improved dramatically in Turkey. The improvement made in the last five years has been more marked. The improvement is closely related to the government's special interest, attention and efforts to prevent and identify the most common health problems and to overcome these problems with appropriate interventions. The government also pays special attention to the socio-economic priority areas of the country and initiates special health programs in these areas first, in order to reduce high regional differences in MCH indices.

Despite these dramatic improvements, one great obstacle is the high maternal and infant mortality and morbidity rates in the country at the time of ratification of the European Social Charter. The success made so for in maternal and child health should not be ignored, but it must be realized that much still remains to be done to improve the MCH level further. *Key words: child health, maternal health, Turkey.*

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Turkey is a Middle Eastern country representing a bridge between Asia and Europe. The country has a surface area of 774,815 square kilometers and, according to the 1995 census, a population of 62,526,000. Ninety-eight percent of the population is Muslim. Turkey is among the 20 most populous countries of the World and is the most populous country in the Middle East.

Turkey has a young population as a result of high fertility and growth rates in the recent past. Thirty-five percent of the population is less than 15 years of age, while the population of the elderly is quite low. Reproductive-age women represent 25 percent of the population.

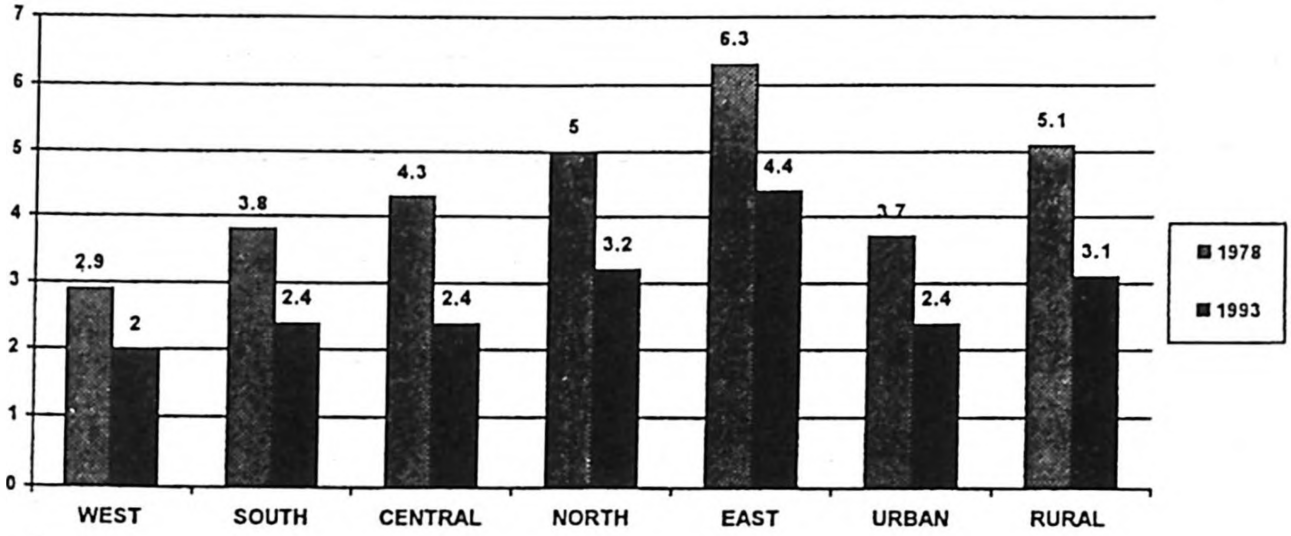
Intercensal estimates of population growth (PGR) have been around 20-25 per thousand since the 1970s. The latest estimate of the PGR was 19 per thousand for the 1990-1995 period. Now it is estimated to beetven lower at 17 per thousand.

Recent decades have witnessed dramatic declines in fertility rates. In the early 1970s the total fertility rate was approximately five children per woman, declining to 2.7 in 1993. The crude birth rate is estimated to be around 23 per thousand now. The crude death rate has also declined from around 30 per thousand in the 1940s to 6.5 per thousand in the 1990s. Life expectancy at birth in Turkey is currently 65.7 for males and 70.3 for females.

The infant mortality rate in the 1960s was approximately 200 per thousand, declining to 67 per thousand during the 1985-1990 period, and 53 per thousand for the period 1988-1993. It was 48 per thousand in 1995 and 42.2 per thousand in 1996, according to the State Planning Organization. The infant mortality rate has been reduced by 35 percent in the last ten years. The mortality rate for children age five was 113.5 per thousand between 1978-1993, 60.9 per thousand in 1993, and is now 50 per thousand.

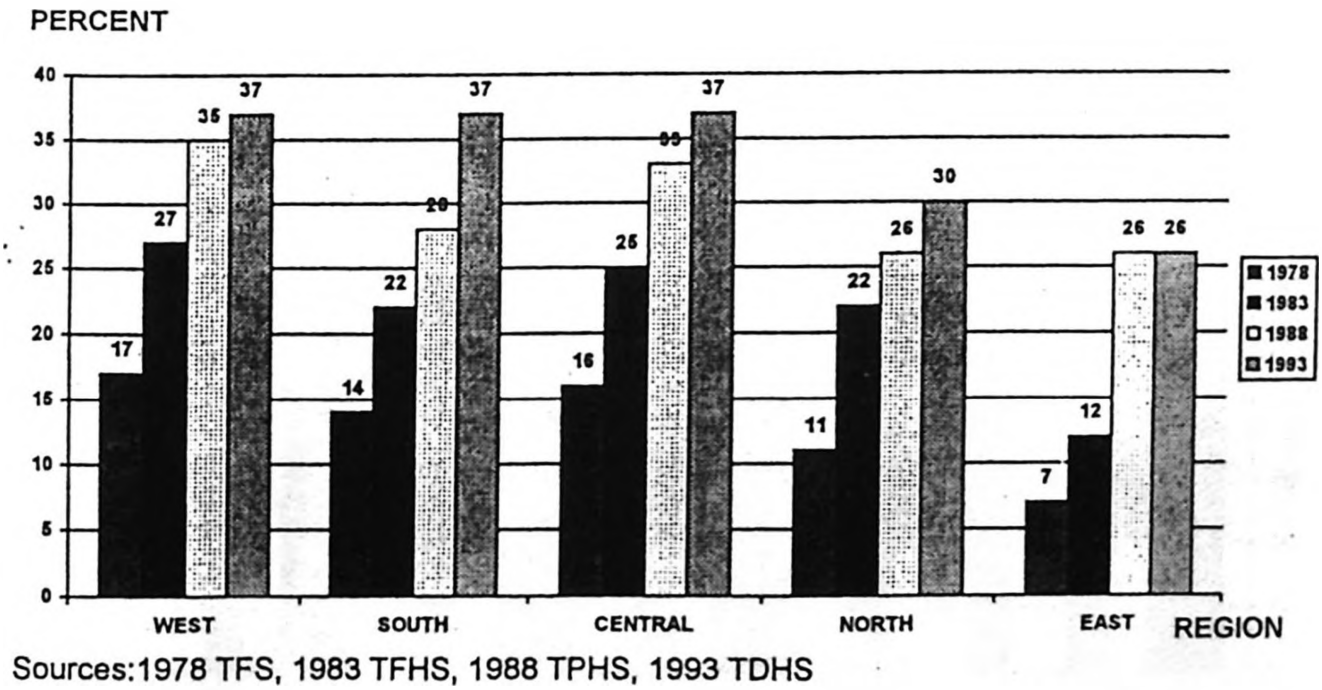
The maternal mortality rate was greater than 200/100,000 live births in 1974. It decreased to 132 in 1981, and was estimated to be 100/100,000 in 1995. A combined community and hospital-based study on maternal and perinatal mortality has been planned in collaboration with WHO.

During the last five years the proportion of women receiving prenatal care has increased from 43 to 63 percent. The proportion of safe deliveries is 76 percent. Overall 39 percent of deliveries are being done at home. The important point here is that the proportion of unsafe deliveries (those assisted by traditional birth attendants) is 24 percent. The leading problem in this situation is accesibility of health services rather than their existence due to certain social and environmental factors in some regions; the low social status of women and/or hard winter conditions very often create problems related to transportation and accessibility.



Sources:
1978 TFS, 1993 TDHS

Fig. 1: Total fertility rate by region and urban-rural residence



Sources:1978 TFS, 1983 TFHS, 1988 TPHS, 1993 TDHS

Fig. 2: Current use of modern contraception by region
(percentage of currently married women age 15-49).

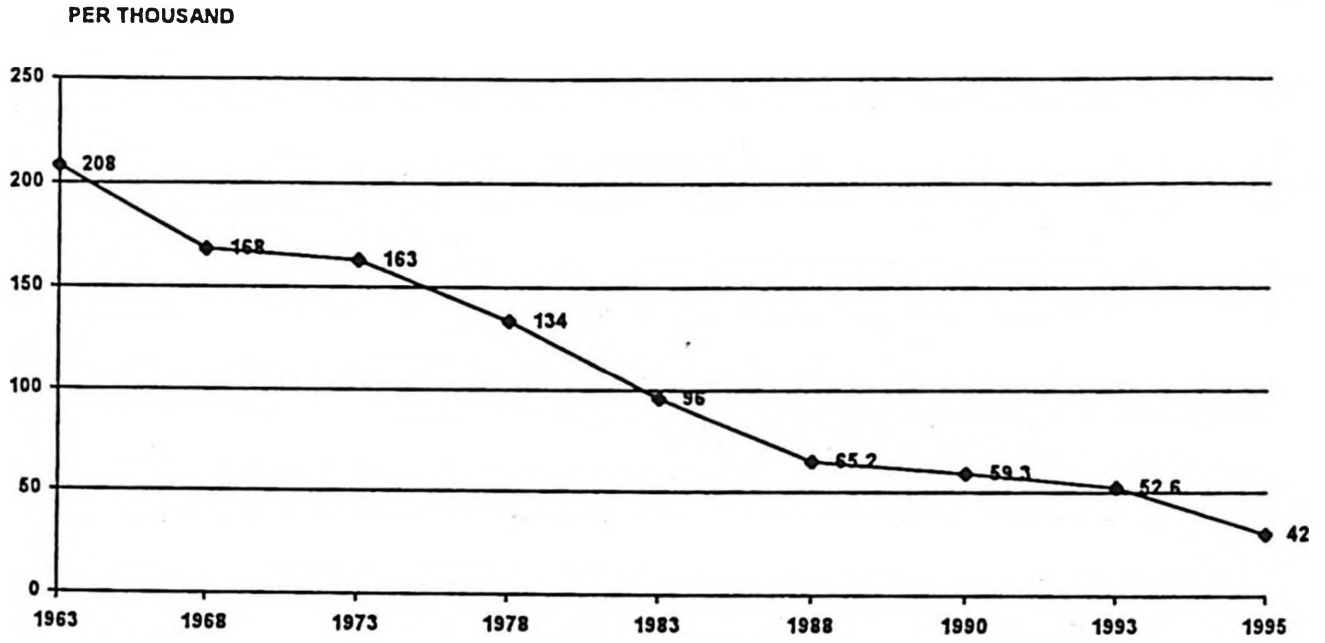


Fig. 3: Infant mortality rate by year.

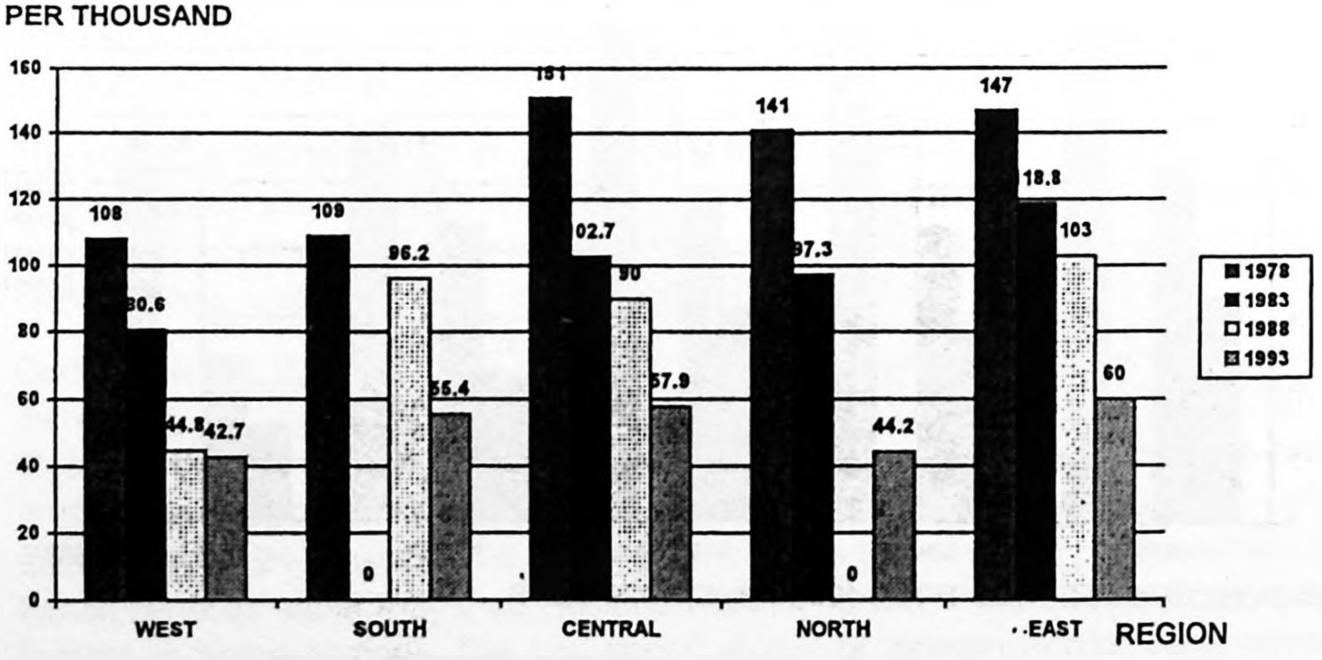
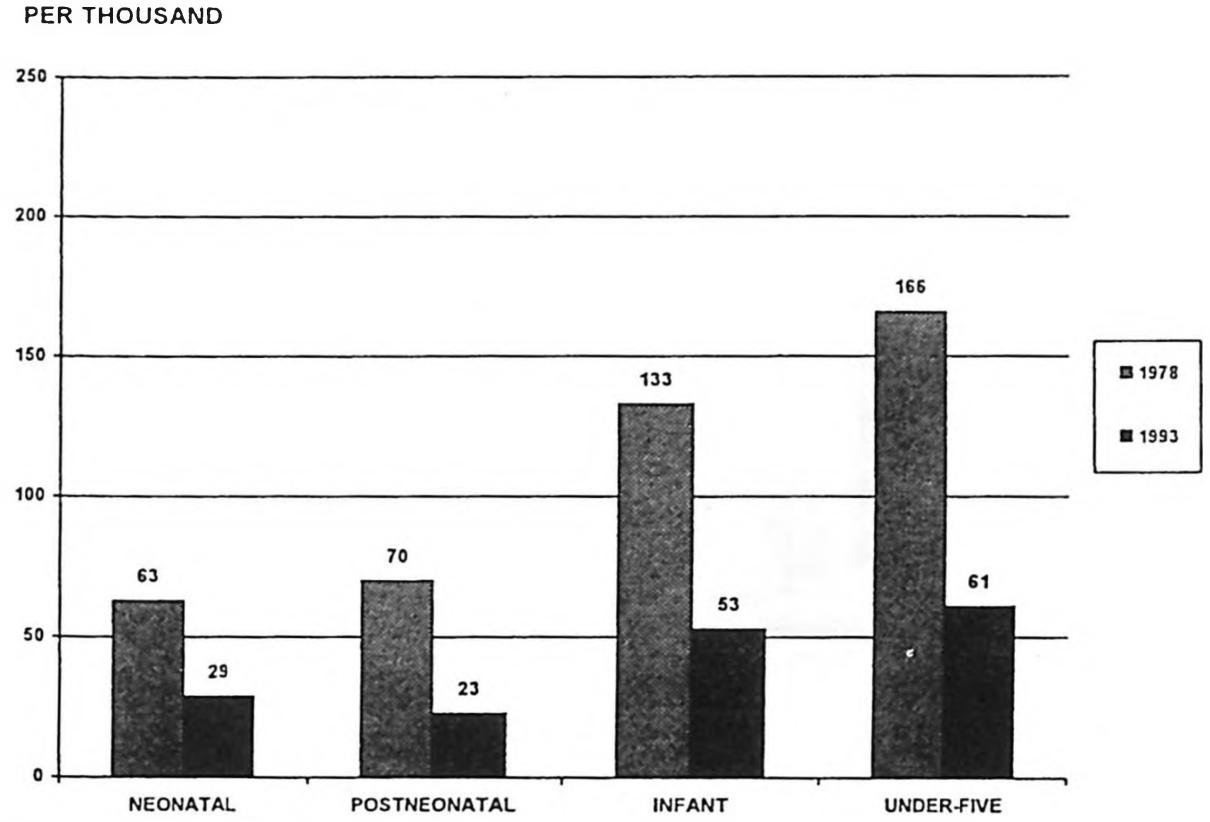


Fig. 4: Infant mortality rate by region.



Sources:
1978 TFS, 1993 TDHS

Fig. 5 Childhood mortality rate 1973-1978 and 1988-1993.

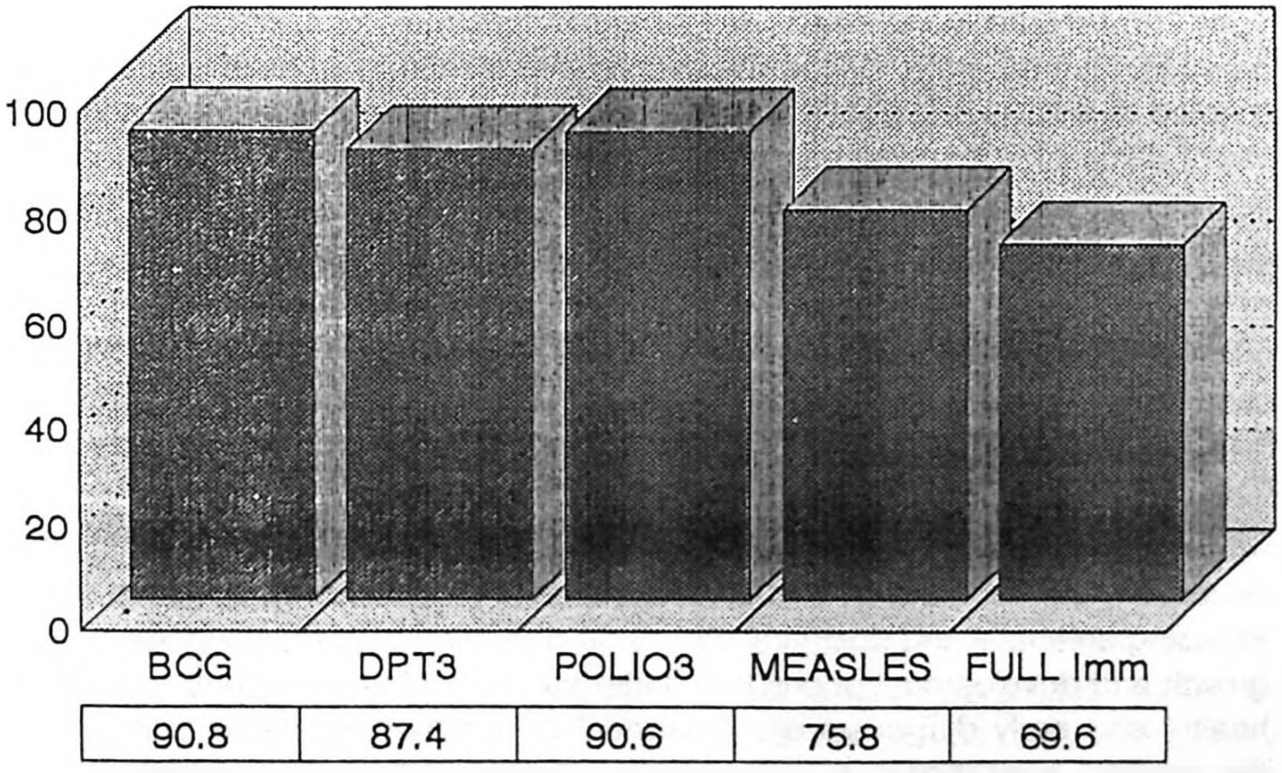


Fig. 6: Immunization rates among children aged 12-23 months in Turkey.

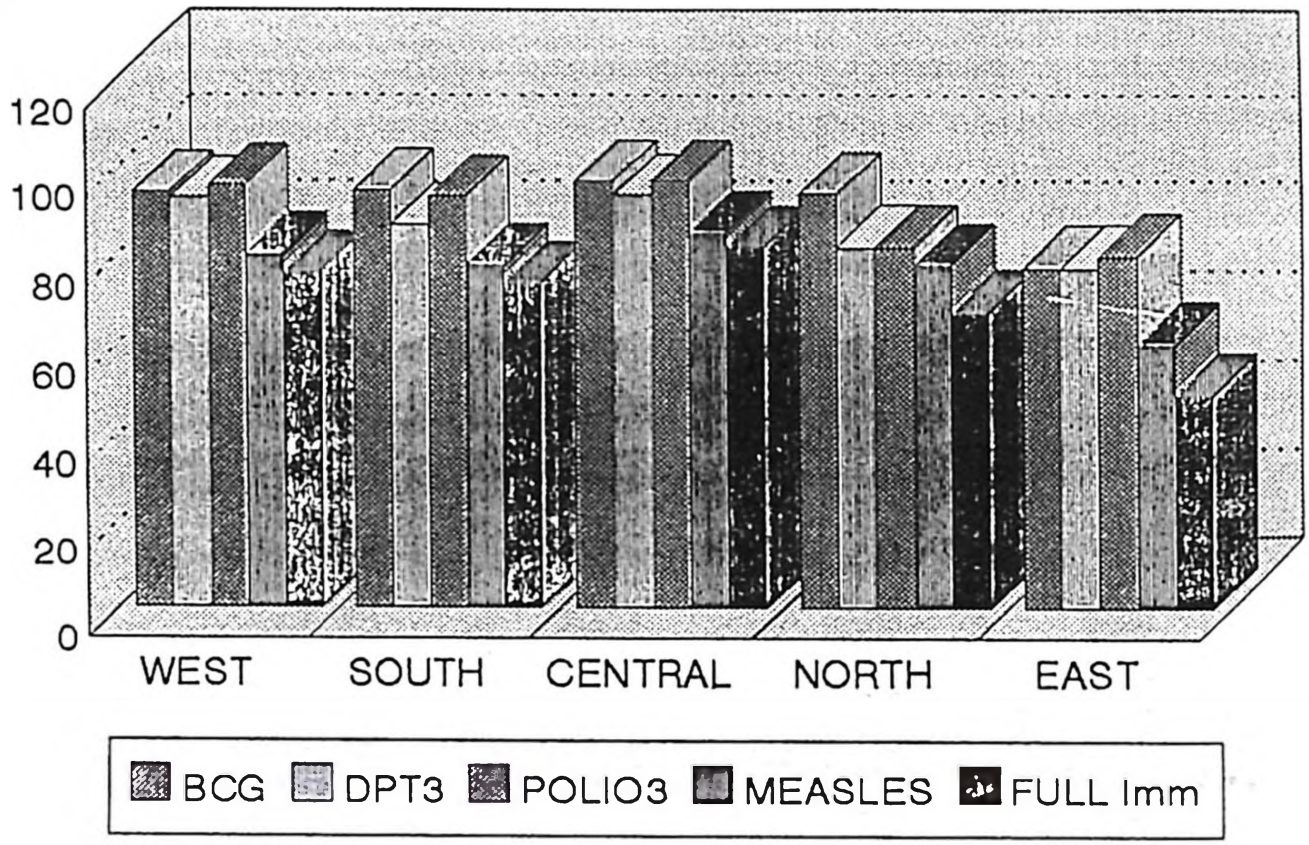


Fig. 7: Immunization rates among children aged 12-23 months according to region.

Mother and Child Health Care Services in Turkey

Family and mother-child health is secured by our main constitutions as one of the basic human rights. Since the law on socialization of health services was enacted in 1961, mother-child health services have been provided as an integral part of primary health care services, and an intersectorial approach is encouraged.

The infrastructure of the National Health Care System is quite favorable for providing these services all over the country. At the grass roots level, there are 11,877 health facilities where nurse-midwives provide MCH/FP services. At the primary health care (PHC) level, there are 4,987 health centers and 270 MCH/FP centers where a health team including specialists, general practitioners and nurse-midwives provide PHC services. Those PHC units are supported by 1,000 hospitals in the country.

At the primary health care level, the services include: counseling, information, education, communication and clinical services related to reproductive health, including antenatal and postnatal care; safe delivery; family planning; monitoring, growth and development of children under six years of age; immunization; family health; and early diagnosis and treatment of common infectious diseases. At the hospital level, besides these services further diagnosis and treatment for risks and complications due to pregnancy, delivery and infancy are provided.

Since mother and child health care services are accepted as integral components of primary health care, the service coverage is intended to meet the needs of not only women but also adolescents and men.

Traditional attitudes of the government toward population growth began to change in the 1950s, mainly due to medical problems (e.g., realization of the existence of high maternal mortality caused by illegal abortions). High urbanization and unemployment problems were also factors contributing to the new anti-natalist policy. The State Planning Organization and the Ministry of Health pioneered the policy change; previous policies became more liberal by allowing provision of contraceptives as well as information, education and counseling (IEC) activities of family planning. The first anti-natalist Population Planning Law was enacted in 1965.

In 1983, the law was revised and a more liberal and comprehensive one was accepted. This new law legalized abortion up to the 10^{th} week of pregnancy plus voluntary surgical contraception. In addition, trained nurses and midwives were authorized to provide effective methods and perform tasks such as IUD insertion. In this law, intersectorial collaboration was also emphasized for the success of family planning services.

The Population Planning Advisory Committee (PPAC) was established to secure conformity to human rights, and to ethical and professional standards in the delivery of health care to mothers, family planning and related reproductive health services and to ensure responsible, voluntary and informed consent. This committee is headed by the Minister of Health. In 1993, the women's Health Advisory Board was established under the PPAC, and includes several representatives of governmental and non-governmental organizations (Ministries, international organizations, NGOs, the State Planning Organization, the State Statistical Institute, Hacettepe Institute of Population Studies, and the Higher Education Council).

The Turkish intersectoral committee for Child Survival and Development was established in 1989. Its name was changed to the "Child Intersectoral Board" in 1990. Its mission is to monitor and supervise Child Health-related activities and report regularly to the President.

Besides routine services, special MCH programs have been implemented by the MOH since 1985 in collaboration with several international organizations such as UNICEF, UNFPA, USAID, JICA and GTZ in order to improve MCH levels where the needs are greatest.

These special program include:

- Expanded immunization program, including: Eradication of poliomyelitis program.
- Fluorine use in oral and dental health
- Safe motherhood and newborn care program

- Elimination of neonatal tetanus program
- Control of acute respiratory infections
- Control of diarrheal diseases.
- Inservice training program for medical staff.
- Management Information Systems.
- Information-education communication support project
- Promotion of Baby Friendly Hospital Initiative.
- Elimination of iodine deficiencies and promotion of iodized salt.
- Phenylketonuria screening.

To minimize the differences between regions and declines in health indicators, the Second Health Project was implemented in 1995 in collaboration with the World Bank, and will continue until 2001. This project covers eastern and southeastern parts of Turkey where the need to improve MCH levels is greatest. Within the framework of this project, all mother-child health and family planning program are being carried out as an integrated package program. It is expected. that the health indicators will improve further within few years.

The safe motherhood approach is considered one of the priority program of the country that will increase the utilization of health care services, especially through, community-based health care activities. The Ministry of Health plans to expand the safe motherhood approach throughout the country.

For in-service training and public education, a number of IEC and source materials have been produced and are being used such as:

- National Family Planning Guidelines
- Mother Health and Family Planning Handbook
- Child Health Handbook
- Situation analysis of mothers and children in Turkey
- Management of Diarrheal Diseases
- Diagnosis and Treatment of Acute Respiratory Infections
- Source books on Safe Motherhood Program
- 1993 Turkish Demographic and Health Survey
- Source book on population-development and the environment
- Prenatal care, breastfeeding, family planning, contraception flipbooks for counseling
- ICPD documents

To improve and strengthen MCH/FP services and to reach the WHO "Health For All" targets by the year 2000, national targets and strategies have been defined and the following two documents have been prepared and are in use now. The main objective is to give these services in more accessible, affordable ways with the highest quality and equality by sharing the responsibilities.

These documents include:

1. National Programme of Action for Children was prepared in 1993 and updated in 1995. Being the product of comprehensive work, the document "National Programme of Action" aims to realize the overall goals of the World Summit for Children at the national level.

2. To help couples and individuals meet their reproductive goals in a framework that promotes optimum health, responsibility and family well-being and respects the dignity of all persons and their right to access qualified reproductive health services, the Women's Health and Family Planning Strategic Plan was prepared in 1995.

The defined targets of the National Plan of Action are as follows:

Maternal Health and Family Planning:

By the year 2000 (based on 1990 rates):

1. Reducing maternal and perinatal mortality by 50 percent.
2. Increasing the proportion of the practice of modern family planning methods to 70 percent.
3. Detecting all pregnancies and ensuring prenatal care.
4. Ensuring all deliveries occur in healthy conditions.
5. Reducing all inter-regional differences in health service indicators by 75 percent.

Child Health:

By the year 2000 (based on 1990 rates):

1. Reducing the infant mortality rate by one-third.
2. Reducing the mortality rate of children under the age of five years by 50 percent.
3. Reducing the mortality rate of children under age five due to diarrheal diseases by 50 percent, and the incidence of diarrheal diseases by 25 percent by the year 2000.
4. Increasing oral rehydration therapy to 80 percent.
5. Reducing the mortality rate of children under age five due to ARI, particularly pneumonia.
6. Reducing the severe and moderate malnutrition of children five years of age by 50 percent.
7. Achieving 95 percent immunization coverage for each vaccine in children than age one.
8. Eradicating polio.
9. Eliminating, neonatal tetanus.
10. Reducing the measles incidence by 90 percent and the mortality rate due to measles by 95 percent.

Conclusion

It is very obvious that during last 15 years the MCH level, especially the child health level, has improved dramatically in Turkey (appendices). The improvement made in the last five years has been more marked. The improvement is closely related to the government's special interest, attention and efforts to prevent and identify the most common health problems, and efforts to overcome these problems with appropriate interventions. Also, the government pays special attention to the socio-economic priority areas of the country and initiates special health programs first in these areas in order to reduce the high regional differences in MCH indices.

Despite the dramatic improvements summarized above, the main obstacle is the initial high maternal and infant mortality and morbidity in the country at the time of ratification of the European Social Charter. So far the success made in MCH should not be ignored, but it should be realized that much still remains to be done to improve the MCH level further.

Turkey Demographic and Health Indicators

Fact Sheet:	
Total population-1995 (millions)	62.2
Urban population (%)	59
Rate of Natural Increase-1994 (%)	1.8
Population Doubling Time (years)	31.9
Crude Birth Rate-1995 (per 1,000 population)	22.4
Crude Death Rate (per 1,000 population)	6.6
Life Expectancy At Birth (years)	65.7
Males	65.7
Females	70.3
Marriage and Other Fertility Determinants (1993)	
Percent of women age 15-49 currently married	64.6
Percent of women age 15-49 ever married	67.1
Median age at first marriage among women age 25-49	19.0
Median duration of breastfeeding (months)	11.9
Median duration of postpartum amenorrhea (months)	3.7
Median duration of postpartum abstinence (months)	1.9
Fertility (1993)	
Total fertility rate	2.7
Mean number of children ever born to women age 40-49	4.6

Desire for Children (1993)	
Percent of currently married women who:	
Want no more children	69.8
Want to delay next birth by at least 2 years	13.9
Mean ideal number of children among women age 15-49	2.4
Percent of women a non numeric response to ideal family size	1.8
Percent of births in the last five years which were:	
Unwanted	20.4
Mis-timed	12.0
Knowledge and Use of Family Planning (1993)	
Percent of currently married women:	
Knowing any method	99.1
Knowing a modern method	98.6
Knowing a modern method and knowing a source for the method	94.6
Who have ever used any method	80.1
Who are currently using any method	62.6
Percent of currently married women currently using:	
Pill	4.9
IUD	18.8
Injection	0.1
Diaphragm, foam, jelly	1.2
Condom	6.6
Female sterilization	2.9
Male sterilization	0.0
Periodic abstinence	1.0
Withdrawal	26.2
Other traditional method	0.9
Mortality and Health (1993)	
Infant mortality rate	52.6
Under-five mortality rate	60.9
Percent of births in which mother	
Received antenatal care	62.3
Received 2 or more tetanus toxoid injections	26.2
Percent of births in which mother was assisted at delivery by a	
Doctor	33.7
Trained midwife/nurse	42.2
Traditional birth attendant	12.9

Percent of children	
0-1 months old who are breastfed	99.0
4-5 months old who are breastfed	79.7
10-11 months old who are breastfed	60.7
Percent of children 12-23 months old who received:	
BCG	89.1
DPT (3 doses)	77.1
Polio (3 doses)	77.2
Measles	77.9
All recommended immunizations	64.7
Percent of children under five years old who:	
Had diarrhea in the 2 weeks preceding the survey	24.8
Had a cough accompanied by rapid breathing in the 2 weeks preceding the survey	12.4
Are chronically undernourished (stunted)	18.9
Are acutely undernourished (wasted)	3.0

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HEPATITIS – B – ASSOCIATED GLOMERULONEPHRITIS IN CHILDREN*

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SUMMARY: Öner A, Tınaztepe K, Demircin G. (Pediatric Nephrology Unit, Dr. Sami Ulus Children's Hospital, and the Nephropathology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Hepatitis-B-associated glomerulonephritis in children. Turk J Pediatr 1997; 39: 239-246.

Three cases with hepatitis B virus (HBV)-related, biopsy-diagnosed glomerulopathies, one of which was membranous glomerulonephritis and the others membranoproliferative glomerulonephritis, are reported, emphasizing the clinical course. Two patients had spontaneous remission after seroconversion to anti-HBe-positivity, while the third patient was lost to follow-up. We reviewed the management of HBV-associated glomerulonephritis and concluded that immunosuppressive drugs should be avoided since spontaneous remission can be expected in these types of glomerulopathies. *Key words: hepatitis-B-associated glomerulopathies, membranous glomerulonephritis, membranoproliferative glomerulonephritis, spontaneous remission.*

The association between hepatitis B virus (HBV) infection and glomerulonephritis (GN) was first described by Combes et al.[1] in 1971, and since that time many other examples of the association have been documented[2-6]. Although the most common presentation of GN associated with HBV infection is membranous glomerulonephritis (MGN), membranoproliferative glomerulonephritis (MPGN) has also been reported[7-13].

The aim of this report was to present three children having hepatitis B surface antigenemia (HBs Ag) associated with glomerulonephritis (two cases with MPGN and one case with MGN), and to emphasize the occurrence of spontaneous remission in these disorders without any immunosuppressive therapy.

Case Reports

Case 1

A three-year-old girl was admitted to the hospital with the chief complaints of swelling of the legs, oliguria and darkness of the urine, which had lasted for a month.

Physical examination on admission revealed edema and hepatomegaly two cm below the right costal margin. Laboratory data revealed a hemoglobin (Hb) level of 11 g/dl and a white blood cell count (WBC) of 12,400/mm^3. Serum

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transaminases, creatinine, total protein and albumin levels were within normal limits. The patient had microscopic hematuria. Daily urinary protein loss was not in the nephrotic range (265 mg/m^2). Third component of complement (C3) (90 mg/dl) and C4 (37 mg/dl) were normal. Abdominal ultrasonography showed diffuse hepatomegaly. Excretory urography was normal. She was followed up for acute GN.

Two months after the initial hematuria, she developed nephrotic syndrome with a serum albumin level of 2.5 g/dl and a daily urinary protein loss of 2.8 g/m^2. Macroscopic hematuria was present. Her liver was enlarged to three cm below the costal margin, and slight splenomegaly was noticed. Liver enzymes were significantly elevated: alanine aminotransferase (SGPT) 1450 IU, aspartate aminotransferase (SGOT) 800 IU. Results of serologic studies for HBV were as follows: HBs Ag (+), antibody to HBs Ag (anti HBs) (-), antibody to hepatitis B core antigen (anti HBc) (-), hepatitis B e antigen (HBe Ag) (+), antibody to HBe Ag (anti HBe) (-). The patient was followed up for HBV-related GN with supportive therapy.

Six months after the development of nephrotic syndrome, her liver enzymes decreased, while anti HBe and anti HBc appeared in the serum. The proteinuria reached 6 g/m^2/day.

Percutaneous renal needle biopsy was performed 10 months after the initial hematuria. Light microscopy showed thickening of the glomerular capillary wall with demonstrable spikes related to the presence of subepithelial and intramembraneous deposits without increased cellularity (Figs. 1, 2). Immunofluorescence studies confirmed rather diffuse granular depositions of C3 (++), C1q (+), IgG (++), IgM (+) and fibrinogen (++) along the glomerular basement membrane zone (BMZ). The patient was diagnosed with MGN, stage III.

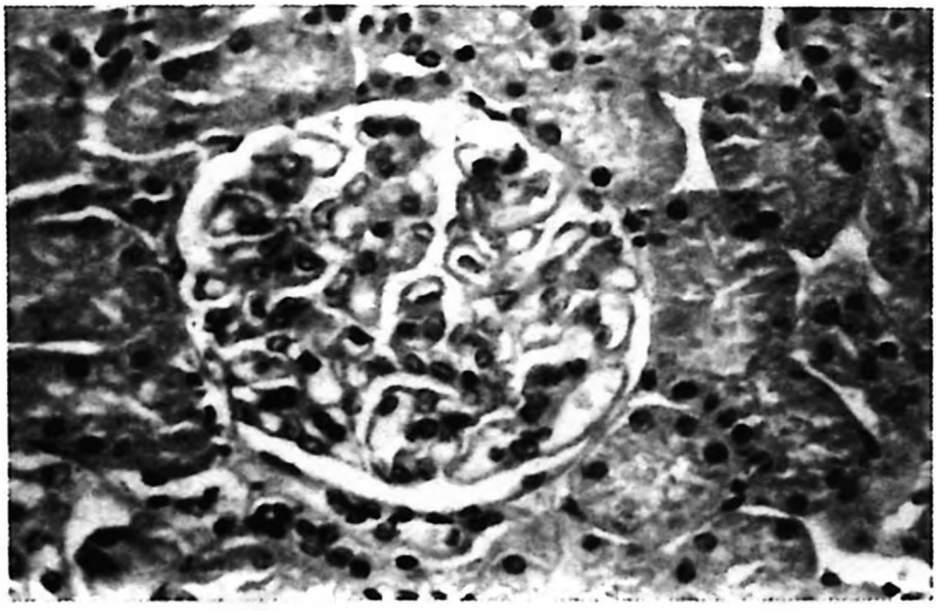

Fig. 1: A glomerulus of a case of MGN showing thickening of the basement membrane without hypercellularity, open capillary luminae (H+E stain X 200, Case 1).

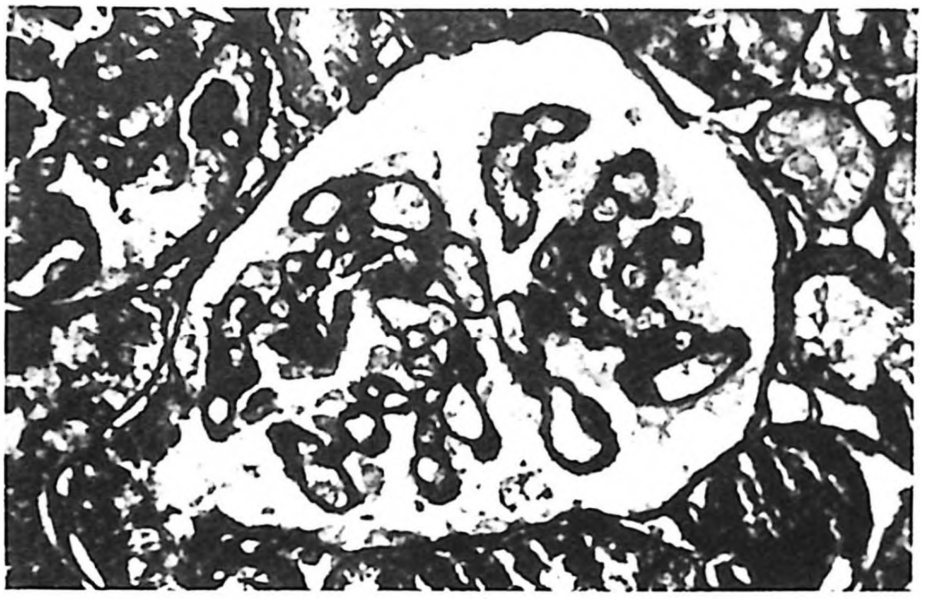

Fig. 2: A glomerulus with subepithelial and intramembranous humps in stage III membranous glomerulonephritis (X 225 Johnes Silver Stain, Case 1).

The patient received albumin infusions and low-dose diuretic therapy for seven months. A year after the renal biopsy, spontaneous remission occurred. She has not developed any relapse for 24 months.

Case 2

A 12-year-old girl was admitted to the hospital because of edema and darkness of the urine.

Physical examination revealed a well-developed child with elevated blood pressure (140/90 mm Hg) and periorbital edema. The laboratory data showed normal complete blood count, total protein and albumin levels, and elevated blood urea nitrogen and creatinine levels (30 mg/dl and 2.5 mg/dl, respectively). Liver enzymes were slightly increased. The levels of C3 (16 mg/dl) and C4 (10 mg/dl) were decreased. Antinuclear antibody (ANA) was found to be negative, while anti DNA was within normal limits (4.5 IU/ml). Serological studies for hepatitis B virus were as follows: HBs Ag (+), anti HBc IgM (-), anti HBc IgG (+), HBe Ag (+), anti-HBe (-). The mother and one sibling of the patient were HBs Ag carriers no hepatic or renal disease. The urinalysis showed hematuria and proteinuria (++). The daily urinary protein loss was 900 mg/m^2. Abdominal ultrasonography demonstrated normal echogenicity of the kidneys and the liver.

Percutaneous renal needle biopsy was performed. Light microscopic examination revealed hypercellular enlarged glomeruli with increment of the mesangial matrix and double countering of the basement membrane in a few places (Fig. 3). Fluorescent microscopic study showed depositions of C3 in the BMZ, without

demonstrable depositions of immunoglobulins. Membranoproliferative glomerulonephritis was diagnosed, and the patient was followed up with supportive therapy. Six months after the initial symptoms, anti-HBe positivity was noted and spontaneous remission occurred within eight months. All the clinical and laboratory findings returned to normal except the HBs Ag positivity which persisted for 18 months.

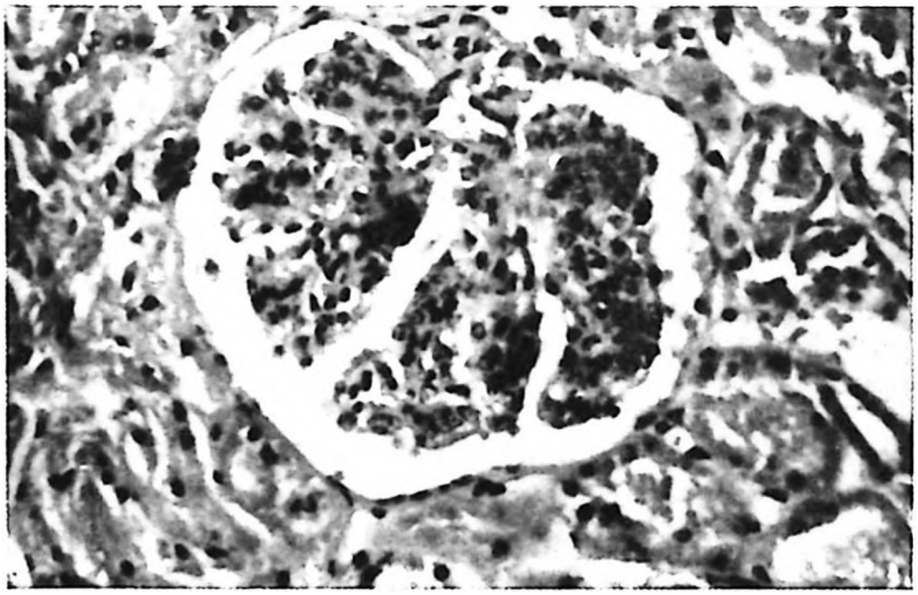

Fig. 3: A glomerulus of a case of MPGN showing hypercellularity with increment of mesangial matrix forming lobulation and obstruction of the majority of the capillary luminae (X 200, H+E, Case 2).

Case 3

A nine-year-old boy was admitted to the hospital with the complaint of edema of the face and legs for the previous month. Similar symptoms had appeared six months prior to admission and his symptoms had disappeared in one month following corticosteroid treatment.

Physical examination revealed marked orbital, scrotal and ankle edema with ascites in the abdomen. The laboratory examination showed normal Hb, WBC, blood urea nitrogen and creatinine levels. Serum albumin was 2.3 g/dl, SGOT: 426 IU, SGPT: 241 IU, the serum electrolytes, C3 and C4 levels were within normal limits. HBs Ag was (+), HBe Ag (+), anti HBe (-), and anti HBc IgM (+). Urinalysis revealed heavy proteinuria (++++) and hematuria. Daily protein loss was 10.6 g/m^2/day. Abdominal ultrasonography showed slightly enlarged kidneys.

Percutaneous renal needle biopsy was performed. Light microscopic study revealed characteristic findings consistent with MPGN. On fluorescent microscopic study, there was a frame-like pattern of glomeruli imitating lobulations with fine granular sticky deposits of C3 (++), C1q (+), IgG (++), IgM (+) and fibrinogen (+) in the BMZ. The patient was diagnosed with MPGN.

The patient was discharged from the hospital after his edema disappeared on diuretic therapy and albumin infusion. Five months after the biopsy the nephrotic syndrome was persisting with proteinuria of 5.78 g/m^2/day, while his liver enzymes were normal (SGOT: 25 IU, SGPT: 15 IU) and HBsAg was (+). The patient was unfortunately lost to follow-up after that time.

The pertinent clinical data of these patients are summarized in Table I.

Table I: Selected Patient Data

	Case 1	Case 2	Case 3
Age at presentation	3 yrs	12 yrs	9 yrs
Sex	female	female	male
Clinical presentation of glomerular disease	Nephrotic + nephritic syndrome	Nephritic syndrome	Nephrotic + nephritic syndrome
Histological diagnosis	MGN (Stage III)	MPGN	MPGN
Treatment	supportive	supportive	steroid + supportive
Outcome	remission	remission	unknown

Discussion

The pathogenesis of HBV-associated GN is not completely understood. Immune complex mechanism including HBs Ag and HBe Ag, are thought to play an important role in the development of glomerular capillary wall damage[4, 5, 8]. The finding of immunoglobulins, complement components and viral antigens (HBs Ag, HBe Ag) within glomerular deposits also supports an immune complex pathogenesis[4, 5, 8]. Takehoshi et al.[14] demonstrated the presence of HBe Ag within glomerular deposits that were not stained with fluorescent anti-HBs and anti-HBc antisera. Ito et al.[15] reported glomerular deposits containing HBe-Ag in four children, and within one of them both HBe-Ag and HBs-Ag were demonstrated. From follow-up studies they concluded that larger HBs Ag immune complexes could be deposited secondarily along glomerular basement membrane damaged by smaller HBe Ag immune complexes, which may act as planted antigens. This hypothesis is compatible with the experimental findings of Adler et al.[16], who demonstrated that the endogenous antigen placed in rat glomerular capillary membranes produced MGN with complement-dependent, neutrophil-independent proteinuria. Spontaneous remission in patients with HBV-associated GN after seroconversion of HBe Ag to anti HBe is another example of the role of immune complex mechanism in the pathogenesis[10]. We also observed spontaneous remission in two of our patients with the appearance of anti HBe. On the other hand, we cannot explain why only Case 2 developed glomerulopathy, while the other two members of her family have no signs of GN although they are also seropositive for HBs Ag.

In most reports, HBV antigens have been detected within subepithelial deposits by immunofluorescence studies[4, 5, 8]. However, Kleinknecht et al.[3] were unable to detect HBs Ag in the kidneys of eight patients studied. We could not perform immunofluorescence studies to demonstrate HBV antigens in the glomerular lesions. Whether our cases represent a true association or a coincidental finding is uncertain. However, in all cases, HBs Ag was associated with elevated levels of transaminase and enlarged liver without jaundice at the onset of the GN. The disappearance of proteinuria was simultaneous with the occurrence of anti-HBe and anti HBc in Case 1 and anti HBe in Case 2, and with the normalization of transaminase levels. These results provide indirect evidence that the immune complex nephritis in these patients may involve the deposition or formation of immune-complex-containing HBV antigens.

Reports on hypocomplementemia and HBV-associated GN have previously been contradictory, but recently the tendency for low C3 and C4 levels in HBV-associated GN has been reported, suggesting the activation of the classical complement pathway[10]. Only one of our patients (Case 2) had low serum C3 and C4 levels during the acute phase of the disease, reaching normal values after remission. On the other hand, the type of MPGN in this case could not be demonstrated since only light microscopic examination was available.

HBV infection is the most frequent cause of MGN in children; however, MPGN and mesengial proliferative GN have been reported[4]. One of our patients had MGN while the other two had MPGN, which is a rare nephrological disease in children.

The optimal treatment for HBV-associated GN remains undefined. Corticosteroid therapy in patients with MGN and persistent HBs antigenemia was concluded to be harmful, as activation of viral replication could occur[17]. There are also several reports showing that different immunosuppressive therapies have been given to patients with MPGN with consistent efficacies[6, 10]. However, Wyszynska et al.[2] found that immunosuppressive treatment did not affect the course and outcome of the disease. The relapse of the symptoms of Case 3 five months after steroid therapy may also suggest that steroid therapy had no advantage in the course of the disease. Recently there have been several reports demonstrating the beneficial effect of recombinant human interferon-alpha treatment in HBV-associated GN[6, 18-21]. On the other hand, improvement of proteinuria in HBV-associated GN after spontaneous seroconversion of serum HBV markers has also been reported[15, 18]. We were not able to follow up Case 3, but the glomerulopathies of Cases 1 and 2 achieved spontaneous remission during the course of the disease after the seroconversion of HBe Ag to anti-HBe positivity, although the MGN of Case 1 was at stage III.

Although we do not have an adequate number of cases, we emphasize that spontaneous remission in HBV-related glomerulopathies can occur. Since

treatment with immunosuppressive drugs such as steroids or azathioprine may prolong the persistence of liver and/or renal disease, besides their other adverse effects, the use of such drugs should be avoided in HBV-associated GN, and patients should be given a chance for spontaneous remission. For patients with persistent infection, recombinant human interferon-alpha may be the treatment of choice.

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HYPOPHOSPHATEMIC VITAMIN-D RESISTANT RICKETS ASSOCIATED WITH EPIDERMAL NEVUS SYNDROME*

A Case Report

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SUMMARY: Tokatlı A, Coşkun T, Özalp İ. (Metabolism and Nutrition Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Hypophosphatemic vitamin-D-resistant rickets associated with epidermal nevus syndrome: a case report. Turk J Pediatr 1997; 39: 247-251.

Epidermal nevus syndrome is characterized by congenital anomalies affecting multiple body systems, especially the skin, skeleton and central nervous system. A form of rickets/osteomalacia that is markedly resistant to treatment with vitamin D has been reported in children with this syndrome. We report the clinical and laboratory observations in a child with epidermal nevi and severe hypophosphatemic rickets/ osteomalacia. *Key words: epidermal nevus syndrome, hypophosphatemic vitamin-D-resistant rickets/osteomalacia.*

Epidermal nevi are organoid nevi arising from the pluripotential germinative cells in the basal layer of the embryonic epidermis[1]. The nevi are classified by their predominant component as sebaceous, verrucous or comedoneous.

The term epidermal nevus syndrome (ENS) refers to the association of epidermal nevi with congenital anomalies affecting multiple body systems[1-3]. Synonyms for this syndrome are Solomon syndrome, linear nevus syndrome or Schimmelpenning-Feuerstein-Mims syndrome. The most frequent association has been central nervous system involvement; abnormalities in other organ systems are seen less frequently. Hypophosphatemic rickets/osteomalacia in ENS has been reported in recent years, and it has been suggested that vitamin-D-resistant rickets/osteomalacia is a variant of tumor-induced rickets/osteomalacia[4,5].

Here we present the clinical and laboratory observations in a child with epidermal nevi and severe hypophosphatemic rickets/osteomalacia.

Case Report

A five-year-old boy was referred to Hacettepe Children's Hospital in 1981 because of vitamin D-resistant rickets. The patient was the first child born to healthy, unrelated parents with no family history of skin or bone disease. At

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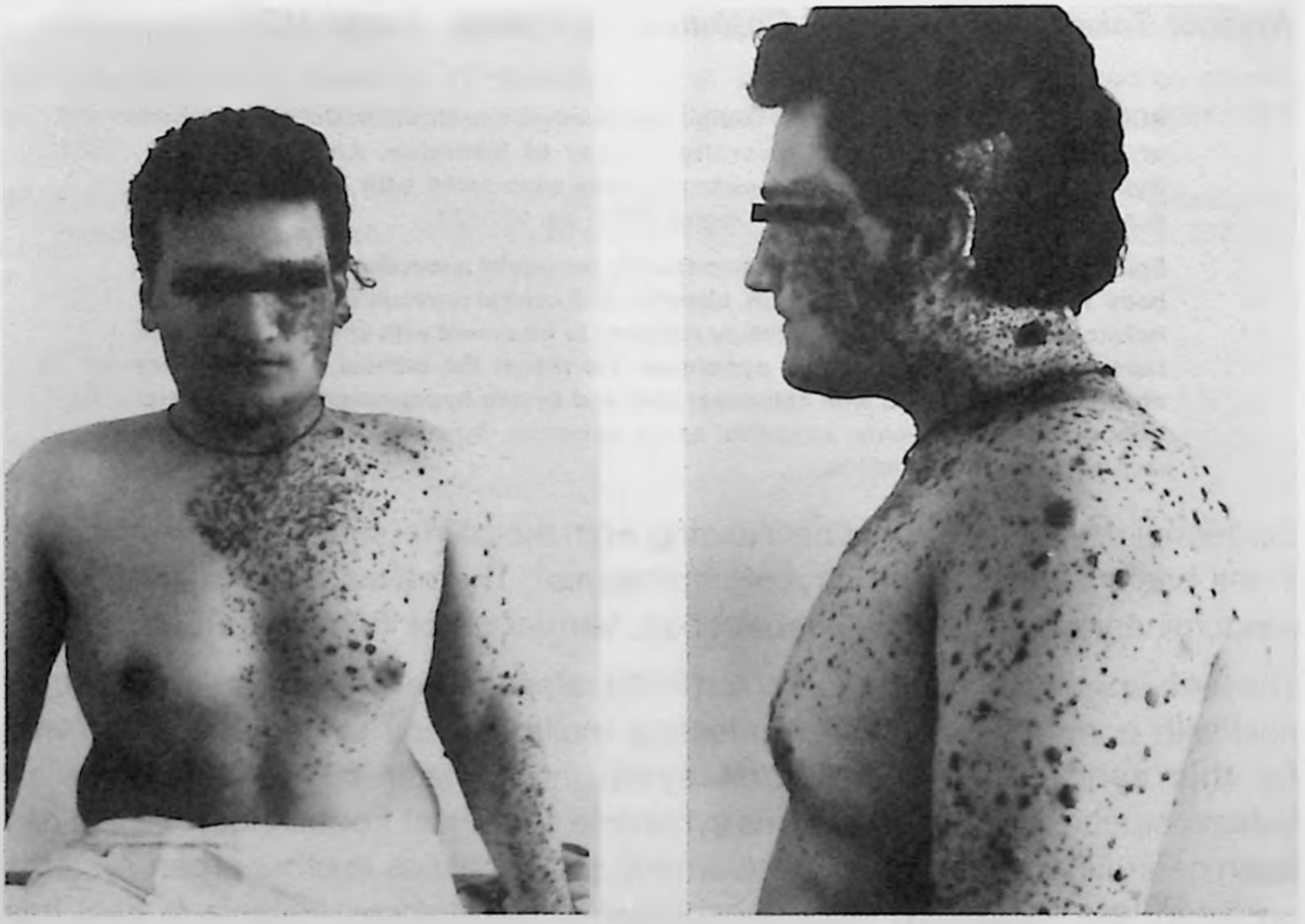
birth, extensive nevi involving the left side of the body were noted, as well as a large verrucous-sebaceus plaque over the left postauricular area, and multiple raised melanotic nevi covering the left side of the face, chest, back, left arm and left leg (Figs. 1-3).



Figs. 1 and 2: Patient at 16 years of age showing nevi on the left side of the body.

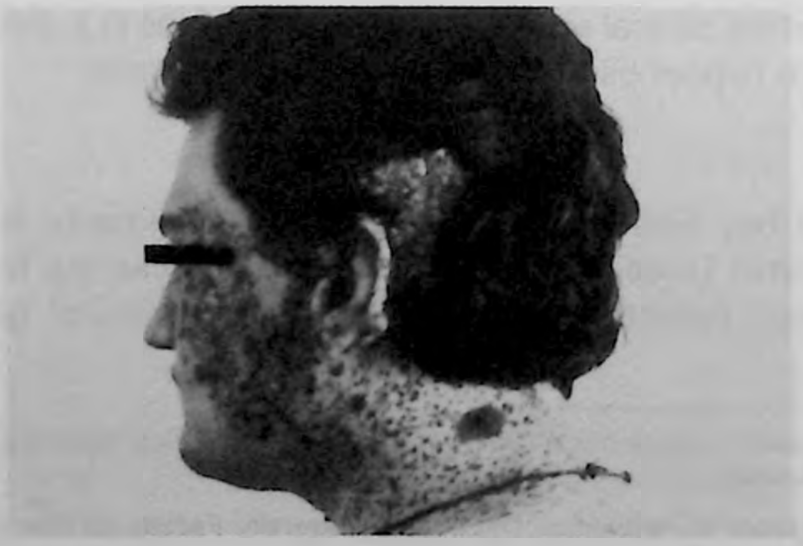


Fig. 3: Close-up view of the large verrucous nevus on the left side of the scalp.

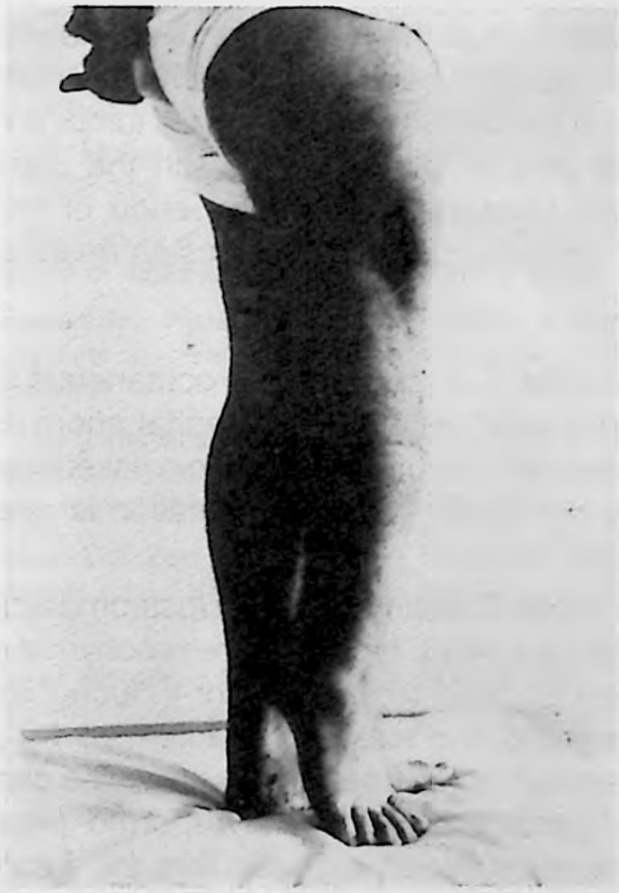


Fig. 4: Note the difference in leg length and the skin lesions on the left leg.

At the age of two years, a difference in the length of the patient's two legs was noticed; his right leg was shorter than the left (Fig. 4). At that time, the patient was investigated elsewhere, the rickets was recognised, and he was prescribed vitamin D several times. At the age of five years, he was referred to our hospital because of vitamin D-resistant rickets. On admission, besides the leg-length difference and the skin lesions which had already been fully noticed at birth, enlargement of the costochondral junctions, wrist and ankles as well as knock-knees were noted. His linear growth had been below the 3 third percentile, but his developmental milestones were normal. The serum calcium concentration was 10.6 mg/dl, serum phosphorus 1.7 mg/dl, and alkaline phosphatase 25 U/L. Active rickets was observed on roentgenograms. At that time, therapy was started with vitamin D 50.000 IU/day and phosphorus 2 g/day p.o. From ages five to 11, the child received vitamin D at doses up to 50.000 IU/day as well as oral phosphorus supplementation with no evidence of radiographic or biochemical healing. He continued to complain of bone pain. After substitution of vitamin D with 1-α hydroxyvitamin D_3, the patient's general condition gradually improved; however, despite phosphorus therapy he was still hypophosphatemic.

Because of the known relation between hypophosphatemic rickets/osteomalacia and epidermal nevi, excision of the skin lesion was planned, but the extensive distribution of the skin lesion in our patient precluded total surgical extirpation. After surgical removal of the verrucous-sebaceous tumor on the scalp, his serum phosphorus level was able to be regulated with the combination of calcitriol [1,25 $(OH)_2D$] and oral phosphorus. Over a period of months, his bone pain disappeared. Biopsy specimens of the scalp lesion revealed nevus sebaceous.

Discussion

Epidermal nevus syndrome is a sporadic neurocutaneous disorder that consists of epidermal nevi and a wide variety of congenital anomalies of the brain, eye, skeleton and other systems[1-3]. Hypophosphatemic rickets/osteomalacia has been described in patients with ENS[4-7]. This combination is rare and has been only sporadically described.

Hypophosphatemic vitamin D-resistant rickets/osteomalacia has been reported in association with various tumors, including mesenchymal and ossifying tumors, and this phenomenon has been termed "tumor-induced rickets/osteomalacia"[8]. Tumor-induced rickets/osteomalacia is characterized by remission of rickets/osteomalacia after resection of the coexisting tumor. The clinical and biochemical reversal of the rickets/osteomalacia in the reported patients with ENS upon surgical removal of the skin lesions suggests that the disorder may be a variant of tumor-induced rickets/osteomalacia. It has been shown that material extracted from the skin lesion of the patient with ENS and rickets/osteomalacia caused phosphaturia in an experimental animal[9]. However, if there is a relation between the skin lesion and rickets-osteomalacia, there is no explanation as to why the complication of rickets/osteomalacia is rare in ENS. Some authors suggest that tumors with different histologic patterns will be found associated with hypophosphatemic vitamin D-resistant rickets/osteomalacia; some types of tumors are responsible for the patient's phosphaturia, but the others do not cause renal phosphate loss which in turn is responsible for the rickets/osteomalacia[9].

The clinical and biochemical findings in our patient were fully compatible with ENS and hypophosphatemic vitamin-D-resistant rickets/osteomalacia. Treatment with vitamin D and phosphorus did not result in complete healing of rickets/osteomalacia. However, removal of parts of skin lesions influenced the rickets/osteomalacia. This result emphasizes that successful therapy for rickets/osteomalacia in ENS cannot be achieved with the mere combination of calcitriol and phosphorus.

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COMPOUND HETEROZYGOSITY FOR HEMOGLOBIN KNOSSOS [$\alpha_2\beta_2$ 27 (B9) Ala – Ser] and IVS I – 1 MUTATION*

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SUMMARY: Gürgey A, Balkan H, İrken G, Gümrük F, Altay S, Kalaycıoğlu A, Öner C, Öner R. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, and Department of Biology, Faculty of Science, Hacettepe University, Ankara, and Hematology Unit, Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Compound heterozygosity for hemoglobin Knossos [$\alpha_2\beta_2$ 27 (B 9) Ala-Ser] and IVS I-1 mutation. Turk J Pediatr 1997; 39: 253-256.

A three-year-old female with compound heterozygosity for Hb Knossos and IVS-I-1 mutation is presented. On physical examination she had no abnormality except for pallor. Hb was 6.9 g/dl, MCV 61 fl, Hb A2 2% and Hb F 38.5%. Acrylamidegel electrophoresis at a pH of 6 revealed the presence of Hb Knossos in the child and her father. DNA studies revealed that the child was compound heterozygous for Hb Knossos and the IVS I-1 mutation. When the clinical expression of this combination in a previously reported patient with Hb Knossos/FSC8 mutation is compared, it is shown that the newly presented patient has a more severe condition, indicating that the mutations in the trans of Hb Knossos may play a role in the phenotypical expression of the disease.
Key words: Hb Knossos, β-thalassemia, mutation, abnormal hemoglobin.

Hemoglobin (Hb) Knossos (β-27 Ala-Ser) results from a mis-sense mutation at codon 27 which causes abnormal RNA processing[1,2]. Although Hb Knossos cannot be shown in routine electrophoretic study, abnormal β-chain can easily be detected by acrylamide gel electrophoresis[1-4]. Hb Knossos was originally reported in a Greek patient with β-thalassemia intermedia[1]. Later it was found in patients from Algeria and Egypt[3,4] and in Turkey in a patient with trans of frame shift codon 8 (FSC8) mutation whose clinical and hematological picture was very mild[5]. Since FSC8 itself is associated with β-thalassemia intermedia,

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it was not clear to us whether the mildness of the diseases was due to Hb Knossos mutation or FSC8, or both. Therefore we believe that comparison of this patient with a patient with compound heterozygosity for Hb Knossos and a severe β-thalassemia mutation would be interesting.

Case Report

A three-year-old girl was referred to Dokuz Eylül University for evaluation of anemia. She was the first product of a nonconsanguineous marriage and had been pale since one year of age. One year previous to her referral, she received transfusion of one unit of blood.

On physical examination at admission, her weight was 14 kg, height 95 cm (both were at the 50th percentile) and there was no organomegaly. In laboratory studies, Hb was 6.9 g/dl, packed red cells 0.19 L/L, MCV 61 fl, Hb A_2 2 percent and Hb F 38.5 percent. The Hb A_2 and Hb F levels of the mother and father are shown in Table I. The presence of a slient mutation was predicted due to the normal Hb A_2 level in the father. Therefore, acrylamide gel electrophoresis was performed, and the presence of Hb Knossos mutation. was detected in the patient and her father. DNA was extracted from the patient's white blood cells and from both parents, according to the procedure described by Poncz et al.[6] The presence of α-thalassemia was studied by digestion of genomic DNA with Bam HI and Bgl II and hybridization to the α-specific gene probe, as described previously[7].

Table I: Hematologic Data of The Family

	Age (Years) and Sex*	Hb g/dl	MCV fl	HbA2 %	HbF %	Genotype
Propositus	3F	6.9	61	2.0	38.5	Hb^{Knos}/IVS-1-1
Mother		11.0	56	4.9	1.7	IVS-1-1/β^A
Father		13.0	72	2.5	0.9	Hb^{Knos}/β^A
Case 2	17M	8.0	77	1.2	46.5	Hb^{Knos}/FSC8 (ref: 5)

* Age at presentation.

In-vitro amplification of the β-globin gene region was performed by two-stage polymerase chain reaction (PCR) described by Saiki et al.[10] with minor modifications using a DNA Thermal Cycler. For the first-stage amplification, a 1860 bp double-stranded fragment of β-globin region was obtained using equal amounts of the primers (100 pmol) at position -158 in the promoter region and at position 128 after the poly A signal in the 3' untranslated region. For the second-stage amplification, the region between position -148 in the promoter region and position 147 in the IVS II region of the β-globin gene was asymmetrically amplified using the 5' primer as the limiting one. Direct sequencing of

asymmetrically amplified DNA was done using the dideoxy terminating method of Sanger[8]. Sequence analysis indicated the presence of compound heterozygosity for Hb Knossos and IVSI-1 G-T mutation in the patient (Fig. 1). The father was heterozygous for Hb Knossos and the mother for IVS I-1.

Discussion

Hb Knossos is one of the hemoglobinopathies that causes β-thalassemia intermedia such as the clinical and hematological picture in homozygous and

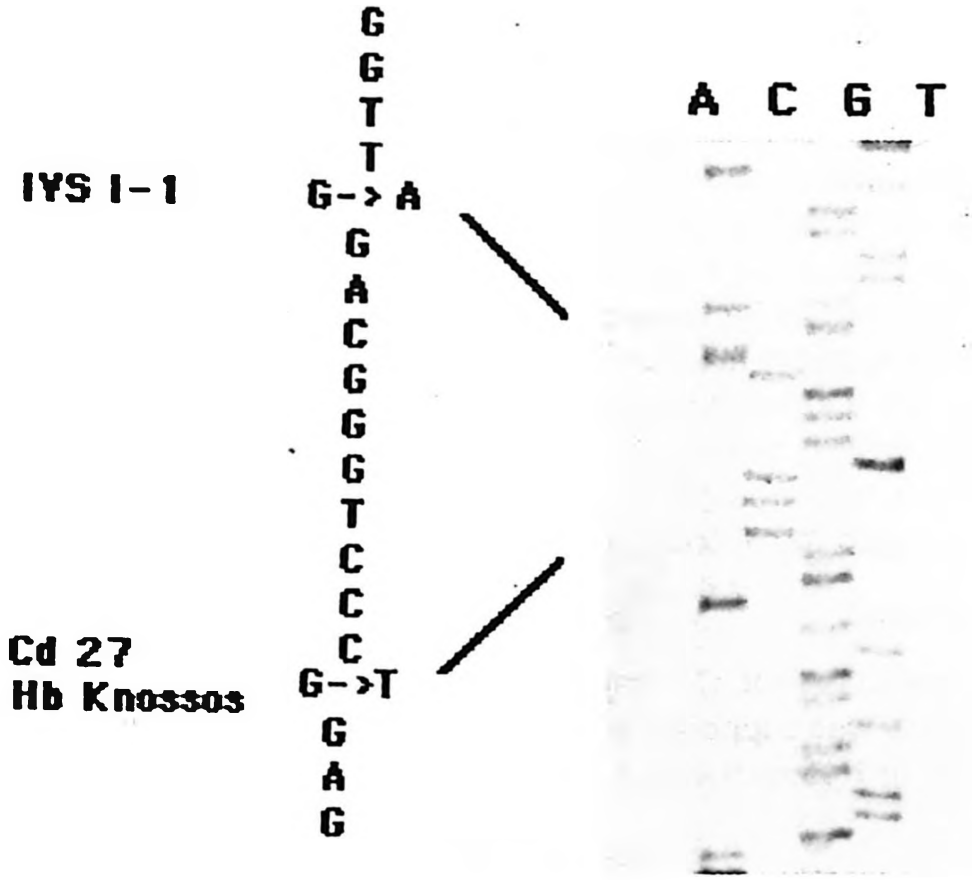

Fig. 1: Sequence analysis for Hb Knossos and IVS-I-I G-T mutation.

compound heterozygous (a β-thalassemia mutation in trans) conditions. Although Hb F slightly increases in homozygous conditions, there is no increase in Hb F and Hb A_2 values in Hb Knossos traits, though an appreciable increase in the Hb F value is shown in compound heterozygotes who have a β-thalassemia-intermedia-like clinical picture. Red-cell indices and the Hb A_2 values of the parents would be useful for detecting Hb Knossos traits that usually have mild microcytosis associated with normal Hb A_2 values[2]. On the contrary, the -101 mutation of the β promotor region and a normal Hb A_2 value are associated with normal red cell indices[9]. Absence of an elevation in Hb A_2 value in Hb

Knossos homozygotes was explained on the basis of a second mutation resulting in δ^o-thalasemia by Baklouti et al.[4] However, a similar mutation could not be found in other subjects with Hb Knossos. Mutations in codon 26 (Hb E) and codon 27 (Hb Knossos) were accepted as thalassemic hemoglobinopathies because they create an abnormality in the processing mechanism[2].

The molecular basis of the β-thalassemia mutations in the trans of Hb Knossos was unknown in the majority of the previously published patients who were compound heterozygous for Hb Knossos and β-thalassemia[1, 3, 4, 10]. Therefore, the effect of β-thalassemia mutations in the trans of Hb Knossos on the clinical and hematological expression of the disease is unknown. Our previously pusblished patients had FSC8 mutation, and the present patient had IVS 1-1 mutation[5]. Although both of these mutations result in β^o-thalassemia, the first one was regarded as one of the well-studied mild β^o-thalassemia mutations. Indeed when the hematological parameters of these two patients were compared, it seemed that the condition associated with Hb Knossos/FSC8 mutation was milder than Hb Knossos/IVSI-1 combination (Table I). However the latter condition could still be regarded as β-thalassemia intermedia (Table I). This indicates that Hb Knossos itself is a very mild thalassemic mutation. We believe that inclusion of acrylamide gel electrophoresis in hematological studies in thalassemia intermedia patients with normal Hb F and Hb A_2 values and heterozygotes with mild microcytosis and normal Hb A_2 values will increase the detection of subjects with the Hb Knossos mutation.

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HIGH – DOSE METHYLPREDNISOLONE – INDUCED DRAMATIC MATURATION OF GRANULOCYTES IN IDIOPATHIC CHRONIC NEUTROPENIA*

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SUMMARY: Yetgin S, Özbek N. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). High-dose methylprednisolone-induced dramatic maturation of granulocytes in idiopathic chronic neutropenia. Turk J Pediatr 1997; 39: 259-263.

Idiopathic chronic neutropenia is characterized by a variable pattern of age at the onset of neutropenia, neutrophil counts, maturational arrest at the promyelocyte/myelocyte stage in the bone marrow, and recurrent pyogenic infections without any underlying disease or drug administration. There have been reports of lack of response to conventional-dose steroid therapy, but in recent studies it has been shown that high-dose corticosteroids are effective in several neutropenic states. Here we report an apparent response to high-dose methylprednisolone in a patient with idiopathic chronic neutropenia. *Key words: idiopathic chronic neutropenia, high-dose methylprednisolone.*

Idiopathic chronic neutropenia (ICN) is a form of neutropenia that may present at any age of life with neutropenia usually <500/mm^3 causing recurrent pyogenic infections involving the skin, mucous membranes, lungs and lymph nodes. Bone marrow examination reveals a variable pattern, usually with maturational arrest at the promyelocyte/myelocyte stage. A differential diagnosis of the several underlying diseases that may cause neutropenia should be done, and drug administration should be excluded in order to confirm the diagnosis of ICN[1,2]. These patients are usually responsive to a granulocyte-colony-stimulating factor. However, recent articles, have reported malignant transformation and some mutations in patients with different forms of neutropenia[3,4]. Although there are reports of a lack of response to conventional steroid therapy[1,2], here we report an apparent response to high-dose methylprednisolone in ICN.

Case Report

A two-year-old boy was admitted to our hospital with the complaints of recurrent bronchopneumonia and cough. On physical examination, his breath sounds were decreased on the left side of the thorax, and minimal hepatosplenomegaly was

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noted. The chest radiogram revealed cardiac enlargement and pleural effusion on the left side. The blood picture was compatible with absolute neutropenia (7% polymorphonuclear lymphocytes, 12% monocytes, 5% eosinophils and 66% lymphocytes). Bone marrow examination showed 24 percent erythroid cells, 46 percent myeloid cells, six percent monocytoid cells and 24 percent lymphoid cells. However, no myeloid element was noted beyond the myelocytic stage. There was no finding relevant to known causes of neutropenia. Because of the relatively late onset (two years of age), Kostmann syndrome was excluded in the differential diagnosis and the patient was finally diagnosed with ICN. He was given high-dose methylprednisolone (HD-MP) by the oral route in one morning dose for four weeks. His neutrophil count increased to normal levels in a week and remained greater than 500/mm^3 during this period. After cessation of therapy he became neutropenic again. To test the effect of short interval therapy, HD-MP (30 mg/kg) was given without any effect on the neutrophil count. After a month, the same four-week schedule was repeated successfully. His neutrophil counts were greater than 500/mm^3 with the dose of HD-MP tapered to 0.5 mg/kg/day every other day (Table I and Fig. 1). Improvement was also shown in the bone marrow. There were no side effects of HD-MP therapy except for a slight Cushingoid appearance.

TABLE I: Effect of HDMP in Different Doses

HDMP TREATMENT		WBC COUNT (mm^3)		PNL COUNT (mm^3)	
Dose (mg/kg)	Duration (weeks)	Initial	Last	Initial	Last
–	–	5800	–	406	–
30	1	3200	6000	128	2520
20	1	6000	5500	2520	1650
10	1	5500	4000	1650	1440
5	1	4000	5000	1440	1020
2	1	5000	4500	1020	1835
–	3	4500	4000	1835	480
–	2	5000	–	250	–
–	2	5600	–	280	–
30	3 days	5600	4800	280	288
–	3	4800	5000	288	250
20	1	5000	5400	250	1296
10	1	5400	5700	1296	2166
5	1	5700	7000	2166	560
2	1	700	5700	560	228
1	1	5700	5200	228	260
–	4	5200	6600	260	330
30	1	6600	4700	330	1786
20	1	4700	7700	1786	4125
10	1	7700	11100	4125	5600
5	1	11100	6100	5600	1880
2	1	6100	5900	1880	1062
1	1	5900	4000	1062	1200
0.5	1	4000	3800	1200	950
0.5*	3	3800	2800	950	634

* Given every other day.

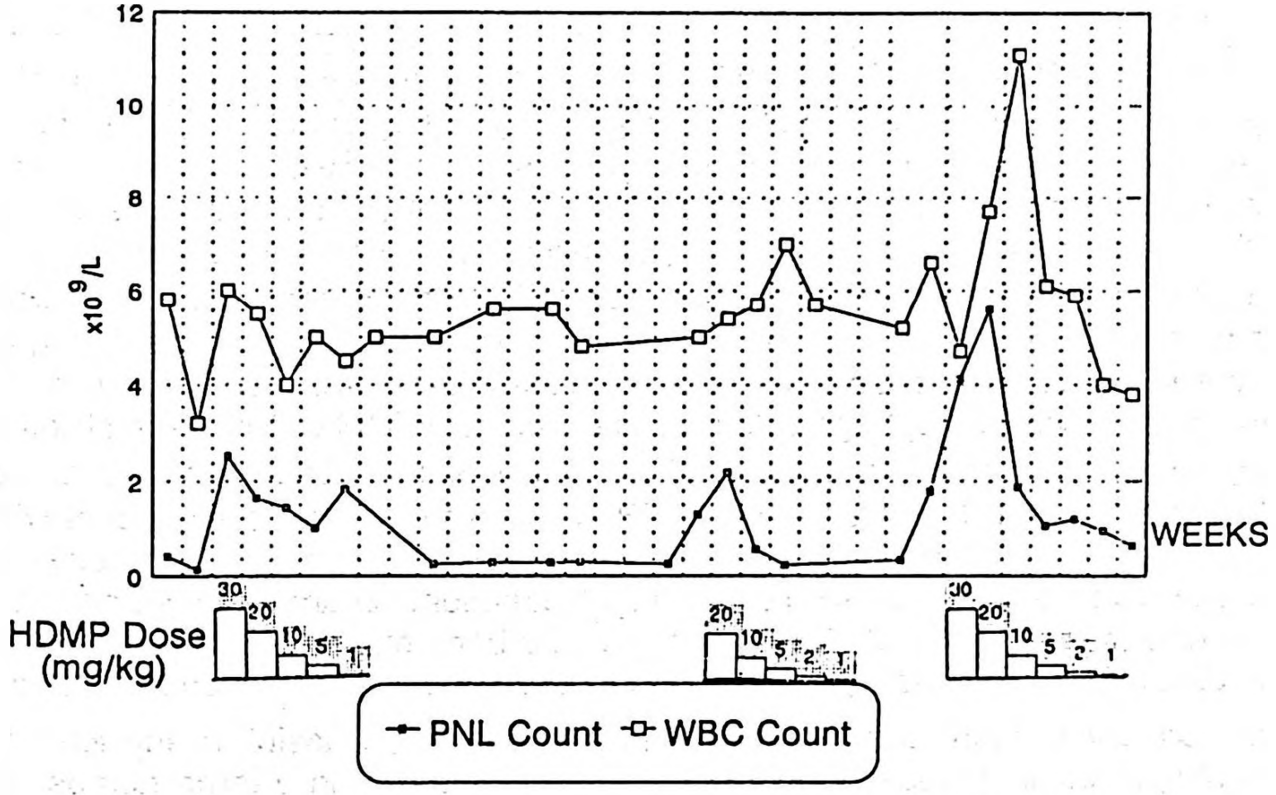

Fig. 1: Changes of WBC and neutrophil counts with HDMP.

Discussion

Chronic neutropenia of childhood is a group of disorders in which the blood neutrophil counts decrease below normal levels for a prolonged period. Although neutropenia can be caused by many definable disorders, there is a large group of patients in whom no clear cause can be identified. In our case, we excluded Fanconi's anemia, dyskeratosis congenita, cartilage-hair syndrome and Schwachman-Diamond syndrome on the basis of the normal phenotype. The age at onset and the course of the disease did not support a diagnosis of severe congenital neutropenia (Kostmann syndrome). Also, neither underlying disease or drug administration could be determined, and the patient was diagnosed to have ICN. Some patients with chronic neutropenia have anti-neutrophil antibodies; we could not study antineutrophil antibodies in our patient. However, in a recent article, it was shown that there was no relationship between the severity of symptoms of neutropenia and the presence or absence of antineutrophil antibodies in a group of patients with chronic neutropenia. Also, no other differences could be detected between groups when the patients were classified according to antineutrophil antibody results[1].

Dexamethasone and prednisolone can induce differentiation of some mouse myeloid leukemic cells into macrophages and granulocytes in vitro[5]. It has been shown that macrophage-granulocyte inducer and steroids have different sites

on myeloid leukemic cells for the induction of differentiation[6-8]. The effect of corticosteroids in increasing colony-stimulating activity in normal subjects[9], patients with aplastic anemia[10] and myelodysplastic syndrome[8-11] has been reported. Corticosteroid-induced leukocytosis is a well-known phenomenon, and leukocyte recovery has been observed in different patient groups such as idiopathic thrombocytopenic purpura, aplastic anemia, myelofibrosis, refractory anemia and acute lymphoblastic leukemia by HD-MP[12]. Hiçsönmez et al[8] showed a potential role of HD-MP in the maturation of myeloid leukemia in children in consecutive studies. It has also been shown that HD-MP treatment may increase the granulocyte-macrophage-colony stimulating factor (GM-CSF) level in children with acute leukemia[13]. In support of the above studies and their results, we have treated an ICN patient with HD-MP. As shown in Table I, the effect of HD-MP on the maturation of granulocytes was clear and depends on the doses of prednisolone and the duration of therapy. Maturational changes were observed not only in the peripheral blood but also in the bone marrow. It was shown that not the conventional dose, but high-dose steroids have an effect on maturation.

In conclusion, high- dose methylprednisolone may be useful in any form of neutropenia. Serial testing of the effect of this therapy on a large number of patients will clarify whether or not this effect is universal.

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INTRACEREBRAL HEMORRHAGE: A RARE LATE MANIFESTATION OF VITAMIN – K DEFICIENCY IN A BREASTFED INFANT*

A Case Report

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SUMMARY: Soylu H, Aslan Y, Sarı A, Erduran E. (Departments of Pediatrics and Radiology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey). Intracerebral hemorrhage: a rare late manifestation of vitamin-K deficiency in a breastfed infant. A case report. Turk J Pediatr 1997; 39: 265-269.

Late hemorrhagic disease of the newborn (HDN) is a rare complication of vitamin-K deficiency and is especially associated with intracranial hemorrhage. It may also occur in infants who received vitamin-K prophylaxis at birth. Here, we reported a case of late HDN with frontal lobe hemorrhage due to vitamin-K deficiency. This form of intracranial hemorrhage of late HDN has been reported in the literature very rarely. We conclude that the efficiency of single-dose vitamin-K prophylaxis should be revaluated. *Key words: intracerebral hemorrhage, vitamin-K deficiency.*

Hemorrhagic disease of the newborn (HDN) consists of hemorrhages from multiple sites in otherwise healthy infants in the absence of trauma, asphyxia or infection[1]. The link between vitamin-K deficiency and spontaneous hemorrhaging was first made by Dam in 1929[1,2]. Hemorrhagic disease of the newborn due to vitamin-K deficiency may occur at three different times:

1. On the first day of life: Drugs taken by the mother may play a part-notably, anticonvulsants (e.g., phenobarbital and phenytoin), rifampicin, isoniazid, anticoagulants (e.g., warfarin) and phenylbutazone[1,2].
2. Between the second and fifth days: This is defined as classic HDN and is characterized by gastrointestinal bleeding, mostly from the stomach, intestine, umbilical stump and needle or operation sites, widespread ecchymoses and, rarely, intracranial bleeding.

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3. Beyond the second week: This form is called late HDN and is particularly associated with intracranial hemorrhage. Mortality and morbidity rates are higher in this group than others[4].

Severe intracranial hemorrhage due to vitamin-K deficiency in young infants may occur suddenly and result in death or severe central nervous system dysfunction. Therefore, it is an extremely serious event[3].

HDN may be associated with a variety of disorders compromising the supply of vitamin-K, including chronic diarrhea, diarrhea of breastfed infants, cystic fibrosis, ∝-1-antitrypsin deficiency, hepatitis, celiac disease and others[1,5]. Because of maternal milk's low vitamin-K content and inhibition to colonization of the intestinal microbial flora capable of synthesing vitamin-K, it has been accused of causing HDN[1-3].

Since the 1970s, through the oral administration of vitamin-K, the incidence of intracranial hemorrhage has markedly decreased from 34.3/100,000 to 10.1/100,000 live births[6]. Also, in our country, 1 mg vitamin-K_1 has been administrated routinely via intramuscular injection at birth. However, late HDN has still occurred rarely.

Here, we report a case of late HDN with intracranial bleeding in an infant who had received vitamin-K prophylaxis at birth.

Case Report

A 37-day-old boy was admitted to our pediatric emergency ward with the complaint of seizures. He had no history of diarrhea or drug use. He had been born at term following a healthy labor and delivery. The patient's mother had not used any medicine during the prenatal or postnatal period. The baby was breastfed. There was not any hemorrhagic diathesis disorder in his family. Vitamin-K_1 (1 mg) had been injected intramuscularly within the first 24 hours following delivery.

Physical examination revealed a temperature of 36 °C, pulse of 152 beats per minute, respirations 44 per minute, and a blood pressure of 64/39 mmHg. Pallor, generalized tonic-clonic seizures with a left predominance, fontanel bulging and grade I papillary stasis of both eyes were observed. The deep tendon reflexes were more active on the left side. The seizures were stopped by intravenous diazepam, and phenobarbital prophylaxis was commenced.

Laboratory findings included hemoglobin 7.4 g/dl, leukocytes 14.1 x 10^9/L, thrombocytes 186 x 10^9/L, bleeding time 4.5 minutes, prothrombin time (PT) 22 sec, activated partial thromboplastin time (aPTT) 34.6 sec, and fibrinogen level 264 g/L. The results of liver function tests were in the normal range. Intracerebral hemorrhage due to vitamin-K deficiency was diagnosed, and vitamin-K_1 (2 mg) was administered intravenously.

Cranial tomography (CT) originally revealed a large hemorrhage surrounded by edema in the right frontal region (Fig. 1). Two weeks later, magnetic resonance imaging (MRI) was done, and the same hemorrhagic lesion was seen in the subacute period (Figs. 2a,b). After observation for 15 days, PT and aPTT were within normal limits and the previous clinical findings had improved. Moreover, the results of both the sweat test for cystic fibrosis and the α-1-antitrypsin level were found in normal ranges. Therefore, the patient was discharged on phenobarbital prophylaxis. He has been followed for six months as an outpatient with no complaints.

Discussion

Intracranial hemorrhage due to vitamin-K deficiency is the most common form of late HDN and accounts for approximately 50 percent of the bleeding episodes at presentation[4,7]. It often occurs in the first to third months of life[4]. In our case, the episode occurred on the 37th day of life. The majority of these infants are breastfed[8-10]; the importance of breastfeeding as a risk factor seems to lie in the low concentrations of vitamin-K in human milk[2,3,7].

Gobel et al[10] found that the serum prothrombin time of infants who were solely breastfed was longer on the fifth day of life than in infants fed formula. Furthermore, in early HDN, drugs taken by the mother during pregnancy, and

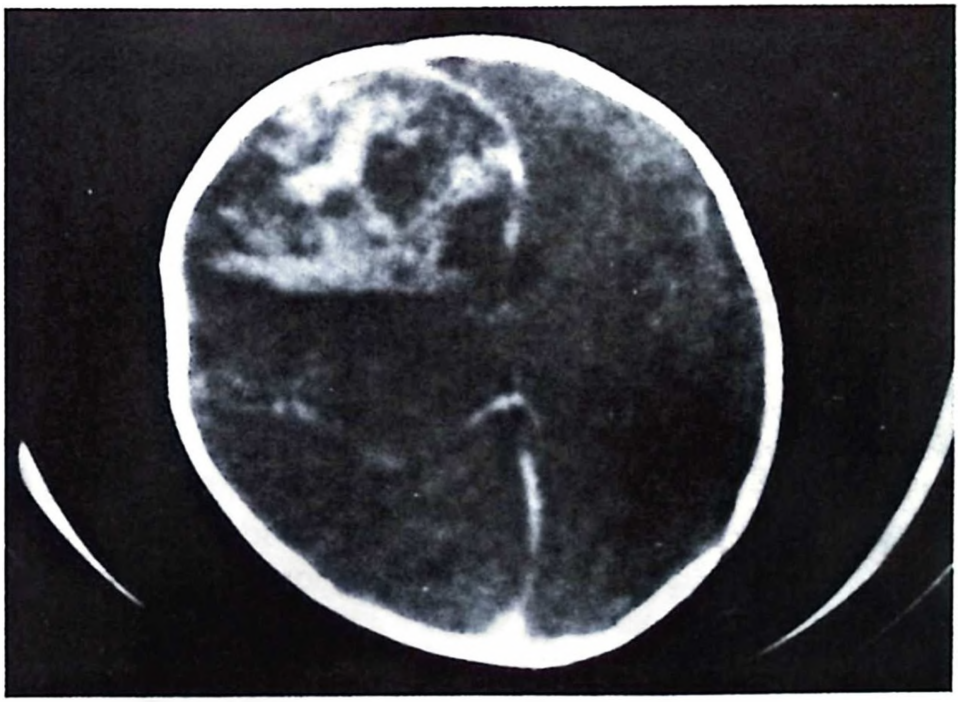

Fig. 1: CT showed a large heterogeneous hyperdensity in the right frontal lobe, indicating intracerebral hemorrhage in the acute-subacute period and shift of interhemispheric fissure to the left side.

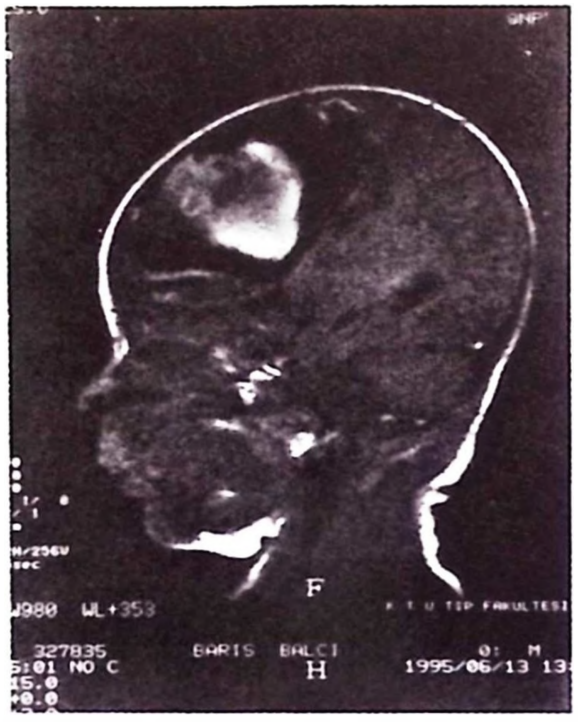

Fig. 2a: T1 sagittal MRI on the 15th day of the hemorrhage, showing the late subacute hematoma in the right frontal lobe surrounded by a hypointense rim.

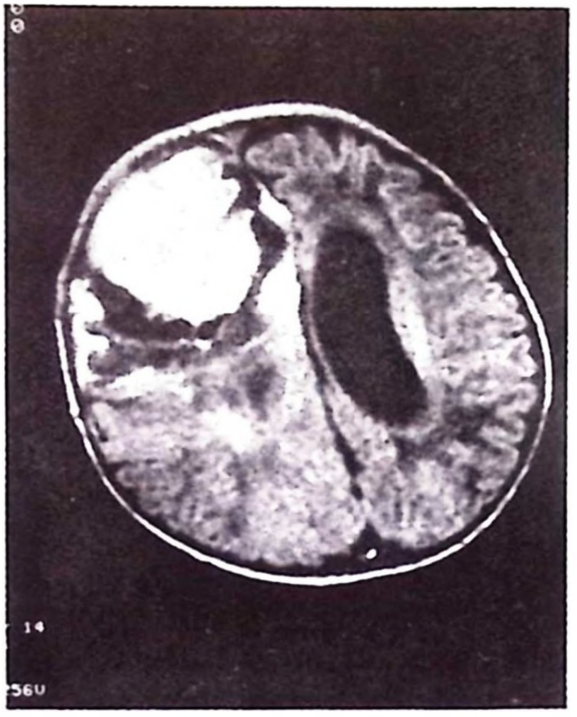

Fig. 2b: Axial proton density image revealed the presence of left lateral ventricle dilatation.

in late HDN, chronic diarrhea, hepatitis, α-1-antitrypsin deficiency and cystic fibrosis may also play roles[1,2,5]. In our case, the patient had no history of gastrointestinal disorder or antibiotic use that might cause vitamin-K deficiency, and the results of α-1-antitrypsin measurement, liver function and the sweat tests were within normal limits. The patient had been solely breastfed.

Sutor et al[11] reported a sudden-onset and severe hemorrhage with prompt improvement after administration of vitamin-K, blood or blood derivates. Similar findings were noted in our case.

The administration of vitamin-K prophylactically in the newborn period has been used widely in many countries. In the past, some clinicians suggested that vitamin-K prophylaxis was not needed for all full-term infants[12]. Consequently, vitamin-K prophylaxis was suspended in some countries[1,7]. This approach resulted in a recurrence of HDN in these countries and proved the effectiveness of vitamin-K statistically, thus advocating the need for vitamin-K prophylaxis[4,6,8,10]. However, the advantage of this prophylaxis for late HDN has been controversial until now. Single-dose intramuscular vitamin-K prophylaxis (1 mg) may not be sufficient to prevent intracranial hemorrhage. A few cases of late-stage intracranial hemorrhage have been reported, despite receiving single-dose vitamin-K prophylaxis at birth, as in our case.

Late HDN occurs most often in the subarachnoid and/or subdural regions (90% and 37.5%, respectively)[13]. In our case, however, the hemorrhage occurred in the patient's frontal lobe, a rarely reported location[13,14].

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AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE I*
A Case Report

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SUMMARY: Cinaz P, Bideci A, Haznedaroğlu A, Ezgü FS, Ataoğlu Ö, Kürşaklıoğlu S. (Department of Pediatric Endocrinology, Gazi University Faculty of Medicine, Ankara, Turkey). Autoimmune polyglandular syndrome type I: a case report. Turk J Pediatr 1997; 39: 271-275.

Autoimmune polyglandular syndrome (APS) type I is a disorder that consists of three primary diseases: hypoparathyroidism (HPT), adrenocortical insufficiency (ACI) and chronic mucocutaneous candidiasis. Several other disorders may be associated. The diagnosis of APS type I was made in a 16-year-old patient with HPT, Hashimato's thyroiditis and ACI in our department. She has been observed for more than four years for other possible endocrine and non-endocrine disorders.
Key words: autoimmune, polyglandular syndrome type I, childhood.

Various autoimmune endocrinologic insufficiencies are described as autoimmune polyglandular syndrome (APS). The detection of autoantibodies against the endocrinologic gland is definitive for the diagnosis; these antibodies choose the normal gland as a target, and hypofunction develops over time[1].

APS type I consists of three primary disorders: hypoparathyroidism (HPT), adrenocortical insufficiency (ACI) and chronic mucocutaneous candidiasis; several other disorders, such as gonadal failure, alopecia, thyroiditis, malabsorption, vitiligo, chronic active hepatitis and kerotopathy may be associated[2,3]. Type II APS also consists of three primary disorders: insulin-dependent diabetes mellitus, ACI and Hashimato's thyroiditis (HT)[4].

The features of a case with type I APS are presented in this report.

Case Report

A 16-year-old girl was referred to Gazi University Hospital because of an 11-month history of fatigue, weakness and recurrent episodes of carpopedal

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spasm. She had a history of oral moniliasis at the age of five, and her menses had begun a year previously. From her family history we learned that her parents were first-degree relatives, her father had died nine years previously and her mother had had a goiter operation. On physical examination, her height and weight were both at the 50th percentile. On admission she had marked carpal spasms with a blood pressure of 90/60 mmHg. The vertical length of the right and the left thyroid lobes was 5 and 4 cm, respectively. There were brown pigmentations and deformities on her teeth (Fig. 1). Chvostek and Trousseau's signs were easily elicited. Pubertal development was graded as stage 5 according to the Tanner scale of development[5].

Laboratory investigations were in the normal range, including complete blood cell count and urinalysis. The skeletal bone age was estimated to be 16 years. Serum sodium, potassium, magnesium, blood urea nitrogen, creatinine, fasting sugar, aspartate aminotransferase, alanine aminotransferase, albumin and cortisol were in the normal ranges. Tubular phosphorus reabsorption, urine calcium excretion and urine free cortisol were also within normal limits, as were serum immunoglobulin levels, vitamin B_{12}, folic acid and antinuclear antibody. In the electrocardiogram, the corrected QT interval was measured as 0.52 seconds (normal < 0.44 second). Serum calcium (ionized) was found to be 1.6 mg/dl (normal: 4.48-4.92 mg/dl), phosphorus 7.8 mg/dl (normal: 2.7-4.5 mg/dl) and parathormone (intact) 4 pg/ml (normal: 10-65 pg/ml).

The thyroid hormones and thyroid stimulating hormone levels were found to be in the normal ranges. Thyroid antimicrosomal and antithyroglobulin antibodies

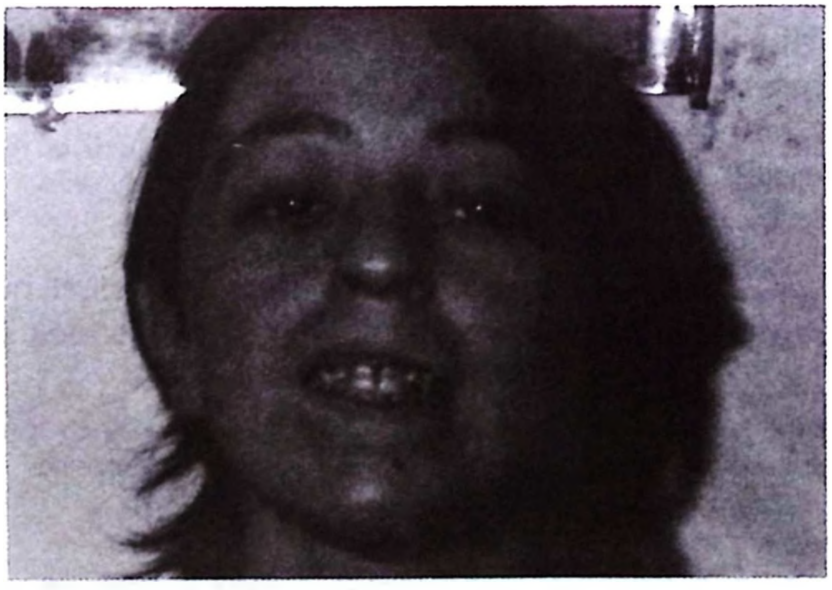

Fig. 1: The appearance of the pigmentation of the patient's teeth.

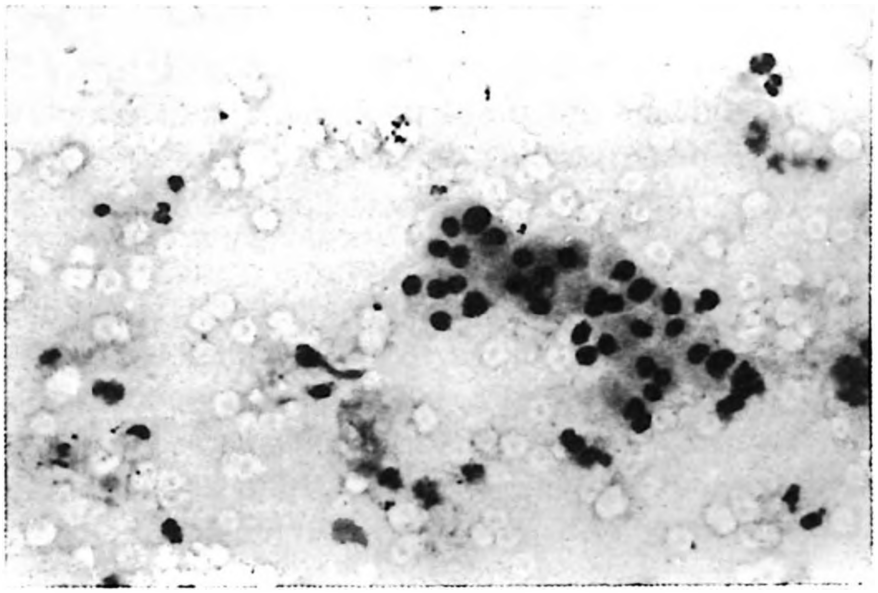

Fig. 2: A cluster of Hurtle cells with abundant granular eosinophilic cytoplasm and uneven nuclei. The background of the smear shows erythrocytes and polymorphonuclear leukocytes of blood origin.
(Aspiration biopsy material taken from thyroid tissue) (x 400, Hemotoxylen and Eosin).

were 287 mg/dl (normal < 50 mg/dl) and 44 mg/dl (normal < 50 mg/dl), respectively. Thyroid ultrasonography showed the right lobe as 17 x 59 x 11 mm, and the left lobe as 17 x 48 x 9 mm without a solid or cystic nodule. Fine needle thyroid aspiration biopsy was consistent with HT (Fig. 2).

The patient's basal adrenocorticotropic hormone (ACTH) value was elevated (84 pg/ml; normal 10-60 pg/ml). Before and after the ACTH stimulation test, cortisol levels measured as 17.79 ng/dl and 18.54 ng/dl, respectively, and these values were much lower than expected. Follicle stimulating hormone, luteinizing hormone, total estriol and dehydroepiandrosterone sulfate were within normal limits. Chest x-ray, brain tomography, adrenal tomography and parathyroid scintigraphy were all reported to be normal.

Intravenous calcium gluconate and oral 1.25-dihydroxy-vitamin D_3 were administered to treat the hypocalcemia and tetany. On the third day of admission, serum calcium and phosphorus levels returned to normal. Our patient was treated daily with hydrocortisone per oral 20 mg/m²/day. L-thyroxine was given at a dose of 100 µg/day to treat the goiter. Five months later, the patient suffered from orthostatic hypotention with a plasma sodium level of 124 mEq/L (normal: 136-146 mEq/L), potassium 5.8 mEq/L (normal: 3.5-5.1 mEq/L) blood renin activity 50 ng/ml (normal < 4.3 ng/ml/hr), and her basal ACTH level was higher than normal. These findings led us to the diagnosis of mineralocorticoid insufficiency, and flurocortisone therapy at adose of 0.1 mg/day was initiated.

Discussion

A rare disorder, type I APS is diagnosed when two or three of HPT, ACI and mucocutaneous candidiasis are present. A number of endocrine and non-endocrine autoimmune disorders such as gonadal failure, alopecia, autoimmune thyroid disorder, vitiligo, chronic active hepatitis and keratopathy are associated with this syndrome[1,3,6].

The initial diagnosis of our case was HPT and goiter. HT was then diagnosed by thyroid fine-needle aspiration biopsy and the patient's high level of thyroid antimicrosomal antibody. In type I APS, HPT is the primary finding in 90 percent of patients, and HT can be seen in 10 percent patients[4,6]. Because of suspected complaints we further investigated probable ACI. In one month glucocorticoid deficiency and in six months mineralocorticoid insufficiency were found. ACI can be seen in two-thirds of cases[4]. Glucocorticoid and mineralocorticoid deficiences can be seen either at the same time as or five years after presentation with the disease[4,7].

Gonadal failure is another finding that can be seen in type I APS, and women are affected more commonly at older ages[8]. The gonadal functions of our patient were considered to be normal.

The most common nonendocrine disturbances seen with this syndrome are deformation of the teeth and nails and mucocutaneous candidiasis[9]. From the patient's history we learned that she had once had white plaque in her mouth, which could have been oral candidiasis, and her teeth were pigmented.

Cellular and humoral immune system disturbances have also been reported in type I APS. High levels of immunoglobulin G and immunoglobulin E and lower levels of immunoglobulin A may be seen[1,10,11]. The increased activity of suppressor T-cells may be responsible for these immunoglobulin disturbances[9,11]. However immunoglobulin levels and T lymphocyte subgroups were normal in our patient, and she had anergy for candida antigen.

It is possible that some autoimmune disorders other than HPT, HT, ACI and endocrine or non-endocrine disorders could be seen at a later age with type I APS. This fact should always be kept in mind during the clinical follow-up of patients with APS.

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A CASE OF JUVENILE ANKYLOSING SPONDYLITIS AND CROHN'S DISEASE*

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*Ali Küpelioğlu MD*****, *Necla T. Çevik MD****

SUMMARY: Ünsal E, Eroğlu Y, Büyükgebiz B, Küpelioğlu A, Çevik NT. (Departments of Pediatrics and Pathology, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). A case of juvenile ankylosing spondylitis and Crohn's disease. Turk J Pediatr 1997; 39: 277-280.

Juvenile ankylosing spondylitis (JAS) is a chronic inflammatory arthritis of the peripheral and axial skeleton, frequently accompanied by enthesitis. About four percent of patients with JAS have ulcerative colitis or Crohn's disease. Crohn's disease is the more common of the two and is diagnosed in 26 percent of patients with chronic spondyloarthropathy. In this paper, a 14-year-old male patient is presented as a typical case of juvenile ankylosing spondylitis and Crohn's disease with low back pain, morning stiffness, limited motion in anterior and lateral flexion and extension, left sacroiliitis, ankylosis in the apophyseal joints of the lumbar vertebrae, abdominal pain, bloody diarrhea, characteristic histopathologic changes of colonic involvement such as lymphoid follicles, fissures, submucosal polymorphonuclear cell infiltration and definite ganglion cells. The current therapy with mesalazin, having fewer side effects than sulfosalazin, and its applicability in combination with naproxen sodium is also discussed. *Key words: ankylosing spondylitis, Crohn's disease, mesalazin.*

Juvenile ankylosing spondylitis (JAS) is a genetically based, chronic inflammatory arthritis of the peripheral and axial skleton, frequently accompanied by enthesitis, and not characterized by rheumatoid factor (RF) or antinuclear antibody (ANA) seropositivity. The sacroiliac joints are eventually affected in all patients with JAS, and radiologic evidence of inflammation of these joints is required for a definitive diagnosis[1]. JAS has distinctive features such as familial aggregation, association with HLA B27, enthesopathy, sacroiliitis and onset mostly after the age of 10 years[2]. About four percent of patients with JAS evaluated by radiology and sigmoidoscopy have ulcerative colitis or Crohn's disease. Crohn's disease is the more common of the two and is diagnosed in 26 percent of patients with chronic spondyloarthropathy[3]. In this paper, a case having both JAS and Crohn's disease is presented.

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Case Report

A 14-year-old male patient was referred to the Pediatric Rheumatology Unit suffering from low back pain, morning stiffness, abdominal pain and bloody diarrhea three times a day. He had had gastrointestinal system complaints for three years and low back pain for two. In the previous two months he had had three fainting and syncope attacks. Physical examination revealed tenderness and pain of the left sacroiliac joint. He also had limited motion in anterior and lateral flexion and extension. He had no signs or symptoms of uveitis. In the laboratory evaluation, the hemoglobin level was 9.7 g/dl with microcytosis and hypochromia, C-reactive protein was strongly positive, erythrocyte sedimentation rate (ESR) was 60 mm/hr, and RF and ANA were negative. X-ray examination showed left sacroiliitis with ankylosis in the apophyseal jounts of the lumbar vertebrae (Fig. 1). Intestinal barium enema was not helpful because of colon gases. Colonoscopy was not available. A diagnostic approach by rectal biopsy revealed lymphoid follicles, fissures, submucosal polymorphonuclear cell infiltration and definite ganglion cells compatible with Crohn's disease (Fig. 2). The patient was given mesalazin 750 mg and naproxen sodium 500 mg a day. In the first month of therapy, significant regression was obtained in the number and the bloody character of stools as well as in the low back pain.

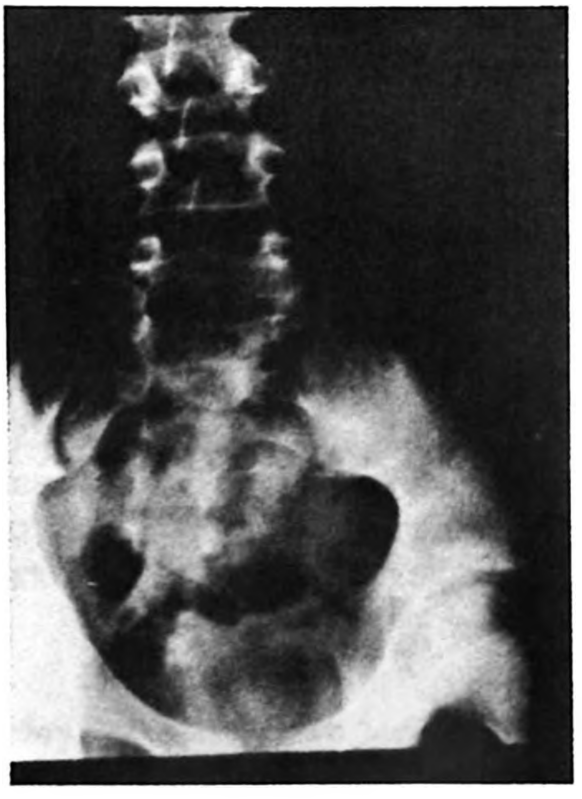

Fig. 1: Ankylosis in the apophyseal joints of the lumbar vertebrae with closed left sacroiliac joint.

Fig. 2: Marked lymphoid tissue in the mucosal layer and hyperplasia of the lymphoid follicle is clearly seen at the right. There is also a fissure tract of the mucosa in the middle. (Hematoxylen + Eosin x 4).

Discussion

Crohn's disease is a chronic inflammatory disease that is characterized by unpredictable exacerbations and remissions in disease activity. The cause is entirely unknown. The currently held hypothesis is that it occurs as a result of a genetically determined response which is probably immunologically mediated. Other theories include alterations in autoprotection due to abnormal metabolism of the prostoglandin-leukotriene system or changes in the mucus layer[4,5]. Increased permeability of the gut wall to known and unknown antigenic material together with a defective local immune regulation are implicated to be sufficient to initiate joint inflammation in genetically predisposed individuals. In Crohn's disease, articular manifestations may precede intestinal signs by years[1]. In this patient, after a year of passing bloody stools, low back pain occurred with limitation of motion in anterior flexion, lateral flexion and extension caused by ankylosis in the apophyseal joints and left sacroiliitis. Findings in the rectal biopsy established the diagnosis of Crohn's disease associated with ankylosing spondylitis.

In a clinical, radiological and immunogenetical study of 51 Crohn's patients, rheumatological disorders were found in 16 (31%) of them[6]. Spondylitis clinically and radiologically indistinguishable from idiopathic ankylosing spondylitis was detected in three to six percent of patients with inflammatory bowel disease in one prospective study[7]. In chronic spondyloarthropathies, and especially in ankylosing spondylitis, gastrointestinal infection also plays a role in which the inflammation of joints is triggered by specific bacterial pathogens.

The therapy of Crohn's disease has been the focus of several multicentered studies. Symptomatic therapy with prednisone, sulfosalazine, metronidazole, azathioprine or 6-mercaptopurine, together with nutritional support are the main goals. Sulfosalazine and its derivative mesalazin, the latter having fewer, side effects, have been shown to be useful in inducing remission in patients with colonic involvement[4]. This patient has been well controlled by mesalazin and naproxen sodium. Interestingly, nonsteroidal antiinflammatory drugs have not been found to be associated with gut lesions[3]. Therefore, in our opinion, a combination of the two drugs is recommended to control symptoms.

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DIABETES INSIPIDUS IN A PEDIATRIC PATIENT WITH SYSTEMIC LUPUS ERYTHEMATOSUS*

A Case Report

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SUMMARY: Tekin N, Kural N, Koçak AK, Köse S. (Department of Pediatrics, Osmangazi University Faculty of Medicine, Eskişehir, Turkey). Diabetes insipidus in a pediatric patient with systemic lupus erythematosus: a case report. Turk J Pediatr 1997; 39: 281-284.

In systemic lupus erythematosus (SLE), the central nervous system (CNS) produces numerous and varying clinical disorders. A 14-year-old girl with the features of cerebral involvement in SLE is presented. The development of diabetes insipidus (DI), which responded to desmopressin, was an interesting finding in this case. Therapy consisting of high-dose methylprednisolone combined with cyclophosphamide was effective in improving the symptoms as well as the DI. *Key words: cerebral involvement, diabetes insipidus, systemic lupus erythematosus.*

The presentation of systemic lupus erythematosus (SLE) varies from an acute, rapidly fatal onset to an insidious chronic disease. Exacerbations may be seen during the course of this multisystemic disease. The basic pathologic lesions of SLE are an immune complex vasculitis and fibrinoid necrosis, inflammatory cellular infiltrates and sclerosis of collagen[1]. Nephritis, pericarditis, thrombocytopenia, episodes of arthritis, splenomegaly and mucocutaneous ulcerations are observed more commonly in children than adults[1].

In this report, a 14-year-old female patient with the clinical symptoms of central nervous system involvement besides the other findings of SLE is presented. The development of diabetes insipidus (DI) during her hospitalization was an interesting clinical manifestation of this case. The criteria for cerebral involvement, the efficacy of high-dose methylprednisolone (HDMP) therapy and probable causes of DI development in SLE are discussed.

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Case Report

A 14-year-old girl presented with arthralgia, headache, rash on her face and hands, and alteration of consciousness. Four months before admission, migratory joint pain associated with edema, and local heat and sun-induced maculopapular rash on her face and hands had been noted. At that time she had been diagnosed first with acute rheumatic fever, and corticosteroid therapy was administered; then, persistance of the symptoms suggested the diagnosis of rheumatoid arthritis and the patient received salicylate therapy. She was noted to have deterioration in mental status with meaningless crying and talking as well as severe headache of four days duration. She was uncooperative and looked chronically ill, with pallor and loss of subcutaneous fat tissue. Examination revealed a fever of 38.5 °C, pulse 150 beats per minute, respiratory rate 30 per minute and blood pressure 100/60 mmHg. The skin of the face, from the nares to the cheeks, erythematous. On cardiovascular examination she had a pansystolic murmur of 4/6 degree over the mitral, tricuspid and pulmonary valves with thrill and marked tachycardia. Edema was present over the knees, elbows and wrists but was not associated with warmth or erythema. Palmar erythema was detected. Her muscles were atrophied. Hypertonicity, spasticity especially of the arms, and hand tremor were present. Deep tendon reflexes were normoactive. Speech was slurred. She was not able to walk, sit or eat meals. Her mental status was disturbed, and sometimes she cried with fear because of hallucinations. The reaction of both pupils to light was normal. The cranial nerves and fundoscopic examination were normal.

Laboratory results were as follows: hemoglobin 10.3 g/dl, hematocrit 31.2%, white blood cell count 6,500/mm^3; platelet count 231,000/mm^3, erythrocyte sedimentation rate 27 mm/hour, BUN 18 mg/dl, Cr 0.8 mg/dl, glucose 83 mg/dl, Na 130 mEq/L, K 4.3 mEq/L, total protein 7.2 g/dl, albumin 3.2 g/dl, ASO 166 Todd U, CRP 24 mg/dl, ANA (+), anti DNA 171.151 (0-6) IU/ml, IgG 2,160 (800-1700) mg/dl, IgM 369 (50-370) mg/dl, C3 20 (50-90) mg/dl and C4 218 (10-40) mg/dl. Urinalysis was normal. Chest roentgenogram revealed cardiomegaly. Mitral insufficiency was detected in echocardiography. Cerebral and cerebellar atrophy were noted on computerized tomography (CT) findings (Fig. 1). Electroencephalogram (EEG) was normal.

On the basis of these findings, the diagnosis of SLE was made. Cerebral findings were prominent. Since cardiomegaly, tachycardia and murmur of 4/6 degree suggested the development of endocarditis due to SLE, heart failure therapy was started. High-dose methylprednisolone therapy was administered for the first three days with standard steroid therapy and cyclophosphamide added to the therapy schedule. A daily urine output of greater than eight liters per day accompanied by a low urine density of les than 1005 suggested the diagnosis of diabetes insipidus. She responded to an injection of 5 µg desmopressin with elevation of urine density to 1020. Desmopressin therapy was given for almost two weeks until the normal urine and density values were obtained during corticosteroid therapy combined with cyclophosphamide. Neurological findings improved partially with the improvement in cardiac symptoms, and she could walk without help.

Discussion

Depending on the system involved during the acute phase of this episodic disease a patient could be misdiagnosed until the correct diagnosis of SLE is established. For this reason, older children commonly have a previous history of intermittent symptoms, such as arthritis, pleuritis, nephritis or dermatitis[1]. Our case had been diagnosed with acute rheumatic fever (ARF) because of the migratory character of the arthritis, and then with rheumatoid arthritis because of the persistence of symptoms. The presence of four criteria out of 11 identified by a committee of the American Rheumatism Association has sensitivity of 96 percent and a specificity of 96 percent for the diagnosis of SLE[1]. In this case the presence of a malar (butterfly) rash, photosensitivity, nonerosive arthritis, encephalopathy, positive antinuclear antibody test and high anti DNA represented six out of 11 criteria.

In our case the most striking finding was the acute organic brain syndrome, which was the reason for admission. Neurological and neuropsychiatric manifestation occur in approximately 60 percent of adult patients with SLE[2,3]. Studies of SLE that involve large groups of children show an incidence in central nervous system (CNS) involvement between nine and 45 percent[2,3]. The records of 37 patients with SLE followed at Children's Hospital of Philadelphia between 1968 and 1978 were reviewed for evidence of CNS involvement[3]. Three criteria were established as objective evidence of CNS involvement: 1) objective neurologic signs; 2) acute organic brain syndrome; and 3) symptoms referable to the CNS with abnormality of one of the following studies: EEG, cerebrospinal fluid (CSF) analysis, and CT scan. In this report, 16 of 37 children had CNS involvement (43%). The presence of one of the three criteria was used to indicate that the patient had CNS involvement. In the neurological examination of our case, the presence of objective neurologic findings such as tremor, spasticity, development of organic brain syndrome with hallucinations, and deterioration in mental status and CT findings were strongly indicative of CNS involvement. Laboratory examinations of cerebral lupus cases may consist of diffuse slowing in EEG, cortical atrophy or cerebral edema in the CT, and pressure and protein elevation in cerebrospinal fluid analysis. EEG examination is usually normal, as it was in our case. The most frequent CT finding is cerebral and cerebellar atrophy, which was also present in this case. Atrophy could be related to active or previous encephalopathy, steroid therapy or some temporary factors. MRI is more sensitive for cerebral edema than CT but was not performed in this case.

In this case DI developed, but responded to desmopressin therapy. The development of DI may be due to autoantibodies which are detected in SLE. Circulating antibodies against AVP neurones were demonstrated in 18 out of 47 patients with central AVP deficiency, suggesting an autoimmune etiology. Of these patients, 13 had other organ-specific antibodies[5]. On the other hand, in three patients with SLE who developed DI, antipituitary antibodies were not detected in their serum. They were accepted as primary empty sella syndrome[6].

The improvement of DI in our case could be due to the cyclophosphamide therapy accompanied by corticosteroid therapy, since the success of cyclophosphamide therapy in cases of diabetes insipidus secondary to Wegener's granulomatosis has been reported[7].

High-dose methylprednisolone (HDMP) therapy has been recommended for CNS involvement, as in cases of renal involvement[4,8]. Recently HDMP pulse therapy in the first three days of therapy followed by standard therapy has been applied to these cases[9,10]. Most symptoms have improved dramatically in the first few days after intravenous rose, approaching the normal range four weeks after pulse therapy[8].

A few cases of SLE associated with DI have been reported. As it is known that cerebral involvement in SLE is manifested by various clinical presentations, more advanced studies are required to identify the etiology and establish the therapy.

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CONGENITAL TRACHEOESOPHAGEAL FISTULA WITHOUT ATRESIA: AN INCIDENTAL FINDING*

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SUMMARY: Oğuzkurt L, Balkancı F, Arıyürek M. (Department of Radiology, Hacettepe University Faculty of Medicine, Ankara, Turkey). Congenital tracheoesophageal fistula without atresia: an incidental finding. Turk J Pediatr 1997; 39: 285-287.

A four-year-old boy who had a long history of upper respiratory tract infections and growth retardation was admitted because of recurrent abdominal pain. During upper gastrointestinal series to search for a gastric or duodenal ulcer, the examiner noticed a minute amount of contrast medium within the trachea. Repeat esophagography on an angiographic table led to the correct diagnosis of a congenital H-type fistula. The patient did not have the classical symptoms of a history of choking and cyanosis after feeding during infancy or recurrent lower respiratory tract infections. The only finding consistent with a fistula was growth retardation, and the diagnosis was established incidentally during a work-up for abdominal pain. *Key words: tracheosophageal fistula, without atresia.*

Congenital tracheoesophageal fistula (TEF) without atresia (H-type fistula) comprises three percent of TEF[1]. Coughing, choking and cyanosis after feeding are the most common presenting features, followed by recurrent pneumonia. It is usually detected within the first year of life, but symptoms may persist until adulthood before the correct diagnosis techniques have been advocated, because identification of the fistula can be elusive and operating on suspicion alone is unwise[3].

An H-type fistula was diagnosed incidentally in a four-year-old boy presenting with recurrent abdominal pain of one-month duration.

Case Report

A four-year-old boy was admitted to the hospital for recurrent abdominal pain of one-month duration. The pain was transient and worse after meals. He did not have nausea or vomiting, and he had normal bowel habits. Physical examination of the chest and abdomen, chest x-ray and routine laboratory findings were normal. He had been followed for growth retardation at this hospital

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for two years. On the present admission, his weight and height were at the third percentile and third to tenth percentile, respectively. His skeletal age was consistent with three years. His history included upper respiratory tract infections with coughing after the first year of life. On each admission for this complaint, physical examination of the chest was normal and chst x-rays were negative for a parenchymal infiltrate.

Upper gastrointestinal series to search for a gastric or duodenal ulcer did not reveal any pathological findings in the stomach and small intestine, but leakage of the contrast medium (barium sulfate) to the trachea was noted after swallowing of the contrast medium. Although this finding can be due to aspiration of the contrast medium, a fistula between the esophagus and trachea was suspected, and esophagography was performed using digital substraction angiography on the following day.

A feeding tube was inserted from the nose into the esophagus to the level of the third thoracic vertebra with the patient lying prone on the table. Thirty mililiters of diluted contrast medium, barium sulfate, was injected manually, and serial films at three exposures per second were obtained. The examination revealed a fistula tract running anteriorly and cranially from the esophagus to the trachea at the level of the second thoracic vertebra (Fig. 1).

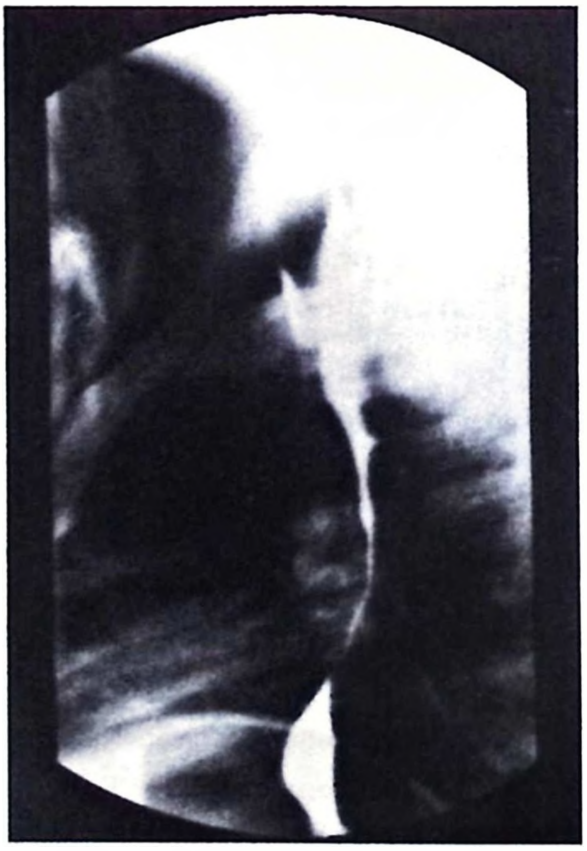

Fig. 1: Fistula tract running cranially from the esophagus to the trachea.

The patient was operated on and the fistula was ligated and excised. He had an uneventful post-operative course and was well with no symptoms at the six-month follow-up.

Discussion

It is a rare occasion for a surgeon to see a patient with an H-type fistula. For this reason, it is important to consider the diagnosis of H-type fistula from the clinical features. H-type fistula should be suspected in an infant with choking, coughing and cyanosis caused by aspiration after feeding, recurrent pneumonia with excessive tracheal secretions and abdominal distention caused by air in the stomach[4]. Relief of symptoms with a gastric tube feeding may occur, and long-term growth retardation is not an unusual finding. Although these symptoms could arouse suspicion of an H-type fistula, patients with aspiration, reflux esophagitis and palatopharyngeal dyskinesia may demonstrate similar findings[5].

The patient presented here was not at the usual age and did not show the classical symptoms and signs of TEF. He had recurrent upper respiratory tract infections with coughing after one year of life, but on each admission, the lungs were clear to percussion and auscultation and the chest x-ray was normal. The only finding consistent with a fistula was growth retardation, the cause of which could not be found for the preceding three years.

Since congenital TEF occurs rarely and may go unnoticed into adulthood, diagnosis is difficult. Esophagography is a simple means of detection for those patients. It may not be diagnostic because of technical limitations, especially if the fistula is a small one, but may lead the radiologist to suspect a fistula. Cineesophagography or esophagography performed on an angiography table is usually diagnostic and should be performed in every child with a history of coughing, choking and growth retardation or a child with a questionable esophagography.

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BREASTFEEDING – THE NATURAL ADVANTAGE

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