THE TURKISH JOURNAL OF PEDIATRICS

PUBLISHED BY
Turkish National Pediatric Society,
Hacettepe University
Institute of Child Health and
The Turkish and International
Children's Center

PRINTED IN
Meteksan Anonim Şirketi, Ankara

ISSN 0010-0161

This publication is included in the abstracting and indexing coverage of Index Medicus, Current Contents, Excerpta Medica Database (EMBASE), Abstracts on Hygiene and Communicable Diseases, CAB Abstracts (International Center for Agriculture and BioSciences), Tropical Diseases Bulletin and Turkish Medical Index.

EDITORIAL OFFICE
Prof. Dr. Turgay Coşkun
Editor
The Turkish Journal of Pediatrics
P.K. 66, Samanpazarı
06240 Ankara, Turkey
Fax: 90 (312) 324 32 84

SUBSCRIPTION ADDRESS
Prof. Dr. A. Murat Tuncer
Director
Hacettepe University
Institute of Child Health
06100 Ankara, Turkey
Fax: 90 (312) 324 32 84

THE TURKISH JOURNAL OF PEDIATRICS

VOLUME: 39 NUMBER: 3 JULY - SEPTEMBER 1997

CONTENTS

The Turkish Journal of Pediatrics 1997; 39: 287-294

HISTIOCYTIC SYNDROMES IN CHILDREN*

*Nazan Çetingül MD**, Senay Öztop MD***, Kaan Kavaklı MD***
*İpek Özunan MD****, Güngör Nişli MD***, Mine Hekimgil MD*****

SUMMARY: Çetingül N, Öztop S, Kavaklı K, Özunan İ, Nişli G, Hekimgil M. (Departments of Pediatrics and Pathology, Ege University Faculty of Medicine, İzmir, Turkey). Histiocytic syndromes in children. Turk J Pediatr 39: 287-294.

The "histiocytes" are a group of proliferative disorders of the mononuclear phagocyte system whose etiologies are basically unknown. The majority of childhood histiocytoses are expressions of excessive numbers of Langerhans cells, representing so-called Langerhans cell histiocytosis. Fifteen patients who were diagnosed with histiocytosis syndrome at the Pediatric Hematology and Oncology Department of Ege University Hospital between October 1986 and January 1995 were included in this study. The majority of the patients had Langerhans cell histiocytosis (LCH), and skeletal involvement was the most common manifestation. A good response to radiotherapy and chemotherapy was obtained by our patients with unifocal and multifocal involvement of LCH. Two patients with disseminated LCH died with progressive disease. In the patient with Rosai-Dorfman disease, a partial response was obtained with prednisone. The patient with malignant histiocytosis died during a relapse at the end of one year. Organ dysfunction and the patient's age are important factors affecting the outcome of the disease. *Key words: Langerhans cell histiocytosis, chemotherapy, radiotherapy, "histiocytosis syndrome".*

The "histiocytes" are a group of proliferative disorders of the mononuclear phagocyte system whose etiologies are basically unknown, although there are selected instances of a clinical association with primary immunodeficiency states. The majority of childhood histiocytoses are expressions of excessive numbers of Langerhans cells, representing so-called histiocytosis X or the preferred appellation, Langerhans cell histiocytosis[1]. Etiologies vary; the response to infection, immunologic stimulation, genetic abnormality and clonal malignant proliferation are all associated with proliferation of histiocytes[2-4]. There are three classes of histiocytic syndromes. Langerhans cell histiocytosis (LCH-Class I) embraces disorders previously referred to as "Histiocytosis X" (including eosinophilic granuloma of bone, Letterer-Siwe and Hand Schüller-Christian syndrome). The hemophagocytic syndromes (Class II) include familial hemophagocytic lymphohistiocytosis, infection-associated hemophagocytic syndrome, and sinus hystiocytosis with massive lymphadenopathy (Rosai-

* From the Department of Pediatrics and Pathology, Ege University Faculty of Medicine, İzmir.
** Associate Professor of Pediatrics, Ege University Faculty of Medicine.
*** Professor of Pediatrics, Ege University Faculty of Medicine.
**** Research Assistant in Pediatrics, Ege University Faculty of Medicine.
***** Associate Professor of Pathology, Ege University Faculty of Medicine.

Dorfman disease). The malignant histiocytosis (MH) syndromes (Class III) comprise acute monocytic leukemia, malignant hystiocytosis and hystiocytic lymphoma[2,3,5].

Classes II and III are rarely seen in childhood. LCH can occur in persons ranging in age from newborn to elderly; the peak incidence occurs between one and four years of age. LCH is probably underdiagnosed, the bone lesions being symptomless[6]. According to the site of infiltration, osteolytic lesions may appear, while various other organs may also be involved in LCH. The role of cytokine expression and the production of LCH cells has also been documented, reflecting the activated state of these cells and probably explaining some of the clinicopathological changes[7-10].

In this study, we investigated the classes of histiocytosis stages of LCH and their treatment and prognosis in our clinic.

Material and Methods

Fifteen patients who had been admitted to the Pediatric Hematology and Oncology Department of Ege University Hospital between October 1986 and January 1995 and diagnosed with histiocytosis syndrome by histopathological examination were retrospectively evaluated for epidemiologic, clinical, therapeutic and prognostic factors.

Radiographic investigations, skeletal surveys, complete blood counts as well as liver and kidney function tests were performed on all patients. Most patients were diagnosed by bone, skin or lymph node biopsy. The patient with malignant histiocytosis was recognized on splenectomy.

Organ dysfunction was evaluated using the criteria of Lahey, as shown below[11]:

Hematopoietic: Hemoglobin < 10 g/dl (in the absence of other causes), neutrophils < 1,500/mm^3, platelets < 100,000/mm^3.

Hepatic: Total bilirubin > 1.5 mg/dl, total protein < 5.5 g/dl, albumin < 2.5 g/dl, edema or ascites.

Pulmonary: Tachypnea, dyspnea or cyanosis, pneumothorax, pleural effusion. The staging and classification of LCH were made as shown in Table I[4-11].

Group IA: Unifocal involvement (those with a single bone lesion, with or without an associated soft tissue mass or regional lymph node involvement).

Group IB: Multifocal involvement (patients with two or more bone or soft tissue lesions, with or without skin rash, diabetes insipidus or both).

Group II: Acute disseminated form (patients with organ dysfunction) Histopathological evaluation was performed in the Department of Pathology, Ege University Faculty of Medicine.

Table I: Clinical Staging System for LCH

Variable	No. of Points
Age at presentation	
> 2 years	0
< 2 years	1
Number of organs involved	
< 4	0
> 4	1
Presence of organ dysfunction	
No	0
Yes	1
Stage	Total points
I	0
II	1
III	2
IV	3

Results

This retrospective study involved 15 patients with histiocytic syndrome. There were six girls and nine boys whose ages ranged between 14 months and 14 years (mean 6 years). Thirteen had class I (LCH Fig. 1), one had class II (NLCH Fig. 2) and one had class III (MH Fig. 3) histiocytosis.

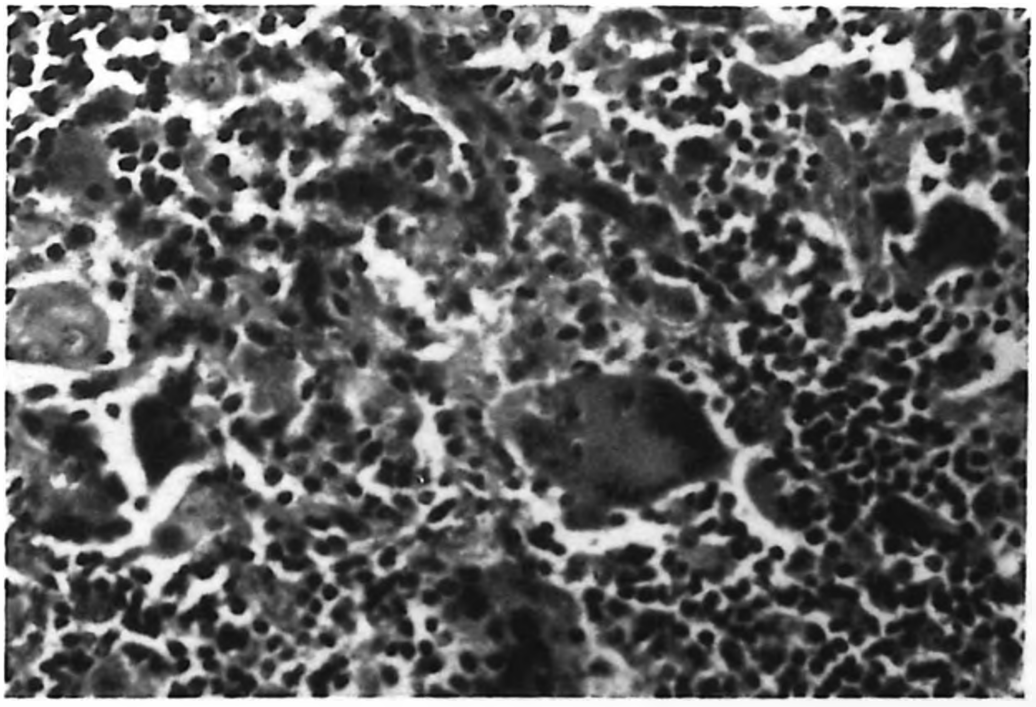

Fig. 1: Langerhans cell histiocytosis (LCH-Class I).

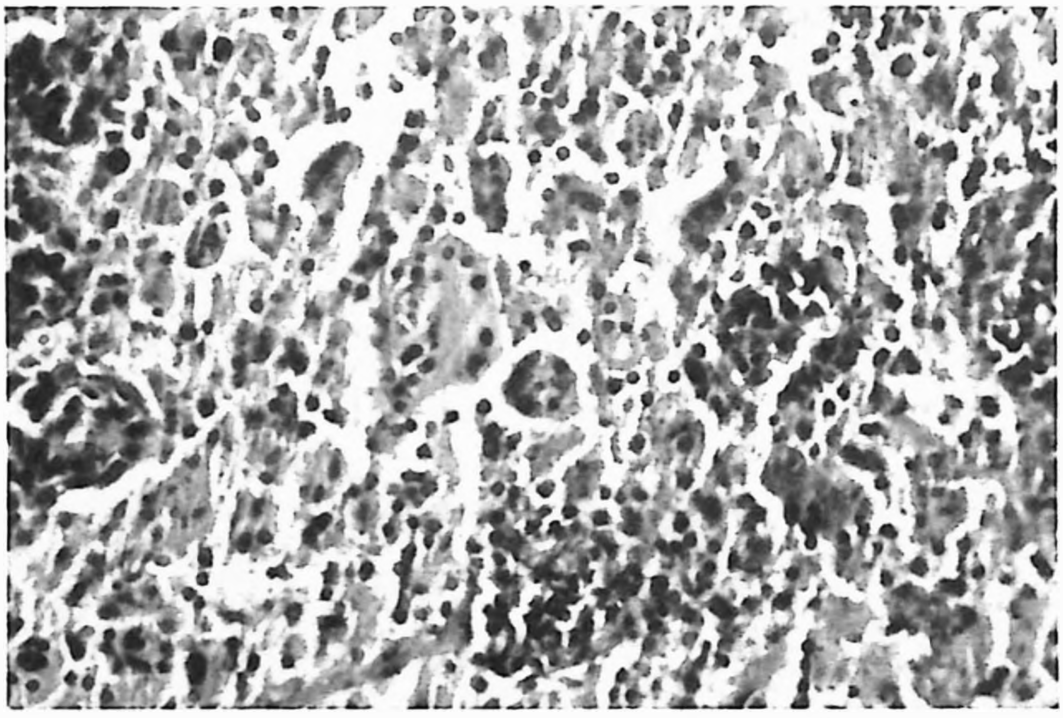

Fig. 2: Rosai-Dorfman disease (sinus histiocytosis with massive lymphadenopathy).

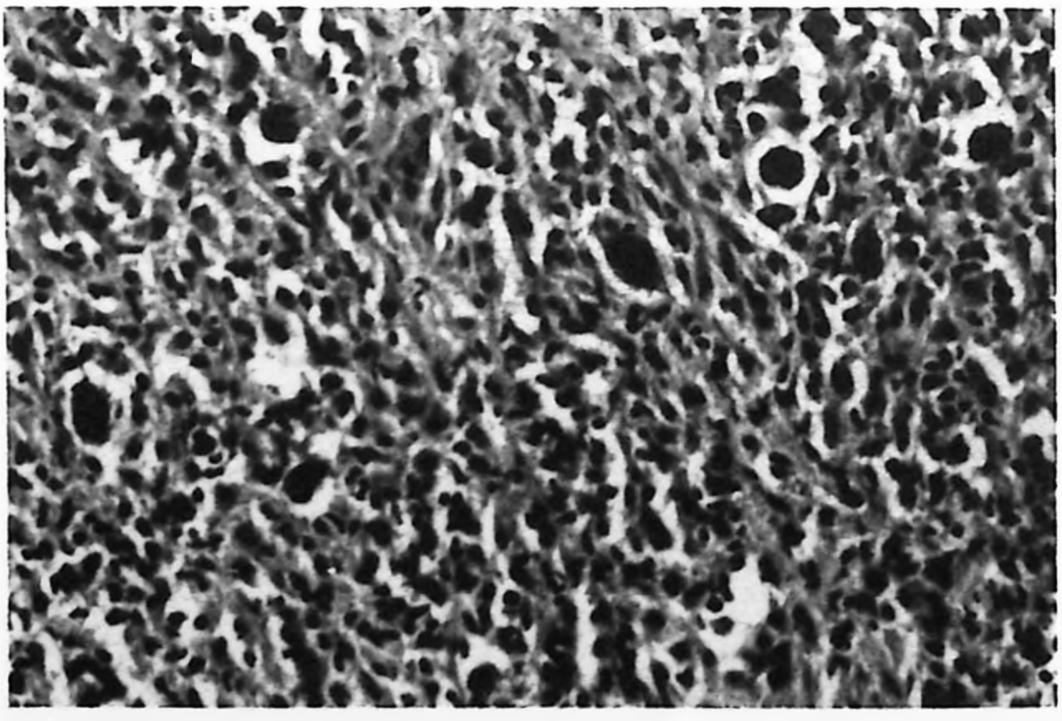

Fig. 3: Malignant histiocytosis (LCH-Class III).

The male-to-female ratio was two to one for the patients with LCH. The majority of patients had multifocal involvement (61.5% Group IB), 23 percent had unifocal involvement (Group IA) and 15.5 percent had disseminated disease (Group II). The clinical manifestations and distribution of bone lesions are listed in Table II.

Surgery, radiotherapy (1600-2000 cGy) or single- or double agent chemotherapy (prednisolone, vinblastine [VBL] or cyclophosphamide [CYC]) was administered for treatment of patients with unifocal involvement in the years 1986 to 1990. Combined chemotherapy (prednisolone + VBL or vincristine [VCR] or CYC) was administered to patients with multifocal involvement. Between 1991 and 1994, single or multiple chemotherapeutic agents including prednisolone, VBL, etoposide (VP-16), methotrexate (MTX), CYC and radiotherapy (if necessary) were administered to patients with LCH. Prednisolone was used for the patient with Rosai Dorfman. The patient with MH was treated with intensive chemotherapy. The results of treatment are shown in Table III.

Table II: Clinical Manifestations of Langerhans Cell Histiocytosis

System	n	(%)
Bone lesions*	10	67
Skin and mucous membranes	2	13
Lymphadenopathy	3	20
Pulmonary manifestations	3	20
Central nervous system (D. insipidus)	2	13
Chronic otitis media	1	7

* Distribution of bone lesions:
Skull 40%, Innominate bone 20%, Ribs 20%, Femur 13%, Vertebra 7%.

Table III: Treatment Results of Patients with Histiocytosis Syndrome

Patients (n)	Results
Class I	
Unisystem unifocal involvement (3)	Remission
Unisystem multifocal involvement (8)	Remission
Systemic involvement (2)	Exitus (6 months and 1 year later)
Class II	
Sinus histiocytosis with massive lymphadenopathy (1)	Partial response
Class III	
Malignant histiocytosis (1)	Exitus (1 year later)

Discussion

The histiocytoses are characterized by infiltration and proliferation of histiocytes. Their biologic diversity extends from the clearly benign to the fulminantly malignant, with uncertain lesions in between[2]. LCH is a nonmalignant disorder of immune regulation. There are several well-established clinical syndromes that

herald the presence of LCH in a child between the newborn period and late adolescence. Regardless of the severity of clinical manifestations, the basic histopathologic findings are essentially identical, whereas the number of affected organs and associated dysfunction are the principal determinants of prognosis[1]. According to the site of infiltration, osteolytic lesions appear, and various other organs may be involved[5, 7, 12]. Patients with localized disease have a good prognosis, and spontaneous healing of the lesions, especially of the skeletal and cutaneous ones, may be observed[3, 5, 7, 13]. Moreover, patients can experience spontaneous remissions and exacerbations. Careful classification and staging would partly overcome the difficulty of comparing the response to therapy in a disease that has variable and heterogeneous clinical manifestations[14].

The majority of our patients had LCH, and skeletal involvement was the most common manifestation (Table II). In addition, nondisseminated involvement was established in 11 of the patients with LCH.

A variety of radiation doses and many different drugs and drug combinations, including vincristine, vinblastine, prednisone, mercaptopurine, methotrexate, cyclosphosphamide, chlorambucil and etoposide have been employed[3-5, 15, 16]. A good response was obtained to radiotherapy and single-agent or double-agent chemotherapy (VCR, VBL, Pred, CYC) in the patients in groups IA and IB. Remission was achieved after six weeks. VP-16 and MTX were added to the chemotherapy regimen of the patients who had recurrences, and complete remission was achieved in this patient group as well. Desmopression was used for the patient with diabetes insipidus. The first evidence of the efficacy of Cyclosporin-A (CSA) in LCH was provided by Mahmoud et al.[17], who showed that all three untreated LCH patients had a favorable response to CSA associated with vinblastine and steroids.

Children with disseminated disease may have a more severe clinical picture, at times, progressing to a life-threatening condition. Despite several therapeutic approaches including steroids and cytotoxic drugs, no individual treatment has proven to be superior. The optimal treatment for disseminated LCH has not yet been established. Disseminated LCH may become life-threatening, and immediate clinical improvement may be critical. CSA is also effective for the treatment of LCH in pretreated children with progressive disease, including life-threatening organ dysfunction[17, 18], as supported by recent evidence of massive cytokine production (IL 1, PGE2, etc.) within the lesional tissue in the course of LCH and from isolated LCH cells[18, 19]. Previous authors have used the specificity of a monoclonal antibody directed against the CD1a antigen on Langerhans cells[6]. In our patient with disseminated disease and lung dysfunction, refractory LCH was pretreated with multiple agents including VBL, MTX, VP-16 and CYC, which led to a partial remission for six months. However, the patient

relapsed at the end of this period and was given CSA. The patient died of progressive disease at the end of the first month. Another patient was less than two years of age at the time of diagnosis. He did not respond to multiagent chemotherapy and died after six months.

Although sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) is not considered a malignant disorder, considerable mortality and morbidity can result. Both radiotherapy and chemotherapy have been used with some success, and in some cases the lesions spontaneously regress[3, 4, 20-22]. Prednisone were given to our patient with Rosai-Dorfman disease; partial remission was obtained, and the patient was still alive with the disease as of this writing.

Malignant histiocytosis is a syndrome of fever, wasting, pancytopenia, hepatosplenomegaly, adenopathy and systemic proliferation of malignant histiocytes. The disease occasionally presents with isolated involvement of the gastrointestinal tract. The majority of affected cases are now known as having Ki-I anaplastic large cell lymphoma or some other hematological disorder[23, 24]. Our patient with MH was treated as a case of histiocytic lymphoma. He had a complete remission, but died during a relapse at the end of the first year.

In conclusion, the outcome of localized LCH and of LCH with multifocal involvement are generally good. The two key factors appear to be age at the time of diagnosis and the degree of organ involvement. Children younger than two years of age at the time of diagnosis have a higher mortality rate than do older children. Involvement of multiple organ systems and the presence of organ dysfunction have been found to be poor prognostic signs. The treatment of patients with organ dysfunction is still a big problem. New prospective clinical trials should be designed to determine the optimal therapy in class I and II patients.

REFERENCES

1. Dehner LP. Morphologic findings in the histiocytic syndromes. Semin Oncol 1991; 18: 8-17.
2. Komp DM, Perry MC. The histiocytic syndromes. Semin Oncol 1991; 18: 1-2.
3. Ladisch S, Jaffe ES. The histiocytoses. In: Pizzo P, Poplack DG (eds). Principles and Practice of Pediatric Oncology (2nd ed.). Philadelphia: JB. Lippincott; 1993: 617-631.
4. Lanzkowsky P. Histiocytosis Syndromes. Manual of Pediatric Hematology and Oncology (2nd ed). New York: Churchill Livingstone; 1995: 493-511.
5. Karadeniz C, Büyükpamukçu M, Sarıalioğlu F. Langerhans cell histiocytosis: a report of 99 cases. Turk J Cancer 1992; 22: 25-38.
6. Egeler RM, D'Angio GJ. Langerhans cell histiocytosis. J Pediatr 1995; 127: 1-11.
7. Anco M, Colella R, Conter V, et al. Cyclosporin therapy for refractory Langerhans cell histiocytosis. Med Pediatr Oncol 1995; 25: 12-16.
8. Chu T, D'Angio GJ, Favara B, et al. Histiocytic syndromes in children and histiocytosis syndromes in children II. Approach to the clinical and laboratory evaluation of children with Langerhans cell histiocytosis. The Histiocyte Society 1993.

9. Komp DM. Concepts in staging and clinical studies for treatment of Langerhans' cell histiocytosis. Semin Oncol 1991; 18: 18-23.
10. Berry DH, Becton DL. Natural history of histiocytosis-X. Hematol/Oncol Clin North Am 1987; 1: 23-34.
11. Lahey ME. Histiocytosis-X: an analysis of prognostic factors. J Pediatr 1975; 87: 184-189.
12. Berry DH, Gresik M, Maybee D, Marcus R. Histiocytosis X in bone only. Med Pediatr Oncol 1990; 18: 292-294.
13. Raney RB Jr, D'Angio GL. Langerhans' cell histiocytosis (histiocytosis-X): experience at the Children's Hospital of Philadelphia, 1970-1984. Med Pediatr Oncol 1989; 17: 20-28.
14. Stathopoulou FT, Xaidara A, Mikraki V, et al. Effect of trimethoprim-sulfamethoxazole in Langerhans cell histiocytosis: preliminary observations. Med Pediatr Oncol 1995; 25: 74.
15. Womer RB, Anunciato KR, Chehrenama M. Oral methotrexate and alternate-day prednisone for low-risk Langerhans cell histiocytosis. Med Pediatr Oncol 1995; 25: 70-73.
16. Lavin PT, Osband ME. Evaluating the role of therapy in histiocytosis-X. Hematol Clin North Am 1987; 1: 35-7.
17. Mahmoud HH, Wang WC, Murphy SB. Cyclosporine therapy for advanced Langerhans cell histiocytosis. Blood 1991; 77: 721-725.
18. Sawamura M, Yamaguchi S, Murayama K, et al. Cyclosporine therapy for refractory Langerhans cell histiocytosis. Blood 1991; 78: 178-179.
19. Nezelof C, Egeler M, Bucsky P, Gogusev J. Malignant histiocytosis in childhood: a disease in guest of new nosological criteria: Med Pediatr Oncol 1995; 25: 67-69.
20. Komp DM. The treatment of sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman Disease). Semin Diagn Pathol 1990; 7: 83-86.
21. Rangwala AF, Zinterhofer LJ, Nyi KM, Ferreira PP. Sinus histiocytosis with massive lymphadenopathy and malignant lymphoma. An unreported association. Cancer 1990; 65: 999-1002.
22. Foucar E, Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenopathy. An analysis of 14 deaths occurring in a patient registry. Cancer 1994; 54: 1834-1840.
23. Esumi N, Hashida T, Matsumura T, et al. Malignant histiocytosis in childhood. Clinical features and therapeutic results by combination chemotherapy. Am J Pediatr Hematol Oncol 1986; 8: 300-307.

The Turkish Journal of Pediatrics 1997; 39: 295-301

GRANULOCYTE AND GRANULOCYTE – MACROPHAGE COLONY – STIMULATING FACTORS, THEIR RECEPTORS AND INTERLEUKIN – 3 LEVELS IN NEWBORNS*

*Mesut Coşkun MSc**, Fırat Kardelen MD***, Nihal Oygür MD****
*Levent Ündar MD*****, Ayşe Savaş MSc**, Olcay Yeğin MD******

SUMMARY: Coşkun M, Kardelen F, Oygür N, Ündar L, Savaş A, Yeğin O. (Departments of Neonatology, Clinical Immunology and Hematology, Akdeniz University Faculty of Medicine, Antalya, Turkey). Granulocyte and granulocyte-macrophage colony-stimulating factors, their receptors and interleukin-3 levels in newborns. Turk J Pediatr 1997; 39: 295-301.

Serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and interleukin-3 (IL-3) as well as neutrophil counts were studied on the first, second and fifth days after birth, in order to elucidate the relations between neutrophil kinetics and these hematopoietic factors. The G-CSF and GM-CSF receptors on neutrophils were also investigated in 16 healthy newborns.

G-CSF and GM-CSF receptor-positive neutrophil percentages were not different from those in the peripheral blood of adult controls. The receptor densities, assessed by mean fluorescence channel number, were also similar in newborn and adult neutrophils.

The mean serum concentrations of G-CSF were high (191 pg/ml) on the first day of life and gradually decreased on the second (128 pg/ml) and fifth (112 pg/ml) days. A statistically significant correlation ($p < 0.05$) was found between G-CSF levels and absolute neutrophil counts (ANC). Furthermore, the percentage of decrease in G-CSF levels correlated significantly with the percentage of decrease in ANC ($p < 0.001$). GM-CSF levels were also high, though less striking, on the first day of life (9.5 pg/ml), remained at high levels on the second (10 pg/ml), and gradually decreased on the fifth (8.8 pg/ml) day. IL-3 levels were high (110 pg/ml) on the first day of life and remained at high levels on the second (138 pg/ml) and fifth (138 pg/ml) days. We found that the IL-3, GM-CSF and G-CSF levels were elevated during the first week of postnatal life. Our findings suggest that significant changes in the levels of the growth factors are likely to be the cause of significant leukocyte blood picture changes during the first week of life. We found normal GM-CSF and G-CSF receptors in uninfected newborns. *Key words: newborn, G-CSF, GM-CSF, IL-3, neutrophil.*

Neutrophil production and function in the neonatal period are immature, and the newborn has a tendency to develop neutropenia during severe infections[1,2]. It is also well known that the leukocyte blood picture changes significantly during

* From the Departments of Neonatology, Clinical Immunology and Hematology, Akdeniz University Faculty of Medicine, Antalya.

** Biologist, Akdeniz University Faculty of Medicine.

*** Research Assistant in Pediatrics, Akdeniz University Faculty of Medicine.

**** Associate Professor of Pediatrics, Akdeniz University Faculty of Medicine.

***** Professor of Internal Medicine, Akdeniz University Faculty of Medicine.

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

This study was supported by the Akdeniz University Research Foundation.

the early days of postnatal life[3-5]. The number and functional activity of neutrophils available for host defense, depends on their production, which is regulated by growth factors granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and interleukin-3 (IL-3). These hematopoietic growth factors exert their biologic activities through binding to their specific receptors. Little is known about the regulation of GM-CSF and G-CSF receptor expression and their status in the neonatal period[6-10].

The levels of G-CSF and GM-CSF receptors on neonatal neutrophils were measured with flow cytometry. The serum levels of GM-CSF, G-CSF and IL-3 and the neutrophil counts on the first, second and fifth days after birth were studied in order to elucidate the possible relations between neutrophil kinetics and these hematopoietic factors.

Material and Methods

Subjects: The peripheral blood of 25 healthy term neonates was studied on the first, second and fifth days after birth. GM-CSF and G-CSF receptors levels were studied in the cord blood of 16 healthy term neonates and the peripheral blood of 10 adult controls. Newborns with complicated deliveries or born to mothers with any sign of infection were not enrolled in the study. Newborns showing any signs of infection during the observation period were also excluded. The study was sanctioned by the Akdeniz University Ethics Committee. Peripheral blood samples were collected in sterile tubes and allowed to coagulate in the refrigerator (+4 °C) for 15-20 minutes; the tubes were then centrifuged at 600 g for 10 minutes, and samples from the serum were removed and kept frozen at -60 °C until they were assayed. White blood cell and differential counts were determined from each sample.

Cytokine Determinations: We used commercially available ELISA (Research and Diagnostics Inc, Minneapolis, MN, USA) kits specific to G-CSF, GM-CSF and IL-3 to assess the levels of these cytokines. All samples were tested in duplicate, and all sera were studied simultaneously.

GM-CSF and G-CSF Receptor Determination: Biotinylated GM-CSF, G-CSF and Avidin-FITC were purchased from British Bio-Technology Ltd, Oxford U.K. Quantitative fluorescence analyses were performed using flow cytometry (Coulter Epics-Profile II, Coulter Electronics, Inc., Hialeah, FL, USA). Then neutrophil population was identified using forward and riht-angle light scatter characteristics. Avidin-FITC was used as a negative control. The percentage of receptor-positive cells was defined as the percentage of gated events with a fluorescence intensity greater than the negative marker. Events within the acquisition gate were more than 95 percent positive for anti-CD13 binding. The mean fluorescence channel number was used as an arbitrary unit of receptor fluorescence intensity, i.e., the amount of receptor on the neutrophil surface.

The data were analyzed by BMDP statistical software. Nonparametric Friedman tests and the Mann-Whitney-"U" test were applied, and a p value below 0.05 was considered significant. Data were presented as mean ± one standard deviation.

Results

Serum levels of G-CSF, GM-CSF and IL-3 as well as absolute neutrophil counts (ANC) are shown in Table I.

Table I: G-CSF, GM-CSF, IL-3 Levels and Absolute Neutrophil Counts (ANC)

n = 25	1st Day	2nd Day	5th Day
G-CSF (pg/ml)*	191 ± 90	128 ± 20	112 ± 17
GM-CSF (pg/ml)**	9.5 ± 1.9	10 ± 2.2	8.8 ± 1.8
IL-3 (pg-ml)**	110 ± 35.5	138 ± 58	138 ± 74
ANC (10^9/L)	14 ± 3.9	7.5 ± 2.2	4.5 ± 1.6

* Adult levels were reported to be less than 30 pg/ml by the manufacturer.
** Adult levels were reported to be nondetectable by the manufacturer.

Mean serum concentrations of G-CSF were high on the first day of life and gradually decreased on the second and fifth days (Table I). A statistically significant correlation ($p < 0.05$) was found between G-CSF levels and ANC, but no correlation between any other parameters could be found. Furthermore, the percentage of the decrease between the first and second, second and fifth, and first and fifth days of life in G-CSF levels correlated significantly with the percentage of decrease in ANC (Fig. 1). Friedmann tests were used for analysis; the p value was 0.0027 and the Kendall coefficient of concordance was 0.3600 for ANC and G-CSF values on the first versus second days. Similar calculations for ANC and G-CSF (first versus fifth days) showed $p = 0.0001$, and the Kendall coefficient of concordance was 0.5776. Calculations for the second and fifth days showed $p = 0.0027$ and the Kendall coefficient of concordance value to be 0.3600.

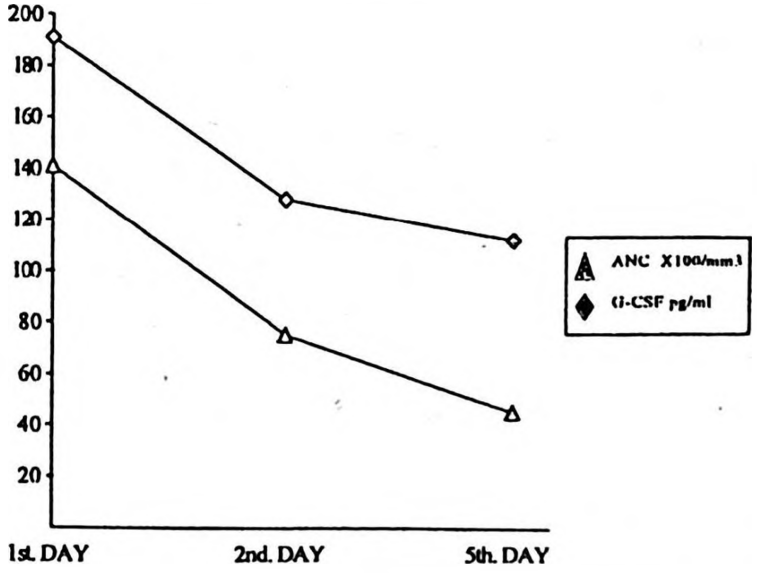

Fig. 1: G-CSF and absolute neutrophil values.

GM-CSF levels were also high, though not as striking as the others, on the first day of life, stayed at high levels on the second day, and decreased slightly on the fifth day. IL-3 levels were high on the first day of life and remained at high levels on the second and fifth days (Table I).

The mean values of the G-CSF receptor-positive neutrophil percentage was found to be 70 ± 4 percent (range 64-78%, n = 16) in cord blood, and it was not different from that in the peripheral blood of adult controls (77 ± 5%, range 69-83%, n = 10 $p > 0.05$). Mean channel numbers also did not differ significant (cord blood = 25.3 ± 1.2, adults = 23.6 ± 0.9, $p > 0.05$).

The GM-CSF receptor-positive neutrophil percentage (89 ± 4%, range: 82-95%, n = 16) in cord blood was not different from that in adult controls (86 ± 4%, range: 76-90%, n = 10, $p > 0.005$). The mean channel values were also similar in cord blood (25 ± 1.5) and adult controls (mean = 24.5 ± 1.0 $p > 0.05$).

Discussion

We observed a peak in the absolute neutrophil counts on the first day and a rapid decrease thereafter, as previously reported[3-5]. This decrease significantly correlated with the decrease in G-CSF levels (Table I). Our study shows that neonates have high levels of G-CSF at birth. Although a rapid decrease was observed on the second and fifth days, it was still higher than the adult levels (Table I). G-CSF levels correlated significantly with absolute neutrophil counts; the percentage of decrease in G-CSF correlated significantly with the percentage of decrease in ANC (Fig. 1). Our findings suggest that significant changes in the levels of growth factors may be the cause of this phenomenon. The lack of correlation between the ANC and the GM-CSF or IL-3 levels could be explained by their relatively wider spectrum of activity on the progenitor cells.

Russel et al.[11] also reported high G-CSF levels on the first day of life and a rapid decline by days 4-9. However, they found no correlation between the G-CSF levels and neutrophil counts. Yurdakök et al.[12] reported elevated G-CSF and GM-CSF levels in preterm neonates within 12 hours after birth. Laver et al.[13] and Ishii et al.[14] also reported significantly high G-CSF levels as compared to adults. Gessler et al.[15] found elevated G-CSF levels which peaked at seven hours after birth and declined on the fourth to seventh days. Our results corroborate these previous studies, but the correlation between the G-CSF levels and absolute neutrophil counts have not been reported before.

In contrast, Schibler et al.[16] and Cairo et al.[17] showed that the G-CSF production and gene expression of monocytes after stimulation was low in preterm and term newborns as compared to adults. Schibler et al.[16] found increased G-CSF serum levels in non-neutropenic newborns (values were very similar to ours).

Interestingly they showed that G-CSF serum concentrations were not elevated in seven neutropenic neonates, a finding which is in contradistinction to Russel et al.'s.[18] findings of significantly elevated pretreatment G-CSF levels in 12 neutropenic preterm neonates.

Medlock et al.[19] reported that maternally administered G-CSF crosses the placenta (although levels were 1000-fold lower than in the mother), and stimulates fetal rat granulopoiesis. (The G-CSF levels returned to baseline levels in 12 hours). It is possible that the increased levels of G-CSF on the first day, observed in our study and in previous studies, might have a maternal or a placental origin, as suggested by Ishii et al.[14], but its persistence on the second and fifth days is in favor of endogenous production. [The half life of serum G-CSF was reported to be 4.4 hours[20]]. Further studies are needed in order to clarify the production site and rate of G-CSF during increased demand in the early days of life.

There is only one study on IL-3 levels in the neonatal period, conducted by Meister et al.[21]. They found elevated levels of IL-3, which persisted for a month, in both premature and mature newborns. Our results confirm this study. Cairo et al.[17] reported decreased IL-3 production of stimulated mononuclear cells in newborn infants.

We observed high GM-CSF levels in neonates, which did not change during the observation period. High GM-CSF levels have been reported previously[12, 13], although Cairo et al.[22] found nondetectable levels of GM-CSF in the sera of cord and adult peripheral blood, and decreased stimulated GM-CSF production of mononuclear cells and GM-CSF gene expression in term newborns. English et al.[23] also reported diminished GM-CSF production of mononuclear cells in the neonatal period. Using an ELISA kit, Meister et al.[21] found low concentrations of GM-CSF in 38 percent of premature and 53 percent of mature neonates. These discrepancies may be explained by the maternal or placental origin of these colony-stimulating factors. It is also possible that the mononuclear cells may not be the main producers of GM-CSF in the neonatal period. Since our knowledge of the physiologic levels of these colony-stimulating factors and their biologic relevance is very limited, the high serum levels of these factors, observed in our study and reported previously, may still be insufficent for the neonatal period.

Laver et al.[13] and Christensen et al.[1, 24] showed that cord blood had increased numbers of circulating hematopoietic progenitors, but the data obtained in rats indicated that neonates might have a low committed progenitor pool per gram of body weight. The observations of high GM-CSF, G-CSF and IL-3 levels and increased proliferative rates of hematopoietic progenitors indicate that the neutrophil production rate is high even in a steady state condition in neonates. This might be a physiological adaptation mechanism to extrauterine life. As Laver et al.[13] suggested, limited numbers of progenitors proliferating at maximal capacity might be unable to respond to the increased demand, and this progenitor pool could become exhausted even in the presence of high levels of growth factors[1, 24, 25].

In our study, GM-CSF and G-CSF receptor-positive neutrophil percentages were found not to be different in cord blood when compared with those in the peripheral blood of adult controls. The receptor densities, assessed by mean fluorescence channel number, were also similar in newborn and adult neutrophils. Neonatal GM-CSF receptor number and affinity were found to be normal by Cairo et al.[22]. Browmeyer et al.[26] also showed that adult and term cord blood myeloid progenitors respond similarly to GM-CSF stimulation, which indirectly shows that neonatal neutrophils have normal receptors and respond to GM-CSF. Our study confirms these reports and shows that G-CSF receptor levels in newborns are also not different from adults. Cairo et al.[17], using a different method, also showed that G-CSF receptors were equal both in number and affinity in term newborns as compared to adult mature effector neutrophils. Our and previous data on these receptors were obtained from uninfected neonates. It has been shown that G-CSF, GM-CSF, IL-3, TNF and bacterial lipopolysaccharide down-modulate the G-CSF receptor expression[8], so further studies are needed on the expression of these receptors in newborns with sepsis.

TNF-alpha and gamma-interferon increase during infections, and they have suppressive effects on myelopoiesis[27, 28]. Their possible effects on neutropenia in septic newborns have not yet been fully investigated. Further studies on the effects of these growth factors, their metabolism and relations with other cytokines during severe infections, are needed.

REFERENCES

1. Christensen RD. Neutrophil kinetics in the fetus and neonate. Am J Pediatr Hematol Oncol 1989; 11: 215-223.
2. Hill HR. Biochemical, structural, and functional abnormalities of polymorphonuclear leukocytes in the neonate. Pediatr Res 1987; 22: 375-382.
3. Coulombel L, Dehan M, Tchernia G, Hill C, Vial M. The number of polymorphonuclear leukocytes in relation to gestational age in the newborn. Acta Paediatr Scand 1979; 68: 709.
4. Gür Güven A, Göl B, Oygür N, Yeğin O. Leukocyte blood picture in early neonatal period in Antalya region of Turkey. Turk J Pediatr 1994; 36: 123-132.
5. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I Reference values for neutrophilic cells. J Pediatr 1979; 95: 89-98.
6. Cairo MS. Review of G-CSF and GM-CSF. Effects on neonatal neutrophil kinetics. Am J Pediatr Hematol Oncol 1989; 11: 238-244.
7. Cannistra SA, Griffin JD. Regulation of the production and function of granulocytes and monocytes. Semin Hematol 1988; 25: 173-188.
8. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. Blood 1991; 78: 2791-2808.
9. Gasson JC. Molecular physiology of granulocyte-macrophage colony-stimulating factor. Blood 1991; 77: 1131-1145.
10. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. N Engl J Med 1992; 327: 28-35.

11. Russel AR, Davies EG, McGuigan S, Scopes GJ, Daly S, Gordon-Smith EC. Plasma granulocyte-colony stimulating factor concentrations (G-CSF) in the early neonatal period. Br J Haematol 1994; 86: 642-644.
12. Yurdakök M, Gürakan B, Ergin H, Kirazlı Ş. G-CSF and GM-CSF in respiratory distress syndrome. Am J Hematol 1994; 46: 155-156.
13. Laver J, Duncan E, Abboud M, et al. High levels of granulocyte and granulocyte-macrophage colony-stimulating factors in cord blood of normal full-term neonates. J Pediatr 1990; 16: 627-632.
14. Ishii E, Masuyama T, Yamaguchi H, et al. Production and expression of granulocyte- and macrophage-colony-stimulating factors in newborns: their roles in leukocytosis at birth. Acta Haematol 1995; 94: 23-31.
15. Gessler P, Kirchmann N, Kientsch-Engel R, Haas N, Lasch P, Kachel W. Serum concentrations of granulocyte colony-stimulating factor in healthy term and preterm neonates and in those with various diseases including bacterial infections. Blood 1993; 82: 3177-3182.
16. Schibler KR, Liechty KW, White WL, Christensen RD. Production of granulocyte colony-stimulating factor in vitro by monocytes from preterm and term neonates. Blood 1993; 82: 2478-2484.
17. Cairo MS, Suen Y, Knoppel E, et al. Decreased G-CSF and IL-3 production and gene expression from mononuclear cells of newborn infants. Pediatr Res 192; 31: 574-578.
18. Russell AR, Davies EG, Ball SE, Gordon-Smith E. Granulocyte colony stimulating factor treatment for neonatal neutropenia. Arch Dis Child Fetal Neonatal Ed 1995; 72 F53-54.
19. Medlock ES, Kaplan DL, Cecchai M, Ulich TR, del Castillo J, Andresen J. Granulocyte colony-stimulating factor crosses the placenta and stimulates fetal rat granulopoiesis. Blood 1993; 81: 916-922.
20. Gillan ER, Christensen RD, Suen Y, Ellis R, van de Ven C, Cairo MS. A randomized, placebo-controlled trial of recombinant human granulocyte colony-stimulating factor administration in newborn infants with presumed sepsis: significant induction of peripheral and bone marrow neutrophilia. Blood 1994; 84: 1427-1433.
21. Meister B, Herold M, Myr A, et al. Interleukin-3, interleukin-6, granulocyte-macrophage colony-stimulating factor and erythropoietin cord blood levels of preterm and term neonates. Eur J Pediatr 1993; 152: 569-573.
22. Cairo MS, Suen Y, Knoppel E, van de Ven C, Nguyen A, Sender L. Decreased stimulated GM-CSF production and GM-CSF gene expression but normal numbers of GM-CSF receptors in human term newborns compared with adults. Pediatr Res 1991; 30: 362-367.
23. English K, Wilson CB. Diminished granulocyte-macrophage colony stimulating factor (GM-CSF) and increased macrophage-CSF (M-CSF) production by neonatal blood mononuclear cells (abstract). Pediatr Res 1989; 25: 162.
24. Christensen RD, Harper TE, Rothstein G. Granulocyte-macrophage progenitor cells in term and preterm neonates. J Pediatr 1986; 109: 1047-1051.
25. Engle WA, McGuire WA, Schreiner R, Yu PL. Neutrophil storage pool depletion in neonates with sepsis and neutropenia. J Pediatr 1988; 113: 747-759.
26. Broxmeyer HE, Douglas GW, Hangoc G, et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci USA 1989; 86: 3828-3832.
27. Broxmeyer HE, Williams DE, Lu L, et al. The suppressive influences of human tumor necrosis factors on bone marrow hematopoietic progenitor cells from normal donors and patients with leukemia: synergism of tumor necrosis factor and interferon gamma. J Immunol 1986; 136: 4487-4495.
28. Raefsky EL, Platanias LC, Zoumbos NC, Young NS. Studies on interferon as a regulator of hematopoietic cell proliferation. J Immunol 1985; 135: 2507-2512.

The Turkish Journal of Pediatrics 1997; 39: 303-311

EVALUATION OF CHIMERISM WITH DNA POLYMORPHISMS IN BONE MARROW TRANSPLANTATION*

*Uğur Özbek MD, PhD**, Burçak Vural MSc***, Sevgi Kalayoğlu MD****
*Teoman Soysal MD*****, Hülya Bilgen MD******, Selim Yavuz MD*******
*Sema Anak MD********, Deniz Sargın MD*********, Gündüz Gedikoğlu MD**********
*Burhan Ferhanoğlu MD***********, Tevfik Akoğlu MD************
*Yücel Tangün MD*********, Tayfun Özçelik MD**********

SUMMARY: Özbek U, Vural B, Kalayoğlu S, Soysal T, Bilgen H, Yavuz S, Anak S, Sargın D, Gedikoğlu G, Ferhanoğlu B, Akoğlu T, Tangün Y, Özçelik T. [Department of Genetics, Institute for Experimental Medicine (DETAE), İstanbul University, İstanbul, Turkey]. Evaluation of chimerism with DNA polymorphisms in bone marrow transplantation. Turk J Pediatr 1997; 39: 303-311.

Evaluation of chimeric status following allogenic BMT is an important tool for monitoring the replacement of host cells with donor cells and for determining the risk of relapse. Polymorphic DNA sequences can be used as powerful markers in identification of donor/recipient genotype differences, even between close relatives. Polymerase chain reaction (PCR) amplification of three variable number of tandem repeat (VNTR) loci and five single-locus polymorphisms (SLP) was used to identify chimerism in 40 recipient-donor pairs. Mixed chimerism was present in 11 patients, and complete chimerism in 29. This PCR method is a rapid and sensitive assay to detect engraftment and evaluate relapse potential, and thus is very useful in the clinical management of BMT patients. *Key words: bone marrow transplantation, chimerism, polymerase chain reaction, polymorphism, VNTR, relapse.*

* From the Department of Genetics, Institute for Experimental Medicine (DETAE), İstanbul University, İstanbul.

** Research Assistant in Genetics, İstanbul University, Institute for Experimental Medicine (DETAE).

*** Biologist in Genetics, İstanbul University, İnstitute for Ewperimental Medicine (DETAE).

**** Specialist in Hematology, İstanbul University, İstanbul Faculty of Medicine.

***** Associate Professor of Internal Medicine, İstanbul University, Cerrahpaşa Faculty of Medicine.

****** Research Assistant in Pediatrics, İstanbul University, İstanbul Faculty of Medicine.

******* Specialist in Internal Medicine, İstanbul University, İstanbul Faculty of Medicine.

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

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

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

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

************ Professor of Internal Medicine, Marmara University Faculty of Medicine, İstanbul.

************* Associate Professor of Genetics, Institute for Experimental Medicine (DETAE), and Department of Molecular Biology and Genetics, Bilkent University, Ankara.

This work was partially suported by the Turkish Scientific and Technical Research Council (TÜBİTAK) Research Fund No: SBAG-1241, and DETAM Resarch Fund No: TUR/CLR/002 (UNDP-TUR/86/012).

Bone marrow transplantation (BMT) is the treatment of choice for some inborn errors of metabolism, and in many hematological and oncological diseases. BMT involves eradication of recipient stem cells by total body irradiation and/or high-dose chemotherapy followed by introduction of healthy donor bone marrow cells. This method creates a chimeric status in the patient's hematopoietic system. The patient may exhibit either "mixed chimerism" in which there is persistence of host hematopoietic cells along with donor cells, or "complete chimerism" in which there is complete conversion to the donor's cell type.

Several techniques have been used to evaluate chimerism following BMT, including analysis of protein polymorphisms, chromosomal studies and DNA typing[1-5]. These techniques have different sensitivities in clinical use. Recently, polymerase chain reaction (PCR) has been used for the rapid and sensitive analysis of human DNA variations that are inherited in a Mendelian fashion[6]. They include highly polymorphic, tandemly repetitive minisatellites and single-locus polymorphisms[7].

This study aimed to evaluate the clinical utility of PCR-based detection of human polymorphisms for the evaluation of chimerism in patients treated by allogenic BMT.

Material and Methods

Patients

A total of 40 recipient-donor pairs who underwent allogenic BMT for different diseases were evaluated for the development of chimerism. Informed consent was obtained from the recipient-donor pairs or parents who served as subjects prior to DNA analysis experiments. The diagnoses were as follows: aplastic anemia (AA) n = 3, thalassemia (T) n = 1, Fanconi anemia (FA) n = 1, chronic myeloid leukemia (CML) n = 14, acute lymphoblastic leukemia (ALL) n = 9, and acute non-lymphoblastic leukemia (ANLL) n = 12. Thirty-nine of the patients were from three different BMT units of İstanbul University. Of these, eight patients (Cases1-8) were from the BMT unit of the Department of Pediatrics, and 17 patients (Cases17-23, 29, 30, 33-35, 37, 39, 40) were from the Department of Internal Medicine at İstanbul Faculty of Medicine. Fourteen patients (Cases11-14, 16, 24, 28, 31, 32, 36, 38) were from the BMT unit of the Department of Internal Medicine at Cerrahpaşa Faculty of Medicine. The remaining patient (Case 9) was from the BMT unit of the Department of Internal Medicine at Marmara University Medical Faculty. All the patients were grafted from HLA-identical siblings. Patient data (disease type, age, sex, conditioning regimen, graft-versus-host disease, phase of BMT); DNA analysis results, such as time of PCR analysis, informative loci and chimeric status; and finally the present status are summarized in Table I.

Table I: Clinical Characteristics of Patients and Results of Molecular Studies

Case	Disease	Age	Sex (D/R)	Conditioning Regimen	GVHD		Phase of BMT	Time of PCR Analysis	Informative Loci	Chimeric Status	Outcome
					Acute	Chronic					
A. Pediatric Age-group											
1. (KIT.5)	β-TM	4	M/M	TBI+CTX	–	–	–	+395	HBGG, GYPA	MC	Alive
2. (KIT12)	AA	16	M/F	TLI+CTX	–	–	–	+365	GYPA	MC	Alive
3. (KIT.7)	AA	14	F/M	TLI+CTX	–	–	–	+385	LDLR, GYPA, GC	MC	Alive
4. (KIT.8)	FA	12	M/F	TLI+CTX	I	–	–	+180	D1S80, ApoB	MC	Died
5. (KIT.6)	ALL	8	F/M	TBI+CTX	–	–	2nd CR	+420	D7S8	MC	Alive
6. (KIT.19)	ANLL	5	M/M	TBI+CTX	I	–	1st CR	+180	ApoB	CC	Relapse
7. (KIT.9)	ANLL	6	M/M	TBI+CTX	–	–	1st CR	+210	PAH	MC	Alive
8. (KIT.20)	CML	10	F/F	TBI+CTX	II	–	CP	+120	PAH, ApoB	MC	Alive
9. (KIT.21)	CML	15	M/F	TBI+CTX	I	–	1st CR	+90	ApoB	CC	Died
B. Adult Age-group											
10. (KIT.13)	AA	24	M/M	ATG+Cy	–	–	–	+48	HBGG, GC	MC	Alive
11. (KIT.37)	ALL	16	F/F	TBI+Cy	–	–	1st CR	+90	LDLR,D7S8,GC,GYPA,Apo B	MC	Alive
12. (KIT.14)	ALL	20	M/M	TBI+Cy	–	–	1st CR	+325	PAH	CC	Alive
13. (KIT.25)	ALL	23	M/F	TBI+Cy	I	–	1st CR	+90	PAH	CC	Alive
14. (KIT.28)	ALL	26	M/F	TBI+Cy	–	–	1st CR	+240	ApoB, PAH	CC	Alive
15. (KIT.17)	ALL	25	M/F	TBI+Cy	I	–	2nd CR	+75	D7S8, GC	MC	Relapse
16. (KIT.27)	ALL	23	M/M	TBI+Cy	–	–	1st CR	+250	D1S80	CC	Alive
17. (KIT.40)	ALL	25	M/M	TBI+Cy	I	–	2nd CR	+720	ApoB	CC	Alive
18. (KIT.46)	ALL	28	M/M	TBI+Cy	–	–	1st CR	+260	D1S80	CC	Died
19. (KIT.16)	ANLL	43	F/M	Bu+Cy	I	–	1st CR	+120	GYPA, D7S8, GC	CC	Alive
20. (KIT.29)	ANLL	37	M/M	Bu+Cy	–	–	1st CR	+45	D1S80	CC	Alive
21. (KIT.31)	ANLL	18	M/M	Bu+Cy	–	–	1st CR	+110	ApoB	CC	Alive
22. (KIT.32)	ANLL	29	M/M	Bu+Cy+VP-16	I	–	Relapse	+90	D1S80	CC	Alive
23. (KIT.36)	ANLL	29	M/F	Bu+Cy+VP-16	I	Lim.	1st CR	+200	D1S80	CC	Alive
24. (KIT.15)	ANLL	18	F/M	TBI+Cy	I	Lim.	1st CR	+330	GYPA, D7S8, GC	CC	Alive
25. (KIT.24)	ANLL	19	F/M	TBI+Cy	I	Lim.	1st CR	+425	ApoB	CC	Alive
26. (KIT.44)	ANLL	23	F/M	TBI+Cy	I	–	1st CR	+210	D1S80	CC	Alive
27. (KIT.49)	ANLL	24	F/F	Bu+Cy	II	–	2nd CR	+120	D1S80	CC	Alive
28. (KIT.50)	ANLL	33	M/F	Bu+Cy	–	–	1st CR	+90	D1S80	CC	Alive
29. (KIT.23)	CML	30	F/M	Bu+Cy	I	Ext.	AP	+119	PAH	CC	Died

Table I: Clinical Characteristics of Patients and Results of Molecular Studies

Case	Disease	Age	Sex (D/R)	Conditioning Regimen	GVHD		Phase of BMT	Time of PCR Analysis	Informative Loci	Chimeric Status	Outcome
					Acute	Chronic					
30. (KIT.26)	CML	30	F/F	TBI+Cy	I	Ext.	2nd CP	+60	ApoB	CC	Alive
31. (KIT.11)	CML	37	F/M	TBI+Cy	–	–	1st CR	+160	PAH	MC	Died
32. (KIT.30)	CML	39	F/F	TBI+Cy	–	–	1st CR	+112	ApoB	CC	Alive
33. (KIT.22)	CML	36	M/F	Bu+Cy I	–	–	CP	+60	ApoB	CC	Alive
34. (KIT.34)	CML	34	M/M	TBI+Cy III	–	–	CP	+68	PAH	CC	Died
35. (KIT.39)	CML	33	F/F	Bu+Cy I	–	–	Relapse	+600	PAH	CC	Alive
36. (KIT.42)	CML	35	F/F	TBI+Cy	–	–	1st CR	+120	PAH	CC	Alive
37. (KIT.43)	CML	35	F/M	TBI+CY	–	–	1st CR	+180	ApoB	CC	Alive
38. (KIT.45)	CML	29	M/M	Bu+Cy I	–	–	CP	+90	PAH	CC	Alive
39. (KIT.38)	CML	23	M/M	Bu+Cy I	–	–	CP	+67	D1S80	CC	Alive
40. (KIT.47)	CML	25	M/M	Bu+Cy I	–	–	CP	+90	D1S80	CC	Alive

D : Donor
R : Recipient
Age of patients : Age in years at BMT
ANLL : Acute non-lymphoblastic leukemia
ALL : Acute lymphoblastic leukemia
CML : Chronic myeloblastic leukemia
AA : Aplastic anemia
FA : Fanconi anemia
β-TM : Beta thalassemia major
well : Disease free
Lim. : Limited
Ext : Extensive

MC : Mixed chimerism
CC : Complete chimerism
+ : Number of days after BMT (on which sample collected for analysis)
CR : Complete remission
CP : Chronic phase
AP : Accelerated phase
TBI : Total body irradiation
TLI : Total lymphoid irradiation
Bu : Busulphan
VP-16 : Etoposide
CTX and Cy : Cytoxane and Cyclophosphamide

Conditioning Regimens

Twenty-six patients were treated with radiotherapy and chemotherapy, and the remaining 14 patients with chemotherapy alone (Table I).

DNA Extraction

Genomic DNA was isolated from peripheral blood samples of donors and recipients (after BMT) by overnight proteinase K and sodium dodecyl sulfate digestion at 56 °C, ammonium acetate extraction and ethanol precipitation as described elsewhere[8]. The original genotype of the recipient was determined from DNA extracted from hair root cells. The hair roots were plucked, cut with scissors approximately 1 cm from the root, and rinsed in distilled H_2O followed by absolute ethanol. After drying they were digested in 0.5 ml lysis buffer (NaCl 100 mM; disodium EDTA 25 mM) containing 50 µg/ml proteinase K and one percent SDS. DNA was extracted with 1.5 M NaCl and chloroform: isoamylalcohol (24:1), and precipitated with isopropanol as described elsewhere[9].

PCR Analysis

As shown in Table I, eight genomic loci were analysed in this study. These include three VNTR and five SLP. The VNTR loci were phenylalanine hydroxylase (PAH) gene[10], apolipoprotein B (ApoB) gene[11], and D1S80 locus[12]. The remaining five loci were two or three allele polymorphisms: low-density lipoprotein receptor (LDLR)[13], glycophorin (GYPA)[14], hemoglobin G gammaglobulin (HBGG)[15], D7S8[16] and human group-specific component (GC)[17]. PCR reactions were performed in an automated thermal cycler (Perkin-Elmer, 480). Primers for PAH, D1S80 and ApoB loci were synthesized by a commercial company (Genomed Ltd.). AmpliType PM kits (Perkin-Elmer) were used for typing the LDLR, GYPA, HBGG, D7S8, and GC loci. For VNTR analysis, 0.5 µg of DNA was amplified in a final volume of 50 µL in the presence of 50 mmol/L KCl, 10 mmol/L tris-HCl pH 8, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 40 pmol of each primer and 0.5 U Taq DNA polymerase (Boehringer-Mannheim). The PCR conditions for the AmpliType PM kit were 60 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C for 32 cycles followed by a seven-minute extension at 72 °C; for PAH locus, 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C for 30 cycles, for D1S80 locus, 60 s at 94 °C, 60 s at 65 °C, and eight minutes at 72 °C for 30 cycles, and for ApoB locus, 60 s at 94 °C and six minutes at 60 °C for 32 cycles. The primers used are cited in the references.

After amplification of the PAH, D1S80 and ApoB loci, 20 µl of the sample volume was electrophoresed through a three percent agarose gel, stained with ethidium bromide and photographed under UV light. For the other five loci, amplified DNA samples were hybridized to strips onto which allele-specific oligonucleotide

probes were blotted. Specifically bound, amplified DNA was visualized by the enzymatic precipitation of biotin-labelled probes that give a color reaction. Determination of the genotypes was carried out as suggested by the manufacturer (Perkin Elmer Cetus, 1990). In order to determine the state of chimerism, the genotype of the recipient's post-BMT genotype was compared to the genotype of the hair root and the genotype of the donor.

Results

In this study, polymorphic DNA markers were used to evaluate chimerism post-BMT in 40 donor-recipient pairs. Nine patients were in the pediatric age-group and 31 were adults. Genotype analyses were done at intervals during the 45 to 720 days post-BMT. Complete chimerism was documented in 29 patients and mixed chimerism in 11 (Table I). Figure 1 shows an example of complete chimerism detected by the PAH-VNTR polymorphism (Case 13).

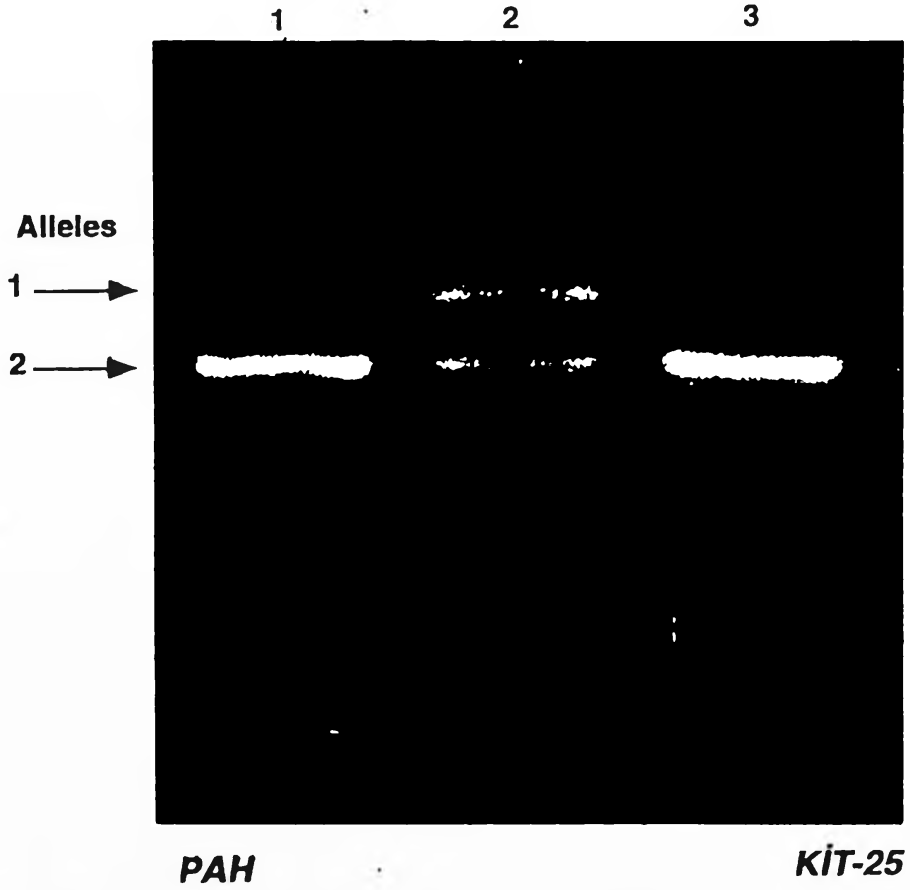

Fig. 1: An example of complete chimerism detected by PAH-VNTR polymorphism (Case 13).

Chimerism was evaluated initially using the VNTR polymorphisms. AmpliType PM analysis was used if the VNTR polymorphisms were not informative. VNTR polymorphism analysis alone was informative for 31 patients, while nine patients required analysis with the single-locus polymorphisms to determine chimerism.

Of the donor-recipient pairs, ApoB was fully informative in 13 pairs, D1S80 in 11 pairs, and PAH in 11 pairs. In nine cases requiring AmpliType PM analysis, GC and GYPA was informative in six pairs, D7S8 in five pairs, HBGG in two pairs, and LDLR in two pairs. In four patients (Cases 4, 8, 11, 14) single-locus typing was only partially informative. The evaluation of chimerism in these four patients required typing with two or more loci.

Discussion

VNTR (ApoB, D1S80, PAH) and AmpliType PM (LDLR, GYPA, HBGG, D7S8, GC) analyses were successful in evaluating post-BMT chimerism in 40 recipient-donor pairs. This represents the largest series of bone-marrow-grafted patients analyzed with DNA markers in Turkey. Twenty-nine patients (72.5%) were mixed chimeric (MC). The CC/MC ratios in the adult and pediatric age-groups were different. Whereas 87 percent of the adult patients were CC, only 22 percent of the pediatric group were CC. Reports from different series of patients indicate the incidence of MC in the early phase of BMT to be approximately 50 percent when PCR is used. Disease type, conditioning regimen, and the time of PCR analysis are the factors that affect the chimeric status of the patients[18]. We believe these factors have been effective in our study group as well since MC/CC ratios differed between the adult and pediatric age-groups.

Several markers have been utilized to evaluate chimerism after BMT, including chromosomes, red blood cell antigens, immunoglobulin allotypes, cell enzymes, HLA typing, in-situ hybridization and DNA polymorphisms[1-5, 19]. PCR-based analysis of DNA polymorphisms is a very sensitive technique that is particularly useful in cases where the number of blood cells is low, for example during the early post-transplantation period and also during graft rejection. In addition, the use of highly polymorphic loci increases the power of discrimination in cases with HLA-identical, sex-matched donor/recipient pairs[1].

An important factor in the DNA-based evaluation of chimerism is the choice of polymorphic loci. Generally, VNTR polymorphisms are more informative and therefore more suitable for molecular analysis. In our study, the ApoB polymorphism was informative in 13 of 17 (76%) donor-recipient pairs, D1S80 was informative in 11 of 16 (68.7%) pairs, and PAH was informative in 11 of 24 (45%) pairs. Our standard protocol involves testing first with the ApoB and D1S80 loci followed by PAH typing if these are not informative. The AmpliType PM loci, although informative, are not routinely used because of their high cost.

Several groups have reported a correlation between mixed chimerism and leukemic relapse[20-22]. Generally the recipient's cells are responsible for the leukemic relapse[23]. Therefore, detection of these cells in the circulation may prove useful in monitoring patients susceptible to leukemic relapse[18]. At present

the significance of persistent low levels of recipient cells detected by PCR is not clear. The evaluation of the prognostic value and clinical significance of the DNA polymorphisms detected by PCR results will require further analysis of the BMT patients participating in this study for longer periods.

In conclusion, PCR-based detection of VNTR and AmpliType PM polymorphisms appears to be a sensitive and powerful technique for the evaluation of chimeric status in patients treated by BMT. Furthermore, polymorphic markers can be used instead of direct mutation-based tests to monitor relapse in hematopoietic malignancies, which represent a group of disorders that have a wide range of disease-specific genetic abberrations[23].

REFERENCES

1. van den Berg H, Beverstock G, Westerhof JP, Vossen JM. Chromosomal studies after bone marrow transplantation for leukaemia in children. Bone Marrow Transplant 1991; 7: 335-342.
2. van Leeuwen JE, van Tol MJ, Joosten AM, et al. Mixed T-lymphoid chimerism after allogeneic bone marrow transplantation for hematologic malignancies of children is not correlated with relapse. Blood 1993; 82: 1921-1928.
3. Martinelli G, Trabetti E, Zaccaria A, et al. In vitro amplification of hypervariable DNA regions for the evaluation of chimerism after allogeneic BMT. Bone Marrow Transplant 1993; 12. 115-120.
4. Suttorp M, Schmitz N, Dreger P, et al. Monitoring of chimerism after allogeneic bone marrow transplantation with unmanipulated marrow by use of DNA polymorphisms. Leukemia 1993; 7: 679-687.
5. Wilborn F, Schmidt CA, Siegert W. Demonstration of chimerism after allogeneic bone marrow transplantation by polymerase chain reaction of Y-chromosome-specific nucleotide sequences-characterization of a new technical approach. Leukemia 1993; 7: 140-143.
6. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988; 239: 487-491.
7. Botstein D, White RL, Skolnick M. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 1980; 32: 314-331.
8. Sambrook J, Fritsch EF, Maniatis J. Molecular Cloning: A Laboratory Manual (2nd ed). New York: Cold Spring Harbor Laboratory; 1989; 9.16-9.23.
9. Higuchi R, von Beroldingen CH, Sensabaugh GF, Erlich HA. DNA typing from single hairs. Nature 1988; 332: 543-546.
10. Goltsov AA, Eisensmith RC, Konecki DS, et al. Associations between mutations and a VNTR in the human phenylalanine hydroxylase gene. Am J Hum Genet 1992; 51: 627-636.
11. Boerwinkle E, Xiong W, Fourest E, Chan L. Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: application to the apolipoprotein B 3' hypervariable region. Proc Natl Acad Sci USA 1989; 86: 212-216.
12. Budowle B, Chakraborty R, Giusti AM, et al. Analysis of the VNTR locus D1S80 by the PCR followed by high resolution PAGE. Am J Hum Genet 1991; 48: 137-144.
13. Yamamoto T, Davis CG, Brown MS, et al. The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. Cell 1984; 39: 27-38.
14. Siebert PD, Fukuda M. Molecular cloning of a human glycophorin B cDNA: nucleotide sequence and genomic relationship to glycophorin A. Proc Natl Acad Sci USA 1987; 84: 6735-6739.

15. Slightom JL, Blechl AE, Smithies O. Human fetal G-gamma and A-gamma-globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes. Cell 1980; 21: 627-638.

16. Horn GT, Richards B, Merrill JJ, Klinger KW. Characterization and rapid diagnostic analysis of DNA polymorphisms closely linked to the cystic fibrosis locus. Clin Chem 1990; 36: 1614-1619.

17. Yang F, Brune JL, Naylor SL, et al. Human group-specific component (Gc) is a member of the albumin family. Proc Natl Acad Sci USA 1985; 82: 7994-7998.

18. Gialt SA, Champlin RA. Leukemia relapse after bone marrow transplantation: a review. Blood 1994; 84: 3603-3612.

19. Bowcock AM, Ray A, Erlich H, Sehgal PB. Rapid detection and sequencing of alleles in the 3' flanking region of the interleukin-6 gene. Nucleic Acids Res 1989; 17: 6855-6864.

20. Mackinnon S, Barnett L, Heller G, O'Reilly RJ. Minimal residual disease is more common in patients who have mixed T-cell chimerism after bone marrow transplantation for chronic myelogenous leukemia. Blood 1994; 83: 3409-3416.

21. Pignon JM, Henni T, Amselem T, et al. Frequent detection of minimal residual disease by use of the polymerase chain reaction in long-term survivors after bone marrow transplantation for chronic myeloid leukemia. Leukemia 1990; 4: 83-86.

22. Sawyers CL, Timson L, Kawasaki ES, et al. Molecular relapse in chronic myelogenous leukemia patients after bone marrow transplantation detected by polymerase chain reaction. Proc Natl Acad Sci USA 1990; 87: 563-567.

23. Chen JH. Molecular basis of hematopoietic malignancy in children. Cancer Bull 1992; 44: 483-486.

The Turkish Journal of Pediatrics 1997; 39: 313-315

THE PREVALENCE OF FACTOR V LEIDEN (1691 G → A) MUTATION IN TURKEY*

*Aytemiz Gürgey MD***, *Lütfiye Mesci BS****

SUMMARY: Gürgey A, Mesci L. (Hematology Unit, Department of Pediatrics, Hacettepe University, Faculty of Medicine, Ankara, Turkey). The prevalence of factor V Leiden (1691 G→A) mutation in Turkey. Turk J Pediatr 1997; 39: 313-315.

Resistance to activated protein C (APC), which is caused by a single point mutation in the gene for factor V, is a common risk factor for thrombosis. In this study, we screened factor V (FV) Leiden mutation in 81 subjects. The mutation in the heterozygous form was found in 7.1 percent of our normal population. This high frequency suggests that screening for the FV mutation should be considered in patients with a family history of thrombosis. *Key words: factor V, activated protein C resistance, factor V Leiden.*

Venous thrombosis is a serious problem causing significant morbidity and mortality. Resistance to activated protein C (APC) has been shown to be one of the major risk factors for venous thrombosis. The molecular basis for APC resistance involves a mutation in the coagulation factor V (FV) gene at nucleotide 1691, which causes the mutation Arg 506 Gln (FV-Leiden)[1-3].

The FV mutation has been demonstrated in 20 to 40 percent of patients with thrombophilia and has a prevalence of five percent in the general population[1,2]. The frequency of FV mutation in Turkey is unknown. We have studied the frequency of the FV mutation.

Material and Methods

Fourty-eight adults and 33 children with no history of familial thrombophilia were screened for the 1691 G→A mutation in the FV gene. Genomic DNA was extracted from white blood cells and amplified by polymerase chain reaction (PCR). The 1691 G→A mutation was detected by loss of a cleavage site for Mnl I digestion. A 267-bp fragment was amplified using 5' primer TGCCCAGTGCTTAACAAGACCA-3' and 3' primer TGTTATCACACTGGTGCTAA-3'. The PCR conditions were as follows: 250 µl DNA was heated to 96 °C for five minutes and 34 °C for 30 seconds, and then held at 60 °C for two minutes and at 72 °C for three minutes together with the amplification solution and the primers as described[3]. Aliquots were digested for 12 hour at 37 °C with Mnl I (1 U). The fragments were separated on two percent agarose gels and visualized with ethidium bromide (Fig. 1).

* From the Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

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

*** Biologist, Hacettepe University Faculty of Medicine.

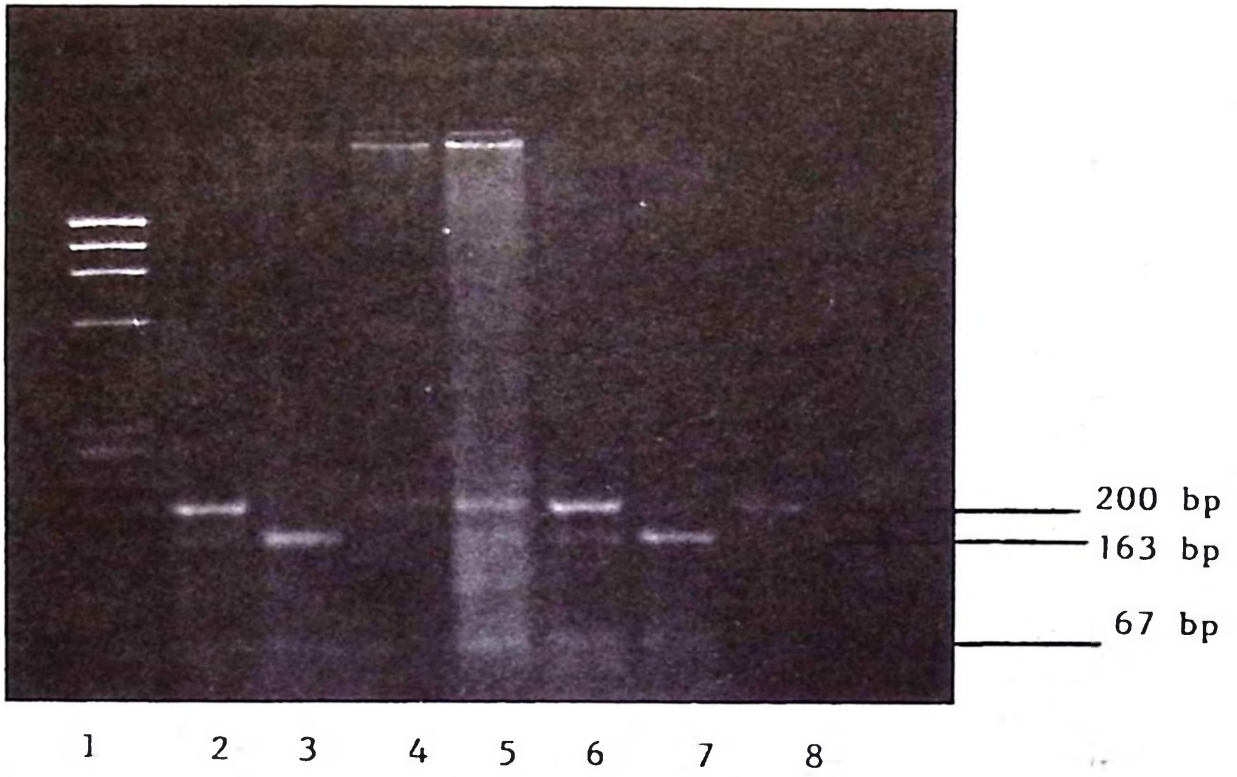

Fig. 1: Restriction analysis with Mnl I. 1: Marker (OX 174), 2, 4, 5, 6 heterozygote, 3, 7 normal.

Results

Among 81 individuals, we found six (7.1%) who were heterozygous for the FV mutation, while the other 75 were normal (Table I).

Table I: Carrier Rate For Factor V Leiden Mutation in Selected Countries

Country	Carrier Rate (%)	References
1. Turkey	7.1	Present study
2. Germany	4.0	5
3. Finland	4.0	7
4. Netherlands	2.9	8
5. United Kingdom	8.8	5
6. Greece	15.0	5
7. Africa	0.0	5

Discussion

The frequency of the FV mutation in Western Europe has been estimated at three to five percent[1,2,4]. The frequency of 7.1 percent found in our series is close to that in the United Kingdom (8.8%) and less than that of Greece (15%)[5] (Table I). It has been suggested that the FV mutation, like heterozygous protein C deficiency, is not enough solely to cause thrombosis, but is associated with

a life-long increased risk of venous thrombosis[3,4]. Recently, the FV mutation has been found in five healthy centenarians. It is interesting that none of them developed thrombotic episodes[6]. Heterozygotes for the FV mutation usually have a five-to 10-fold increased risk of venous thrombosis, and homozygotes are 50- to 100-fold more susceptible[7-9].

The FV mutation accounts for 21 percent of deep vein thromboses and up to 50 percent of familial thromboses[2,7]. The mutation was shown in eight (50%) of 16 children with thrombosis in our previous publication[10]. Deficiencies of protein C, protein S, antithrombin III and dysfibrinogenemia were together accountable for only five to 10 percent of cases, indicating the FV mutation to be at least 5 times more common than any of the other known genetic defects[1].

In conclusion, because of the approximately seven percent prevalence of FV Leiden in our population, it is suggested that family members of any subject with APC resistance should be screened to identify those who should receive prophylactic anticoagulant therapy during periods of increased risk of thrombosis, such as major surgery, pregnancy or the use of oral contraceptives.

Acknowledgement

We thank Dr. C. Altay for her continuous support.

REFERENCES

1. Sensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994; 330: 517-522.
2. Koster T, Rosendaal FR, de Ronde H, et al. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet 1993; 342: 1503-1506.
3. Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64-67.
4. Beauchamp NJ, Daly ME, Hampton KK, et al. High prevalence of a mutation in the factor V gene within the U.K. population: relationship to activated protein C resistance and familial thrombosis. Br J Haematol 1994; 88: 219-222.
5. Rees DC, Cox Clegg JB. World distribution of factor V Leiden. Lancet 1995; 346: 1133-1134.
6. Dari D, Manucci PM, Duca F, Bertolini S, Franceschi C. Mutant factor V (Arg 506 Gln) in healthy centenarians. Lancet 1996; 347: 1044.
7. Dahlback B. Inherited thrombophilia: resistance to activated protein C as a pathogenic factor of venous thromboembolism. Blood 1995; 85: 607-614.
8. Petaja J, Jalanko H, Holmberg C, et al. Resistance to activated protein C as an underlying cause of recurrent venous thrombosis during relapsing nephrotic syndrome. J Pediatr 1995; 127: 103-105.
9. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 1995; 85: 1504-1508.
10. Gurgey A, Mesci L, Renda Y, Olcay L, Kocak N, Erdem G. The factor VQ 506 mutation in children with thrombosis. Am J Hematol 1996; 53: 37-39.

The Turkish Journal of Pediatrics 1997; 39: 317-324

CORRELATION OF LABORATORY AND CLINICAL FINDINGS WITH THE LOCATION OF Xp21 DELETION IN DUCHENNE MUSCULAR DYSTROPHY*

*H. Ali Taşdemir MD**, Haluk Topaloğlu MD***, Pervin Dinçer PhD****
*Safiye Göğüş MD***, Esin Kotiloğlu MD*****, Emine Özdirim MD***
*Kalbiye Yalaz MD****

SUMMARY: Taşdemir HA, Topaloğlu H, Dinçer P, Göğüş S. Kotiloğlu E, Özdirim E, Yalaz K. (Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Correlation of laboratory and clinical findings with the location of Xp21 deletion in Duchenne muscular dystrophy. Turk J Pediatr 1997; 39: 317-324.

Laboratory and clinical features of 28 Duchenne muscular dystrophy patients were evaluated. Positive family history was present in only two cases (7.1%). Dystrophin I-positive fibers were present in 33 percent of the cases with the deletion close to the 5' end of the gene. In the cases with deletion concerning the central part of the gene, all fibers were dystrophin I-negative. In five of the six cases with short stature, the deletion was close to the 5' end of the gene, and short stature was especially seen together with 8th and 13th exon deletion. Statistical analysis concerning the age at which the patient began to have difficulty in standing up and at which he could not walk, did not correlate with the clinical severity and deletion zone, location or extent. *Key words: Duchenne muscular dystrophy, Xp21 deletion, dystrophin, laboratory findings, clinical features.*

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder transmitted by female carriers to their male offspring[1-5]. On rare occasions, it occurs in females[2-4]. It is well-known that deletions on the Xp21 chromosome result differently in the quality and quantity of dystrophin, leading to muscle weakness[6-8]. However, it has been shown that the presence of dystrophin-positive (+) fibers does not correlate with clinical severity[9]. Mental retardation (MR), cardiomyopathy (CMP), respiratory disorders and obesity are seen in some but not all cases of DMD[2, 3, 10]. Some DMD patients cannot walk at the age of seven, and others at 12 years of age[3, 10, 11]. Factors contributing to these different clinical pictures are being researched by several investigators.

This study was performed to shed light on the relationship between the clinical and laboratory findings and the location of the deletions.

* From the Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.
** Associate Professor of Pediatric Neurology, Atatürk University Faculty of Medicine, Erzurum.
*** Professor of Pediatrics, Hacettepe University Faculty of Medicine.
**** Assistant Professor of Medical Biology, Hacettepe University Faculty of Medicine.
***** Associate Professor of Pathology, Hacettepe University Faculty of Medicine.

Material and Methods

The study was completed between January 1990 December 1993 and included 28 patients diagnosed with DMD with Xp21 deletion found by DNA analysis. The patients' family histories were taken and a thorough physical examination performed. Short stature was identified as length below three percent of the value appropriate for age obesity was defined as weight above 20 percent of the value appropriate for length[12, 13]. The total muscle strength score (TMSS) was calculated according to the guidelines of the Medical Research Center (MRC)[14]. Intelligence tests were given to the patients, their IQ levels were determined and MR was scored[15]. The presence of cardiomyopathy (CMP) was determined according to the findings of cardiologic examination, radiography, electrocardiography (EKG) and echocardiography. Left ventricle functions were evaluated by ejection fraction (EF) and contraction fraction (CF)[16, 17]. Tests for respiratory functions were performed, and vital capacity (VC) as a representative was measured[18-20].

Histopathologic evaluation included immunohistochemical study of open biopsy samples by using dystrophin I (mid-rod domain), dystrophin II (carboxyl terminal) and dystrophin III (amino terminal) antibodies, and the ratio of dystrophin-positive [Dys(+)], partially positive (±) and negative (-) fibers were calculated.

Patient DNAs were amplified by multiplex polymerase chain reaction (PCR). Deletion analysis was performed by using the 9-exon multiplex primer PCR set (4, 8, 12, 17, 19, 44, 45, 48, 51 exons) and 5-exon multiplex PCR set (Pm, 13, 43, 50, 52 exons)[21, 22]. The exact deletion extent was able to be determined in 13 cases.

Statistical analysis was done by SPSS for Windows V5.01.

Results

In this study, the mean age was 115.6 ± 42.1 months, with a range of 33 to 220 months. Positive family history was detected in two patients (7.1%) whose uncles had DMD. Family history was negative in the majority of cases (26 out of 28, 92.9%). Nine patients (32.1%) were obese and six of them could not walk. Six patients (21.4%) had short stature. Intelligence tests were given to 24 of the 28 patients, of whom 11 (45.8%) had normal or high and 13 (54.2%) had low IQ levels. Respiratory function tests were performed in 16 cases, and five of them (31.3%) had VC with predicted values below 80 percent. Overall, 14 patients (50%) had CMP, and two (7.1%) had left ventricle dysfunction. Nineteen patients (67.9%) began to have difficulty in standing before four years of age, and nine patients (32.1%) after that age. Thirteen patients could not walk, and this symptom occurred before nine years of age in seven of them (53.9%) and after nine years of age in six of them (46.2%).

Dys I (+) fibers were present in three (10.7%), Dys I (±) in five (17.9%), Dys III (+) in four (14.3%) and Dys III (±) in two (7.1%) cases. Dys II was negative in all cases.

Deletions found in our cases were focused in two regions of the dystrophin gene: 4-19 exons zone and 45-52 exons zone (Table I). In nine cases (32.1%), deletions were close to the 5' end (4-19 exons) and in 18 cases (64.3%) close to the 3' end (45-52 exons). One case had a large deletion beginning from the 5' end and extending to the central zone (4-44 exons). The most frequently deleted exons were the 47th, 48th and 49th exons, detected in eight cases (28.6%). Deletion of the 8th exon was seen in seven cases (25%).

Table I: Deleted Zones and Extent of Deletion in 28 DMD Patients

Case No.	Deletion Detected	Exact Extent of Deletion	Case No.	Deletion Detected	Exact Extent of Deletion
1	8-13		15	45-50	45-50
2	8-13		16	48-50	
3	13-19		17	45	45
4	13-19		18	45-52	45-52
5	8		19	47	
6	8		20	46-47	46-47
7	50-52	50-52	21	48-52	
8	45-50		22	47-48	47-48
9	46-47	46-47	23	45	45
10	45-50	45-50	24	48	
11	19		25	51	51
12	51-52		26	50	50
13	48	48	27	8	
14	51	51	28	4-44	

There was no correlation between the extent of deletion and other parameters [TMSS, EF, CF, VC pred %, IQ levels and Dys I, III (+) or (±) fiber ratios] (Table II). However, when the location of the deletion was taken into account, the ratio of Dys I (+) fibers was significantly different in the two groups ($p < 0.002$). The results of other laboratory findings did not differ significantly (Table II). Another interesting observation concerning the location of the deletion was that five of the six (83.3%) short patients had a deletion close to the 5' end (4-19 exons zone), especially in the 8th and 13th exons (Table III).

Walking capability is such an important parameter that it is included in the diagnostic criteria[23]. Hence, the relationship between the location of the deletion and the age at which the patient could not walk was analyzed by a more detailed (x^2 test) analysis (Table IV), and no correlation was found.

Figure 1 depicts a graph of the relationship between age and walking capability. The aim was to show that the deleted zone has no effect on the age at which the patient could not walk, and to reveal the possible walking ability of a DMD patient at a proposed age.

Table II: Correlation Between Deleted Zones and Selected Clinical and Laboratory Findings (Covariant Analysis)

	4-19 Exons Deletion		45-52 Exons Deletion			p*
	Mean	Corrected Mean	Mean	Corrected Mean	Age	Deletion Groups
Number of cases		9		18		
Age (months)	101.2 ± 49.9	–	121.6 ± 38.3	–	–	0.25
TMSS	54.4 ± 21.8	52.2	50.7 ± 12.9	52.9	0.001	0.84
EF	66.8 ± 8.0	66.4	63.6 ± 6.8	64.0	0.23	0.44
CF	36.4 ± 6.0	36.2	34.9 ± 6.3	35.2	0.42	0.70
Dystrophin I (+) %	0.17 ± 0.25	0.18	–	–	0.06	0.002
Dystrophin I (±) %	1.17 ± 1.95	1.19	1.00 ± 4.00	0.98	0.91	0.89
Dystrophin III (+) %	–	–	0.42 ± 0.91	0.44	0.60	0.17
Dystrophin III (±) %	–	–	1.17 ± 4.71	1.28	0.54	0.40
IQ	84.6 ± 14.8	84.2	84.8 ± 20.1	85.2	0.53	0.91

* The difference is significant if $p < 0.05$
CF : Contraction fraction
EF : Ejection fraction
TMSS: Total muscle strength score

Table III: Statistical Comparison (p values) of the Frequencies of Signs and Symptoms in the Presence and Absence of Different Exon Deletions (x^2 Analysis)

	Symptoms and Findings					
Deletion Zone Deleted Exon	Difficulty in Standing Before 4 Years	Short MR	CMP	VC < 80%	Obesity	Stature
Deletion zone 4-19/45-52	0.61	0.68	0.72	0.64	0.22	0.008
Deleted 8th exon	0.78	0.23	0.46	0.56	0.69	0.02
Deleted 13th exon	–	0.23	0.55	0.36	0.45	0.05
Deleted 45th exon	0.51	0.22	0.36	0.48	0.21	0.62
Deleted 47th exon	0.29	0.58	0.62	0.38	0.59	0.43
Deleted 48th exon	0.44	0.55	0.73	0.52	0.59	0.43
Deleted 49th exon	–	0.42	0.66	0.92	0.57	0.62
Deleted 50th exon	0.44	0.62	0.39	0.73	0.59	0.43
Deleted 51st exon	–	0.12	0.42	0.32	0.43	0.62
Deleted 52nd exon	–	0.37	0.44	0.18	0.24	0.64

MR : Mental retardation
CMP : Cardiomyopathy
VC : Vital capacity

Table IV: Cox Regression Model Where Age and Deleted Exon or Exon Zone are Covariates, and Age at Which the Patient cannot Walk is Independent Variable (*)

Deleted Zone Deleted Exon	Effect of Deletion Zone (p)	Effect of Age (p)	Relative Risk	95% Confidence Limits of Relative Risk	
4-19/45-52	0.99	0.40	1.01	0.26	3.91
			45-52-4-19		
8th exon	0.91	0.46	0.92	0.24	3.49
45th exon	0.83	0.44	0.87	0.25	3.03
47th exon	0.47	0.61	0.60	0.15	2.42
48th exon	0.49	0.62	0.62	0.16	2.42
50th exon	0.52	0.48	1.49	0.44	5.07

* 2nd column shows p values representing the relation between deletion zone and age at which the patient could not walk; 3rd column shows p values representing the relation between the present age of the patient and the age he could not walk.

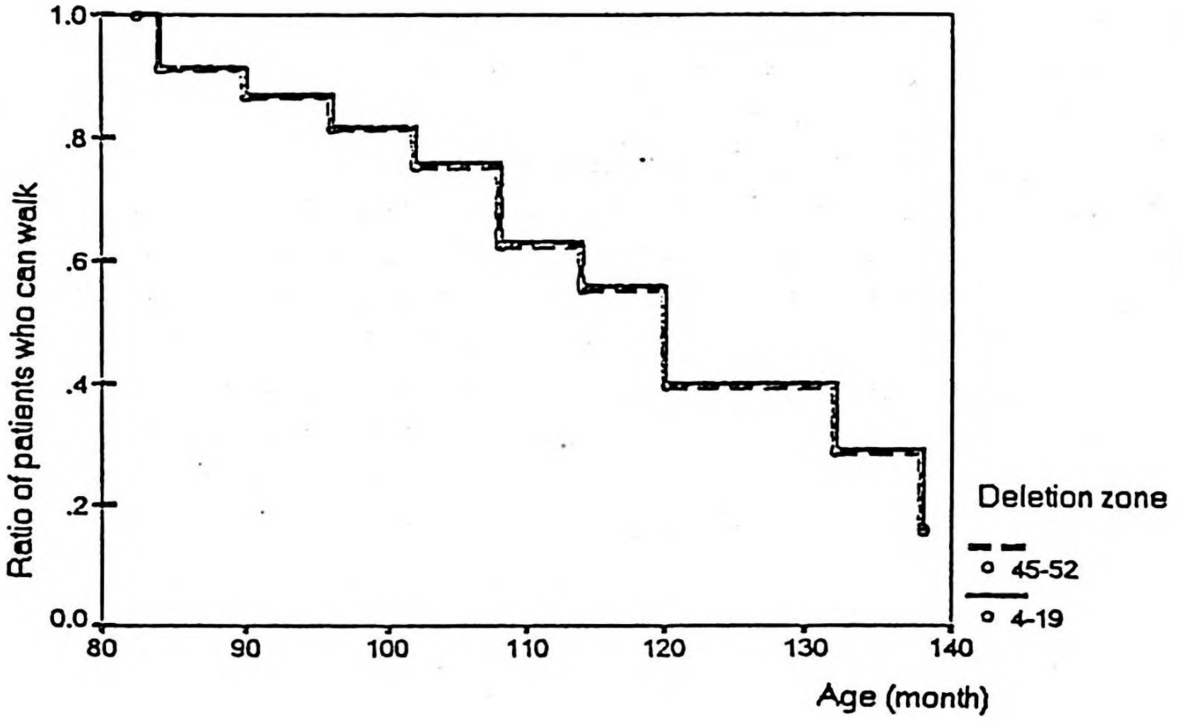

Fig. 1: Graph showing the relationship between age and walking ability in patients with deletions of 4-19 and 45-52 exons.

Discussion

In one-third of the DMD cases, there was a positive family history; another one-third were offspring of possible mutant carriers not diagnosed earlier, while the remainder were due to new mutations[2, 5, 6]. In our study group, a positive family history was present only in 7.1 percent of cases.

There was no difference in TMSS, EF, KF, IQ, Dys I (±) or Dys III (+) or (±) fiber ratios between the groups with 4-19 exons deletion and 45-52 exons deletions (Table II). On the other hand, three of nine cases (33.3%) with the deletion close to the 5' end (4-19 exons) had Dys I (+) fibers, in contrast to all cases with central exon deletions and Dys (-) fibers (Table II).

In our group, the location of the deletions or certain deleted exons did not affect the presence of MR, CMP, obesity or disturbances in VC (Table III). Hodgson[24] found that IQ levels were normal in seven of the 12 DMD patients and abnormal in the remaining five cases (41.7%). However, in the series of Rappaport et al.[25], the MR rate was much higher. Among the 27 cases in their group, 19 (70.4%) patients were mentally retarded.

In our study group, five out of six patients with short stature (83.3%) had a deletion close to the 5' end, especially deleted 8^{th} and 13^{th} exons (Table III). No correlation was found between short stature and the location of the deletion by Hodgson et al.[26].

Since 1988, studies have focused on mapping of the dystrophin gene in order to formulize the suggestion that deletions of certain regions of the gene lead to a certain clinical severity of DMD or Becker muscular dystrophy (BMD)[27, 28-30]. Medori et al.[28] reported that 8 of 11 mild DMD cases had deletions of the 5' end, and that none of the classical DMD cases had similar deletions. There have been further studies showing the regions responsible for BMD and DMD[26, 28]. Read et al.[30] tried to define specific deletions for BMD and DMD. However, there are several reports showing no correlation between the clinical severity and the location extent of the deletion[31, 32-34]. Similar results were achieved in our study with the detailed statistical analysis, using age at the onset of walking and standing disturbances as a parameter of clinical severity (Tables III-IV, Fig. 1).

REFERENCES

1. Swaiman KF. Pediatric Neurology. Missouri: CV Mosby Company; 1989: 1067-1079, 1139-1149.
2. Asbury AK, McKhan GM, McDonald WI. Diseases of the Nervous System Clinical Neurobiology. Philadelphia: WB. Saunders; 1992: 123-132.
3. Walton SJ. Disorders of Voluntary Muscle. New York: Churchill Livingstone; 1988: 32-65, 109-137, 519-540.
4. Aicardi J. Diseases of the Nervous System in Childhood. London: MacKeith Press; 1992: 1172-1178.
5. Adams JH, Duchen LW. Greenfield's Neuropathology. London: Edward Arnold; 1992: 1456-1460.
6. Hoffman EP, Schwartz L. Dystrophin and disease. Mol Aspect Med 1991; 12: 175-194.
7. Love DR, Byth BC, Tinsley JM, et al. Dystrophin and dystrophin-related proteins: a review of protein and RNA studies. Neuromuscul Disord 1993; 3: 5-21.
8. Matsumura K, Campbell KP. Deficiency of dystrophin-associated proteins: a common mechanism leading to muscle cell necrosis in severe childhood muscular dystrophies. Neuromusc Disord 1993; 3: 109-118.

9. Fanin M, Danieli GA, Vitiello L, Senter L, Angelini C. Prevalence of dystrophin-positive fibers in 85 Duchenne muscular dystrophy patients. Neuromusc Disord 1992; 2: 41-45.

10. Iannaccone ST. Current status of Duchenne muscular dystrophy. Pediatr Clin North Am 1992; 39: 879-894.

11. Dubowitz V. Muscle Disorders in Childhood (2nd ed). London: WB. Saunders; 1978: 19-49.

12. Judith J, Henry RN. Routine growth monitoring and assessment of growth disorders. J Ped Health Care; 1992; 6: 291-301.

13. Hung W. Clinical Pediatric Endocrinology. St. Louis: CV Mosby; 1992: 356.

14. Brett EM, Lake BD. Neuromuscular disorder: I. Primary muscle disease and anterior horn cell disorders. In: Brett EM (ed). Pediatric Neurology (2nd ed). London: Churchill Livingstone; 1991: 53-68.

15. American Association on Mental deficiency Adaptive Behaviour Scale. 1974 Manual. Washington, D.C.

16. Nigro G, Comi LI, Politano L, Bain RJ. The incidence and evolution of cardiomyopathy in Duchenne muscular dystrophy. Int J Card 1990; 26: 271-277.

17. D'Orsogna L, O'Shea JP, Miller G. Cardiomyopathy of Duchenne muscular dystrophy. Pediatr Cardiol 1988; 9: 205-213.

18. Burke SS, Grove NM, Houser CR, Johnson DM. Respiratory aspects of pesudohypertrophic muscular dystrophy. Am J Dis Child 1974; 121: 230-234.

19. Inkley SR, Oldenburg FC, Vignos PJ. Pulmonary function in Duchenne muscular dystrophy related to stage of disease. Am J Med 1974; 56: 297-306.

20. Hapke EJ, Meek JC, Jacops J. Pulmonary function in progressive muscular dystrophy. Chest 1972; 61: 41-47.

21. Chamberlain JS, Gibbs RA, Rainer JE, Caskey CT. Multiplex PCR for the diagnosis of DMD. In: Innis MA, Gelfand DH, Sninski J, White T (Eds.). PCR Protocols: A guide to Methods and Applications. San Diego: Academic Press; 1990: 272-281.

22. Dinçer P, Topaloğlu H, Ayter Ş, Özgüç M, Taşdemir HA, Renda Y. Molecular deletion patterns in Turkish Duchenne and Becker dystrophy patients. Brain Dev 1996; 18: 91-94.

23. Jennekens FGI, ten kate LP, de Visser M, Wintzen AR. Diagnostic criteria for Duchenne and Becker muscular dystrophy and myotonic dystrophy. Neuromuscul Disord 1991; 1: 389-391.

24. Hodgson SV, Abbs S, Clark S, et al. Correlation of clinical and deletion data in Duchenne and Becker muscular dystrophy with special reference to mental ability. Neuromuscul Disord 1992; 2: 269-276.

25. Rapaport D, Passos-Bueno MR, Brandao L, et al. Apparent association of mental retardation and specific patterns of deletions screened with probes cf56a and cf23a in Duchenne muscular dystrophy. Am J Med Genet 1991; 39: 437-441.

26. Hodgson S, Hart K, Abbs S, et al. Correlation of clinical and deletion data in Duchenne and Becker muscular dystrophy. J Med Genet 1989; 26: 682-693.

27. Beggs AH, Hoffman EP, Snyder JR, et al. Exploring the molecular basis for variability among patients with Becker muscular dystrophy: dystrophin gene and protein studies. Am J Hum Genet 1991; 49: 54-67.

28. Medori R, Brooke MH, Waterston RH. Genetic abnormalities in Duchenne and Becker dystrophies: clinical correlations. Neurology 1989: 39: 461-465.

29. Koenig M, Beggs AH, Moyer M, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. Am J Genet 1989; 45: 498-506.

30. Read AP, Mountford RC, Forrest SM, et al. Patterns of exon deletions in Duchenne and Becker muscular dystrophy. Hum Genet 1988, 80: 152-156.

31. Darras BT, Blattner P, Harper JF, et al. Intragenic deletions in 21 DMD/BMD families studied with the dystrophin cDNA: location of break points on Hind III and Bg III exon-containing fragment maps, meiotic and mitotic origin of the mutations. Am J Hum Genet 1988, 43: 620-629.

32. Baumbach LL, Chamberlain JS, Ward PA, Farwell NJ, Caskey CT. Molecular and clinical correlations of deletions leading to Duchenne and Becker muscular dystrophies. Neurology 1989; 39: 465-474.

33. Davies KE, Smith TJ, Bundey S, et al. Mild and severe muscular dystrophy associated with deletions in Xp21 of the human X chromosome. J Med Genet 1988; 25: 9-13.

34. Norman AM, Thomas NS, Kingston HM, Harper PS. Becker muscular dystrophy: correlation of deletion type with clinical severity. J Med Genet 1990; 27: 236-239.

The Turkish Journal of Pediatrics 1997; 39: 325-334

EARLY DETERMINATION OF NUTRITIONAL PROBLEMS IN PEDIATRIC CANCER PATIENTS*

*Zafer Kurugöl MD***, Ayten Egemen MD****, Nazan Çetingül MD******
*Kaan Kavaklı MD*****, Güngör Nişli MD****, Senay Öztop MD*****

SUMMARY: Kurugöl Z, Egemen A, Çetingül N, Kavaklı K, Nişli G, Öztop S. (Deparment of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Early determination of nutritional problems in pediatric cancer patients. Turk J Pediatr 1997; 39: 325-334.

Mild and marginal malnutrition must be identified to prevent the development of severe protein-energy malnutrition in pediatric cancer patients. We aimed to evaluate nutritional status and determine daily energy, protein and micronutrient intake to identify mild or marginal malnutrition in pediatric cancer patients. Daily energy, protein and micronutrient intake, anthropometric measurements and biochemical indices were studied in 45 patients (25 in remission, 20 newly diagnosed or relapsed) who consumed energy, protein, vitamins and minerals below the recommended guantities. According to the weight-for-height index, 23 children (51.1%) were determined to be malnourished. Absolute and relative prealbumin values were 19.4 ± 7.2 mg/dl and 74.3 ± 29.1 mg/dl in the remission group, and 14.8 ± 5.1 mg/dl and 58.1 ± 23.3 in the active disease group, respectively ($p < 0.05$). Relative prealbumin values were found to be low in 63.6 percent of nonmalnourished children, and 80 percent of children with mild malnutrition. We conclude that malnutrition is common in pediatric cancer patients, and prealbumin is a reliable and sensitive indicator of mild and marginal malnutrition. Determining prealbumin values and assessing the deficiency of micro- and macronutrients before malnutrition is detected by anthropometric measures may provide a warning that nutritional problems may occur. *Key words: pediatric oncology, prealbumin, malnutrition.*

Increased metabolic rates associated with illness, malabsorption, mechanical gastrointestinal problems and cachexia caused by elevated levels of tumor necrosis factor may cause nutritional problems in pediatric cancer patients[1-3]. Nausea and vomiting associated with aggressive multimodal chemotherapy, and side effects of radiation therapy such as stomatitis, dysphagia, diarrhea and somnolence, may produce further nutritional deficits. Behavioral and environmental factors such as poor eating habits, decreased palatability of hospital food and noncompliance with dietary regimens may also contribute to poor nutrition[4]. Cancer itself may cause poor oral energy and protein intake. Therefore, pediatric cancer patients represent a high-risk group for protein energy malnutrition[5,6]. Malnutrition occurs more frequently in patients diagnosed with Ewing's sarcoma, Wilms' tumor, head and neck tumors, advanced lymphomas and neuroblastoma[7].

* From the Department of Pediatrics, Ege University Faculty of Medicine, İzmir.
** Pediatrician, Ege University Faculty of Medicine.
*** Professor of Pediatrics, Ege University Faculty of Medicine.
**** Associate Professor of Pediatrics, Ege University Faculty of Medicine.

Malnutrition decreases chemotherapy tolerance, increases the tendency for infections due to deterioration in cell-mediated immunity, and is associated with poor prognosis[1-4, 6]. Therefore, determination of the nutritional status of pediatric cancer patients and early detection of their nutritional problems is as important as the regulation of chemotherapy regimens. In patients in whom assessment of nutritional status has been studied, anthropometric variables (weight, height, weight for height, triceps skinfold thickness, midarm circumference) and biochemical indices (albumin, transferrin) have been measured[2, 8, 9]. These measurements, however, are not sensitive enough to detect mild or moderate protein-energy malnutrition[5]. In pediatric cancer patients, mild and marginal malnutrition must be identified to prevent the development of severe protein-energy malnutrition.

The aim of this study was to evaluate the nutritional status and determine the consumption of nutrients (oral intake of energy, protein and micronutrients) to identify mild or marginal malnutrition in pediatric cancer patients, and to compare the nutritional status and oral intake of patients with active disease to patients in remission.

Material and Methods

Forty-five patients (27 male and 18 female) followed at Ege University's Pediatric Oncology and Hematology Department participated in this study. The patient group consisted of 20 patients with leukemias, 13 with bone tumors and 12 with other solid tumors. Twenty-five of these children were in remission (remission group), and 20 were newly diagnosed or relapsed (active disease group). The mean age of the children in remission was 10.3 ± 5.2 years, and in the active disease group 10.7 ± 5.5 years (Table I). No significant difference in sex or age was found between the two groups.

Table I: Selected Characteristics of Pediatric Cancer Patients

Characteristics	Remission Group (n = 25)	Active Disease Group (n = 20)	Total (n = 45)
Age (years)	10.3 ± 5.2*	10.7 ± 5.5*	10.5 ± 5.4*
Sex			
Male	16 (64%)	11 (55%)	27 (60%)
Female	9 (36%)	9 (45%)	18 (40%)
Tumors			
Leukemia	13 (52%)	7 (35%)	20 (44%)
Bone tumors	7 (28%)	6 (30%)	13 (28%)
Other tumors	5 (20%)	7 (35%)	12 (16%)

* Values are mean ± SD. No significant age differences were found between groups.

Dietary intake history over three consecutive days, and daily energy, protein and micronutrient intake were calculated. Patients' daily energy and nutrient intake were evaluated according to 1989 Recommended Dietary Allowances (RDAs)[10].

Because the allowances for pediatric cancer patients are 15 percent greater than the allowances for healthy children, energy and protein intake in these patients was compared to the RDAs increased by 15 percent[6]. None of the patients in the study group received enteral or parenteral nutrition. Also, the percentage of patients consuming macro- and micronutrients below the recommended levels was evaluated.

Anthropometric measurements of all patients were determined by a clinician. Children were weighed once wearing a light gown and no shoes on an electronic scale to the nearest 100 g. Height was measured with a standard stadiometer in children older than two years and in the supine position to the nearest 0.1 cm in children less than two years of age.

Percentiles for weight and height were compared with the data of the 50th percentile of the National Center for Statistics (NCHS), and the nutritional status of patients was evaluated according to the weight-for-height index[11].

The weight-for-height index was calculated by dividing the observed weight by the expected weight of a child of the same height and sex at the same percentile. A weight-for-height index of less than 0.85 signified advanced malnutrition, an index of 0.85 to 0.90 signified moderate malnutrition, and an index of 0.90 to 0.95 signified mild malnutrition. A weight-for-height index of greater than 0.95 was indicative of normal nutritional status.

Two milliliters of venous blood was obtained from the patients. Serum urea, creatinine and albumin levels were measured by an autoanalyser (A. Menarini diagnostics), and prealbumin and transferrin levels were measured by immunonephelometry (Beckman Protein Array). Serum albumin levels less than 3.5 g/dl, and serum transferrin levels less than 200 mg/dl were considered as abnormal, as proposed in the literature[12]. Since prealbumin values vary with age and sex, observed values were converted to relative values as follows: observed prealbumin/expected prealbumin x 100[13]. Relative prealbumin values below 80 percent of expected levels were considered abnormal since this level corresponds to the mean -1 SD of the referenced values for age and sex. While prealbumin levels were measured, the axillary temperature of all children was determined to be less than 37 °C; infection was not detected in any children.

For the statistical evaluation, Student's t-test and fisher exact test were used.

Results

The oral protein and energy intake of children of various ages with cancer are shown in Table II. Children in all age-groups consumed half of the energy that was recommended. There was no significant difference in energy intake between the remission and active disease groups.

Table II: Daily Energy and Protein Intake in Pediatric Cancer Patients

Age Group (Years)	Daily Energy Intake (Kcal/day)			Daily Protein Intake (g/day)		
	Remission Group	Active Disease Group	Recommended*	Remission Group	Active Disease Group	Recommended*
1-3	765 ± 166	542 ± 23	1495	26.2 ± 4.8	15.3 ± 3.4	18.4
4-6	1219 ± 469	1108 ± 463	1920	42.2 ± 16.1	36.3 ± 8.0	27.6
7-10	1476 ± 301	1097 ± 710	2300	54.9 ± 9.9	36.3 ± 28.2	32.2
11-14 (male)	1537 ± 362.5	1439 ± 150	2875	51.6 ± 12.3	48.9 ± 5.2	51.8
11-14 (female)	1821 ± 131	1779 ± 100	2530	55.0 ± 1.0	30.3 ± 3.1	52.9
15-18 (male)	1571 ± 172	1330 ± 625	3600	48.4 ± 20.8	45.6 ± 25.3	67.9
15-18 (female)	1236 ± 124	1112 ± 220	2530	47.8 ± 3.6	39.9 ± 4.3	50.6

* Values are expressed as mean ± SD.
** Patients' daily energy and nutrient intakes were evaluated according to 1989 RDAs.

We found that children with cancer accepted protein much better than energy. Children in remission except those 15-18 years old consumed protein at proper levels. However, children over age 11 in the active disease group consumed protein below recommended levels.

The vitamin and mineral intake of patients is displayed in Tables III and IV. Patients in both the active disease and remission groups consumed vitamin A and B and minerals below recommended levels. We found that all patients consumed half of the recommended amounts of calcium, iron, zinc and copper, independent of the activity of disease (Table IV).

Table III: Daily Vitamin Intake in Pediatric Cancer Patients

Vitamins	1-3 Years	4-6 Years	7-10 Years	11-14 Years (M)	11-14 Years (F)	15-18 Years (M)	15-18 Years (F)
Vitamin A (IU/day)							
Remission group	1989 (95)	2467 (117)	3142 (82)	2606 (54)	4059 (84)	4405 (88)	3662 (73)
Active disease group	862 (41)	2036 (97)	1904 (50)	1832 (38)	3888 (80)	1239 (25)	2562 (51)
Thiamin (mg/day)							
Remission group	0.3 (43)	0.4 (44)	0.6 (60)	0.6 (46)	0.7 (64)	0.7 (47)	0.6 (55)
Active disease group	0.2 (29)	0.4 (44)	0.5 (50)	0.6 (46)	0.4 (36)	0.7 (47)	0.5 (45)
Riboflavin (mg/day)							
Remission group	0.7 (88)	0.9 (82)	1.0 (83)	0.8 (62)	1.4 (108)	0.9 (50)	0.7 (54)
Active disease group	0.4 (50)	0.5 (45)	0.9 (75)	1.3 (87)	0.6 (46)	0.9 (50)	0.7 (54)
Niacin (mg/day)							
Remission group	5.0 (56)	8.1 (68)	10.9 (84)	13.4 (89)	12.7 (85)	10.2 (51)	9.1 (61)
Active disease group	2.1 (23)	5.9 (49)	5.2 (40)	9.9 (66)	7.5 (50)	10.5 (53)	8.4 (56)
Vitamin C (mg/day)							
Remission group	35.7 (88)	41.5 (92)	41.1 (91)	127.7 (255)	185.3 (231)	95.2 (159)	70.5 (118)
Active disease group	24.7 (62)	20.9 (46)	87.5 (194)	53.4 (107)	49.9 (100)	94.6 (153)	64.5 (108)

The values in parenthesis show the percentage of the vitamin consumed according to 1989 RDAs.

Table IV: Daily Minerals Intake in Pediatric Cancer Patients

Minerals	1-3 Years	4-6 Years	7-10 Years	11-14 Years (M)	11-14 Years (F)	15-18 Years (M)	15-18 Years (F)
Calcium (mg/day)							
Remission group	346 (43)	496 (62)	489 (61)	557 (46)	746 (62)	528 (44)	427 (36)
Active disease group	238 (30)	161 (20)	513 (64)	359 (30)	254 (21)	374 (31)	315 (26)
Iron (mg/day)							
Remission group	3.9 (39)	5.6 (56)	7.4 (74)	6.3 (42)	9.4 (63)	8.4 (70)	8.0 (53)
Active disease group	2.5 (25)	5.8 (58)	4.7 (47)	9.9 (66)	9.3 (62)	9.1 (76)	5.4 (36)
Zinc (mg/day)							
Remission group	3.4 (34)	4.4 (44)	6.1 (61)	4.8 (40)	4.8 (40)	4.8 (32)	4.6 (38)
Active disease group	1.8 (18)	3.1 (31)	4.0 (40)	4.4 (37)	3.3 (28)	2.8 (19)	3.8 (32)
Copper (mg/day)							
Remission group	0.3 (33)	0.6 (46)	0.9 (60)	1.1 (55)	0.9 (45)	1.1 (55)	0.7 (35)
Active disease group	0.3 (33)	0.6 (46)	0.6 (40)	0.8 (67)	0.5 (28)	0.7 (35)	0.8 (40)

The values in parenthesis show the percentage of the mineral consumed according to 1989 RDAs.

Ninety-five percent of patients consumed energy and 35.5 percent consumed protein at less than recommended levels (Fig. 1). We showed that 51.1, 84.6, 53.4, 62.1 and 36.7 percent of patients consumed vitamin A, thiamin, riboflavin, niacin, and vitamin C, respectively, at less than RDA levels. Most of the patients consumed minerals at less than RDA levels. Calcium, iron and zinc were consumed at less than recommended levels by 88.2, 75.8 and 89.1 percent of patients, respectively. There was no significant difference in the consumption of vitamins and minerals between the remission and active disease groups.

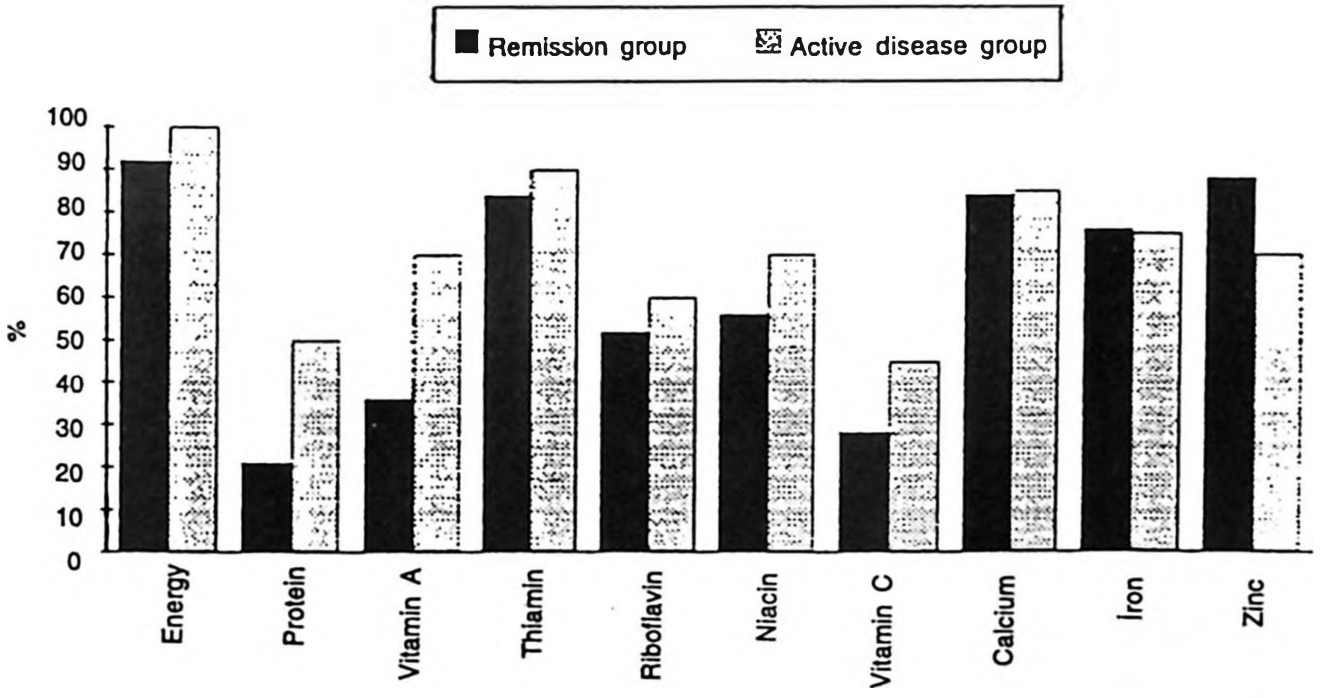

Fig. 1: The percentage of patients who consumed energy, protein, vitamins and minerals at lower than recommended levels in the remission and active disease groups.

Micro and macronutrients were consumed ineffectively by various tumor groups. No significant difference was found in the daily consumption of nutrients between groups.

The weight and height percentiles of our patients varied from the 3rd to the 97th percentile of NCHS growth charts. According to the weight-for-height index, 23 children (51.1%) were determined to be malnourished. Thirteen children (28.9%) displayed advanced malnutrition, five (11.1%) moderate malnutrition and five (11.1%) mild malnutrition. Twenty-two (48.9%) of these children had a normal nutritional status. Fifty-six percent of the children in remission were normal in nutritional status, whereas 44 percent of them had malnutrition (12% mild, 8% moderate, and 24% advanced). In the active disease group, 40 percent were normal in nutritional status, while 60 percent of them had malnutrition (10% mild, 15% moderate, and 35% advanced). The frequency of malnutrition in the active disease group was not significantly different from that of the remission group. Children with advanced malnutrition consumed 30 percent of the energy recommended, whereas children with mild malnutrition consumed 49.3 percent.

Children with malnutrition consumed significantly less energy and protein compared to children with normal nutritional status. However; the children with normal nutritional status according to anthropometric measurements consumed 52.7 percent of the energy recommended.

The biochemical measurements of pediatric cancer patients are summarized in Table V. Serum albumin levels of all children were greater than 3.5 g/dl. Serum albumin levels of patients in the active disease group were found to be significanty lower than those in the remission group ($p < 0.05$).

Table V: Biochemical Measurements in Pediatric Cancer Patients

Biochemical Measurements	Remission Group (n = 25)	Active Disease Group (n = 20)	Total (n = 45)
Creatinine (mg/dl)	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
Albumin (g/dl)	4.7 ± 0.6	4.3 ± 0.6*	4.5 ± 0.6
Transferrin (mg/dl)	253 ± 87	255 ± 68	254 ± 78
Prealbumin (mg/dl)	19.4 ± 7.2*	14.8 ± 5.1*	16.8 ± 5.3
Relative prealbumin (%)	74.3 ± 29.1*	58.1 ± 23.3*	63.0 ± 19.9

Relative prealbumin = observed prealbumin/expected prealbumin x 100; excepted prealbumin values for age and sex are those of Bevegna et al.[13].

* The means of the active disease group were significantly different from those of the remission group for prealbumin and relative prealbumin according to Student's t test ($p < 0.05$).

Seventy-three percent of the patients in the study group had relative prealbumin values below 80 percent of the expected vlaues for age and sex. Relative prealbumin values were found to be low in 63.6 percent nonmalnourished children, 80 percent of those with mild malnutrition, 80 percent of those with

moderate malnutrition and 92.3 percent of patients with advanced malnutrition, according to the weight-height index (Fig. 2). Relative prealbumin values were significantly higher in patients who/had received prednisone (75.2 ± 28.1 mg/dl) compared to those who had not (65 ± 25.3 mg/dl).

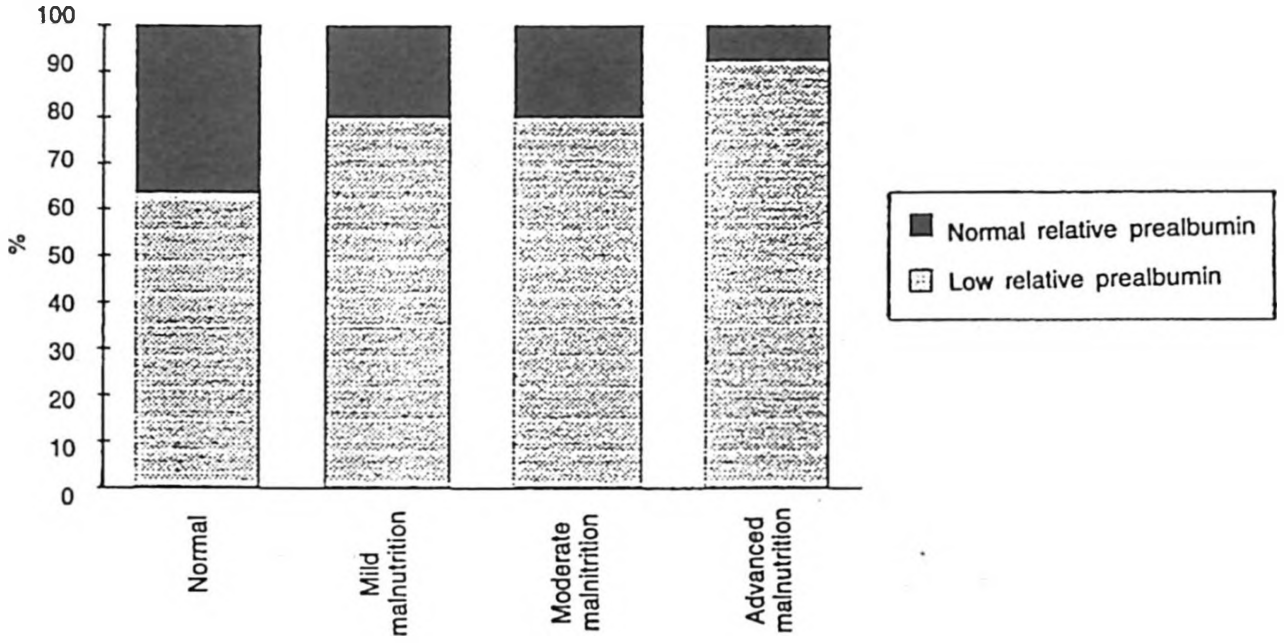

Fig. 2: The percentage of patients whose relative prealbumin values were less than 80 percent of the recommended values for age and sex.

Discussion

In developed countries it has been estimated that malnutrition is seen in eight to 32 percent of pediatric cancer patients[14]. In Turkey there are many problems related to educational status, traditional nutritional habits of families and environmental conditions. Therefore, malnutrition continues to be a major problem in Turkey, although its frequency is decreasing[15].

Especially with chronic diseases such as cancer, the risk of malnutrition is increasing. In this study, malnutrition according to the weight for height index was determined in 51.1 percent of pediatric cancer patients, a higher ratio than that in developed countries.

Malnutrition has been diagnosed by biochemical and anthropometric measurements in previous studies[2, 8, 9]. Some researchers have reported that weight-for-height ratios are the most reliable measurement of nutritional status in pediatric cancer patients, whereas others have suggested that these ratios may underestimate malnutrition[16-18]. Serum albumin levels of less than 3.2 mg/dl have also been identified as a threshold to warrant malnutritional intervention, although some studies report that low serum albumin levels may reflect an acute

metabolic response to fever and infection rather than true depletion of body mass[12]. Although in our study group malnutrition was common, as determined by weight-for-height ratios, serum albumin levels of patients were found to be normal. However, serum albumin levels were lower in the active disease group than in the remission group. These results suggested that albumin is not a reliable indicator in the early diagnosis of malnutrition in pediatric cancer patients, but may reflect acute metabolic response to active disease.

There is controversy about transferrin as an index of malnutrition. Some studies report transferrin as an index of malnutrition, while others state that it is not a reliable indicator[19,20]. Seventeen percent of our malnourished patients had low transferrin levels.

Prealbumin is considered to be a sensitive indicator of protein energy malnutrition, also reflecting short-term changes in nutritional status[19,21]. Changes in prealbumin levels are reported to occur at an earlier stage than changes in anthropometric measurements[19]. Yu et al.[5] reported that prealbumin was the most sensitive indicator of visceral protein status in leukemic patients. In our study group, 80 percent of patients with mild malnutrition had low relative prealbumin values, verifying that prealbumin is a reliable and sensitive indicator of mild and marginal malnutrition. According to anthropometric measurements, 69.6 percent of patients with normal nutritional status had low prealbumin values. This finding suggests that prealbumin may be found low before malnutrition is detected by anthropometric measurements, and low prealbumin values may indicate the need for nutritional intervention. Children in the active disease group had lower prealbumin values than children in the remission group, suggesting that the disease activity may affect the nutritional state of children with malignancy.

Prealbumin is affected by any inflammation or infection. In this study, prealbumin levels could not have been affected since infection was not observed in any of the patients.

It has been reported that prednisone increases the hepatic synthesis of prealbumin and diminishes its fractional metabolism, thus increasing prealbumin levels[22]. However, Yu et al.[5] found that serum prealbumin levels in children with leukemia receiving prednisone were higher than in patients not receiving prednisone, and suggested that increased prealbumin levels were due to better caloric and protein intake, since prednisone usually improves appetite. We also observed that prealbumin levels in children receiving prednisone were significantly higher than in those not receiving.

Nutritional support after malnutrition has occurred is often insufficient, as it is usually difficult to correct the deficiency. Therefore, it is important to prevent malnutrition before it occurs. We observed that patients with normal nutritional

status consumed only 52.7 percent of the recommended energy. This observation suggests that nutritional deficiency occurred in our patients before malnutrition was detected by anthropometric measures. Determining prealbumin values and assessing the deficiency of micro-and macronutrients before malnutrition is detected by anthropometric measures, may give a warning that nutritional problems are likely, allowing earlier nutritional intervention in pediatric cancer patients.

REFERENCES

1. Mauer AM, Burgess JB, Donaldson SS, et al. Special nutritional needs of children with malignancies: a review. J Parenter Enteral Nutr 1990; 14: 315-324.
2. Rickard KA, Grosfeld JL, Kirksey A, Ballantine TVN, Bacher RL. Reversal of protein-energy malnutrition in children during treatment of advanced neoplastic disease. Ann Surg 1979; 190: 771-781.
3. Saarinen UM, Koskelo EK, Teppo AM, Siimes MA. Tumor necrosis factor in children with malignancies. Cancer Res 1990; 50: 592-595.
4. Tyc VL, Vallelunga L, Mahoney S, Smith BF, Mulhern RK. Nutritional and treatment-related characteristics of pediatric oncology patients referred or not referred for nutritional support. Med Pediatr Oncol 1995; 25: 379-388.
5. Yu LC, Kuvibidila S, Ducos R, Warrier RP. Nutritional status of children with leukemia. Med Pediatr Oncol 1994; 22: 73-77.
6. Singher L, Lukens JN, Ablin AR. Nutritional support. In: Ablin AR (ed). Supportive Care of Children with Cancer. Baltimore: Johns Hopkins University Press; 1993: 107-113.
7. Rickard KA, Coates TD, Grosfeld JL, Weetman RM, Baehner RL. The value of nutrition support in children with cancer. Cancer 1986; 58: 1904-1910.
8. Harvey BK, Bothe J, Blackburn GL. Nutritional assessment and patient outcome during oncological therapy. Cancer 1979; 43: 2065-2069.
9. Gumey JM, Jelliffe DB. Arm anthropometry in nutritional assessment: nomogram for rapid calculation of muscle and fat areas. Am J Clin Nutr 1973; 26: 912-915.
10. Monsen ER. The 10th edition of the Recommended Dietary Allowances: what's new in the 1989 RDAs? J Am Diet Assoc 1989; 89: 1748-1752.
11. Hamill PW, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM. Physical growth: national Center for Health Statistics percentiles. Am J Clin Nutr 1979; 32: 607-629.
12. Haider M, Haider SQ. Assessment of protein-calorie malnutrition. Clin Chem 1984; 30: 1286-1299.
13. Bevenga S, Deluca F, Vermiglio F, Pandullo E, Bartalena L, Trimarchi F. Changes in radioimmunoassayable prealbumin (TBPA) serum levels from birth to adulthood. Horm Metab Res 1986; 18: 73.
14. Coates TD, Rickard KA, Grosfeld JL, Weetman RM. Nutritional support of children with neoplastic diseases. Surg Clin North Am 1986; 66: 1197-1212.
15. Egemen A, Merdal T, Şensoy F. Malnutrition prevalence trends in Çubuk. XXI. Congress of UMEMPS, 24-27 October, İzmir, 1993.
16. Smith DE, Stevens MC, Booth IW. Malnutrition at diagnosis of malignancy in childhood: common but mostly missed. Eur J Pediatr 1991; 150: 318-322.
17. Carter P, Carr D, van Eys J, Coody D. Nutritional parameters in children with cancer. J Am Diet Assoc 1983; 82: 616-622.

18. Merritt RJ, Kalsch M, Roux LD, Ashley-Mills J, Siegel SS. Significance of hypoalbuminemia in pediatric oncology patients-malnutrition or infection? J Parenter Enteral Nutr 1985; 9: 303-306.

19. Polberger SK, Fex GA, Axelsson IE, Raiha NC. Eleven plasma proteins as indicators of protein nutritional status in very low birth weight infants. Pediatrics 1990; 86: 916-921.

20. Georgieff MK, Amaranth UM, Murphy EL, Ophoven JJ. Serum transferrin levels in the longitudinal assessment of protein-energy status in preterm infants. J Pediatr Gastroenterol Nutr 1989; 8: 234-239.

21. Yoder MC, Anderson DC, Gopalakrishna GS, et al. Comparison of serum fibronectin, prealbumin, and albumin concentrations during nutritional repletion in protein-calorie malnourished infants. J Pediatr Gastroenterol Nutr 1987; 6: 84-88.

22. Openheimer JH, Werner SC. Effect of prednisone on thyroxin-binding proteins. J Clin Endocr 1966; 26: 715-721.

The Turkish Journal of Pediatrics 1997; 39: 335-339

RENAL FUNCTION IN CHILDREN WITH HYPERCALCIURIA*

*Neslihan Tekin MD**, Nurdan Kural MD***, Mübeyyen Torun MD****

SUMMARY: Tekin N, Kural N, Torun M. (Department of Pediatrics, Osmangazi University Faculty of Medicine, Eskişehir, Turkey). Renal function in children with hypercalciuria. Turk J Pediatr 1997; 39: 335-339.

Hypercalciuria is a common problem causing symptoms such as abdominal pain, hematuria and enuresis, and leading to stone formation. It results from a renal tubular calcium "leak" or intestinal hyper-reabsorption of calcium. This study was performed to determine whether renal functional impairment was present in children with hypercalciuria. The study group comprised 298 children who were screened for hypercalciuria by means of urinary calcium/creatinine (UCa/UCr) ratio. The renal functions of 18 children (6.4%) detected as having hypercalciuria with Ca/Cr ratios of greater than 0.18 in their spot urines were evaluated. Results were compared with those of the healthy control group. The rate of hypercalciuria did not vary significantly between the boys and girls ($p > 0.05$). The mean value of daily calcium excretion was 6.42 + 3.93 mg/kg/day in the children with hypercalciuria, which was significantly different from that of the control group ($p < 0.01$). When the values of creatinine, osmolar and free water clearances, fractional excretion of sodium and tubular reabsorption of phosphorus were compared between the patient and control groups, the difference was not significant ($p > 0.05$). Urinary N-acetyl-β-D-glucosaminidase (NAG) excretion, which was described as the creatinine ratio, was significantly higher in the children with hypercalciuria. These findings suggest that in the presence of normal renal functional studies in children with hypercalciuria, tubular injury can be detected by NAG, which is a more sensitive marker of renal tubular injury. *Key words: hypercalciuria, renal function, N-acetyl-β-D-glucosaminidase.*

Hypercalciuria is more common in children than adults and causes symptoms such as hematuria, dysuria, enuresis, colic and growth failure and leads to urolithiasis[1,2]. The incidence of asymptomatic idiopathic hypercalciuria has been reported as 2.2-6.4 percent among children[3,4]. Idiopathic hypercalciuria was found in 30 percent of patients who peresented with hematuria and five to seven percent of patients who had urolithiasis[3-5]. Hypercalciuria can be due to impaired renal reabsorption of calcium or excessive gastrointestinal absorption of dietary calcium. In childhood, renal tubular reabsorption of calcium is more likely to be impaired, as reported previously[6]. Previous studies in adult patients with renal hypercalciuria have described various defects in either proximal or distal renal tubular function. In most previous pediatric studies, renal and absorptive hypercalciuria were not distinguished; thus, renal tubular defects associated with a tubular abnormality in renal hypercalciuria may not have been apparent. In

* From the Department of Pediatrics, Osmangazi University Faculty of Medicine, Eskişehir.

** Assistant Professor of Pediatrics, Osmangazi University Faculty of Medicine.

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

**** Pediatrician, Manavgat, Antalya.

this study we evaluated renal function in a group of 18 children with idiopathic hypercalciuria. The proximal tubulus was also evaluated by means of N-acetyl-β-D-glucosaminidase (NAG), a lysosomal enzyme which is present in high concentrations in renal tubular cells[7]. The results were compared with those of the control group.

Material and Methods

The study group comprised 298 children (220 boys and 78 girls) between four and 17 years in an orphanage. All children were screened for hypercalciuria, and those whose urinary calcium/creatinine ratios (U_{Ca}/U_{Cr}) were below 0.18 in their spot urines were excluded. A 24-hour urine specimen was collected for the determination of daily calcium excretion in the goup whose urine was examined twice and U_{Ca}/U_{Cr} values were found to be greater than 0.18. Eighteen children with urine calcium values greater than 4 mg/kg/day were included in the study, while 21 healthy children (13 girls and 8 boys) were taken as the control group. All the children were questioned regarding the presence of abdominal pain, dysuria, hematuria, enuresis and increased frequency of urination as well as any drug use. Physical examinations were done and the patients' weights, heights and blood pressures were recorded. Blood calcium (Ca), phosphorus (P), alkaline phosphatase, blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), potassium (K) and osmolarities were determined in both groups. A 24-hour urine specimen was collected for Na, Ca, Cr, P and NAG. Clearances of Cr (C_{Cr}), fractional excretion of sodium (FE_{Na}), tubular reabsorption of phosphorus (TRP), and clearances of potassium (C_K), osmolarity (C_{osm}), and free water (C_{H2O}) were calculated. Urinary NAG activity was determined spectrophotometrically[8]. The enzyme activity in each urine sample was expressed as the NAG U/mmol creatinine. NAG excretion was determined as U_{NAG}/U_{Cr} mmol. U_{Ca}/U_{Cr} urinary sodium/creatinine ratios (U_{Na}/U_{Cr}) were determined from fasting urines. Urinalysis was performed in all of the children for density, pH, proteinuria and microscopic examination. Urine cultures were taken. Direct roentgenogram of the abdomen was evaluated for calculi and bone density.

Statistical analyses were performed using Student's t test. The values are reported as mean ± SE.

Results

Twelve boys (5.5%) and six girls (7.7%) comprised the study group, which consisted of those 18 children (6.0%) who were detected as having hypercalciuria with U_{Ca}/U_{Cr} values of greater than 0.18 in two fasting urines, and daily Ca excretion of greater than 4 mg/kg. The rate of hypercalciuria was similar among boys and girls ($p > 0.05$, Table I). Eight children (44.4%) had abdominal and/

or groin pain, while two children (11.1%) had enuresis nocturna, and four children (2 boys, 2 girls) had hematuria. No pathogenic microorganism was detected in their urine cultures. Biochemical analyses of blood for BUN, Cr, Na, K, Ca, P and alkaline phosphatase were within normal limits, and when compared with the control group the difference was not statistically significant ($p > 0.05$). The mean value of U_{Ca}/U_{Cr} was 0.34 ± 0.20 in the fasting urines and 0.25 ± 0.06 mg/mg in the patients' second urines; when compared with the U_{Ca}/U_{Cr} values of the control group (0.12 ± 0.04 mg/mg) the difference was statistically significant ($p < 0.01$, Table II). Ca excretion in the 24-hour collected urine in the study group (6.42 ± 3.93 mg/kg/day) was higher than in the control group (1.92 ± 0.78), and the difference was significant ($p < 0.001$). Clearances of creatinine, osmolar and free water as well as values of FE_{Na} and TRP were normal, and there was no significant difference between the two groups. The U_{Na}/U_{Cr} ratio was not different between the two groups ($p > 0.05$). U_{NAG}/U_{Cr} values were higher in the study group (242.86 ± 82.74) than the control group (133.15 ± 98.69), and the difference was statistically significant ($p < 0.001$, Table II). Roentgenograms of the abdomen and bone densities were normal.

Table I: The Rate of Hypercalciuria Among Girls and Boys

Sex	Hyercalciuria (+)	%	Hyercalciuria (-)	%	Total
Female	6	7.69	72	92.31	78
Male	12	5.45	208	94.55	220

Table II: Renal Function of the Patients and the Control Group

Renal Function	Patients	Control	p
U_{Ca}/U_{Cr}	0.25 ± 0.06	0.12 ± 0.04	< 0.01
U_{Ca}/U_{Na}	0.21 ± 0.39	0.04 ± 0.02	< 0.001
U_{Na}/U_{Cr}	2.49 ± 1.83	2.72 ± 1.66	> 0.05
C_{Cr}	87.39 ± 8.01	98.05 ± 12.75	> 0.05
FE_{Na}	1.39 ± 1.08	1.16 ± 0.62	> 0.05
TRP	92.53 ± 2.54	95.47 ± 2.75	> 0.05
C_{osm}	2.37 ± 1.23	3.16 ± 1.01	> 0.05
C_{H20}	-1.67 ± 1.01	-1.87 ± 1.10	> 0.05
U_{NAG}/U_{Cr}	242.8 ± 82.74	133.15 ± 98.69	< 0.001

Discussion

The long morbidity associated with hypercalciuria is due to the development of renal stones[9]. This requires clinicians both to attempt to prevent it and to identify those children already affected at presentation. The incidence of

hypercalciuria varies between 2.2 and 6.0 percent in different countries[3, 4]. Turkey ranks among the countries with a high incidence of urolithiasis. The incidence was found to be 4.2 percent in a study done in Ankara[10]. In our study the rate of hypercalciuria was 6.0 percent among 298 children, with 7.7 percent of girls and 5.5 percent of boys affected. In the literature there are studies demonstrating the incidence of hypercalciuria to be higher in males[5, 11], while in other series no difference was found between the two sexes[9]. In some studies an autosomal dominant inheritance of hypercalciuria has been demonstrated[12, 13]. Because our study was performed in an orphanage, we could not interpret the inheritance, but there are two siblings among our patients.

Renal tubular reabsorption of calcium is more likely to be impaired in childhood[11, 14]. Because the U_{Ca}/U_{Cr} values of the fasting urines of 18 children were greater than 0.18, the absorptive type of hypercalciuria was eliminated. In our study serum levels of calcium, phosphorus and alkaline phosphatase were within normal limits, and there was no statistically significant difference between the study and control groups; moreover, radiological evaluation of bone mineralization was normal. For that reason renal hypercalciuria was considered to be more likely.

Renal tubular transport of calcium is an energy-requiring process and Ca-Mg-ATP-ase dependent. More than 50 percent of reabsorption takes place in the proximal tubulus, parathyroid hormone while (PTH) increases Ca reabsorption in the distal segments of the tubulus[6]. Recently proximal and distal tubulus damage due to prolonged crytalluria has been reported in adults[15]. On the other hand, Stapleton et al.[6] evaluated the renal functions of 10 children with hypercalciuria and detected impairment in the concentration ability of the kidney. In our study, the values of C_{Cr}, FE_{Na}, TRP, C_{osm} and C_{H20} were normal and statistically similar to the control group values. Because the children with hypercalciuria were selected as the study group, U_{Ca}/U_{Cr} values were higher than among controls. In a previous study, dysfunctional proximal renal tubular sodium transport was suggested in idiopathic hypercalciuria (IH), but dietary sodium was not controlled in that study[16]. Stapleton and Miller[6] restricted sodium in their study group with IH and pointed out that daily sodium excretion closely approximated dietary sodium intake. In our study, values of FE_{Na} and U_{Na}/U_{Cr} were not different from those of the control group.

NAG is a lysosomal enzyme with a molecular weight that precludes its filtration at the glomerulus, and urinary NAG excretion has been found to be a sensitive marker of renal tubular injury[17]. It has been reported that increased excretion of calcium causes renal damage by way of microcalculi focuses and leads to renal tubular injury. For that reason we studied urinary NAG excretion in both the study and control groups. U_{NAG}/U_{Cr} values of the children with hypercalciuria were much

higher than in the control group ($p < 0.001$), as was reported in previous studies[16, 17]. In conclusion, in our study tubular dysfunction was confirmed by means of NAG, which was grossly abnormal in the group with hypercalciuria.

REFERENCES

1. Stapleton FB, Noe HN, Roy S, 3d, Jerkins G. Hypercalciuria in children with urolithiasis. Am J Dis Child 1982; 136: 675-678.
2. Roy S, 3d, Stapleton FB, Noe HN, Jerkins G. Hematuria preceding renal calculus formation in children with hypercalciuria. J Pediatr 1981; 99: 712-715.
3. Moore ES, Coe FI, McMann G, Favus MJ. Idiopathic hypercalciuria in children: prevalence and metabolic characteristics. J Pediatr 1978; 92: 906-910.
4. Coe FL, Canterburry JM, Firpo JJ. Evidence for hyperparathyroidism in idiopathic hypercalciuria. J Clin Invest 1973; 52: 134-142.
5. Stapleton FB, Roy S 3d, Noe HN, Jerkins G. Hypercalciuria in children with hematuria. N Engl J Med 1984; 310: 1345-1348.
6. Stapleton FB, Miller LA. Renal function in children with idiopathic hypercalciuria. Pediatr Nephrol 1988; 2: 229-235.
7. Kunin CM, Chesney RW, Craig WA, England AC, De Angelis C. Enzymuria as a marker of renal injury and disease: studies on N-acetyl-β-D-glucosaminidase in the general population and in patients with renal disease. Pediatrics 1978; 62: 751-760.
8. Horak E, Hopfer MS, Sundermann WF. Spectrophotometric assay for urinary N-acetyl-β-D-glucosamiindase activity. Clin Chem 1983; 29: 1717-1719.
9. Effect of hydrochlorothiazide on renal hypercalciuria. Child Nephrol Urol 1992; 12: 6-9.
10. Buyan N, Saatçi Ü, Bakkaloğlu A, Beşbaş N. Okul çocuklarında asemptomatik hiperkalsiüri: epidemiyoloji ve patogenez. Çocuk Sağlığı ve Hastalıkları Dergisi 1989; 32: 43-50.
11. Langman CB, Moore ES. Hypercalciuria in clinical pediatrics. A review. Clin Pediatr (Phila) 1984; 23: 135-137.
12. Coe FL, Parks JH, Moore ES. Familial idiopathic hypercalciuria. N Engl J Med 1979; 300: 337-340.
13. Buyan N, Saatçi Ü, Bakkaloğlu A, Beşbaş N. Familial idiopathic hypercalciuria. Turk J Pediatr 1988; 30: 145-151.
14. Carvera A, Corral FJ, Gomez C. Idiopathic hypercalciuria in children: classification, clinical manifestations and outcome. Acta Paediatr Scand 1987; 76: 271-278.
15. Lemann J Jr, Adams ND, Oray RW. Urinary calcium excretion in human beings. New Engl J Med 1979; 301: 535-541.
16. Aladjem M, Modan M, Lusky A, et al. Idiopathic hypercalciuria: a familial generalized renal hyperexcretory state. Kidney Int 1983; 24: 549-554.
17. Stapleton FB, Chesney RW, Behrmann AT, Miller LS. Increased urinary excretion of renal N-acetyl beta glucosaminidase in hypercalciuria. Am J Dis Child 1985; 139: 950-95.

The Turkish Journal of Pediatrics 1997; 39: 341-345

INTRAVENOUS IMMUNOGLOBULIN FOR SEPSIS PREVENTION IN PRETERM INFANTS*

*Fatoş Tanzer MD***, *Nursel Yazar MD****, *Yahya Hakgüdener PhD*****
*Gülden Kafalı MD******

SUMMARY: Tanzer F, Yazar N, Hakgüdener Y, Kafalı G. (Department of Pediatrics, Cumhuriyet University Faculty of Medicine, Sivas, Turkey). Intravenous immunoglobulin for sepsis prevention in preterm infants. Turk J Pediatr 1997; 39: 341-345.

The aim of this study was to investigate whether intravenous immunoglobulin (IVIG) can prevent sepsis in premature newborn infants. The study group consisted of 80 preterm newborn infants, who were divided into two groups: 40 preterm newborns received IVIG prophylactically (group A) and the control group (group B, n = 40) did not receive IVIG. IVIG was given at a dose of 500 mg/kg to infants. weighing greater than 1500 g, and 700 mg/kg to those weighing less than 1500 g at birth on days one, two and eight of life.

By two, eight and 12 days of age, the treatment group had significantly greater IgG concentrations than the control group. Mortality was 7.5 percent (3/40) in group A and 27.5 percent (11/40) in group B ($p < 0.01$). Bacteremia was determined in three blood cultures in group A and eight in group B, particularly S.aureus and S.enteritis.
Key words: immunoglobulin, preterm infants, sepsis.

As a result of inadequate placental transport of maternal IgG, preterm neonates are more susceptible to severe infections than are term neonates[1]. In recent years, intravenously administered immunglobulin (IVIG) has been used for the prevention or treatment of neonatal sepsis[2-5].

The present study was conducted in Sivas, an eastern province of Turkey where socioeconomic conditions are unsatisfactory, and undernourished mothers have frequent pregnancies. The aim of this study was to investigate whether IVIG prevents sepsis in premature newborn infants.

Material and Methods

Eighty preterm newborn infants were included in this study and were divided into two groups on the basis of order of admission; the treatment group received IVIG (group A, n = 40) prophylactically and the control group did not receive IVIG (group B, n = 40).

* From the Department of Pediatrics, Cumhuriyet University Faculty of Medicine, Sivas.
** Professor of Pediatrics, Cumhuriyet University Faculty of Medicine.
*** Pediatrician, Ereğli.
**** Professor of Microbiology, Cumhuriyet University Faculty of Medicine.
***** Assistant Professor of Pediatrics, Cumhuriyet University Faculty of Medicine.

Conditions at birth that precluded enrollment of infants in the study included multiple life-threatening congenital anomalies, maternal infection and cardiopulmonary arrest. IVIG (Sandoglobulin R) was given at a dose of 500 mg/kg to infants weighing greater than 1500 g, and 700 mg/kg to those weighing less than 1500 g at birth on days one, two and eight of life. Blood samples were obtained to monitor IgG concentrations before every infusion of immunoglobulin and at 12 days of age. Serum IgG concentrations were identified by Behring Nor-Partigen (R) IgG HC radial immunodiffusion plates.

On the first day of life, the following laboratory eaminations were performed: cultures, of blood, stool, throat and the umbilicus; white blood cell count, band/total neutrophil ratio, platelet count and C-reactive protein. The cases having a positive blood culture were classified as "proved sepsis" and the others as "suspected sepsis". These examinations were repeated if sepsis was suspected[6].

Student's and Chi Square tests were used in the statistical analysis[7].

Results

Clinical features of the two groups are shown in Table I. The sex distribution, gestational age, weight, Apgar score and antibacterial treatment were identical in the two groups. Serum IgG concentrations are shown in Table II. The mean IgG concentrations on the first day of life were not significantly different between the two groups ($p > 0.01$). By two, eight and 12 days of age, the treatment group had significantly greater IgG concentrations than the control group. Sepsis developed in 12.5 percent (5/40) of cases in group A (3 proved, 2 suspected sepsis), and 40 percent (16/40) in group B (8 proved, 8 suspected) ($p < 0.02$, Table III). Bacteremia was determined in three blood cultures in group A and eight in group B, particularly S. aureus and S. enteritis (Table IV). The sepsis-related mortality rate was 7.5 percent (3/40) in group A and 27.5 percent (11/40) in group B ($p < 0.02$, Table V). The length of hospitalization was 8.32 ± 1.17 days in group B ($p < 0.05$).

No adverse effect related to IVIG administration in these neonates was observed.

Table I: Clinical Features in Treated and Control Infants

	Group A (IVIG)	Group B (No IVIG)	P
Gestational age	36.18 ± 0.17	35.58 ± 0.19	> 0.05
Birth weight (grams)	1.85 ± 0.07	1.67 ± 0.07	> 0.05
Mean Apgar score at 5 minutes	8.08 ± 0.17	7.83 ± 0.19	> 0.05
Sex (M/F)	24/16	18/22	
Use of antibiotic (+)	36 (90%)	37 (92.5%)	
Use of antibiotic (-)	4 (10%)	3 (7.5%)	

Table II: Serum IgG Concentrations

Days	Serum IgG (mg/dl) Group A (IVIG) n: 40 $\bar{X} \pm$ SD	Group B No (IVIG) n: 40 $\bar{X} \pm$ SD	P
1	775.54 ± 51.48	679.96 ± 49.88	> 0.01
2	1211.86 ± 75	742.31 ± 49.95	< 0.01
8	1075 ± 85.60	713.10 ± 45.11	< 0.01
12	1039.32 ± 37.10	744.51 ± 33.29	< 0.01

Table III: Sepsis in Each Group

	Group A (IVIG) n: 40	Group B (No IVIG) n: 40	X^2	P
Proved sepsis	3	8	3.84	p = 0.0501
Suspected sepsis	2	8	5.31	p = 0.020
Total sepsis	5	16	7.92	p = 0.0190

Table IV: Organisms Isolated from Blood the in Two Groups

Organism Isolated	Blood Culture Group A (IVIG)	Group B (No IVIG)
S.enteritis	2	–
S.aureus	–	3
E.coli	1	1
E.cloacea	–	2
Pseudomonas species	–	1
Corynobacterium species	–	1

Table V: Mortality Rate in Each Group

Group	Number	%
A (IVIG)	3	7.5
B (No IVIG)	11	27.5
$X^2 = 5.54$	$p < 0.02$	

Discussion

Infections are one of the most important problems of the newborn, particularly preterm infants. Preterm infants have a lack of IgG, because transplacental transport of IgG occurs predominantly in the latter half of the third trimester of pregnancy[1]. Besides, preterm infants may have certain defects in some steps of cellular and humoral immunity. For this reason, the incidence of sepsis is approximately 1/250 in preterms as compared to 1/1000 in term infants[8]. Therapy

with polivalent immunoglobulin concentrates has been used successfully. Sidiropoulos et al.[4,5] and Lindquists et al.[9] have shown a significant reduction in mortality using intravenous immunoglobulin for the treatment of sepsis.

Haque et al.[10] were the first investigators to use IVIG as a prophylactic agent. Their sepsis incidence was four percent (4/100) in newborns weighing less than 1500 g and 12 percent (6/50) in the control group. In a study by Chirico et al.[11], the incidence rates were seven percent (3/43) and 20 percent (8/40); Bussel et al.[12] found rates of 15 percent (9/61) and 25 percent (16/65), respectively.

In our study, the sepsis incidence in newborns was 12.5 percent (5/40) in preterm infants given IVIG, and 40 percent (16/40) among preterm newborn controls.

Clapp et al.[13] observed that no case of sepsis occurred in a study of 56 preterm infants administered IVIG compared with the control group, who had an incidence of 12 percent. The rate found by Baker[14] (46%) in controls is close to our result (40%), but Baker[14] found the sepsis incidence to be 34 percent with IVIG administration.

There have been other studies showing that the incidence of sepsis did not change when IVIG was administered[15]. Conway et al.[16] put forth that IVIG administered at a dose of 200 mg/kg might be insufficient for prophylaxis. The insufficiency of prophylaxis in the Weisman et al.'s[17] study may be due to the one-dose application of IVIG.

Kyllonen et al[18] stated that establishment of the dosage needed to reach target (700 mg/dl) levels would be useful. Our data demonstrated a reduced incidence of bacterial infection in the IVIG-treated group maintained at the serum IgG concentration of 700 mg/dl and greater (Table II). This is in accordance with Kyllonen's study.

Most of the studies show that IVIG administered in appropriate dosages to preterm and low-birth-weight babies is useful in preventing the occurrence of sepsis. In our study we showed a significant difference in the sepsis and mortality rates between the group treated with immunoglobulin (group A) and the group that remained untreated (Tables III-V). This finding corroborates that of previous studies.

The role of antibiotics in infections is undoubtedly important. In our study antibiotics were used in both group A + B, in 36 and 37 patients, respectively. The administration of antibiotics with IVIG was shown to lower the sepsis and mortality rates. In our study, microorganisms were detected in the blood culture of three patients in group A and eight patients in group B. Of the gram-positives, Staphylococcus aureus was predominant among the gram-positives, and the Salmonella species among the gram-negatives. Another important point is the short length of the hospital stay when IVIG was administered ($p < 0.05$).

We conclude that IVIG replacement therapy at an optimum dosage and longer intervals is both safe and effective for the prophylaxis of infection in high-risk, preterm, very-low-birth-weight infants with low levels of IgG.

REFERENCES

1. Wilson CB. Developmental immunology and the role of host defenses in neonatal susceptibility. In: Remington JS, Klein JO (eds). Infectious Diseases of The Fetus and Newborn Infant (3rd ed). Philadelphia: Saunders Co.; 1990; 17-66.
2. Fischer GW, Weisman LB, Hemming VG, et al. Intravenous immunoglobulins in neonatal GBS disease. Pharmakokinetic and safety studies in monkeys and humans. Am J Med 1984; 76: 117-123.
3. Sidiropoulos D, Herrmann U Jr, Morell A, et al. Transplacental passage of intravenous immunoglobulin in the last trimester of pregnancy. J Pediatr 1986; 109: 505-508.
4. Sidiropoulos D, Boehme U, von Muralt G, et al. Immunoglobulin substitution in the treatment of neonatal sepsis. Schweiz Med Wochenschr 1981; 111: 1649-1655.
5. Sidiropoulos D, Boehme U, Von Muralt G, et al. Immunoglobulin supplementation in prevention or treatment of neonatal sepsis. Pediatr Infect Dis J 1986; 5S: 193-194.
6. Töllner U. Early diagnosis of septicemia in the newborn. Clinical studies and sepsis score. Eur J Pediatr 1982; 138: 331-337.
7. Dawson C, Saunders B, Trapp RG. Basic and Clinical Bioistatistics. Norwalk CT: Appleton and Lange; 1990; 111-123.
8. Burgio GR. IVIG can save many preterm infants. In: Imbach P (ed). Immunotherapy with Intravenous Immunoglobulins. Proceedings of a conference held at Interlaken (Switzerland), May 6-9, 1990. London: Academic Press; 1991: 59-64.
9. Lindquist L, Lundenberg P, Massing R. Pepsin-treated human gamma globulin in bacterial infections. Vox Sang 1981; 40: 329-337.
10. Haque KN, Zaidi MH, Haque SK, et al. Intravenous immunoglobulin for prevention of sepsis in preterm and low birth weight infants. Pediatr Infect Dis 1986; 5: 622-625.
11. Chirico G, Rondini G, Plebani A, et al. Intravenous gammaglobulin therapy for prophylaxis of infection in high-irsk neonates. J Pediatr 1987; 110: 437-442.
12. Bussel J, Gueliano M, La Gamma EF. Prophylaxis of neonatal sepsis with intravenous gammaglobulin. In: Morell A, Nydegger UE (eds). Clinical use of intravenous immunoglobulins. London: Academic Press; 1986: 171-178.
13. Clapp DW, Kliegman RM, Baley JE, et al. Use of intravenously administered immunoglobulin to prevent nosocomial sepsis in low birth weight infants: report of a pilot study. J Pediatr Infect Dis 1989; 115: 973-978.
14. Baker CJ. Neonatal sepsis: an overview. In: Imbach (ed). Immunotherapy with Intravenous Immunoglobulins. Proceedings of a conference Interlaken (Switzerland), May 6-9, 1990. London: Academic Press; 1991: 65-74.
15. Erdem G, Yurdakök M, Tekinalp G, Ersoy F. The use of IgM-enriched intravenous immunoglobulin for the treatment of neonatal sepsis in preterm infants. Turk J Pediatr 1993; 35: 277-281.
16. Conway SP, Ng PC, Howel D, Maclain B, Gooi HC. Prophylactic intravenous immunoglobulin in preterm infants: a controlled trial. Vox Sang 1990; 59: 6-11.
17. Weisman E, Stoll B, Kueser T, et al. Intravenous immunoglobulin (IVIG) prophylaxis of late-onset septicemia in neonates (abstract). Pediatr Res 1990; 27: 227A.
18. Kyllonen KS, Clapp DW, Kliegman RM, et al. Dosage of intravenously administered immuneglobulin and dosing interval required to maintain target levels of immunoglobulin G in low-birth-weight neonates. Rev Infect Dis J Pediatr 1989; 115: 1013-1016.

The Turkish Journal of Pediatrics 1997; 39: 347-352

IMMUNOGLOBULIN ISOTYPES AND IgG SUBCLASSES IN RECURRENT INFECTIONS*

*Yavuz Coşkun MD***, *Ziya Bayraktaroğlu MD****

SUMMARY: Coşkun Y, Bayraktaroğlu Z. (Department of Pediatrics, Gaziantep University Faculty of Medicine, Gaziantep, Turkey). Immunoglobulin isotypes and IgG subclasses in recurrent infections. Turk J Pediatr 1997; 39: 347-352.

The frequency of major immunoglobulin isotype and IgG subclass deficiencies among 74 children aged six months to 14 years with recurrent infections was studied. All children had at least five to six episodes of respiratory tract infections, while recurrent diarrhea had occurred in eight. Two selective and six partial IgA deficiencies were detected. IgG4, IgG3 and IgG2 deficiencies, either isolated or combined, were found in 13, nine and one patient respectively. Among these there were two combined deficiencies of IgA + IgG4, three of IgA + IgG3, one of IgA + IgG2 + IgG4, one of IgG1 + IgG3 and two of IgG1 + IgG4. There was one patient with panhypogammaglobulinemia. Our results, similar to those of other studies, showed that the occurrence of the Ig isotype, particularly subclass deficiencies, is not uncommon in children with frequent infections. *Key words: recurrent infections, Ig isotypes, IgG subclass.*

Recurrent infection is a relatively common problem in pediatric practice. Primary B-cell immunodeficiencies are associated with recurrent or chronic bacterial infections involving notably the respiratory tract and/or gastrointestinal system[1, 2]. The most common abnormality among primary B-cell immune deficiencies is selective IgA deficiency. The disease is characterized by its clinical variability associated with allergic, hematologic and autoimmune diseases, malignancies and other imunodeficiencies, and can be detected even in asymptomatic individuals[3, 4]. On the other hand, IgG subclass deficiencies account for recurrent infections in some children with or without major immunoglobulin deficiencies[5-9].

IgA deficiency is the most common one to be accompanied by IgG subclass deficiency[10, 11]. The purpose of the present study was to evaluate immunoglobulin isotypes and IgG subclasses in patients with recurrent sinopulmonary infections.

Material and Methods

Seventy-four patients, 40 boys (55%) and 34 girls (45%) between six months and 14 years in age (mean four years), who were admitted to the pediatric outpatient clinic between February 1994 and April 1995 were included in the study. Twenty-three out of 74 had a history of seven to eight episodes of upper and lower respiratory tract infections (URTI, LRTI) including sinusitis, otitis,

* From the Department of Pediatrics, Gaziantep University Faculty of Medicine, Gaziantep.

** Associate Professor of Pediatrics, Gaziantep University Faculty of Medicine.

*** Assistant Professor of Pediatrics, Gaziantep University Faculty of Medicine.

This study was supported by the Gaziantep University Research Fund (project: TF. 92.02).

tonsillitis, pharyngitis, laryngitis and pneumonia. Additionally, eight of these 23 patients had frequent diarrhea. Fifty-one patients out of 74 had a history of five to six episodes of URTI and LRTI. In this group seven patients also had a history of one of the following disorders: septic arthritis, abetalipoproteinemia, pulmonary tuberculosis, sepsis and asthma (in two).

All patients were tested for serum immunoglobulin levels and complete blood count (CBC). Major immunoglobulin classes (IgG, IgA, IgM) and IgG subclass levels were measured by radial immunodiffusion using the plates and reference sera of Behringwerke AG, Marburg, Germany and Binding Site Ltd, Birmingham, UK, respectively. When low concentrations were detected in the first measurement, it was repeated to confirm its accuracy. Immunodiffusion ring diameters were read on the electronic rid-reader (Binding Site Ltd, Birmingham, UK). The serum IgA concentrations below the limit of detection of standard immunodiffusion plates were retested by means of low level plates (Behringwerke AG, Marburg, Germany, LC partigen).

Age-stratified normal values for IgG, IgA, IgM, and IgG subclasses in healthy Turkish children were used as controls[12, 13]. This age-appropriate range was established in a healthy population, except for IgG[4]. Patients with serum IgA levels of less than 0.05 g/L, and serum IgA levels below two standard deviations (2SD) but above 0.05 g/L were accepted as selective and partial IgA deficiency, respectively.

For IgM, IgG and IgG subclass levels, patients who had levels below 2SD of normal values for his/her age were considered to be deficient. Patients with IgG4 concentrations equal to or below an arbitrarily chosen value of 0.03 g/L were accepted as deficient.

Results

The sum of IgG1, G2, G3 and G4 subclasses correlated well with the total IgG concentration (linear regression analysis, $r = 0.88$; $p < 0.001$). Various major immunoglobulin isotypes and/or IgG subclass deficiencies were found in 26 of 74 patients (35%) studied (Table I). One patient (no. 26) had panhypogammaglobulinemia. Low IgG levels were observed in two patients (nos. 24, 25); one had mainly decreased IgG1 and IgG3 concentrations, and the other decreased IgG1 and IgG4 levels.

Selective IgA deficiency occurred in two children (2.7%) combined with IgG3 deficiency in one and IgG4 deficiency in the other (nos: 1, 2). There were six patients with partial IgA deficiency (8%), three of whom also had IgG subclass deficiencies (nos: 3-8). Deficiency of IgG4 was found in 13 patients (17.5%); nine of these (nos: 14-22) were isolated. Two patients with IgG4 deficiency combined with IgG1, one with selective IgA deficiency and one with partial IgA + IgG2 deficiency (the only IgG2 deficiency found) were detected. There were nine IgG3-deficient patients (12%), five isolated (nos. 9-13) and four combined (nos. 1, 6, 7, 25) with IgA, IgG1 or IgG4. No lymphopenia or leukopenia was detected in any patient.

Table I: Clinical Features and Serum Immunoglobulin Levels (g/L) of Children with Immunoglobulin Deficiencies

Patient No.	Age (Months)	Clinical Presentation	Deficiency	IgA	IgM	IgG	IgG1	IgG2	IgG3	IgG4
1	42	RSP	S-IgA+G3	< 0.05	1.52	10.70	9.72	0.96	0.22**	0.21
2	36	RSP	S-IgA+G4	< 0.05	2.26	9.63	6.36	1.26	0.46	0.01x
3	32	RSP	P-IgA	0.17*	1.38	9.08	6.88	1.26	0.46	0.11
4	84	RSP	P-IgA	0.50*	1.60	12.50	9.42	1.80	0.41	0.57
5	108	RSP, Asthma	P-IgA	0.50*	0.77	8.55	5.37	1.92	0.88	0.27
6	20	RSP, ROM	P-IgA+G3	0.15*	1.99	8.02	6.36	1.16	0.24**	0.06
7	36	RSP, GE	P-IgA+G3	0.18*	1.84	10.70	8.83	0.67	0.28*	0.34
8	24	RSP	P-IgA+G2+G4	0.17*	0.89	7.00	5.37	0.22**	0.48	0.00x
9	24	RP	IgG3	1.09	1.45	10.70	7.15	1.26	0.26**	0.34
10	72	RSP, Giardias	IgG3	1.71	3.09	9.63	6.60	1.47	0.26**	0.31
11	36	RSP	IgG3	0.62	1.60	9.08	7.69	1.47	0.22**	0.16
12	168	RSP	IgG3	2.41	1.76	9.08	5.13	1.69	0.34**	0.06
13	16	RSP	IgG3	0.21	0.65	6.02	4.43	0.76	0.002**	0.15
14	9	RSP	IgG4	0.77	2.52	13.7	11.30	0.67	0.63	0.03x
15	10	RSP, ROM	IgG4	0.70	3.81	8.55	7.97	0.68	0.74	0.01x
16	36	ROM	IgG4	0.93	1.09	11.30	6.10	1.26	0.72	0.03x
17	18	RSP	IgG4	0.77	4.83	10.70	9.12	0.68	0.44	0.01x
18	15	RSP, ROM	IgG4	2.20	3.59	14.30	9.72	1.26	0.64	0.00x
19	36	RSP	IgG4	0.85	1.76	12.50	4.43	0.76	0.53	0.01x
20	6	RSP	IgG4	0.12*	1.38	5.08	3.99	0.68	0.50	1.01x
21	6	RSP	IgG4	0.16*	2.71	7.00	4.43	1.69	0.82	0.01x
22	10	RSP	IgG4	0.15	2.61	7.51	6.10	0.68	0.85	0.03x
23	24	RSP, ROM	IgG1+G4	1.09	2.00	6.51	3.57*	0.96	1.20	0.02x
24	48	RSP, ROM	IgG, G1+G4	0.55	1.16	5.55*	3.78*	0.76	0.48	0.03x
25	11	ROM	IgG, G1+G3	0.42	0.65	2.91**	2.02**	0.67	0.16**	0.04
26	33	RSP, ROM, GE	Hypo	0.13*	0.53*	3.32**	2.20**	0.25**	0.43	0.01x

RSP : recurrent sinopulmonary infection
GE : gastroenteritis
ROM: recurrent otitis media
S : Selective
P : Partial

Hypo : hypogammaglobulinemia
* : two standard deviations below the mean
** : three standard deviations below the mean
x : serum IgG4 level ≤ 0.03 g/L

Discussion

Children who experience recurrent or chronic respiratory tract or other infections pose difficult problem[8] for parents and pediatricians. A careful history, physical and laboratory examination, and review of medical records are very important in the evaluation of these patients.

This study demonstrated a high proportion (35%) of cases with various immunoglobulin deficiencies. A higher percentage of immunodeficiency among recurrent infections was reported in the studies of Gross et al.[14] and Moss et al.[15] In terms of deficient immunoglobulin isotype, Gross et al.[14] found 33 percent IgA, 10 percent IgG4, 6 percent IgG3 and 13 percent IgG2 deficient patients, while Moss et al.15 reported that IgG4 deficiency (17%) was the most common followed by IgG1 (6.5%) and IgG2 (6.5%). Surprisingly, only three IgA-deficient cases in a group of 123 patients with recurrent infections were detected in Moss's[15] study. The most common abnormalities in our patients were IgG4 (17.5%) and IgA (10.8%) deficiencies. These ratios of IgG4 and IgA deficiencies are very similar to the findings of Moss et al.[15] and Gross et al.[14], respectively. In the majority of studies, IgG4 deficiency was found to be common[8, 14-16]. But it should be emphasized that because a very low IgG4 level, particularly with radial immunodiffusion, can be detected even in healthy subjects (ranging from 7-30%), it is difficult to consider these patients as IgG4-deficient[15, 17, 18]. We found two selective and six partial IgA deficiencies in 74 patients. Concurrent deficiency of IgG3 occurred in two IgA-deficient patients in this study. There was also one patient with a combined deficiency of partial IgA-IgG2 + IgG4. IgG2 deficiency is the most common subclass deficiency that may be associated with IgA deficiency in most reports[10, 11, 17, 19-21]. However, we detected only one patient with a combined deficiency of IgA + IgG2 who was also IgG4 deficient. The frequency of IgG3 deficiency accompanied by IgA deficiency was also high (2/8) in this study, unlike in similar previous studies[10, 11, 17, 18, 21].

Although the exact cause of the concurrence of IgA and IgG subclass deficiencies is not well understood, it might be related to the close linkage of the genes that encode these heavy chains[6].

IgG subclass concentrations lower than 2SD below the mean for age-matched normal levels are accepted as subclass deficiency in most reports (as in ours) while others define this situation when the concentration is less than 3SD[14, 15, 18]. We detected nine (12%) cases of IgG3 deficiency (five isolated and four combined), which is the second most common IgG subclass deficiency followed by IgG4. This finding is also similar to the results of Gross et al.[14] We observed a low level of total IgG, primarily IgG1 (below 2SD) in three patients (nos: 23-25). Further studies could not be performed to determine the nature of the primary

defect in these three and the hypogammaglobulinemic patients. Except for these four patients, in all age groups the mean IgM, IgA and IgG concentrations tended to be higher than those of healthy subjects, probably due to recurrent infections.

In patients with IgA and subclass deficiency, serum IgA and IgG subclass levels may show a considerable increase with age[22,23]. Therefore, measurement of serum Ig levels at periodic intervals is necessary.

The true prevalence of IgG subclass deficiency is unknown. Controversy exists concerning the frequency of immunoglobulin subclass deficiencies in various studies. This may be due in part to differences in the method used for measurements, cut-off levels and clinical heterogeneity of the patients included in the studies. Children and even adults with recurrent infections should be evaluated for Ig isotype deficiencies, which are not so rare.

REFERENCES

1. Hong R. Recurrent infections. Pediatr Rev 1989; 11: 180-183.
2. Stiehm ER. They're back: recurrent infections in pediatric practice. Contemporary Pediatrics 1990; 7: 20-40.
3. Hong R, Ammann AJ. Disorders of the IgA system. In: Stiehm ER (ed). Immunologic Disorders in Infants and Children. Philadelphia: Saunders; 1989: 329-342.
4. Ammann AJ. Antibody (B cell) immunodeficiency disorders. In: Stites DP, Terr AI, Parslow TG (eds). Basic and Clinical Immunology. Connecticut: Appleton Lange; 1994: 266-278.
5. Jefferis R, Kumararatne DS. Selective IgG subclass deficiency: quantification and clinical relevance. Clin Exp Immunol 1990; 81: 357-367.
6. Oxeilus VA. Immunoglobulin G (IgG) subclasses and human disease. Am J Med 1984; 76: 7-18.
7. Shackelford PG. IgG subclasses: importance in pediatric practice. Pediatr Rev 1993; 14: 291-296.
8. Debaets F, Kint J, Pauwels R, Leroy J. IgG subclass deficiency in children with recurrent bronchitis. Eur J Pediatr 1992; 151: 274-278.
9. Oxelius VA. Quantitative and qualitative investigations of serum IgG subclasses in immunodeficiency diseases. Clin Exp Immunol 1979; 36: 112-116.
10. Oxelius VA, Laurell AB, Lindquist B, et al. IgG subclasses in selective IgA deficiency: importance of IgG2-IgA deficiency. N Engl J Med 1981; 304: 1476-1477.
11. Robertson DM, Colgan T, Ferrante A, Jones C, Mermelstein N, Sennhauser F. IgG subclass concentrations in absolute, partial and transient IgA deficiency in childhood. Pediatr Infect Dis J 1990; 9: 41-45.
12. Berkel AI, Ersoy F, Sanal O, Yeğin O. Serum immunoglobulin and complement levels in healthy Turkish children. Turk J Pediatr 1985; 28: 89-102.
13. Berkel AI, Tezcan I, Ersoy F, Sanal O. Serum immunoglobulin G subclass values in healthy Turkish children and adults. Turk J Pediatr 1994; 36: 197-204.
14. Gross S, Blaiss MS, Herrod HG. Role of immunoglobulin subclasses and specific antibody determinations in the evaluation of recurrent infection in children. J Pediatr 1992; 121: 516-522.
15. Moss RB, Carmack MA, Esrig S. Deficiency of IgG4 in children: association of isolated IgG4 deficiency with recurrent respiratory tract infection. J Pediatr 1992; 120: 16-21.

16. Verini M, Chiarelli F, Verrotti A, Morgese G. IgG subclass deficiency and recurrent respiratory infections in children. Riv Eur Sci Med Farmacol 1989; 11: 251-255.

17. Plebani A, Monafo V, Avanzini MA, Ugazio AG, Burgio GR. Relationship beetween IgA and IgG subclass deficiencies; a reappraisal. Monogr Allergy 1986; 20: 171-178.

18. Beard LJ, Ferrante A. IgG4 deficiency in IgA-deficient patients. Pediatr Infect Dis J 1989; 8: 705-709.

19. French MA, Harrison G. Serum IgG subclasses in patients with an increased susceptibility to respiratory tract infections. Aust NZJ Med 1987; 17: 402-406.

20. Shackelford PG, Polmar SH, Mayus JL, Johnson WL, Corry CM, Nahm MH. Spectrum of IgG2 subclass deficiency in children with recurrent infections: prospective study. J Pediatr 1986; 108: 647-653.

21. Ugazio AG, Out TA, Plebani A, et al. recurrent infections in children with "selective" IgA deficiency: association with IgG2 and IgG4 deficiency. Birth Defects 1983; 19: 169-171.

22. de Laat PC, Weemaes CM, Bakkeren JA. Immunoglobulin levels during follow-up of children with selective IgA deficiency. Scand J Immunol 1992; 35: 719-725.

23. Shackelford PG, Granoff DM, Polmar SH, et al. Subnormal serum concentrations of IgG2 in children with frequent infections associated with varied patterns of immunologic dysfunction. J Pediatr 1990; 116: 529-538.

The Turkish Journal of Pediatrics 1997; 39: 353-362

MANAGEMENT OF FOREIGN BODY ASPIRATION IN INFANCY AND CHILDHOOD*

A Life – Threatening Problem

*Işık Şenkaya MD**, Kadir Sağdıç MD***, Cengiz Gebitekin MD*****

*Mert Yılmaz MD**, Hayati Özkan MD*****, Mete Cengiz MD******

SUMMARY: Şenkaya I, Sağdıç K, Gebitekin C, Yılmaz M, Özkan H, Cengiz M. (Department of Cardiothoracic and Vascular Surgery, Uludağ University Faculty of Medicine, Bursa, Turkey). Management of foreign body aspiration in infancy and childhood: a life-threatening problem. Turk J Pediatr 1997; 39: 353-362.

It is well known that young children have tendency to place objects in their mouths, frequently leading to aspiration of foreign bodies (FBs) into the tracheobronchial tree (TBT).

The patient group comprised 596 patients with a history of suspected aspiration of FBs into the TBT who were bronchoscoped for diagnosis and treatment. There were 306 male (51.3%) and 290 female (48.7%) patients, with a mean age of 2.4 years (range 3 months-13 years). Sunflower seeds and hazelnuts were the most common FBs that were extracted using an open-tube rigid bronchoscope (Storz, Germany) and suitable coaxial forceps (Storz, Germany). Patients admitted within 48 hours following the aspiration numbered 341 (57.2%). The distribution of FBs between the right and left lung and trachea was 53, 37 and five percent, respectively. The aspirated material was visible on the chest x-ray in only 10 percent cases, which facilitated in making the diagnosis. Despite a history of aspiration, bronchoscopy was negative in 21 (3.4%) of the cases. Thoracotomy and subsequent bronchotomy was the treatment of choice in seven (1.5%) and lobectomy in two (0.3%) cases. Cardiorespiratory arrest was observed in five (0.8%) cases, three of whom (0.5%) died during bronchoscopy (2 cases) or thoracotomy (1 case).

In conclusion, patients with FB aspiration are rapidly recognized from their histories and easily treated by bronchoscopy and extraction of the aspirated foreign body. A high index of suspicion is crucial for early diagnosis. However, education is the best preventive measure for decreasing the incidence of this problem. *Key words: foreign body, aspiration, bronchoscopy, childhood.*

Foreign bodies in the tracheobronchial tree (TBT) are commonly encountered in infants and young children and occasionally in adults. Although it is rare, suffocation and subsequent death may be seen in some patients. If the foreign body (FB) is not removed, complications may include various lung disease such as acute respiratory distress, atelectasis, chronic pulmonary infections, abscess,

* From the Department of Cardiothoracic and Vascular Surgery, Uludağ University Faculty of Medicine, Bursa.

** Assistant Professor of Cardiothoracic and Vascular Surgery, Uludağ University Faculty of Medicine.

*** Research Assistant in Cardiothoracic and Vascular Surgery, Uludağ University Faculty of Medicine.

**** Thoracic Surgeon, Uludağ University Faculty of Medicine.

***** Professor of Cardiothoracic and Vascular Surgery, Uludağ University Faculty of Medicine.

bronchiectasis or asthma[1]. In most children, aspiration usually produces acute respiratory symptoms such as choking, coughing, wheezing and dyspnea. Using current techniques, FBs may be removed without significant morbidity and mortality. In addition, improved instruments play an important role in the diagnosis and management of this catastrophe. Bronchoscopy is the technique of choice for diagnosis and treatment. In experienced hands, FB extraction can be simple and almost free of complications. The presentation may occasionally be more subtle and the FB may be left undetected, usually leading to chronic or recurrent lung infections. We presented 10 years of experience with 596 patients diagnosed and treated at this center.

Material and Methods

The study involved a retrospective review of 596 patients who were admitted and underwent bronchoscopic examination of the TBT owing to suspected FB aspiration between January 1985 and December 1995 at the University of Uludağ, Department of Cardiothoracic and Vascular Surgery. Only the children less than 13 years of age were included in the study.

A history of sudden choking or coughing, often followed by wheezing, stridor and respiratory distress, prompted further evaluation. Acute signs of aspiration included cyanosis and chest wall retractions. Most children were initially evaluated by chest x-ray taken on insprium in cases sent by the emergency department, and taken on insprium and exprium in elective cases sent by the Department of Pediatrics. If the child was in acute respiratory distress, the procedure was performed on an urgent basis without chest x-ray. Computerized tomography was also used in chronic cases. Bronchoscopies were performed under general anesthesia induced by intravenous thiopental sodium administration and inhalation of a mixture of oxygen, nitrous oxide as well and halothane. An intravenous (IV) route was kept open in order to administer the medication. In addition, succinylcholine chloride was used to facilitate muscle relaxation. Heart rate, blood pressure, oxygen saturation and electrocardiogram were monitored in all patients. The appropriate-sized pediatric ventilating rigid bronchoscope (Stortz, Germany) was inserted under direct vision, and FB extraction was attempted using coaxial forceps (Storz, Germany) in all cases. If the procedure was unsuccessful, other techniques were also used, such as insertion of a Fogarty balloon catheter (Baxter, USA), thoracotomy and bronchotomy.

In most cases, particularly following the removal of a nutty substance, the TBT was washed with 0.9 percent saline solution and aspirated. During bronchoscopy, prednisolone at a dose of 2 mg/kg was administered via the IV route in order to prevent post-bronchoscopy edema. Nebulization of sterile water with a bronchodilator (salbutamol) was used routinely in almost all cases for 6-12 hours

after bronchoscopy. A routine chest x-ray was taken in all cases following the procedure, and the patients were discharged 24 hours following an uneventful recovery. All patients who died during transport and were resuscitated unsuccessfully were excluded from the study.

Results

The patient group consisted of 596 patients, 85 percent of whom were less than six years of age (Table I). Only 20 patients were between 10 and 13 years of age. There were 306 male (51.3%) and 290 female (48.7%) patients, with a mean age of 2.4 years (range 3 months-13 years). FBs were detected in 575 patients, whereas in 21 cases bronchoscopy were carried out for suspected aspiration but no FB was found (Table II). Owing to the regression of symptoms, bronchoscopy was not repeated in such cases. Three hundred and forty-one cases (57.2%) were admitted within 48 hours of the aspiration, the main symptom being respiratory distress. The other group of 152 patients (25.5%) having mild respiratory distress and signs of pulmonary infection were admitted to the hospital three to seven days following aspiration. Sixty-nine patients (11.5%) who had chronic pulmonary or tracheobronchial infections were admitted to the hospital eight days to one month following FB aspiration. The clinical presentation generally resembled chronic bronchitis or bronchiectasis in the 28 children (4.8%) admitted more than one month following aspiration (Table III).

On chest x-ray, hyperaeration was seen in 190 (32%) and atelectasis in 96 cases (16%, Fig. 1). The aspirated object was visible in only 10 percent of the children (Fig. 2). Chest x-rays were evaluated as normal in 250 (42%) cases.

Bronchoscopy was the first treatment of choice and was performed under general anesthesia in all cases. Coaxial forceps were used successfully for the removal of all but 13 cases (2.2%). Fogarty balloon catheter was successfully used in four (0.7%) patients with firmly lodged objects, and thoracotomy as well as bronchotomy was performed (6 cases) in nine (1.5%) cases in whom all other procedures failed to remove the FB or the bronchoscopy was complicated by a ruptured bronchus (3 cases). A right lower lobectomy had to be performed due to obstructive bronchiectasis in only two (0.3%). cases. Bronchoscopy was

Table I: Age Distribution of Children with Suspected Foreign Body Aspiration

	Age groups (years)				
	0<1	1-3	4-5	6-9	10-13
No. of cases	134	346	51	45	20
%	22.5	58	8.5	7.5	3.5

Table II: Localization of the Foreign Body in the Tracheobronchial Tree

	n		%
Right side	303		51.0
main bronchus	193	32.5	
lower lobe bronchus	88	14.7	
upper lobe bronchus	12	2.2	
middle lobe bronchus	10	1.6	
Left side	212		35.6
main bronchus	138	23.2	
lower lobe bronchus	72	12.2	
upper lobe bronchus	2	0.2	
Trachea	30		5.0
Bilateral	30		5.0
Absent	21		3.4
Total	596		100.0

Table II: Time Interval Between Aspiration of Suspected Foreign Body and Endoscopy

Time Interval	No.	%	Manifestation
0-48 Hours	341	57.2	Coughing, choking, respiratory distress
3-7 days	152	25.5	Respiratory distress, infection
8-29 days	69	11.5	Bronchitis, pneumonia
1 month-5 years	28	4.8	Chronic bronchitis bronchiectasis
6 years and longer	6	1.0	Chronic bronchitis, bronchiectasis, abscess
Total	596	100.0	

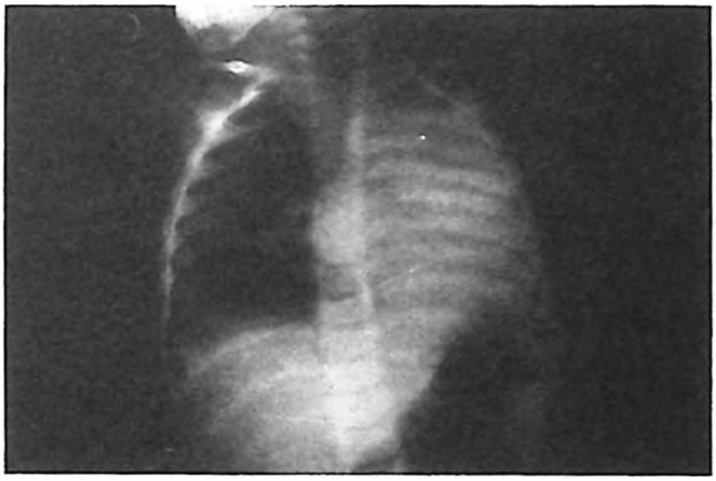

Fig. 1: Atelectasia on the chest x-ray of a case with sunflower seed aspiration.

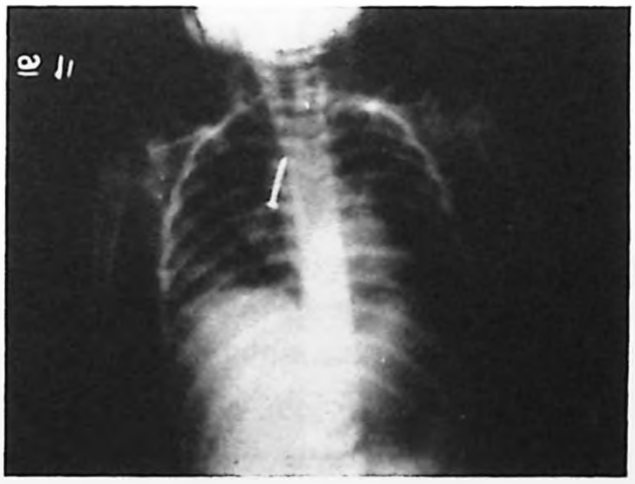

Fig. 2: The chest x-ray of a case with delayed (37 days) presentation of radiopaque screw aspiration.

repeated in 22 (3.7%) cases owing to persistent symptoms. These cases were delayed due to an infection in the TBT. In one case, bronchoscopy was performed twice with an interval of four months due to two separate aspirations. Two children were admitted with cardiorespiratory arrest due to total occlusion of the trachea caused by a dried bean; thus, they were excluded from the study.

Sunflower seeds and hazelnuts were the most common FBs, observed in 33.5 percent of the children (Table IV). The location was the right lung in 303 cases (53%), the left lung in 212 (37%) and the trachea in 30 (5%) cases. Furthermore, bilateral aspiration was observed in 30 (5%) cases (Table III).

Three cases died (0.5%) during the hospital stay. The cause of death was sudden cardiorespiratory arrest during bronchoscopy in two (0.3%) cases and thoracotomy in one (0.2%) case.

Table IV: Nature of the Aspirated Foreign Bodies

	No	%
Sunflower seeds	138	23.0
Hazelnuts	63	10.5
Watermelon seeds	52	8.8
Roasted chick peas	55	9.2
Peanuts	40	6.7
Pumpkin seeds	36	6.1
Beans	23	4.0
Plastic objects	26	4.5
Maize	28	4.8
Metal pins and wires	16	2.5
None found	21	3.4
Miscellaneous	98	16.5
Total	596	100

Discussion

Aspiration of foreign bodies is common in children less than five years of age[3,4]; we treated only 20 cases greater than ten years old. Boys are affected much more than girls[5] because they are more active than girls during childhood and they are more likely to play with toys containing small parts.

More than half of the children (57.2%) were admitted to the hospital in the first 48 hours following aspiration. The presentation was respiratory distress with symptoms of bronchial irritation such as severe cough. In these patients, cyanosis and respiratory distress may be more prominent and require urgent bronchoscopic removal of the FB in order to relieve respiratory failure. Such symptoms are usually due to blockage of the trachea or main bronchi caused by large tracheal or multiple bilateral FBs. Dried beans cause a special airway obstruction as they, in almost all cases, settle in the lower trachea or the orifice of the main bronchi. Within a few hours, dried beans are swollen rapidly with bronchial secretions and may cause total blockage as observed by Hoeve et al.[6], as well as in our study. Therefore, if the history of dried bean aspiration is given, immediate bronchoscopy must be carried out even if the symptoms are mild. Unfortunately, two of our patients were admitted with cardiorespiratory arrest due to aspiration of such material.

It was shown by Wolach et al.[3] and Hoeve et al.[6] that three to seven days following aspiration of material such as sunflower seeds, peanuts or hazelnuts, symptoms of respiratory distress may be significant due to persistent pulmonary infection. This was also observed in our study. The most commonly aspirated material in our series was the sunflower seed. Although most children are immediately admitted to the hospital, some cases are seen 24 hours or more following aspiration. If the diagnosis is delayed more than seven days following aspiration, the clinical picture may resemble pulmonary infection such as bronchitis, pneumonia or bronchiectasis[7]. Plastic or metallic materials may remain for many years in some cases[7].

As shown by Kain et al.[8], the diagnosis may in some cases be easily confirmed by chest x-ray, radiopaque material is aspirated. Hyperaeration and/or atelectasis with a history of aspiration observed on chest x-ray is also diagnostic in such cases. However, even utilizing special techniques such as inspiration-expiration films, bilateral decubitis films and fluoroscopy, the diagnosis can only be confirmed in a limited number of cases by means a of chest x-ray[3,6,9]; thus prolonged radiographic diagnostic efforts are not worthwhile. Prebronchoscopic chest x-ray was diagnostic in 10 percent of the children in our series.

Our policy requires only a high index of suspicion together with a positive history of suspected FB aspiration for investigation and subsequent bronchoscopy. We have decided to perform bronchoscopy so readily because of the potential complications of FBs. Our criteria for performing bronchoscopy are:

1. History of suspected aspiration of FB,
2. Physical findings suggesting the possibility of FB aspiration, or
3. Recurrent unexplained pulmonary infection, atelectasis or hemoptysis in a child.

In our opinion, any of the above three findings is sufficient to warrant the performance of bronchoscopy. A history of playing with small objects, ingestion of nuts, seeds or other foods causing cough, choking, wheezing, dyspnea and cyanosis are very important guides for such aspiration. Physical examination may produce a finding such as wheezing, diminished breath sounds or unilateral rhonchi. In such conditions, early bronchoscopy is the only safe way to diagnose and treat, as reported by Abdulmajid et al.[10] and Black et al.[11].

If the patient is admitted with respiratory distress or cardiorespiratory arrest, emergency bronchoscopy must be performed even without anesthesia. We carried out urgent bronchoscopy in two cases in whom cardiorespiratory arrest had occurred on the way to the operating room. Open-tube ventilating bronchoscopes have been used in many centers for FB extraction[12-14]. Cunanon[15] suggests that a flexible fiberoptic bronchoscope has advantages in only mentally retarded and physically handicapped patients with skeletal deformities, particularly of the cervical spine or pharynx. We used an open-tube ventilating bronchoscope and coaxial forceps in our patients.

Extraction of nutty substances with forceps may be difficult because of fragmentation or slippage, even with improved instruments and bronchoscopic techniques. Kosloske[16] suggested that the use of the Fogarty balloon technique is useful for the removal of nuts and other spherical substances. We performed this technique in only four cases. Extraction of nutty substances was a major problem in our department because of fragmentation and melting. However, the removal of seeds, the most commonly aspirated substance in our series, was easy with the use of coaxial forceps. Likewise, the Dormier basket (Stortz, foreign body basket) is excellent for the removal of stones and pointed objects from the tracheobronchial tree, as suggested by Kosloske[16]; we used it in only two cases.

Whenever a FB is removed, examination of the entire TBT must be carried out to ensure that all bronchial orifices are patent. An unusual presentation of the aspirated material is encountered rarely, most likely due to severe cough following aspiration. Black et al.[11] suggested that when the bronchoscope and FB are removed together, a second look with the bronchoscope must be performed in order to check for a FB fragment, to aspirate retained secretions for culture as well as to evaluate the severity of bronchitis. In most of our patients, the entire TBT was washed out with 0.9 percent isotonic saline solution and aspirated after extraction of the FB, especially for nutty substances.

Some authors[17,18] have suggested that physiotherapy and postural drainage may be effective for expectoration of the aspirated material, although we believe these to be ineffective, and expensive method associated with complications time-consuming in the treatment of such conditions. In a series by Kosloske[13], the success rate with postural drainage was only 25 percent; however, the efficacy of the bronchoscopic removal exceeded 98 percent. In our series, bronchoscopy was successful in all but nine cases (98.4%) in whom foreign bodies were endoscopically visualized. The complication rate was only 1.3 percent, which was similiar to that of other series[2,7,10]. Five cardiac arrests and three bronchial ruptures occurred during bronchoscopy, requiring thoracotomy.

Dudgeon et al.[14] suggested that simple removal of the FB may be sufficient to reverse chronic pulmonary changes so that resection may not be necessary. Surgical resection should be applied if obvious irreversible changes have occurred. In our series, only two patients required pulmonary resection due to delayed presentation. Irritative effects of nutty substances were more frequent than from metallic or plastic substances. Mu et al.[1] showed that, in chronic cases, bronchial edema, pus and granulation tissue may obscure the FB, as we also observed in delayed cases. Bronchoscopy may be repeated in such cases due to persistent symptoms, as experienced by 29 (4.9%) cases in this series.

In 12 patients, despite a history and signs of aspiration, no material was detected in the TBT. This finding was thought to be the result of either a liquified FB or mucous crusts. In these children, removal of such material, washing out of the TBT with 0.9 percent isotonic saline solution, nebulization, antibiotics and physiotherapy cured the disease, as described by Dudgeon et al[14].

Cardiopulmonary arrest may occur before or during bronchoscopic removal in one to two percent of cases[2]. In two series, mortality was reported at zero[19] and 1.8 percent[2]. In our series five cardiac arrests occurred during bronchoscopy (0.8%). Cardiopulmonary resuscitation was successful in two cases; this, three patients died during bronchoscopy (0.5%).

In conclusion, bronchoscopy is the treatment of choice for the removal of all tracheobronchial FBs. Preventive measures, however, continue to remain the best means of protection of these children. Parents should be educated as to the potential dangers of allowing infants to handle small objects and food material that they are not able to chew properly, such as seeds and peanuts. With improved education the incidence of this potentially fatal condition will decrease.

REFERENCES

1. Mu L, He P, Sun D. The causes and complications of late diagnosis of foreign body aspiration in children. Report of 210 cases. Arch Otolaryngol Head Neck Surg 1991; 117: 876-879.
2. Aytaç A, Yurdakul Y, İkizler C, Olga R, Saylan A. Inhalation of foreign bodies in children: reports of 500 cases. J Thorac Cardiovasc Surg 1977; 74: 145-151.
3. Wolach B, Raz A, Weinberg J, Mikulski Y, Ben Ari J, Sadan N. Aspirated foreign bodies in the respiratory tract of children: eleven years experience with 127 patients. Int J Pediatr Otorhinolaryngol 1994; 30: 1-10.
4. Linegar AG, von Oppell UO, Hegemann S, de Groot M, Odell JA. Tracheobronchial foreign bodies. Experience at Red Cross Children's Hospital, 1985-1990. S Afr Med J 1992; 82: 164-167.
5. Paşaoğlu İ, Doğan R, Demircin M, Hatipoğlu A, Bozer AY. Bronchoscopic removal of foreign bodies in children: retrospective analysis of 822 cases. Thorac Cardiovasc Surg 1991; 39: 95-98.
6. Hoeve LJ, Rombout J, Pot DJ. Foreign body aspiration in children. The diagnostic value of signs, symptoms and pre-operative examination. Clin Otolaryngol 1993; 18: 55-57.
7. Banks W, Postic W. Elusive unsuspected foreign bodies in the tracheobronchial tree. Clin Pediatr 1977; 16: 31-35.
8. Kain ZN. O'Connor TZ, Berde CB. Management of tracheobronchial and esophageal foreign bodies in children: a survey study. J Clin Anesth 1994; 6: 28-32.
9. Moazam F, Talbert JL, Rodgers BM. Foreign bodies in the pediatric tracheobronchial tree. Clin Pediatr 1983; 2: 148-150.
10. Abdulmajid OA, Ebeid AM, Motaweh MM, Kleibo IS. Aspirated foreign bodies in the tracheobronchial tree: report of 250 cases. Thorax 1976; 31: 635-640.
11. Black RE, Johnson DG, Matlak ME. Bronchoscopic removal of aspirated foreign bodies in children. J Pediatr Surg 1994; 29: 682-684.

12. O'Neil JA Jr, Holcomb GW Jr, Neblett WW. Management of tracheobronchial and esophageal foreign bodies in childhood. J Pediatr Surg 1983; 18: 475-479.

13. Kosloske AM. Tracheobronchial foreign bodies in children: back to the bronchoscope and a balloon. Commentaries. Pediatrics 1980; 66: 321-323.

14. Dudgeon DL, Parker FB, Frittelli G, Rabuzzi DD. Bronchiectasis in pediatric patients resulting from aspirated grass inflorescences. Arch Surg 1980; 115: 979-983.

15. Cunanon OS. The flexible fiberoptic bronchoscope in foreign body removal: experience in 300 cases. Chest 1978; 73: 725-726.

16. Kosloske AM. Bronchoscopic extraction of aspirated foreign bodies in children. Am J Dis Child 1982; 136: 924-947.

17. Burrington JD, Cotton EK. Removal of foreign bodies from the tracheobronchial tree. J Pediatr Surg 1972; 7: 119-122.

18. Cotton EK, Abrams G, Vanhoutte J, et al. Removed of aspirated foreign bodies by inhalation and postural drainage. A survey of 24 cases. Clin Pediatr 1973; 12: 270-276.

19. Holinger PH, Holinger LD. Use of the open-tube bronchoscope in the extraction of foreign bodies. Chest 1978; 73: 721-724.

The Turkish Journal of Pediatrics 1997; 39: 363-371

CEREBROSPINAL FLUID SHUNT COMPLICATIONS*

*Elif Güzelbağ MD**, Yusuf Erşahin MD***, Saffet Mutluer MD*****

SUMMARY: Güzelbağ E, Erşahin Y, Mutluer S. (Division of Pediatric Neurosurgery, Department of Neurosurgery, Ege University Faculty of Medicine, İzmir, Turkey). Cerebrospinal fluid shunt complications. Turk J Pediatr 1997; 39: 363-371.

We report our experience with cerebrospinal fluid shunt procedures performed on 306 patients between 1983 and 1993. Patients were between the ages of one day and 15 years (average 14.9 months) on admission. Three hundred and thirty-six shunt placements and 274 revisions were done. The first complication occurred in the first postoperative month in 52 patients and within the first six months following surgery in 97 patients. Age was determined as a statistically significant factor in only infection and the slit ventricle syndrome (SVS). Shunt types and systems were not significant factors causing complications. The level of consciousness of the patients at the time of surgery influenced the rate of complications; patients with impaired consciousness at the time of surgery had higher complication rates than those operated on in a normal state of consciousness (41% and 8.5%, respectively). *Key words: cerebrospinal fluid shunts, complication, hydrocephalus, child.*

Many attempts have been made over the years to treat hydrocephalus surgically[1,2]. Since shunting systems became a therapeutic procedure for hydrocephalus in the late 1950s, significant progress has been made. However, this success has been overshadowed by a high incidence of serious complications.

The aim of this study was to present a series of 612 shunt procedures in 306 children and discuss their complications.

Materials and Methods

Three hundred and thirty-eight new procedures and 274 shunt revisions were performed on 306 patients at the Division of Pediatric Neurosurgery between 1983 and 1993 (Table I). Hydrocephalus was due to myelomeningocele in 109 patients, congenital hydrocephalus in 51, meningitis in 48, tumors in 26, aqueduct stenosis in 17, encephalocele in 16, Dandy-Walker in 12, intraventricular hemorrhage in 11, arachnoid cyst in eight, trauma in five and porencephaly in three patients. The age of the patients on admission ranged from one day to 15 years (average 14.9 months). There were 165 boys and 141 girls. In this study, 612 procedures were performed not including external ventricular drainage

* From the Division of Pediatric Neurosurgery, Department of Neurosurgery, Ege University Faculty of Medicine, İzmir.

** Research Assistant in Neurosurgery, Ege University Faculty of Medicine.

*** Associate Professor of Neurosurgery, Ege University Faculty of Medicine.

**** Professor of Neurosurgery, Ege University Faculty of Medicine.

Table I: Number of Procedures Performed on 306 Children

CSF shunting procedures	No.
VP shunt	297
VA shunt	24
CP shunt	17
Shunt revision	274
Total	612

VP: ventriculo-peritoneal, VA: ventriculo-atrial, CP: cyst-peritoneal.

and shunt removals. The patients' medical records were reviewed retrospectively. The follow-up period of the patients ranged from six months to 10 years (average 102.4 months).

All shunt placements or revisions were performed by four neurosurgeons and 28 research assistants. The shunt types used were as follows: a multicomponent shunt was placed in 281, one-piece design in 26, and a flow-regulating shunt in two patients.

Two hundred and ninety-seven ventriculo-peritoneal (VP), 24 ventriculo-atrial (VA) and 17 cyst-peritoneal (CP) shunt systems were used. The majority of the VP shunts were multicomponent system (95%), consisting of a ventricular and a peritoneal catheter and an interposed valve reservoir or flushing device. Five percent of the VP shunts were the one-piece design. Standard shunt placement techniques were performed and prophylactic antibiotics were given in all cases. Fourteen VA shunts were inserted as the first procedure while seven were a second procedure after a recurrent abdominal pseudocyst or distal infection was observed in VP shunts. The results regarding shunt infection are not presented in detail, since data pertaining to shunt infections has been previously elucidated and published[3]. Chi-squared test was used in the statistical analysis.

Results

Complications developed in 45 percent of the shunting procedures during the course of review, and 55 percent of the procedures remained free of complications. Two hundred and seventy-seven complications occurred in 133 patients. Obstruction developed in 22.8 percent of all shunt procedures and was the most common complication (49%) among shunt malfunctions. Proximal obstructions and distal obstructions occurred in 33.4 and 15.6 percent of the patients, respectively. Age was determined to be a significant factor in infection and the slit ventricle syndrome (SVS). The infection rate was 16 percent under one year of age, whereas it was six percent over the age of one year (x^2=5.3284,

p<0.025). SVS was not seen under one year of age, and all seven children with SVS were greater than one year old (x^2=15.388, p<0.000). Of the 24 patients in which a VA shunt was inserted, 11 (46%) had one or more revisions. Twenty-eight revisions were done in this group, with an average of 2.5 revisions, and 13 patients (54%) were not revised at all. Of 297 patients in which a VP shunt was inserted, 154 patients (52%) underwent one or more revisions. In this group 112 revisions were done, with an average of 0.7 revisions. No revision was done in 143 patients (48%) with VP shunts. Of 17 patients with a CP shunt, eight had one or more revisions (47%), while nine had none (53%). Eighteen revisions were done, with an average of 2.2 revisions for each patient. It appears that VA and CP shunts have a higher rate of revisions than do VP shunts. This finding was not statistically significant (x^2=0.444, p=0.801). Table II illustrates the total revision rates in relation to shunt systems (x^2=0.357, p=0.986).

Mechanical complications represented the most common type of shunt failure (28.4%). Obstruction of the ventricular catheter occurred in 95 cases (15.5%), obstruction of the distal catheter in 45 (7.3%), migration of the shunt device in 13 (2.1%), disconnection in 12 cases (1.9%), bowel perforation in five (0.8%), misplacement of the distal catheter and extrusion of the distal catheter in two cases (0.3%), and bladder perforation in one patient (0.1%) (Table III). Misplacement of the distal catheter was not observed in VA shunts. Shortening and extrusion of the peritoneal catheter from the abdominal cavity were observed in four patients with VP shunts. In 19 patients (3.1% of the complicated cases), subdural collection due to overdrainage was seen. We compared the burr-hole localization in terms of VP shunt complications. On CT scan, the tip of the ventricular catheter was observed in the opposite frontal horn in most cases in which a parietal burr hole had been used to place the ventricular catheter. The ventricular catheter was inserted through a parietal burr hole in 172 patients. In 42 of 172 patients, 60 ventricular obstructions were observed. The catheter was placed through a burr hole in the posterior parietal region in 122 patients. Twenty-five of these children (20%) had 34 ventricular obstructions (x^2=0.422, p=0.516).

Two hundred and eighty-four patients were operated on in a state of normal consciousness. Variable degrees of consciousness from drowsiness to deep

Table II: Revision Rates in Relation to VP, VA and CP Shunts

Revision No.	VP shunt (n=297)	VA shunt (n=24)	CP shunt (n=17)
0	143 (48%)	13 (54%)	9 (53%)
≥1	154 (52%)	11 (46%)	9 (47%)

VP: ventriculo-peritoneal, VA: ventriculo-atrial, CP: cyst-peritoneal.

Table III: CSF Shunt Complications

Type of complication	VP shunt	VA shunt	CP shunt	Total No.	%
Proximal catheter obstruction	78	16	1	95	15.5
Distal catheter obstruction	37	5	3	45	7.3
Infection	37	3	6	46	7.5
Subdural collection	16	1	2	19	3.1
Migration	9	–	4	13	2.1
Disconnection	11	1	–	12	1.9
Shortening of distal catheter	9	–	2	11	1.7
Abdominal pseudocyst	10	–	–	10	1.6
Slit ventricle syndrome	4	1	2	7	1.1
Bowel perforation	5	–	–	5	0.8
Postoperative hematoma	3	1	–	4	0.6
Wound dehiscence	2	–	–	2	0.3
Misplacement of distal catheter	2	–	–	2	0.3
Extrusion of distal catheter	2	–	–	2	0.3
Double compartment hydrocephalus	1	–	–	1	0.1
Abdominal CSF fistula	1	–	–	1	0.1
Isolated lateral ventricle	1	–	–	1	0.1
Bladder perforation	1	–	–	1	0.1
Total	292	28	20	277	45.2

VP: ventriculo-peritoneal, VA: ventriculo-atrial, CP: cyst-peritoneal, CSF: cerebrospinal fluid.

coma were described in 22 patients. Out of 612 shunt procedures, 292 were elective (95.4%) and 14 (4.6%) were performed in emergency situations. The level consciousness of the patients at the time of operation influenced the complication rates; the patients with impaired consciousness at the time of surgery had higher complication rates than did those who were alert (41% and 8.5%, respectively, $x^2=19.112$, $p=0.000$). Abdominal insertion of the peritoneal catheter was performed by a trocar in 95 percent of cases (290) and by an open technique in the rest because of an abdominal problem.

Fifty-nine deaths were recorded during the ten-year period, 39 of which were due to a shunt complication. The overall mortality rate was 9.6 percent while the shunt-dependent mortality rate was 6.3 percent. However, progression of the original disease, such as tumor, meningitis, pneumonia, or head injury, was the cause of death in 20 patients. Shunt infection was responsible for 27 deaths, thus constituting the most severe complicatin ($x^2=0.696$, $p=0.706$). We compared the causes of death following VP, VA and CP shunts. Although the shunt-related mortality rate was 5.3 percent in VP, 0.5 percent in VA and 0.5 percent in CP shunted patients, the differences were not found statistically significant ($x^2=0.696$, $p=0.706$).

Discussion

The development of effective cerebrospinal fluid (CSF) shunts represents a landmark achievement in neurosurgery. However, this success has been tempered by a high incidence of serious complications. With relation to the cause of revisions, shunt malfunctions were more frequent (175 patients) than other complications (102 patients) in our series, comparable to rates reported in the literature[4,5]. The most frequent cause of shunt malfunction was obstruction, constituting 49 percent of all shunt malfunctions, 22.8 percent of all shunt procedures and 50.5 percent of all complications, as reported by many authors[4-14].

We determined on histopathologic examination of the obstructing material that the most commonly found tissues were glial, granulomatous and choroid plexus. Choroid plexus, fibrin, brain debris, blood clot and germs were found as obstructing material[8,14-16]. The tip of the ventricular catheter should be pointed away from the choroid plexus. Whether this is best accomplished by placement in the frontal horn remains controversial[11,17,18]. Saint-Rose[19] reported a slightly lower risk of proximal obstruction for ventricular catheter tips located in the occipital horn of the lateral ventricles than for those located frontally. We compared the burr hole locations in terms of the route of insertion, but the results did not have statistical significance, as Bierbrauer et al.[11] stated in a prospective study. The revision rate for VA shunts in large series varied between 46 and 78 percent[10,20,21] as compared to 17 and 58 percent for VP shunts[6,21,22]. A very high percentage of VP procedures (88%) compared to VA (7%) and CP (5%) procedures did not allow reliable comparative evaluation of different types of surgical procedures, and we found the incidence of revisions was slightly lower in VA shunts (46%) and CP shunts (47%) than in VP shunts (52%). The differences among them was not significant.

Mazza et al.[5] pointed out in his series of 165 children with nontumoral hydrocephalus that VA and VP shunts required 1.8 and 2.3 revisions per patient, respectively. In our study, we determined that there were 2.5, 2.2 and 0.7 revisions per patient in VA-, CP- and VP-shunted patients, respectively. It seems that VP shunts are associated with a lower rate of complications and revisions as compared to VA and CP shunts.

Griebel et al.[12] reported that host variables are one of the most elusive factors; neither the patient's age, sex nor the underlying condition appeared to influence the shunt complication rate or survival. Raimondi et al.[4] reported that both overall complication and infection rates were higher prior to three months of age. In our study, we found that age was a statistically significant factor in infection and SVS, but not in other types of complications. Steinbok[23] found an increased incidence of all complications in patients with spina bifida.

SVS is a well-known complication in the treatment of hydrocephalus by shunting. The symptoms may be due to "overdrainage" of CSF over either the short or generally long term, the effects of low pressure or intermittent shunt obstruction as a noncompliant ventricular system, and a growing brain that fills the intracranial space, leading to the development of increased intracranial pressure[24,25]. SVS is not seen in all patients who have anatomic slit ventricles, although the radiologic appearance of slit ventricles reaches high percentages[26-29], and up to 80 percent of shunted patients overall are at risk for the development of slit ventricles. Walker et al.[30] stated that only 11.5 percent of patients with slit ventricles on computed tomographic scan had been symptomatic and classified as having SVS, and 55 percent of those patients (6.5% of the total population) needed surgical intervention. In our study, seven patients required surgical treatment for SVS. All seven children with symptomatic SVS were greater than one year old (x^2=15.388, p=0.000). The initial treatment we use is upgrading of the shunt valve pressure (to high) in order to achieve a chronic increase in ventricular size and/or adding an antisiphon device at the same time. This therapy effectively reduced the negative intracranial pressures and treated all patients, as stated by many authors previously[30-34].

Griebel et al.[12] pointed out a marked difference between the complication rates of various surgeons in their series, testifying to the importance of this operative variable. He also demonstrated that the length of the surgical procedure did not have a statistically significant influence. We could not calculate the complication rates of different surgeons, because the revisions were performed by 32 different staff physicians or resident. Also, the duration of the surgical procedures was not noted on the charts; thus we were not able to conclude whether or not it influenced the results. We found 10 cases of abdominal pseudocyst, eight of which occurred in patients with multiple shunt revisions and previous shunt infections[35]. Symptoms generally consisted of focal abdominal fullness and discomfort as well as nausea and vomiting. Physical signs included generalized or focal abdominal tenderness, distension, decreased bowel sounds and a palpable mass. Pseudocysts were detected by abdominal ultrasound in all cases. In the absence of CSF infection, shunt removal and conversion of the shunt system to VA was the treatment of choice. Disconnection was found in 12 patients, five of which (60%) were due to normal growth. Disconnection was manifested by the appearance of a soft fluctuant mass along the shunt tract in most cases, as previously reported[16]. The reported incidence of subdural hematoma and hygroma varies between 4.5 and 21 percent[36-38]. We observed subdural collection in 19 cases.

Every structure that the shunt catheter bypasses can be penetrated or eroded. Perforations are uncommon complications, and most of the reports are case

studies[39-42]. The clinical manifestation of a hollow viscus perforation varies. We observed peritoneal irritation signs that prompted evaluation of the patient and surgical exploration, as stated by others[43,44]. However, in some cases, perforations occur without signs of peritoneal inflammation as stated by Rubin et al[44]. We noted one bladder and five bowel perforations in our study. The catheter tip was observed extending from the anus in two cases, both of whom died from intractable gram-negative infections. The risk factors determined in our study were comparable to those previously reported, such as a higher risk of complications in the youngest age group, in congenital hydrocephalus, after emergency operations and after operations performed in patients with an impaired level of consciousness. In this study, 277 shunted children (43%) experienced shunt complications, and in 39 of them the complication ended in death (14% of the patients with complications). In the VP-shunted patients, there were 33 deaths following shunt-related complications (21% of VP-shunted patients presenting with complications), while there were three deaths each in VA- and CP-shunted patients (27% of VA-shunted and 37% of CP-shunted patients presenting with complications). The absolute mortality for shunt-related complications in each group was 11 percent in VP, 13 percent in VA and 18 percent in CP-shunted patients. Of 39 shunt-related deaths, 27 were due to infection (not statistically significant). Keucher and Mealey[8] reported that 31 percent of surviving patients in their series still had their original VP shunt, compared to only two percent still having a virgin VA shunt. In our series, 48 percent of VP-shunted and 54 percent of VA-shunted patients still had their original shunts.

In conclusion, although the prognosis for hydrocephalic children has improved remarkably during the last 30 years, since the introduction of shunt surgery, it is still not possible to stop all the harmful complications. However, the treatment of hydrocephalus and its complications is a highly complex and difficult matter and needs close attention, knowledge and experience. We believe that complications can be avoided to a great extent in experienced hands.

REFERENCES

1. Davidoff LM. Treatment of hydrocephalus. Historical review and description of a new method. Arch Surg 1929; 18: 1737-1762.
2. Scarff J. Treatment of hydrocephalus: a historical and critical review of methods and results. J Neurol Neurosurg Psychiatry 1963; 26: 1-26.
3. Erşahin Y, Mutluer S, Güzelbağ E. Cerebrospinal fluid shunt infections. J Neurosurg Sci 1994; 38: 161-165.
4. Raimondi AJ, Robinson, JS, Kuwamura K. Complications of ventriculoperitoneal shunting and critical comparison of the three-piece and one-piece systems. Child's Brain 1977; 3: 321-342.
5. Mazza C, Pasqualin A, Da Pian R. Results of treatment with ventriculoatrial and ventriculo-peritoneal shunt in infantile nontumoral hydrocephalus. Child's Brain 1980; 7: 1-14.
6. Ignelzi RJ, Kirsch WM. Follow-up analysis of ventriculoperitoneal and ventriculoatrial shunts for hydrocephalus. J Neurosurg 1975; 42: 679-682.

7. Illingworth RD, Logue V, Symon L, Uemura K. The ventriculocaval shunt in the treatment of adult hydrocephalus. Results and complications in 101 patients. J Neurosurg 1971; 35: 681-685.
8. Keucher TR, Mealey J. Long-term results after ventriculoatrial and ventriculoperitoneal shunting for infantile hydrocephalus. J Neurosurg 1979; 50: 179-186.
9. Little JR, Rhoton AL, Mellinger JF. Comparison of ventriculoperitoneal and ventriculoatrial shunts for hydrocephalus in children. Clin Proc 1972; 47: 396-401.
10. Overton MC, Snodgrass SR. Ventriculovenous shunts for infantile hydrocephalus. A review of five year experience with this method. J Neurosurg 1965; 23: 517-521.
11. Bierbrauer KS, Storrs BB, McLone DG, et al. A prospective, randomized study of shunt function and infections as a function of shunt placement. Pediatr Neurosurg 1990; 16: 287-291.
12. Griebel R, Khan M, Tan L. CSF shunt complications: an analysis of contributory factors. Childs Nerv Syst 1985; 1: 77-80.
13. Sainte-Rose C, Piatt JH, Renier D, et al. Mechanical complications in shunts. Pediatr Neurosurg 1991-92; 17: 2-9.
14. Sekhar LN, Moossy J, Guthkelch AN. Malfunctioning ventriculoperitoneal shunts. Clinical and pathological features. J Neurosurg 1982; 56: 411-416.
15. Collins P, Hockley AD, Woollam DH. Surface ultrastructure of tissue occluding ventricular catheters. J Neurosurg 1978; 48: 609-613.
16. Gower DJ, Lewis JC, Kelly DL Jr. Sterile shunt malfunction. A scanning electron microscopic perspective. J Neurosurg 1984; 61: 1079-1084.
17. Sainte-Rose C, Hooven MD, Hirsch JF. A new approach in the treatment of hydrocephalus. J Neurosurg 1987; 66: 213-226.
18. Albright AL, Haines SJ, Taylor FH. Function of parietal and frontal shunts in childhood hydrocephalus. J Neurosurg 1988; 69: 883-886.
19. Sainte-Rose C. Shunt obstruction: a preventable complication? Pediatr Neurosurg 1993; 19: 156-164.
20. Becker DP, Nulsen FE. Control of hydrocephalus by valve-regulated venous shunt. Avoidance of complications in prolonged shunt maintenance. J Neurosurg 1968; 28: 215-226.
21. Lajat Y, Lebatard-Sartre R, Guihard D, et al. Etude comperative des complications observe'es dans les derivations ventriculoatriales et ventriculoperitoneales. Neuro-Chirurgie 1975; 21: 147-161.
22. Robertson JS, Maraqa MI, Jennett B. Ventriculoperitoneal shunting for hydrocephalus. Br Med J 1973; 1: 289-292.
23. Steinbok P, Thompson GB. Complications of ventriculovascular shunts: computer analysis of etiological factors. Surg Neurol 1976; 5: 31-35.
24. Epstein FJ. "Slit ventricle syndrome": etiology and treatment. Pediatr Neurosci 1988; 14: 5-10.
25. Epstein FJ. Increased intracranial pressure in hydrocephalic children with functioning shunts: a complication of shunt dependency. In: Shapiro K, Marmarou A, Portnoy H (eds). Hydrocephalus. New York: Raven Press; 1985: 315-321.
26. Foltz EL, Blanks JP. Symptomatic low intracranial pressure in shunted hydrocephalus. J Neurosurg 1988; 68: 401-408.
27. Kiekens R, Mortier W, Pothmann R, et al. The slit-ventricle syndrome after shunting in hydrocephalic children. Neuropediatrics 1982; 13: 190-194.
28. Pudenz RH, Foltz EL. Hydrocephalus: overdrainage by ventricular shunts. A review and recommendations. Surg Neurol 1991; 35: 200-212.
29. Serlo W, Heikkinnen E, Saukkonen AL, von Wendt L. Classification and management of the slit ventricle syndrome. Child's Nerv Sys 1985; 1: 194-199.

30. Walker ML, Fried A, Petronio J. Diagnosis and treatment of the slit ventricle syndrome. Neurosurg Clin Am 1993; 4: 707-714.

31. Fox JL, Portnoy HD, Schulte RR. Cerebrospinal fluid shunts: an experimental evaluation of flow rates and pressure values in the anti-siphon valve. Surg Neurol 1973; 1: 299-302.

32. Gruber R. The Relationship of ventricular shunt cmoplications to the chronic overdrainage syndrome: a follow-up study. Z Kinderchir 1981; 34: 346-352.

33. Gruber R, Jenny P, Herzog B. Experiences with the anti-siphon device (ASD) in shunt therapy of pediatric hydrocephalus. J Neurosurg 1984; 61: 156-162.

34. Portnoy HD, Schulte RR, Fox JL. Antisiphon and reversible occlusion valves for shunting in hydrocephalus and preventing post-shunt subdural hematomas. J Neurosurg 1973; 38: 729-738.

35. Parry SW, Schumacher JF, Llewellyn RC. Abdominal pseudocysts and ascites formation after ventriculoperitoneal shunt procedures: report of four cases. J Neurosurg 1975; 43: 476-480.

36. Pudenz RH, Foltz EL. Hydrocephalus: overdrainage by ventricular shunts. A review and recommendations. Surg Neurol 1991; 35: 200-212.

37. Faulhauer K, Schimitz P. Overdrainage phenomena in shunt treated hydrocephalus. Acta Neurochir (Wien) 1978; 45: 89-101.

38. Samuelson S, Long DM, Chou SN. Subdural hematoma as a complication of shunting procedures for normal pressure hydrocephalus. J Neurosurg 1972; 37: 548-551.

39. Oi SZ, Shose Y, Asano N et al. Intragastric migration of a ventriculoperitoneal shunt catheter. Neurosurgery 1987; 21: 255-257.

40. Portnoy HD, Croissant PD. Two unusual complications of a ventriculoperitoneal shunt: case report. J Neurosurg 1973; 39: 775-776.

41. Sakoda TH, Maxwell JA, Bracket CE: Intestinal volvulus secondary to a ventriculoperitoneal shunt: case report. J Neurosurg 1971; 35: 95-96.

42. Whittle IR, Johnston IH. Extrusion of peritoneal catheter through neck incision: a rare complication of ventriculoperitoneal shunting. Aust NZ J Neurosurg 1983; 53: 177-178.

43. Schulhof LA, Worth RM, Kalsbeck JE. Bowel perforation due to peritoneal shunt. Surg Neurol 1975; 3: 265-269.

44. Rubin RC, Ghatak NR, Visudhipan P. Asymptomatic perforated viscus and gram-negative ventriculitis as a complication of valve-regulated ventriculoperitoneal shunts. J Neurosurg 1972; 37: 616-618.

The Turkish Journal of Pediatrics 1997; 39: 373-378

RISK FACTORS ASSOCIATED WITH CORRECTIVE SURGERY IN CONGENITAL SCOLIOSIS WITH TETHERED CORD*

*Emre Acaroğlu MD***, *Ahmet Alanay MD****, *Nejat Akalan MD*****
*Bülent Atilla MD****, *Erhan Bulutçu MD******, *Adil Surat MD*******

SUMMARY: Acaroğlu E, Alanay A, Akalan N, Atilla B, Bulutçu E, Surat A. (Departments of Orthopedics, Neurosurgery, and Anesthesiology, Hacettepe University Faculty of Medicine, Ankara, Turkey). Risk factors associated with corrective surgery in congenital scoliosis with tethered cord. Turk J Pediatr 1997; 39: 373-378.

Severe neglected congenital scoliosis with tethered cord presents major difficulties in management. The primary objective of this study was to identify the risk factors associated with corrective scoliosis surgery in neglected cases presenting with severe deformity.

Six patients who presented with such problems draw attention to the importance of a staged anterior and posterior approach in order to obtain satisfactory functional results. However, corrective surgery is associated with major neurologic and systemic complications, and every effort should be made to intervene with the progression of the deformity before corrective measures becomes necessary. *Key words: tethered cord, diastematomyelia, congenital scoliosis, surgery.*

The close relationship between neural and vertebral development in the early embryological period leads to a high incidence of neurologic malformations co-existing with congenital scoliosis. Scoliosis associated with tethered cord may be due to diastometamyelia or other occult intraspinal anomalies and tight filum terminale, hence the tethering of the cord, or to congenital anomalies of the vertebral column, or to paralysis associated with spinal cord tethering[1-3]. The goal of treatment for these patients is to obtain a stable spine in reasonably good alignment over a level pelvis with preservation of normal sagittal alignment, and ideally should be performed before any neurologic problem occurs. Treatment in such cases consists of early untethering of the cord in a child with a significant growth potential, and early recognition and fusion of the spinal segments that are likely to be involved in a significant spinal deformity that may in turn lead to spinal column inbalance in the frontal or sagittal planes.

* From the Departments of Orthopedics, Neurosurgery and Anesthesiology, Hacettepe University Faculty of Medicine, Ankara.

** Associate Professor of Orthopedics, Hacettepe University Faculty of Medicine.

*** Research Assistant in Orthopedics, Hacettepe University Faculty of Medicine.

**** Associate Professor of Neurosurgery, Hacettepe University Faculty of Medicine.

***** Anesthesiologist, Hacettepe University Faculty of Medicine.

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

Treatment after skeletal maturity remains controversial. Untethering of the spinal cord seldom leads to improvement in neurologic symptoms and is usually unnecessary[1, 3, 4]. Surgical correction of a spinal deformity is often very difficult even in the most experienced hands and may be associated with severe complications.

The objective of this study was to analyze the results and complications associated with surgical correction of a severe imbalance of the spinal column due to a neglected spinal deformity in a group of patients with tethered spinal cord.

Material and Methods

Nine cases of scoliosis associated with tethered cord that were operated on between 1992 and 1995 were prospectively analyzed. Another case who had corrective surgery in 1985 was added to this population. Four of these cases were skeletally immature patients who had staged untethering as well as anterior and posterior fusion operations without correction, aiming to arrest the progression of the severe deformity and neurologic involvement. The follow-up period ranged from one to 10 years for the five patients that had corrective surgery, while the sixth patient died in the third postoperative month because of respiratory problems.

The study group consisted of six patients who had corrective surgery with instrumentation for congenital scoliosis associated with tethered cord. The average age of patients was 13.8 (9-17) years. The cause of tethering was identified as diastematomyelia in four and tight filum terminale in two patients. Five patients (three with diastematomyelias and two with tight filum terminales) had untethering operations prior to corrective surgery. One patient with diastematomyelia did not have surgery for excision of the bony spur.

The average preoperative Cobb measurement was 74.8 (50-100) degrees. Fixed pelvic obliguity was present in five cases, averaging 9.3 (5-15) degrees. T1 tilt to one side averaged 23 degrees in four patients. All of the patients had frontal imbalance measured as the distance from the center of S1 to the gravity line passing from T1, and averaged 23 (12-33) mms. One patient had fixed hyperlordosis in the thoraco-lumbar area with a shift of the gravity line 27 mm posterior of the center of S1.

Two patients had only posterior surgeries, while four had both anterior and posterior. One Harrington and one Texas Scottish Rite Hospital (TSRH) instrumentation was used for the cases with only posterior surgeries with extension of the instrumentation down to the pelvis in the latter patient. ISOLA™ instrumentation was used for the other four cases with anterior and posterior multiple osteotomies, and none had to be extended down to the pelvis. The osteotomies were performed as closing wedges of the convex side of the

deformity so as not to elongate the spinal column and stretch the spinal cord, and did not coincide with the level of the diastematomyelia in any of the cases. Use of sublaminar wires was avoided in most cases unless the spinal canal could be demonstrated to be normal in diameter during surgery (Fig. 1).

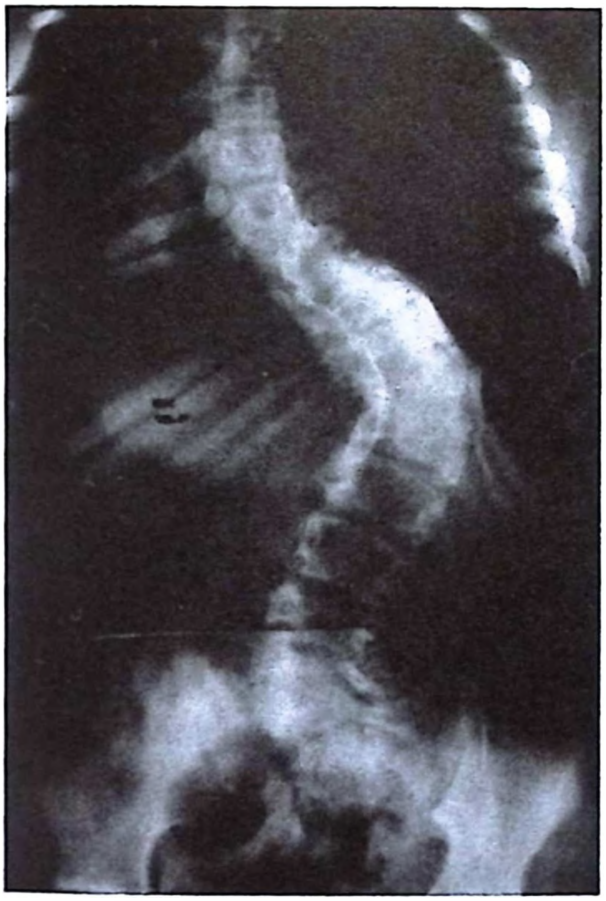

Fig. 1a: A/P radiographs of a patient who had a severe thoracolumbar curve (Th9-L3; 86 degrees).

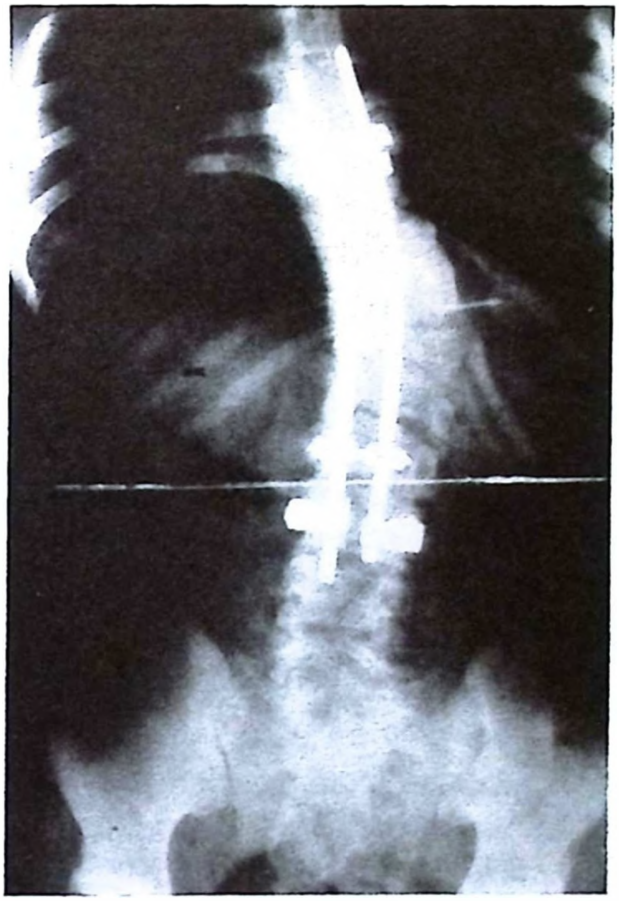

Fig. 1b: The postoperative result. Curve was corrected to 38 degrees. The spinal canal configuration in this particular case allowed for sublaminar wire application.

Results

The average postoperative Cobb angle was 40.5 degrees, with an average correction of 45.9 percent Fixed pelvic obliguity was corrected to 5.7 (0-10) degrees. T1 tilt was measured to be 23 degrees on average for the entire study group and could be corrected to 8.6 degrees in those patients with preoperative tilt, with an average correction of 63 percent. Frontal imbalance could be corrected to an average of 4.4 (0-12) mm. The sagittal balance of the patient with hyperlordosis improved to 7 mms from the plumb line.

Complications were seen in two patients. One patient had late deep infection of the instrumentation that necessitated hardware removal with mild loss of

correction (from 45 degrees to 57 degrees). Another patient wo had the most severe deformity in this group presented with a curve of 100 degrees and a forced vital lung capacity of 850 ccs. She had undergone sequential anterior-posterior surgery and demonstrated monoparesis of the left lower extremity after this intervention. Despite prompt removal of the instrumentation allowing collapse of the spinal column, her neurologic status gradually deteriorated to complete paraplegia in one week. This complication could not be attributed to specific pathology except very poor oxygenation of the cord because of respiratory problems following surgery that necessitated continuous mechanical ventilation via a tracheostomy for one week; she was weaned of the respirator in six weeks. This patient died three months after her discharge because of an acute upper respiratory tract infection.

Discussion

Diastematomyelia is the most common cause of dysraphism among the occult intraspinal anomalies coexisting with congenital scoliosis, and it is the leading cause of all spinal cord tethering[1,2]. It has been estimated that five to 16 percent of patients with congenital scoliosis have diastematomyelia. Other less common anomalies presenting with tethered cord include: neuroenteric, epidermoid and dermoid cysts; teratoma; neurofibroma; lipofibroma; fibrous bands; and a tight filum terminale.

The goal of the treatment of scoliosis associated with tethered cord, with or without neurologic problems, should be prevention of the progression of any deformity[1,3-5]. Severe congenital spinal deformities with spinal cord tethering are clinically associated with impairment of pulmonary function, compression of the abdominal viscera, and poor sitting and standing posture. Pain, increasing spasticity, and decreasing motor function of the lower extremities are indications for operative release of the tethered cord as well as spinal arthrodesis[5]. While there is ongoing controversy regarding the treatment of tethered cord without neurologic problems, it is generally accepted that symptomatic spinal cord tethering presenting with severe scoliotic deformity should be released before any attempt is made to correct the scoliosis[1,5-8]. As the presence of intraspinal abnormalities limits the ability of the spinal cord to move within the spinal canal, which is likely to occur during correction of the deformity, distraction may cause serious neurologic complications.

Formation or segmentation anomalies of the vertebrae are prone to produce an imbalance in the longitudinal growth of the spine and result in progressive congenital scoliosis. All patients with congenital curves should be monitored closely for progression of the existing deformity. Accurate assessment of neurological status should be made by serial examinations. Progression of the deformity should never be permitted and should be prevented by early fusions,

Winter et al.[2,3] suggested that in-situ arthrodesis of the spine is satisfactory for most patients presenting with congenital curves. However, for neglected cases of congenital scoliosis presenting with severe deformity and imbalance, an attempt to correct the deformity surgically may be considered. Anterior and posterior releases realised by sequential osteotomies and instrumentation may allow for major correction of the spinal deformity as well as stabilization of pulmonary function[5]. Patients with severe neglected congenital deformities present a very difficult problem surgically as well as ethically.

When dealing with the severe curves associated with congenital vertebral or neurologic anomalies, aiming to shorten the convexity of a curve, as was done in the majority of cases in this series, appears to be safer than aiming to lengthen the concavity. The only patient with harrington distraction instrumentation appeared to have been operated on before the commencement of modern imaging techniques and only luckily escaped severe neurologic complications, probably because of the modest amount of correction that could be obtained. Correction of a severely deformed spinal column is a very demanding procedure and should be performed by experienced surgeons. These operations have been associated with a high incidence of complications such as neurologic deterioration, loss of correction due to pseudoarthrosis, sepsis, and cardiopulmonary or urinary system problems. Routine intraoperative monitoring of cord function during corrective surgery is essential to avoid neural complications[3-5].

The severe complication that occurred in one patient in our series needs to be emphasized. The occurrence of neurologic involvement despite the routine wake-up test during surgery and its progression to complete paraplegia during the postoperative period while the patient was in severe respiratory distress suggest the posibility of poor spinal cord oxygenation coupled with surgical trauma as the cause. This patient, as stated above, was the most recently operated case in this series and eventually died due to respiratory problems. Our experience with this patient suggests that the general respiratory status of the patient may be the most important prognostic factor, and any severe restriction of pulmonary function before corrective surgery for congenital scoliosis should be evaluated very carefully; moreover, both the patient's and the medical center's ability to withstand such major surgery should be taken into consideration.

In conclusion, for severe cases of congenital spinal deformity associated with tethering of the spinal cord, corrective surgery remains a procedure associated with a high complication rate. Six patients who presented with such problems drew attention to the importance of a staged anterior and posterior approach in order to obtain satisfactory results. The general circulatory and respiratory condition of the patient may be major factors affecting the neurologic and overall prognosis of the patient and should be taken into consideration before surgery.

REFERENCES

1. McMaster MJ. Occult intraspinal anomalies and congenital scoliosis. J Bone Joint Surg 1984; 66: 588-601.
2. Bradford DS, Heithoff KB, Cohen M. Intraspinal abnormalities and congenital spinal deformities: a radiographic and MRI study. J Ped Orthop 1991; 11: 36-41.
3. Winter RB, Lonstein JE. Congenital spine deformity. J Bone Joint Surg 1995; 78: 300-311.
4. Winter RB, Moe JH, Lonstein JE. Posterior spinal arthrodesis for congenital scoliosis. An analysis of the cases of two hundred and ninety patients, five to nineteen years old. J Bone Joint Surg 1984; 66: 1188-1197.
5. Beaty JH, Canale ST, Roach JW, et al. Current concepts review. Orthopaedic aspects of myelomeningocele. J Bone Joint Surg 1990; 72: 626-630.
6. McLone DG, Herman JM, Gabrielli AP, Dios L. Tethered cord as a cause of scoliosis in children with myelomeningocele. Pediatr Neurosurg 1990-1991; 16: 8-13.
7. Conner AN, Young DG, Hide TA. Occult intraspinal anomalies and congenital scoliosis. J Bone Joint Surg 1984; 66: 1319.
8. Reigel DH, Tchernoukha K, Bazmi B, Kortyna R, Rotenstein D. Change in spinal curvature following release of tethered spinal cord associated with spina bifida. Pediatr Neurosurg 1994; 20: 30-42.

The Turkish Journal of Pediatrics 1997; 39: 379-385

ACUTE PANCREATITIS IN A PATIENT WITH GLUTARIC ACIDEMIA TYPE II*

*Turgay Coşkun MD**, *Safiye Göğüş MD***, *Zuhal Akçören MD****
*Ayşegül Tokatlı MD*****, *İmran Özalp MD***

SUMMARY: Coşkun T, Göğüş S, Akçören Z, Tokatlı A, Özalp İ. (Nutrition-Metabolism and Pathology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Hacettepe-Ankara, Turkey). Acute pancreatitis in a patient with glutaric acidemia type II. Turk J Pediatr 1997; 39: 379-385.

We describe a two-year-old girl who presented with coma following an upper respiratory tract infection. Nonketotic hypoglycemia, metabolic acidosis and mild hyperammonemia were detected. The urinary organic acid profile was consistent with glutaric aciduria type II. Pancreatitis was diagnosed at autopsy. Although pancreatitis has been described in a number of inborn errors of metabolism including organic acidemias, to the best of our knowledge this is the first report of acute pancreatitis occurring in glutaric acidemia type II. It was stressed, therefore, that this complication should be searched for in organic aciduria patients, and the measurement of plasma amylase and lipase levels should be added to the battery of laboratory investigations in such cases. *Key words: glutaric acidemia type II, pancreatitis.*

The number of reports on the occurrence of acute and chronic pancreatitis in patients with inborn errors of metabolism has been increasing in recent years. Among the metabolic diseases reported with accompanying pancreatitis are maple syrup urine disease[1], isovaleric acidemia[1], methylmalonic acidemia[1], propionic acidemia[2], 3-OH-3-methyl glutaric aciduria[3], homocystinuria[4], Pearson syndrome[5], cytochrome c oxidase deficiency[6], glycogen storage disease type I[7,8] and carnitine palmitoyltransferase II deficiency[9]. Glutaric acidemia type I was added to the list most recently[10]. To our knowledge, acute pancreatitis in glutaric acidemia type II or multiple acyl CoA dehydrogenation deficiency has not previously been described. In this report we describe acute pancreatitis occurring in a two-year-old girl with glutaric aciduria type II.

Case Report

The patient, a two-year-old female, was admitted to Hacettepe University İhsan Doğramacı Children's Hospital in a coma following a brief period of seizure. She had an upper respiratory tract infection characterized by fever, nasal discharge

* Nutrition-Metabolism and Pathology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

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

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

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

and cough that started two days prior to her admission, and was hospitalized with the presumptive diagnosis of Reye's syndrome or encephalitis. Her parents were first cousins, and two siblings were healthy. She had reportedly been hospitalized at a local hospital for approximately one week with a suspected diagnosis of "aseptic meningitis" when she was six months old.

On physical examination, the patient's general condition was poor and she was unconscious. She had grunting respirations. The body temperature was 36.5 °C, pulse 120, and respirations 28/minute. The liver was palpable four centimeters below the right costal margin. Deep tendon reflexes were found to be slightly increased, and the Babinski sign was positive bilaterally. Her body weight was 15 kg ($> 90^{th}$ percentile), length 85 cm ($< 50^{th}$ percentile), and head circumference 46 cm ($< 10^{th}$ percentile).

Laboratory values included Hb: 10.4 g/dl, Hct: 30.7%, WBC: 8.700/mm^3 with 90% polymorphonuclear leukocytes, 8% lymphocytes and 2% band forms. Urinalysis was normal. Serum electrolytes were sodium: 135 mEq/L, potassium: 6.2 mEq/L, chloride: 87 mEq/L, and HCO_3^-: 11 mEq/L. Serum glucose was 11 mg/dl, BUN: 24 mg/dl, calcium: 9.5 mg/dl, phosphorus: 7 mg/dl, SGOT: 702 U/L, SGPT: 401 U/L, alkaline phosphatase: 202 U/L, total bilirubin: 0.6 mg/dl (conjugated fraction: 0.4 mg/dl), total protein: 7.1 g/dl, albumin 4.7 g/dl, creatine phosphokinase: 3,829 U/L, and arterial pH: 7.41. The blood ammonia level was 180 µg/dl (normal: 20-120 µg/dl), lactate: 27.3 mg/dl (normal: 10-14 mg/dl), and pyruvate: 2.8 mg/dl (normal: 0.5-1.0 mg/dl). The prothrombin and partial thromboplastin times were 15.5 and 30 seconds, respectively. EEG revealed a widespread suppression of the cerebral bioelectric activity. Cranial computed tomography demonstrated brain edema. A fatty liver was found on abdominal ultrasonography. Gas chromatographic-mass spectrometric analysis of urine was consistent with glutaric aciduria type II.

Despite mechanical ventilation and parenteral administration of dextrose, fluids, fresh frozen plasma, bicarbonate, vitamin K, antibiotics and acyclovir, the patient died on the sixth day of admission.

Autopsy revealed a pale liver weighing 540 g (normal: 430 g). The brain was edematous with a weight of 1125 g (normal: 1012 g). The pancreas (weight: 48 g, normal: 19.9 g) was also edematous and had hemorrhagic areas. No congenital anomaly was detected.

Fatty infiltration of the liver with both small- and large-sized lipid vacuoles was found on microscopic examination (Fig. 1). Frozen sections of the liver showed diffuse staining with oil red O. Microscopic examination of the pancreas disclosed an acute, diffuse and hemorrhagic pancreatitis and necrosis of the peripancreatic fat tissue (Fig. 2). Sections of the brain revealed edema and anoxic changes. There was

necrosis of the granular layer of the cerebellum. Spongiosis was detected between the gray and white matter in many sections of the cerebral cortex (Fig. 3). Bronchopneumonia and intraalveolar hemorrhage were present in the lungs.

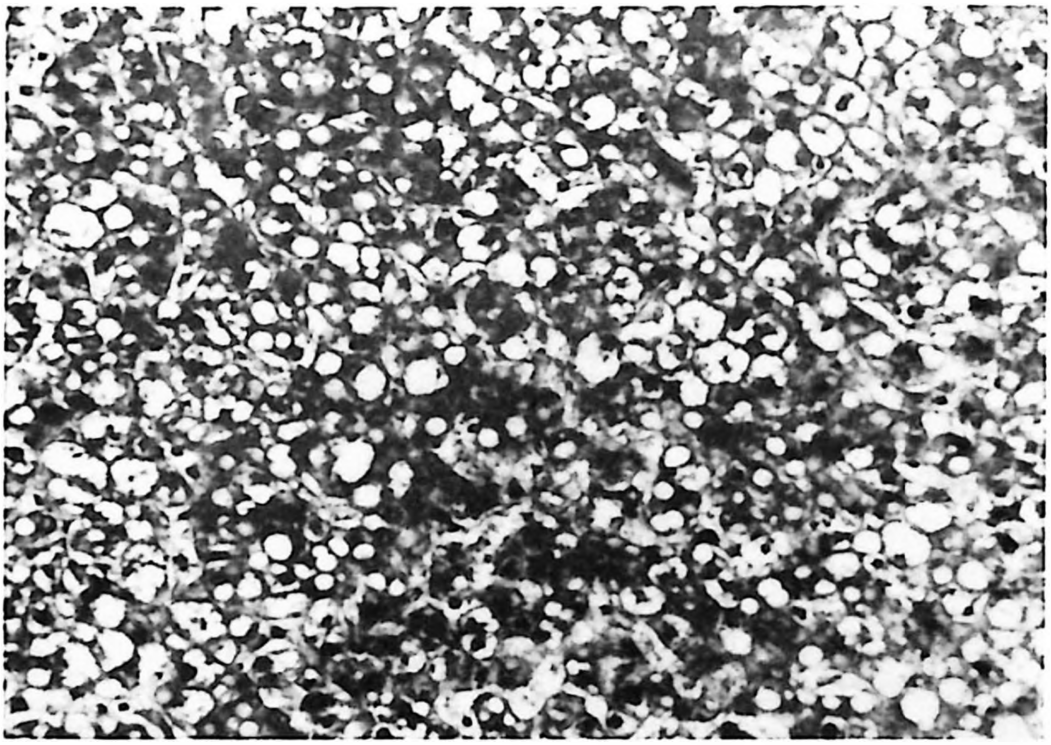

Fig. 1: Small and large lipid vacuoles in the liver. H-E, x66.

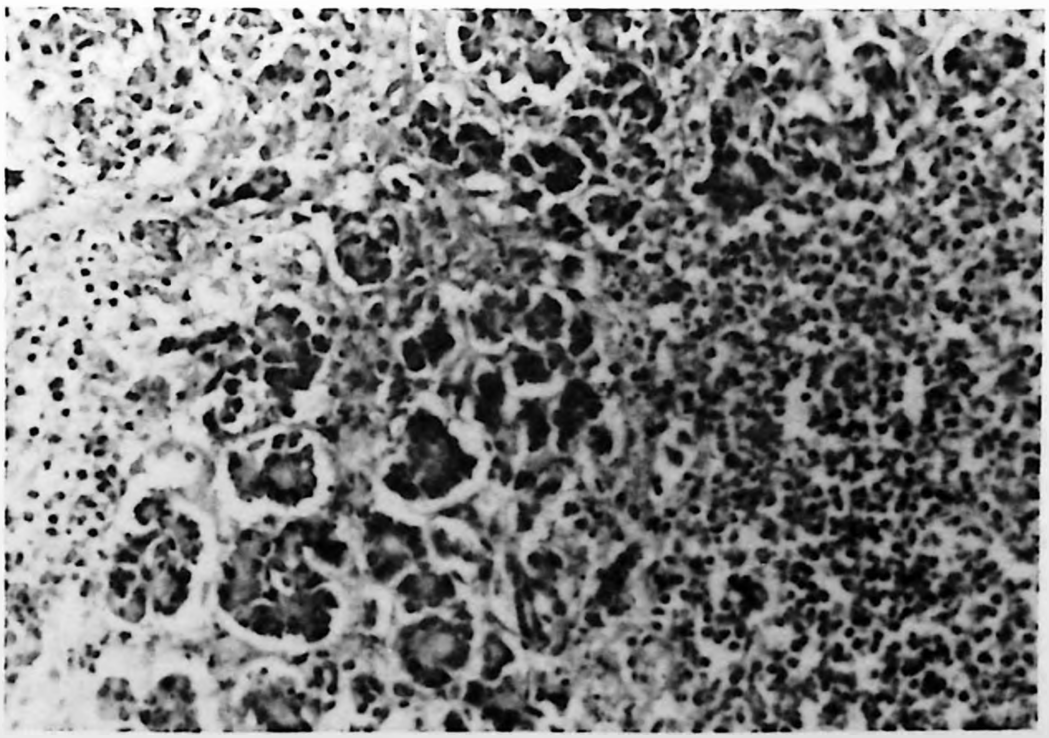

Fig. 2: Necrosis and inflammatory reaction in the pancreas. H-E, x66.

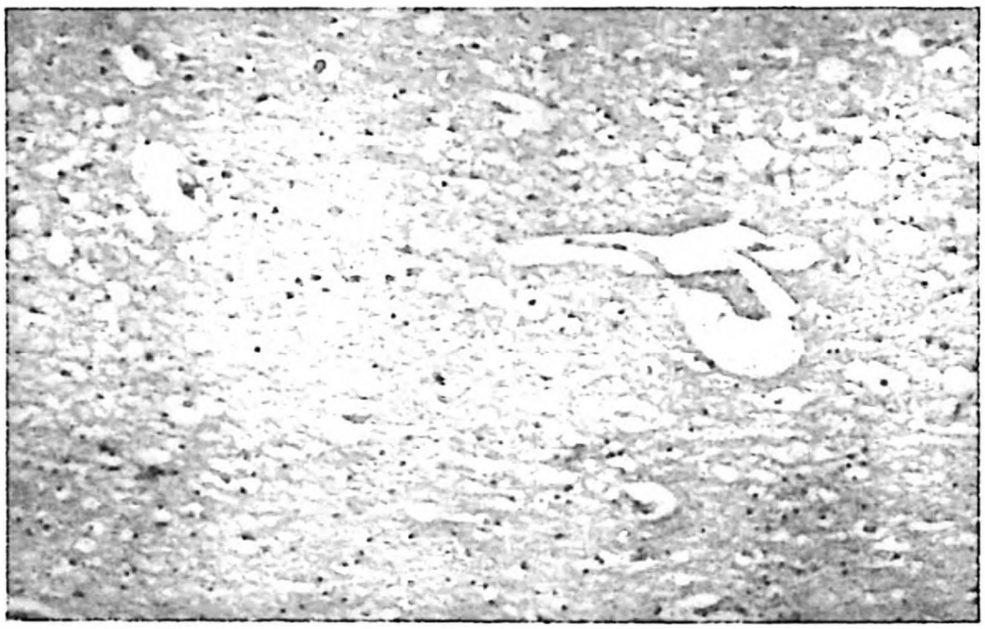

Fig. 3: Spongiosis between gray and white matter of the cerebral cortex. H-E, x33.

Discussion

Glutaric acidemia type II is an inborn error characterized by hypoketotic or nonketotic hypoglycemia, metabolic acidosis, hyperammonemia and organic aciduria. The underlying metabolic defect that causes the disease is a deficiency of either electron transport flavoprotein (ETF) or electron transport flavoprotein oxoreductase (ETF-QO). The mode of inheritance is autosomal-recessive. The disease is heterogenous both clinically and biochemically. Some affected infants present with life-threatening illnesses in the neonatal period, with or without congenital anomalies, while others may have the mild- or late-onset form and become symptomatic later in life[11]. The clinical picture of glutaric acidemia type II, particularly that of the neonatal form, may well mimic Reye's syndrome[12]. Many affected patients have facial dysmorphism, abnormalities of the external genitalia, polycystic kidneys with or without dysplastic changes, and symmetrical warty dysplasia of the cerebral cortex[11, 13-16]. Our patient, presenting with metabolic acidosis, nonketotic hypoglycemia, slightly increased blood ammonia and hepatomegaly at two years of age, belongs to the second group. Although no further information was available, we assumed that the episode experienced at six months by our patient and diagnosed as "aseptic meningitis" might well have been a mild attack of the disease. The typical urinary organic acid pattern established the diagnosis of glutaric acidemia type II in the patient.

Organic acidemias account for a considerably large group among the inborn errors of metabolism, and a number of these have been reported to be associated with either acute or chronic pancreatitis[1]. It was found that nine out of 108 children with organic acidemia had pancreatitis in a study covering five institutions, a

finding that gave further support to the hypothesis that the occurrence of pancreatitis in organic acidemias is not fortuitous and points to a causal link[1].

Pancreatitis in glutaric acidemia type I but not type II has previously been reported[10]. To our knowledge, this is the first report of acute pancreatitis in glutaric acidemia type II. Cases reported earlier led the current authors to think that pancreatitis develops in organic acidemias mostly due to impaired metabolism of branched-chain compounds[1]. In fact, several other mechanisms have been hypothesized. These include a direct toxic effect of the accumulated compounds, particularly that of triglycerides on the acinar cell membrane, lack of ATP, excess free-radical formation and deficiencies of carnitine, methionine and antioxidants, and a low-protein high-lipid diet[1, 10].

Acute pancreatitis is rather uncommon in childhood and difficult to diagnose[1]. Its diagnosis is even more difficult in organic acidemia patients, since the conditions share certain common gastrointestinal manifestations, such as feeding refusal, nausea, vomiting and abdominal pain. Moreover, amylase and lipase determinations are not usually included in the battery of laboratory investigations done during acute decompensation in such disorders. This was the case in our patient, and the diagnosis of pancreatitis was made only at autopsy.

A literature search disclosed postmortem examinations of fewer than 30 cases with glutaric acidemia type II[13-15, 17, 18]. Similar to that found in our case, fatty degeneration of the liver parenchymal cells characterized by small-, medium- and large-sized droplets were the most common findings in all the reported glutaric acidemia type II cases to date. These fatty changes in the liver differed from those in Reye's syndrome[13, 15]. Hypoplasia of the ducts and ductules was detected in two cases[16]. Fatty degeneration of the renal tubular epithelium and myocardium has also been reported in glutaric acidemia type II[11-16, 18]. Oil red O stain was not positive in the frozen sections of the formalin-fixed tissues of the kidneys and myocardium in our case. Neuropathologic findings described in several previously reported cases included edema, warty dysplasia, microgyria, Alzheimer type II changes, disordered neuronal migration, hydrocephalus and several other nonspecific changes. Spongiosis of the cerebrum has not previously been described. Only Kamiya et al.[15] mentioned spongioform changes in the gray matter of the spinal cord in their report and hypothesized that these changes might be secondary to hypoxia, hypoglycemia and severe acidosis. The above-mentioned factors may have played a role in our case as well.

Whatever the underlying mechanism, our case when considered together with the lengthening list of previously reported cases points to a causal relationship between organic acidemias and pancreatitis rather than the association of these two being mere chance. Physicians caring for patients with organic acidemias,

particularly those resembling the clinical picture of Reye's syndrome, should be aware of this possibility. Only awareness of this possibility can lead to the diagnosis of pancreatitis in patients with organic acidemias during acute decompensation, or the diagnosis of pancreatitis in the absence of a known cause may lead to the diagnosis of an inborn error of metabolism.

Acknowledgement

We thank Dr. J.G.M. Huijmans, Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands, for GC-MS analysis of the patient's urine sample.

REFERENCES

1. Kahler SG, Sherwood WG, Woolf D, et al. Pancreatitis in patients with organic acidemias. J Pediatr 1994; 124: 239-243.
2. Burlina AB, Dionisi-Vici C, Piovan S, et al. Acute pancreatitis in propionic acidaemia. J Inherit Metab Dis 1995; 18: 169-172.
3. Wilson WG, Cass MB, Sovik O, Gibson KM, Sweetman L. A child with acute pancreatitis and recurrent hypoglycemia due to 3-hydroxy-3-methyl glutaryl-CoA lyase deficiency. Eur J Pediatr 1984; 142: 289-291.
4. Collins JE, Brenton DP. Pancreatitis and homocystinuria. J Inherit Metab Dis 1990; 13: 232-233.
5. Rötig A, Cormier V, Blanche S, et al. Pearson's marrow-pancreas syndrome: a multisystem mitochondrial disorder in infancy. J Clin Invest 1990; 86: 1601-1608.
6. Kato S, Miyabayashi S, Ohi R, et al. Chronic pancreatitis in muscular cytochrome c oxidase deficiency. J Pediatr Gastroenterol Nutr 1990; 11: 549-552.
7. Kikuchi M, Hasegawa K, Handa I, Watabe M, Narisawa K, Tada K. Chronic pancreatitis in a child with glycogen storage disease type I. Eur J Pediatr 1991; 150: 852-853.
8. Michels VV, Beaudet AL. Hemorrhagic pancreatitis in a patient with glycogen storage disease type I. Clin Genet 1980; 17: 220-222.
9. Tein I, Christodoulou J, Donner E, McInnes RR. Carnitine palmitoyltransferase II deficiency: a new cause of recurrent pancreatitis. J Pediatr 1994; 124: 938-940.
10. Lemire EG, Moroz S, Pollock B, Postuma R, Greenberg CR. Acute pancreatitis in a patient with glutaric acidemia type I. J Pediatr 1996; 128: 589-590.
11. Frerman FE, Goodman SI. Nuclear-encoded defects of the mitochondrial respiratory chain, including glutaric acidemia type II. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Bases of Inherited Disease (7^{th} ed). Vol I. New York: McGraw-Hill, Inc., 1995: 1611-1629.
12. Bennett MJ, Curnock DA, Engel PC, et al. Glutaric aciduria type II: biochemical investigation and treatment of a child diagnosed prenatally. J Inherit Metab Dis 1984; 7: 57-61.
13. Colevas AD, Edwards JL, Hruban RH, Mitchell GA. Valle D, Hutchins GM. Glutaric acidemia type II. Comparison of pathologic features in two infants. Arch Pathol Lab Med 1988; 112: 1133-1139.
14. Loehr JP, Goodman SI, Frerman E. Glutaric acidemia type II: heterogeneity of clinical and biochemical phenotypes. Pediatr Res 1990; 27: 311-315.
15. Kamiya M, Eimoto T, Kishimoto H, et al. Glutaric aciduria type II: autopsy study of a case with electron transferring flavoprotein dehydrogenase deficiency. Pediatr Pathol 1990; 10: 1007-1019.

16. Böhm N, Uy J, Kiebling M, Lehnert W. Multiple acyl-CoA dehydrogenation deficiency (glutaric aciduria type II), congenital polycystic kidneys, and symmetric warty dysplasia of the cerebral cortex in two newborn brothers. Eur J Pediatr 1982; 139: 60-65.

17. Rhead WJ, Wolff JA, Lipson M, et al. Clinical and biochemical variation and family studies in the multiple acyl-CoA dehydrogenation disorders. Pediatr Res 1987; 21: 371-376.

18. Ip WC, Hammond JW, Wilcken B. Neonatal multiple acyl-CoA dehydrogenase deficiency: essentially absent fatty acid oxidation activity in proband but normal activity in parental cultured skin fibroblasts. J Inherit Metab Dis 1996; 19: 379-380.

The Turkish Journal of Pediatrics 1997; 39: 387-393

LEPRECHAUNISM IN TWO TURKISH PATIENTS*

*Aytemiz Gürgey MD***, Safiye Göğüş MD***, Ümit Saatçi MD***
*Nihat Bilginturan MD***, Nurşen Yordam MD***, Turgay Coşkun MD***
*Süheyla Özkutlu MD***, Nilüfer Şahin MD****

SUMMARY: Gürgey A, Göğüş S, Saatçi Ü, Bilginturan N, Yordam N, Coşkun T, Özkutlu S, Şahin N. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Leprechaunism in two Turkish patients. Turk J Pediatr 1997; 39: 387-393.

We report two cases of Leprechaunism with the classical features. The first case had hyperglycemia and severe hyperinsulinemia. The postmortem examination of the second child revealed enlargement of both ovaries, islet cell hyperplasia in the pancreas, and cholestasis and paucity of bile ducts in the liver. Cystic changes were noted in the ovaries, and the kidneys contained a few small cortical cysts. Both patients died at early ages.
Key words: leprechaunism, insulin resistance, Donohue syndrome, islet cell hyperplasia.

Leprechaunism is a rare autosomal-recessive syndrome characterized by a gnome-like appearance, decreased subcutaneous adipose tissue, hirsutism, enlarged genitalia, and intrauterine and neonatal growth retardation[1-10]. Associated metabolic abnormalities include insulin resistance with hyperinsulinemia and postprandial glucose intolerance[11-18]. Mutations in the insulin receptor gene have been described in patients with leprechaunism, and most have homozygote missense or compound heterozygote mutations[15-17]. However, homozygous nonsense mutation in the insulin receptor gene has also been described in an infant with leprechaunism[15]. These patients are also subject to episodes of paradoxical fasting hypoglycemia, which may be due to poor availability of gluconeogenic substrates[1]. As far as we know, this phenomenon is rare in Turkey[18].

Here, we report two unrelated Turkish patients with this syndrome, one of whom was diagnosed with leprechaunism on postmortem examination.

Case Reports

Case 1

A three-month-old male was referred to Hacettepe Children's Hospital for evaluation of hyperglycemia. His history revealed that he was the third product of a marriage between first cousins, and his sister had died of an ovarian cyst following surgery at the age of eight months. His birth weight was 1800 g. Physical examination revealed findings of leprechaunism including growth retardation, widely spaced eyes, depressed nasal bridge, large ears, gum hypertrophy,

* From the Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.
** Professor of Pediatrics, Hacettepe University Faculty of Medicine.
*** Research Assistant in Pediatrics, Hacettepe University Faculty of Medicine.

prominent nipples, hypertrichosis, lack of subcutaneous fat, decreased muscle mass, distended abdomen and large genitalia (Fig. 1). His weight was 2700 g, length 51 cm and head circumference 34.5 cm, all being below the 5th percentile. The liver was palpated four centimeters below the costal margin. The left ventricular posterior wall thickness was found to be nine mm (normal 2.3-3.7 mm), the interventricular septal thickness 11 mm (normal 2.4-3.6 mm), and the left ventricular systolic functions were normal on two-dimensional echocardiography. Doppler echocardiography revealed mitral regurgitation (3.28 m/sn). Renal ultrasound revealed bilaterally enlarged kidneys and the presence of medullary nephrocalcinosis. He also had mild hypertension. His bone age corresponded to his chronological age. Investigations including complete blood count, total bilirubin, cholesterol, alkaline phosphatase, total protein, albumin, erythrocyte sedimentation rate, vanyl mandelic acid (VMA), creatine clearance, lymphocyte subpopulation, complement components and serum immunoglobulins were normal. The skeletal survey revealed no bony abnormality.

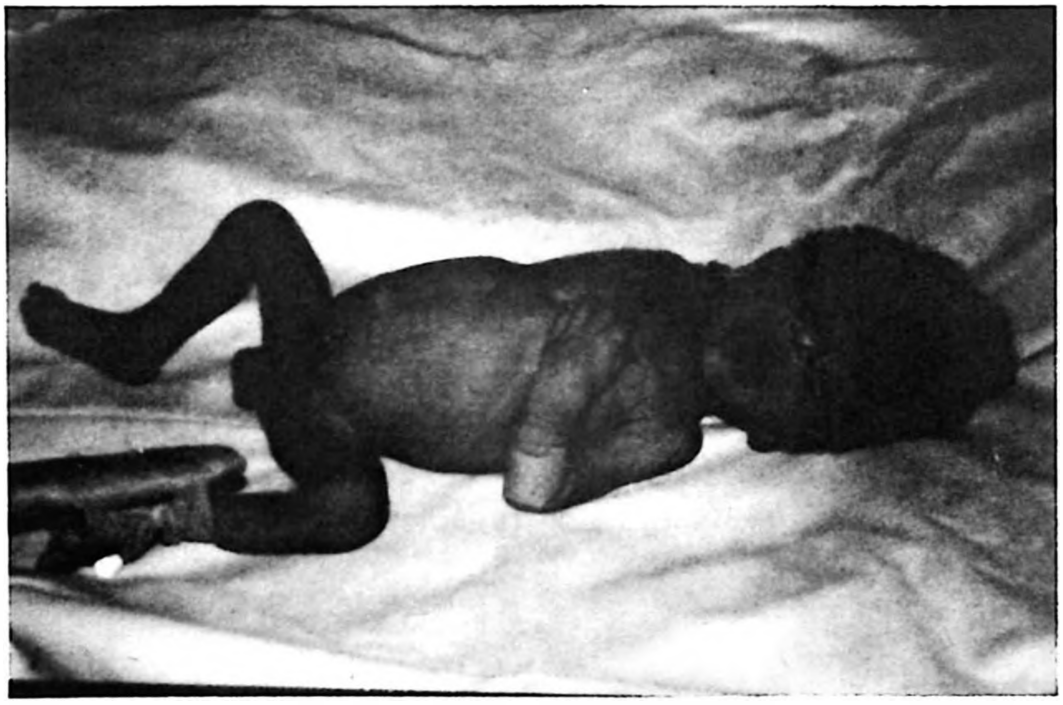

Fig. 1: Appearance of Case 1.

His fasting blood glucose was 348 mg/dl with a simultaneous insulin level of 125 μu/ml (normal 25 < μu/ml). C-peptide was 1.70 pmol/ml (normal < 1.30 pmol). The triglyceride level was 464 mg/dl. He was treated with regular insulin subcutaneously (1 u/kg/day) with no response. Insulin was then administered by continuous infusion intravenously for two days. His insulin requirement decreased gradually and later was given by subcutaneous injection (Fig. 2). The patient unfortunately developed septicemia and died at the age of four months.

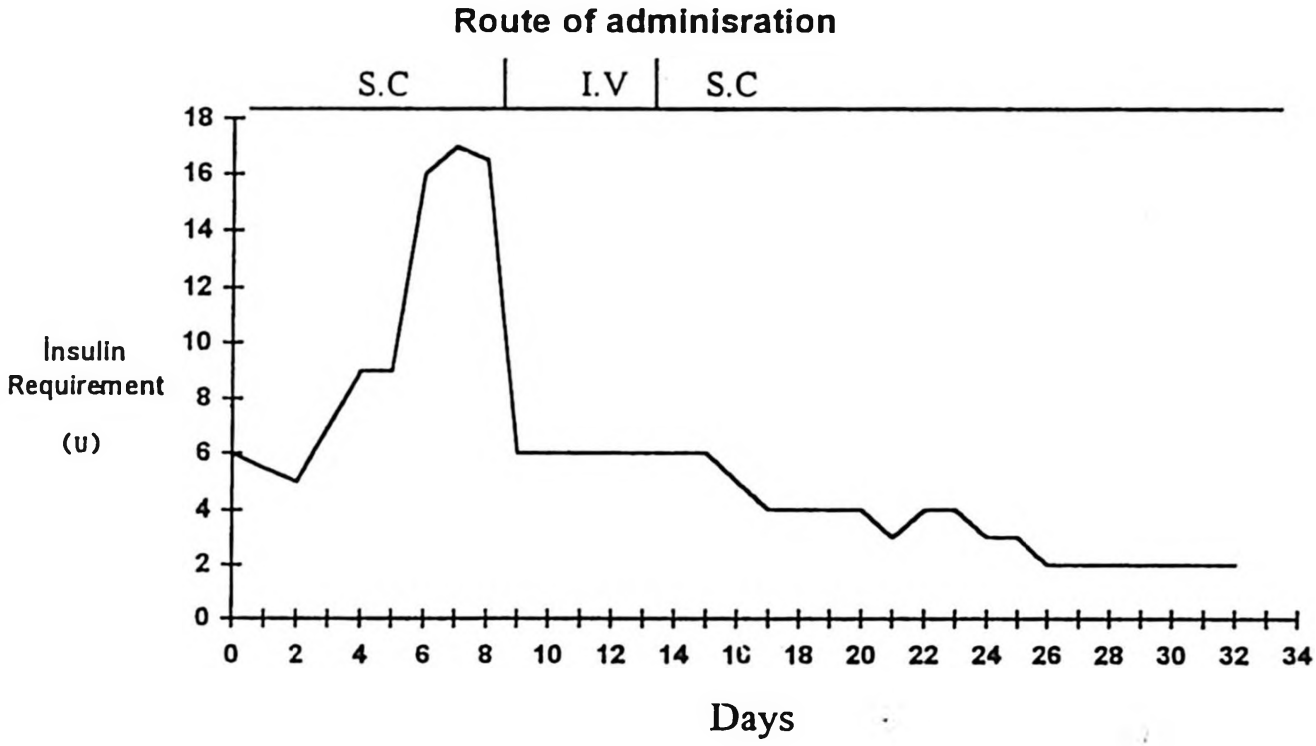

Fig. 2: Insulin requirement of Case 1.

Case 2

A 21-day-old girl was admitted to Hacettepe Children's Hospital for evaluation of a two-week history of abdominal distention and acholic feces. She had been born as the second child to healthy unrelated parents after an uncomplicated term pregnancy and normal delivery. Her birth weight was 1800 g. The first child of the family was healthy and length years old. On physical examination, her weight was 2300 g (< 5^{th} percentile), Length was 43 cm (< 5^{th} percentile) and head circumference was 35 cm (< 10^{th} percentile). She had a peculiar appearance with widely spaced eyes, depressed nasal bridge, facial hypertrichosis especially of the forehead, and decreased subcutaneous tissue. Her eyelids were edematous, and pretibial edema was noted. She also had a distended abdomen and an umbilical hernia.

Results of Laboratory Investigations were as Follows:

Serum total protein 4 g/dl (albumin 2 g/dl), blood glucose 70 mg/dl, alkaline phosphatase 18.9 IU/L (normal 1.5-4.5 IU/L), SGOT 110 IU/L, SGPT 55 IU/L, total serum bilirubin 2.5 mg/dl and direct reacting bilirubin 1.7 mg/dl. The remainder of laboratory investigations including complete blood count, urinalysis, total lipids, cholesterol, blood urea, amino acids and serum α_1 antitrypsin levels were within normal limits. There was a generalized nonspecific aminoaciduria. Prothrombin and partial thromboplastin times were prolonged. Paracentesis

revealed clear ascitic fluid with a protein leves of 1.8 g/dl. Direct Coombs was negative. A search for intrauterine infections was found to be negative. The patient developed a local skin infection and died 10 days after admission. A complete autopsy was performed.

Autopsy findings: Macroscopically there were double renal arteries in the right kidney. The weight of the kidneys was 34 g (normal 18.3 g), while the weight of the pancreas was 5 g (normal 2.1 g). Both ovaries were large for age (Fig. 3). Microscopically emphysema and bronchopneumonia were detected in the lungs. Moreover, hepatocellular and canallicular cholestasis, pseudoglandular transformation of the hepatocyte, extramedullary hematopoiesis, and a mild to moderate increase of fibrous tissue in the portal areas were noted. Paucity of the bile duct was also detected in the portal areas. Iron pigments were present in both hepatocytes and Kupffer cells. Prominent islet cell hyperplasia and hypertrophy of the pancreas were prominent (Fig. 4). There were various degrees of maturation in the ovarian follicles with some cystic changes. Edema was found in the brain tissue. The spleen was congested, extramedullary hematopoiesis and iron pigment were detected and lymphoid follicles were present. There was lymphocyte depletion as well as many cystic and clacified Hassall's corpuscles in the thymus. Lymphoid tissue depletion was not present in the other tissues. Hypertrophy was not found in the myocardial fibris. The kidneys had a few small cortical cysts and congestion; otherwise the histologic appearance was normal. Subcutaneous fat tissue was decreased. No microscopic pathology was detected in the other internal organs. The patient fulfilled the clinical and pathological findings for the diagnosis of leprechaunism.

Fig. 3: Gross appearance of the ovary in Case 2 as compared to age-matched control.

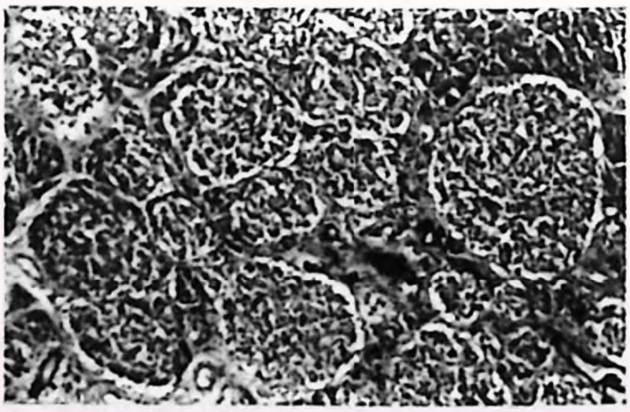

Fig. 4: Hyperplastic islets of Langherhans in the panceras (Case 2). HE x 33.

Discussion

Leprechaunism (Donohue Syndrome) was initially described as dysendocrinism by Donohue in 1948[1]. In 1954, a similar child was born to the same family, and

Donohue et al.[2] proposed the term "leprechaunism" for this disorder. Since then only a few patients with the syndrome have been studied extensively. Our patients had clinical, biochemical and pathological findings for the diagnosis of leprechaunism. Another genetic syndrome with insulin resistance is congenital generalized lipoatrophy[20]. Although these two syndromes share some features such as hypertrichosis, cardiomyopathy, hypertriglyceridemia, hyperglycemia and growth retardation, a syndrome is defined based on the presence or absence of specific clinical features. For example, in lipoatrophic diabetes, there is atrophy of subcutaneous fat. Fasting hypoglycemia, hyperglycemia/hyperinsulinemia after meals, and multiple abnormal features are specific to leprechaunism[21].

At least 52 patients with this syndrome had been reported as of 1993[11]. There are fever than 30 autopsy reports in the literature[1-10, 13, 21-22]. A common pathologic finding is the presence of large, cystic ovaries in females[1-10]. Enlargement of the testis and penis in some male patients and hyperplasia of pancreatic islet cells have been described[1-10]. In a review of 19 autopsy cases of leprechaunism, 12 cases (63%) had pancreatic islet cell hyperplasia[20]. The ovaries and pancreas of Case 2 had these histopathologic features while the sister of Case 1 had died following surgery for ovarian cysts. Hepatic pathology in leprechaunism is not uniform. The histologic appearance of the liver may be normal[3, 5], although, cholestasis bile duct proliferation and hemosiderosis have been detected in the liver of some cases[1, 2, 4, 6-8]. In this disorder, multiple small nodules composed of large, pale foamy hepatocytes with large quantities of glycogen and little fat and iron have been also described in the liver[2, 6]. David and Goodman[22] reported four infants who had hepatic pathology characterized by marked hemosiderosis, cholestasis with severe diminution of the number of portal intrahepatic bile ducts and formation of bile lakes and pseudoglandular structures in the hepatic lobules. The liver of Case 2 also showed cholestasis, hemosiderosis, increased fibrous tissue and paucity of bile ducts in the portal areas. Recently a case of leprechaunism with a congenital bilateral tumors of the ovaries and cytomegalovirus hepatitis was reported[23].

Kidney enlargement as well as histologically tubular dilatation, calcification with intratubular granules or both, have also been described in leprechaunism[2-4, 6, 10]. Medullary nephrocalcinosis and kidney enlargement were present in Case 1. Recently a 13-year-old girl with leprechaunism who developed hypertension and albuminuria was described, and renal and glomerular enlargement as well as morphometric glomerular changes similar to those seen in patients with diabetic nephropathy were observed[24]. Although the weight of the kidneys in our Case 2 was increased, the histologic appearance was normal apart from congestion and the peresence of a few small cortical cysts. Myocardial hypertrophy has also been described in some cases[10, 12], and was present in Case 1 but not

Case 2. Lymphoid tissue depletion in the lymphoid organs and cystic Hassall's corpuscles have been described in some patients[3-5, 7, 8]. The thymus of Case 2 showed lymphocyte depletion and cystic changes of Hassall's corpuscles.

At the molecular level, leprechaunism is associated with defects in insulin binding, insulin receptor autophosphorylation, and kinase activity[14].

However, recently various point mutations in the insulin receptor gene have been detected, and some patients also have functional abnormalities of receptors for insulin-like growth factor 1(IGF)[14, 15]. It has been suggested that different defects of the insulin receptor and receptor-signaling mechanism may be responsible for this phenotypic heterogeneity of leprechaunism[16]. The selective action of insulin on some tissues may be releated to the density or affinity of the IGF-1 receptor. The ovary, heart, muscle vascular endothelium and kidney have IGF-1 receptors[16, 17]. This could explain the ovarian enlargement, myocardial hypertrophy, kidney enlargement and severe intrauterine growth retardation which are reported in some patients with leprechaunism.

REFERENCES

1. Donohue WL. Dysendocrinism. J Pediatr 1948; 32: 739-748.
2. Donohue WL, Uchida I. Leprechaunism. A euphemism for a rare familial disorder. J Pediatr 1954; 45: 505-519.
3. Salmon MA, Weeb JN. Dystrophic changes associated with leprechaunism in a male infant. Arch Dis Child 1963; 38: 530-535.
4. Kallo A, Lakatos I, Szijarto I. Leprechaunism (Donohue's syndrome). J Pediatr 1965; 66: 372-379.
5. Summitt RL, Favora BE. Leprechaunism (Donohue's syndrome). A case report. J Pediatr 1969; 74: 601-610.
6. Ordway NK, Stout LC. Intrauterine growth retardation, jaundice and hypoglycemia in a neonate. J Pediatr 1973; 83: 867-874.
7. Hartdegen RG, Dogliotti M, Rabinoitz, Bartlett R. Leprechaunism. A case report in a black African child. Br J Dermatol 1975; 93: 587-591.
8. Rosenberg AM, Haworth JC, Degroot GW, et al. A case of leprechaunism with severe hyperinsulinemia. Am J Dis Child 1980; 134: 170-175.
9. Kashiwa H, Kawaguchi S, Takeda M, et al. Insulin resistance in an infant with leprechaunism. Acta Paediatr Scand 1984; 73: 701-704.
10. Patterson JH, Watkins WL. Leprechaunism in a male infant. J Pediatr 1992; 60: 730-739.
11. D'Ercole AJ, Underwood LE, Groelke J, et al. Leprechanunism: studies of the relationship among hyperinsulinism, insulin resistance, and growth retardation. J Clin Endocrinol Metab 1979; 48: 495-502.
12. Algazali LI, Khalil M, Devadas K. Syndrome of insulin resistance resembling leprechaunism in five sibs of consanguineous parents. J Med Genet 1993; 30: 470-475.
13. Geffner ME, Kaplan SA, Bersch N, et al. Leprechaunism: in vitro insulin action despite genetic insulin resistance. Pediatr Res 1987; 22: 286-291.

14. Grigorescu F, Herzberg V, King G, et al. Defects in insulin binding and autophosphorylation of erythrocyte insulin receptors in patients with syndromes of severe insulin resistance and their parents. J Clin Endocrinol Metab 1987; 64: 549-556.

15. Krook A, Brueton L, O'Rahilly S. Homozygous nonsense mutation in the insulin receptor gene in infant with leprechaunism. Lancet 1993; 342: 277-278.

16. Kuzuya H, Matsuura N, Sakamota M, et al. Trial of insulin-like growth factor 1 therapy for patients with extreme insulin resistance syndromes. Diabetes 1993; 42: 696-705.

17. Flier JS. Lilly Lecture: Syndromes of insulin resistance. From patient to gene and back again. Diabetes 1992; 41: 1207-1219.

18. Backeljauw PF, Alves C, Eidson M, et al. Effect of intravenous insulin-like growth factor 1 in two patients with leprechaunism. Pediatr Res 1994; 36: 749-754.

19. Tokatlı A, Özsoylu S, Özme S. Leprechaunism (Donohue's syndrome): a case report. Turk J Pediatr 1993; 35: 319-322.

20. Senior B, Gellis SS. The syndromes of total lipodystrophy and of partial lipodystrophy. Pediatrics 1964; 33: 593-612.

21. Szilagyi PG, Corsetti J, Callahan CM, McCormick K, Metlay LA. Pancreatic exocrine aplasia, clinical features of leprechaunism, and abnormal gonadotropin regulation. Pediatr Pathol 1987; 7: 51-61.

22. David R, Goodman RM. Leprechaunism in 4 sibs: new pathologic and electron microscopic observations. Hum Hered 1977; 27: 172A.

23. Brisigotti M, Fabbretti G, Pesce F, et al. Congenital bilateral juvenile granulosa cell tumor of the ovary in leprechaunism: a case report. Pediatr Pathol 1993; 13: 549-588.

24. Ellis EN, Kemp SF, Frindik JP, Elders MJ. Glomerulopathy in a patient with Donohue syndrome (leprechaunism). Diabetes Care 1991; 14: 413-414.

The Turkish Journal of Pediatrics 1997; 39: 395-401

IgA NEPHROPATHY OCCURRING IN TWO SIBLINGS OF THREE FAMILIES*

*Caner Kabasakal MD***, *Ahmet Keskinoğlu MD***
*Sevgi Mir MD****, *Gülçin Başdemir MD*****

SUMMARY: Kabasakal C, Keskinoğlu A, Mir S, Başdemir G. (Department of Pediatric Nephrology, Ege University Faculty of Medicine, İzmir, Turkey). IgA nephropathy occurring in two siblings of three families. Turk J Pediatr 1997; 39: 395-401.

There have been suggestions in the literature that IgA nephropathy may be familial. Genetic factors may influence the development of disease in an association between HLA antigens and IgA nephropathy. At the present time, no conclusion can be drawn. Here, two siblings with typical IgA nephropathy in three families are presented. A relation between HLA and IgA nephropathy was not detected in these family studies. The first family involved a 14-year-old girl and her brother, who at the age of 12 years were admitted to the hospital with macroscopic hematuria. All of the investigations, including serum IgA levels (104 mg/dl and 102 mg/dl respectively) showed normal kidneys with IgA deposition in the mesangium of the glomeruli. In the second family, a 15-year-old boy and his brother at nine years of age were admitted with macroscopic hematuria and gross hematuria, respectively. Laboratory investigations were normal. The serum IgA level (287 mg/dl) was normal in the first patient but the second was elevated at 485 mg/dl. IgA deposits were observed in the glomerular mesangium in these patients. The third family consisted of a 15-year-old boy and his nine-year-old sister who were both admitted with microscopic hematuria. Serum IgA levels (193 and 131 mg/dl, respectively) and laboratory investigations were normal. Renal biopsy specimens revealed C3 and IgA depositions in the glomerular mesangium of both siblings. In the first family the patients were HLA identical, while in the others the siblings were one-haplotype identical. Although IgA nephropathy and HLA antigens are strongly associated in the literature, we could not find this association. *Key words: IgA nephropathy, familial, HLA, lymphocyte subsets.*

Primary IgA nephropathy (pIgAN) is a clinical entity characterized by recurrent hematuria, asymptomatic microhematuria and/proteinuria in the absence of systemic disease, liver disease or lower urinary tract diseases. Inherited factors are known to be important in the pathogenesis of IgA nephropathy[1,2]. Recently, the occurrence of the disease in siblings, or one or more family members of patients, has been reported[3,4]. In this report, two siblings with typical IgAN in three families are presented. Routine biopsy of the siblings at the onset of disease revealed extensive IgA deposition in the mesangium; six patients, two parents and four healthy family members appear not to have a single identical HLA

* From the Department of Pediatric Nephrology, Ege University Faculty of Medicine, İzmir.
** Pediatrician, Ege University Faculty of Medicine.
*** Professor of Pediatrics, Ege University Faculty of Medicine.
**** Associate Professor of Pathology, Ege University Faculty of Medicine.

antigen, and have some immunological abnormalities. We speculated that these findings indicate that the pathogenesis of IgA nephropathy may be under genetic control in some patients.

Case Reports

Family 1

A 14-year-old girl and her 12-year-old brother were admitted to the hospital with macroscopic hematuria. Both were found to have normal blood pressure (100/70 mmHg and 110/80 mmHg). All of the laboratory investigations, including serum IgA levels (104 mg/dl and 102 mg/dl respectively) were normal (Table I).

Table I: Clinical and Laboratory Findings

	Family 1		Family 2		Family 3	
	Boy	Girl	Boy	Boy	Boy	Girl
Age at onset (years)	14	12	15	9	13	8
Follow-up period (years)	7	2	5	1.50	2	0.50
Blood pressure (mmHg)	100/70	110/80	120/80	95/70	120/80	95/70
Urine analysis						
Osmolarity (mOsm/kg)	915	825	625	886	545	950
Proteinuria (g/day)	0.21	0	1.05	0	0	0
Urine sediment (RBC/hpf)	100	50-100	100	100	100	50-100
Dysmorphism (%)	55	60	75	70	50	50
Ca/Creatinine ratio	0.01	0.05	0.08	0.07	0.11	0.13
Blood						
Na (mEq/L)	148	140	138	142	140	140
K (mEq/L)	5.00	4.00	4.50	3.90	4.20	4.60
Ca (mEq/L)	5.50	5.00	5.20	5.40	4.70	5.10
P (mg/dl)	4.60	4.60	3.80	4.00	4.40	4.80
ALP (KAU)	14.70	21.30	14	12.70	14	19
BUN (mg/dl)	10	10	10	10	14	8
Creatinine (mg/dl)	0.50	1.1	0.50	1.20	0.90	0.50
GFR (ml/min/1.73m^2)	183	146	175	71	100	184
Cryoglobulin (mg/dl)	(–)	(–)	(–)	(–)	(–)	(–)
C3 (mg/dl)	104	155	124	90	105	117
IgA (mg/dl)	104	102	287	485	193	101
Radiological investigations (US, IVI, VCU)	N	N	N	N	N	N

RBC/hpf: red blood cells per high-power field, ALP: alkaline phosphatase, BUN: blood urea nitrogen, GFR: glomerular filtration rate, US: renal ultrasound scan, IVU: intravenous urography, VCU: voiding cycto-urethrography.

Immunofluorescence studies of kidney biopsies showed deposition of IgA and C3 in the mesangium of the glomeruli in both siblings. Histological findings revealed mesangial, endothelial cell proliferation with crescent formation in four of 28 glomeruli in the sister (grade II according to the World Health Organization classification) (Fig. 1), and a mild increase in the mesangial matrix in the brother (grade I).

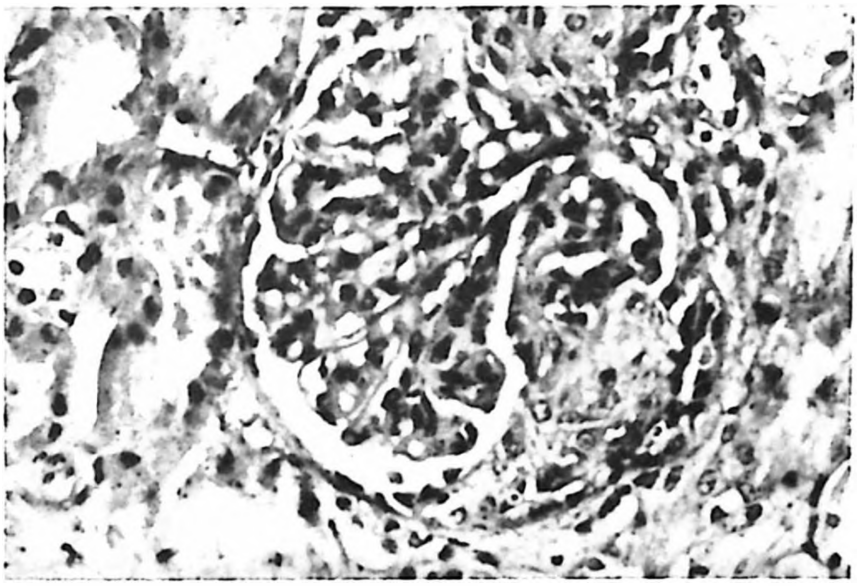

Fig. 1: One of four glomeruli with crescent formation showing a global mesangial increase in the renal biopsy from the female patient with IgA nephropathy in the first family. (H.E. x 40).

Family 2

A 15-year-old boy and his nine-year-old brother were admitted with macroscopic hematuria and gross hematuria, respectively. Blood pressures were normal (120/80 mmHg and 95/70 mm/Hg). The serum IgA levels were normal in the first patient (287 mg/dl) and elevated in the second (485 mg/dl). The other laboratory investigations were normal (Table I). On immunofluorescence studies of renal biopsy specimens, C3 and IgA depositions were observed in the glomerular mesangium in both patients. Light microscopy showed a segmental increase in matrix with cell proliferation in the first boy (grade II, Fig. 2) and segmental cell proliferation in his younger brother (grade I).

Family 3

A 15-year-old boy and his nine-year-old sister were admitted with microscopic hematuria. Blood pressures were normal (120/70 mmHg and 90/70 mmHg). Serum IgA levels (193 mg/dl and 131 mg/dl, respectively) were normal, as were

Fig. 2: Glomerulus in the renal biopsy from the first male child of the second family shows segmental minimal mesangial cell proliferation (arrows) and increased matrix (arrowhead). (H.E. x 40).

the other laboratory investigations (Table I). Immunofluorescence studies of renal biopsy specimens revealed C3 and IgA depositions in the glomerular mesangium in both siblings. On light microscopy studies an increase in matrix with segmental and global cell proliferation in the brother and a mild increase in matrix at some sites in the sister were observed (grade II).

HLA Characteristics

HLA typing was performed in all six patients, and in the fathers and two healthy siblings in the first and second families. In the first family the mother had died; the mother in the second family and the parents and healthy siblings in the third family were unwilling to participate in the investigation. Although there were no HLA-identical siblings in the second family, we observed identity/similarity in HLA antigens [HLA A2-A3-B52(5)-B35-DR11-DR13-DQ6-DQ7] in the first family and [HLA A68(28)-B27-DR4-DQ7] in the third family (Table II).

The pedigrees for the families are shown in Figure 3.

Immunological Findings

The lymphocyte subsets of peripheral blood were examined in the patients by flow cytometry. No significant change in T cell, CD4 or CD8 levels or in the CD4/CD8 ratio were observed in flow cytometry. Increased expression of IL-2 receptors and HLA-DR (+) T cell on T lymphocytes were observed during the

Table II: HLA Characteristics of the Patients and their Relatives

Family 1	HLA A	HLA-B	HLA-DR	HLA-DQ
Male patient	A2, A3	B35, B52	DR11, DR13	DQ6, DQ7
Female patient	A2, A3	B35, B52	DR11, DR13,	DQ6, DQ7
Sister	A1, A10	B16, B50	DR4, DR7	DQ2, –
Father	A1, A3	B50, B52	DR11, DR7	DQ2, DQ7
Mother	Exitus			
Family 2				
Elder male patient	A2, –	B5, –	DR4, DR13,	DQ2, DQ7
Young male patient	A2, –	B44, B35	DR4, DR11	DQ2, DQ7
Brother	A2, A33	B44, B22	DR11	DQ7
Daughter	A2, A24	B5, B44	DR4, DR11	
Father	A2, A24	B5, B22	–	–
Family 3				
Male patient	A68, A29	B8, B27	DR17, DR4	DQ2, DQ7
Female patient	A24, A68	B27, B35	DR4, DR11	DQ4, DQ7

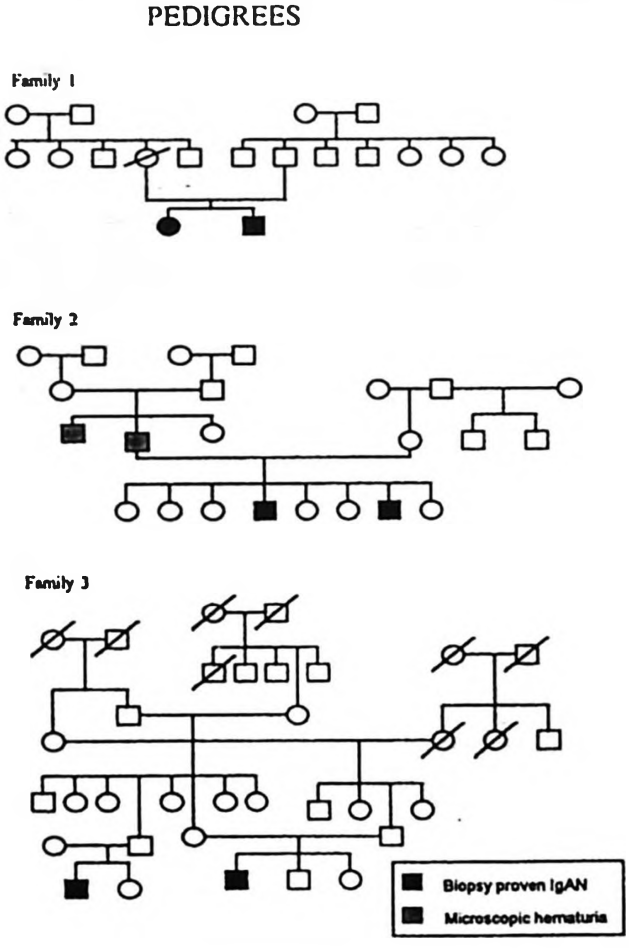

Fig. 3: Pedigrees of three families.

acute phase. High serum IgA levels and an increased number of IgA-bearing B lymphocytes were observed in the patient with IgAN. Immunological findings are shown in Table III.

Table III: The Findings of Cell-mediated Immunity in the Patients with IgAN

	Family 1		Family 2		Family 3		Controls
	Boy	Girl	Boy	Boy	Boy	Girl	n=16
CD3+T cell (%)	73	81	54	68	54	71	69.39
CD19+B cell (%)	11	9	11	8	5	8	7.36
CD4+T cell (%)	44	48	26	38	27	49	42.31
CD8+T cell (%)	36	38	33	31	39	32	31.44
CD4/CD8	1.40	1.30	0.80	1.20	0.70	1.50	1.42
HLA-DR(+) T cell (%)	5	9	10	4	3	12	4.72
IL-2R (%)	11	5	4	6	3	11	3.52
IgA binding B cell (%)	49	64	63	59	56	67	9
IgG binding B cell (%)	14	17	11	13	16	39	13.81
IgM binding B cell (%)	8	6	7	4	5	9	6.50

Discussion

Although intensive efforts have been made toward elucidating the pathogenesis of pIgAN, it remains unclear[4,5]. However, observing the occurrence of the disease in siblings and in renal allografts of patients with pIgAN, many investigators have reported the presence of familial pIgAN[3]. On the other hand, since no serologic marker has been found, diagnosis of the disease depends on an invasive procedure such as renal biopsy, even in family members or in patients with minimal urinary findings[6]. In the absence of any environmental factor, the pathogenesis of pIgAN has been suggested to include a genetic mechanism[1,7]; however, the mode of inheritance cannot be defined[1,2]. An association between pIgAN and DR4, B35 has been postulated in some countries since 1976[8] although more recent report have not confirmed a definite correlation between HLA and pIgAN[9,10]. In this study HLA A2-B35-B5-DR4-D11 and DR13 were found to be the most frequent HLA antigens. However, HLA A2-B35-B5 and A24 are the most common HLA class I antigens, but neither class II antigen is common in western Turkey[11]. Therefore, we could not accept the presence of these antigens as an association with pIgAN.

In conclusion, the existence of two siblings with IgA nephropathy in three families may support the hypothesis that genetic factors are important in the pathogenesis

of pIgAN in some patients. Some immunological abnormalities, such as high serum levels of IgA, an increased number of IgA-bearing B lymphocytes, increased expression of IL-2 receptors and HLA-DR (+) T cells, indicate that at least some determinants of pIgA may be under genetic control. Furthermore, some environmental factors may have effects on this familial tendency.

REFERENCES

1. Welch TR, Beischel LS, Choi EM. Molecular genetics of C4B deficiency in IgA nephropathy. Hum Immunol 1989; 26: 353-363.
2. Hiki Y, Kobayashi Y, Ookubo M, Obata F, Kashiwagi N. Association of HLA-DQw4 with IgA nephropathy in the Japanese population. Nephron 1991; 58: 109-111.
3. Julian BA, Quiggins PA, Thompson JS, Woodford SY, Gleason K, Wyatt RJ. Familial IgA nephropathy. Evidence of an inherited mechanism of disease. N Engl J Med 1985; 312: 202-208.
4. Waldo FB, Beischel L, West CD. IgA synthesis by lymphocytes from patients with IgA nephropathy and their relatives. Kidney Int 1986; 29: 1229-1233.
5. Noel LH, Deschamps B, Jungers P, et al. HLA antigens in three types of glomerulonephritis. Clin Immunol Immunopathol 1978; 10: 19-23.
6. Croker BP, Dawson DV, Sanfilippo F. IgA nephropathy. Correlation of clinical and histologic features. Lab Invest 1983; 48: 19-24.
7. Hiki Y, Kobayashi Y, Tateno S, Sada M, Kashiwagi N. Strong association of HLA-DR4 with benign IgA nephropathy. Nephron 1982; 32: 222-226.
8. MacDonald IM, Dumble LJ, Kincaid Smith P. HLA and glomerulonephritis. In: HLA and Disease. Paris: INSERM; 1976: 203.
9. Fauchet R, Le Pogamp P, Genetet B, et al. HLA DR4 antigen and IgA nephropathy. Tissue Antigens 1980; 16: 222-226.
10. Fauchet R, Guegen M, Genetet B, et al. HLA DR antigens in IgA nephropathy (Berger's disease). N Engl J Med 1980; 302: 1033.
11. Mir S, Kütükçüler N, Kavaklı K, Tekgül H, Cura A. A study of HLA-A, -B, -C and -DR antigens in 630 cases resident in western Turkey. Med J Ege University 1991; 1: 95-97.

The Turkish Journal of Pediatrics 1997; 39: 403-407

AKINETIC MUTISM DUE TO DIPHENYLHYDANTOIN TOXICITY*

*Sarenur Tütüncüoğlu MD***, *Mehmet Kantar MD****
*Hasan Tekgül MD*****, *Can Candan MD******

SUMMARY: Tütüncüoğlu S, Kantar M, Tekgül H, Candan C. (Neurology Unit, Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Akinetic mutism due to diphenylhydantoin toxicity. Turk J Pediatr 1997; 39: 403-407.

Akinetic mutism (AM) is a rare, specific, unconscious state. An AM patient seems to be awake, lacks mental activity, is unable to speak, and does not respond to any environmental stimulus. Cyclical sleep and awake states are maintained, and incontinence is present. Various factors such as tumor, vascular events, drug use and radiotherapy are responsible for the development of AM.
A 12-year-old epileptic patient displayed AM and diphenylhydantoin toxicity (DPH). She seemed awake, was unable to speak or to understand, and had no movements with either spontaneous or noxious stimuli. Her serum DPH level was greater than 40 µg/ml. Magnetic resonance imaging (MRI) showed mild cerebellar atrophy. All known causes of AM were excluded. The AM state in this patient was considered to be due to toxic DPH levels. She regained her motor and mental activity within two months after carbamazepine was administered to replace DPH. She was symptom-free when examined at the two-year follow-up. No similar adverse effect of DPH has been reported to date. *Key words: diphenylhydantoin, akinetic mutism, adverse effect.*

Akinetic mutism (AM) is a rare, specific, unconscious state. An AM patient appears to be awake, lacks mental activity, is unable to speak, and does not respond to any environmental stimulus. Cyclical sleep and awake states are maintained, and incontinence is present. Possible causes of AM include cerebrovascular accident, craniopharyngioma, cardiac arrest, severe hypoglycemia, carbon monoxide intoxication, several drugs, central pontine myelinolysis (CPM), radiotherapy, trauma, tumor, hydrocephaly, Wernicke-Korsakoff disease and tuberculous endocarditis[1-7]. A case with AM possibly due to diphenylhydantoin (DPH) toxicity is presented.

Case Report

A 12-year-old female had been taking DPH at a dose of 300 mg/d for generalized convulsions. She had recently been admitted to a state hospital for gait disorder

* From the Neurology Unit, Department of Pediatrics, Ege University Faculty of Medicine, İzmir.
** Associate Professor of Pediatrics, Ege University Faculty of Medicine.
*** Research Assistant in Pediatrics Ege University Faculty of Medicine.
**** Pediatrician and Fellow in Pediatric Neurology, Ege University Faculty of Medicine.
***** Research Assistant in Neurology, Trakya University Faculty of Medicine, Edirne.

and ataxia, having deteriorated over the previous three months. She had dysarthria, truncal ataxia and horizontal nystagmus on examination. DPH was discontinued as the serum level was greater than 40 µg/ml. The patient experienced fever on day 10 of admission, which was considered to be due possibly to a lung infection. Later she was admitted to our hospital. On admission she appeared to be awake, but was unable to speak or to understand any speech and uncooperative. No extremity movements were observed with either spontaneous or noxious stimuli. She had orofacial dyskinesia and incontinence, and displayed cyclical sleep/awake states. Other physical findings were normal.

Laboratory examinations including routine blood and urine analysis, liver enzymes, ammonia, urea, lipid fractions, LDH, immunoglobulins, thyroid hormone levels, lactic acid, serum B_{12} level, and serum and urine aminoacid chromatographies were normal. Group agglutination tests, monospot test, HBsAg, anti-HCV, anti HIV I-II antibodies, VDRL test, anti-CMV antibodies, and anti-HSV antibody in cerebrospinal fluid (CSF) were negative. The CSF-IgG index and CSF-myelin basic protein (MBP) were normal. The ECG, EEG (on day 1,5, and 25), EMG, VEP, SEP recordings and Tc^{99m} HMPAO-Brain SPECT were normal. Cranial MRI (on days 1,7 and 21) showed only cerebellar atrophy (Figs. 1, 2). Brainstem auditory evoked potential (BAEP) recording showed prolonged bulbopontine transmission and normal wave 1 latency. The serum folic acid level was 1.1 ng/ml (normal: 2.5 ng/ml). Megaloblastic changes were not observed. No clinical or laboratory data compatible with lung infection were present. After

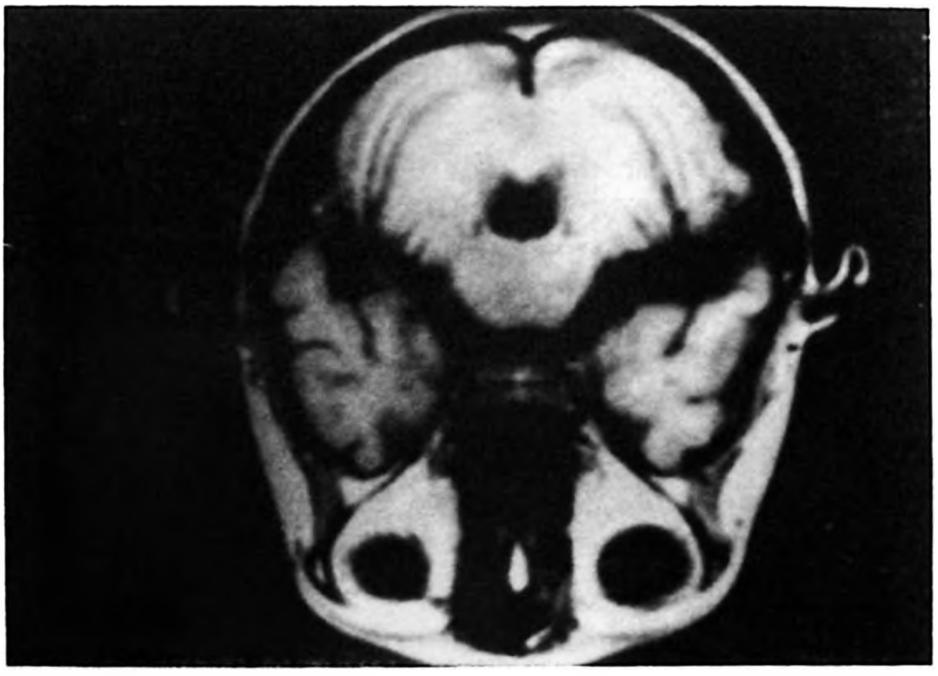

Fig. 1: Cerebellar foliae are prominent (Cranial MR-T1W1).

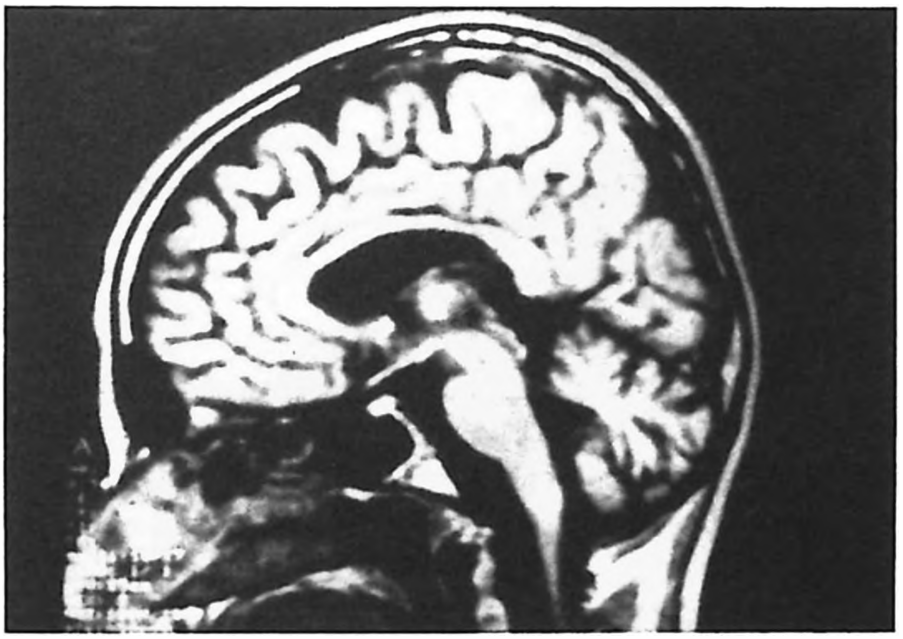

Fig. 2: Note mild cerebellar atrophy (Cranial MR-T1W1).

cessation of DPH, its serum level declined quickly. Carbamazepine administration at a dose of 300 mg/dl, and folic acid at 5 mg/d were instituted. Her spontaneous limb movements began on day 5, and orofacial dyskinesia was resolved within one week. She was able to walk and obey commands within the second month and to pronounce single words in the 10th week after admission. At the end of the 2nd month she had no cerebellar dysfunction. Four months later she was able to speak and had no seizures. Her repeated BAEP response was found to be normal.

Discussion

Diphenylhydantoin is a well-known anticonvulsant drug which has several adverse effects. These effects include nausea, vomiting, nystagmus, diplopia, ataxia, drowsiness, encephalopathy (depressed mental function, increase in seizures), depression, confusion, dyskinesia, myoclonus, dystonia, total external ophthalmoplegia, myasthenic state, and progressive or transient hemiparesis[8]. Akinetic mutism is characterized by a lack of responsiveness in the presence of preserved vigilance caused by many factors[2-7]. AM due to DPH use has not been reported to date.

Our patient displayed the AM state. All known vascular, cardiac, toxic, traumatic, infectious, tumoral and demyelinating factors that may cause AM were excluded. Our patient had only mild cerebellar atrophy and a low serum folic acid level,

which were attributable to long-term DPH use. Central pontine myelinolysis (CPM) may also cause AM[3]. Our patient had a normal CSF-IgG index, MBP levels and cranial MRIs. She only showed an abnormal BAEP response, which itself was not sufficient to make the diagnosis of CPM without other supportive tests. She had been taking DPH for 11 years, and her serum DPH level was greater than 40 µg/ml. She had cerebellar findings and orofacial dyskinesia, which are compatible with toxic DPH levels.

No association between DPH and AM has been defined in recent years. Folic acid depletion in the presence or absence of anemia has also not been reported to be related with AM. The anticonvulsant action of DPH pertains to the movement of sodium and calcium across cellular membranes by either passive or active transport. The pathophysiological mechanisms responsible for the AM state are not well understood. However, reduced arousal of cortical functions due to lesions at or rostral to the mesodiencephalic junction or impaired activation of the motor system following bilateral damage to the frontal lobes have been considered in the pathogenesis[9]. SPECT analysis has been found to demonstrate decreased blood supply to the frontal lobes[10]. Although the exact pathophysiological implications are not yet known, clinical trials with bromocriptine, a dopamine receptor agonist, have been promising and indicate the involvement of dopaminergic pathways[11]. Our patient had a normal SPECT analysis, demonstrating the absence of an organic, frontal lobe lesion. She also recovered spontaneously without taking bromocriptine.

We were unable to detect any known cause of AM in our patient. She displayed some signs of toxic DPH levels. We concluded that DPH may have been responsible for AM in our patient.

REFERENCES

1. Plum F, Posner JB. Diagnosis of Stupor and Coma (3rd ed). Philadelphia: Davis; 1980: 1-13.
2. Parmar MS. Akinetic mutism after baclofen. Ann Intern Med 1991: 115: 499-500.
3. Bird GL, Meadows J, Goka J, et al. Cyclosporin-associated akinetic mutism and extrapyramidal syndrome after liver transplantation. J Neurol Neurosurg Psychiatr 1990; 53: 1068-1071.
4. Thompson CB, June CH, Sullivan KM, Thomas ED. Association between cyclosporin neurotoxicity and hypomagnesaemia. Lancet 1984; 2: 1116-1120.
5. Gütling E, Landis T, Kleihues P. Akinetic mutism in bilateral necrotizing leucoencephalopathy after radiation and chemotherapy: electrophysiological and autopsy findings. J Neurol 1992; 239: 125-128.
6. Mann MW, Degos JD. Akinetic mutism in Wernicke-Korsakoff disease: a case report. J Neurol Neurosurg Psychiatry 1988; 51: 588-590.
7. Fesenmeier JT, Kuzniecky R, Garcia JH. Akinetic mutism caused by bilateral anterior cerebral tuberculous obliterative arteritis. Neurol 1990; 40: 1005-1006.

8. Mervyn JE, Tyrer JH. Anticonvulsant Therapy: Pharmacological Basis and Practice (3rd ed). Edinburgh: Churchill Livingstone; 1989: 98-100.
9. Ackermann H, Ziegler W. Akinetic mutism—a review of the literature. Fortschr Neurol Psychiatr 1995; 63: 59-67.
10. Satomi K, Terashima Y, Goto K, Arakawa M, Ito H. Capsular pseudobulbar mutism in a patient of lacunar state. Rinsho Shinkeigaku 1990; 30: 299-303.
11. Muller U, von-Cramon DY. The therapeutic potential of bromocriptine in neuropsychological rehabilitation of patients with acquired brain damage. Prog Neuropsychopharmacol Biol Psychiatry 1994; 18: 1103-1120.

The Turkish Journal of Pediatrics 1997; 39: 409-413

NETHERTON'S SYNDROME AND NEONATAL HYPERNATREMIA*

A Case Report

*Hacer Ergin MD**, *İlknur Kılıç MD****, *Gülsevin Tekinalp MD*****

SUMMARY: Ergin H, Kılıç İ, Tekinalp G. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Netherton's syndrome and neonatal hypernatremia. A case report. Turk J Pediatr 1997; 39: 409-413.

Netherton's syndrome is characterized by ichthyosiform desquamation, bamboo hair and often atopic diathesis. It is transmitted as an autosomal-recessive trait. In this paper we report a baby with Netherton's syndrome who developed hypernatremia during the neonatal period. This complication should be remembered in erythrodermic infants as a preventable cause of neonatal morbidity. *Key words: Netherton's syndrome, newborn, hypernatremia.*

In 1958, Netherton's syndrome was first described as a hair shaft defect (bamboo hair), pruritis and congenital ichthyosiform erythroderma (CIE)[1,2]. Since then, Netherton's syndrome has come to indicate the triad of the hair shaft defect, an atopic diathesis and either ichthyosis linearis circumflexa (ILC) or CIE. It is transmitted as an autosomal-recessive trait[3-5]. In addition to these diagnostic criteria, some patients have been noted to have hypernatremia in the newborn period, aminoaciduria, mental deficiency, neurologic defects, delayed growth, recurrent infections, and hyper-or hypoglobulinemia[2].

In this report, we present a newborn with a rare combination of Netherton's syndrome, CIE and hypernatremia.

Case Report

A seven-day-old girl was admitted to our hospital with erythematous, peeling skin. Her parents were first-degree relatives who were both healthy. She was born by normal vaginal delivery at term (39 weeks) following an uneventful pregnancy. At birth she weighed 2550 g (10^{th}-25^{th} percentile) and had an Apgar score of seven at five minutes. It was learned that the patient's three-year-old brother had died of degenerative brain disease (unknown origin). The patient's

* From the Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Assistant Professor of Pediatrics and Neonatologist, Pamukkale University Faculty of Medicine, Denizli.

*** Assistant Professor of Pediatrics, Pamukkale University Faculty of Medicine.

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

other brother, who had had similar skin lesions, had also died in the newborn period. She had one healthy sister. Her three nephews, whose parents had first-degree consanguinity, had ichthyosis. One of them was a stillbirth, and the others had died with similar skin lesions at 1.5 and two months of age. Until our patient was admitted to our hospital she was breastfed. She had no history of vomiting or diarrhea.

Physical examination revealed a small, hypoactive and spactic infant with a generalized erythroderma and lamellar scaling. She did not have eyelashes (Fig. 1). She had purulent discharge from the umbilicus, and her subcutaneous fat tissue was diminished. We could not evaluate the skin turgor of the patient because of the presence of the skin lesions and the loss of subcutaneous fat tissue. Her anterior fontanel was depressed, and her eyes were sunken. We accepted the patient as having a moderate degree of dehydration.

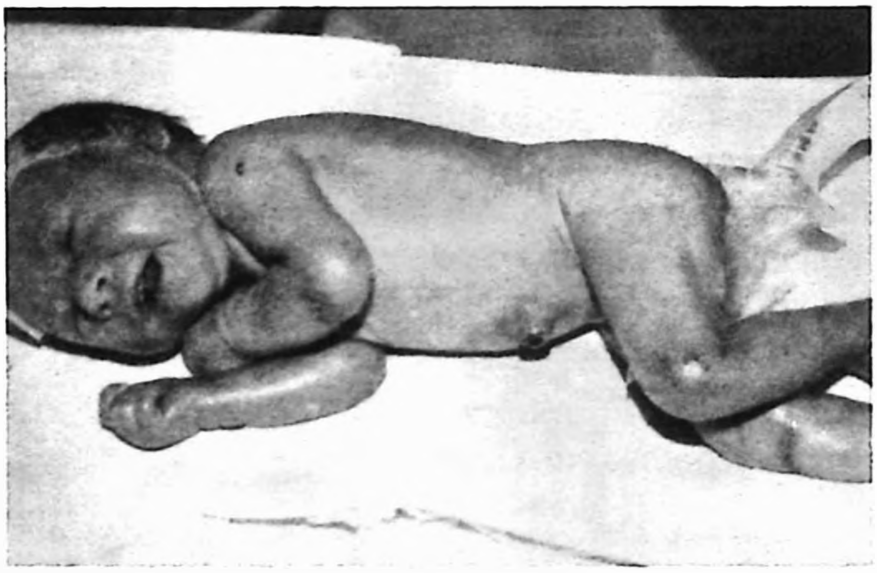

Fig. 1: The infant with generalized erythroderma and lamellar scaling.

Microscopically, the patient's, her parents' and her healthy sister's hair was normal. Her hemoglobin was 14.2 g/dl, hematocrit 47 percent, and the white blood cell count was 7,500/mm^3 with 60 percent neutrophils, 36 percent lymphocytes, and four percent band forms. Red blood cells were normochromic and macrocytic. Urine analysis was normal excluding mild proteinuria. The urinary specific gravity was 1025. Other studies indicated serum sodium 174 mEq/L, potassium 5.8 mEq/L, chloride 98 mEq/L, total protein 6.6 g/dl, albumin 4.4 g/dl, BUN 50 mg/dl, uric acid 8.6 mg/dl, creatinine 1.2 mg/dl, calcium 8.7 mg/dl and phosphorus 7 mg/dl. Abdominal and cranial ultrasonic examination,

plasma and urinary amino acid analyses, and scalp hair on light microscopic examination were normal. Staphylococcus aureus was isolated from the umbilical culture.

In order to correct the fluid and electrolyte imbalance, we began administering intravenous fluids to the patient (160 ml/kg/day, 1/5 isotonic saline in five percent dextrose solution). We treated the omphalitis with penicillin (100,000 U/kg/day) and netilmycin (5 mg/kg/day). We administered phenobarbital for spasticity and five percent lactic acid in vaseline for the skin lesions. After the fluid and electrolyte imbalance was corrected, her mild proteinuria resolved the spasticity was corrected and the biochemical values became normal. Lamellar scaling cleared up and the erythema diminished after treatment with five percent lactic acid in vaseline. After one week of treatment in the hospital, the patient was discharged. It was learned that she died at home at the age of 40 days. The cause of death was not detected.

Discussion

Netherton's syndrome has a clinical spectrum ranging from congenital ichthyosiform erythroderma (CIE) to ichthyosis linearis circumflexa (ILC). The skin lesions in the newborn period are characterized by lamellar ichthyosis; in adults these lesions change to ichthyosis linearis circumflexa[6]. Our patient with diffuse erythroderma and lamellar ichthyosis can be defined as having CIE. Beginning with a collodion baby appearance is a very rare condition, was not seen in a series of 69 patients with Netherton's syndrome[7]. We also did not detect a collodion baby appearance in our patient. Hair shaft anomalies in Netherton's syndrome are different manifestations of the same keratinization defect. These hair anomalies represent a large spectrum such as trichorrhexis nodosa, pili torti, monilethrix, angle-bent hair, and trichorrhexis invaginata, which is the latest stage[8]. Different hair shaft defects can be detected in the same family or in different regions of the same patient[9]. Hair shaft dysplasia is very rare in the newborn period, and can be due to the absence of dysplasia and a later presentation or to insufficient examination. In a series of 90 newborns with Netherton's syndrome, a hair shaft defect was noted in four patients[8]. Bamboo hair and other hair shaft defects were not detected in either the parents or our patient. We think that further investigations for hair dysplasia, would be beneficial.

In the literature, severe hypernatremia in the newborn period was reported in 13 of 99 patients without gastrointestinal and renal diseases[2,8,10]. It has been reported that the CIE phenotype of Netherton's syndrome is associated with severe hypernatremia[8]. Hypernatremia, rarely seen in the neonatal period, was also found in our patient. The causes of hypernatremia, including diarrhea, high

fever, polyuria, insufficient water intake due to poor sucking, and treatment with $NaHCO_3$, were not detected in our case. The urinary specific gravity showed a concentrating ability, making a diagnosis of diabetes insipidus unlikely. A diagnosis of hypernatremia, which has been recognized in premature infants born between 24 and 30 weeks of gestation, was not considered because our patient was a term baby. The mechanism of hypernatremia in Netherton's syndrome is not clear. Abnormal renal sodium handling, which is thought to be one etiology of hypernatremia, has not been found in patients with Netherton's syndrome. The most probable cause of hypernatremia in patients with Netherton's syndrome is the loss of transepidermal water. On electron microscopic examinations, the finding of basal membrane thickness supports this hypothesis[11]. However, if this hypothesis is correct, the question arises as to why hypernatremia is not reported more often in neonates who are erythrodermic for another reason, not just as a result of Netherton's syndrome.

In the neonatal period, in the lamellar ichthyosis form of Netherton's syndrome, the skin loses its ability to form an effective barrier. Both skin water homeostasis and temperature regulatory function are defective. Garty et al.[10] reported hypothermia in four of five patients with hypernatremic dehydration and congenital lamellar ichthyosis. We did not detect hypothermia in our patient during her hospitalization.

In the follow-up of patients with Netherton's syndrome, 26 percent of cases have been found to have staturo-ponderal retardation, 16 percent mental retardation, and 13 percent atopic diathesis. Intestinal malabsorption also has a role in staturo-ponderal retardation[8]. We observed our patient only in the newborn period, so her mental development, atopic status and staturo-ponderal state could not be evaluated.

Oral vitamin A, zinc, topical tretinoin, topical steroids, propylene glycol and five percent lactic acid lotions have been used and found ineffective in the treatment of Netherton's syndrome[12]. In other studies, five percent[13] and 12 percent lactic acid solutions[12] were reported as effective. Photochemotherapy and chemotherapy (cyclophosphamide) have also been used in therapy[9,14]. We treated our patient's skin lesions with five percent lactic acid in vaseline.

The possible diagnoses of Netherton's syndrome and hypernatremia should be remembered in newborns with lamellar ichthyosis. Early recognition of hypernatremia and prompt institution of corrective fluid and electrolytes may prevent avoidable neonatal morbidity in these erythrodermic neonates.

REFERENCES

1. Netherton EW. A unique case of trichorrhexis nodosa. "Bamboo hairs." Arch Dermatol 1958; 78: 483-487.

2. Jones SK, Thomason LM, Surbrugg SK, Weston WL. Neonatal hypernatremia in two siblings with Netherton's syndrome. Br J Dermatol 1986; 114: 741-743.

3. Wilkinson RD, Curtis GH, Hawk WA. Netherton's disease: tricorrhexis invaginata (bamboo hair), congenital ichthyosiform erythroderma and atopic diathesis; a histopathological study. Arch Dermatol 1964; 89: 46-54.

4. Altman J, Stroud J. Netherton's syndrome and ichthyosis linearis circumflexa: a psoriasiform ichthyosis. Arch Dermatol 1969; 100: 550-558.
5. Greene SL, Muller SA. Netherton's syndrome. Report of a case and review of the literature. J Am Acad Dermatol 1985; 13: 329-337.
6. Dupre A, Bonafe JL, Carrere S. Comel's linear circumflex ichthyosis and Netherton's syndrome. General conceptions based on a study of 4 cases. Ann Dermatol Venereol 1978; 105: 49-54.
7. Larrégue M, Ottavy N, Bressieux JM, Lorette G. Collodion baby: 32 new case reports. Ann Dermatol Venereol 1986; 113: 773-785.
8. Plantin P, Delaire P, Guillet MH, Labouche F, Guillet G. Netherton's syndrome: current aspects. Apropos of 9 cases. Ann Dermatol Venereol 1991; 118: 525-530.
9. Kassis V, Nielsen JM, Klem-Thomsen H, Dahl-Christensen J, Wadskov S. Familial Netherton's Disease. Cutis 1986; 38: 175-178.
10. Garty BZ, Wiseman Y, Metzker A, Reisner SH, Nitzan M. Hypernatremic dehydration and hypothermia in congenital lamellar ichthyosis. Pediatr Dermatol 1985; 3: 65-68.
11. Larrégue M, Bressieux JM, Bonafe JL, et al. TI et érythrodermie acrofigurée: Hétérogénéite du syndrome dit de "Netherton". Ann Dermatol Venereol 1989; 116: 685.
12. Wehr RF, Hickman J, Krochmal L. Effective treatment of Netherton's Syndrome with 12% lactate lotion. J Am Acad Dermatol 1988; 19: 140-142.
13. Erdem G. Treatment of collodion babies with a topical preparation containing lactic acid. Turk J Pediatr 1982; 24: 97-101.
14. Nagata T. Netherton's syndrome which responded to photochemotherapy. Dermatologica 1980; 161: 51-56.

The Turkish Journal of Pediatrics 1997; 39: 415-420

A CASE OF BRACHYOLMIA*

*Nalan Karabıyık MD**, Fatma Oğuz MD***, Müjgan Sıdal MD****
*Nezih Hekim MD*****, Hülya Kayserili MD******

SUMMARY: Karabıyık N, Oğuz F, Sıdal M, Hekim N, Kayserili H. (Department of Pediatrics, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey). A case of brachyolmia. Turk J Pediatr 1997; 39: 415-420.

Brachyolmia refers to a form of skeletal dysplasia characterized by general platyspondyly without significant epiphyseal, metaphyseal or diaphyseal changes in long bones. Three, possibly four, types of brachyolmia have been defined: Type I-Hobaeck-Toledo type, Type II-Maroteaux and Type III. We report a patient with brachyolmia and present the clinical and radiological findings. A 15-year-old boy presented to our Outpatient Department because of his short stature. His height, weight, head circumference and arm span were 127 cm (<3rd percentile), (3rd percentile) 39 kg, 55 cm (50th-75th percentile), and 142 cm respectively, and his upper segment/lower segment ratio was 0.91. His neck and trunk were short. He had severe kyphoscoliosis. Slit-lamp examination was normal. Radiologic features included platyspondyly in cervical, thoracic and lumbar vertebrae as well as kyphoscoliosis. Bilateral coxa valga and mild acetabular irregularities were noticed on pelvic radiographies. Levels of chondroitin and heparan sulphate as well as the glycosaminoglycan/creatinine ratio were elevated in the 24-hour urine specimen. The activities of N-acetylgalactosamine-6-sulphatase, β-galactosidase and β-hexosaminosidase were all normal in fibroblast culture. Although the x-ray findings of this patient are consistent with both Types I and III, recessive inheritance and glycosaminoglycan anomalies point to Type I brachyolmia. *Key words: skeletal dysplasia, brachyolmia, dwarfism.*

The brachyolmia are associated with short-trunk dwarfism, and major radiographic changes are present in the spine. Usually there are no epiphyseal, metaphyseal or diaphyseal changes in the long bones. There are three, possibly four, types of brachyolmia. Type I (Toledo-Hobaek Type), Type II (Maroteaux Type), and Type III (Dominant Type). In this paper, we describe a male patient with the radiological features of brachyolmia.

* From the Department of Pediatrics, İstanbul University İstanbul Faculty of Medicine, İstanbul.
** Research Assistant in Pediatrics, İstanbul University İstanbul Faculty of Medicine.
*** Associate Professor of Pediatrics, İstanbul University İstanbul Faculty of Medicine.
**** Professor of Pediatrics, İstanbul University İstanbul Faculty of Medicine.
***** Associate Professor of Biochemistry, Pakize Tarzı Maternity Hospital, İstanbul.
****** PhD Student in Medical Genetics, İstanbul University Institute of Child Health, İstanbul.

Case Report

The patient, a 15-year-old male, was the third child of first-degree-cousin parents (G6 P6 Ab0). The pedigree is shown in Figure 1. The second, fourth, fifth and sixth children were an 18-year-old girl, a 14-year-old boy, a 13-year-old boy and a 6-year-old girl with heights of 166 cm (75^{th} percentile), 134 cm ($<3^{rd}$ percentile), 116 cm ($<3^{rd}$ percentile), and 98 cm ($<3^{rd}$ percentile), respectively. Except for the second child, all the children were described as having bowed legs (Fig. 2). The father was 178 cm (75^{th} percentile) and the mother was 158 cm tall (50^{th} percentile). Although we could not examine the siblings who lived far away from our hospital, their photographs and heights were available.

The family noticed our patient's short stature when he was seven years old. His birth length was normal, as was his somatic and mental development. When he was 15 years old, his height was 127 cm ($<3^{rd}$ percentile), weight was 39 kg (3^{rd} percentile), head circumference was 55 cm (50^{th}-75^{th} percentile), arm

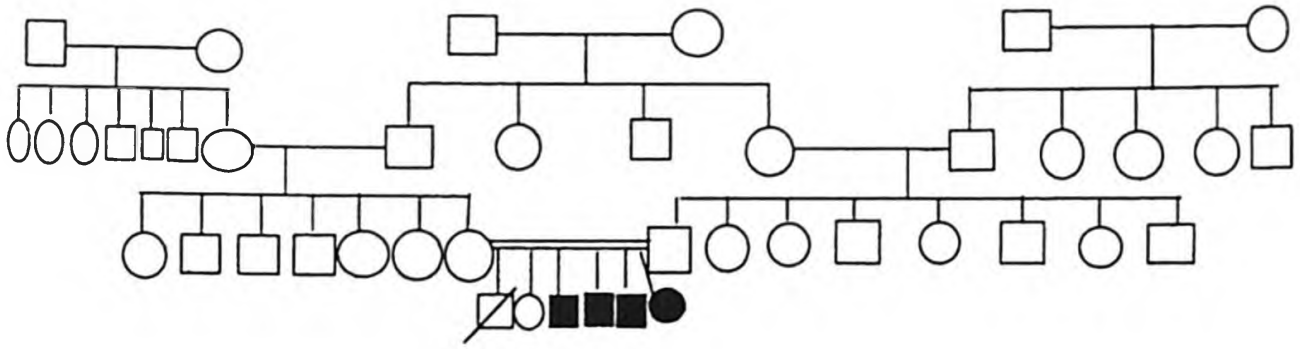

Fig. 1: Our patient's pedigree.

Fig. 2: Our patient's three siblings with short stature and bowed legs.

span was 142 cm and the upper segment/lower segment ratio was 0.91. His facial features appeared somewhat coarse with a wide nasal bridge. He had broad hands, a short neck, a short trunk and kyphoscoliosis but no organomegaly (Fig. 3). Slit-lamp examination did not reveal corneal clouding or punctations.

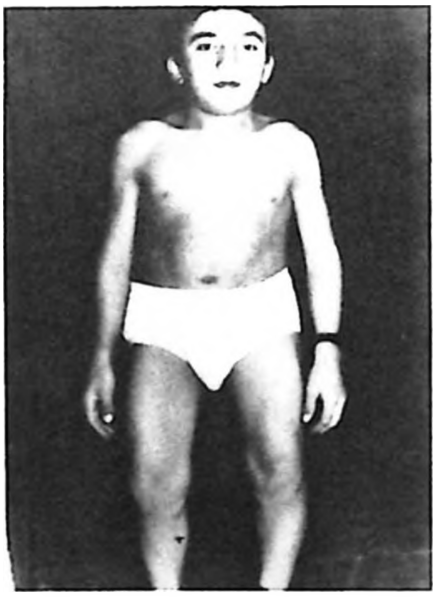

Fig. 3: Our patient: short-trunk dwarfism, coarse face and short neck are evident.

Radiological Features

His bone age was 14 years (Fig. 4). The thoracic and lumbar vertebrae showed definite signs of platyspondyly and severe kyphoscoliosis (Figs. 5a and 5b). The intervertebral spaces were markedly narrowed. Cervical vertebrae also showed platyspondyly (Fig. 5c). The skull was normal. Bilateral coxa valga and

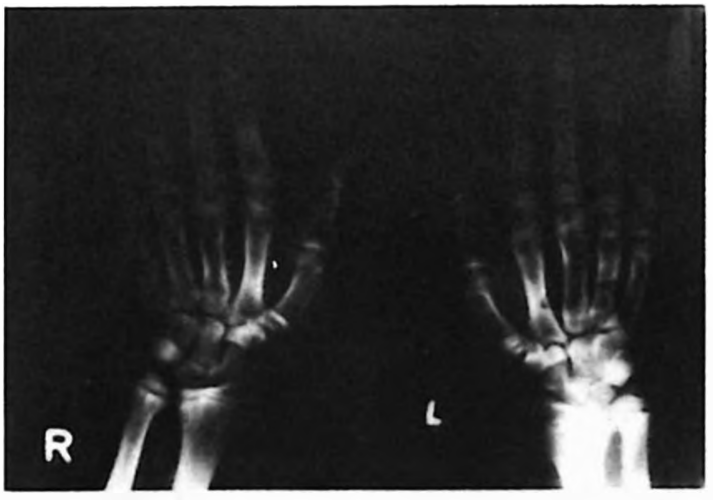

Fig. 4: Our patient's radiograph of hand and wrist.

mild irregularity of the acetabular roof without any epiphyseal or metaphyseal changes were noticed (Fig. 6). There was no precocious calcification of the falx cerebri or costal cartilage.

Fig. 5a: Lateral radiograph of the spine showing marked platyspondyly, kyphosis and rectangular vertebral bodies.

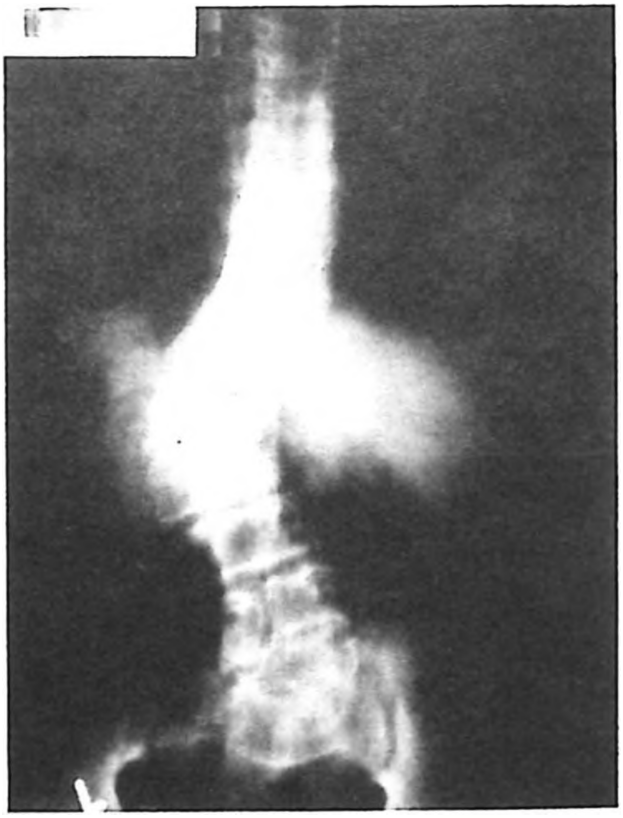

Fig. 5b: Anteroposterior raoliograph of the spine shawing scoliosis, platyspondyly and narrow entervertebral spaces.

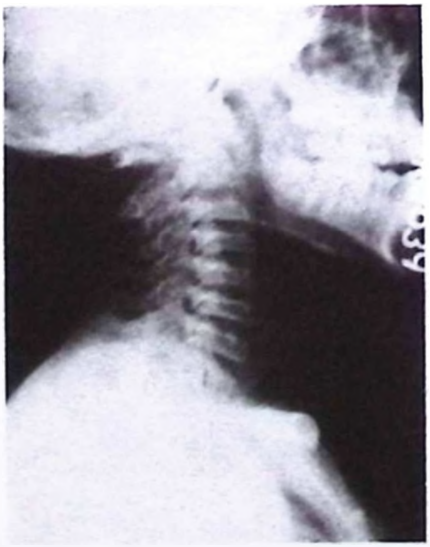

Fig. 5c: Lateral radiograph of the cervical spine showing severe flattening of the vertebral bodies.

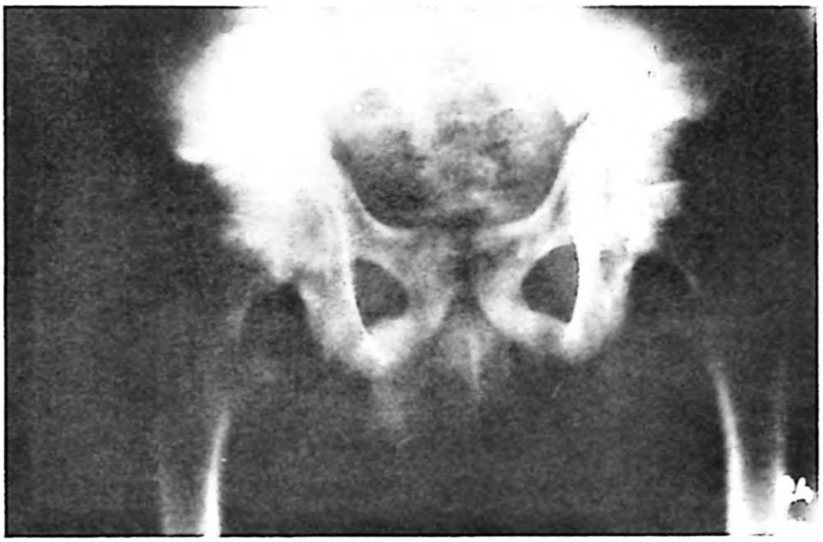

Fig. 6: Anteroposterior radiograph of pelvis and hips showing coxa valga and mild acetabular irregularities.

Laboratory Findings

Calcium, phosphorus and alkaline phosphatase levels were normal. A 24-hour urine glycosaminoglycan electrophoresis revealed increased levels of chondroitin sulphate and heparan sulphate with an elevated urine glycosaminoglycan/ creatinine ratio of 8 (London Hospital for Sick Children). N-acetylgalactosamine-6-sulphatase, β-galactosidase and β-hexoaminidase activities were normal in fibroblast culture.

Discussion

The term "brachyolmia" comes from the Greek word for short trunk. In 1969 Maroteaux[1] used the term brachyolmia to describe short-trunk dwarfism and generalized platyspondyly. Shohat et al[2] have defined at least three distinct types of brachyolmia. Type I (Hobaek Type) is inherited as an autosomal-recessive trait and is associated with squared-off platyspondyly, narrow intervertebral spaces, marked endplate irregularity and close-set pedicles on anteroposterior view. Elongated rectangular vertebral bodies are seen on lateral radiographic views. The greatest flattening is in the thoracic region. The ilia may be somewhat shortened, but the acetabular and femoral heads are normal. The femoral neck may be short and broad. Scoliosis may also be present, although it is not as common or severe as in other types. The Hobaek type seems to be most common type of brachyolmia[2,3]. In 1978 Toledo et al[4] described a family with similar radiographic changes and qualitative glycosaminoglycans (GAG) anomalies, mostly involving chondroitin-6-sulphate in the urine, but the affected patients

also had corneal opacities and precocious anterior rib calcification. Later, Sewell et al[5] also reported qualitative glycosaminoglycans anomalies in one case with Type I brachyolmia.

Type II (Maroteaux Type) cases differ from Type I in having rounded vertebral edges, mild endplate irregularities, and normal or broadened intervertebral disc spaces. Mild facial anomalies have been described. Precocious falx cerebri calcification can be seen. This condition is transmitted as an autosomal-recessive trait[2].

Type III has been described in a mother and son in which there was severe involvement of the vertebrae similar to that in Type II, as well as severe cervical platyspondyly. Type III was described in this family, suggesting autosomal-dominant inheritance. These patients also had the most severe scoliosis and demonstrated marked cervical vertebral flattening and irregularity[2]. A report from South Africa in 1994[6] described a mother and her son as having autosomal-dominant type brachyolmia, but they differed from previously documented autosomal-dominant cases of brachyolmia in terms of the severity of their platyspondyly and the shape of their vertebral bodies.

There is some controversy concerning the classification of this disorder. In our case, a first-degree-cousin marriage, body habitus similar to siblings and some of the radiological findings suggest Hobaek-type brachyolmia, which is an autosomal-recessive disorder. However, the severe kyphoscoliosis and cervical vertebrae findings suggest Type III brachyolmia, which is an autosomal-dominant disorder. These radiological and genetic differences support genetic heterogenity of brachyolmia. As a result, our patient may be an example of this heterogenity. We conclude that further investigation is needed to classify brachyolmia.

REFERENCES

1. Maroteaux P. Spondyloepiphyseal dysplasias and metatropic dwarfism. Birth Defects Original Article Series; 1st Conference on the Clinical Delineation of Birth Defects. Part IV: Skeletal Dysplasia. National Foundation March of Dimes, 1969: 34-44.
2. Shohat M, Lachman R, Gruber HE, Rimoin DL. Brachyolmia: radiological and genetic evidence of heterogeneity. Am J Med Genet 1989; 33: 209-219.
3. Horton WA, Langer LO, Collins DL, Dwyer C. Brachyolmia recessive type (Hobaek): a clinical, radiographic, and histochemical study. Am J Med Genet 1983; 16: 201-211.
4. Toledo SP, Mouaro PA, Lamego C, et al. Recessively inherited late onset spondylar dysplasia and peripheral corneal opacity with anomalies in urinary mucopolysaccharides: a possible error of chondroitin-6-sulphate synthesis. Am J Med Genet 1978; 2: 385-395.
5. Sewell AC, Wern C, Pontz BF. Brachyolmia: a skeletal dysplasia with an altered mucopolysaccharide excretion. Clin Genet 1991; 40: 312-317.
6. Gardner J, Beighton P. Brachyolmia: an autosomal dominant form. Am J Med Genet 1994; 49: 308-312.

The Turkish Journal of Pediatrics 1997; 39: 421-427

RADIOFREQUENCY CATHETER ABLATION TREATMENT OF A CHILD WITH DILATED CARDIOMYOPATHY SECONDARY TO CHRONIC ECTOPIC ATRIAL TACHYCARDIA*

Uğur Kemal Tezcan MD, Erdem Diker MD*, Murat Özdemir MD*
Gülümser Heper MD*, Sengül Çehreli MD***, Siber Göksel MD****

SUMMARY: Tezcan UK, Diker E, Özdemir M, Heper G, Çehreli S, Göksel S. (Cardiology Department, Yüksek İhtisas Hospital, Ankara, Turkey). Radiofrequency catheter ablation treatment of a child with dilated cardiomyopathy secondary to chronic ectopic atrial tachycardia. Turk J Pediatr 1997; 39: 421-427.

Ectopic atrial tachycardia (EAT) is a rare but reversible cause of dilated cardiomyopathy (DCMP). The diagnosis and the definite control of the arrhythmia are essential for the regression of DCMP. Unfortunately, conventional antiarrhythmic drugs usually fail to control the arrhythmia, and the results of surgery or direct current ablation are suboptimal. Recently, radiofrequency (RF) catheter ablation has been evolving as a safe and effective therapy for EAT.

This report describes the RF ablation treatment of a 14-year-old boy with DCMP secondary to chronic EAT. Activation mapping was used for the purpose of identifying the focus origin located just anterior to the coronary sinus os. RF energy applied at this focus successfully terminated the tachycardia. No complications related to the procedure were observed. RF ablation not only caused elimination of the EAT but also led to improvement in left ventricular function as early as two weeks after the procedure, and complete resolution of DCMP in three months. *Key words: ectopic atrial tachycardia, dilated cardiomyopathy, radiofrequency catheter ablation.*

Ectopic atrial tachycardia (EAT) is among the few reversible causes of dilated cardiomyopathy (DCMP)[1]. The arrhythmia usually presents in children or young adults[2]. When the tachycardia is terminated, ventricular function normalizes over weeks to months[3,4]. EAT is generally difficult to control with antiarrhythmic medications[5], and the results of cardiac surgery are suboptimal[6,7]. Radiofrequency (RF) ablation is now evolving as a safe and effective therapy for EAT[8-10].

This report describes the diagnosis and RF ablation treatment of a child with DCMP due to chronic EAT.

* From the Department of Cardiology, Yüksek İhtisas Hospital, Ankara.
** Research Assistant in Cardiology, Yüksek İhtisas Hospital.
*** Associate Professor of Cardiology, Yüksek İhtisas Hospital.
**** Professor of Cardiology, Yüksek İhtisas Hospital.

Case Report

A 14-year-old boy was admitted to the hospital with the diagnosis of DCMP. He had had progressive dyspnea on effort for one year before admission. In other centers he had been treated with digitalis, diuretics, angiotensin-converting enzyme inhibitors, intravenous dobutamine and prednisolone, with the diagnosis of DCMP secondary to myocarditis. However, his functional capacity had improved little despite this intensive treatment regimen. Review of the past medical history and reports revealed that he had resting tachycardia at a rate of 160 bpm, diagnosed as sinus tachycardia, S 3 gallop rhythm and an ejection fraction (EF) of 15 percent as well as diffuse left ventricular hypokinesia on two dimensional echocardiograms.

His functional capacity was New York Heart Association (NHYA) class 2-3 on admission. His heart rate was 150 bpm. Auscultation of the heart revealed an S 3 gallop rhythm. The electrocardiogram (ECG) showed a regular rhythm at a rate of 150 bpm, with inverted P waves in the inferior leads (Fig. 1). The chest radiogram revealed cardiomegaly (Fig. 2). The echocardiographic examination showed diffuse left ventricular hypokinesia, and an EF of 16 percent.

Upon noting the inappropriately high pulse rate and inverted P waves in the inferior leads on the ECG, the patient was taken to the electrophysiology laboratory with the presumptive diagnosis of EAT, which was verified by standard electrophysiologic criteria. The location of the ectopic focus was identified by activation mapping, where the earliest local atrial electrogram relative to the

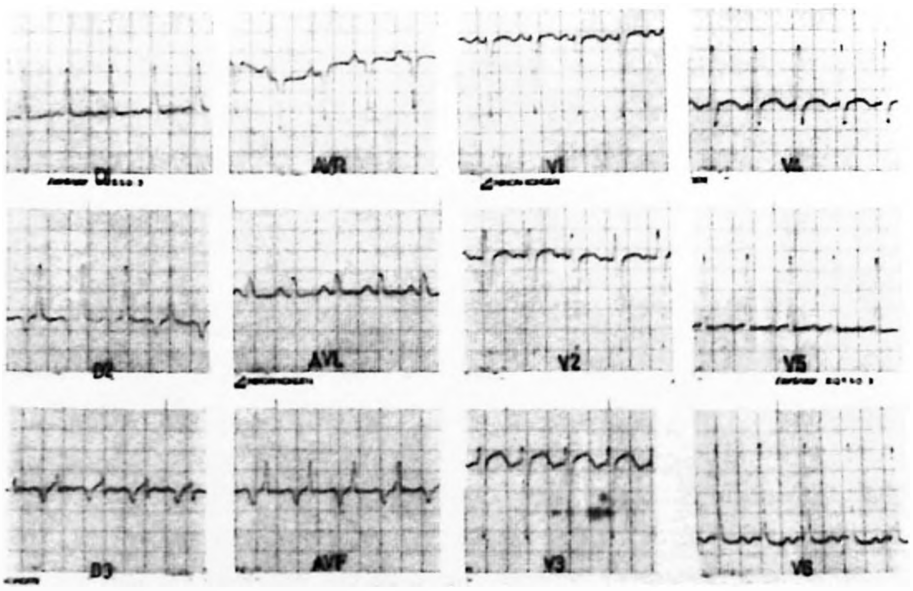

Fig. 1: Electrocardiagram obtained before ablation, with inverted P waves in the inferior leads.

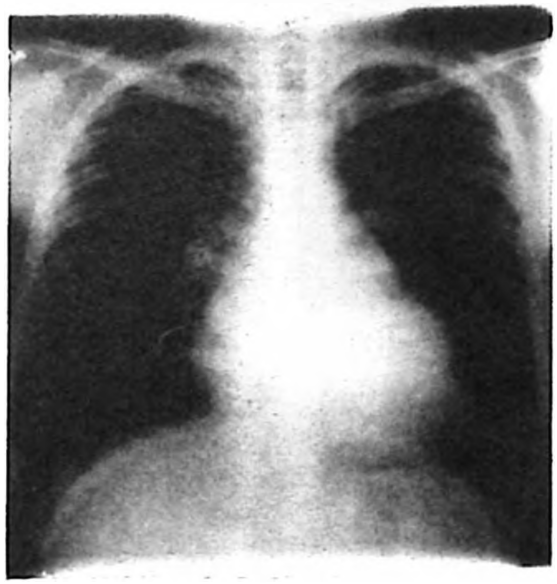

Fig. 2: The chest radiogram before ablation.

surface P wave was sought (Fig. 3). The ectopic focus, which was shown to be located just anterior to the coronary sinus os, was successfully ablated by using RF energy in the same session (Figs. 4 and 5). No complication related to the procedure was observed.

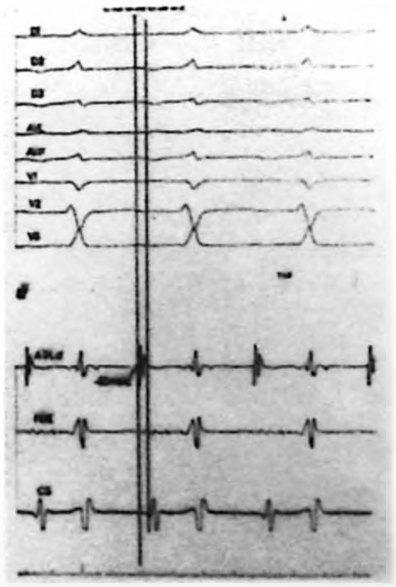

Fig. 3: The intracardiac electrograms along with the surface electrocardiogram obtained at the successful ablation site. ABLd: The distal electrode of the ablation catheter, HBE: His-Bundle electrogram, CS: Coronary sinus.

Echocardiographic examination performed two weeks, three months and one year after the ablation revealed EF of 31, 55 and 57 percent, respectively. In accordance with these improvements in the EF, the cardiothoracic ratio decreased in serial chest radiograms taken after the ablation procedure (Figs. 6 and 7). The patient is now NYHA functional class 1, and surviving a normal life without any medications.

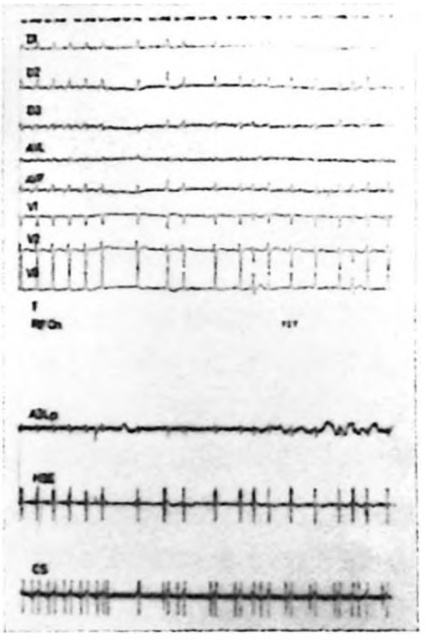

Fig. 4: Termination of tachycardia during radiofrequency energy application. ABLp: The proximal electrode of the ablation catheter, HBE: His-Bundle electrogram, CS: Coronary sinus.

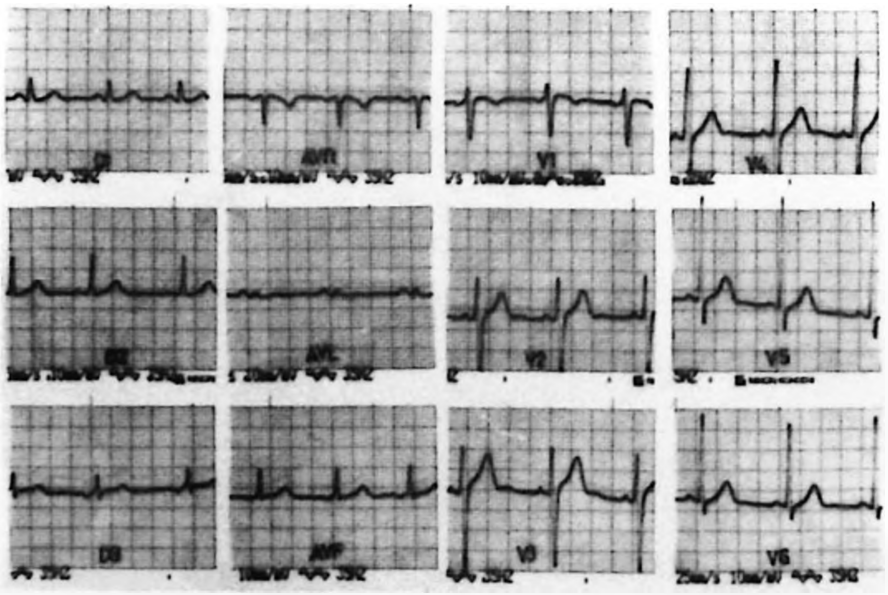

Fig. 5: Electrocardiogram obtained after the ablation. Note the normal, sinus-initiated, P-wave morphology.

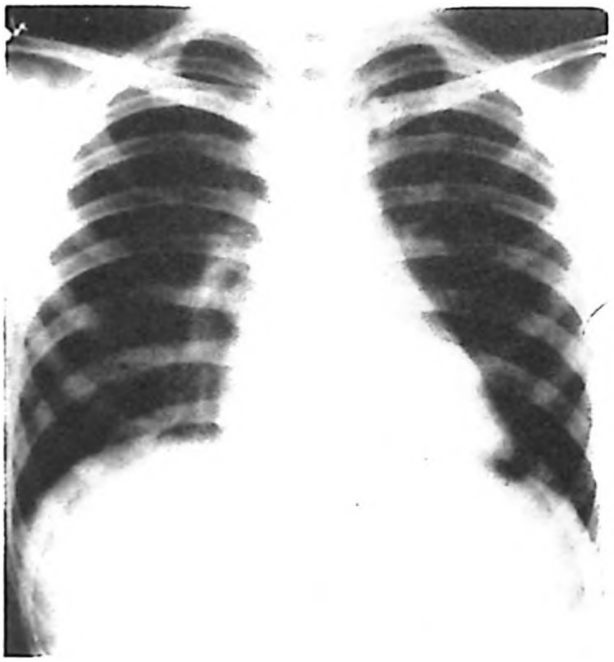

Fig. 6: The chest radiogram three months after ablation.

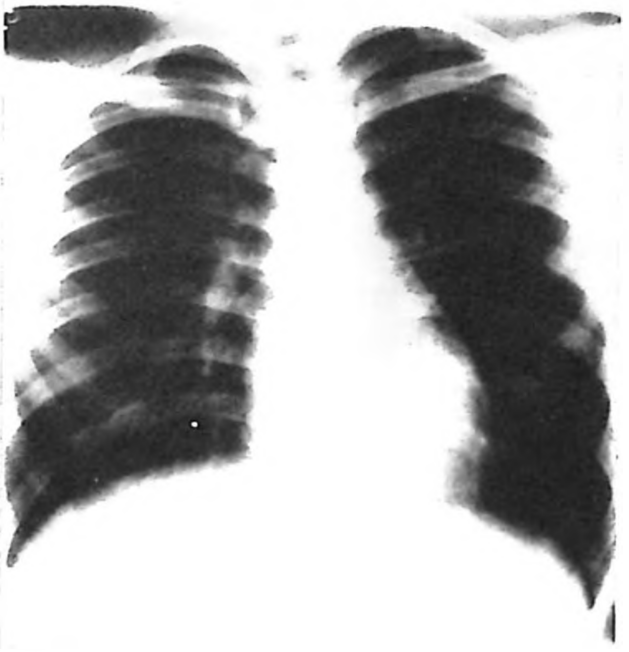

Fig. 7: The chest radiogram one year after ablation.

Discussion

In children and young adults, incessant or continuous EAT may lead to progressive cardiac dilatation[1]. This tachycardia-induced cardiomyopathy is potentially reversible upon control of the arrhythmia[3,4]. Indeed, chronic tachycardias such as EAT are among the few reversible causes of DCMP. Chronic tachycardias associated with DCMP other than EAT include a permanent form of junctional reciprocating tachycardia and some forms of incessant ventricular tachycardia[11].

Patients with incessant EAT may be asymptomatic for many years, and the development of DCMP may be the first presentation, as was the case in our patient. The presenting complaint is related to DCMP in over 50 percent of cases[8]. Our patient never complained of palpitations but of progressive effort dyspnea.

The diagnosis can usually be made on the basis of a surface ECG, which reveals a single abnormal P-wave morphology and heart rates varying from 120 to 240 bpm that are inappropriately rapid for age and physiological state[11]. If the atrial focus is in the high right atrium, it may have P-wave morphology similar to that of the sinus node-initiated P wave; however, more commonly an atrial focus takes place in the low right atrium or left atrium, with negative P waves in the inferior leads[12]. In addition to the abnormal P-wave morphology, a variable PR

interval and the existence of atrioventricular (AV) block despite continuation of supraventricular tachycardia, confirms the diagnosis of atrial tachycardia[12]. In our case the surface electrogram revealed inverted P waves in the inferior leads, but a variable PR interval or the existence of AV block despite continuation of the supraventricular tachycardia was not observed. In this instance a through electrophysiologic study was performed to disclose an atrioventricular accessory pathway or atrioventricular nodal reentry, and ectopic atrial tachycardia originating from a focus just anterior to the coronary sinus os was confirmed by standard electrophysiologic techniques.

The use of antiarrhythmic drugs in the therapy of EAT is limited because of their in effectiveness and negative inotropic side effects[5,13]. Likewise, associated morbidity and technical difficulties limit the use of surgery and direct current catheter ablation[3,14]. is now evolving as a safe and effective therapy for a variety of cardiac arrhythmias, including tachycardias utilizing accessory pathways, atrioventricular nodal reentry, and EAT[8-10,15]. RF energy is a form of electrical energy generated from a high-frequency (300-750 Khz) alternating current. Tissue lesions are created with the formation of coagulation necrosis, primarily produced through resistive heating. Acute success with this method is achievable in 80 to 95 percent of cases of EAT[8,9,16]. Recurrence rates of less than 10 percent have been reported[8]. EAT did not recur in our patient in 12 months of follow-up.

In conclusion, RF ablation appears to be a safe and effective therapy for EAT and may be the treatment of choice for patients with ventricular dysfunction secondary to the arrhythmia.

REFERENCES

1. Gillette PC, Smith RT, Garson A Jr, et al. Chronic supraventricular tachycardia. A curable cause of congestive cardiomyopathy. JAMA 1985; 253: 391-392.
2. Registry PESRCA. Radiofrequency catheter ablation in children and adolescents: early results in 189 patients from 13 centers (abstract). PACE 1992; 15: 589.
3. Olsson S, Bloomstrom P, Sabel KG, William-Olsson G. Incessant ectopic atrial tachycardia: successful surgical treatment with regression of dilated cardiomyopathy picture. Am J Cardiol 1984; 53: 1465-1466.
4. Packer DL, Bardy GH, Warley SJ, et al. Tachycardia-induced cardiomyopathy: a reversible form of left ventricular dysfunction. Am J Cardiol 1986; 57: 563-570.
5. Gillette PC, Garson A Jr. Electrophysiologic and pharmacologic characteristics of automatic ectopic atrial tachycardia. Circulation 1977; 56: 571-575.
6. Ott DA, Gillette PC, Garson A Jr, Cooley DA, Reul GJ, McNamara DG. Surgical management of refractory supraventricular tachycardia in infants and children. J Am Coll Cardiol 1985; 5: 124-129.
7. Garson A Jr, Moak JP, Freidman RA, Perry JC, Ott DA. Surgical treatment of arrhythmias in children. Cardiol Clin 1989; 7: 319-329.

8. Walsh EP, Saul JP, Hulse JE, et al. Transcatheter ablation of ectopic atrial tachycardia in young patients using radiofrequency current. Circulation 1992; 86: 1138-1146.
9. Kay GN, Chong F, Epstein AE, Dailey SM, Plumb VJ. Radiofrequency ablation for treatment of primary atrial tachycardias. J Am Coll Cardiol 1993; 21: 901-909.
10. Tracy CM, Swartz JF, Fletcher RD, et al. Radiofrequency catheter ablation of ectopic atrial tachycardia using paced activation sequence mapping. J Am Coll Cardiol 1993; 21: 910-917.
11. Walsh EP. Ablation of ectopic atrial tachycardia in children. In: Huang SK (ed). Radiofrequency Catheter Ablation of Cardiac Arrhythmias: Basic Concepts and Clinical Applications. New York: Futura Publishing Co; 1995: 421-443.
12. Naccarelli GV, Shih HT, Jalal S. Sinus node reentry and atrial tachycardias. In: Zipes DP, Jalife J (eds). Cardiac Electrophysiology: From Cell to Bedside *(2nd ed)*. Philadelphia: WB Saunders Co; 1995: 607-619.
13. Prager NA, Cox JL, Lindsay BD, et al. Long-term effectiveness of surgical treatment of ectopic atrial tachycardia. J Am Coll Cardiol 1993; 22: 85-92.
14. Gillette PC, Wampler DG, Garson A Jr, Ott D, Cooley D. Treatment of atrial automatic tachycardia by ablation procedures. J Am Coll Cardiol 1985; 6: 405-409.
15. Fisher JD. Catheter ablation for cardiac arrhythmias: clinical applications, personnel and facilities (ACC Position Statement). JACC 1994; 24: 828-833.
16. Lesh M, Van Hare GF, Epstein L, et al. Radiofrequency catheter ablation of atrial arrhythmias. Results and mechanism. Circulation 1994; 89: 1074-1089.

The Turkish Journal of Pediatrics 1997; 39: 429-432

BICUSPID AORTIC VALVE AND COARCTATION OF AORTA*

*Ali Sarıgül MD***, *Yurdakul Yurdakul MD****, *Selim İsbir MD*****
*Şükrü Mercan MD******, *Alpay Çeliker MD*******

SUMMARY: Sarıgül A, Yurdakul Y, İşbir S, Mercan Ş, Çeliker A. (Department of Thoracic and Cardiovascular Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey). Bicuspid aortic valve and coarctation of aorta. Turk J Pediatr 1997; 39: 429-432.

Bicuspid aortic valve (BAV) and coarctation of aorta (COA) are frequently seen together. It is believed that these malformations result from a single developmental diathesis. A case is presented of COA, aortic stenosis and aneurysm of the ascending aorta corrected by patch aortoplasty and commisurotomy. An aneurysm at the site of the coarctation repair can develop as late as 20 to 25 years after surgery. Almost all of the aneurysms described have occurred in patients undergoing patch aortoplasty. We do not recommend this technique except in special cases. *Key words: bicuspid aortic valve, coarctation of aorta, aneurysm.*

Bicuspid aortic valve has long been known to be common in patients with coarctation. It was found in 23 percent of the cases studied by Abbott[1] and in 25 percent of the series of Benkwitz and Hunter[2]. Tawes and associates[3] note in their report that among 250 living children with long-term follow-up, 32 (13%) had clinical evidence of aortic valve disease (mainly stenosis but also incompetence).

McKusick[4] suggested that in patients with coarctation of aorta (COA) and bicuspid aortic valve (BAV) cystic medial necrosis occurs due to aortic wall weakness. Aneurysm, dissection and rupture may also develop in these patients due to surgical repair[5].

We present here a patient who developed an ascending aortic aneurysm 11 years after repair of aortic coarctation with patch aortoplasty.

* From the Department of Thoracic and Cardiovascular Surgery, Hacettepe University Faculty of Medicine, Ankara.

** Assistant Professor of Thoracic and Cardiovascular Surgery, Hacettepe University Faculty of Medicine.

*** Professor of Thoracic and Cardiovascular Surgery, Hacettepe University Faculty of Medicine.

**** Research Assistant in Thoracic and Cardiovascular Surgery, Hacettepe University Faculty of Medicine.

***** Professor of Pediatrics and Pediatric Cardiologist, Hacettepe University Faculty Medicine.

Case Report

A 1.5-year-old male patient was first referred to our hospital in 1977 with tachypnea and feeding problems. On physical examination his heart rate was 145/min and his blood pressure was 140/85 mmHg on the right and 130/85 mmHg on the left side. A III/VI systolic ejection murmur was heard along the left sternal edge. Lower extremity peripheral arterial pulses were absent. His chest x-ray showed a moderate cardiomegaly (Fig. 1). Echocardiography demonstrated aortic stenosis, aortic coarctation and minimal dilatation of the ascending aorta (Fig. 2). A presumptive diagnosis of COA and BAV was made. In 1986, surgical repair for COA was performed by dacron patch aortoplasty.

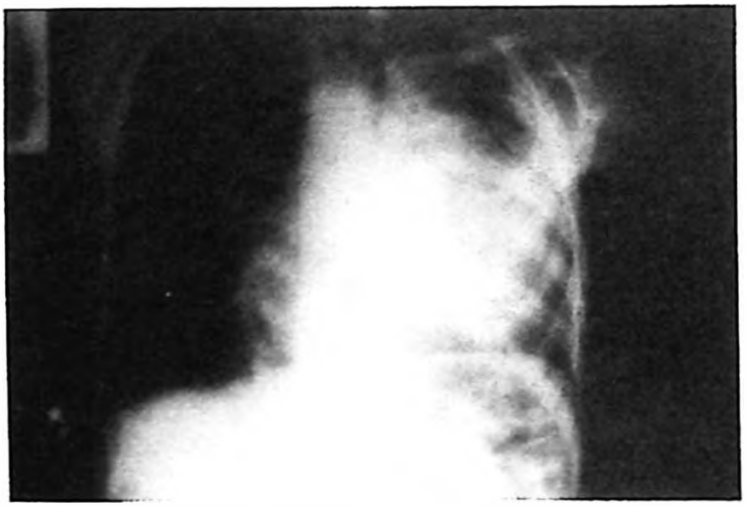

Fig. 1: Chest x-ray showed marked cardiomegaly.

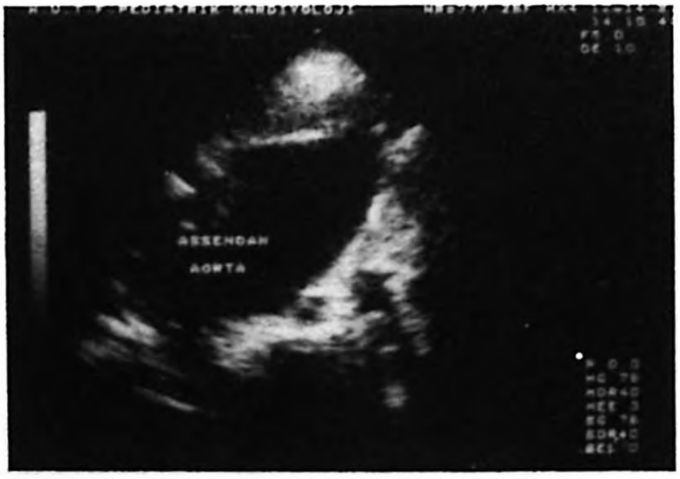

Fig. 2: Echocardiogram demonstrated aortic stenosis, aortic coarctation and minimal dilatation of the ascending aorta.

In 1988 cardiac catheterization and echocardiography were performed in order to confirm the ascending aortic dilatation and aortic stenosis (Fig. 3) The diameter of the ascending aorta (45 mm) was also observed to be greater than the diameter of the descending aorta.

With these manifestations, surgery was performed in 1988 when the patient was 12 years old. The heart was exposed through a median sternotomy and the patient was placed on total cardiopulmonary bypass via right femoral arterial and standard bicaval cannulation.

The aorta was found to be quite dilated. Intra-operatively a vertical incision was made through the aorta. The valve was in bicuspid morphology. Valvotomy was performed by dividing the commissures with a knife to within one mm of the aortic wall. The segment that revealed aneurysmatic dilatation was resected. The aorta was then closed primarily with 4/0 polypropylene suture (Fig. 4). The postoperative period was uneventful, and the patient was discharged two weeks later.

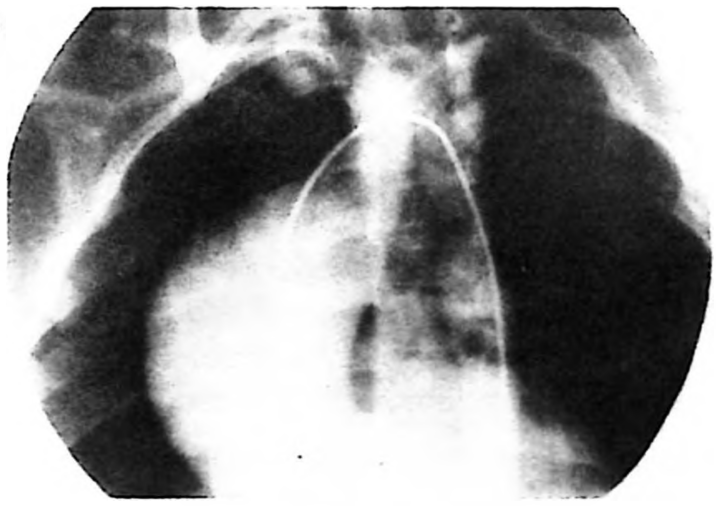

Fig. 3: Cardiac catheterization showed ascending aortic dilatation and aortic coarctation.

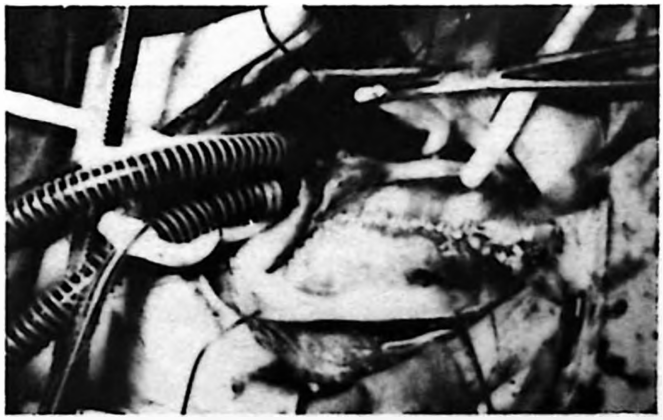

Fig 4: Aneurysmatic dilatation was resected and the aorta was closed primarily with 4/0 polypropylene suture.

Discussion

Valvar heart disease may complicate the long-term management of patients who have undergone coarctation repair, and occasionally prevents a good outcome[6]. In one study, surgery for aortic stenosis was required at three to seven years of age in seven percent of patients whose coarctation was repaired in infancy[7].

A significantly large number of patients with bicuspid valves will require surgery for calcific aortic stenosis in the fifth or sixth decade of life.

A true or false aneurysm may occur late postoperatively after repair of COA. Because the stiff patch transmits additional tension to the adjacent elastic aortic wall, a true aneurysm after a Dacron patch repair has always been associated with a false (suture line) aneurysm. In Barrat-Boynes' series[4] the incidence of aneurysm was 10 percent but increased to 25 percent in the eight patients followed 15 years or more. In the same report, aneurysm had not occurred in patients operated on before 10 years of age. Our patient was 11 years old at the time of his first operation. Dissection may occasionally occur in either the ascending or descending aorta, proximal or distal to the coarctation repair site, which may also lead to aneurysm formation.

We conclude that in cases of childhood aortic coarctation, if the patient is greater than 10 years old, patch aortoplasty can be performed instead of end-to-end anastomosis because of the low incidence of recurrent coarctation. However, because almost all aneurysms described have occurred in patients undergoing patch aortoplasty, we do not recommend this technique.

COA can be well repaired from an anterior midline approach through median sternotomy using cardiopulmonary bypass if the patient has a concomitant intracardiac defect. The advantage of this option is that reoperation may be avoided.

REFERENCES

1. Abbott ME. Coarctation of the aorta of the adult type. Am Heart J 1928; 3: 381-421, 574-618.
2. Benkwitz KB, Hunter WC. Combined infantile and adult coarctation of aorta coincident with occlusion of vena cava superior. Report of a case. Am J Pathol 1937; 13: 289-310.
3. Tawes RL Jr, Berry CL, Aberdeen E. Congenital bicuspid aortic valves associated with coarctation of the aorta in children. Br Heart J 1969; 31: 127-128.
4. McKusick VA. Association of congenital bicuspid aortic valve and Erdheim's cystic medial necrosis. Lancet 1972; 1: 1026-1027.
5. Schlatmann TJM, Becker AE. Histologic changes in the ascending aorta: implication for dissecting aortic aneurysm. Am J Cardiol 1977; 39: 13-20.
6. Bergdahl L, Bjork VO. Jonasson R. Surgical correction of coarctation of the aorta. Influence of age on late results. J Thorac Cardiovasc Surg 1983; 85: 532-536.
7. Kirklin JW, Barrat Boynes. Coarctation and bicuspid aortic valve. In: Kirklin JW (ed). Cardiac Surgery (2nd ed) Vol. 2. New York: Churchill Livingstone; 1993: 1279.

ANNOUNCEMENTS

JERUSALEM, 6-10 JULY 1997

INTERNATIONAL CONGRESS PEDIATRICS IN THE COMMUNITY - 2000+

Inquiries to: Congress Secretariat
ISAS International Seminars
PO Box 34001
91340 Jerusalem
ISRAEL
Phone : 972-2-652 05 74
Fax : 972-2-652 05 58
E-mail : isas@netvision.net.il

TURKU, 16-20 JULY 1997

XXIII[rd] ISPAD ANNUAL SCIENTIFIC MEETING
INTERNATIONAL SOCIETY FOR PEDIATRIC AND ADOLESCENT DIABETES

Inquiries to: Hans Akerblom
Children's Hospital Stenbackintaku 11,
Helsinki 00290
FINLAND
Phone : 358-0-471-2701
Fax : 358-0-471-4704

EDINBURGH, 20-24 JULY 1997

IV[th] INTERNATIONAL CONGRESS ON ESSENTIAL FATTY ACIDS AND EICOSANOIDS

Inquiries to: Vicky Grant or Wendy Adesegun
c/o Meeting Makers,
50 George St., Glasgow G1 1QA,
Scotland
UNITED KINGDOM
Phone : 44-0-141-553-1930
Fax : 44-0-141-553-0511
E-mail : mm@meetingmakers.co.uk

MONTREAL, 20 JULY - 1 AUGUST 1997

16[th] INTERNATIONAL CONGRESS OF NUTRITION

Inquiries to: Congress Secretariat
IUNS '97
National Research Council of Canada
Building M-19
Montreal Road, Ottawa, ON
CANADA K1A OR6
Phone : 613 993 7271
Fax : 613 993 7260

BERLIN, 4-6 SEPTEMBER 1997

IVth INTERNATIONAL WORKSHOP: "DIABETIC ANGIOPATHY IN CHILDREN"
INTERNATIONAL SOCIETY FOR PEDIATRIC AND ADOLESCENT DIABETES

Inquiries to: Stuart Brink, MD
Secretary-General
International Society for Pediatric
and Adolescent Diabetes
c/o New England Diabetes and
Endocrinology Center
40 Second Ave., Suite 170
Waltham, MA 02154-1132
U.S.A.
Phone : 617-890-3610
Fax : 617-890-3612
E-mail : sbrink@opal.tufts.edu

BAKU, 21-25 SEPTEMBER 1997

IVth REGIONAL CONGRESS OF PEDIATRIC SOCIETIES OF CENTRAL ASIAN AND OTHER TURKISH-SPEAKING COUNTRIES WITH INTERNATIONAL PARTICIPATION

Inquiries to: Prof.Dr. Namazova Adile Avazkızı
Kiçik Deniz Küçesi 4
Sahiyye Nazirliyi
Baku
AZERBAIJAN
Phone : 994-12-939612
Fax : 994-12-988559

SYDNEY, 23-25 OCTOBER 1997

BREASTFEEDING-THE NATURAL ADVANTAGE

Inquiries to: Nursing Mothers' Association of Australia
NMAA 1997 Conference
PO Box 3124, Tamarama, NSW, 2026
AUSTRALIA
Phone : +61 3 9877 5011
Fax : +61 2 9389 4504

İZMİR, 30-31 OCTOBER 1997

IVth NATIONAL METABOLIC DISEASES AND NUTRITION SYMPOSIUM

Inquiries to: Prof.Dr. Benal Büyükgebiz
Dokuz Eylül Üniversitesi
Tıp Fakültesi
Çocuk Sağlığı ve Hastalıkları
Anabilim Dalı
35340 İnciraltı, İzmir
TURKEY
Phone : +90-0-232-277 77 77
Fax : +90-0-232-278 84 13 Ext. 3608

CHA - AM, PETCHABURI 15-18 NOVEMBER 1998

III[rd] ASIA-PACIFIC REGIONAL MEETING OF THE INTERNATIONAL SOCIETY FOR NEONATAL SCREEN

NEONATAL SCREENING IN THE 21[st] CENTURY

Inquiries to: The Congress Secretariat
3[rd] APRM of the ISNS
c/o Department of Obstetrics & Gynecology
Faculty of Medicine, Ramathibodi Hospital
Rama 6 Road, Phayathai, Bangkok 10400
THAILAND
Phone : (662) 245 5828, 201 1416, 201 1412
Fax : (662) 201 1416, 246 0233

NOTICE TO AUTHORS

The Turkish Journal of Pediatrics is an English publication of original articles, case reports, reviews of the literature, case of the month, short communications, clinicopathological exercises and letter to the editor in the field of pediatrics, and will consider papers from authors of all countries. Original articles should report scientific findings within the pediatric field of interest. Case reports should contain accounts of rare syndromes, new diagnostic tools and methods, new kinds of treatment and laboratory research with foreseeable practical application. Review articles should contain an objective account of different points of view and an internationally representative bibliography. Authors considering submission of review articles of medical progress are encouraged to consult with the Editor before submission. Papers should in general not exceed 15 written pages for original articles or 7 for case reports and reviews of the literature, including tables, figures and illustrations. All papers are subject to editorial revision for the purpose of conformity to the style adopted by *The Turkish Journal of Pediatrics,* and authors may be requested to shorten or revise their papers.

Statements and opinions expressed in the articles are those of the author(s) and not necessarily those of the Editor or publisher; the Editor and publisher disclaim any responsibility or liability for such material. Neither the Editor nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication; neither do they guarantee any claim made by the manufacturer of such product or service.

We recommend that all manuscripts be approved for submission by the department chairman or head of the institution in which the study has been done. All manuscripts must be accompanied by the following **written statement,** signed by the author(s): "The author(s) transfer(s) all copyright ownership of the manuscript entitled (title of article) to *The Turkish Journal of Pediatrics* in the event the work is buplished. The author(s) warrant(s) that the article is original, is not for consideration by another journal, has not been previously published (except in a congress report), and has been prepared according to the manuscript rules" (as described below). Papers describing research involving human subjects should indicate that informed consent was obtained from the parents or guardians of the children who served as subjects and, when appropriate, from the subjects themselves.

Preparation of Manuscript

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles and may result in rejection of otherwise acceptable manuscripts. Manuscripts should be sent in triplicate (original and two photocopies acceptable) to the Editorial Secretary, *Turkish Journal of Pediatrics,* P.K. 66, 06240 Samanpazarı, Ankara, Turkey. Manuscripts, whether rejected or published, are not returned to the authors, but original illustrations which are rejected are returned upon request of the author.

Format Manuscripts should be typed, double-spaced, on one side of the paper only, with wide margins.

Title Page A separate title page should include authors' names and academic degrees; departmental and institutional affiliations of each author, and sources of financial assistance, if any. Designate one author as the correspondent, and provide an address as well as business and home telephone numbers. Galley proofs and order forms for reprints will be sent to the corresponding author, at a later date.

Contents Each original article should contain the following sections: Introduction, Material and Methods, Results, Discussion and Summary, and each Case Report should contain an Introduction, Case Report, Discussion and Summary.

Abbreviations Abbreviations must conform to accepted standards. Laboratory slang, clinical jargon and colloquialisms must be avoided.

Tables Tables should be numbered with Roman numerals according to the order of mention in the text. Each table must be typed double-spaced on a separate sheet of paper, and a concise title supplied for each. Tables should be self-explanatory and supplement, not duplicate the text. If a table or any data therein have been previously published, a footnote must give full credit to the original source.

Figures Figures and illustrations should be of direct interest to the subject of the paper and numbered with Arabic numerals according to order of reference in the text. Figures should be drawn on white paper and photographs must be clear prints. The name of the first author and the number of the figure or illustration must be written in pencil on the back of each, with an arrow indicating the top side. Original drawings or graphs should be prepared in black India ink or typographic (press-apply) lettering. Typewritten or freehand lettering is unacceptable. All lettering must be professional and in proportion to the drawing, graph, or photograph. Do not send original art work, x-ray films or ECG strips. A list of legends for the figures and illustrations must be provided on a separate page.

Summary / Abstract An informative summary not exceeding 150 words must accompany each manuscript, typed on a separate sheet. Unexplained abbreviations and references are not allowed.

References References to the literature should be limited to those cited by the author, arranged and numbered according to order of mention in the text. All authors of an article or book should be listed where there are six or fewer. If more than six, the first three names should be given followed by the indication "et al". In a manuscript underlining indicates italics.

References to journal articles:

Example: 1. Bier DM, Fulginiti VA, Garfunkel JM, et al. Duplicate publication and related problems. Pediatrics 1990; 86: 997-998.

Names of journals are to be abbreviated in accordance with the style of *Index Medicus.*

References to books:

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

References to chapters in books:

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

Key Words Authors are requested to list on a separate sheet of paper key words related to their article. These will bu used in compiling an index for the volume of the journal in which the article appears.

Proofs and Reprints Galley proofs will be sent to the corresponding (or principal) author by the editor. The master proof should be returned to the Managing Editor together with the manuscript within two days, and by air mail if received by air mail.

Announcements Announcements of scheduled meetings, symposia, or postgraduate courses may be sent to the publisher for consideration at least 5 months in advance of the meeting date.

Books for Review Books and monographs, domestic or foreign, may be sent to the Editor for consideration for review. No books are returned, nor acknowledgement made of books received.

Advertising Communications of a business nature and all advertising communications should be addressed to the Journal's Editor.

Checklist

Please check your article as described below before sending it to the Editor.

- Letter of submission
- Signed "Statement" (which is written on a separate paper) as described above
- Three copies of article (one original and two photocopies will be acceptable).
- Title page
  - Title of article
  - Short running title
  - Name(s), academic degrees, and affiliations of author(s)
  - Name, address, and business and home telephone numbers of corresponding author
- Article proper
  - Original articles: Introduction, Material and Methods, Results, Discussion, Summary
  - Case Reports: Introduction, Case Report, Discussion, Summary
  - Key words
- References on a separate sheet
- Tables on separate sheets
- Legends on separate sheets
- Illustrations properly labelled (one set of glossy prints and one set of photocopies).