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: 4 OCTOBER - DECEMBER 1997

CONTENTS

The Turkish Journal of Pediatrics 1997; 39: 433

AWARD OF THE WORLD HEALTH ORGANIZATION HEALTH – FOR – ALL GOLD MEDAL TO İHSAN DOĞRAMACI

Following are excerpts from the statement made by Dr. Hiroshi Nakajima, Director General of WHO, when he presented the Health-for-All Gold Medal to Professor İhsan Doğramacı at a ceremony in İstanbul on 15 September 1997.

İhsan Doğramacı is one of the most distinguished leaders in international health, in addition to having made major contributions to many areas of health development in his own country. His contributions to pediatrics and child health and to inter-university relations in Europe have been significant.

Professor Doğramacı is the only surviving signatory of the World Health Organization Constitution, an event that occurred at the conclusion of the International Health Conference held in New York in June-July 1946. In addition to heading the Turkish Delegation to six successive World Health Assemblies (1976-1981), he served with distinction as a member of WHO's Executive Board from 1979 to 1982, as a member of what was then known as the Advisory Committee on Medical Research (1979-1983) and-for a period of 30 years-as a member of the WHO Expert Advisory Panel on Health Manpower. Professor Doğramacı has been a Member of the UNICEF Executive Board for no less than 38 years.

He served as President of the International Pediatric Association from 1968 to 1977, and as its Executive Director from 1977 to 1993.

As founder of Hacettepe University, he introduced an integrated and community-oriented teaching system at the Faculty of Medicine and Health Sciences which has been internationally acknowledged and replicated in many countries.

The WHO Health-for-All Gold Medal has been awarded to Professor İhsan Doğramacı for his outstanding contributions during the past 50 years to the advancement of international health cooperation, particularly in the field of child health and medical education, both crucial to the achievement of the social goal of Health-for-All.

The Turkish Journal of Pediatrics 1997; 39: 435-445

BENIGN VASCULAR TUMORS AND VASCULAR MALFORMATIONS IN CHILDHOOD: A RETROSPECTIVE ANALYSIS OF 1127 CASES*

*Canan Akyüz MD***, *Nilgün Yarış MD****, *M. Tezer Kutluk MD*****
*Münevver Büyükpamukçu MD******

SUMMARY: Akyüz C, Yarış N, Kutluk MT, Büyükpamukçu M. (Oncology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Benign vascular tumors and vascular malformations in childhood: a retrospective analysis of 1127 cases. Turk J Pediatr 1997; 39: 435-445.

Vascular lesions in childhood are classified as vascular malformations and hemangiomas. Vascular malformations are congenital abnormalities thought to arise from defects during embryological development of vascular tissue. Hemangiomas are benign tumors of vascular endothelium and can spontaneously become involuted in almost all cases.

One thousand one hundred and twenty-seven patients with vascular lesions were followed by the Department of Pediatric Oncology, Hacettepe University, for 19 years. Diagnosis was based mainly on history, clinical condition and follow-up data in 98.2 percent of cases. The distribution of the vascular lesions was as follows: 969 patients had hemangiomas, 120 had lymphatic malformations, 18 had combined vascular malformations, 11 had venous malformations, six had port-wine stain, and three had angiokeratoma. Except for ten cases with Klippel-Trenaunay Weber syndrome, vascular malformations were not accompanied by any syndrome. *Key words: hemangioma, vascular malformation, benign tumors, vascular tumors, vascular birthmarks.*

The issue of whether vascular lesions should be accepted as benign tumors, malformations or hamartomas has been discussed for a long time. Previously all vascular lesions had been mistakenly discussed under the heading of hemangioma and were generally accepted as benign tumors of vascular tissue[1-4]. Mulliken and Glowacki[5] proposed a new classification of vascular lesions on the basis of their histopathological and clinical features. Vascular malformations and hemangiomas are the major categories of this classification (Table I). These authors emphasized that hemangiomas have appeared in the first months of life, showed a rapid postnatal growth and very slow involution. Unlike hemangiomas, vascular malformations may generally be recognized at birth, grow commensurately with the child and never regress[5].

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

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

*** Pediatrician and Fellow in Pediatric Oncology, Hacettepe University Faculty of Medicine.

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

Table I: Classification of the Vascular Lesions

I) Hemangiomas
II) Vascular malformations
1. Capillary vascular malformations
a) Port-wine stain
b) Angiokeratoma
2. Telangiectasias
3. Venous malformations
4. Lymphatic malformations
5. Combined forms

Hemangiomas are the lesions made up of hyperplasic, mature endothelial cells which are coated by multilaminated basement membrane. Proliferation of the endothelium may be present in the superficial dermis (capillary hemangioma), lower dermis (cavernous hemangioma), both of these layers (mixed hemangioma) or in some other tissue (muscle, liver, larynx, etc.)[5,6]. In contrast, vascular malformations are formed by ectatic, malformed channels lined by flat endothelium adjacent to a thin basement membrane[5,6].

Except in the case of some complications, such as compression or obstruction of important structures, Kasabach-Merritt syndrome and heart failure, it is accepted that the best management of cases with hemangiomas is clinical observation without treatment. Indications for the treatment of vascular malformations are cosmetic or functional[7].

The aim of the present study is to classify the vascular lesions in cases referred to our clinic for nineteen years using history and physical examination without any sophisticated laboratory techniques; to analyze the natural histories, and clinical and epidemiological features of the patients; to discuss treatment modality; and to underline the differential diagnosis of these lesions.

Material and Methods

From December 1971 to December 1990, 1127 cases with vascular lesions were referred to the Pediatric Oncology Unit of Hacettepe University Children's Hospital. Follow-up registrations and photographs of cases were retrospectively investigated. Patients were evaluated with regard to sex, age, complaints and the characteristics of the lesions. Diagnosis was made on the basis of natural history, clinical condition and follow-up data. According to these findings, all patients were classified as hemangioma or vascular malformation.

We accepted the pale-pink macular or telangiectatic lesions present at birth as the first signs of the nascent hemangioma. In later weeks and months, we observed that the lesions became darker in color and simultaneously enlarged.

Lesions which became raised from the skin, have sharp edges, bright red-purple color, and a smooth or rough surface are described as the capillary type. Those of normal skin color or bluish soft masses with a smooth surface are accepted as the cavernous type. When both types of lesions were present together, we classified it as mixed type. We observed that lesions started to fade and diminish in size after a stable phase. They finally disappeared with wrinkles and atrophy, or without leaving any trace on the skin.

We classified the red-purple, smooth-surfaced macular lesions which generally become darker and rough with age as port-wine stains. Dark red, hyperkeratotic, warty-like papular lesions with a tendency to bleed were considered to be angiokeratomas. Lesions characterized by venectasia, varicosities, and soft, non-pulsatile spongy masses with a bluish hue are accepted as venous malformations. Soft, immobile, normal skin colored masses ranging from a few centimetres to extremely large dimensions are defined as lymphangiomas. In the natural history of cases with port-wine stains, angiokeratomas or venous and lymphatic malformations, we observed that the lesions were generally present at birth and never showed rapid growth or spontaneous regression.

Diagnosis was made on the basis of clinical condition and natural history in 98.2 percent of patients. Histopathological examination was performed in 104 patients. Ninety-three of these underwent surgical intervention for therapeutic or cosmetic reasons. Diagnosis was confirmed by angiography in 10 patients with venous and combined vascular malformations and by scintigraphy in one patient with hemangioma and two patients with lymphangiectasia.

Results

Of the 5504 cases with benign tumors or malformations and malignant tumors who were referred to our oncology clinic over a period of 19 years, 1127 had vascular lesions. Of these, 158 were considered to be vascular malformations. The remaining 969 cases had hemangiomas (Fig. 1).

There were 673 females and 296 males with hemangiomas (F/M = 2.3). Mature hemangioma lesions were present at birth in 30.7 percent of cases and were recognized in the first month of life in 58.1 percent of cases (Fig. 2). Rapid growth and slow regression phases were determined in hemangioma lesions from the history and clinical follow-up. The lesions of 969 hemangioma cases were located on skin (961 cases), in the liver (4 cases), in the parotid glands (3 cases) and in the larynx (1 case).

Multiple lesions were seen in 24.2 percent of cases with skin hemangiomas. The total number of skin lesions in these 961 cases was 1293, and most were located in the head/neck regions of the patients (Table II). Six hundred and ninety-four (53.7%) of skin hemangiomas were capillary, 243 (18.8%) were cavernous and 356 (27.5%) were mixed type.

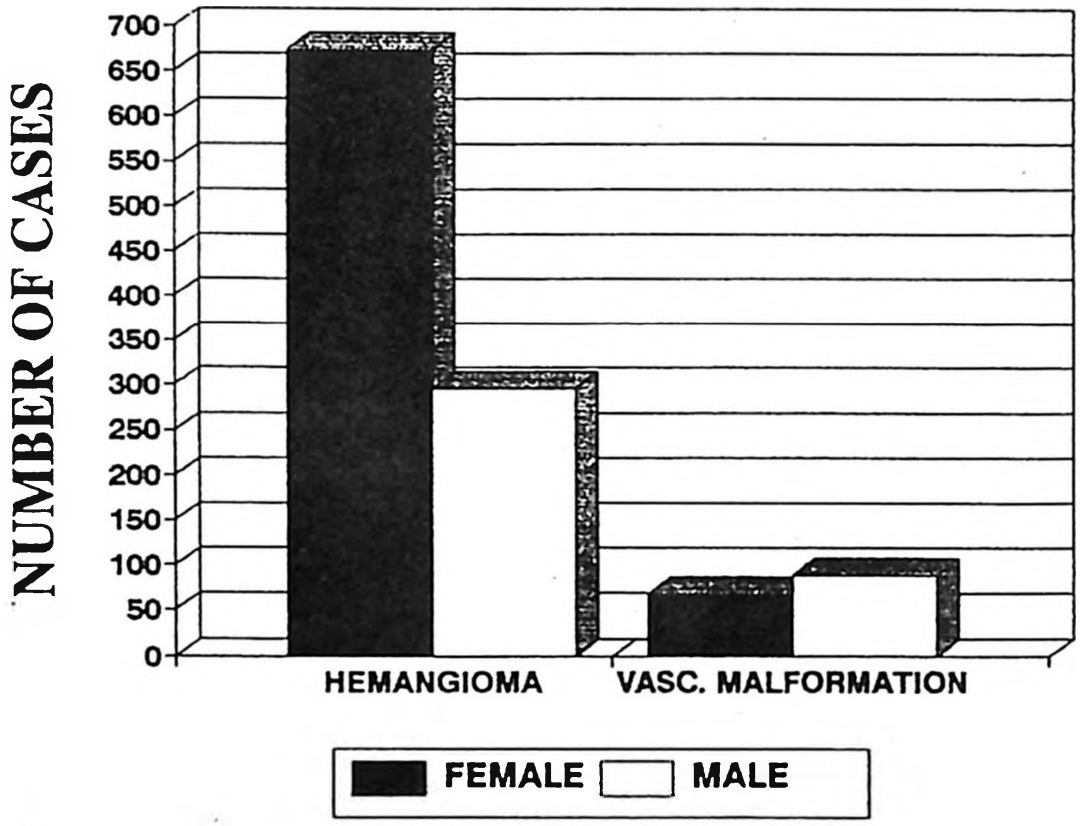


Fig. 1: Sex distribution in 969 hemangioma (female/male = 2.3) and 158 vascular malformation (female/male = 0.8) cases [Vasc. Malformation: Vascular Malformation].

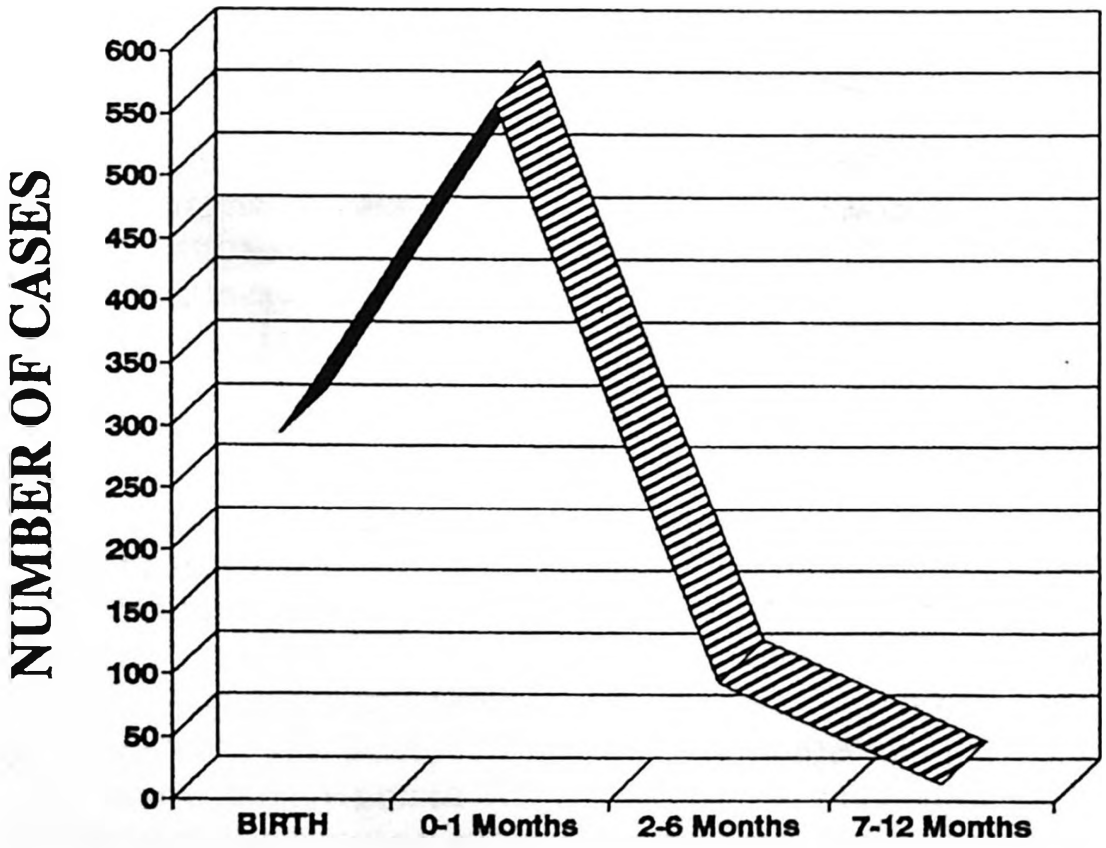


Fig. 2: The distribution of recognition ages of 961 skin hemangiomas.

Table II: Localizations of Hemangioma Lesions

Localization		Number of Cases	%
Skin		961	99.0
	Number of Skin Lesions (*) / %		
Head and neck	788 / 60.9		
Extremity	243 / 18.8		
Trunk	204 / 15.8		
Genital region	38 / 2.9		
Extend over more than one region	20 / 1.6		
Total	1293 / 100.0		
Liver		4	0.4
Parotid gland		3	0.3
Larynx		1	0.3
Total		969	100.0

* Multiple lesions were seen insome of the cases with skin hemangioma.

One hundred and two out of 969 hemangioma cases required treatment because of life-threatening complications. Parents of the 660 patients with small, uncomplicated hemangiomas were informed about the disease and advised to have their cases followed by their local doctors. The remaining 207 cases were followed by our clinic for one to 15 years (median 4.5 years). Ninety-one of these patients showed complete regression, 37 almost complete regression, and 15 approximately 50 percent regression during this period. Complete regression was observed by the age of five in 67.5 percent of cases and by the age of nine in 89.6 percent of cases.

Among the 158 patients diagnosed with vascular malformations, 68 were female and 90 were male (F/M = 0.75). We found that the lesions were present at birth in 70 percent of cases, and appeared between the ages of one to 15 years in the remaining patients (Fig. 3). The distribution of the lesions according to the sub-types of vascular malformations is summarized in Figure 4.

Six cases (4 female and 2 male) of the 158 vascular malformations had port-wine stain. The lesions were present at birth in all six cases and were located on the face in four and distributed over the body in two. In patients whose lesions were located on the face, the lesions were disseminated through the dermatomes of the maxillary branch in two, maxillary and ophthalmic branches in one, and all three branches of the trigeminal nerve in one case. The average follow-up period of these cases was 9.5 years (5-15 years). During this period, none of the lesions involuted and the symptoms of Sturge-Weber syndrome were not observed in any patient.

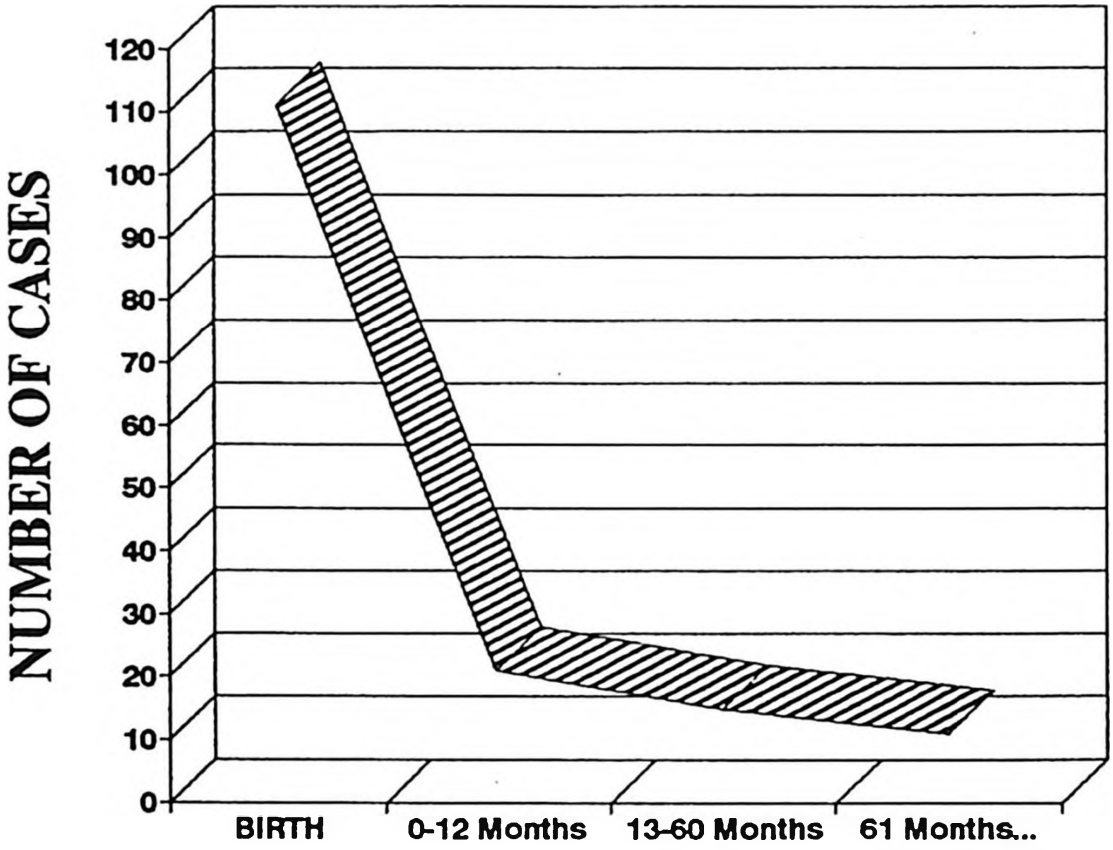


Fig. 3: The distribution of recognition ages of 158 vascular malformatilons.

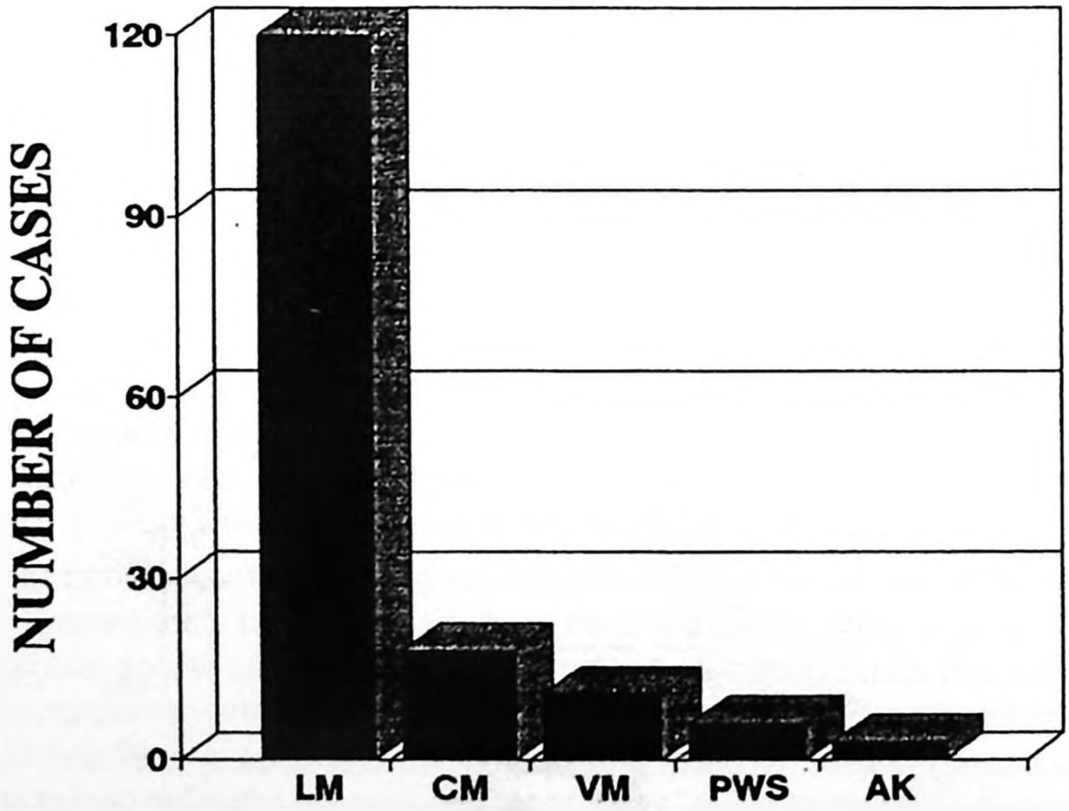


Fig. 4: The distribution of the vascular malformations according to the subtypes [LM: Lymphatic Malformations, CM: Combined Malformations, VM: Venous Malformations, PWS: Port-Wine Stain, AK: Angiokeratoma].

Angiokeratoma, another vascular malformation, was determined in three cases (2 female, 1 male). Lesions, which were recognized at birth, were located in the lower extremities in two patients and the trunk in the third. All the patients suffered from pain and bleeding. Lesions of two patients were surgically excised because of frequent bleeding.

Eleven patients (4 female, 7 male) had venous malformations. The lesions were recognised at birth in nine cases and by five years of age in two cases. These lesions were located in the extremities in eight cases, and widely distributed in the extremities, trunk and face in the remaining three cases. In seven patients, the diagnosis was confirmed by angiography which showed ectatic veins and prolonged pooling of contrast in the venous phase.

Lymphatic malformations (120 cases) was the largest group of vascular malformations in our series. Out of 120 cases (51 female, 69 male), 118 were lymphangiomas and two were intestinal lymphangiectasias. In 11 cases with lymphangioma, multiple lesions were observed. The total number of lymphangiomas was 133, and their localizations are shown in Table III. Sixty-

Table III: Localizations of Lymphangioma Lesions

Localization		Number of Lesions (*)	%
Head and neck		61	45.8
Neck	35		
Tongue	8		
Periorbital	4		
Floor of mouth	2		
Other parts	12		
Extremity		29	21.8
Trunk		23	17.3
Axilla		9	6.7
Intestinal mesentery		5	3.7
Mediastinum		3	2.3
Liver		1	0.8
Spleen		1	0.8
Salivary gland		1	0.8
Total		133	100.0

* Multiple lesions were seen in some of the cases with lymphangioma.

nine (51.8%) out of 133 lymphangiomas were of the cystic type. The most prominent complaint (84.7%) of these patients was mass and swelling in various regions of the body. However, 6.7 percent had macroglossia, 3.3 percent had respiratory distress, and 4.9 percent had feeding and/or speech problems.

Lymphangiomas were present since birth in 62 percent of cases and appeared up to the fifth year of age in 90.7 percent. Two cases presented with complaints of abdominal pain, distention and diarrhea; a diagnosis of intestinal lymphangiectasia, which causes malabsorption and protein-losing enteropathy, was confirmed by lymphoscintigraphy and intestinal biopsy. Lymphangiomatous masses in different localizations were excised totally in 50 cases and partially in six cases. Recurrence was observed in 12 and four cases, in totally and partially excised groups, respectively.

There were 18 patients with combined malformations, in which different combinations of lymphatic venous and capillary vascular malformations were present. Five of these patients had capillary and venous malformations eight had capillary and lymphatic malformations, three had venous and lymphatic malformations and two had all three types of malformations in the same localization. The malformations were located on an extremity in 15 patients, on the trunk in one patient, and on both an extremity and the trunk in one patient. Klippel-Trenaunay-Weber syndrome (KTWS) was diagnosed in 10 of these cases in which hypertrophy of the skeleton and soft tissue was present. Venography was performed in three of these 10 patients and occlusion in the proximal branches of the vena femoralis was demonstrated.

Discussion

Benign tumors of vascular endothelium and vascular malformations are the main lesions of vascular tissue in childhood. It has been reported that 21.7 percent of all malformative lesions and all benign tumors in childhood originated from vascular tissue[8].

In the present study, a total of 1127 cases with either hemangioma, lymphangioma, venous malformation, port-wine stain, angiokeratoma or combined vascular malformations were retrospectively evaluated. Hemangioma, a benign tumor of vascular endothelium, is the most frequent tumor in childhood[9, 10]. The number of all patients with benign tumors followed by our department for 19 years was 1175 and 82.5 percent of them were hemangiomas. The incidence of vascular malformations varies with respect to the sub-type of the malformation. The incidence of port-wine stain is approximately 0.3 percent[11] and that of angiokeratoma is extremely small[12, 13]. It has been reported that only five of 3000 new applications to a surgery clinic per year were lymphangiomas[14]. One hundred and eighteen out of 5504 new cases referred to our clinic over 19 years were cases with lymphangiomas.

The diagnosis was based only on the history, clinical condition and follow-up data in 97.9 percent of our cases, on histopathological examinations in 0.9 percent

and on radiological methods in 1.2 percent. Finn et al.[15] stated that hemangiomas and vascular malformations could be clinically diagnosed and distinguished without any histopathological or laboratory examination in 96 percent of cases.

Hemangiomas are more frequent in females than males, but there is no difference between sexes for vascular malformation[5, 9, 14, 15]. In our study, hemangiomas were 2.5 times more frequent in females, while vascular malformations were slightly more predominant in males. In the present study, we observed that hemangiomas generally appeared in the first few weeks of the postnatal period, and showed rapid growth and slow involution phases. In completely regressed hemangioma cases, we saw that regression had occurred in 67.5 percent by the age of five years and in 89.6 percent by the age of nine years. Vascular malformations were generally present at birth, grew proportionally with the child and never involuted. These findings are consistent with the data presented by some authors[5, 15].

Therapeutic intervention for hemargiomas should be considered only in rare cases, because the majority of lesions undergo spontaneous involution. Approximately 10-20 percent of hemangioma lesions cause problems, such as bleeding, infections, compression or obstruction[7, 9, 10, 16]. Only 10.5 percent of our cases with hemangiomas required treatment. The remaining 867 cases were followed-up by our clinic or by their local doctors.

The risk factors and management of vascular malformations vary according to sub-type[7, 16, 17]. Cases with port-wine stain should be treated because of cosmetic and psychological problems[18]. The lesions can be camouflaged by cosmetic creams. Laser has been used to treat port-wine stains for over 15 years. Good or excellent response to dye laser treatment can be expected in 60-90 percent of patients[16, 18, 19]. We did not use any treatment modality in our patients with port-wine stain. Treatment of angiokeratomas is usually symptomatic. Electrocoagulation, cryotherapy, laser therapy and surgical excision have been recommended for control of the symptoms[12, 13, 17]. Two of our cases with angiokeratomas underwent surgical treatment because of frequent bleeding. Therapy for venous malformations is usually undertaken for cosmetic and functional reasons. Radiotherapy, electrocoagulation, sclerotherapy, and compression therapy can be used, but usually venous malformations cannot be treated successfully[7, 17]. In the present study, compression therapy with elastic bandage was used in patients with venous malformations on their extremities. The recommended treatment for lymphangiomas is surgical excision. Total excision of these lesions should be carried out if it is possible, but it is not always feasible due to certain anatomical relationships[7, 17, 20, 21]. Heether et al.[22] stated that patients whose lesions were partially excised had remained free of complication and rarely required re-operation. Other modalities such as sclerotherapy and radiotherapy have been proposed for the treatment of

lymphangiomas but the results of these methods were not satisfactory[7, 20, 23]. In our series, 56 of 118 patients with lymphangiomas underwent surgical treatment. Lesions were totally excised in 42.3 percent of cases and partially in five percent. Recurrence rate was found to be 28.6 percent. Six of 16 recurrent lymphangioma cases required re-operation.

Sturge-Weber syndrome is described as the port-wine stain located on the face, ipsilateral vascular malformation in the leptomeninges, and choroid plexus of the eye[24]. This syndrome was seen in 11 percent of port-wine stain cases whose lesions were located in the head/neck region and 29 percent of cases with port-wine stain on the dermatomes of the trigeminal nerve[25]. We had four cases with a port-wine stain on the dermatomes of the trigeminal nerve, but none of these cases was defined as having Sturge-Weber syndrome, possibly because of the low number of port-wine stain cases.

KTWS is characterized by large capillary malformations, varicose veins, lymphatic malformations and skeletal-soft tissue hypertrophy[1, 24]. Capillary malformations on the skin have previously been incorrectly termed hemangiomas[25]. Servelle[26] showed an elongation of the extremity in 86 percent of 786 cases with KTWS, atrophy in 10 percent, varicosity in 36 percent, and capillary malformation in 32 percent of the cases. In the same study, it was reported that the findings of KTWS were seen on the lower extremities in 79.6 percent of cases, upper extremities in 13 percent, both upper extremities in 3.2 percent both lower extremities in 1.9 percent, and all extremities in 2.3 percent[26]. Elongation of the extremity was present in 20 percent, atrophy in 10 percent, varicosity in 40 percent, and capillary malformation in 70 percent of the 10 KTWS cases in our series. Findings were present in the lower extremity in eight, upper extremity in one, and upper and lower extremities together in one case.

Servelle[26] showed some malformations such as agenesis, hypoplasia, and fenestration in veins and the perivenous sheath. Such malformations were defined as the causes of KTWS by Servelle[26], but Baskerville[27] regarded them as the components rather than the causes of KTWS. In three of the 10 KTWS cases in the present study, occlusion in the veins was shown by venography.

It is important to evaluate a vascular lesion present in childhood, whether it is a vascular tumor or a vascular malformation. A capillary hemangioma can easily be underestimated as a port-wine stain and the cavernous type as a lymphangioma. The correct diagnosis can be made with a careful history, physical examination and clinical follow-up. Because over 90 percent of hemangiomas regress, the current accepted management remains careful observation and the education of parents. Families must be properly enlightened in order to alleviate any anxiety and prevent some unnecessary interventions.

REFERENCES

1. Lister WA. The natural history of strawberry nevi. Lancet 1938; 1: 1429-1434.
2. MacCollum DW, Martin LW. Hemangiomas in infancy and childhood. Surg Clin North Am 1956; 36: 1647-1663.
3. Simpson JR. Natural history of cavernous hemangiomata. Lancet 1959; 12: 1057-1059.
4. Martin LW. Angiomas in infancy and children. Am J Surg 1964; 107: 511-517.
5. Mulliken JB, Glowacki J. Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. Plast Reconstr Surg 1982; 69: 412-422.
6. Pearson JM, McWilliam LJ. A light microscopical, immunohistochemical, and ultrastructural comparison of hemangiomata and lymphangiomata. Ultrastruct Pathol 1990; 14: 497-504.
7. Enjolras O, Mulliken JB. The current management of vascular birthmarks. Pediatr Dermatol 1993; 10: 311-333.
8. Anderson DH. Tumors of infancy and childhood. Cancer 1951; 4: 890-906.
9. Hartley AH, Rasmussen JE. Hemangiomas and Spitz nevi. Pediatr Rev 1990; 11: 262-267.
10. Cohen BA. Hemangiomas in infancy and childhood. Pediatr Ann 1987; 16: 17-26.
11. Jacobs AH, Walton RG. The incidence of birthmarks in neonates. Pediatrics 1976; 58: 218-222.
12. Imperial R, Helwig EB. Angiokeratoma: a clinicopathological study. Arch Dermatol 1967; 95: 166-175.
13. Sehgal VN, Ghorpade A, Koranne RV. Angiokeratoma corporis naevi forme. Dermatologica 1984; 168: 144-146.
14. Bill A, Sumner DS. A unified concept of lymphangioma and cystic hygroma. Surg Gynecol Obst 1965; 120: 79-86.
15. Finn MC, Glowacki J, Mulliken JB. Congenital vascular lesions: clinical application of a new classification. J Pediatr Surg 1983; 18: 894-900.
16. Silverman RA. Hemangiomas and vascular malformations. Pediatr Clin North Am 1991; 38: 811-834.
17. Fishman SJ, Mulliken JB, Hemangiomas and vascular malformations of infancy and childhood. Pediatr Clin North Am 1993; 40: 1177-1200.
18. Wagner KD, Wagner RF Jr. The necessity for treatment of childhood port-wine stains. Cutis 1990; 45: 317-318.
19. Tan OT, Gilchrest BA. Laser therapy for selected cutaneous vascular lesions in the pediatric population: a review. Pediatrics 1988; 82: 652-662.
20. Curningham MJ. Management of congenital neck masses. Am J Otolaryngol 1992; 13: 78-92.
21. Fonkalsrud EW. Surgical management of congenital malformations of lymphatic system. Am J Surg 1974; 128: 152-159.
22. Heether J, Whalen T, Doolin E. Follow-up of complex unresectable lymphangiomas. Am Surg 1994; 60: 840-841.
23. Ogita S, Tsuto T, Nakamura K, Deguchi E, Iwai N. OK-432 therapy in 64 patients with lymphangioma. J Pediatr Surg 1994; 29: 784-785.
24. Enjolras O, Riche MC, Merland JJ. Facial port-wine stains and Sturge-Weber syndrome. Pediatrics 1985; 76: 48-51.
25. Lindenauer SM. The Klippel Trenaunay syndrome. 768 operated cases. Ann Surg 1965; 162: 303-314.
26. Servelle M. Klippel and Trenaunay's syndrome. Ann Surg 1985; 201: 365-373.
27. Baskerville PA, Ackroyd JS, Browse NL. The etiology of the Klippel-Trenaunay syndrome. Ann Surg 1985; 202: 624-627.

The Turkish Journal of Pediatrics 1997; 39: 447-452

ALLELE DISTRIBUTION OF D5S125, MAP1B5' AND D5S679 MICROSATELLITE MARKERS IN TURKISH SPINAL MUSCULAR ATROPHY FAMILIES*

*Hayat Erdem PhD***, *Sacide Pehlivan MS****, *Haluk Topaloğlu MD*****
*İnci Togan PhD******, *Meral Özgüç PhD*******

SUMMARY: Erdem H, Pehlivan S, Topaloğlu H, Togan İ, Özgüç M. (Department of Medical Biology and Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Allele distribution of D5S125, MAP1B5' and D5S679 microsatellite markers in Turkish spinal muscular atrophy families. Turk J Pediatr 1997; 39: 447-452.

Spinal muscular atrophy (SMA) is an autosomal recessive disease and one of the most common genetic causes of death in childhood. The gene for SMA has been mapped to chromosome 5q11.2-13.3. Chromosomal distribution of the alleles of D5S125, MAP1B5' and D5S679 polymorphic microsatellite markers in 14 unrelated Turkish SMA families have been determined. It is observed that the A9 allele of D5S679 has a significant (χ^2: 3.41 p: 0.065) non-random association with mutant chromosomes.
Key words: spinal muscular atrophy, microsatellite, polymorphism, non-random association.

Spinal muscular atrophy (SMA) is the second most common lethal, autosomal recessive disease in Caucasians that affects the α-motor neurons, leading to symmetrical weakness and wasting of voluntary muscles. There are three childhood onset forms of SMA: acute Werdnig-Hoffmann disease (type I), intermediate SMA (type II), and Kugelberg-Welander (type III)[1,2]. It has been shown that all three forms of the disease are due to mutations in the same region (5q11.2-q13.3)[3-6].

Recent studies have shown that this region contains multiple copies of genes and pseudogenes[7,8]. Two candidate genes, neuronal apoptosis inhibitory protein (NAIP) and survival motor neuron (SMN), have been reported as the cause of childhood SMA, and these genes frequently show homozygous deletions in SMA patients. The SMN gene is present in two copies within the SMA-candidate region. Single base substitutions in exons 7 and 8 distinguish the telomeric and the centromeric copy of SMN. The telomeric copy has been found to be deleted

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

** Associate Professor of Medical Biology, Hacettepe University Faculty of Medicine.

*** Research Assistant in Medical Biology, Hacettepe University Faculty of Medicine.

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

***** Professor of Biology, Middle East Technical University, Ankara.

****** Professor of Medical Biology, Hacettepe University Faculty of Medicine.

This work was partially supported by the Turkish Scientific and Technical Research Council (TUBİTAK) Research Fund No: SBAG-AYD-10.

in 95-98 percent of all forms of the disease[9-13]. The NAIP gene shows deletions in about 50 percent of type I SMA patients and 18 percent of type II and III patients[14, 15].

On the other hand, several multicopy dinucleotide markers which show strong linkage disequilibrium with the disease have been identified and used for molecular typing of SMA families[16-20]. Genetic linkage analyses have demonstrated that with respect to CA-repeat markers, the SMA gene lies within the interval (4cM) between D5S125 and MAP1B5'. Microtubule-associated protein (MAP1B5') locus has two CA-repeat regions at 5' and 3' direction of the gene[21-23]. Microsatellites corresponding to these loci exhibit variability in their length because of the differences in their number of repeat units. D5S125, MAP1B5' and D5S679 have 2, 6 and 10 different alleles, respectively[24, 25].

Material and Methods

In 14 families, SMA was diagnosed according to the telomeric SMN gene (exons 7 and 8). Deletions and patients were classified by the standard criteria presented by the international SMA consortium[26]. The clinical classification of the 14 SMA families was of type I and II. These families were unrelated. To determine the haplotypes of the normal and mutant chromosomes with respect to D5S125, MAP1B5' and D5S679 loci, 10 ml blood samples were taken under consent from the parents and children. DNA samples from leukocytes were obtained by salt precipitation method[27].

Dinucleotide repeat polymorphisms were analyzed by polymerase chain reaction. Each genomic DNA sample (200 ng) was amplified in a 50 µl reaction consisting of 10 mM Tris, 200 µM dGTP, 200 µM dCTP, 200 µM dTTP, 200 µM dATP, 1.5 mM $MgCl_2$, 50 mM KCl, 110 pmol of each primer, and 2.5 units of Taq DNA Polymerase (Promega). After an initial denaturation at 94 °C for five minutes, the samples were cycled through 94 °C 1 min, 62 °C 1 min (D5S125 and MAP1B5'), 53 °C 1 min (D5S679) and 72 °C 1 min D5S125 and D5S679 PCRs were cycled 30 times and MAP1B5' PCRs were cycled 23 times. After cycling, the reactions were incubated for 7 min at 72 °C to ensure complete elongation of all products.

The primer sequences used for amplifications are:

D5S125:
F: AAG AGG ACT CCC ATG CTT GTT G
R: TAG AAA ATA CTA CAG TCT TCT TGG G

MAP1B5':
F: TTC CTT CTT CCA AAA CCA GGG TGA AGC CTC
R: AAA TTT CTA GGA TGC TTG CGG GAT CTC TTC

D5S679:
F: TTG CAT TTG GGA AAG AG
R: GCA ACT TCT TTA CTC TC

PCR products of D5S125 and MAP1B5' were analyzed on 12 percent acrylamide (19:1) nondenaturing gels (7h, 340 V) and D5S679 was analyzed on 6 percent acrylamide (19:1) denaturing gel (5h, 36 W). The results were visible with silver staining technique[28]. Gels are fixed by incubation in 10 percent ethanol, 0.5 percent acetic acid for three minutes twice, stained in 0.1 percent aqueous solution of silver nitrate for 15 minutes. After incubating the gel in 1.5 percent NaOH and 0.1 percent formaldehyde solution for 20 minutes, it is fixed with 0.75 percent solution of Na_2CO_3.

To detect the possible significant association between the mutant and normal chromosomes, a chi-square test was carried out[29].

Results

Consanguinity was observed in 64 percent of the families. This was taken into consideration in normalizing the allele counts. Normal and mutant chromosomes were analyzed with respect to D5S125, MAP1B5' and D5S679 markers. The haplotype of each individual was determined by the CA-repeat length patterns that each carries. As an example: in Figure 1, genetic structures of the individuals based on D5S679 locus is shown. The chromosomal distributions of the allele types of the markers and the results of the chi-square test are presented in Table I.

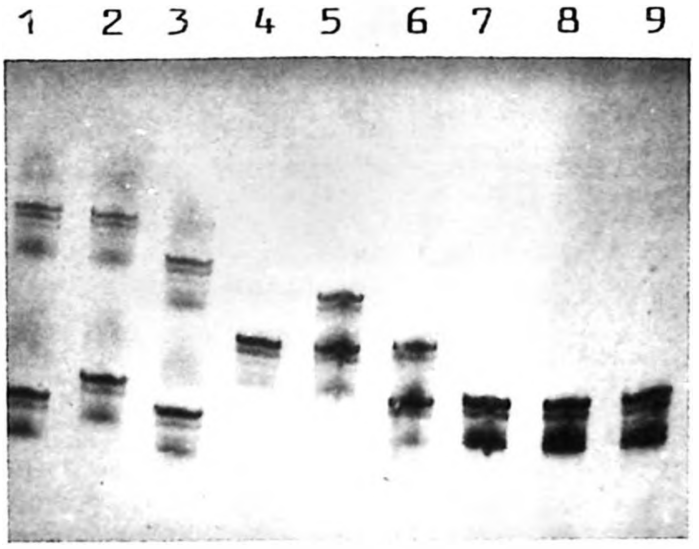


Fig. 1: Segregation of D5S679 polymorphism in 3 families with SMA.
Lane 1 (Patient 1): 4/10
Lane 2 (Father 1) : 4/9
Lane 3 (Mother 1): 5/10
Lane 4 (Patient 2): 7/7
Lane 5 (Father 2) : 6/7
Lane 6 (Mother 2): 7/9
Lane 7 (Patient 3): 9/9
Lane 8 (Father 3) : 9/9
Lane 9 (Mother 3): 9/9

Table I: Chromosomal Distribution of Microsatellite Allele Types and χ^2 Values Obtained by Chi-Square Test

Microsatellite Marker	Alleles	Allele Size (bp)	Normal Chromosomes	Mutant Chromosomes	χ^2 - Values
D5S125					
	A1	143	21	18	
	A2	147	7	3	0.083
MAP1B5'					
	A1	139	1	–	
	A2	137	4	3	
	A3	135	3	7	
	A4	133	9	7	
	A5	131	9	10	
	A6	129	1	1	1.05
D5S679					
	A1	222	–	–	
	A2	220	2	–	
	A3	212	3	–	
	A4	210	3	–	
	A5	208	–	–	
	A6	198	1	–	
	A7	196	4	4	
	A8	190	–	1	
	A9	184	11	15	
	A10	180	3	2	3.41*

*: Statistically significant (χ^2: 3.41 p: 0.065).

Eleven of the 14 families were informative for all three markers simultaneously. In Table II, distribution of the alleles of these three markers on normal and mutant chromosomes are given.

Table II: Haplotype Determination Using D5S679, D5S125 and MAP1B5' Markers

Family No.	Normal Chromosome	Mutant Chromosome
1	3-2-5	9-1-2
	9-1-1	9-1-5
2	9-1-5	9-1-3
	9-1-5	9-1-4
3	6-2-5	7-1-4
	9-2-3	7-1-2
4	7-1-4	7-1-5
	3-2-2	7-1-5
5	9-1-4	9-2-3
	7-1-4	9-1-5
6	4-1-5	9-1-5
	4-1-4	3-1-3
7	2-1-4	9-1-2
	9-2-2	9-1-3
8	9-2-5	9-1-3
	2-1-3	9-1-4
9	9-1-2	9-1-3
	10-1-3	9-1-4
10	4-1-4	10-2-5
	9-1-4	9-1-5
11	8-1-5	9-1-4
	9-1-5	9-1-5

Discussion

In a previous study, it was shown that 92 percent of Turkish SMA families had deletion type of mutations[13]. The main purpose of the present study is to define the haplotypes associated with SMN gene deletions in Turkish SMA patients using microsatellite markers.

The markers lie with the order of D5S679, D5S125/SMA/MAP1B5' from the centromeric to telomeric position. A1 of D5S125 and A5 of MAP1B5' are the most common alleles observed on both normal and mutant chromosomes. Allelic distributions of D5S125 and MAP1B5' markers on normal and mutant chromosomes are not significantly different from each other. The A9 allele of D5S679 is the predominant allele, with significant selection for the mutant chromosomes.

These results indicate that the markers used to analyze the allele segregations are polymorphic in the normal Turkish population and that a risk haplotype for the mutant alleles cannot be stated.

REFERENCES

1. Dubowitz V. Muscle Disorders in Childhood. Philadelphia: W.B. Saunders Comp; 1978: 146-148.
2. Pearn JH. Incidence, prevalence and gene frequency studies of chronic childhood spinal muscular atrophy. J Med Genet 1978; 15: 409-413.
3. Brzustowicz LM, Lehner T, Castilla LH, et al. Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q11.2-13.3. Nature 1990; 344: 540-541.
4. Gilliam TC, Brzustowicz LM, Castilla LH, et al. Genetic homogeneity between acute and chronic forms of spinal mscular atrophy. Nature 1990; 345: 823-825.
5. Melki J, Abdelhak S, Sheth P, et al. Gene for chronic proximal spinal muscular atrophies maps to chromosome 5q. Nature 1990; 344: 767-768.
6. Melki J, Sheth P, Abdelhak S, et al. Mapping of acute (type I) spinal muscular atrophy to chromosome 5q12-q14. Lancet 1990; 326: 271-273.
7. Soares VM, Brzustowicz LM, Kleyn PW, et al. Refinement of the spinal muscular atrophy locus to the interval between D5S435 and MAP1B. Genomics 1993; 15: 365-371.
8. Lewin B. Gene for SMA: multum in parvo. Cell 1995; 80: 1-5.
9. Hahnen E, Forkert R, Marke C, et al. Molecular analysis of candidate genes on chromosome 5q11 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. Hum Mol Genet 1995; 4: 1927-1933.
10. Lefebvre S, Bürglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. Cell 1995; 80: 155-165.
11. van der Steege G, Grootscholten PM, van der Vlies P, et al. PCR-based DNA test to confirm clinical diagnosis of autosomal recessive spinal muscular atrophy. Lancet 1995; 345: 985-986.
12. Rodrigues NR, Owen N, Talbot K, et al. Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. Hum Mol Genet 1995; 4: 631-634.
13. Erdem H, Pehlivan S, Topaloğlu H, et al. Deletions in the survival motor neuron gene in Turkish spinal muscular atrophy patients. J Inherit Metab Dis 1996; 19: 724-728.

14. Roy N, Mahadevan MS, McLean M, et al. The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. Cell 1995; 80: 167-178.

15. Thompson TG, Di Donato CJ, Simart LR, et al. A novel cDNA detects homozygous microdeletions in greater than 50% of type I spinal muscular atrophy patients. Nat Genet 1995; 9: 56-62.

16. Clermont O, Burlet P, Burglen L, et al. Use of genetic and physical mapping to locate the spinal muscular atrophy locus between two new highly polymorphic DNA markers. Am J Hum Genet 1994; 54: 687-694.

17. Burghes AH, Ingraham SE, McLean M, et al. A multicopy dinucleotide marker that maps close to the spinal muscular atrophy gene. Genomics 1994; 21: 394-402.

18. Brahe C, Velona I, van der Steege G, et al. Mapping of two new markers within the smallest interval harboring the spinal muscular atrophy locus by family and radiation hybrid analysis. Hum Genet 1994; 93: 494-501.

19. Wirth B, Pick E, Leutner A, et al. Large linkage analysis in 100 families with autosomal recessive spinal muscular atrophy (SMA) and 11 CEPH families using 15 polymorphic loci in the region 5q11.2-q13.3. Genomics 1994; 20: 84-93.

20. Melki J, Burlet P, Clermont O, et al. Refined linkage map of chromosome 5 in the region of the spinal muscular atrophy gene. Genomics 1993; 15: 521-524.

21. Sherington R, Mankoo B, Curtis D, et al. Seven new dinucleotide polymorphisms at 5q11.2-13.3 and RFLP for the 5HT1a receptor gene. Cytogenet Cell Genet 1991; 58: 1902.

22. Morrison KE, Daniels RJ, Suthers GK, et al. Two novel microsatellite markers for prenatal prediction of spinal muscular atrophy (SMA). Hum Genet 1993; 92: 133-138.

23. Simard LR, Prescott G, Rochette C, et al. Linkage disequilibrium analysis of childhood-onset spinal muscular atrophy (SMA) in the French-Canadian population. Hum Mol Genet 1994; 3: 459-463.

24. Brzustowicz LM, Kleyn PW, Boyce FM, et al. Fine-mapping of the spinal muscular atrophy locus to a region flanked by MAP1B and D5S6. Genomics 1992; 13: 991-998.

25. Mankoo BS, Sherrington R, De La Cocha A, et al. Two microsatellite polymorphisms at the D5S39 locus. Nucleic Acids Res 1991; 19: 1963.

26. Munsat TL. International SMA collaboration. Neuromusc Disorder 1991; 1: 81.

27. Miller SA, Dykes DP, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.

28. Lenk U, Hanke R, Kräft U, et al. Non-isotopic analysis of single strand conformation polymorphism (SSCP) in the exon 13 region of the human dystrophin gene. J Med Genet 1993; 30: 951-954.

29. Wayne WD. Biostatistics: A Foundation for Analysis in the Health Sciences. New York: John Wiley and Sons Inc.; 1995.

The Turkish Journal of Pediatrics 1997; 39: 453-457

DEFECTIVE SERUM OPSONIZATION ACTIVITY IN CHILDREN AGED 6 – 48 MONTHS HAVING ACUTE PURULENT OTITIS MEDIA*

*İlhan Tezcan MD PhD**, *Yeşim Yılmaz MD****, *Filiz Öner PhD*****
*Leman Yel MD******, *Özden Sanal MD*******, *Fügen Ersoy MD********
*Metin Önerci MD*********, *A. İzzet Berkel MD*********

SUMMARY: Tezcan İ, Yılmaz Y, Öner F, Yel L, Sanal Ö, Ersoy F, Önerci M, Berkel Aİ. (Immunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Defective serum opsonization activity in children aged 6-48 months having acute purulent otitis media. Trk J Pediatr 1997; 39: 453-457.

Serum opsonization of yeast (Saccharomyces) was investigated in 51 patients whose ages were between six and 48 months (median 15 months) with acute purulent otitis media and in an age-matched control group (median 13 months). Opsonization was assessed by measuring yeast particle uptake in an assay based on an electronic count of the unphagocytosed particles in serum by polymorphonuclear leukocytes. Despite normal levels of CH50 and serum immunoglobulins, a defective opsonization was determined in 13.7 percent of the patients (7 in 51). The corresponding figure was 2.9 percent in 103 healthy controls ($p < 0.001$). On the other hand, 218 percent (5 in 23) of the children having a history of recurrent purulent otitis media showed defective opsonization ($p < 0.001$). Previously, the presence of an opsonization defect has been linked to low levels of mannan binding lectin (MBL), a calcium dependent serum lectin that acts as an opsonin. Therefore, our findings indirectly support the idea that MBL has an important role as host defense, particularly in the earlier period of life when the antibody repertoire is restricted. *Key words: common opsonic defect, acute otitis media, mannan binding lectin.*

Acute otitis media (AOM) is one of the most common infectious diseases of childhood[1,2]. The peak incidence of AOM occurs in the second half of the first year of life, with a second, lower peak, between the ages of four and five years. Most of the children have their first episode of AOM in their first two years of life. Opsonophagocytosis is the key immune mechanism against encapsulated bacteria (S. pneumonia, H. influenzae, etc.) the leading cause of AOM[3]. During the early years of life, the antibody repertoire is restricted. Members of the

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

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

*** Pediatrician.

**** Professor of Pharmacology, Hacettepe University Faculty of Pharmacy.

***** Research Fellow, Department of Immunology, St. Jude Children's Hospital USA.

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

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

collectin family, for instance mannan binding lectin (MBL), may play a critical role in opsonization of sugar-containing bacteria[4-6]. Super et al.[5] showed that common opsonic defect is linked with low serum levels of MBL.

Evaluation of the host defense and risk factors, such as bottle feeding, parental smoking, and dysfunction of the eustachian tube, are of utmost importance for the treatment of both AOM and its possible complications. In this study, we investigated serum opsonization activity in 51 children with AOM to determine whether common opsonic defect is an important risk factor for acute purulent otitis media when the antibody repertoire is restricted.

Material and Methods

The study group consisted of 51 patients aged between six and 48 months (median 15 months, 28 male, 23 female) who were diagnosed as AOM without apparent underlying immunodeficiency in Hacettepe University Children's Hospital during the period December 1993-May 1994.

The diagnostic criteria of AOM were fever, earache, purulent discharge in the external ear canal, and perforation and hyperemia of the eardrum. The patients having three or more attacks of acute purulent otitis media during the six months prior to admission were defined as the recurrent otitis media group. (ROMG, n: 23).

Each case was examined by an ear, nose and throat (ENT) specialist to exclude from the study the cases with hypertrophic adenoids.

The study also consisted of a control group of 103 healthy children aged between six and 46 months (median 13 months, 57 male, 46 female).

Serum samples were obtained two to three weeks after the acute illness and stored at -30 °C until the analysis. Quantitative serum immunoglobulins, CH50, and IgG subclasses of the patients were studied by conventional methods.

Measurement of serum opsonic activity: An objective semi-quantitative method for killed baker's yeast (S. cerevisiae) opsonization by normal neutrophils was used[7]. Briefly, 10 µl patient's serum, 50 µl 0.9 percent NaCl, 50 µl yeast particles (108/ml) and 100 µl healthy neutrophils (PMN) (1 x 10^7/ml) were mixed together in a plastic tube and incubated at 37 °C for 30 minutes on a mixer. In controls, 50 µl yeast particles made up to the same reaction volume of only saline were similarly incubated. After completion of the incubation, the reaction mixture was diluted 1:500 in Isoton (Coulter Electronics Ltd.). The number of yeast particles left unphagocytosed were counted in a Coulter Counter, set to count particles 3-5 µm. Result are expressed as percentage uptake of yeast by normal PMNs and are calculated as follows. Percentage uptake: The number of yeast particles added to the reaction (A)-the number of yeast particles left unphagocytosed: A x 100.

The deficient serum was defined as the value less than two standard deviations (-2SD) from the mean measured in the healthy control group.

Student's t and Mann-Whitney U tests were used for the statistical analysis; p value below 0.05 was considered significant.

Results

The percentage of yeast opsonization in the healthy controls was 60.9 ± 13.6 percent (Fig. 1). The deficiency level was calculated as less than 33.5 percent. The percentages of defective opsonization in the healthy group and the patient group were 2.9 percent (3 in 103) and 13.7 percent (7 in 51), respectively ($p < 0.001$) (Fig. 2). Among the cases with recurrent otitis media (ROMG), the percentage of defective opsonization was found to be much higher (21.8%, 5 in 23) in comparison to the controls ($p < 0.001$).

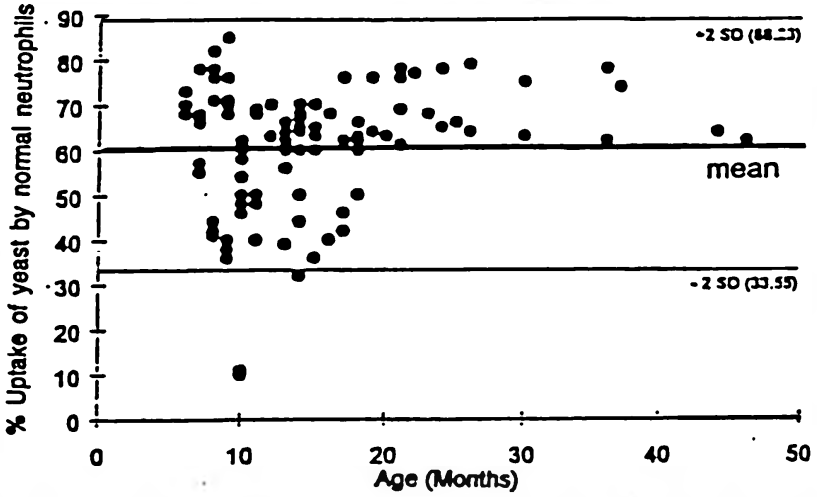
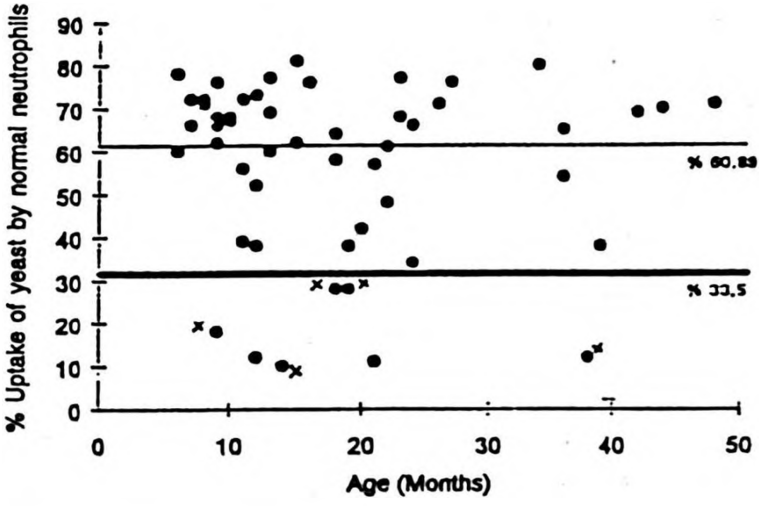


Fig. 1: The distribution of opsonization activity in healthy children.



* The cases with defective opsonization activity from recurrent otitis media group.

Fig. 2: The distribution of opsonization activity in children with acute otitis media.

Other immunological tests (serum immunoglobulins, IgG subclasses and CH50) of the patients were within normal ranges.

Discussion

Miller et al.[8] first defined the failure of serum to opsonize baker's yeast for phagocytosis by normal neutrophils in an infant with severe recurrent infections, diarrhea and failure to thrive. The defect was also shown in a group of children with chronic diarrhea[9]. In 1983, Richardson et al.[10] reported that 26 healthy term babies with defective yeast opsonization activity had a tendency to develop acute otitis media, gastroenteritis and atopy during a one year follow-up period. The defect occurs in five to eight percent of the normal population[6, 11]. In our study, 2.9 percent of the healthy subjects were shown to have defective opsonization activity. The defect was found to be significantly higher in both AOM and ROM groups, when compared to the healthy age-matched controls (13.7 and 21.8% vs 2.9%, respectively). Since all patients with defective opsonization had a history of three to 15 attacks of otitis media, our results may suggest that common opsonic defect could be considered as a contributing factor for the recurrence of acute otitis media in children under the age of four years.

In 1989, Super et al.[5] reported that common opsonic defect is linked with low serum levels of mannan binding lectin (MBL), which is a member of the collectin family of proteins with a collagenous region and a lectin domain. It has various features, including binding a wide range of substrates with multiple carbohydrate binding sites, acting as an opsonin by coating sugar-rich microbial surfaces and also initiating activation of the complement system[12]. It is likely to be a very important component in the innate immune system, particularly in the early steps of inflammation.

Recently, Garred et al.[13] reported that a higher frequency of homozygotes for abnormal MBL alleles associated with a low serum MBL level was observed in 228 unrelated patients suspected of non-HIV related immunodeficiencies, when compared to homozygosity for abnormal alleles in healthy controls (8.3 vs 0.8 percent). In a study conducted to determine MBL levels in plasma of children with recurrent otitis media, the same authors reported no difference in MBL levels in the healthy and diseased children[14]. However, it should be noted that the median age of the patient group was a rather late age of 51.5 months, at which time many defense mechanisms of the immune system have already been developed.

In a recent report, Summerfield et al.[15] showed that the prevalence of MBL gene mutations in children admitted to the hospital due to various infections was about twice that in children without infection. Mutations in the MBL gene which cause a common opsonic defect are associated with the infections. We believe that prospective controlled studies conducted on larger patient populations with various infections will contribute significantly to a better understanding of the role of abnormal alleles of MBL in the distinct susceptibility of children to various types of infections, including AOM.

REFERENCES

1. Teele DW, Klein JO, Rosner B. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study J Infect Dis 1989; 160: 83-94.
2. Hoekelman RA. Infectious illness during the first year of life. Pediatrics 1977; 59: 119-124.
3. Klein JO, Teele DW, Pelton SI. New concepts in otitis media: results of investigations of the Greater Boston Otitis Media Study Group. Adv Pediatr 1992; 39: 127-156.
4. Turner MW. Mannose binding lectin: the pluripotent molecule of the innate immune system. Immunol Today 1996; 17: 532-540.
5. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan binding protein with a common defect of opsonisation. Lancet 1989, 2: 1236-1239.
6. Sumiya M, Super M, Tabona P, et al. Molecular basis of opsonic defect in immunodeficient children. Lancet 1991; 337: 1569-1570.
7. Levinsky RJ, Harvey BAM, Paleja S. A rapid objective method for measuring the yeast opsonisation activity of serum. J Immunol Methods 1978; 24: 251-256.
8. Miller ME, Seals J, Kaye R, Levitsky LC. A familial plasma-associated defect of phagocytosis. Lancet 1968; ii: 60-63.
9. Soothill JF, Harvey BAM. Defective opsonisation: a common immunity deficiency. Arch Dis Child 1976; 51: 91-99.
10. Richardson VF, Larcher VF, Price JF. A common congenital immunodeficiency predisposing to infection and atopy in infancy. Arch Dis Child 1983; 58: 799-802.
11. Kerr MA, Falconer JS, Bashey A, Beck JS. The effect of C3 levels on yeast opsonization by normal and pathological sera: identification of a complement independent opsonin. Clin Exp Immunol 1983; 54: 793-800.
12. Turner MW, Super M, Singh S, Levinsky RJ. Molecular basis of a common opsonic defect. Clin Exp Allergy 1991; 21 (Suppl 1): 182-188.
13. Garred P, Madsen HO, Hofmann B, Svejgaard A. Increased frequency of homozygosity of abnormal mannan-binding-protein alleles in patients with suspected immunodeficiency. Lancet 1995; 346: 941-943.
14. Garred P, Brygge K, Sprensen CH, Madsen HO, Thiel S, Svejgaard A. Mannan-binding protein-levels in plasma and upper-airways secretions and frequency of genotypes in children with recurrence of otitis media. Clin Exp Immunol 1993; 94: 99-104.
15. Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. Br Med J 1997; 314: 1229-1232.

The Turkish Journal of Pediatrics 1997; 39: 459-464

SERUM ERYTHROPOIETIN IN CHILDREN WITH IRON DEFICIENCY ANEMIA*

*Mualla Çetin MD**, *Aytemiz Gürgey MD****, *Fatma Gümrük MD*****
*Çiğdem Altay MD*****

SUMMARY: Çetin M, Gürgey A, Gümrük F, Altay Ç. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Serum erythropoietin in children with iron deficiency anemia. Turk J Pediatr 1997; 39: 459-464.

Serum erythropoietin (EPO) levels were determined in 30 children with iron deficiency anemia. The mean age of the children, hemoglobin (Hb) levels, serum EPO levels and log EPO values were 4.7 ± 5 years, 6.7 ± 1.7 g/dl, 2284 ± 3177 mU/ml and 2.81 ± 0.82, respectively. In 83 percent of the patients poor diet was the determined cause of iron deficiency and in the remaining 17 percent, chronic blood loss. A significant negative correlation was found between the log EPO values and Hb values ($r = 0.62$, $p < 0.01$). There was no significant correlation between log EPO values and the other parameters [sex, age, mean corpuscular volume (MCV) red blood cell (RBC) red cell distribution width (RDW), serum iron, iron binding capacity]. There was a significant difference in the age of the patients with an Hb value < 5.5 g/dl and those with a value ≥ 5.5 Significant differences were also observed in log EPO levels among these patients ($p < 0.004$). The mean Hb value of patients with log EPO values ≥ 3 was lower than that in patients with log EPO values < 3 ($p < 0.003$). In 20 percent of the patients, serum EPO levels were much lower than the values expected from their Hb level. Serum EPO levels were high in all five patients with a history of chronic blood loss.
Key words: erythropoietin, iron deficiency anemia.

The serum erythropoietin (EPO) level increases in anemia of various etiologies except for those which result from inefficient endogenous production of EPO, such as that seen in patients with renal failure, anemia in premature infants and anemia associated with chronic infection[1-3]. Although an inverse correlation between EPO and hemoglobin (Hb) levels is observed in general, EPO levels in iron deficiency anemia give heterogeneous results. In some studies the EPO level was not found to be as high as expected from the Hb value[4]. The age of the patients, duration (chronicity) of anemia, and underlying pathology leading to anemia might contribute a great deal of influence on the heterogeneous distribution of EPO levels in iron deficiency anemia. In order to assess possible

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

** Pediatric Hematologist, Hacettepe University Faculty of Medicine.

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

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

This study was supported in part by TUBA.

effects of these factors on serum EPO levels, we studied quite a high number of patients belonging to various age groups and with various underlying pathologies leading to iron deficiency anemia.

Material and Methods

A total of 30 patients with iron deficiency anemia were the subjects of this study. Twelve healthy children with a mean age of 8.1 ± 8.5 years (0.8-23) were used as controls. All patients were questioned regarding their dietary history and history of bleeding. Diagnosis of iron deficiency anemia was based on the presence of an Hb value ≤ 10 g/dl associated with microcytosis, hypochromia, low serum iron and increased iron-binding capacity. Stools were examined for occult blood. routine hematological evaluations were made by standard procedures. EPO level was measured by radioimmune assay.

Results

The mean age and mean hematological parameters at diagnosis are given in Table I. Twelve of the 30 patients were older than three years of age. The mean Hb, EPO and log EPO values were 6.7 ± 1.7 g/dl, 2284 ± 3177 mU/ml, and 2.81 ± 0.82, respectively. The mean EPO and log EPO values in the control group were 17.8 ± 5.9 mU/ml and 1.24 ± 0.12, respectively. A correlation study indicated the presence of a significant negative correlation between log EPO and Hb values ($r = 0.62$, $p < 0.01$) (Fig. 1). There was a significant difference between the age of the patients with an Hb value < 5.5 g/dl and those with a value ≥ 5.5. A significant difference was also observed in the log EPO levels among these patients ($p < 0.004$) (Table II). There was no significant correlation between the other parameters given in Table I. In three patients (patients 1-3) with a Hb value less than 3.6 g/dl or a hematocrit (Htc) less than 16.2 percent, EPO levels were higher than in the rest of the patients (Table I) (Fig. 1). When the patients were divided according to their log EPO values being ≥ 3 (12 patients) and (18 patients), a significant difference in the mean Hb values ($p < 0.003$) was noted (Table III). In five of the 12 (40%) patients with a value ≥ 3, disorder was present (patients 1, 7, 14, 15, 17) (Table I). In three of them, chronic idiopathic thrombocytopenic purpura (ITP) was the underlying disorder (patients 1, 15, 17). In patient seven, occult blood was positive in the stool examination (Table I) without any bleeding disorder or gastrointestinal pathology. In the remaining 25 patients, anemia was chronic and resulted from an iron-poor diet. In 6 (20%) of the 30 patients, the serum EPO level was much lower than the value expected from their hemoglobin levels (patients 7, 8, 9, 20, 21 and 28) (Table I). Two of these six patients were infants.

Table I: Some of the Clinical and Laboratory Findings

No.	Age (yr)	Sex	Hb g/dl	Hct %	MCV fl	SI µg/dl	SIBC µg/dl	Log EPO	Etiology
1.	12	F	2.6	9.7	59	10	479	4.01	Chronic ITP-rectal bleeding
2	3	F	3.4	11	49.9	10	400	4.01	PD
3	1	M	3.5	16	68.6	10	396	3.94	PD
4	1.5	M	5.2	18	53	30	342	3.89	PD
5	4	M	5.5	19	52	12	317	3.98	PD
6	5	M	5.2	18	68	13	408	3.55	PD
7	11	M	4.7	16.3	50.3	27	396	2.28	occult blood in stool
8.	15	F	4.8	15	64	32	371	2.42	PD
9	12	M	5.2	16	58.8	24	279	2.46	PD
10	2	F	6.3	22	55.4	10	283	2.86	PD
11	5	F	7.5	24	60.6	10	330	2.86	PD
12	1	M	7.3	27	47.6	55	400	2.85	PD
13	15/12	M	7.3	26	48.3	10	321	2.87	PD
14	1.5	M	6.5	28	46.6	25	427	3.25	epistaxis
15	13	F	7	25	58	12	386	3.24	Chronic ITP
16	1.5	F	7.5	25	52.6	18	304	3.22	PD
17	13	F	7.2	24	58	10	383	3.36	Chronic ITP
18	7/12	M	6.9	21	50.1	12	479	3.52	PD
19	2	M	7.2	23	46.9	32	400	3.59	PD
20	1	M	6.3	22	50.2	15	408	1.81	PD
21	10/12	M	6.7	24	51.5	13	429	1.92	PD
22	15/12	F	7.6	25	50.9	12	333	2.17	PD
23	10/12	M	8.3	27	45.8	13	383	2.75	PD
24	8/12	M	8.6	28	60.9	10	300	2.51	PD
25	10	F	8.2	27	63.6	10	342	2.49	PD
26	1.5	M	8.3	29	56.8	33	292	2.16	PD
27	1.5	M	8.8	23	53.2	23	346	1.90	PD
28	14	F	7.8	26	52.8	10	396	1.10	PD
29	1.5	F	9.9	30	57.1	12	354	1.20	PD
30	1.5	M	8.5	28	65	20	350	2.16	PD
	4.7 ± 5		6.7 ± 1.7	22.4 ± 5.4	55.2 ± 6.4	17 8 ± 10.5	368 ± 53	2.81 ± 0.82	

PD : poor diet.
ITP : Idiopathic thrombocytopenic purpura.

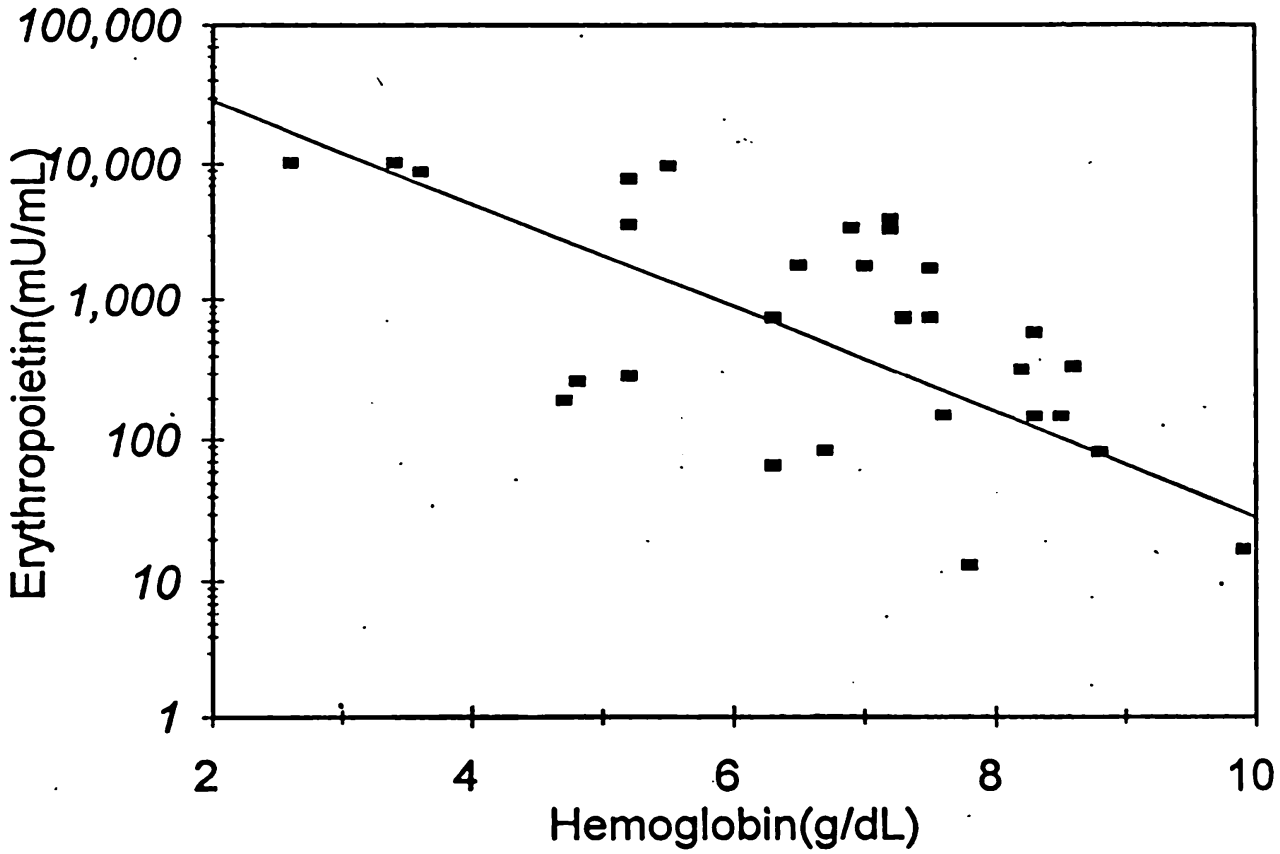


Fig. 1: Relationship between the hemoglobin concentration and the logarithm of the serum EPO level in patients with iron deficiency anemia.

Table II: Comparison of Some of the Parameters in Patients with Hb Values

Number	Hb < 5.5 g/dl	Hb ≥ 5.5 g/dl	p
	9	21	
Age (yr)	7.2 ± 5.3*	3.6 ± 4.6	0.03
	1-15**	0.7-14	
MCV (ft)	58.2 ± 7.4	53.9 ± 5.7	0.047
	49.9 ± 68.6	45.8-65	
Serum iron	18.7 ± 9.4	17.4 ± 11.2	0.38
(µg/dl)	10-32	10-55	
Iron-binding	376 ± 58	364 ± 51	0.28
capacity (µg/dl)	279-408	283-479	
Log EPO	3.39 ± 0.77	2.56 ± 0.72	0.004
	2.28-4.01	1.1-3.59	

* mean ± ISD.
** minimum and maximum values.

Table III: Comparison of Some of the Parameters in Patients with log EPO Levels ≥3 and log EPO <3

	Log EPO <3	Log EPO ≥3	p
Number	18	12	
Age (yr)	4.5 ± 5.2	4.8 ± 4.9	0.44
Hb (g/dl)	7.3 ± 1.4	5.6 ± 1.7	0.003*
MCV (fl)	55.2 ± 6.0	55.2 ± 7.3	0.48
RBC $(X10)^6$	4.2 ± 0.9	3.6 ± 1.3	0.07
RDW	22.6 ± 3.0	20.7 ± 2.8	0.05*
SI (µg/dl)	19 ± 12	16 ± 8	0.25
SIBC (µg/dl)	351 ± 47	377 ± 57	0.09

SI : Serum iron.
SIBC: Serum iron-binding capacity.

Discussion

Presence of a significant negative correlation between the EPO level and Hb value supports previously published studies. However, the EPO values in our patients seemed quite high in comparison to the values in some of the previously published studies[1,3]. This probably resulted from the severity of anemia in our group of patients. It was suggested that in severe anemia oxygen-binding heme protein fails to work, probably due to an abnormality in its heme-containing component[4,5]. Contrary to this prediction, our study, as was reported previously, indicated that the highest EPO value was found in patients with severe anemia as shown Figure 1, and Tables I and II. This indicates that even in severe iron deficiency anemia organisms either conserve the heme component of oxygen-sensing protein or some other mechanism replaces the function of oxygen-sensing protein in control of EPO production. In some studies, the EPO level was not as high as predicted from the severity of iron deficiency anemia[4]. It was shown that the EPO level returns to normal within a few days after administration of iron, long before the rise in Hb value or before restoration of iron depletion[6]. This observation indicates that either the patients or their parents should be questioned regarding and any medication before EPO determination. It is well documented that in infection, EPO levels dietary history fall below normal[7]. Therefore, it is not unlikely that in patients with iron deficiency anemia, the EPO level decreases when the patient acquires an infection. This indicates the importance of establishing a patient's health status during EPO determinations.

Our study indicated that as a whole, the age of the patients is not an important factor in modification in the EPO level in iron deficiency anemia. However, the mean Hb value was lower, and serum EPO value higher, in patients in the older age group. In infancy, the duration of anemia is several months, whereas in

older age groups, its duration is usually several years. Absence of any significant changes in EPO values in infants and older patients may indicate that the duration of anemia is not an important modifying factor in EPO levels. Presence of chronic blood loss in 40 percent of patients with a high EPO level and absence of any bleeding history in the rest of the patients suggested to us that chronic blood loss usually results in a higher serum EPO level.

This study indicated that in iron deficiency anemia in any age group, prior to any iron supplementation either as medication or as a dietary supplement and in the absence of any coexistent infection, serum EPO levels rise to a very high level and a significant correlation between Hb levels and EPO values can be expected.

REFERENCES

1. de Klerk G, Rosengarten PC, Vet RJ, Goudsmit R. Serum erythropoietin (EST) titers in anemia. Blood 1981; 58: 1164-1170.
2. Erslev AJ. Erythropoietin. N Engl J Med 1991; 324: 1339-1344.
3. Boyd HK, Pappin TR. Erythropoietin deficiency in the anaemia of chronic disorders. Eur J Haematol 1991; 46: 198-201.
4. Bray GL, Taylor B, O'Donnell R. Comparison of the erythropoietin response in children with aplastic anemia, transient erythroblastopenia, and iron deficiency. J. Pediatr 1992; 120: 528-532.
5. Goldberg MA, Dunning SP, Bunn HF. Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. Science 1988; 242: 1412-1415.
6. Cazzola M, Beguin Y. New tools for clinical evaluation of erythron function in man. Br J Haematol 1992; 80: 278-284.
7. Krafte-Jacobs B, Levetown ML, Bray GL, Ruttimann UE, Pollack MM. Erythropoietin response to critical illness. Crit Care Med 1994; 22: 821-826.

The Turkish Journal of Pediatrics 1997; 39: 465-471

ABSORPTION OF IRON FROM GRAPE – MOLASSES AND FERROUS SULFATE: A COMPARATIVE STUDY IN NORMAL SUBJECTS AND SUBJECTS WITH IRON DEFICIENCY ANEMIA*

Yakup Aslan MD, Erol Erduran MD***, Hilal Mocan MD****
*Yusuf Gedik MD*****, Ayşenur Ökten MD***, Hanifi Soylu MD******
*Orhan Değer PhD*******

SUMMARY: Aslan Y, Erduran E, Mocan H, Gedik Y, Ökten A, Soylu H, Değer O. (Departments of Pediatrics and Biochemistry, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey). Absorption of iron from grape-molasses and ferrous sulfate: a comparative study in normal subjects and subjects with iron deficiency anemia. Turk J Pediatr 1997; 39: 465-471.

We compared the absorption of iron from grape molasses (GM) and ferrous sulfate (FS) using the post-absorptive serum iron increase method (non-radioactive). The study involved 56 subjects, aged 6-36 months. Group I consisted of 30 subjects with iron deficiency anemia (IDA) and group II, 26 non-anemic subjects. Each group was subdivided randomly into two equal subgroups to be given either GM or FS. The absorption of the iron from GM was monitored in 15 infants with IDA and in 13 non-anemic infants, and the absorption of iron from FS was tested in the rest of the subjects in each group.

In those infants in each group given GM or FS, there was no significant difference in before-test values for serum iron (SI) and total iron binding capacity (TIBC) ($p > 0.05$). In the group with IDA, the mean after-test SI value in FS-given infants was higher and the mean TIBC value lower than those of GM-given infants ($p < 0.05$). However, in the non-anemic group, there was no significant difference in the mean after-test SI and TIBC values in either GM- or FS-given infants ($p > 0.05$).

The mean increase of serum iron in GM-given infants with IDA was 27.0 ± 13.4 µg/dl and in FS-given infants, 60.6 ± 17.0 µg/dl ($p < 0.05$). In the non-anemic group, the mean increase of serum iron of GM-given infants was 23.0 ± 4.3 µg/dl, and that of FS-given infants, 23.8 ± 10.0 µg/dl ($p > 0.05$).

We determined that in non-anemic subjects, the absorption of iron from GM was comparable to that from FS. Furthermore, we believe that grape molasses is an effective iron source in preventing iron deficiency anemia in infancy. *Key words: iron absorption, grape molasses, ferrous sulfate.*

* From the Departments of Pediatrics and Biochemistry, Karadeniz Technical University Faculty of Medicine, Trabzon.

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

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

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

***** Pediatrician, Karadeniz Technical University Faculty of Medicine.

****** Professor of Biochemistry, Karadeniz Technical University Faculty of Medicine.

Iron deficiency remains the most common cause of anemia despite improvements in diet and the widespread use of well-absorbed forms of iron-fortified foods[1]. Infants who, from early infancy, are fed cow milk[2] or formula that is not iron-fortified[3] remain especially at risk for the development of iron deficiency.

The most important factors contributing to the development of iron deficiency in infants are rapid growth and an insufficient amount or absorption of dietary iron[1,4]. Growth and mental and motor development during infancy is highly dependent on the bioavailability of dietary iron. Therefore, prevention of nutritional iron deficiency is required[5,6].

A balanced weanling diet is the key to preventing iron deficiency anemia in children[7]. A rapidly growing weanling becomes susceptible to iron deficiency when neonatal iron stores have been consumed after the first few months following birth[8]. Experience has also shown that fortification of infant foods with iron is a more reliable and effective measure than providing iron medication[9].

In this study, we compare the absorption of iron in grape molasses (GM), which is traditional infant food in Turkey, to that in ferrous sulfate (FS), and form an opinion about whether or not grape molasses is an alternative to iron-containing drugs in the prevention of iron deficiency anemia (IDA) in infancy.

Material and Methods

The study was performed on 56 subjects, aged 6-36 months, after informed consent was obtained from their parents. Group I consisted of 30 subjects with IDA (hemoglobin < 11 g/dl, transferrin saturation < 10%, ferritin < 10 ng/ml). Group II consisted of 26 non-anemic subjects (hemoglobin > 12 g/dl, transferrin saturation > 15%, ferritin > 10 ng/ml). Each group was subdivided randomly into two equal subgroups. In half of the subjects in each group (i.e. 15 in group I, 13 in group II), the absorption of iron in GM was determined, and in the other half of each group, the absorption of iron in FS was tested. Subjects known to have malabsorption syndromes, acute or chronic infections, or malignancies and those taking non-steroidal anti-inflammatory drugs or having chronic blood losses were excluded.

The iron content of GM used in the study was 9.8 mg per 100 g, which was measured by the spectrophotometrical ferrozine method (Pointe Scientific Inc) with 1/25 dilution.

The post absorptive serum iron increase method (non-radioactive) was used determine the absorption of iron from GM and FS. To avoid the well-known iron absorption inhibiting effect of the many iron complexing compounds in foods, tea, milk etc., the subjects did not take any food or beverage for six hours before and three hours after oral application. Only tap water was allowed during this period. The serum iron studies started at 8 a.m. After drawing an initial venous blood sample, GM or FS, containing 1 mg/kg iron, was given orally. The second

venous blood samples were drawn after three hours. All blood samples were analyzed for serum iron (SI) and total iron binding capacity (TIBC) by routine colorimetric methods, and the increase of serum iron was calculated. Transferrin-Fe-saturation was calculated using total iron and TIBC. The serum ferritin levels were only analyzed in initial blood samples in each group. Hemoglobin, SI, TIBC, serum ferritin, and transferrin-Fe-saturation were used as the diagnostic criteria for the separation into IDA and non-anemic groups.

Statistical analyses were performed using the Wilcoxon matched-pairs signed ranks, Mann-Whitney U, and chi-square tests and Kruskal Wallis variance analysis[10].

Results

There were no statistically significant differences between the two groups in the sex and mean age of the subjects (Table I), nor among the subgroups of each group in the mean hemoglobin values ($p > 0.05$). The mean transferrin-Fe-saturation and serum ferritin level were 25.5 ± 7.1% and 48.4 ± 21.3 ng/ml, respectively, in nonanemic subjects, and 6.9 ± 1.7% and 5.2 ± 2.6 ng/ml respectively, in anemic subjects (the values are mean ± SD). Hematologic results of the subjects with IDA (before and after test) are shown in Table II, and those of non-anemic subjects in Table III. There were no significant differences in before-test SI and TIBC values in either GM- or FS-given subjects in each group ($p > 0.05$). In the group with IDA, the mean after-test SI value in FS-given infants was higher, and the mean TIBC value lower, than those of GM-given infants ($p < 0.05$). However, in the non-anemic group, there were no significant differences between the mean before- and after-test values of SI and TIBC in the GM- and FS- given subjects ($p > 0.05$).

Table I: Sex and Age of the Subjects

		Sex (Male/Female)	Age (X ± SD) (Months)
Subjects with IDA	Grape molasses (n = 15)	6/9	20.5 ± 8.9 (6-36)*
	Ferrous sulfate (n = 15)	6/9	21.4 ± 9.7 (6-36)*
Non-anemic Subjects	Grape molasses (n = 13)	6/7	20.7 ± 9.4 (6-36)*
	Ferrous sulfate (n = 13)	6/7	22.2 ± 9.4 (6-36)*

* Ranges.

Table II: Hematological Data of the Subjects with Iron Deficiency Anemia (the Values are Mean ± SD and Parenthesis Represents Minimum-Maximum Values)

		Before Test	After Test	p
Serum iron (mg/dl)	Grape molasses	32.2 ± 7.0 (18-45)	59.3 ± 18.0 (30-81)	< 0.05
	Ferrous sulfate	30.0 ± 7.6 (19-42)	90.0 ± 19.9 (56.5-120)	< 0.05
p		> 0.05	< 0.05	
TIBC (mg/dl)	Grape molasses	425 ± 59 (363-572)	361 ± 73 (245-485)	< 0.05
	Ferrous sulfate	436 ± 69 (270-557)	295 ± 58 (216 ± 352)	< 0.05
p		> 0.05	< 0.05	

Table III: Hematological Data of the Non-Anemic Subjects (the values are mean ± SD and parenthesis represents minimum-maximum)

		Before Test	After Test	p
Serum iron (mg/dl)	Grape molasses	72.2 ± 21.0 (50-119)	95.0 ± 17.5 (76-136)	< 0.05
	Ferrous sulfate	70.0 ± 17.2 (52-104)	96.0 ± 19.6 (65-120)	< 0.05
p		> 0.05	> 0.05	
TIBC (mg/dl)	Grape molasses	327 ± 32 (250-357)	287 ± 30 (224-318)	< 0.05
	Ferrous sulfate	319 ± 34 (247-370)	280 ± 34 (220 ± 320)	< 0.05
p		> 0.05	> 0.05	

The mean increase of serum iron in GM-given infants with IDA was 27.0 ± 13.4 (3-47) µg/dl and in FS-given infants with IDA, 60.6 ± 17.0 (35-91) µg/dl ($p < 0.05$). On the other hand, in the non-anemic group, the mean increase of serum iron of the GM-given subjects was 23.0 ± 4.3 (17.30) µg/dl and that of the FS-given subjects, 23.8 ± 10.0 (9-40) µg/dl ($p > 0.05$).

Discussion

Despite major advances in our knowledge of iron nutrition in infancy, iron deficiency remains a significant problem, especially in developing countries[11, 12]. Nutritional iron deficiency appears after the age of six months and is undoubtedly linked to inappropriate feeding practices[12, 13].

The Committee on Nutrition of The American Academy of Pediatrics has recommended that iron supplementation (to a maximum of 15 mg per day from one or more sources) sohuld start no later than four months of age in term infants and no later than two months of age in pre-term infants[1,4]. At present, iron-containing drops and foods fortified with iron, such as infant cereals, commercial milk formulas and enriched flour and other cereal products, have been widely used as iron sources in infants[1,4].

The bioavailability of iron in a diet is more important than the amount[1,14]. There is no doubt that absorbable forms of iron in foods will improve iron balance and prevent iron deficiency[1,14]. Recently it has been reported that micronutrient-fortified rusk[15], adapted formula with bovine lactoferrin[16], modified cow milk[17] and cereal fruit products[18] are effective in preventing iron deficiency anemia in infancy.

Grape molasses (which contains 10 mg of iron, 70.6 g of carbohydrate, 0.6 g of protein, 400 mg of calcium, and 293 kcal of energy per 100 g) is boiled grape juice[19,20]. In Turkey, GM is a traditional and inexpensive food, which is widely used in all socoeconomic groups and has been suggested by physicians as a source of iron. To our knowledge, there has been no previous study reported investigating the bioavailability or absorption of iron in grape molasses. Fomon et al.[18] determined erythrocytic incorporation of the stable isotope, Fe^{58}, after feeding the following foods (extrinsically labelled with Fe^{58}) which he claimed seemed nutritionally important as iron sources: cereal-vegetable products, grape juice fortified with ferrous sulfate, and a vitamin-, mineral-, and protein-enriched rice cereal fortified with ferrous fumarate.

In the present study, the absorption of iron in GM and FS was compared using a non-radioactive method (post-absorptive serum iron increase method). In the group with IDA, the mean increase of serum iron which was measured three hours after the oral administration of GM was very low compared to the increase measured after the administration of FS ($p < 05$). However, there was no statistically significant difference between the mean increase of serum iron from GM or FS in the non-anemic group ($p > 0.05$). We were not able to determine why the mean increase of serum iron in GM-given infants was lower than that of FS-given infants in the group with IDA. However, this may be related to the amount of GM.

The post-absorptive serum iron increase method has been used since 1934 to detect the bioavailability of the therapeutic oral iron preparations[21]. This procedure cannot be used for quantitative estimation of iron absorption in individual subjects, but it is useful for comparison of several iron preparations in a sufficient number of subjects (at least 10)[21]. In this study, this method was used for comparison of the iron absorptions of two iron sources in 56 subjects. Bioavailability is not a simple synonym of iron absorption but describes both the fractional iron absorption as well as recreation of a suitable iron reserve[22]. Furthermore, Özsoylu

and Özbek[23] emphasized that iron absorption does not always correspond to its utilization for heme synthesis, which is a better criterion of iron bioavailability.

Finally, we believe that grape molasses, which is inexpensive and readily available, is a reliable and effective iron source in preventing iron deficiency anemia in infancy, but it is not effective in the treatment of IDA. Thus, grape molasses may be recommended for infants after six months of age as an artifical iron source to prevent the development of iron deficiency anemia, but only if the infants have been breastfed within the first four to six months of their lives. If an infant has been fed with artificial foods during the first four to six months, grape molasses may be recommended earlier than six months of age. However, new clinical studies about the long-term effect of grape molasses on iron balance are needed.

REFERENCES

1. Dallman PR, Yip R,Oski FA. Iron deficiency and related nutritional anemias. In: Nathan DG, Oski FA (eds). Hematology of Infancy and Childhood. Philadelphia: WB Saunders Co; 1993: 413-450.
2. Tunnessen WW Jr, Oski FA. Consequences of starting whole cow milk at 6 months of age. J Pediatr 1987; 111: 813-816.
3. Hertrampf E, Cayazzo M, Pizarro F, Stekel A. Bioavailability of iron in soy-based formula and its effect on iron nutriture in infancy. Pediatrics 1986; 78: 640-645.
4. Lee Gr. Iron deficiency and iron-deficiency anemia. In: Lee GR, Bithell TC, Foerster J, Athens JW, Lukens J (eds). Wintrobe's Clinical Hematology. Philadelphia: Lea-Febiger Co; 1993: 808-839.
5. Filer LJ Jr. Iron needs during rapid growth and mental development. J Pediatr 1990; 117 (2 Pt 2): S: 143-146.
6. Idjradinata P, Pollitt E. Reversal of developmental delays in iron deficient anaemic infants treated with iron. Lancet 1993; 341: 1-4.
7. Palmer G. Weaning. Any old iron. Health Visit 1993; 66: 248-249, 252.
8. Dallman PR. Iron deficiency in the weanling: a nutritional problem on the way to resolution. Acta Paediatr Scand 1986; 323 (Suppl): 59-67.
9. Dallman PR. Progress in the prevention of iron deficiency in infants. Acta Paediatr Scand 1990; 365: (Suppl) 28-37.
10. Siegell S. Nonparametric statistic for the behavioral sciences. New York: McGraw Hill Book Co; 1956.
11. Irigoyen M, Davidson LL, Carriero D, Seaman C. Randomized, placebo-controlled trial of iron supplementation in infants with low hemoglobin levels fed iron-fortified formula. Pediatrics 1991; 88: 320-326.
12. Fairweather-Tait SJ. Iron deficiency in infancy; easy to prevent is it? Eur J Clin Nutr 1992; 46 (Suppl 4): S9-14.
13. Pizarro F, Yip R, Dallman PR, Olivares M, Hertrampf E, Walter T. Iron status with different infant feeding regimens: relevance to screening and prevention of iron deficiency. J Pediatr 1991; 118: 687-692.
14. Hulten L, Gramatkovski E, Gleerup A, Hallberg L. Iron absorption from the whole diet. Relation to meal composition, iron requirements and iron stores. Eur J Clin Nutr 1995; 49: 794-808.
15. Liu DS, Bates CJ, Yin TA, Wang WB, Lu CQ. Nutritional efficacy of a fortified weaning rusk in a rural area near Beijing. Am J Clin Nutr 1993; 57: 506-511.

16. Chierici R, Sawatzki G, Tamisari L, Volpato S, Vigi V. Supplementation of an adapted formula with bovine lactoferrin. 2 Effects on serum iron, ferrittin and zinc levels. Acta Paediatr 1992; 81: 475-479.

17. Hertrampf E, Pizarro F, Pereyra A, Vega V. A new milk formula: iron bioavailability and effect on prevention of iron deficiency in infants. Rev Child Pediatr 1991; 62: 94-98.

18. Fomon SJ, Ziegler EE, Rogers RR, et al. Iron absorption from infant foods. Pediatr Res 1989; 26: 250-254.

19. Food Composition Tables For Use in Middle East (Publication No: 20). Lebanon: American University of Beirut; 1964.

20. Türkiye Diyetisyenler Derneği. Besinlerin Bileşimi. Ankara: Yeniçağ Basım Yayın; 1991.

21. Heinrich HC, Fischer R. Correlatilon of post absorptive serum iron increase and erythrocyte-59 Fe-incorporation with the whole body retention of absorbed 59 Fe. Klin Wonchenschr 1982; 60: 1493-1496.

22. Kaltwasser JP, Werner E, Niechzial M. Bioavailability and therapeutic efficacy of bivalent and trivalent iron preparations. Arzneimittelforschung 1987; 37 (1A): 122-129.

23. Özsoylu Ş, Özbek N. Bioavailability of iron. Exp Hematol 1991; 19: 1065.

The Turkish Journal of Pediatrics 1997; 39: 473-481

RECOMBINANT HUMAN GRANULOCYTE – MACROPHAGE COLONY – STIMULATING FACTOR THERAPY AND ENDOGENOUS PLASMA GM – CSF, IL – 3, IL – 4 CONCENTRATIONS IN PEDIATRIC PATIENTS WITH SOLID TUMORS*

*Nazan Çetingül MD***, *Necil Kütükçüler MD***, *Mehmet Kantar MD****
*Kaan Kavaklı MD***, *Güngör Nişli MD*****, *Suat Çağlayan MD*****
*Senay Öztop MD******

SUMMARY: Çetingül N, Kütükçüler N, Kantar M, Kavaklı K, Nişli G, Çağlayan S, Öztop S. (Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Recombinant human granulocyte-macrophage colony-stimulating factor therapy and endogenous plasma GM-CSF, IL-3, IL-4 concentrations in pediatric patients with solid tumors. Turk J Pediatr 1997; 39: 473-481.

Ten pediatric patients with solid tumors and chemotherapy-induced neutropenia were given recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM CSF). The duration of the neutropenic phase was then compared with the results obtained from eight patients also with solid tumors, but not treated with rHuGM-CSF. It was found that rHuGM-CSF treatment significantly decreased the duration of the neutropenic phase. Endogenous plasma GM-CSF, IL-3, and IL-4 levels were also measured in the study group and in healthy children. No significant correlation has been found between plasma GM-CSF concentrations and absolute neutrophil counts. However, IL-3 levels of the neutropenic patients positively correlated with platelet counts. Furthermore, IL-4 concentrations were positively correlated with the GM-CSF level in the same individual. Plasma GM-CSF, IL-3, and IL-4 levels in the neutropenic solid tumor group were found to be significantly higher than those in healthy children. Plasma IL-4 levels were significantly elevated in patients with osteosarcoma as compared to patients with other solid tumors. Although rHuGM-CSF has a half-life of only two to three hours, one day after rHuGM-CSF therapy, plasma GM-CSF levels were found to be higher than initial values. In contrast, plasma IL-4 values decreased significantly after administration of rHuGM-CSF. The probable mechanisms for the changes in cytokine levels are discussed. *Key words: rHuGM-CSF, plasma GM-CSF concentration, IL-3, IL-4, neutropenia, solid tumor.*

Recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM-CSF) is currently used in various neutropenic conditions, both iatrogenic and disease related[1,2]. rHuGM-CSF accelerates neutrophil recovery after chemotherapy, leading to a reduction in the duration of the neutropenic phase and, consequently, antibiotic usage and duration of hospitalization[1-3]. However, little is known about the endogenous hemopoietic growth factor (HGF) response or plasma

* From the 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.

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

concentrations of some cytokines in neutropenic patients. Plasma levels of granulocyte colony-stimulating factor (G-CSF) and GM-CSF in some pathological conditions were measured and it was reported that changes in the peripheral neutrophil count almost paralleled those in the plasma G-CSF level, but not in the GM-CSF level[4,5].

Besides the suportive role of recombinant colony-stimulating factors (CSFs) had interleukin-3 (IL-3) in cancer therapy, some cytokines were thought to have direct and indirect anti-tumor effects[2,6]. Recent in vitro studies have indicated that IL-4 had anti-tumor activity[2,7,8]. On the other hand, IL-4 has also been found to be an active factor in myelopoiesis[9,10]. In addition, alterations in the immune status of patients with various cancers may result in the release of cytokines in circulation[11]. Some tumor cells have also been found to secrete cytokines, such as the secretion of IL-4 by malignant cells in Hodgkin's disease[12].

This study is composed of two parts. In the first part, the neutrophil response to rHuGM-CSF and the duration of the neutropenic phase in children with solid tumors are evaluated and compared with another group of patients also with solid tumors, but who were not treated with rHuGM-CSF. In the second part, endogenous plasma GM-CSF, IL-3, and IL-4 levels were measured in patients before and after rHuGM-CSF therapy and in healthy children. These plasma levels were compared with each other. The correlations between plasma GM-CSF, IL-3 levels and peripheral blood neutrophil and platelet counts and also between plasma levels of IL-4 and GM-CSF were analyzed. Although rHuGM-CSF has a very short half-life, its effects on post-treatment plasma IL-3 and IL-4 concentrations were investigated.

Material and Methods

Ten patients (6 girls, 4 boys) with different solid tumors in the active period were included in the study. The age of the patients ranged from two to 18 years with a mean 13.9 ± 4.9. The diagnosis was çonfirmed by histopathological findings. Five of ten children had osteosarcoma (osteoblastic type), while three cases were diagnosed with Ewing's sarcoma. Rhabdomyosarcoma and anaplastic astrocytoma were the other solid tumors. The clinical features of these patients are listed in Table I. None of the patients had clinical or laboratory signs or symptoms of a bacterial or fungal infection before or after rHuGM-CSF treatment. rHuGM-CSF (Leucomax, Sandoz), 5 µg/kg, was administered subcutaneously to the 10 patients with neutropenia. The median rHuGM-CSF treatment duration was seven days (range 5-12 days). The treatment was started in eight patients after their chemotherapy (CT) and/or radiotherapy (RT) schedule and in two patients during their CT/RT schedule (Table II). These patients were not transfused with any blood or blood-derived products during rHuGM-CSF treatment.

Table I: Clinical Features of the Patients (CT: Chemotherapy, RT: Radiotherapy)

Patient No/Sex	Age (Year)	Diagnosis	Treatment
1 (F)	16	Osteosarcoma	CT
2 (F)	18	Osteosarcoma	CT
3 (F)	18	Osteosarcoma	CT
4 (F)	15	Osteosarcoma	CT
5 (M)	17	Osteosarcoma	CT
6 (F)	16	Ewing's sarcoma	CT + RT
7 (M)	14	Ewing's sarcoma	CT + RT
8 (M)	16	Ewing's sarcoma	CT
9 (M)	2	Rhabdomyosarcoma	CT
10 (F)	1	Anaplastic astrocytoma	CT

Table II: The Neutrophil Response to rHuGM-CSF Treatment and Side Effects

		Absolute Neutrophil Count (ANC)/mm^3			
Patient No.	Administration of rHuGM-CSF	Before rHuGM-CSF	Time to Rise Above 1000/mm^3 (Day)	Peak Value/Day	Side Effects
1	After CT	744	1	6468 (6th)	No
2	After CT	1400	–	5016 (2nd)	No
3	After CT	900	3	5360 (3nd)	Fever, itching
4	During CT	392	1	7684 (10th)	Thrombocytopenia
5	After CT	1444	–	22320 (6th)	Fever
6	After CT+During RT	700	1	4255 (2nd)	No
7	During CT+RT	200	Never	385 (9th)	Thrombocytopenia
8	After CT	416	5	2500 (5th)	No
9	After CT	300	4	2838 (7th)	No
10	After CT	966	3	2508 (4th)	Fever
Median		722	3	4636 (5.5)	

Ten healthy children aged 10.3 ± 3.7 years (control group A) and eight children with neutropenia and solid tumors but not treated with rHuGM-CSF, aged 7.2 ± 4.0 years (control group B), were included in the study as control groups. The solid tumors of control group B were osteosarcoma (3 cases), neuroblastoma (4 cases) and Askin's tumor (1 case). The neutrophil response to rHuGM-CSF and the duration of the neutropenic phase in children with solid tumors were compared with control group B. Endogenous serum GM-CSF, IL-3, and IL-4 levels were measured in patients before and after rHuGM-CSF therapy and in control group A.

The peripheral blood samples for analyzing plasma GM-CSF, IL-3, and IL-4 concentrations were collected one day before and after rHuGM-CSF treatment. The plasma GM-CSF (Amersham, UK), IL-3 (Amersham), and IL-4 (Amersham) levels were analyzed using enzyme-linked immunosorbent assay (ELISA). The samples were stored at -30 °C until use. The minumum detectable concentrations of plasma GM-CSF, IL-3 and IL-4 by this method were 0.3 pg/ml, 1.5 pg/ml and 0.6 pg/ml, respectively.

Hematological parameters including white blood cell (WBC), absolute neutrophil count (ANC) and platelet count were investigated with a hemocounter (Serono Baker System 9000, Allentown, PA, USA) and through peripheral blood smear analysis. Liver and renal function tests were performed in order to determine toxic side effects. The patients were also observed for clinical side effects such as fever.

The differences and correlations between different cytokines and between cytokines and hematological parameters were analyzed using Mann-Whitney U test and regression analysis.

Results

Results related to neutrophil response and side effects after rHuGM-CSF treatment:

Before rHuGM-CSF therapy, ANCs of two patients were above 1000/mm^3 (patient nos, 2 and 5). Thus, prompt neutrophil recovery occurred in seven of eight children (Table II). Patient no. 7 did not show any increase in ANC because of the severe myelosuppression. The ANC rose above 1000/mm^3 after three days (median) of the rHuGM-CSF treatment (range 1-5 days) (Table II). However, the median time for ANC to rise above 1000/mm^3 in control group B was seven days (range 5-12 days).

These results showed that rHuGM-CSF treatment significantly decreased the duration of the neutropenic phase in children with solid tumors ($p < 0.001$).

Both groups of patients with solid tumors (treated with or without rHuGM-CSF) had no clinical signs of severe infection during the follow-up periods. Clinical and laboratory side effects of the rHuGM-CSF treatment are listed in Table II.

Results related to endogeneous plasma GM-CSF, IL-3 and IL-4 concentrations in study group and in healthy controls: The median values and ranges of endogenous plasma GM-CSF, IL-3, and IL-4 concentrations in rHuGM-CSF treated patients with solid tumors and in healthy controls are listed in Table III.

Table III: Median Plasma Levels of GM-CSF, IL-3 and IL-4 in the Study Group and in Healthy Children (pg/ml)

	GM-CSF	IL-3	IL-4
Before rHuGM-CSF	3.1 (< 0.3-455)	< 1.5 (< 1.5-50)	5.1 (0.6-41.5)
After rHuGM-CSF	15.6 (3.1-28.1)	< 1.5 (< 1.5-15.6)	0.6 (0.6-20.8)
Healthy controls	< 2.6 (< 0.3-7.3)	< 1.5 (< 1.5-1.5)	< 0.6 (< 0.6)
p value	1-2: $p < 0.001$ 1-3: $p < 0.05$ 2-3: $p > 0.05$	1-2: $p > 0.05$ 1-3: $p < 0.05$ 2-3: $p > 0.05$	1-2: $p < 0.001$ 1-3: $p < 0.05$ 2-3: $p > 0.05$

No significant correlation has been found ($p > 0.05$) between plasma GM-CSF concentration and ANC or platelet count in the peripheral blood of the study group. However, IL-3 levels of this neutropenic group positively correlated with the platelet count (r: 0.680, $p < 0.01$), but not with the ANC.

Plasma IL-4 concentrations, however, were positively correlated with the level of GM-CSF in the same individual (r: 0.87, $p < 0.001$).

The endogeneous plasma GM-CSF and cytokine levels before rHuGM-CSF treatment were compared to those in control group A (healthy controls). Not only the plasma GM-CSF level, but also IL-3 and IL-4 levels in the neutropenic solid tumor group were found to be significantly higher than those in healthy children ($p < 0.05$).

The median plasma GM-CSF level increased from 3.1 pg/ml to 15.6 pg/ml after rHuGM-CSF treatment (Table III), and there was a significant difference ($p < 0.01$) in plasma GM-CSF levels obtained before and after treatment. In contrast, plasma IL-4 values were found to be significantly lower ($p < 0.001$) than initial values after the administration of GM-CSF exogenously.

In addition, cytokine levels of the patients with osteosarcomas (n: 5) and patients with the other tumours (n: 5) were compared in the study group and plasma IL-4 levels were found to be significantly elevated in the osteosarcoma group ($p < 0.05$).

Discussion

CSFs both G-CSF and GM-CSF, are supportive care measures designed to reduce the likelihood of neutropenic infections that result from chemotherapy[13, 14]. Recently, investigators have started to use rHuGM-CSF or rHuG-CSF in pediatric solid tumors in order to accelerate neutrophil recovery. Weinthal et al.[15] reported that exogenous G-CSF therapy when used as an adjunct to aggressive chemotherapy in children with solid tumors reduced both the duration of neutropenia and of infection. On the other hand, Fink et al.[3] treated septic neutropenic pediatric cancer patients with rHuGM-CSF, and an ANC above 500/mm^3 was reached after five days (median). In our study, median time for the ANC to rise above 1000/mm^3 after rHuGM-CSF therapy was three days. When compared to the other pediatric solid tumor patients not treated with rHuGM-CSF, this therapy significantly reduced the duration of the neutropenic phase.

rHuGM-CSF reduces the frequency of infections, antibiotic usage and the duration of hospitalization because it enhances not only the number and functions of neutrophils but also the cytotoxic functions of monocytes and macrophages[1, 16, 17]. None of the patients in the study or in the control groups had any infections. As a result, no significant differences were observed with regard to duration of antibiotic usage and hospitalization and no conclusion could be made about the reduction of the frequency of infections.

rHuGM-CF was well tolerated were no documented cases of bone pain or of local irritation at the site of injection. rHuGM-CSF-induced fever was observed in three cases (30%). Significant differences in platelet counts have been reported in 1-12 percent of patients with various diseases under rHuGM-CSF treatment. In our study, thrombocytopenia was observed in 20 percent of the patients after rHuGM-CSF therapy. There was no indication of inadvertent side effects from serum levels of bilirubin, aspartate-aminotransferase (AST), protein, creatinine, or from urine analyses.

Despite the growing clinical use of the CSFs, there is limited information available about the pattern of endogenous cytokine responses in patients with solid tumors. It has been reported that after intensive chemotherapy (CT) and/or radiotherapy (RT), GM-CSF was released into the serum[18]. Galdiero et al.[19] found an increased release of IL-4 from lymphocytes after minimum doses of irradiation. In our study, before rHuGM-CSF therapy, the endogenous plasma GM-CSF, IL-3, and IL-4 levels in patients who had solid tumors and were treated with CT/RT were found to be significantly higher than those in healthy controls. In patient no. 7, who had both chemotherapy and radiotherapy, the plasma GM-CSF level was extremely high (455.0 pg/ml). The increase in GM-CSF, IL-3, and IL-4 levels may be due to CT/RT. On the other hand, IL-4, G-CSF, IL-1, IL-5, IL-6, and tumor necrosis factor alpha (TNF-α) have been shown to be secreted by malignant cells in Hodgkin's disease[12]. Statistically significantly higher levels of IL-2, soluble IL-2 receptor, IL-6, IL-8, IL-10, and TNF-alpha were observed in patients with non-Hodgkin's lymphoma and acute lymphoblastic leukemia as compared to controls[20,21]. It has been demonstrated that osteosarcoma cell lines were potent producers of IL-11 which is an important component of the cytokine network[22]. With regard to these findings, the increase in plasma GM-CSF, IL-3 and IL-4 levels in pediatric patients with solid tumors, including osteosarcomas, may be due to the secretion of GM-CSF and these cytokines by tumor cells. Furthermore, IL-4 has an anti-tumor activity[2,6,8]. IL-4 and GM-CSF as well as TNF and IL-2 have been reported to activate macrophages for the destruction of tumor cells[23]. The increased production of GM-CSF and IL-4 by lymphocytes and macrophages in patients with solid tumors may be a part of host defense against malignancy.

IL-4 modulates the activity of a variety of lymphoid, hemopoietic and mesenchymal cells, but little is known about its influence on bone cells[24]. In our study, IL-4 levels of the patients with osteosarcomas were significantly higher than those of patients with other tumors. Elevated IL-4 levels in our patients with osteosarcomas may have exerted a modulatory effect on osteosarcoma cells, as was described in Riancho et al.'s[24] study.

Omori et al.[4] and Cebon et al.[5] reported that the peripheral neutrophil count correlates with the serum G-CSF level, but not with GM-CSF. The results of our study supported the data of these studies, because no significant correlation has

been found between plasma GM-CSF level and ANC and platelet count in the peripheral blood of the patients. On the other hand, IL-3 is a multi-colony stimulatory factor and is involved in proliferation of pluripotent stem cells to produce all types of hemopoietic cells, including megakaryocytes and platelets[25]. In our study, plasma IL-3 levels were found to be positively correlated with the platelet count.

GM-CSF is secreted by T lymphocytes, macrophages, fibroblasts, endothelial cells and dendritic cells, whereas IL-4 is produced by activated T helper cells[25,26]. T helper cells are the sources of both GM-CSF and IL-4. Some of the cytokines that control hematopoiesis are present at all times (G-CSF, erythropoietin) whereas others are produced in response to specific stimuli (IL-3, IL-4, GM-CSF)[27]. In this study, plasma IL-4 concentrations were positively correlated with the GM-CSF level in the same individual, suggesting that they are produced in response to the same stimuli.

rHuGM-CSF has been known to be eliminated from the serum with in two or three hours after its subcutaneous administration, and serum levels decrease to undetectable values after 24 hours[17]. Therefore, theoretically, rHuGM-CSF treatment does not increase endogenous plasma GM-CSF levels one day after its administration. However, there was a significant difference in plasma GM-CSF levels obtained before and after rHuGM-CSF treatment. Because of its pharmacokinetic properties, this elevation was not thought to be related to rHuGM-CSF therapy. The half-life for E.coli derived GM-CSF administered subcutaneously in a dose of 5.5 μg/kg had been reported to be 2.1 hours by Nissen and Hovgaard[28].

Plasma IL-4 values were found to be significantly lower than intial values after administration of rHuGM-CSF. There is only one other study in which cytokine levels were measured before and after chemotherapy and rHuGM-CSF treatment. In this study, cytokine levels progressively declined to normal ranges in responding patients[20]. The decline in plasma IL-4 values in our study was similar with Stasi et al.'s[20]. data.

In conclusion, rHuGM-CSF treatment significantly decreased the duration of the neutropenic phase in children with solid tumors. There is no correlation between endogenous plasma GM-CSF concentrations and absolute neutrophil count, but IL-3 concentrations were found to be correlated with platelet counts. The endogenous plasma GM-CSF, IL-3, and IL-4 levels were significantly higher in pediatric patients with solid tumors. The increased productions might be related to their CT-RT regimen or to secretion by malignant cells. These results demonstrate that cytokine measurements can provide valuable information for better management of pediatric solid tumors. In the future, they may be useful in monitoring disease activity or may be used as prognostic indicators.

REFERENCES

1. Diaz-Rubio E, Adrover E. Use of granulocyte growth factors in solid tumors. Eur J Cancer 1994; 30A: 120-122.
2. Henschler R, Brugger W, Kanz L, Mertelsmann R. Haematopoietic growth factors in clinical medicine. Eur J Cancer 1994; 30A: 118-119.
3. Fink FM, Maurer-Dengg K, Fritsch G, et al. Recombinant human granulocyte-macrophage colony-stimulating factor in septic neutropenic pediatric cancer patients: detection of circulating hematopoietic precursor cells correlates with rapid granulocyte recovery. Med Pediatr Oncol 1995; 25: 365-371.
4. Omori F, Okamura S, Shimoda K, Otsuka T, Harada M, Niho Y. Levels of human granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor under pathological conditions. Biotherapy 1992; 4: 147-153.
5. Cebon J, Layton JE, Maher D, Morstyn G. Endogenous haemopoietic growth factors in neutropenia and infection. Br J Haematol 1994; 86: 265-274.
6. Brennscheidt U, Lindemann A. Cytokines in clinical cancer treatment trials: methodoligical aspects [news]. Eur J Cancer 1994; 30A: 412-414.
7. Stein RC, Dalgleish AG, Immunomodulatory agents: the cytokines. Eur J Cancer 1994; 30A: 400-404.
8. Nagai N, Kashiwamura S, Nakanishi K, Shinka S. [Interleukin-4 (IL-4)]. Gan To Kagaku Rhoyo 1994; 21: 1099-1108.
9. Siitonen T, Zheng A, Savolainen ER, Koistinen P. The effect of interleukin-4 with or without mast cell growth factor on peripheral blood granulocyte-macrophage colony-forming cells in healthy controls and in myeloproliferative disorders. Ann Hematol 1995; 70: 203-207.
10. Ferrajoli A, Zipf TF, Talpaz M, Felix EA, Estrov Z. Growth factors controlling interleukin-4 action on hematopoietic progenitors. Ann Hematol 1993; 67: 277-284.
11. Raziuddin S, Sheikha A, Abu-Eshy S, al-Janadi M. Circulating levels of cytokines and soluble cytokine receptors in various T-cell malignancies. Cancer 1994; 73: 2426-2431.
12. Hsu SM, Waldron JW Jr, Hsu PL, Hough AJ Jr. Cytokines in malignant lymphomas: review and prospective evaluation. Hum Pathol 1993; 24: 1040-1057.
13. American Society of Clinical Oncology. Recommendations for the use of hematopoietic growth factors: evidence based clinical practice guidelines. J Clin Oncol 1994; 12: 2471-2508.
14. Bennet CL, Smith TJ, Weeks JC, et al. Use of hematopoietic colony-stimulating factors: the American Society of Clinical Oncology survey. J Clin Oncol 1996; 14: 2511-2520.
15. Weinthal J, Gillan E, Hoder F, et al. G-CSF significantly reduces the nadir of neutropenia, hospitalizations and costs during intensive chemotherapy in children with solid tumors (abstr). Proc Am Soc Clin Oncol 1992; 11: 362.
16. Cebon JS, Bury RW, Lieschke GJ, Morstyn G. The effects of dose and route of administration on the pharmacokinetics of granulocyte-macrophage colony-stimulating factor. Eur J Cancer 1990; 26: 1064-1069.
17. Gisselbrecht C. Hematopoietic growth factors in practice. Bull Acad Natl Med 1994; 178: 781-792.
18. Millar BC, Bell JB, Millar JL, et al. Colony-stimulating activity in the serum of patients with hemopoietic malignancies after intensive chemotherapy/radiotherapy: its augmentation by GM-CSF in vivo and interleukin-4 in vitro. Exp Hematol 1992; 20: 209-215.
19. Galdiero M, Cipollaro de l'Ero G, Folgore A, Cappello M, Giobbe A, Sasso FS. Effects of irradiation doses on alterations in cytokine release by monocytes and lymphocytes. J Med 1994; 25: 23-40.

20. Stasi R, Zinzani L, Galieni P, et al. Clinical implications of cytokine and soluble receptor measurements in patients with newly-diagnosed aggressive non-Hodgkin's lymphoma. Eur J Haematol 1995; 54: 9-17.

21. Çetingül N, Yener E, Öztop S, Nisli G. Serum soluble interleukin-2 receptors and tumor necrosis factor-alpha in hematological malignancies of childhood. Acta Paediatr Jpn 1994; 36: 49-52.

22. Elias JA, Tang W, Horowitz MC. Cytokine and hormonal stimulation of human osteosarcoma interleukin-11 production. Endocrinology 1995; 136: 489-498.

23. Kiyotaki C. [Mechanisms of macrophage activation and macrophage-mediated tumoricidal activity]. Gan To Kagaku Ryoho 1989; 16: 3386-3391.

24. Riancho JA, Zarrabeitia MT, Olmos JM, Amado JA, Gonzales-Macias J. Effects of interleukin-4 on human osteoblast-like cells. Bone Miner 1993; 21: 53-61.

25. Report of a WHO Scientific Group. Primary immunodeficiency diseases. Clin Exp Immunol 1995; 99 (Suppl 1): 2-24.

26. Monroy RL, Davis TA, MacVittie TJ. Granulocyte-macrophage colony-stimulating factor: more than a hemopoietin. Clin Immunol Immunopathol 1990; 54: 333-346.

27. Oppenheim JJ, Ruscetti FW, Faltynek C. Cytokines. In: Stites DP, Terr AI, Parslow TG (eds). Basic and Clinical Immunology (8^{th} ed). London: Appleton and Lange; 1994: 105-123.

28. Nissen NI, Hovgaard D. Overview of cytokines in clinical practice. In: Proceedings from an International Symposium. Lucerne, Switzerland: September 1993; 3-7.

The Turkish Journal of Pediatrics 1997: 39: 483-489

INTRADERMAL ADMINISTRATION OF HEPATITIS B VACCINE IN INFANTS AND PRESCHOOL CHILDREN*

*Zafer Kurugöl MD***, *Ayten Egemen MD****, *Selda Erensoy MD*****
*Altınay Bilgiç MD******, *Necil Kütükçüler MD*******

SUMMARY: Kurugöl Z, Egemen A, Erensoy S, Bilgiç A, Kütükçüler N. (Departments of Pediatrics and Microbiology, Ege University Faculty of Medicine, İzmir, Turkey). Intradermal administration of hepatitis B vaccine in infants and preschool children. Turk J Pediatr 1997; 39: 483-489.

One of the ways to administrate hepatitis B vaccination is the intradermal (id) route. The aim of this study is to evaluate the immunologic response of various age groups of children who received three 2 µg id doses of recombinant hepatitis B vaccine. One hundred and eighty-seven children (86 infants, 101 preschool children) were administered a 2 µg dose of recombinant hepatitis B vaccine (Gen Hevac B) intradermally zero, one and six months. Eight weeks after the third vaccination, the gometric mean titers (GMT) of antibody to hepatitis B surface antigen (anti-HBs) of infants was 753 IU/L; that of preschool children was 799 IU/L. There was no statistically significant difference between the anti-HBs GMT of infants and preschool children. However, infants were less likely to have developed protective anti-HBs (≤ 10 IU/L) than preschool children (93% vs 100%, p = 0.009). In 8.1 percent of infants and 3.9 percent of preschool children, local reactions were observed. The 2 µg recombinant vaccine by id route is safe and suitable for immunization of preschool children. The id route is technically difficult to administrate in infants and protective seroconversion rates are lower than in preschool children. *Key words: intradermal hepatitis B vaccine, infant, preschool children.*

Hepatitis B virus (HBV) infection, with its chronic sequelae of cirrhosis of the liver and hepatocellular carcinoma, constitutes a major health problem[1,2]. In areas of high and intermediate endemicity, large-scale immunization programs aimed at mass immunization of all infants, adolescents, and other individuals at high risk should be applied where economically feasible[1]. Turkey has an intermediate endemicity for HBV infection. In Turkey, hepatitis B surface antigen (HBsAg) and the antibody to HBsAg (Anti-HBs) positivity ranges are reported to be 3-15 percent and 26.2-68.8 percent, respectively[3-10]. Vertical transmission is probably not the major mode of transmission in Turkey[8]. HBsAg positivity is more prevalent in children under five years of age, suggesting an early exposure

* From the Departments of Pediatrics and Microbiology, Ege University Faculty of Medicine, İzmir.

** Pediatrician, Ege University Faculty of Medicine.

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

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

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

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

of young children and horizontal transmission in Turkey[4]. Therefore, screening and immunization of babies born to carrier mothers would not be an effective strategy. If the mass vaccination program is not available, immunization of all preschool children in Turkey can prevent horizontally transmitted HBV infection.

One of the ways to apply hepatitis B vaccination is the intradermal (id) route. To date, various studies have been made to evaluate the anti-HBs response in adults after administration of various hepatitis B vaccines by the id route[11-19]. However, there has been only one study that evaluates the response following an id plasma-derived hepatitis B vaccine in infants[20]. no study has been found which assesses the effectiveness of id recombinant hepatitis B vaccine in infants or children.

The aim of this study is to evaluate the immunologic response of various age groups of children who received three 2 µg id doses of recombinant hepatitis B vaccine.

Material and Methods

One hundred and eighty-seven healthy children (97 female, 90 male) were enrolled in this study. Eighty-six were infants of two months of age, and 101 were preschool children between one and six years of age. Infants were full-term and healthy with a mean birth weight of 3375 ± 426 grams. Mothers of infants, infants and preschool children were all negative for HBsAg, anti HBs, and anti-HBc.

A recombinant hepatitis B vaccine containing 20 µg HBsAg per 0.5 ml (Gen Hevac, Pasteur Mériux) was used in this study. In many studies, 2 µg doses of various hepatitis B vaccines were administrated by id route[11-20]. Infants were administered a 2 µg dose id vaccine in the deltoid region at two, three, and eight months of age (0, 1, 6 months scheme). At two and three months of age, all infants also received regularly scheduled doses of diphtheria-tetanus-pertussis (DTP) and oral polio vaccines. Hepatitis B vaccines were administrated in the right arm and the DTP vaccine in the left arm. Preschool children were administered the same dosages of vaccines in the same region, on a schedule similar to that for the infants (at 0, 1, 6 months). During their follow-up in the out-patient clinic of the Social Pediatric Unit, all vaccines were observed for side effects. The parents were informed to record side effects such as local tenderness, erythema, induration, hyperpigmentation, fever, and irritability. If any side effect was to occur they were to bring their children to our clinics. Persistent induration and hyperpigmentation were assessed after a month of each dose.

Postvaccinal blood samples were collected from all infants and preschool children eight weeks after their last vaccination and were tested for anti-HBs levels by microparticle enzyme immunoassay (IMx, Abbott Diagnostics, USA) quantitatively[21].

Immunologic response of the children was determined by the ratio of protective seroconversion and the geometric mean titer (GMT) of anti-HBs obtained by id vaccine following the third vaccination. These parameters were also used in order to assess

the influence o sex and age on anti-HBs response. Sufficient seroconversion for protection was defined as a concentration of anti-HBs ≥ 10 IU/L[22].

The GMTs for anti-HBs were compared using the Mann-Whitney U test. The Fisher's exact test was used to compare seroconversion rates. Correlation analysis was used to examine the relation of vaccine and sex and age.

Results

The GMT for anti-HBs of infants and preschool children, measured eight weeks after the third vaccination, are shown in Table I and Figure 1. The GMT of infants was 753 IU/L; that of preschool children was 799 IU/L. There was no statistically significant difference between the anti-HBs GMT of infants and preschool children. The GMT of female infants (789 IU/L) was similar to that of male infants (717 IU/L). The GMT of female preschool children (996 IU/L) was also not significantly different from that of male preschool children (626 IU/L).

Table I: The Geometric Mean Titer (GMT) for Anti-HBs Measured Eight Weeks After Three 2 µg Doses Intradermal HB Vaccination

Groups	n	GMT (IU/L)
Infants	86	753.0*
Male	44	717.6
Female	42	789.2
Preschol children	101	799.3*
Male	48	626.3
Female	53	996.7

* There was no statistically significant difference between the anti-HBs GMT of infants and preschool children.

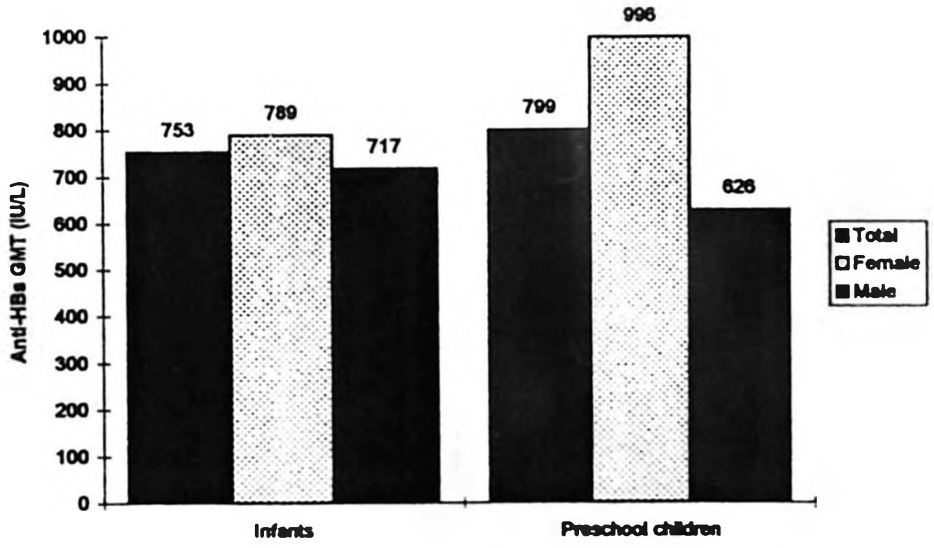


Fig. 1: The geometric mean titer (GMT) for anti-HBs of infants and preschool children measured eight weeks after third vaccination

The percentage of different anti-HBs titers in infants and preschool children are shown in Table II and Figure 2. Eight weeks after completion of vaccination, anti-HBs responses and protective anti-HBs responses (≥ 10 IU/L) developed in 94.2 and 93.0 percent of infants, respectively. Seroconversion to protective anti-HBs levels was achieved in all preschool children. The protective seroconversion rates of preschool children (100%) were significantly higher than those of infants (93%) (p = 0.0086). Seventy-two percent of infants and 91.1 percent of preschool children had anti-HBs titers higher than 100 IU/L. Furthermore, 43.0 percent of infants and 47.5 percent of preschool children had anti-HBs titers higher than 1000 IU/L.

Table II: The Percentage of Children (Infants and Preschool) with Different Anti-HBs Titers Eight Weeks After Third Vaccination

	Anti-HBs Titers (IU/L)			
Groups	≥ 1	≥ 10	≥ 100	≥ 1000
Infants (n = 86)	94.2	93.0	72.1	43.0
Male (n = 44)	93.2	90.9	69.1	42.8
Female (n = 42)	95.2	95.2	75.0	43.2
Preschool children (n = 101)	100	100	91.1	47.5
Male (n = 48)	100	100	85.4	39.6
Female (n = 53)	100	100	96.2	54.7

The protective seroconversion rates of preschool children were significantly higher than those of infants (p = 0.0086).

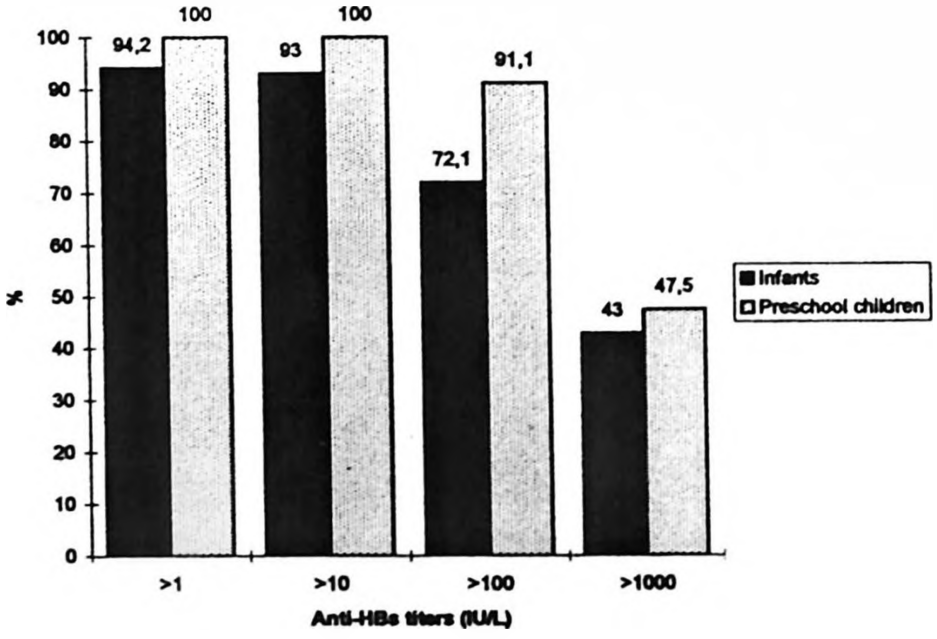


Fig. 2: The percentage of different anti-HBs titers of infants and preschool children eight weeks after third vaccination.

The GMT for anti-HBs and protective seroconversion rates of preschool children in various age groups are summarized in Table III. The GMT for anti-HBs and protective seroconversion rates of the various age groups were similar. No effect of sex and age on anti-HBs response of preschool children was determined.

Table III: The Geometric Mean Titer (GMT) of Anti-HBs and the Proportion of Preschool Children of Various Ages Who Achieved the Protective Level

			Seroconversion Rate	
Age Years	n	Anti-HBs GMT (IU/L)	≥ 10 IU/L	≥ 100 IU/L
0	86	753.4	93.0	72.1
1	11	694.3	100.0	91.0
2	19	881.1	100.0	94.8
3	12	741.3	100.0	91.7
4	23	649.5	100.0	87.0
5	15	1082.3	100.0	100.0
6	21	961.6	100.0	89.8

The GMT for anti-HBs and protective seroconversion rates were not found significantly different among the age groups.

There were no serious adverse experiences attributable to the vaccine, and no infant or preschool child was excluded from the study because of a serious reaction. Mild local tenderness and erythema were observed in 8.1 percent of infants. Persistent induration was detected in only one infant and hyperpigmentation was noted in 5.7 percent. Systemic complaints such as fever and hyperirritability were observed in 12.1 and 38.1 percent of infants respectively after the first or second doses, which were usually given at the same time as the DTP vaccine. However, these side effects were observed in only one infant after the third dose of hepatitis B vaccine when the DTP vaccine was not given.

Mild local tenderness and erythema were noted in 3.9 percent of preschool children and hyperpigmentation in 4.9 percent. Fever and irritability were observed in only one preschool child.

Discussion

Some investigators have reported that protective anti-HBs levels were achieved in 67-100 percent of adults with three or four low-dose id hepatitis B vaccines[11-19]. In the only study which was carried out in Turkey, the use of recombinant 4µ hepatitis B vaccine by id route at zero, one and six months in 101 adult volunteers achieved a 91 percent protective response rate[12]. However, there have not been enough observations and studies which reflect similar results in infants and children. According to these results, all preschool children achieved protective anti-HBs levels by administration of three doses of a 2 µg recombinant hepatitis

B vaccine by id route. However, the duration of protective levels is still unclear, although recent evidence suggests that a person who develops a higher anti-HBs titer is more likely to have long-term protective levels of the antibody two to three years longer than the ones who have lower anti-HBs titers[11, 23]. In this study, a high proportion of preschool children (91.1%) developed anti-HBs titers higher than 100 IU/L.

Therefore, it has been thought that long-term protection has been achieved in preschool children by using the id technique and low-dose recombinant hepatitis B vaccine. However, there is a need for large-scale and long-term prospective comparative trials to substantiate the economic and epidemiologic value of the id hepatitis B vaccination in preschool children.

The lower response of the infants to the hepatitis B vaccine compared with older children can be attributed to the immaturity of the immune system. So far, only one study has been carried out concerning id hepatitis B vaccine administration in infants. In that study, protective anti-HBs levels were achieved in 91 percent of the infants, and the anti-HBs GMT for infants was 312 IU/L following an id plasma-derived hepatitis B vaccine[20]. In our study, a recombinant hepatitis B vaccine was used and 93 percent of infants reached protective anti-HBs levels after id vaccination. The anti-HBs GMT for infants (753 IU/L) was extremely high. The differences observed in antibody responses may have been due to the different types of vaccines (recombinant vs plasma-derived hepatitis B vaccine).

Demographic factors such as age and sex may affect antibody response to the id vaccine[19]. It has been reported previously that the female responded better than the male[12]. However, anti-HBs responses of females were found similar to those of males in this study.

Adverse skin reactions such as local pruritus, pain, erythema, induration, and hyperpigmentation were reported after the id vaccine administration[11, 19, 20]. The thin epidermis of the infants makes id immunization technically difficult, and may cause adverse skin reactions to occur. In this study, the vaccines were well tolerated and there were no serious side effects in infants or preschool children. In 8.1 percent of infants and in 3.9 percent of preschool children, local reactions were observed. No patient was excluded from the study because of side effects.

It was concluded that the 2 µg recombinant vaccine by id route is safe and suitable for immunization of preschool children. The id route is technically difficult to administrate in infants and protective seroconversion rates are lower than these observed preschool children.

REFERENCES

1. Maynard JE, Kane MA, Alter MJ, Halder SC. Controls of hepatitis B by immunization: global perspectives. In: Zuckerman AJ (ed). Viral Hepatitis and Liver Diseases. New York: Alan R. Liss Inc; 1988: 967-969.
2. Report of a WHO Meeting. Prevention of liver cancer. Technical Report 691, World Health Organization, Geneva 1983: 8-9.

3. Bilgiç A, Sezer N, Uçarcı A. İzmir ili kan vericilerinde HBsAg'nin iki ayrı serolojik yöntemle araştırılması. Ege Üniv Tıp Fak Der 1982; 21: 717-720.
4. Uç A, Özsoylu Ş. Age-qrevalence of HBsAg positivity in children seen at Hacettepe Children's Hospital. Turk J Med Res 1992; 10: 264-266.
5. Değertekin H, Kestellioğlu F. The prevalence of HBsAg in healthy people and several liver diseases in Turkey. Asian Med J 1986; 29: 125-127.
6. Turhanoğlu M, Arıkan E. Güney Doğu Anadolu Bölgesinde değişik gruplardaki HBsAg ve antikorunun saptanması. D Ü Tıp Fak Der 1987; 1: 28-40.
7. Arıoğul S, Akalın E, Kanra T. HBsAg among Turkish blood donors. Infection 1987; 15: 456.
8. Kuru Ü, Şenli S, Türel L, Kuru N, Başkent A, Ulucaklı Ö. Age-specific seroprevalence of hepatitis B virus infection. Turk J Pediatr 1995; 37: 331-338.
9. Çakaloğlu Y, Ökten A, Yalçın S. Türkiye'de hepatit B virusu enfeksiyonu seroepidemiyolojisi. Turk J Gastroenterohepatol 1990; 1: 49-52.
10. Badur S. Ülkemizde viral hepatitlerin durumu (Viral hepatitle savaşım derneği raporu). In: Kılıçturgay K (ed). Viral Hepatit'94. İstanbul: Nobel Tıp Kitapevleri Ltd. Şti.; 1994: 15-37.
11. King JW, Taylor EM, Crow SD, et al. Comparison of the immunogenicity of hepatitis B vaccine administered intradermally and intramuscularly. Rev Infect Dis 1990; 12: 1035-1043.
12. Tekeli E, Balık İ, Kurt H, Kandilci S, Şenbil O. Intramuscular and intradermal administration of a recombinant hepatitis B vaccine. Türkiye Tıp Dergisi 1995; 1: 23-26.
13. Mok Q, Underhill G, Wonke B, Aldouri M, Kelsey M, Jefferies D. Intradermal hepatitis B vaccine in thalassaemia and sickle cell disease. Arch Dis Child 1989; 64: 535-540.
14. Toffler WL, Olenick JS, Wolf NE, Retzlaff ZH, Swanson JR, Kenny TA. The immunogenicity and safety of intradermal hepatitis B vaccine. J Fam Pract 1991; 33: 149-154.
15. Ronish RH, Diniega BM, Kelley PW, et al. Immunogenicity achieved by the intradermal hepatitis B vaccination programme for US Army soldiers in Korea. Vaccine 1991; 9: 364-368.
16. Sirinavin S, Muchacheap T, Khupulsup K, Inthraphuwasak W, Petchclai B. Intradermal hepatitis B virus immunization: immunogenicity and reactogenicity. Southeast Asian J Trop Med Public Health 1991; 22: 577-580.
17. Gonzalez ML, Usandizaga M, Alomar P, et al. Intradermal and intramuscular route for vaccination against hepatits B. Vaccine 1990; 8: 402-405.
18. Morris CA, Oliver PR, Reynolds F, Selkon JB. Intradermal hepatitis B immunization with yeast derived vaccine: serological response by sex and age. Epidemiol Infect 1989; 103: 387-394.
19. Rivey MP, Peterson J. Intradermal hepatitis B vaccination. DICP 1991; 25: 628-634.
20. Coberly JS, Townsend T, Repke J, Fields H, Margolis H, Halsey NA. Suboptimal response following intradermal hepatitis B vaccine in infants. Vaccine 1994; 12: 984-987.
21. Abbott Laboratories, Diagnostic Division Abbott Park, IL 60064, November 1991, List No. 2262.
22. Dienstag JL, Werner BG, Polk BF, et al. Hepatitis B vaccine in health care personnel: safety, immunogenicity, and indicators of efficacy. Ann Intern Med 1984; 101: 34-40.
23. Stevens CE, Toy PT, Taylor PE, Lee T; Yip HY. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long-term protection. Pediatrics 1992, 90· 170-173.

The Turkish Journal of Pediatrics 1997; 39: 491-498

INCIDENCE AND SEVERITY OF ARRHYTHMIAS AND CONDUCTION DISTURBANCE AFTER REPAIR OF TETRALOGY OF FALLOT*

*Nazlıhan Günal MD**, Kürşad Tokel MD***, Öz Kahramanyol MD**
*Sema Özer MD****, Alpay Çeliker MD****, Enver Ekici MD*****
*Yurdakul Yurdakul MD******, Ali Kutsal MD*******

SUMMARY: Günal N, Tokel K, Kahramanyol Ö, Özer S, Çeliker A, Ekici E, Yurdakul Y, Kutsal A. (Pediatric Cardiology Unit, Ankara Social Insurance Hospital of Children, Ankara, Turkey). Incidence and severity of arrhythmias and conduction disturbance after repair of tetralogy of Fallot. Turk J Pediatr 1997; 491-498.

Ventricular and supraventricular arrhythmias and conduction disturbances were evaluated by routine electrocardiography and 24-hour ambulatory monitoring in 31 patients who underwent correction of tetralogy of Fallot. The interval from operation to the study was 1 month to 14 years (mean 4.8 ± 2.8). Complete right bundle branch block occured in 22 (71%) patients and incomplete right bundle branch block in 9 (29%) patients. Bifascicular block with right bundle branch block and left axis deviation (LAD) occurred in one patient. Two patients had second degree type II atrioventricular block. Twenty-four-hour ambulatory electrocardiographic monitoring was performed in all patients and they were divided in two groups according to the frequency of ventricular arrhythmias (Lown classification). Group 1 included the 23 patients who had no arrhythmia or rare ventricular arrhythmias (Lown grade 0-1). Group 2 was comprised of eight patients (26%) with significant ventricular arrhythmias (Lown grade 2-5). Twelve patients (39%) had supraventricular arrhythmias, three patients rare supraventricular tachycardia attacks, and seven patients occasional supraventricular ectopies. One patient had bradycardia-tachycardia attacks and one patient had junctional tachycardia. There was no correlation between age at the time of surgery and ventricular arrhythmias. Of the patients who had ventricular and supraventricular arrhythmias of various degrees on ambulatory monitoring, two had significant arrhythmias on routine electrocardiogram. Symptoms were rare in these patients. In conclusion, both supraventricular and ventricular arrhythmias were found in considerable frequency in our patients. As ventricular arrhythmias may be the cause of sudden death and supraventricular arrhythmias are a main cause of morbidity, it is important to evaluate ventricular and supraventricular arrhythmias by ambulatory monitoring in patients who have undergone correction of tetralogy of Fallot.
Key words: ventricular arrhythmia, supraventricular arrhythmia, tetralogy of Fallot, conduction disturbance, ambulatory monitoring, electrocardiography.

* From the Pediatric Cardiology Unit, Ankara Social Insurance Hospital of Children, Ankara.
** Pediatrician and Pediatric Cardiologist, Ankara Social Insurance Hospital of Children.
*** Pediatrician and Pediatric Cardiologist, Başkent University Faculty of Medicine, Ankara
**** Professor of Pediatrics and Pediatric Cardiologist, Hacettepe University Faculty of Medicine, Ankara.
***** Professor of Pediatrics and Pediatric Cardiologist, Başkent University Faculty of Medicine.
****** Professor of Cardiothoracic Surgery, Hacettepe University Faculty of Medicine.
******* Assistant Professor of Cardiothoracic Surgery, Başkent University Faculty of Medicine.

Postoperative ventricular arrhythmia and conduction disturbances are common after repair of tetralogy of Fallot[1-3]. Tetralogy of Fallot (TF) is the most comon cause of sudden death in children among the other postoperative congenital heart diseases[4]. Sudden death is related to ventricular tachyarrhythmias or progressive abnormalities of atrioventricular conduction and complete atrioventricular block[5]. While on routine electrocardiograms (ECG), 10 percent of patients have ventricular arrhythmias, this prevalence increases to 50 percent on Holter ECG[2,6,7]. Right bundle branch block (RBBB) is the most common conduction disturbance after intracardiac repair of many of the cardiac defects[8]. Although this condition in itself is benign, RBBB and left anterior hemiblock together may have a poor prognosis, with a significantly increased incidence in this group of late complete heart block and sudden death[9]. Supraventricular arrhythmias in postoperative TF have rarely been mentioned in previous reports and thus the pathologic significance of these arrhythmias is not well documented.

The purpose of this study is to evaluate the prevalence of ventricular and supraventricular arrhythmias and conduction defects, in addition to the factors that can effect them, in patients with repair of TF.

Material and Methods

Patients: The study involved 31 patients who underwent correction of TF at Başkent and Hacettepe University Hospitals between 1982-1995. The age of patients at the time of corrective surgery was 1.5 to 13 years (mean 4.4 ± 2.6). The interval from operation to the study was 1 month to 14 years (mean 4.8 ± 2.8). Two patients had previously undergone a Blalock-Taussig operation. No patient had undergone reoperation. In all patients, the conventional operative method was closure of the ventricular septal defect (VSD) with a teflon patch with right ventriculotomy and reconstruction of the pulmonary outflow tract. Physical examination and echocardiographic study were performed in all patients for evaluation of presence of residual VSD, pulmonary valve insufficiency or right ventricular outflow obstruction. All patients had 12-lead electrocardiograms during the follow-up period. The tracings were analyzed for the presence of ventricular and atrial arrhythmias and conduction disturbances. QRS intervals in the right precordial leads greater than 120 milliseconds with terminal conduction delay were defined as right bundle branch block (RBBB). Incomplete right bundle branch block (IRBBB) was diagnosed when the QRS duration was less than 120 milliseconds with terminal conduction delay. QRS axis between -30 and -120 was defined as left axis deviation. Twenty-four-hour ambulatory electrocardiographic monitoring was performed in all patients with Danica Holter System. Severity of ventricular arrhythmias (VA) was classified according to Lown Criteria (10): Grade 0: absence of ventricular extrasystoles in 24 hours, grade

1: uniform ventricular extrasystoles less than 30 in an hour, grade 2: more than 30 uniform ventricular extrasystoles in any hour, grade 3: multiforme ventricular extrasystoles less than 30 in an hour, or couplets (two consecutive ventricular extrasystoles), grade 4: couplets, or multiforme ventricular extrasystoles more than 30 in an hour, and grade 5: ventricular tachycardia (more than 3 consecutive ventricular extrasystoles). Patients were divided in two groups according to the significance of VA. Group I included 23 patients who had no arrhythmias or ventricular arrhythmias in Lown grade I. Group II was composed of eight patients with significant VA (Lown grade 2-5).

Statistical analysis was performed using the student's t test for comparison of the values between the two groups.

Results

Clinical Status

Group I was composed of 23 patients with grade 0-1 VA. Group II was composed of eight patients with grade 2-5 VA. There was no significant difference between the two groups regarding age at the time of corrective surgery and interval from surgery to this study (Table I), although ventricular ectopy was more often seen in patients who had undergone surgery more than five years before. According to echocardiographic findings, there was no difference between the two groups. Five patients (16%) had symptoms such as chest pain, fatigue or palpitation (4 of them were in Group II). Symptoms were attributed to arrhythmias, as none of the patients had significant hemodynamic disturbance.

Table I: Clinical Differences Between the Two Groups

	Group I (n: 23)	Group II (n: 8)	p
age at op. (yr)	4.5 ± 2.5	4.4 ± 2.7	> 0.05
interval from op. (yr)	4.4 ± 3.1	5.5 ± 2	> 0.05
symptom free	22 (95%)	4 (50%)	< 0.01

Electrocardiograms

Postoperative resting ECGs showed RBBB in 71 percent of the patients and IRBBB in 29 percent of the patients. Bifascicular block (RBBB and LAD) occurred in one patient. Neither ventricular nor supraventricular ectopy was detected in any of the patients. In Group I, one patient had first degree and one patient second degree, type II atrioventricular block. Bradycardia-tachycardia attacks were detected in one patient on surface ECG. There was no progressive conduction defect during follow-up period. Postoperative conduction defects on ECG are shown in Table II.

Table II: Conduction Defects on Post Operative Electrocardiograms of the Two Groups

Conduction Defects	Group I (n: 23) no. of pts.	Group II (n: 8) no. of pts.
RBBB	16*	6**
IRBBB	7	2
RBBB + LAD	1	0
AVB	2	0
SND	–	1

* These patients also include the patient with RBBB + LAD and the patients with first degree and second degree AVB.

** These patients include the patient with SND.

RBBB : complete right bundle branch block.
IRBBB : incomplete right bundle branch block.
LAD : left axis deviation.
AVB : atrioventricular block.
SND : sinus node dysfunction.

Ambulatory Monitoring

Eight patients (26%) had significant VA of grade 2-5. Eight patients had VA of grade 1 and 15 patients had no VA (grade 0) in 24-hour monitoring. Supraventricular arrhythmias were detected in 12 patients (39%). Three patients had rare supraventricular tachycardia attacks and seven patients had supraventricular ectopy. Sinus pause less than 2.6 seconds was detected in two patients with supraventricular ectopy. One patient had bradycardia-tachycardia attacks (sinus node dysfunction), and junctional tachycardia was detected in one patient (Figs 1, 2).

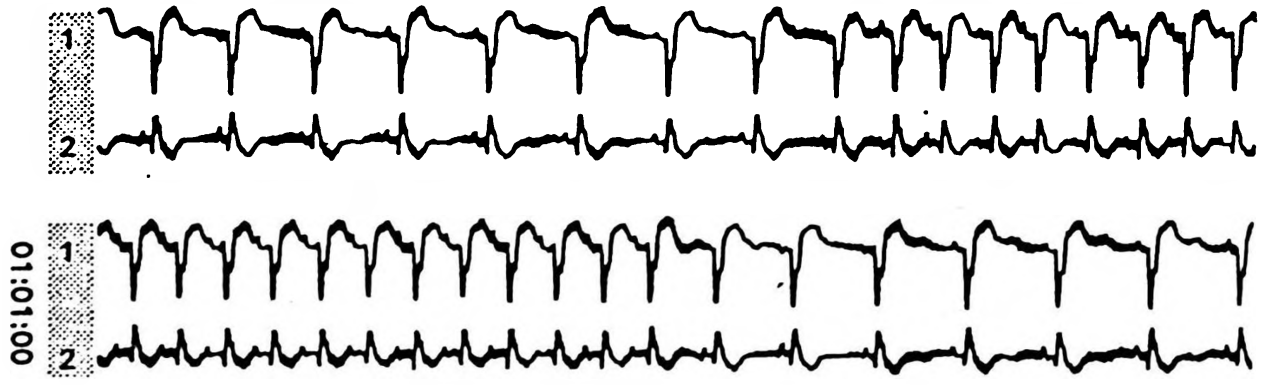


Fig. 1: Bradycardia-tachycardia attacks of a patient.

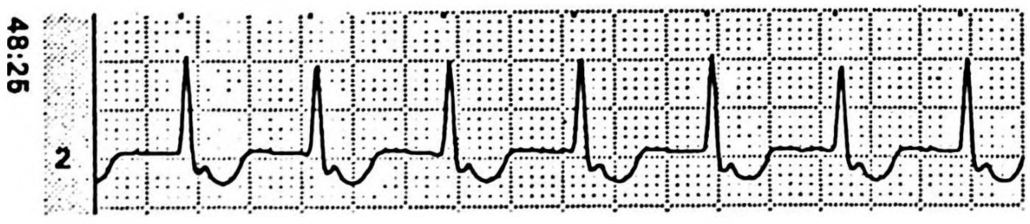


Fig. 2: Nodal (junctional) tachycardia. Note the inverted P waves after each QRS complex, with a heart rate of 130 beats per minute.

Lown classification of VA related to supraventriclar arrhythmias is shown in Table III: Five patients with grade 0; five patients with grade 1, and two patients with grade 5 also had supraventricular arrhythmias. Supraventricular arrhythmias were not found to be related to the frequency of VA.

Table III: Lown Classification of Ventricular Arrhythmias Related to Supraventricular Arrhythmias

	Lown Classification of VA					
	0	1	2	3	4	5
SVET		3				
SVE	3	2				2
SND	1					
Nodal t.	1					

VA : Ventricular arrhythmia. SVT : Supraventricular tachycardia.
SVE : supraventricular ectopy. SND: sinus node dysfunction.
Nodal t.: nodal tachycardia.

Discussion

Patients who have undergone repair for TF have a higher incidence of VA than the normal population, and these patients are at an increased risk for sudden death[11, 12]. Late sudden death caused by VA has been reported in up to five percent of cases where patients have undergone surgical repair of TF[13]. While earlier studies suggested that late cardiac arrest was related to the progression of bifascicular block to complete heart block[9, 14], in recent years, late sudden death after the correction of TF has been attributed to ventricular dysrhythmias[6, 15]. In recent reports, ventricular extrasystoles during ambulatory monitoring or exercise test, presence of ventricular late potentials, inducible ventricular tachycardia[16-18] and prolonged QRS duration[19] are all considered indicators of increased risk for life-threatening VA inpostoperative tetralogy.

Spontaneous VA occurs during ambulatory ECG monitoring in 20-50 percent of patients with surgically corrected TF[10]. In this study, 38 percent of the patients had various degrees of VA on ambulatory monitoring. Significant VA was found in 26 percent patients (Group II). This result is almost the same as in previous reports[2, 3, 7].

In our study, age at the time of surgery and the interval from surgery to the study did not differ statistically between the two groups. Previous reports suggest that the onset of VA is age related. Bove et al.[20] reported that age at the time of surgery was higher in patients with VA than in those without VA. James et al.[21] and Kato et al.[22] also showed increased frequency of VA in older patients who underwent correction of TF. These findings may suggest progressive fibrotic change in the right ventricle[23, 24].

In previous reports, VA and sudden death were correlated with hemodynamic disturbances and raised right ventricular pressure[7, 25]. However, we did not find such a correlation between VA and hemodynamic status, as none of our patients had significant hemodynamic disturbance. In the pediatric population, RBBB pattern is the most common interventricular conduction defect following open heart surgery. It has been reported to occur in 60-100 percent of cases, following repair of TF[8, 26].

The mechanism leading to RBBB in most of these patients was proximal disruption of right bundle due to closure of VSD or distal injury caused by right ventriculotomy[27, 28]. In our study, RBBB was detected in 71 percent and incomplete RBBB in 29% percent of the patients. In the present study, supraventricular arrhythmias were detected on ambulatory monitoring in 12 (39%) patients and these were not considered as hemodynamically significant. Previous studies have stressed the incidence and role of ventricular arrhythmias in post operative TF, with supraventricular arrhythmias rarely mentioned. Recently, Hesselink et al.[29] detected atrial arrhythmias in one-third of their adult patients who underwent correction of TF. Ventricular arrhythmias are well known to be the cause of sudden death, while supraventricular arrhythmias are not related to death, but are considered as the main cause of morbidity in the patients who underwent correction of TF.

In conclusion, we found various degrees of VA in 51 percent and supraventricular arrhythmias in 39 percent of our patients on Holter monitoring. The majority of these patients were asymptomatic. Although there was no significant difference between the two groups in age at the time of surgery and the time interval from surgery to this study, ventricular ectopy was more often seen in patients who had undergone surgery five years or more before. Because both types of arrhythmias are not always present on surface ECGs, it is important to evaluate arrhythmias in these patients during follow-up by ambulatory monitoring.

REFERENCES

1. Deanfield JE, McKenna WJ, Hallidie-Smith KA. Detection of late arrhythmia and conduction disturbance after correction of tetralogy of Fallot. Br Heart J 1980; 44: 248-253.
2. Kavey RE, Blackman MS, Sondheimer HM. Incidence and severity of chronic ventricular dysrhythmias after repair of tetralogy of Fallot. Am Heart J 1982; 103: 342-350.
3. Chandar JS, Wolff GS, Garson A Jr, et al. Ventricular arrhythmias in post operative tetralogy of Fallot. Am J Cardiol 1990; 65: 655-661.
4. James FW, Kaplan S, Chou TC. Unexpected cardiac arrest in patients after surgical correction of tetralogy of Fallot. Circulation 1975; 52: 691-695.
5. Garson A Jr, McNamara DG. Sudden death in pediatric cardiology population, 1958 to 1983: relation to prior arrhythmias. J Am Coll Cardiol 1985; 5: 134B-137B.
6. Garson A Jr, Gillette PC, Gutgesell HP, Mc Namara DG. Stress-induced ventricular arrhythmia after repair of tetralogy of Fallot. Am J Cardiol 1980; 46: 1006-1012.

7. Kobayashi J, Hirose H, Nakano S, et al. Ambulatory electrocardiographic study of the frequency and cause of ventricular arrhythmia after correction of tetralogy of Fallot. Am J Cardiol 1984; 54: 1310-1313.
8. Dunnigan A, Pritzker MR, Benditt DG, Benson DW Jr. Life-threatening ventricular tachycardias in late survivors of surgically corrected tetralogy of Fallot. Br Heart J 1984; 52: 198-206.
9. Wolff GS, Rowland TW, Ellison RC. Surgically induced right bundle-branch block with left anterior hemiblock: an ominous sign in post-operative tetralogy of Fallot. Circulation 1972; 46: 587-594.
10. Ryan M, Lown B, Horn H. Comparison of ventricular ectopic activity during 24-hour monitoring and exercise test in patients with coronary heart disease. N Engl J Med 1975; 292: 224-229.
11. Vaksmann G, Fournier A, Davignon A, Ducharme G, Houyel L, Fouron JC. Frequency and prognosis of arrhythmias after operative "correction" of tetralogy of Fallot. Am J Cardiol 1990; 66: 346-349.
12. Cullen S, Celermajer DS, Franklin RC, Hallidie-Smith KA, Deanfield JE. Prognostic significance of ventricular arrhythmia after repair of tetralogy of Fallot: a 12-year prospective study. J Am Coll Cardiol 1994; 5: 1151-1155.
13. Murphy JG, Gersh BJ, Mair DD, et al. Long-term outcome in patients undergoing surgical repair of tetralogy of Fallot. N Engl J Med 1993; 329: 593-599.
14. Marin-Garcia J, Moller JH: Sudden death after operative repair of tetralogy of Fallot. Br Heart J 1977; 39: 1380-1386.
15. Garson A, Nihill MR, McNamara DG, Coley DA. Status of the adult and adolescent after repair of tetralogy of Fallot. Circulation 1979; 59: 1232-1236.
16. Waksmann G, Elkohen M, Lacroix D, Kacet S, Breviere GM. Influence of clinical and hemodynamic characteristics on signal-averaged electrocardiograms in post operative tetralogy of Fallot. Am J Cardiol 1993; 71: 317-321.
17. Zimmermann M, Friedli B, Adamec R, Oberhänsli I. Ventricular late potentials and induced ventricular arrhythmias after surgical repair of tetralogy of Fallot. Am J Cardiol 1991; 67: 873-878.
18. Giroud D, Zimmermann M, Adamec R, Oberhänsli I, Friedli B. Ventricular late potentials and spontaneous ventricular arrhythmias after surgical repair of tetralogy of Fallot: do they have prognostic value? Br Heart J 1994; 72: 580-583.
19. Gatzoulis MA, Till JA, Somerville J, Redington AN. Mechanoelectrical interaction in tetralogy of Fallot QRS prolongation relates to right ventricular size and predicts malignant ventricular arrhythmias and sudden death. Circulation 1995; 92: 231-237.
20. Bove EL, Byrum CJ, Thomas FD, et al. The influence of pulmonary insufficiency on ventricular function following repair of tetralogy of Fallot. Evaluation using radionuclide ventriculography. J Thorac Cardiovasc Surg 1983; 85: 691-696.
21. James FW, Kaplan S, Schwartz DC, Chou T, Sandker M, Naylor V. Response to exercise in patients after total surgical correction of tetralogy of Fallot. Circulation 1976; 54: 671-679.
22. Kato M, Kawashima Y, Fujita T, Mori T, Manabe H. Right ventricular hypertrophy in tetralogy of Fallot. Recent advances in studies on cardiac structure and metabolism. Recent Adv Stud Cardiac Struct Metab 1976; 12: 149-155.
23. Jones M, Ferrans VS. Myocardial degeneration in congenital heart disease. Comparison of morphologic findings in young and old patients with congenital heart disease associated with muscular obstruction to right ventricular outflow. Am J Cardiol 1977; 39: 1051-1055.
24. Misaki T, Tsubota M, Watanabe G, et al Surgical treatment of ventricular tachycardia after surgical repair of tetralogy of Fallot. Relation between intraoperative mapping and histological findings. Circulation 1994; 90: 264-271.

25. Karademir S, Özkutlu S, Özme Ş, Saraçlar M, Özer S, Bilgiç A. Tüm düzeltme uygulanan Fallot tetralojili hastaların hastane mortalitesi, postoperatif rezidüel defekt ve komplikasyonları. Türk Kardiyol Dern Arş 1992; 20: 55-59.

26. Ehud K. Prognosis for patients with congenital heart disease and post-operative intraventricular conduction defects. Circulation 1978; 57: 867-869.

27. Titus JL, Daughterty GW, Kirklin JW, Edwards DE. Lesion of the atrioventricular conduction system after repair of ventricular septal defect: relation to heart block. Circulation 1963; 28: 82-88.

28. Horowitz LW, Simson MB, Spear JF. The mechanism of apparent right bundle-branch block after transatrial repair of tetralogy of Fallot. Circulation 1979; 59: 1241-1252.

29. Hesselink JR, Perlroth MG, McGhie J, Spituels S. Atrial arrhythmias in adults after repair of tetralogy of Fallot. Circulation 1995; 91: 2214-2219.

The Turkish Journal of Pediatrics 1997; 39: 499-503

TREATMENT OF CONGENITAL DISLOCATION OF THE HIP*

Results of Closed Reduction and Immobilization in the Hip Spica Cast

*M. Talat Göğüş MD***, *M. Cemalettin Aksoy MD****, *Ö. Ahmet Atay MD****
*R. Emre Acaroğlu MD*****, *Adil Surat MD***

SUMMARY: Göğüş MT, Aksoy MC, Atay ÖA, Acaroğlu RE, Surat A. (Department of Orthopedics and Traumatology, Hacettepe University Faculty of Medicine, Ankara, Turkey). Treatment of congenital dislocation of the hip. Results of closed reduction and immobilization in the hip spica cast. 1997; 39: 499-503.

Treatment of congenital dislocation of the hip (CDH) by closed reduction and immobilization in the hip spica cast is one of the accepted methods for use in patients under one year of age. We report 74 congenitally dislocated hips treated with premanipulation skin traction, closed reduction under anesthesia, adductor tenotomy and immobilization in the hip spica cast. Satisfactory results were obtained in 60 hips. In seven hips, avascular necrosis of the capital femoral epiphysis was observed with careful amangement, closed reduction and immobilization in a spica cast provides good results for treatment of CDH. *Key words: congenital dislocation of the hip, closed reductilon.*

Closed reduction of a congenitally dislocated hip was probably first performed by Pravez in 1838[1]. Today, closed treatment of congenital dislocation of the hip (CDH) is still the preferred method of treatment for patients under one year of age. Many methods of treatment have been described, but all have complications and result in a number of hips which develop imperfectly[2]. For example, Renshaw[3], Ferguson[4], Ludloff[5] and Mau et al.[6] have all recommended open reduction for a hip which is slightly lateralized after closed reduction. Thus, there is no real consensus in literature regarding the best method of treatment. The purpose of this study is to determine the effectiveness of this method of treatment and to investigate the rate of complications in this age group.

Material and Methods

We retrospecfively evaluated the hospital records of patients under one year of age diagnosed with congenital dislocation of the hip, who were treated between 1976-1992 at Hacettepe University Faculty of Medicine, Department of Orthopedics and Traumatology. The study group consisted of 60 children (48 female, 12 male) with 74 congenitally dislocated hips. There were 12 dislocations initially seen and treated in patients under three months of age,

* From the Department of Orthopedics and Traumatology, Hacettepe University Faculty of Medicine, Ankara.

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

*** Instructor in Orthopedics, Hacettepe University Faculty of Medicine.

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

28 dislocations in patients between three and six months, and 34 dislocations in patients between six and twelve months. Cases associated with other disorders or syndromes and those involving hips with subluxation or dysplasia, without frank dislocations, were excluded.

First, preliminary skin traction was used on all patients, at home, for two to four weeks. Closed reduction was then performed under general anesthesia; subartaneous adductor tenotomies were necessary in 40% of the cases. Adductor tenotomies were performed if the hip adductors were contracted, since they would resist the abduction of the hip that is essential for reduction. Patients were then immobilized in hip spica casts for three months in the human position. In this position, hips are flexed more than 90° and abducted 30-60°. After three months of immobilization in a plaster cast, if full abduction was possible there was no instability, and plain radiographs showed perfect reduction of the femoral head, the teatment in the cast was discontinued and a splint was applied. The splint allowed flexion and extension but kept the hips moderately abducted. If acetabular remodeling was not considered satisfactory, at that time, another cast was applied for three more months. If there was any question of stability or depth of reduction, an arthrogram was performed. The maximum period of immobilization time for these patients period was nine months.

The average age of patients at the onset of treatment was 5.2 months and the average duration of the treatment in cast was 5.7 months. The mean follow-up was 6.8 ± 3.535 (range, 2-15) years.

Cases of avascular necrosis (AVN) the most noted complication of this treatment, were categorized as described by Bucholz and Ogden[7]. Types 1 and 4, compatible with normal growth, and leading to eventual minimal deformity, are classified as mild AVN. Types 2 and 3 are associated with more severe subsequent disturbances and are classified as severe AVN.

Four criteria were proposed as desirable radiographic findings at the time of the cessation of the treatment. They were: an acetabular angle less than 25°, a center edge (CE) angle greater than 20°, an intact Shenton line and no AVN (Table I).

Table I: Results of Treatment of 74 Congenitally Dislocated Hips

	Severin Grade		
End of Treatment	I	II	III-VI
All criteria met	58	4	0
3 criteria met	2	2	0
2 criteria met	0	0	4
1 criteria met	0	0	3
0 criteria met	0	0	1

Final results were evaluated according to Severin's classification[8] (Table II) with grades being determined using the criteria described above.

Table II: Severin's Classification

Severin Grade	
I	Well developed hip joints
II	Moderate deformity of the femoral head, neck, or acetabulum
III	Dysplasia but not subluxation
IV	Subluxation
V	Femoral head articulating with secondary acetabulum
VI	Redislocation

Results

According to Severin's classification, 60 hips were Severin grade-I, six were Severin grade-II, six were Severin grade-III and two were Severin grade-VI. Thus, of the 74 hips treated with closed reduction and immobilized in hip spica cats, 60 had good or excellent results, six had acceptable results and eight had poor results or failure. The mean acetabular index, 39° ± 3.092 before treatment, improved to 23° ± 2.427 at the final control. The CE angle, which describes the femoral head coverage more meaningfully than the acetabular index after six years of age, was 21° ± 2.582 at the final control. Seven patients had AVN at the final examination (2 had Type-3 and 5 had Type-1).

Discussion

Treatment of CDH in patients under one year of age is difficult, but with careful management, good results can be obtained. An adequate period of premanipulation traction, gentle closed reduction, an adductor tenotomy if needed, and avoidance of extreme positions (excessive abduction) for immobilization are the important requisites of this method of treatment. The main challenge is to avoid AVN during treatment. Premanipulation traction may help by stretching the contracted soft tissues and the muscles spanning the hip, intraarticular pressure is diminished[9, 10]. Forcible positioning of the hip that would lever the femoral head into the acetabulum with a taut adductor muscle acting as a fulcrum is another factor causing AVN. In the series of Gage and Winter[11], incidence of AVN was reduced from 30 to 15 percent with preliminary traction and adductor tenotomy. Salter et al.[12], in their article, emphasize the importance of the adductor tenotomy for reducing the incidence of AVN in the closed treatment of CDH. Avoiding extreme positions during immobilization also helps to reduce the incidence of AVN. Immobilization in a less-forced position (the human position) would diminish the probability of extra-capsular

compression of vessels. Schonecker et al.[13] demonstrated decreased blood flow to the immature femoral head when the hip was placed in extreme positions. Baki et al.[14] in their article also advocate the human position for avoiding AVN. In our series, the AVN rate was 9.2 percent (5% severe and 6.7% mild). This result, when compared to that in found literature, should be accepted as successful. In addition, the final acetabular index and CE angle show that acetabular remodeling was satisfactory.

In cnoclusion, we advocate the following program for the treatment of CDH in a child under one year of age: After a period of skin traction at home, a closed reduction should be performed under general anesthesia, with adductor tenotomy performed if the adductor muscles are contracted. Immobilization should be in the human position in a hip spica cast until the hip becomes stable, plain radiographs demonstrate a perfect reduction of the femoral head, and the acetabular index reaches 25°. Our results indicate that a satisfactory rate of favorable outcomes can be obtained with this treatment.

REFERENCES

1. Severin E. Contributions to the knowledge of congenital dislocation of the hip joint. Late results of closed reduction and arthrographic studies of recent cases. Acta Chir Scand (Suppl) 1941; 63.
2. Willamson DM, Benson MK. Late femoral osteotomy in congenital dislocation of the hip. J Bone Joint Surg (Br) 1988; 10: 614-618.
3. Renshaw TS. Inadequate reduction of congenital dislocation of the hip. J Bone Joint Surg (Am) 1981; 63A: 1114-1121.
4. Ferguson AB. Primary open reduction of congenital dislocation of the hip using a median approach. J Bone Joint Surg (Am) 1973; 55: 671-689.
5. Ludloff K. The open reduction of the congenital hip dislocation by an anterior incision. Am J Orthop Surg 1913; 10: 438-454.
6. Mau H, Dorr WM, Henkel I, Lutsche J. Open reduction of congenital dislocation of the hip by Ludloff's method. J Bone Joint Surg (Am) 1971; 53: 1281-1288.
7. Bucholz RW, Ogden JA. Pattern of ischemic necrosis of the proximal femur in non-operatively treated congenital hip disease. In: Proceedings of the sixth open scientific meeting of the hip society. St. Louis: CV Mosby; 1978: 43-63.
8. Severin E. Congenital dislocation of the hip. Development of the joint after closed reduction. J Bone Joint Surg (Am) 1950; 32: 507-518.
9. Buchanan JR, Greer RB 3rd, Cotler JM. Management strategy for prevention of avascular necrosis during treatment of congenital dislocation of the hip. J Bone Joint Surg (Am) 1981; 63A: 140-146.
10. Weiner DS, Hayt WA, O'Dell HW. Congenital dislocation of the hip. The relationship of premanipulation traction and age to avascular necrosis of femoral head. J Bone Joint Surg (Am) 1977; 59A: 306-311.
11. Gage JR, Winter R. Avascular necrosis of capital femoral epiphysis as a complication of closed reduction of congenital dislocation of the hip. J Bone Joint Surg (Am) 1972; 54A: 373-388.

12. Salter RB, Kostuik J, Dallas S. Avascular necrosis of the femoral head as a complication of treatment for congenital dislocation of the hip in young children. Canadian J Surg (Am) 1969; 12: 44-61.

13. Schonecker PL, Bitz DM, Whiteside LA. The acute effect of position of immobilization on capital femoral epiphyseal blood flow. A quantitative study using the hydrogen washout technique. J Bone Joint Surg (Am) 1978; 60A: 899-904.

14. Baki C, Tokgözoğlu N, Akkoyunlu Ü, Memikoğlu Ş. Douştan kalça çıkığı konzervatif tedavisinin geç sonuçları. V. Milli Türk Ortopedi ve Travmatoloji kongre kitabı 1978; 110.

The Turkish Journal of Pediatrics 1997; 39: 505-510

ARTHROSCOPIC PARTIAL RESECTION OF THE DISCOID MENISCUS IN CHILDREN*

*Özgür Ahmet Atay MD***, *Mahmut Nedim Doral MD****

*Mehmet Cemalettin Aksoy MD***, *Onur Tetik MD*****, *Gürsel Leblebicioğlu MD******

SUMMARY: Atay ÖA, Doral MN, Aksoy MC, Tetik O, Leblebicioğlu G. (Department of Orthopedics and Traumatology, Hacettepe University Faculty of Medicine, Ankara Turkey). Arthroscopic partial resection of the discoid meniscus in children. 1997; 39: 505-510.

Thirteen children with 14 lateral discoid menisci were reviewed at an average follow-up of 2.7 years. Their average age at the time of the operation was 12.8 years. Most of the children had vague and intermittent painful symptoms, and the classical "clunk" was demonstrable in nine of the 13 patients in clinical examinations. Thirteen children underwent arthroscopic partial meniscectomy for symptomatic discoid lateral meniscus, by performing partial resection. This procedure, modifying the discoid lateral meniscus to the normal semilunar shape, was indicated only when the capsular attachment was intact.

The results were excellent both clinically and radiologically. Furthermore, rehabilitation time was considerably shorter than the time required after open procedures.

Arthroscopic discoid meniscus surgery performed by experienced and skilled hands gives better results. According to the literature and our experiences, it is better to perform open techniques in patients with stiff knees. Additionally, it is technically feasible to use small joint instruments in the pediatric age group. *Key words: arthroscopy, discoid meniscus.*

Young[1], in 1889, was the first to describe a discoid lateral meniscus in a cadaver specimen, and the term "snapping knee syndrome" was attributed to it by Kroiss[2]. The vague and intermittent symptoms associated with this anomaly may cause difficulty and delay in the diagnosis. Arthroscopy allows more precise diagnosis of the lesion, confirms the clinical diagnosis, and can identify an associated tear[3, 4].

The purpose of this study was to report our experience with the arthroscopic evaluation and with the performance of partial resection of the discoid lateral meniscus, and to determine the short-term results of operative treatment.

Material and Methods

Between 1990 and 1993, 13 children (6 boys, 7 girls) with 14 lateral discoid menisci were treated at Hacettepe University Faculty of Medicine, Department of Orthopedics and Traumatology, by arthroscopic means. Their average age

* From the Department of Orthopedics and Traumatology, Hacettepe University Faculty of Medicine, Ankara.

** Instructor in Orthopedics, Hacettepe University Faculty of Medicine.

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

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

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

at surgery was 12.8 years (range 11 to 20). The mean follow-up was 2.7 years (range two to five). The right knee was involved in five cases and the left in seven. One child had the condition in both knees.

Pain was the predominant symptom in the majority of cases. Ten children (11 knees) complained of a click or clunk in their knees. However, on examination, the "classical" clunk so characteristic of the discoid meniscus was demonstrable in only nine knees.

Details of the pre-operative clinical and physical signs are shown in Table I.

Table I: Preoperative Clinical and Physical Symptoms

Symptoms	Number of Knees
Pain	14
Clunk	11
Swelling	8
Locking	6
Giving way	4

Routine anteroposterior, lateral and intercondylar (tunnel) views were taken. No widening of the lateral joint space was seen. None of the radiographs showed squaring off of the lateral femoral condyle.

Arthroscopy was performed on all patients in an operating room under general anesthesia. Adequate muscle relaxation was considered essential to performing this type of surgery. A tourniquet was placed on the thigh. The fiberoptic scope and a highly sensitive, high-quality video camera were used throughout the study.

Several portals were required for adequate visibility and probing of the discoid meniscus. A hook was used to determine the stability of the discoid meniscus and whether or not it was torn.

The accepted method of classification for the discoid lateral meniscus, that of Watanabe et al.[5], was used. They described three types: complete (Fig. 1), incomplete, or wrisberg's ligament type, based on the degree of coverage of the lateral tibial plateau and the presence or absence of the normal posterior meniscotibial attachment. The management of the meniscus was based on this system of classification. During arthroscopic inspection, six lateral complete discoid menisci and eight lateral incomplete discoid menisci with tears were revealed; none of the discoid menisci was Wrisberg's ligament type.

Complete and incomplete discoid menisci with tears should be partially resected to a stable peripheral rim. All children underwent arthroscopic partial resection. This procedure modified the discoid meniscus to the normal semilunar shape. During the course of these procedures in six knees, partial resection was performed with the help of a shaver or laser (Fig. 2).

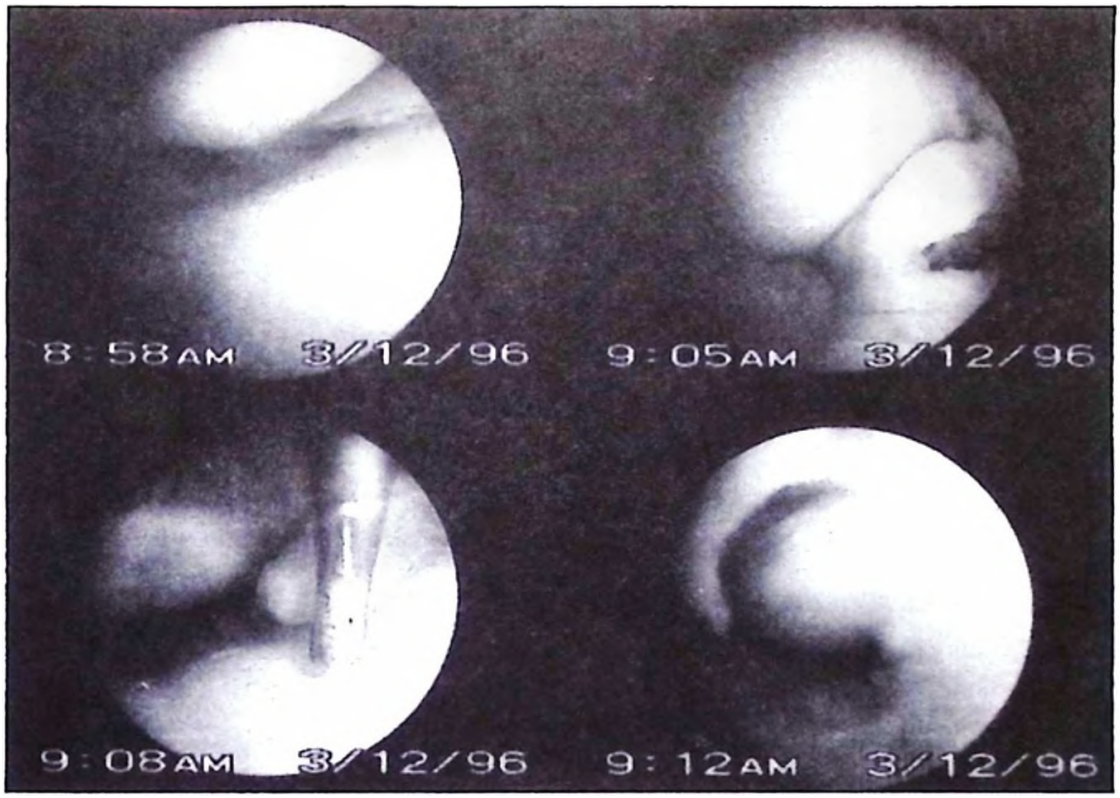


Fig. 1. Arthroscopic view of complete lateral discoid meniscus.

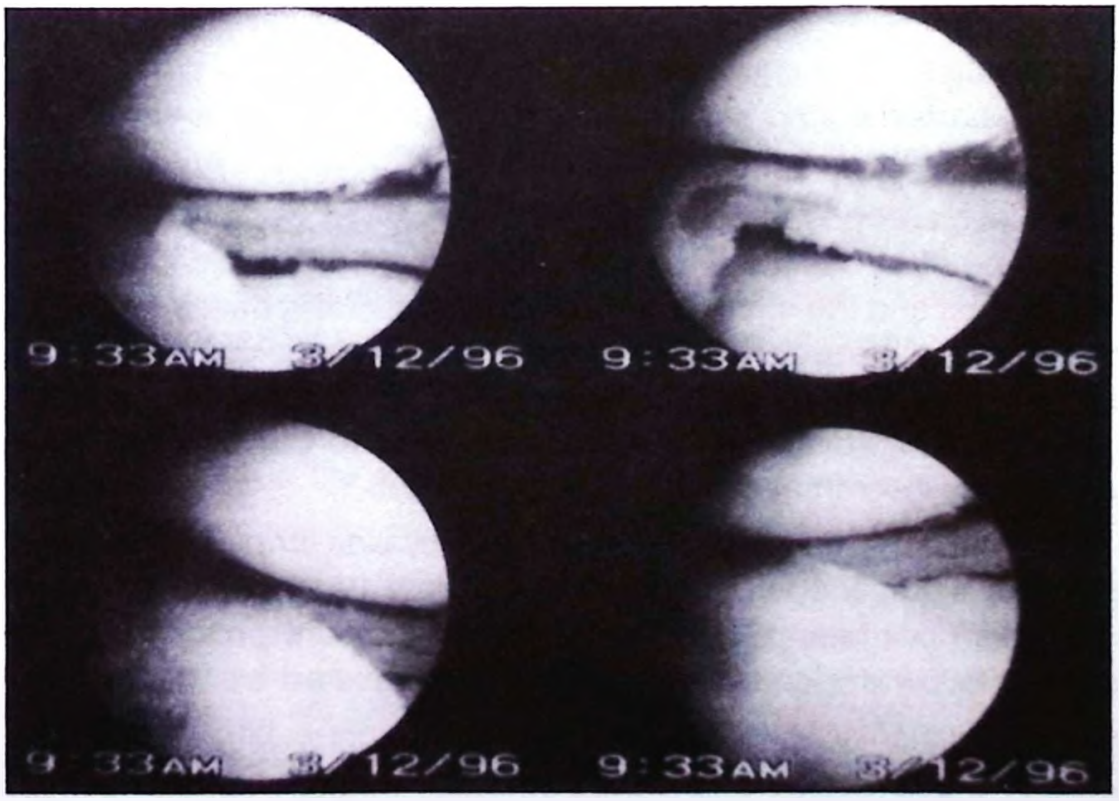


Fig. 2: Arthroscopic partial resection of complete lateral discoid meniscus and views of partially resected meniscus.

No infections or episodes of significant bleeding were encountered.

After the operatilon, aggressive proprioceptive physiotherapy was performed. Overall, clinical and radiological controls of 13 children were reviewed at a mean of 2.7 years.

Results

The clinical results were analyzed according to the IKDC (International Knee Documentation Committee), Tegner activity scale[6], and Lysholm scores[7]. According to IKDC, all patients were in group A. Through the Tegner activity scale, eight were found as the mean (range 7-10), and by the Lysholm score, a mean of 94 (range 88-100) was found.

No degenerative changes were seen in the lateral compartment and radiographs showed no narrowing of the joint space in the mean 2.7 years of follow-up.

The results of partial resection of the discoid meniscus were excellent both clinically and radiologically in all 13 children. Furthermore, the period of rehabilitation after this procedure was much shorter than the time required after open procedures.

Discussion

The present series has revealed several points of interest. Pain was the most common pre-operative symptom rather than the conventially described "clunk"[8]. The pathomechanism of the pain is not well understood, but could be related to free nerve endings[9].

Contrary to Picard[10], we do not feel that radiographs play a significant role in diagnosis. In our series, no knee had the classical lateral joint space widening which has been described in previous reviews[11, 12].

The classification proposed by Watanabe[5] has gained worldwide popularity. In fact, Watanabe et al.'s is the accepted method of classification for the discoid lateral meniscus, and the management of the meniscus is based on this system. Total meniscectomy is recommended for the Wrisberg's ligament type of discoid because the posterior meniscotibial attachment is deficient[11, 13]. Most authors agree that the intact discoid lateral meniscus, of the complete or incomplete type, should not be removed[11, 13].

In the earlier part of the series, before arthroscopy was employed, differentiation between complete, incomplete and Wrisberg's ligament types of discoid meniscus was not made. Since then, our management protocol has evolved and we now follow the classification system suggested by Watanabe et al.[5]

In our study, difficulty in introducing the arthroscope laterally was the first indication of the anomaly. Various authors have recommended arthroscopic partial or total meniscectomy for discoid lateral menisci[3, 4, 11, 12]. This is a difficult technique requiring considerable skill and experience.

In children, total resection of a discoid meniscus is performed only when it is unavoidable, although it has been said that the prognosis after total meniscectomy is better in children than in adults[14]. However, significant degenerative changes are inevitable, especially after a lateral discoid meniscectomy[14, 15].

Recent biomechanical research has highlighted the importance of the meniscus in compensating for the incongruity between femur and tibia, in load transmission, in providing for rotation, as a shock absorber, in the provision of stability and in the elastohydrodynamic lubrication of joint surfaces[15].

Partial resection or repair of peripheral tears of the meniscus have been performed in adults in order to preserve these functions of the meniscus[15].

In children, we perform partial resection of the free edge of the discoid meniscus, modifying it to the normal semilunar shape, for the following reasons: to allow the residual meniscus to function in a manner similar to that of the normal meniscus, to prevent some of the instability resulting from the meniscectomy, to minimize the extent of operative intervention, and to shorten the rehabilitation period.

We recommend an arthroscopic partial lateral meniscectomy for torn, complete or incomplete discoid menisci with a stable posterior meniscotibial attachment. However, in children with Wrisberg's ligament type or hypermobile discoid menisci, we believe the partial meniscectomy to be inappropriate, as it leaves an unstable rim of meniscus which may cause symptoms in the future. Therefore, a total meniscectomy should be recommended in such circumstances.

There are, however, some doubts concerning the long-term results of partial resection of the discoid meniscus. The arrangement of collagen fibers is different in the semilunar and the discoid cartilage and it is possible that further injury or degeneration of the residual meniscus may occur after the operation. However, should the long-term results be as good as the early results, this arthroscopic partial resection would be advisable, in experienced an skilled hands, for treatment of symptomatic discoid meniscus in children. In cases of patients with stiff knees, however, according to both the literature and our experiences, it is better to perform open techniques. Additionally, it is technically feasible to use small joint instruments in the pediatric age group.

REFERENCES

1. Young RB. The external semilunar cartilage as a complete disc. In: Cleland J, Mackay JY, Young RB (eds). Memoirs and Memoranda in Anatomy. London: Williams and Norgate; 1889: 179.
2. Kroiss F. Die Verletzungen der Kniegelenkoszwischenknorpel und ihrer Verbindungen. Beitr Z Klin Chir Tubing 1910; 66: 598-801.
3. Hayashi LK, Yamaga H, Ida K, Miura T. Arthroscopic meniscectomy for discoid lateral meniscus in children. J Bone Joint Surg (Am) 1988; 10A: 1495-1500.
4. Ikeuchi H. Arthroscopic treatment of the discoid lateral meniscus. Technique and long-term results. Clin Orthop 1982; 167: 19-28

5. Watanabe M, Takeda S, Ikeuchi H. Atlas of Arthroscopy (3rd ed). Berlin: Springer-Verlag, 1979.
6. Tegner Y, Lysholm J. Rating systems in the evaluation of knee ligament injuries. Clin Orthop 1985; 198: 43-49.
7. Lysholm J, Gillquist J. Evaluation of knee ligament surgery results with special emphasis on use of a scoring scale. Am J Sports Med 1982; 10: 150-154.
8. Aicroth PM, Patel DV, Marx CL. Congenital discoid lateral meniscus in children. A following study and evolution of management. J Bone Joint Surg (Br) 1991; 73: 932-936.
9. Doral MN. Anterior cruciate ligament reconstruction using solvent dehydrate B-Pt-B allograft in functional instability. Poster exhibition. ESSKA Budapest. Abstract Book: 1996; 191.
10. Picard JJ. Aspects radiologiques des menisques discoides. J Radiol Electr 1964; 45: 839-841.
11. Dickhaut SC, DeLee JC. The discoid lateral-meniscus syndrome. J Bone Joint Surg (Am) 1982; 64-A: 1068-1073.
12. Bellier G, Dupont JY, Larrain M, Caudron C, Carlioz H. Lateral discoid menisci in children. Arthroscopy 1989; 5: 52-56.
13. Griffin PP. The lower limb. In: Lowell WW, Winter RB (eds). Paediatric Orthopaedics (2nd ed). Philadelphia: JB Lippincott; 1986: 887.
14. Kobayashi A. Discoid meniscus of the knee joint. Clin Orthop Surg Jap 1975; 10: 10-24.
15. McGinty JB, Geuss LF, Marvin RA. Partial or total meniscectomy. J Bone Joint Surg (Am) 1977; 59A: 763-766.

The Turkish Journal of Pediatrics 1997; 39: 511-517

ULTRASONOGRAPHIC EVALUATION OF URINARY TRACT IN CHILDREN WITH CHRONIC FUNCTIONAL CONSTIPATION*

*Cahit Karakelleoğlu MD***, *Zerrin Orbak MD****, *Bahri Şen MD*****
*İnan Hülür MD******, *Handan Alp MD***, *Recep Akdağ MD***
*Haydar Taşdemir MD***

SUMMARY: Karakelleoğlu C, Orbak Z, Şen B, Hülür İ. Alp H, Akdağ R, Taşdemir H. (Departments of Pediatrics and Radiology, Atatürk University Faculty of Medicine, Erzurum, Turkey). Ultrasonographic evaluation of urinary tract in children with chronic functional constipation. Turk J Pediatr 1997; 39: 511-517.

Urinary tract anomalies were prospectively investigated with ultrasound in 31 children with chronic functional constipation. These children were compared with 29 healthy controls without constipation both before and after treatment. Bladder residue and upper renal tract dilatation after micturition were significantly more common in the group with constipation than in the improved after-treatment and control groups.
Key words: functional constipation, pelvicalyceal dilatation, residue after micturition, urinary tract infection, ultrasonographic evaluation, children.

Constipation refers to both the frequency of defecation and the consistency of the stool. Functional constipation, defined as constipation without evidence of an underlying abnormality, is common and underdiagnosed pediatric problem[1, 2].

In patients with non-organic constipation, attention has been focused on the colons, with little thought being given to the association of urinary tract pathology. Kottmeier and Clattworthy[3] noted four incidental cases of megaureter in 13 children with functional magacolon[5], but only three cases of megaureter in 27 children with aganglionic megacolons. They presumed the cause to be a mechanical pressure phenomenon. O'Regan et al.[4] reported a series of 47 girls with recurrent urinary tract infection (UTI) and large fecal reservoirs. Intravenous urograms revealed that all had abnormal uninhibited bladder contractions and normal upper tracts.

We undertook a prospective controlled study using ultrasound to evaluate urinary tracts in patients with chronic functional constipation. We observed the effects of treatment and compared the children with controls.

* From the Departments of Pediatrics and Radiology, Atatürk University Faculty of Medicine, Erzurum.
** Associate Professor of Pediatrics, Atatürk University Faculty of Medicine.
*** Assistant Professor of Pediatrics, Atatürk University Faculty of Medicine.
**** Research Assistant in Radiology, Atatürk University Faculty of Medicine.
***** Research Assistant in Pediatrics, Atatürk University Faculty of Medicine.

Material and Methods

This study population consisted of 31 patients (18 females and 13 males) with chronic functional constipation with or without encopresis, and 29 control children (16 males and 13 females). Without treatment, these 31 patients passed less than three stools per week. All constipated children had infrequent and abnormal stools for six months or more. All constipated children had a very large amount of stools present in the rectal ampulla. Heights and weights were appropriate for age in all subjects. We excluded from this study organic constipation induced by drugs (diuretics, anticonvulsants, codeine-containing cough syrups, hypotensives), neurogenic constipation (Hirschsprung's disease, paraplegia, myelomeningocele, diabetic neuropathy), constipation secondary to anal lesion (fistula, abscess, hemorrhoids), and constipation secondary to endocrine disorder (hypothyroidism, hypercalcemia, hypopituitarism). Mental retardation (demonstrated by clinical evidence of psychomotor retardation) was, likewise, a cause of exclusion[1,2].

The control subjects volunteered for this study. These children had between two bowel movements per day to one every other day. They had no history of previous gastrointestinal tract disease, and their physical examinations yielded normal results.

Urograms, urine cultures, and ultrasonographies were performed on all cases (constipated and control cases). Urinary tract infection (UTI) was confirmed as all pure growths of more than 10^5 CFU/ml of a single urinary pathogen from mid-stream urine and a bag specimen, and all pure growths of more than 50×10^3 CFU/ml of a single urinary pathogen in urine collected by catheterization[5]. The children were given a large drink 30-45 minutes before the ultrasound study, which was undertaken initially with a full bladder and then repeated after the bladder was emptied. The same consultant radiologist evaluated bladders, kidneys, and ureters using a standard technique by a 3.5 MHz probe on ultrasound machine (Toshiba SAL 77A). Bladder volume was estimated from three dimensional measurements and the diameters of the pelvicalyceal systems and upper ureters were measured. The measurements were repeated after micturition. Pelvicalyceal diameters greater than 7 mm were accepted as pelvicalyceal dilatation[6].

The 31 constipated patients received treatment. Treatment was initiated by emptying the rectal vault with an enema. Once the rectum had been cleared of stool, to prevent fecal impection and soiling and to allow the rectum to return to normal size, lactulose (1-2 ml/kg body weight daily, as necessary to induce one to two bowel movements per day) was used. In addition, these cases received a high-fiber diet, and instruction bowel training techniques[1,2]. The patients were followed up approximately one year later.

Urine cultures and ultrasound studies were repeated after treatment when a regular bowel habit had been established.

Cooperating patients with pelvicalyceal dilatation underwent voiding cystourethrograms (VCUG). VCUG was performed according to standard technique using a cystographic contrast agent instilled by catheter under gravity.

Results were compared before and after treatment and with controls using the χ^2, and Wilcoxon matched-pairs signed-ranks tests.

Results

Thirty-one constipated and twenty-nine control cases were included in this study. Their ages ranged from two to 14 years. The mean ages of the constipated children (5.6 years) and of the control children (6.8 years) were not significantly different.

Chronic recurrent abdominal pain was one of symptoms associated with constipation, and it occurred in 15 patients (48.4%). Five children (29.4%) of 17 children more than five years old suffered from nocturnal enuresis, and 12 children (60.0%) of 20 children more than four years old suffered from encopresis before treatment. After treatment, cases of enuresis and encopresis improved to 23.5% and 15.0%, respectively (Table I). While there was a statistically significant difference in the decrease of encopresis, there was no significant difference in the decrease of nocturnal enuresis.

Table I: Occurrence (Number and Percentage) of Encopresis and Nocturnal Enuresis in Children with Constipation (Before and After Treatment)

	Constipated Children				
	Before Treatment		After Treatment		
	n	%	n	%	p
Encopresis	12	60	3	15	< 0.01
Nocturnal Enuresis	2	29	4	24	> 0.05

Twenty-two percent of the constipated patients had urinary tract infections at initial assessment, a finding significantly different from controls and improved patients ($p < 0.05$, $p < 0.01$, respectively) (Table II).

Table II: Occurrence (Number and Percentage) of a Residue After Micturition and Urinary Tract Infection in Child with Constipation (Before and After Treatment) and in Controls

	Constipated Children					
	Before Treatment		After Treatment		Controls	
	n	%	n	%	n	%
A residue after micturition	12	39*,**	3	10***	4	14
Urinary tract infection	7	22*,**	0	0***	1	3

* Before treatment vs controls $p < 0.05$.
** Before treatment vs after treatment $p < 0.01$.
*** After treatment vs controls $p > 0.05$.

A residue after micturition was detected in 12 of 31 children (38.7%) at initial assessment and three of 31 children (9.7%) at assessment after treatment, compared with four of 29 controls (13.8%) (Table II). The residue after micturition was significantly more common in constipated children before treatment compared with controls ($p < 0.05$), and was significantly reduced by treatment ($p < 0.01$). After treatment, the patients showed no more significant a residue after micturition than that shown in the controls ($p > 0.05$).

Pelvicalyceal dilatation was present in 13 of 31 (41.9%) constipated children before treatment, compared with three of 29 (10.3%) controls ($p < 0.05$). There was a reduction in the number of children with pelvicalyceal dilatation after treatment and these improvements reached statistical significance ($p < 0.01$) (Table III).

Table III: Occurrence of Pelvicalyceal Dilatation in Children with Constipation (Before and After Treatment) and in Controls

	Constipated Children		
Pelvicalyceal Dilatation	Before Treatment	After Treatment	Controls
Right kidney	8	3	2
Left kidney	7	2	2
Bilateral	2	1	1
Total	13 (41.9%)*,**	4 (12.9%)***	3 (10.3%)

* Before treatment vs controls $p < 0.05$.
** Before treatment vs after treatment $p < 0.01$.
*** After treatment vs controls $p > 0.05$.

Five of the 13 patients with pelvicalyceal dilatation had a residue after micturition. There was no significant difference between the residue after micturition and pelvicalyceal dilatation ($p > 0.05$). The number of patients with pelvicalyceal dilatation increased with the duration of constipation. Pelvicalyceal dilatation was present in three (21.4%) of 14 patients who had a duration of constipation of less than one year, and in 10 (58.8%) of 17 patients who had a duration of constipation of more than one year ($\chi^2 = 3.86$, $p < 0.05$). The number of patients with a residue after micturition also increased with the duration of constipation. A residue after micturition was present in three (35.7%) of 14 patients who had a duration of constipation of less than one year and in nine (52.9%) of 17 patients who had duration of constipation of more than one year ($\chi^2 = 4.12$, $p < 0.05$). Six of seven patients with UTI had pelvicalyceal dilatation, and there was a significant difference between UTI and pelvicalyceal dilatation ($p < 0.01$).

Significant differences between a residue after micturition and nocturnal enuresis or UTI were also found ($p < 0.05$).

None of the children with pelvicalyceal dilatation was shown to have vesicoureteral reflux during micturating cystourethrography.

Discussion

Constipation represents a common problem in children, accounting for three percent of visits to the pediatric outpatient clinic and for 25 percent of visits to the pediatric gastroenterology clinic[7]. Chronic functional constipation is the most common cause of constipation with or without encopresis (90-95%)[2, 7]. Although the associated gastrointestinal and psychological symptoms are well recognized, the effects on the urinary tract are not so widely appreciated. Constipation may not be suspected when a child has a urinary tract infection and, conversely, urinary tract abnormalities are easily overlooked when a family faces the major anxiety of a child with constipation and encopresis.

In literature, the most common symptom associated with constipation is chronic recurrent abdominal pain, which occurs in about 60 percent of the patients. Enuresis is reported in about 30 percent of the cases of children with encopresis. Many have daytime as well as nocturnal enuresis, which resolve when the constipation is treated[1]. In our study, the most common symptoms were encopresis and abdominal pain. While there was a statistically significant difference in the decrease of encoprdesis. there was no significant difference in the decerease of nocturnal enuresis.

Literature on the association of urinary tract abnormalities and constipation in children is scarce. Bladder and ureteric abnormalities have been described in both organic and non-organic groups[3, 8, 9]. It is generally agreed that there is a higher incidence of urinary tract infection in both groups. The urinary tract evaluation must be performed as it plays a crucial part in management. Kottmeier and Clattworthy[3] noted megaureter in 30.7 percent of children with functional constipation and presumed the cause to be a mechanical pressure phenomenon. Shopfner[9] found 12 cases of urinary tract abnormalities in 39 children with severe functional constipation. Although he found no evidence of mechanical obstruction, he found a high incidence of bladder abnormalities, including a cone-shaped bladder base plate, elongated urethra and mural irregularity localized to the pressure area from the fecal mass. In the same study, there was normal ureteric peristalsis and emptying. Hence, he found no supporting evidence of an obstructive cause, but could not exclude mild dilatation secondary to chronically raised intravesical pressure[9]. In the study performed by Hebetko and Hyde[8], the incidence of dilatation of the upper renal tract and UTI was 12 percent and 31.4 percent, respectively, in the patients with non-organic constipation. In the same study, they observed a higher incidence of dilatation of the upper urinary tract in the organic constipation group (67%) than in the functional group (12%)[8].

The anatomic proximity of the bladder and urethra to the rectum, and the similar spinal innervation of the urethral and anal sphincters, make it likely that abnormalities in the gastrointestinal system may affect the urinary tract. Indeed, Shopfner[9] has shown that a fecally impacted rectum will cause irregularities in

the bladder wall. In constipated children, the pelvicalyceal dilatation had likely arisen from the mechanical compression of the urinary tract by the distended rectum. This could occur at the level of the urethra, bladder, or the vesicoureteric junction.

Although it was not intended in this study to determine the incidence of constipation in children with UTI, we showed that urinary tract abnormalities often occur in children with functional constipation and that these abnormalities appear to be reversible after adequate treatment. In our study, significant differences between a residue after micturition and nocturnal enuresis or UTI were found ($p < 0.05$). Inappropriate detrusor muscle contraction in children with constipation was noted[4]. Incomplete bladder emptying creates a reservoir for bacteria and may predispose one to UTI.

Similar to our findings, Dohil et al.[6] found that the bladder residue and upper renal tract dilatation after micturition were significantly more common in the group with constipation than in the improved after treatment group. Also, in the same study, none of the children with upper tract dilatation exhibited vesicoureteral reflux at micturating cystourethrography.

The number of patients with pelvicalyceal dilatation or with a residue after micturition both increased with the duration of constipation. However, there was no significant difference between the residue after micturition and pelvicalyceal dilatation ($p > 0.05$). We found that there was a significant difference between UTI and pelvicalyceal dilatation ($p < 0.01$), and significant differences between a residue after micturition and nocturnal enuresis or UTI ($p < 0.05$). We could not find any information in this regard in literature.

Our study showed that renal abnormalities are relatively common in children with functional constipation and that those are reversible. The etiology of upper tract dilatation may be related to increased bladder volume or pressure. We feel it is important to be aware that there might be an association with constipation. A case can be made for investigating through ultrasound the urinary tract system of all children with chronic constipation.

REFERENCES

1. Klish WJ. Functional constipation and encopresis. In: Oski FA, DeAngelis CD, Feigin RD, McMillan JA, Warshaw JB (eds). Principles and Practice of Pediatrics (2nd ed). Philadelphia: JB Lippincott; 1994: 1843-1845.
2. Loening-Baucke V. Encopresis and soiling. Pediatr Clin North Am 1996; 43: 279-298.
3. Kottmeier PK, Clattworthy HW. Aganglionic and functional megacolon in children-a diagnostic dilemma. Pediatrics 1965; 36: 572-582.
4. O'Regan S, Yazbeck S, Schick E. Constipation, bladder instability, urinary tract infection syndrome. Clin Nephrol 1985; 23: 152-154.
5. Hellerstein S. Urinary tract infection: old and new concept. Pediatr Clin North Am 1995; 42: 1433-1457.

6. Dohil R, Roberts E, Jones KV, Jenkins HR. Constipation and reversible urinary tract abnormalities. Arch Dis Child 1994; 70: 56-57.
7. Loening-Baucke V. Chronic constipation in children. Gastroenterology 1993; 105: 1557-1564.
8. Herbetko J, Hyde I. Urinary tract dilatation in constipated children. Br J Radiol 1990; 63: 855-857.
9. Shopfner CE. Urinary tract pathology associated with constipation. Radiology 1968; 90: 865-877.

The Turkish Journal of Pediatrics 1997; 39: 519-531

DOWN SYNDROME AND LEUKEMIA – AN OVERVIEW OF CYTOGENETIC AND MOLECULAR EVENTS*

*Süreyya Savaşan MD**, *Jeffrey W. Taub MD***, *Yaddanpudi Ravindranath MD****

Down syndrome (DS) is the most common trisomic disorder seen in humans with an incidence of 1/700-1/1000 live births. In addition to its characteristic phenotypic features, heart defects, gastrointestinal system abnormalities, premature aging, and hematological disorders, including an increased risk of leukemia, are associated with DS. The relation between DS and leukemia has been known for many years and has continued to be a focus of interest not only for the probable role of trisomy 21 in the development of leukemia at the molecular level but also for the unique features o fthe leukemia. These include the predominance of acute myeloid leukemia (AML) in younger children with the megakaryoblastic subtype and spontaneous regression of myeloproliferation in newborns.

Acute Leukemia

Down syndrome patients have a 10-to 20-fold increased risk of developing both acute lymphoblastic leukemia (ALL) and AML compared to non-DS children; one percent of DS individuals will develop leukemia during their lifetime[1]. Although most cases of leukemia in DS individuals occur early in childhood, the risk spans into adulthood[2]. AML predominates in DS patients under three years of age, while the relative proportions of ALL and AML are the same in older children with or without DS[3-4]. A large proportion of AML cases in DS children have been classified as "megakaryoblastic" (AML-M7) by positive electron microscopic detection of platelet peroxidases and/or staining with monoclonal antibodies against platelet antigens[5]. In DS patients, the incidence of AML-M7 is approximately 600 times higher than that in non-DS children with AML[6]. AML-M7 is usually associated with a myelodysplastic phase either in the neonatal period or immediately preceding the development of AML, and myelofibrosis is common in patients with DS[3]. Although extramedullary involvement, especially bone infiltration, is relatively common in non-DS patients with AML-M7, the development of granulocytic sarcoma is very rare in DS patients with AML-M7[7].

A significant number of AML patients with DS have additional cytogenetic changes similar to the cytogenetic abnormalities of de novo cases of AML in non-DS patients[4, 8-9]. Trisomies of chromosomes 1q, 8, and monosomy 7 are the most

* From the Barbara Ann Karmanos Cancer Institute, Division of Hematology/Oncology, Children's Hospital of Michigan, Wayne State University School of Medicine, Detroit, Michigan, USA.

** Pediatric Hematologist, Wayne State University School of Medicine.

*** Professor of Pediatric Hematology, Wayne State University School of Medicine.

common numerical abnormalities, while tetrasomy 21 is the most frequent quantitative microscopic abnormality in AML-M7 in DS[8-10]. Non-DS AML-M7 is associated with t (1;22) (p13;q13), while this is not seen in DS cases[11-12]. However, numerical abnormalities of chromosome 21 have been demonstrated in AML-M7 patients without Down syndrome as either primary or nonrandom secondary changes. Favorable treatment outcomes have been reported in DS patients with ALL using intensive regimens which were associated with excessive methotrexate toxicity[13]. DS patients with AML have a superior response rate to intensive chemotherapy with high dose cytosine arabinoside (AraC) containing regimens compared to non-DS patients[9]. Low dose AraC therapy has also been reported to be effective in some DS patients with myelodysplastic syndrome or AML-M7[6, 14].

Transient Myeloproliferative Disorder

Transient myeloproliferative disorder (TMD), also known as transient leukemia, transient leukemoid reaction, or transient abnormal myelopoiesis, occurs in neonates with DS. Cases of TMD may not be recognized due to its mild symptoms, uncomplicated course and self-limiting nature; some cases may even resolve in utero. For these reasons, it is very hard to estimate the true incidence of this disorder in the DS population. It is usually recognized during the first month of life; prenatal diagnosis has also been reported[3, 15]. The most fascinating characteristic of this disorder is that spontaneous regression occurs within several months with supportive care alone, which may include red cell and platelet transfusions. TMD is frequently suspected when a high white blood cell (WBC) count with blasts are observed on the peripheral blood smear. The bone marrow findings of TMD show increased blasts without evidence of myelofibrosis, while the ratio of the blasts in the bone marrow is lower compared to that in the peripheral blood. Skin involvement which occurs frequently in neonatal AML, does not occur in TMD.

Although TMD is a self-limiting disorder, infants can have life-threatening complications, including hyperviscosity secondary to hyperleukocytosis, disseminated intravascular coagulation and pericardial tamponade, which all require rapid intervention such as exchange transfusions[4, 16-17]. Hepatofibrosis, a rare complication, has been the leading cause of death in TMD[18-19]. Hydrops observed in some DS fetuses is hypothesized to be due to hepatofibrosis associated with TMD[3]. The majority of DS neonates with TMD do not have clonal chromosomal abnormalities which is the opposite of DS patients with AML[8-20]. The most frequent cytogenetic abnormality observed in infant leukemias, 11q23 rearrangement, has not been reported in TMD. Cases of TMD have also been reported in phenotypically normal infants with trisomy 21 mosaicism and in neonates without any constitutional cytogenetic abnormalities[17, 21-23]. Interestingly, in the latter

cases, trisomy 21 has been identified in the blast cells alone while following resolution of TMD, trisomy 21 is no longer identified in either peripheral or bone marrow cells. These findings strongly suggest a relationship between TMD and trisomy 21. Although spontaneous resolution of the hematological abnormalities occurs in DS patients with TMD, about one-fourth of these patients later develop AML. There is no clear-cut evidence of clonal evolution of TMD to AML, though it may be likely in view of the increased risk of developing particularly AML-M7.

The blast cells of TMD morphologically resemble AML-M7 cells with cytoplasmic projections and bluish staining of the cytoplasm with the Giemsa stain. These cells have been shown to express some platelet antigens and contain platelet associated enzymes. Erythroid antigens are expressed in some cases and, recently, the same expression pattern of the transcription factors GATA-1 and GATA-2 in normal erythroid precursors has been reported in blasts of TMD and AML-M7 patients with DS[24]. These findings suggest that TMD and AML-M7 arise from a common megakaryocytic-erythroid progenitor or from a myeloid progenitor with the capacity to differentiate into erythroid and megakaryocytic lineages. Studies with fluorescence in situ hybridization in MDS patients with DS further support the erythroid origin of malignant cells by showing the trisomy 8 in blasts and normoblasts but not in myeloid and lymphoid cells[25]. Leukemic blasts from DS patients with AML-M7 produce both megakaryocyte and basophil/mast cell precursors in culture[26]. The observation of scattered hemopoietic cells in fibrous tissues, including pleomorphic megakaryocytes in the livers of patients with liver fibrosis, provided evidence of a mechanisms to explain hepatic fibrosis seen in some DS patients with TMD. Japanese investigators proposed that liver fibrosis in TMD results from the secretion of the cytokines platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and platelet factor 4, which stimulate collagen synthesis, by abnormal hemopoietic cells in the liver and may be analogous to myelofibrosis in AML-M7[27]. Recently, slight fibrotic changes in the bone marrow of two DS patients with TMD and hepatic fibrosis have been reported[28].

Is TMD a true neoplastic process? Despite the monoclonal nature of TMD in DS which has been shown by analysis of the methylation patterns of the X chromosomes using restriction fragment length polymorphisms in female patients, Miyauchi et al.[27] proposed that TMD was not a true malignancy based on the following observations: i) TMD undergoes spontaneous regression in most cases without chemotherapy, ii) trisomy 21 is the only chromosomal abnormality in most TMD cases, while DS patients with either MDS or AML often have additional chromosomal abnormalities, iii) extramedullary hematopoiesis consists of both blast cells and mature trilineage hematopoietic cells as opposed to a homogenous blast infiltration in AML cases, and iv) mature hematopoietic colonies with normal features which are usually absent in acute leukemia grow

in conjunction with the abnormal blast colonies in vitro[29-30]. As a result, TMD can be defined as a self-limited, monoclonal proliferation of hematopoietic progenitors with trisomy 21 which undergoes spontaneous maturation in tissues.

Hematological Abnormalities in Down Syndrome

Newborn infants with DS frequently have hematological abnormalities besides TMD including polycythemia, macrocytosis, and thrombocytosis. Polcythemia is common soon after birth, while macrocytosis and thrombocytosis may develop later in infancy[31]. Thrombocytosis usually subsides by the end of the first year of life, while DS individuals continue to have higher red cell mean corpuscular volumes (MCV) throughout their lives[31-32]. Increased red blood cell (RBC) turnover secondary to accelerated cell membrane aging or other unexplained intrinsic defects giving rise to a younger red cell population has been proposed as a mechanism to explain high MCV in DS patients. A recent study suggests that DS patients with high MCV have a normal RBC life span as RBC hexokinase activity and creatine levels were normal[33]. It has also been speculated that alterations in the folate remethylation pathway secondary to increased cystathionine β-synthase (CBS) activity (localized to 21q22.3) may play a part. These hematological changes suggest that trisomy 21 has an effect on hematopoiesis, particularly the erythroid and megakaryocytic lineages, especially during the perinatal period.

Leukemogenesis and Trisomy 21

How does trisomy 21 contribute to the development of neoplasia? It is known that patients with constitutional trisomies of chromosomes 8, 9, 13, 18, and 21 are at an increased risk of developing various hematological malignancies and solid tumors compared to the general population[34]. This suggests a relationship between the underlying mechanism giving rise to trisomy and the development of the neoplastic process. This may not be true based on the following arguments. A high percentage of patients with constitutional trisomies do not develop cancer; this may be because additional mutational events (hits) must occur for the neoplasia to develop. The types of tumors observed in constitutional trisomies more frequently affect certain organs (leukemia and cutaneous syringoma in trisomy 21, kidney and liver tumors in trisomy 18, kidney tumors and neuroblastoma in trisomy 13). This suggests that an increased gene dosage of genes located on a particular trisomic chromosome may play a central role in the development of neoplasia rather than the underlying mechanisms leading to trisomy itself. This is further supported by the fact that acquired trisomies of the same chromosomes are occasionally detected in patients with the same types of tumors without constitutional abnormalities. But it must be kept in mind that there may be significant differences in the contribution to the development of neoplasia by the trisomic chromosome in a constitutionally trisomic cell and in a cell with an acquired trisomy.

Oncogenes and Chromosome 21

The oncogenes AML1, ERG and ETS-2 have been localized to chromosome 21 and play an important role in the neoplastic processes. The AML1 gene is associated with AML-M2 with the t(8;21) (q22;q22) cytogenetic abnormality and with the cryptic TEL-AML1 chimeric transcript, which has been identified in 25 percent of cases of pediatric B-precursor ALL[35-37]. These observations suggest that the AML1 gene may be involved in both TMD and leukemia in DS patients. AML1 is a transcription factor which binds to the enhancer core motif in the transcriptional regulatory region of the granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and the colony stimulating factor 1 (CSF-1) receptor. The 8;21 translocation of AML-M2 results in the fusion of the AML1 gene to the transcription factor ETO gene, located on chromosome 8, and production of the AML-ETO chimeric protein. This protein interacts with the enhancer core DNA sequence and can alter the transcriptional regulation of AML1 target genes via interference with AML1-dependent transactivation, leading to leukemogenesis.

AML1-deficient mice (generated through gene knock-out targeting in embryonal stem cells), have complete absence of fetal liver derived hematopoiesis[38]. This finding strongly suggests that the AML1 gene plays an essential role in definitive hematopoiesis along with the other transcription factors[39]. Linkage analysis studies have identified that the gene of a familial platelet disorder (FPD), characterized by both quantitative and qualitative platelet abnormalities with a propensity to develop myeloid malignancies, is localized to 21q22[40]. This gene lies in close proximity to the AML1 gene on chromosome 21 and the hematological abnormalities observed in FPD resemble the clinical picture of DS patients with TMD in several aspects.

Genomic Imprinting, Chromosome 21 and Cancer

Since a large proportion of patients with DS or other constitutional trisomies do not develop cancer, it can be postulated that additional mutational steps are required for the multistep process of carcinogenesis. This hypothesis raises the following question. what are those additional hits? Studies have focused on the characteristics of the extra copy of chromosome 21 in cases of DS with leukemia. The increased incidence of TMD or leukemia in DS is not associated with increasing maternal age. "Atypical" constitutional karyotypes, including isochromosome 21, double-ring 21, and mosaic trisomy, each of which generates identical copies of the entire long arm of chromosome, have been reported with increased frequency in DS patients with TMD and AML-M7[41]. This condition, known as "disomic homozygosity", in which two identical copies of a single allele are inherited, may have some important implications in the development of the neoplastic process.

Some of the characteristics of DS may be due to overexpression of a critical susceptibility allele of a gene by disomic homozygosity (the presence of two identical copies of a gene from the parent of origin of the trisomic chromosome) as proposed by Feingold et al[42]. Trisomy 21 arising from mitotic nondisjunction would result in disomic homozygosity of the entire chromosome 21, while nondisjunction occurring during either meiosis I or II would result in disomic homozygosity of a region on the chromosome depending upon the extent of crossovers between the nondisjoined chromosomes (Fig. 1). In accordance with

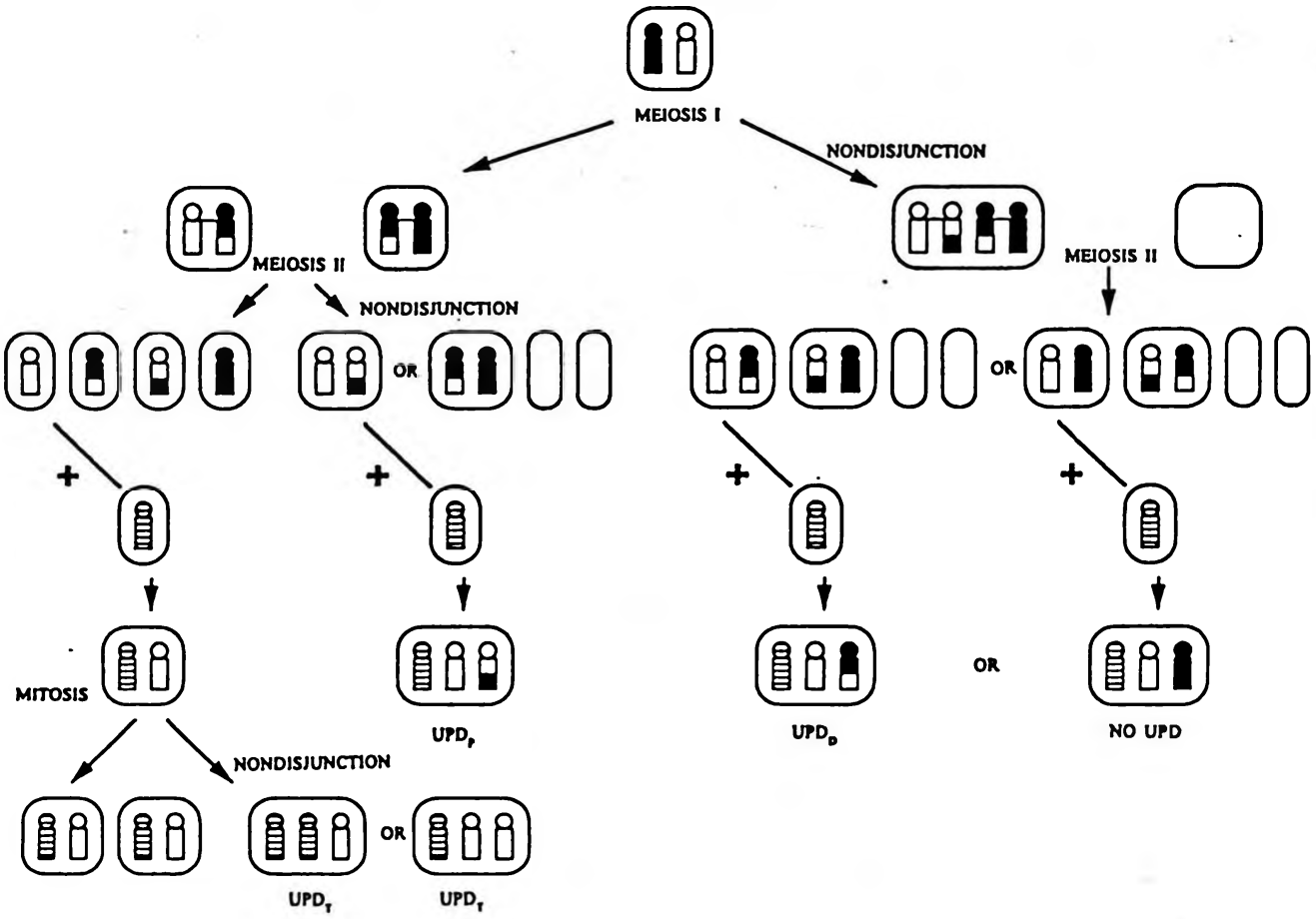


Fig. 1: Generation of uniparental disomy (UPD) in different types of nondisjunction. Abbreviations: UPD_T: uniparental disomy-total; UPD_P: uniparental disomy-proximal; UPD_D: uniparental disomy-distal.

the above hypothesis, earlier studies showed that the copies of chromosome 21 from the parent of origin were identical at the centromere (interpreted as being consistent with the meiosis II or mitotic nondisjunction) in TMD patients with DS[43-44]. A more recent study provided additional evidence of an increased frequency of disomic homozygosity in DS individuals with TMD and AML-M7, especially in the proximal 21q region[41]. These findings suggest a possible contributory role of disomic homozygosity in some TD or AML patients with DS.

Genomic imprinting is the differential expression of a gene on a chromosome which is dependent on whether the gene is inherited from the father or mother.

The parental origin of the extra chromosome in trisomy 21 is similar in patients with or without leukemia[45-46]. Maternal and paternal uniparental disomies for chromosome 21 result in normal phenotype, providing evidence that genomic imprinting on chromosome 21 does not occur[47-48]. These two lines of evidence argue against genomic imprinting as a possible mechanism contributing to the development of neoplasia in DS.

Alternatively, the significance of the disomic homozygosity can be based on the genomic imprinting mechanism giving rise to inactivation of several critical genes with identical base-pair sequences located on chromosome 21. This would lead to decreased expression of these genes while leaving a possibly defective allele on the third copy of chromosome 21 dominant (Fig. 2). Evidence against this assumption is that meiosis II and mitotic nondisjunctions ending in identical or near-identical copies of chromosome 21 are known to occur in both parents of infants with TMD and also AML patients with DS[43-44].

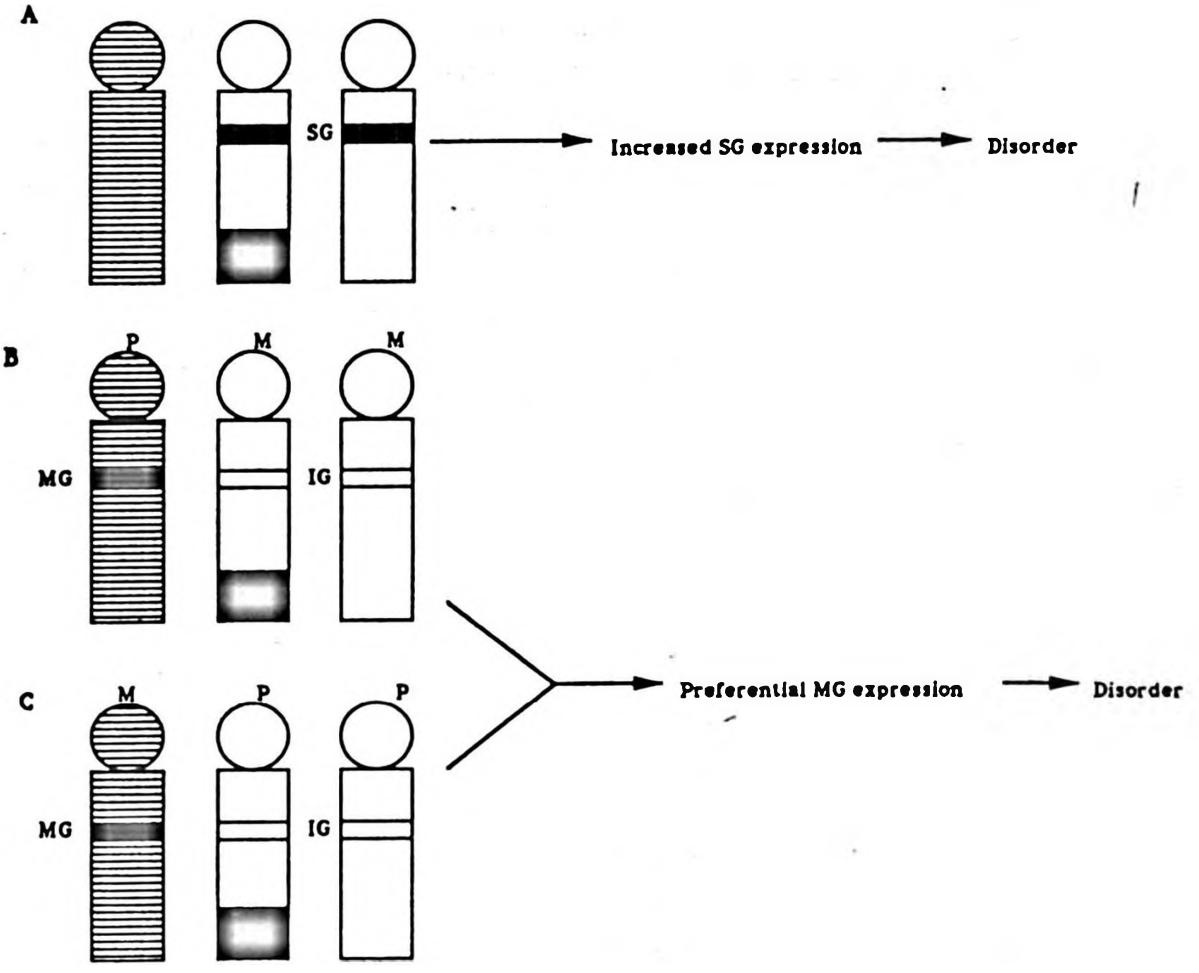


Fig. 2: Different pathophysiologic pathways for the contribution of uniparental disomy to the development of myeloproliferation. Abbreviations: SG: susceptibility gene; MG: mutated gene; IG: imprinted gene; P: paternal; M: maternal.

TMD, AML and Neonatal Tumors

Miyauchi et al.[27] proposed that abnormal blasts seen in TMD may originate from the fetal liver due to the frequent finding of a higher percentage of blast cells in the peripheral blood compared to bone marrow. AML1 appears to be a crucial transcription factor in fetal liver hematopoiesis[38]. Carolyn et al.[40] hypothesized that patients with a certain subtype of familial platelet disorder may have a defective AML1 gene which results in qualitative and quantitative platelet abnormalities and may lead to the development of AML after additional genetic defects are acquired. This suggests that an increased gene dosage of genes such as AML1 plays an important role in the development of TMD. In contrast to FPD, increased expression of the AML1 gene in TMD may lead to overstimulated proliferation of critical progenitor cells in the presence of additional submicroscopic abnormalities involving genes regulating erythro-megakaryocytopoiesis, without affecting their capacity to differentiate into mature blood cells. The expression of these genes with hematopoiesis-controlling properties may be regulated in an age-dependent manner. This can explain the "transient" nature of TMD, the responsible gene(s) being expressed late in fetal life or through the early months of the postnatal period.

Spontaneous regression of neonatal tumors is not confined to trisomy 21-carrying neoplasias and has been reported in neonatal fibrosarcoma, congenital myofibromatosis, and stage IV-S neuroblastoma patients without constitutional genetic abnormalities[49-51]. A striking, common pattern has been observed in patients with certain types of neonatal tumors, particularly in patients with constitutional trisomies. As pointed out by Satge and Van den Berghe[34], spontaneous resolving tumors are characteristic of the perinatal period in which the tumors are either in situ or incompletely developed; spontaneous resolution of well-developed tumors does not occur in children and adults as seen in the case of TMD and AML-M7 in DS in which overproduction of immature fetal tissues is due to a dysregulation of normal embryological development. A similar scenario was proposed to explain intratubular germ cell neoplasia seen in trisomy 21 fetuses and the excess of testicular tumors seen in DS children or adults[52-53]. The influence of the intrauterine milieu and qualitative and quantitative changes of the developing immune system following birth must be taken into consideration in the development and the regression of these tumors. This theory may be very relevant in the case of DS. Down syndrome patients have immunological abnormalities and the interferon α, β, and γ receptor genes are located on chromosome 21. Several hypotheses have been proposed to illuminate the relation between immune dysfunction and trisomy 21[54-55]. The observation of lymphoid depletion in two fatal cases of DS with TMD and visceral fibrosis further supports the role of an antileukemic mechanism of the immune system in TMD[18].

In other words, in some DS patients with TMD, a severely disturbed immune system may not be able to control the proliferation of clonal cells causing fibrosis of visceral organs. A hypothetical schema of development of clonal proliferative disorders in DS is sumarized in Figure 3.

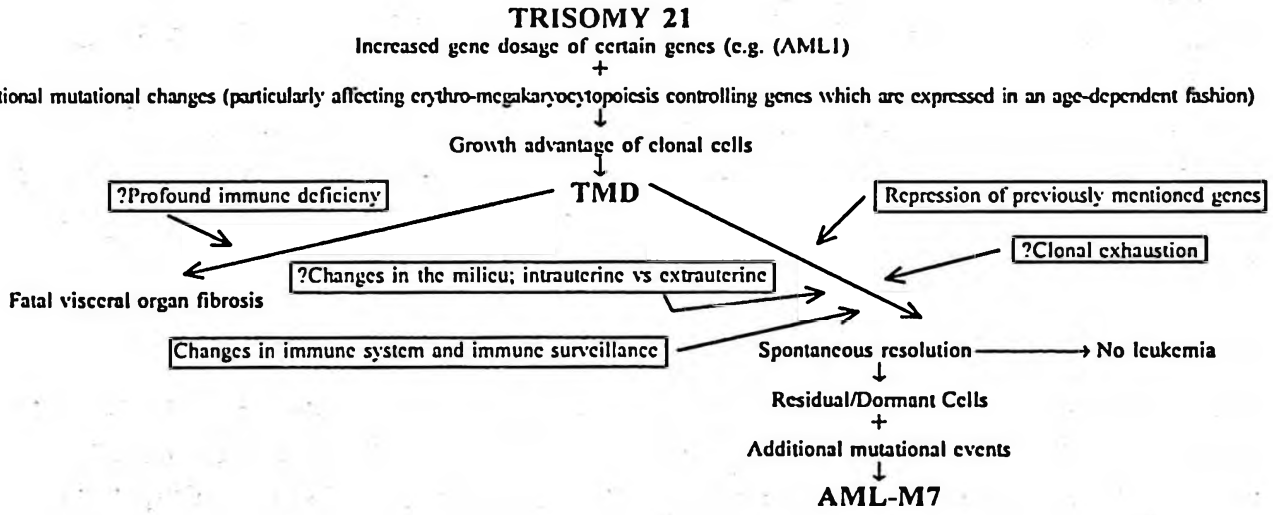


Fig. 3: Hypothetical steps in the development of TMD and AML-M7 in Down syndrome.

Recent Developments

The development of AML-M7 in 20 to 25 percent of TMD patients with DS may support the hypothesis that some residual cells may progress into a true malignant neoplasm by gaining new genetic abnormalities. Development of AML-M7 following TMD in a patient without Down syndrome can be explained by the same mechanism[56]. It is impossible to refute the central role played by different cytogenetic abnormalities in the development and progression of particular cancer, though the relative significance and contribution of each mutation can be quentioned, especially in the temporal relationship to the cancer. The persistence of multipotent progenitors expressing AML1/ETO transcripts in long-term remission patients with t(8;21) AML; spontaneous regression of congenital acute monoblastic leukemia with t(8;16) (p11;p13), which is associated with a poor prognosis; detection of t(14;18) (characteristic cytogenetic abnormality in follicular lymphomas); and expression of BCR-ABL (hallmark of chronic myeloid leukemia) in some healthy adults support the above concept[57-60].

Recent progress in the field of molecular genetics and molecular biology has prompted investigators to take advantage of these leukemogenic mutations as targets for therapy. All-trans retinoic acid has been used successfully for induction therapy of patients with acute promyelocytic leukemia characterized by the 15;17 translocation, which involves the retinoic acid receptor-α gene on chromosome 15[61]. Favorable results have been achieved with anti-sense oligonucleotide therapy directed to specific gene sequences involved in that particular cancer[62]. This

approach is based on the genetic abnormalities associated with the oncogenic mutation. It has been shown that patients with AML-M4 with inversion of chromosome 16 have a better prognosis if the multi drug resistance associated protein gene (MRP), localized to chromosome 16p13, was deleted during the process of chromosomal inversion[63]. The favorable response to intensive regimens and the moderate-to-severe methotrexate toxicity in ALL patients with DS have prompted investigators to study drug metabolism in AML patients with DS. Recently Taub et al.[64] showed that enhanced metabolism of AraC in DS cells is a contributing factor in the superior outcome of AML patients with DS. The gene dosage effect of genes localized to chromosome 21, such as cystathionine β-synthase, was hypothesized to be a mechanism which leads to altered drug metabolism.

Conclusion

The field of oncogenesis has expanded tremendously in the last decade, yet there are still several unanswered questions. Recent molecular oncologic inventions not only shed light on the pathogenesis of the neoplastic disorders but also contribute to the treatment of these disorders. The development of TMD and AML in patients with DS (characterized by trisomy 21) is an interesting model for studying the relationship between molecular changes and leukemogenesis. Despite the significant progress in the field of oncology, the treatment of non-DS AML patients remains a challenge[65]. Solutions may arise from observations which initially appear straightforward.

REFERENCES

1. Robinson L. Down syndrome and leukemia 1992; 6 (Suppl 1): 5-7.
2. Fong CT, Brodeur GM. Down's syndrome and leukemia: epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis. Cancer Genet Cytogenet 1987; 28: 55-76.
3. Zipursky A, Poon A, Doyle J. Leukemia in Down syndrome: a review. Pediatr Hematol Oncol 1992; 9: 139-149.
4. Avet-Loiseau H, Mechinaud F, Harousseau JL. Clonal hematologic disorders in Down syndrome. J Pediatr Hematol Oncol 1995; 17: 19-24.
5. Kojima S,, Matsuyama T, Sato T, et al. Down's syndrome and acute leukemia in children: an analysis of phenotype by use of monoclonal antibodies and electron microscopic platelet peroxidase reaction. Blood 1990; 76: 2348-2353.
6. Zipursky A, Thorner P, DeHarven E, et al. Myelodysplasia and acute megakaryoblastic leukemia in Down's syndrome. Leuk Res 1994; 18: 163-171.
7. Slavc I, Urban C, Haas OA, et al. Acute megakaryocytic leukemia in children. Clinical immunologic, and cytogenetic findings in two patients. Cancer 1991; 68: 2266-2272.
8. Litz CE, Davies S, Brunning RD, et al. Acute leukemia and the transient myeloproliferative disorder associated with Down syndrome: morphologic, immunophenotypic and cytogenetic manifestations. Leukemia 1995; 9: 1432-1439.
9. Ravindranath Y, Abella E, Krischer JP, et al. Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. Blood 1992; 80: 2210-2214.

10. Lu G, Altman AJ, Benn PA. Review of cytogenetic changes in acute megakaryoblastic leukemia: one disease or several? Cancer Genet Cytogenet 1993; 67: 81-89.

11. Carroll A, Civinn C, Schneider N, et al. The t(1;22) (p13;q13) is nonrandom and restricted to infants with acute megakaryoblastic leukemia: a Pediatric Oncology Group Study. Blood 1991; 78: 748-752.

12. Lion T, Haas OA. Acute megakaryocytic leukemia with the t(1;22) (p13;q13). Leuk Lymphoma 1993; 11: 15-20.

13. Abdel-mageed AH, Ragab AJ, Shuster J, et al. Children with Down syndrome and acute lymphoblastic leukemia (DS-ALL). Presenting features and outcome of therapy: a Pediatric Oncology Group Study. Cancer 1991; 67: 1057-1063.

14. Tcherina G, Lejeune F, Boccara J-F, et al. Erythroblastic and/or megakaryoblastic leukemia in Down syndrome: treatment with low-dose arabinosyl cytosine. Pediatr Hematol Oncol 1996; 18: 59-62.

15. Zerres K, Schwanitz G, Niesen M, et al. Prenatal diagnosis of acute non-lymphoblastic leukaemia in Down syndrome. Lancet 1990; 335: 117.

16. Nakagawa T, Nishida H, Arai T, et al. Hyperviscosity syndrome with transient abnormal myelopoiesis in Down syndrome. J Pediatr 1988; 112: 58-61.

17. Jones GR, Weaver M, Laug WE. Transient blastemia in phenotypically normal newborns. Am J Pediatr Hematol Oncol 1987; 9: 153-157.

18. Becroft DM, Zwi LJ. Perinatal visceral fibrosis accompanying the megakaryoblastic leukemoid reaction of Down syndrome. Pediatr Pathol 1990; 10: 397-406.

19. Becroft DM. Fetal megakaryocytic dyshemopoiesis in Down syndrome: association with hepatic and pancreatic fibrosis. Pediatr Pathol 1993; 13: 811-820.

20. Hayashi Y, Eguchi M, Sugita K, et al. Cytogenetic findings and clinical features in acute leukemia and transient myeloproliferative disorder in Down's syndrome. Blood 1988; 72: 15-23.

21. Ridgway D, Benda GI, Magenis E, et al. Transient myeloproliferative disorder of the Down type in the normal newborn. Am J Dis Child 1990; 144: 1117-1119.

22. Hanna MD, Melvin SL, Dow LW, et al. Transient myeloproliferative syndrome in a phenotypically normal infant. Am J Pediatr Hematol Oncol 1985; 7: 79-81.

23. Faed MJ, Robertson J, Todd AS, et al. Trisomy 21 in transient myeloproliferative disorder. Cancer Genet Cytogenet 1990; 48: 259-264.

24. Ito E, Kasai M, Hayashi Y, et al. Expression of erythroid-specific genes in acute megakaryoblastic leukemia and transient myeloproliferative disorder in Down's syndrome. Br J Haematol 1995; 90: 607-614.

25. Zipursky A, Wang H, Brown EJ, Squire J. Interphase cytogenetic analysis of in vivo differentiation in the myelodysplasia of Down syndrome. Blood 1994; 84: 2278-2282.

26. Suda T, Suda J, Miura Y, et al. Clonal analysis of basophil differentiation in bone marrow cultures from a Down's syndrome patient with megakaryoblastic leukemia. Blood 1985; 66: 1278-1283.

27. Miyauchi J, Ito Y, Kawano T, et al. Unusual diffuse liver fibrosis accompanying transient myeloproliferative disorder in Down's syndrome: a report of four autopsy cases and proposal of a hypothesis. Blood 1992; 80: 1521-1527.

28. Yagihashi N, Watanabe K, Yagihashi S. Transient abnormal myelopoiesis accompanied by hepatic fibrosis in two infants with Down syndrome. J Clin Pathol 1995; 48: 973-975.

29. Kurahashi H, Hara J, Yumura-Yagi K, et al. Monoclonal nature of transient abnormal myelopoiesis in Down's syndrome. Blood 1991; 77: 1161-1163.

30. Alarcon PA, Patil S, Golberg J, et al. Infants with Down's syndrome. Use of cytogenetic studies and in vitro colony assay for granulocyte progenitor to distinguish acute nonlymphocytic leukemia from a transient myeloproliferative disorder. Cancer 1987; 60: 987-993.

31. Kivivuori SM, Rajantie J, Siimes MA. Peripheral blood cell counts in infants with Down's syndrome. Clin Genet 1996; 49: 15-19.

32. Roizen NJ, Amarose AP. Hematologic abnormalities in children with Down syndrome. Am J Med Genet 1993; 46: 510-512.

33. David O, Fiorucci GC, Tosi MT, et al. Hematologic studies in children with Down syndrome. Pediatr Hematol Oncol 1996; 13: 271-275.

34. Satge D, Van den Berghe H. Aspects of the neoplasms observed in patients with constitutional autosomal trisomy. Cancer Genet Cytogenet 1996; 87: 63-70.

35. Nucifora G, Rowley JD. AML1 and the 8;21 and 3;21 translocations in acute and chronic myeloid leukemia. Blood 1995; 86: 1-14.

36. Romana SP, Poirel H, Le Coniat M, et al. High frequency of t(12;21) in childhood B lineage acute lymphoblastic leukemia. Blood 1995; 86: 4263-4269.

37. Shurtleff SA, Buijs A, Behm FG, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. Leukemia 1995; 9: 1985-1989.

38. Okuda T, van Deursen J, Hiebert SW, et al. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell 1996; 84: 321-330.

39. Ramesh AS, Orkin SH. The transcriptional control of hematopoiesis. Blood 1996; 87: 4025-4039.

40. Carolyn YH, Otterud B, Legare RD, et al. Linkage of a familial platelet disorder with a propensity to develop myeloid malignancies to human chromosome 21q22.1-22.2. Blood 1996; 87: 5218-5224.

41. Shen JJ, Williams BJ, Zipursky A, et al. Cytogenetic and molecular studies of Down syndrome individuals with leukemia. Am J Hum Genet 1995; 56: 915-925.

42. Feingold E, Lamb NE, Sherman SL. Methods for genetic linkage analysis using trisomies. Am J Hum Genet 1995; 56: 475-483.

43. Abe K, Kajii T, Niikawa N. Disomic homozygosity in 21-trisomic cells: a mechanism responsible for transient myeloproliferative syndrome. Hum Genet 1989; 82: 313-316.

44. Iselius L, Jacobs P, Morton N. Leukaemia and transient leukaemia in Down syndrome. Hum Genet 1990; 85: 477-485.

45. Lorber BJ, Freeman SB, Hassold T, et al. Characterization and molecular analysis of nondisjunction in 18 cases of trisomy 21 and leukemia. Genes Chromosom Cancer 1992; 4: 222-227.

46. Antonarakis SE. Parental origin of the extra chromosome in trisomy 21 as indicated by analysis of DNA polymorphisms. Down Syndrome Collaborative Group. N Engl J Med 1991; 324: 872-876.

47. Créau-Goldberg N, Gegonne A, Delabar J, et al. Maternal origin of a de novo balanced t(21q21q) identified by ets-2 polymorphism. Hum Genet 1987; 76: 396-398.

48. Blouin JL, Avramopoulos D, Pangalos C, Antonarahis SE. Normal phenotype with paternal uniparental isodisomy for chromosome 21. Am J Hum Genet 1993; 53: 1074-1078.

49. Madden NP, Spicer RD, Allibone EB, Lewis IJ, Spontaneous regression of neonatal fibrosarcoma. Br J Cancer 1992; 18 (Suppl): S72-75.

50. Jenkins EA, Cawley MI. Infantile myofibromatosis: a cause of severe bone pain in a neonate. Br J Rheumatol 1993; 32: 849-852.

51. Guglielmi M, De Bernardi B, Rizzo A, et al. Resection of primary tumor at diagnosis in stage IV-S neuroblastoma: does it affect the clinical course? J Clin Oncol 1996; 14: 1537-1544.

52. Jacobsen GK, Henriques UV. A fetal testis with intratubular germ cell neoplasia (ITGCN). Mod Pathol 1992; 5: 547-549.

53. Satge D, Jacobsen GK, Cessot F, et al. A fetus with Down syndrome and intratubular germ cell neoplasia. Pediatr Pathol 1996; 16: 107-112.

54. Cuadrado E, Barrena MJ. Immune dysfunction in Down's syndrome: primary immune deficiency or early senescence of the immune system? Clin Immunol Immunopathol 1996; 78: 209-214.

55. Zihni L. Down's syndrome, interferon sensitivity and the development of leukaemia. Leuk Res 1994; 18: 1-6.

56. Brissette MD, Duval-Arnould BJ, Gordon BG, Cotelingan JD. Acute megakaryoblastic leukemia following transient myeloproliferative disorder in a patient without Down syndrome. Am J Hematol 1994; 47: 316-319.

57. Miyamoto T, Nagafuji K, Akashi K, et al. Persistence of multipotent progenitors expressing AML1/ETO transcripts in long-term remission patients with t(8;21) acute myelogenous leukemia. Blood 1996; 87: 4789-4796.

58. Sainati L, Bolcato S, Cocito MG, et al. Transient acute monoblastic leukemia with reciprocal (8;16) (p11;p13) translocation. Pediatr Hematol Oncol 1996; 13: 151-157.

59. Liu Y, Hernandez AM, Shibata D, Cortopassi GA. BCL2 translocation frequency rises with age in humans. Proc Natl Acad Sci USA 1994; 91: 8910-8914.

60. Biernaux C, Sels A, Huez G, et al. Very low level of major BCR-ABL expression in blood of some healthy individuals. Bone Marrow Transplant 1996; 17 (Suppl 3): S45-47.

61. Warrell RPJr, de The H, Wang ZY, Degos L. Acute promyelocytic leukemia. N Engl J Med 1993; 329: 177-189.

62. Askari FK, McDonnel WM. Antisense-oligonucleotide therapy. N Engl J Med 1996; 334: 316-318.

63. Kuss BJ, Deeley RG, Cole SP, et al. Deletion of gene for multidrug resistance in acute myeloid leukaemia with inversion in chromosome 16: prognostic implications. Lancet 1994; 343: 1531-1534.

64. Taub JW, Matherly LH, Stout ML, et al. Enhanced metabolism of 1-β-D-Arabinofuranosylcytosine in Down syndrome cells: a contributing factor to the superior event free survival of Down syndrome children with acute myeloid leukemia. Blood 1996; 87: 3395-3403.

65. Ravindranath Y, Yeager AM, Chang MN, et al. Autologous bone marrow transplantation versus intensive consolidation chemotherapy for acute myeloid leukemia in childhood. New Engl J Med 1996; 334: 1428-1434.

The Turkish Journal of Pediatrics 1997; 39: 533-540

THE WORLD'S CHILDREN*

*Reiko Niimi***

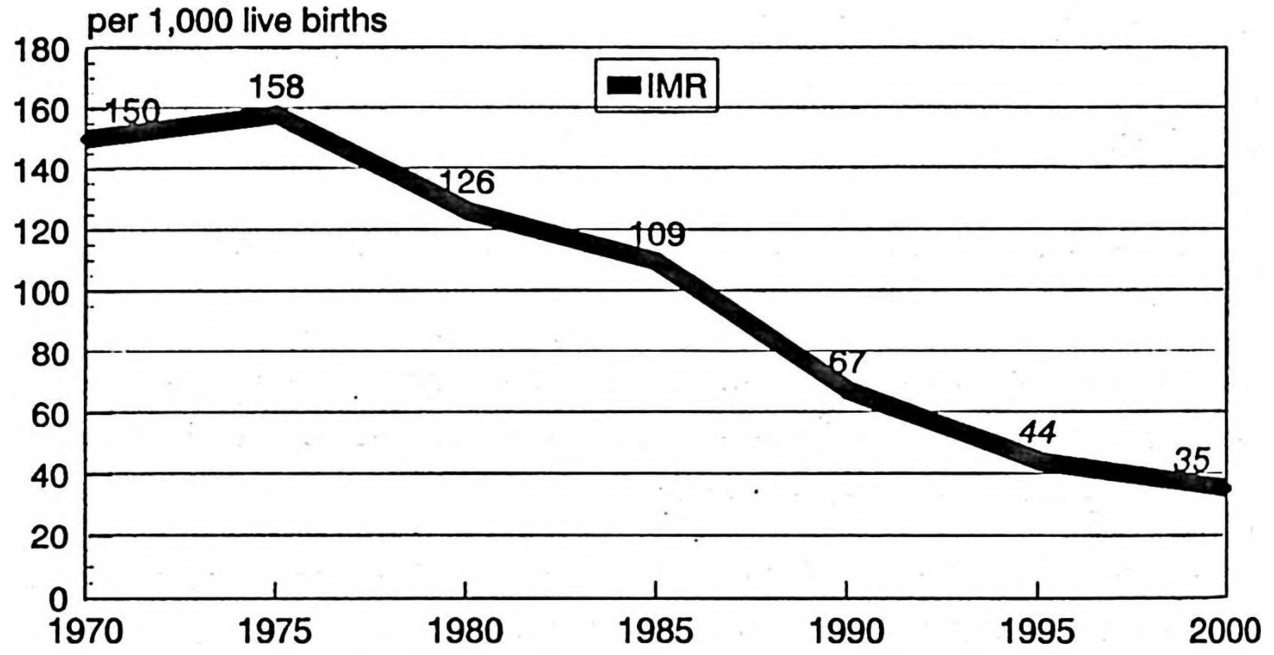
It has been over six years since the World Summit for Children in 1990, when world leaders from 159 countries agreed to specific goals which would reduce child and maternal mortality and give every child access to basic education, clean water and proper sanitation by the year 2000. Dramatic progress has been achieved in most countries and can be considered "a victory for children and all of humanity". Over 80 percent of the world's children are now immunized against diphtheria, tetanus and whooping cough. The lives of more than one million children are being saved each year through the increased use of oral rehydration therapy to treat diarrheal dehydration. Polio and guinea worm disease are on the verge of eradication. Since 1990, an additional 1.5 milion people are using iodized salt, protecting around 12 million infants each year from iodine deficiency, the world's leading cause of preventable mental retardation. There has also been significant progress in the provision of universal access to safe drinking water.

In Turkey, the infant mortality rate used to be between 300 to 350 per thousand live births in the urban areas, where reports were available in the 1940s. Conditions in isolated villages must have been far worse at that time. Scientific developments in pediatrics, strengthening of national health services, and utilization of cost-effective methods like immunization, oral rehydration therapy, promotion of breastfeeding and growth monitoring have contributed to the dramatic decrease in Turkey's infant mortality rate now 42 per thousand live births nationwide. The first round of National Immunization Days for this year, to eradicate polio in Turkey, just ended last week, by the way. And yet, 42 children out of 1000 live births still die each year in Turkey before their first birthday (Fig. 1). The rates continue to be high in eastern and southeast Turkey. Surely, some of these deaths (and sorrow to their families) can be prevented at low cost.

---

* This lecture was given on the occasion of the 40th anniversary of the foundation of Hacettepe University İhsan Doğramacı Children's Hospital and the 30th anniversary of the foundation of Hacettepe University, on May 5, 1997.

** UNICEF Representative, Ankara.



Source: SIS

**Figures for 1995 and onwards are based on estimations*

Fig. 1: Infant mortality trends in Turkey.

New Developments in Public Health

Permit me to mention a few concrete areas where we can immediately make a difference to the chance of children's survival. A new and comprehensive study on immunization -the "State of the World's Vaccines and Immunization" that was launched jointly by WHO and UNICEF in September 1996- predicts that over the next 15 years, revolutionary new vaccines could save the lives of some eight million children who die each year from infectious diseases. That is if sustained efforts are made to ensure that these vaccines are affordable in the developing world. The study also urges the international community to continue supporting scientific research and global immunization.

Recent research and discoveries in the Central Asian Republics show that anemia caused by iron deficiency among women and adolescents can be tackled in a cost-effective way. They have started to fortify their flour and to prescribe weekly iron tablets instead of daily tablets.

Beyond the creation of baby-friendly hospitals that encourage mothers to breastfeed instead of bootlefeed, we must look at existing legal codes. The International Code of Marketing of Breastmilk Substitutes is a practical aid to governments in fulfilling each country's obligation to protect young infants.

The Convention on the Rights of the Child

What happens to those children who have been immunized and survive childhood diseases? By ratifying the Convention on the Rights of the Child (CRC), in December 1994, the Turkish Government has pledged to protect the best interests and rights of the child until age 18. By signing the Convention, Turkey recognizes every child's right to develop physically, mentally and socially to his or her fullest potential, to express his or her opinions freely, and to participate in decisions affecting his or her future. This new ethic goes for girls as well as boys; it applies to children living in rural and hard-to-reach areas; it is as valid for children whose families and communities are poor, as for those who are better-off; and it should benefit children with disabilities and those of racial, ethnic, or religious minorities as well as those from the mainstream of society.

The cause of child rights has been gaining ground throughout the century, but recognition of the proposition that children have human rights of their own has developed with decisive force in recent years. The adoption of the 1989 Convention on the Rights of the Child by the UN General Assembly and its subsequent near universal ratification (except for the USA, Somalia and the Cook Islands) marks a new era in the cause of children worldwide.

In its 50th year, UNICEF's new Mission Statement recognizes that the pursuit of children's rights is its fundamental purpose. UNICEF's work on behalf of children is now guided by the principles and standards established by the Convention for overall protection of childhood. We are mandated by the UN General Assembly to advocate for the protection of children's rights, to help meet their basic needs and to expand their opportunities to reach their full potential. We are now working to establish children's rights as enduring ethical principles.

The fundamental rationale for UNICEF's work has, until now, been the existence of gross poverty in large parts of the world, and the consequent deprivation in nutrition, health, education, and other essential human needs suffered by many children in developing countries. The organization's roots were quintessentially humanitarian and its structure and the deployment of the resources available to it have primarily reflected the overall aim of combating child distress by short- and long-term means. The goals for the year 2000, agreed upon at the World Summit for Children in 1990, correspond to commitments for "human development" strategies or "meeting basic needs".

The emergence of children's rights and their elaboration in the Convention implies a shift in the rationale for our work, from meeting basic needs to fulfilling basic human rights. The idea that every human being has basic rights -which it is the duty of an earthly power, usually the state or the government, to respect and to fulfill- goes back to the French Revolution and beyond. The international

expression of these rights begins with the Universal Declaration of Human Rights and has been followed by the other declarations and conventions. Most of the rights covered in these instruments have been expressed as civil liberties, political freedoms and protection from various forms of abuse and exploitation; others as aspirational social and economic rights.

UNICEF believes that the time has now come to put the needs and the rights of children at the very center of every country's development strategy. This argument is based neither on institutional vested interest nor on sentimentality about the young. It is based on the fact that childhood is the period when minds and bodies, values and personalities are being formed and during which even temporary deprivation is capable of inflicting lifelong damage and distortion on human development. It follows that, whether the threat be war and violence or economic marginalization, children should, as far as humanly possible, be protected from the worst mistakes and malignancies of the adult world.

For this reason, the vital, vulnerable years of childhood should be given a first call on societies' concerns and capacities. This commitment should be maintained in good and bad times. A child has only one chance to develop, and the protection of that one chance therefore demands the kind of commitment that though there will always be something more immediate, there will never be anything more important.

Meeting children's basic needs and investing in their healthy development will help accelerate finding solutions to the main problems that vex and threaten humankind on the threshold of the 21st century: the problems of poverty, overpopulation, and environmental degradation feed off of one another in a downward spiral that brings instability and strife in its wake. CHILDREN ARE AN EXTRAORDINARY LEVER FOR PROGRESS; Let us not lose this opportunity!

In this region of the world we like to think that the situation for children is constantly improving. Yet, many families are having to cope with spiraling inflation, unemployment, and continuing deterioration in their material conditions. Poverty and social dislocation have put enormous burdens on families who often have limited capacity or experience in taking responsibility for their children's welfare-traditionally the task of state authorities. Since child monitoring and checking mechanisms, including those normally expected within school and health systems, have been eroded and are in need of reform, children are suffering.

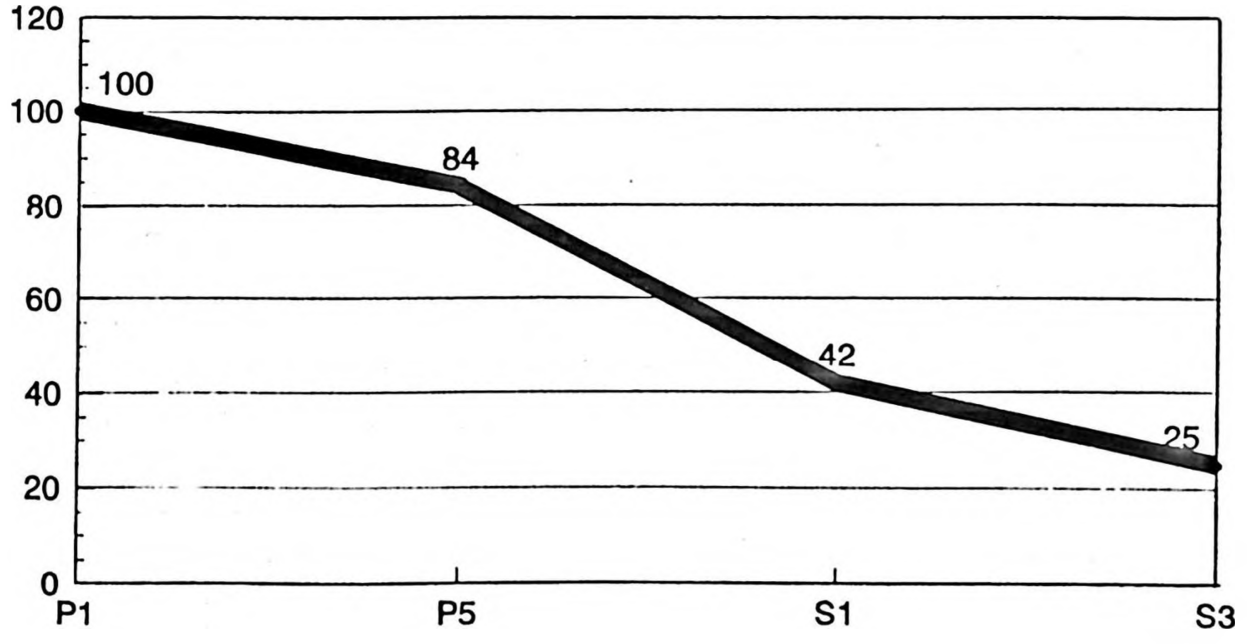
Poor nutrition, alcoholism, smoking, stress, and increasing violence are also taking their toll on families. Parents are having to take greater responsibility for the development of their children at a time when they are less able to do so, and when child health and education needs are rising. Former full enrollment in primary schools has ceased to exist, and the growing cost of schooling (even in official "free" systems) means that children from poorer households face problems

of access to education, extracurricular activities and remedial help. Deprived of social protection mechanisms, children and adolescents in particular are falling prey to drug abuse, alcohol and tobacco addiction, violence, crime, child prostitution and sexually-transmitted diseases, including HIV/AIDS.

Because Turkey is a rapidly urbanizing country, I would also like to mention the relationship between the rights of the child and risks in the urban environment. Many psycho-social problems, such as drug abuse, child abuse, delinquency, rape and assault are associated with unsatisfactory housing conditions, especially overcrowding and noise. Children living in low-income settlements or on the streets are disadvantaged in gaining access to education. And if they do, they are disadvantaged again by increased ill-health and lack of conducive environments. These negatively influence the children's future economic opportunities and their ability to contribute to the future development of society. Obviously, children are not the problem, but innocent victims. They can contribute to solutions, however.

Education and Child Development

Children's organizations are beginning to claim the right to better participation in decision-making on issues that concern their own welfare. It is their right to question the relevance of school curricula and to request a stimulating school environment. Most importantly, we must develop educational programs that first encourage all girls to stay in school, and then help ensure their academic success once there. Out of every 100 students who enroll in primary school, only 84 complete five years and 42 enroll in secondary school. By age 14-15, only 23 percent have completed their basic education in Turkey (Fig. 2). We must



Source: MICS 1995

Fig. 2: Schooling attrition rates in Turkey, 1995.

also realize the direct links between education and child labor, as well as the socio-economic dimensions of child labor. The rights of working children and disabled children must also be taken into consideration.

Child Protection and the CRC

The existence of measurable goals, deadlines and proven strategies in the areas of health, nutrition, education, water and sanitation, and family planning paves the way for accelerated action for children. But due to the lack of comparable goals, deadlines and strategies in the areas of CHILD PROTECTION and PARTICIPATION, we risk neglecting or marginalizing children's rights in these equally vital areas. We must not let it happen. Especially since the International Committee on the Rights of the Child has found serious and widespread problems of child abuse, exploitation and neglect in many of the countries whose reports it has already reviewed. Turkey is submitting its first report to this Committee in May of this year. Let Turkey's report not neglect to cover the implementation of all rights accorded to Turkish children through the Convention.

Many of the rights elaborated in the CRC have to do with areas of children's lives on which UNICEF has not traditionally focused its attention and resources. These include civil rights [e.g. right to a name and nationality (Article 7), right to freedom of thought (Article 14), and right not to be subject to arbitrary invasion of privacy (Article 15)] and rights to be protected from various forms of exploitation, abuse and neglect, such as protection from economic exploitation and hazardous work (Article 32), protection from sexual abuse and exploitation (Article 34) and protection from life imprisonment (Article 37).

Ladies and gentlemen, permit me to review some of the protection issues facing the world's children, including those of Turkey:

Children in armed conflict: Last year's Graça Machel Report on The Impact of Armed Conflict on Children describes the suffering of children caught up in wars around the world, and is the most comprehensive analysis of the subject ever undertaken. According to the report, two million children have died in armed conflicts in the past decade, and three times as many have been seriously injured or permanently disabled. Many more die as a result of the indirect effects of war, such as the destruction of health centers and water supplies. Countless others have been forced to witness or even take part in horrifying acts of violence. The report recommends the banning of the recruitment into the armed forces of children under the age of 18; the prosecution of rape and sexual torture as war crimes; a complete ban on the production, use, trade and stockpiling of land mines; close monitoring by the international community of the impact of sanctions on children; and a stricter implementation of existing international treaties which protect children and civilians.

Furthermore, in September 1996, UNICEF, Radda Barnen and the Sudan Relief and Rehabilitation Association helped more than 168 unaccompanied children reunite with their families in southern Sudan in the first reunification program into areas controlled by the Sudan People's Liberation Army. Many of the children, aged ten to eighteen, had not seen their parents for as many as three years. As part of the reunification program, each child received a "going home" kit, containing cooking equipment, clothing, school stationery, fishing equipment, soap, salt and mosquito nets.

Dramatic events in the Great Lakes Region in late 1996 led to the sudden return to Rwanda of more than 700,000 refugees from eastern Zaire and almost 500,000 refugees from Tanzania. The massive population movement continues even as I talk. In addition to ongoing support for rebuilding basic services in Rwanda, UNICEF provides assistance to returning refugees through water supplies, sanitation, health, nutrition, the reunification of unaccompanied children with their families, and the care and protection of vulnerable children. UNICEF also provides emergency assistance to vulnerable children and families still in eastern Zaire.

Child Labor: Another protection issue is that of exploitative child labor. The 1997 State of the World's Children Report identifies six steps to tackle the root causes of child labor and to ease the plight of an estimated 250 million working children worldwide: 1. the immediate elimination of hazardous and exploitative child labor; 2. free and compulsory education for every child; 3. stringent child labor laws and their vigorous enforcement in each country; 4. registration of all children at birth; 5. data collection and monitoring; and 6. codes of conduct and procurement policies. UNICEF's most fundamental demand is the immediate end to the most intolerable forms of child labor, such as bonded labor and prostitution, which are as grave an abuse of human rights as slavery.

Children in Turkey start working to supplement their families' income at a very young age. Among children aged six to nine, 18.5 percent are working, and among those aged ten to eleven, 31.6 percent, or nearly one in three (Fig. 3). What impact does this have on their development? We are concerned about those whose education and development is threatened by work.

The sale of children (bonded labor), child prostitution, child pornography, and child abuse are issues which were raised during the 1996 World Congress Against Commercial and Sexual Exploitation of Children. This Congress has begun to have a global impact. National plans are being devised for measures including legal reforms, training of law enforcement personnel and the implementation of advocacy and rehabilitation programs. The first breakthrough is for broader public recognition that this is a problem in our own neighborhoods, and often among people we know. It is not just a phenomenon in "other" countries.

We need to further strengthen the safety net for children. Eliminating the suffering of children worldwide remains an enormous task. The CRC envisages a role in

the implementation of child rights for the whole of civil society, starting with children's parents and families. By civil society, I mean the inclusion of universities such as Hacettepe, non-governmental organizations, private sector, religious institutions, labor unions, professional associations, minority groups, and the media-in effect, any organized manifestation of civil society whose activity has a bearing on the fulfillment of child rights. To paraphrase an old Chinese saying, those who have helped to save the lives of children such as yourselves now have a responsibility for their development and protection.

Hepinize başarılar diler, Teşekkür ederim.

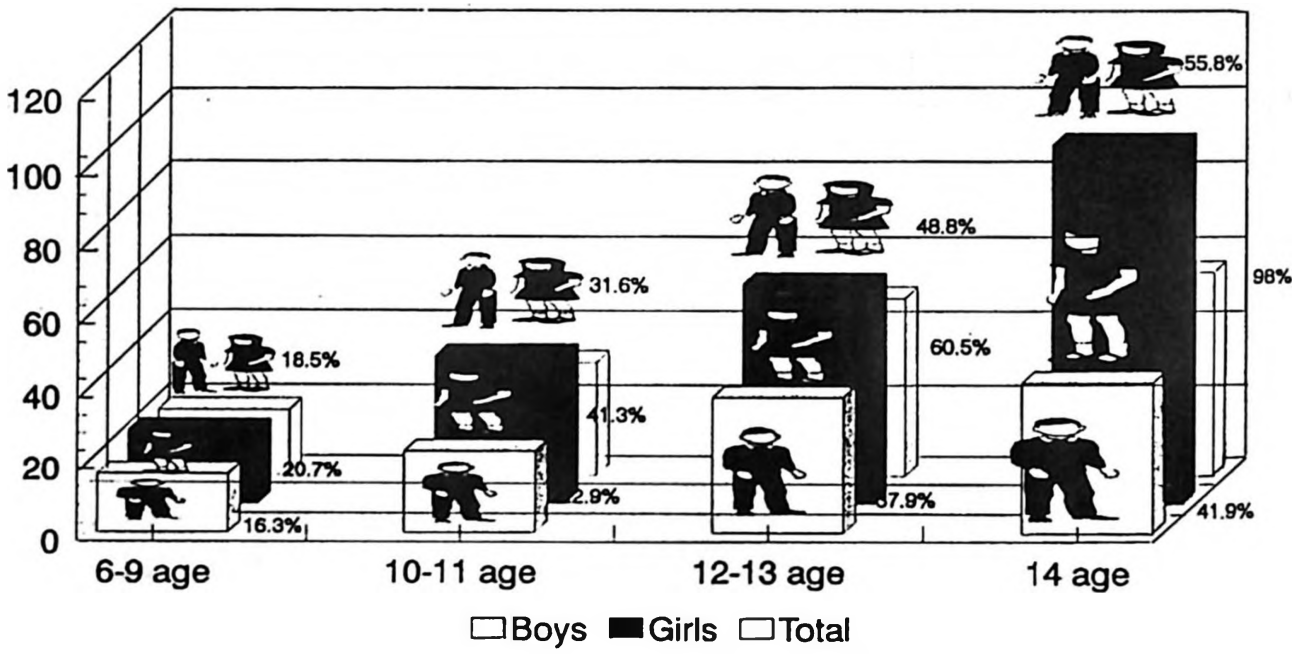


Fig. 3: Child work in Turkey, 1994 (6-14 age group).

The Turkish Journal of Pediatrics 1997; 39: 541-545

RECURRENT PNEUMONITIS IN A 10 – YEAR – OLD GIRL WITH PIGEON BREEDER'S DISEASE*

Embiya Dilber MD, Uğur Özçelik MD*, Ayhan Göçmen MD*
Zeynep Mısırlıgil MD*, Nural Kiper MD*

SUMMARY: Dilber E, Özçelik U, Göçmen A, Mısırlıgil Z, Kiper N. (Chest Disease Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Recurrent pneumonitis in a 10-year-old girl with pigeon breeder's disease. Turk J Pediatr 1997; 39: 541-545.

Hypersensitivity pneumonitis is an allergic disease resulting from sensitization to inhaled organic dust, usually manifest with recurrent bouts of cough, dyspnea and fever. Most of the reported cases concern the adult population. The disease is rarely seen in the pediatric age group, as stated by most of the reviews on the subject. In this article, a case with hypersensitivity pneumonitis is described. Precipitating antibodies to avian protein were demonstrated in serum from a girl with recurrent bouts of pneumonitis which was otherwise unexplained. *Key words: hypersensitivity pneumonitis, pigeon breeder's disease, precipitating antibodies.*

Hypersensitivity pneumonitis or extrinsic allergic alveolitis is an allergic disease resulting from the sensitization and recurrent exposure to any of a wide variety of inhaled organic dusts[1]. Pigeon breeder's disease is the most common form[2, 3]. Although it is rarely encountered in the pediatric age group, it should be considered in any child with recurrent or persistent and unexplained respiratory symptoms[2, 4].

Case Report

A 10-year-old girl with a history of recurrent pneumonitis was referred to our hospital from another center. She had no serious health problems until three months previously when she had developed pneumonitis with fever, dry cough, dyspnea, tachypnea and congestive heart failure. Chest x-ray, taken at the time of pneumonitis, disclosed diffuse nodular infiltrates with patchy infiltration along the peripheral areas (Fig. 1). She was then hospitalized in a local institute, treated with antibiotics, and was discharged upon full recovery. One month later, her symptoms recurred and she was hospitalized in the same institute for a second time. During the second attack, a computerized tomography (CT) scan of the

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

** Pediatrician, Hacettepe University Faculty of Medicine.

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

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

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

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

thorax was done which revealed diffuse miliary infiltrates in addition to several patchy consolidations (Fig. 2). The patient once again responded to the nonspecific management with antibiotics and supportive therapy with full recovery. After her recovery from the second bout, she was sent to us for the etiological investigation of severe recurrent pneumonitis which seemed to be quite unexplained.

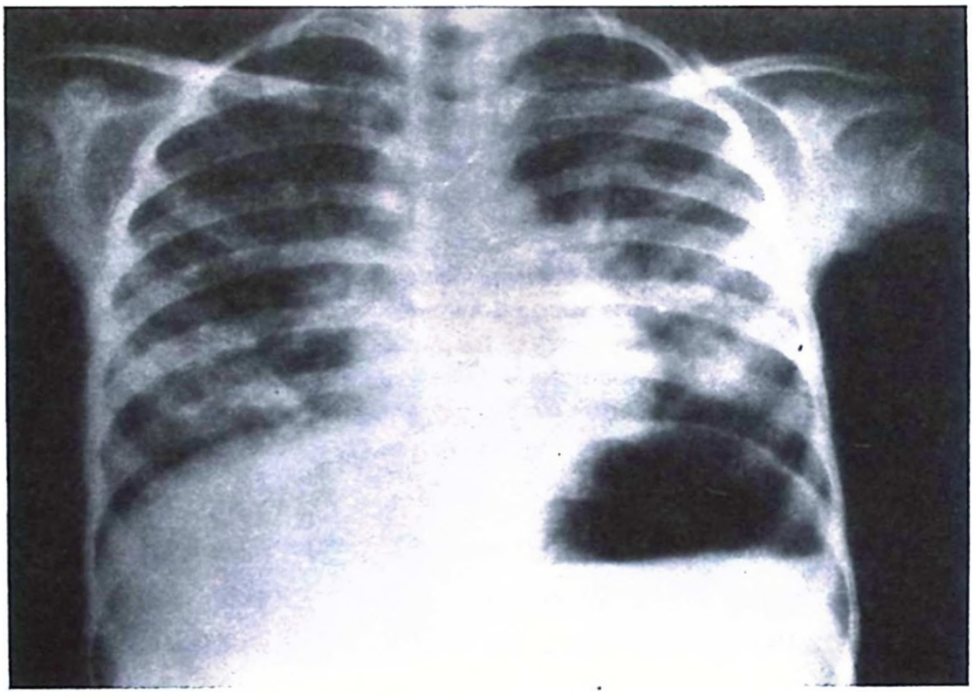


Fig. 1: Widespread nodular infiltrates and patchy infiltration prominent at the lung base.

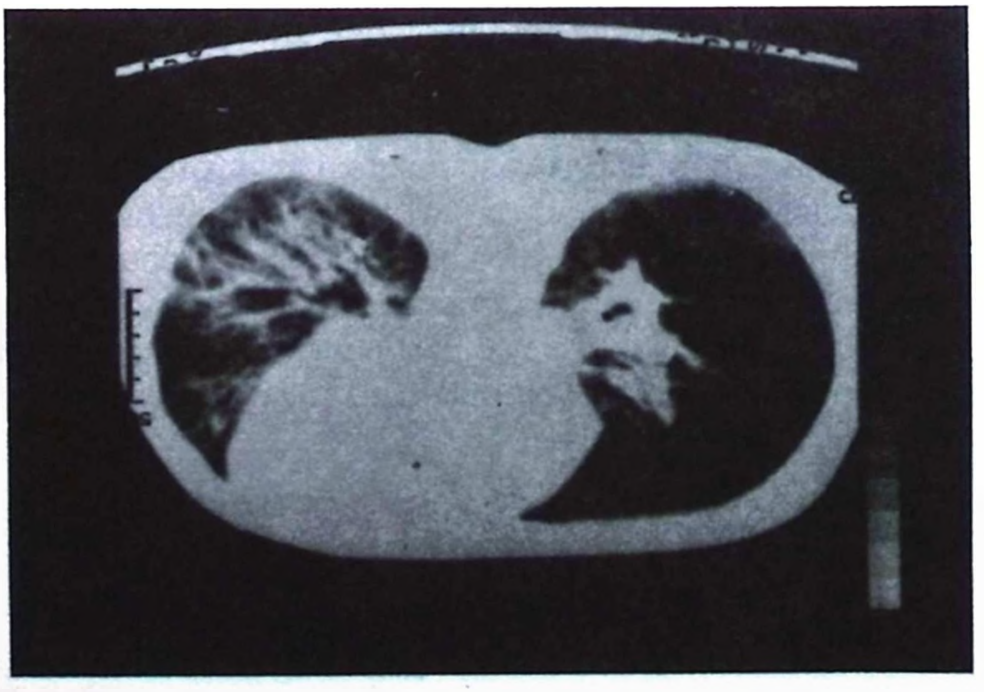


Fig. 2: Coronal section of thoracal computed tomography (CT). Diffuse miliary infiltrates are present, especially on the right side, with patchy consolidation.

Upon admission at our hospital she was in good health and was without symptoms. Her chest x-ray had reverted to normal (Fig. 3). Results of pulmonary function tests and serum imunoglobulin levels were all within normal limits. Upon inquiry, the family reported a history of pigeon breeding in the attic, dating back six months before the intiation of the patient's symptoms. Although the patient normally did not have direct contact with the birds as part of her daily activities, she did report having direct contact three to four hours prior to her symptoms on both occasions. Precipitating antibodies to avian protein were present in her serum (Fig. 4). The patient has been well since the removal of the birds from her house (12 months before the writing of this report).

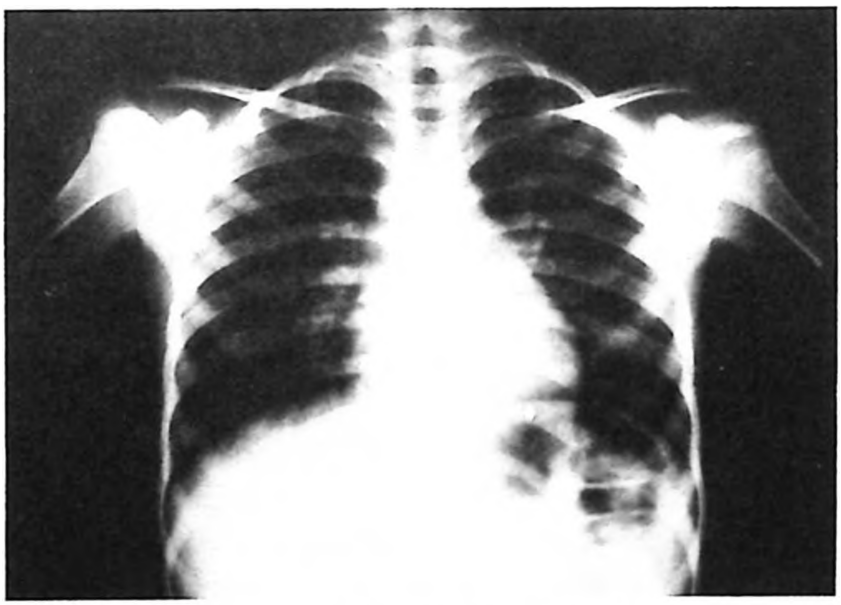


Fig. 3: Chest x-ray of the patient after referral to us.

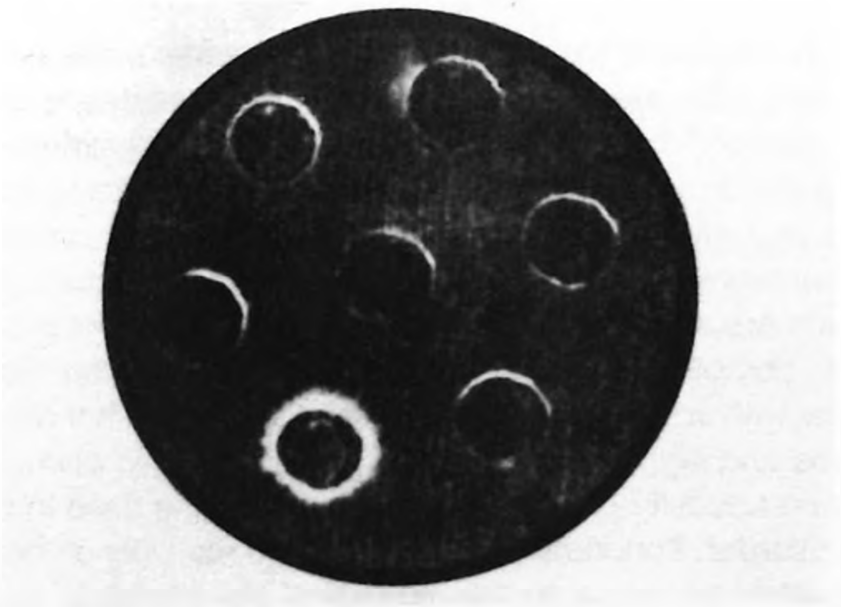


Fig. 4: Ouchterlony plate showing precipitating antibodies to avian protein in the patient's serum.

Discussion

Pigeon breeder's lung in children was first described by Reed et al.[5] Exposure to a wide range of pigeon-derived materials, including extracts from droppings, serum, egg white, and feathers, induces a hypersensitivity pneumonitis. The disease is a diffuse, predominantly mononuclear inflammation of the lung parenchyma, particularly the terminal bronchioles, interstitium, and alveoli. The inflammation often organizes into granulomas and may progress to fibrosis[1,3,4].

It appears to be two distinct syndromes[1,2,4-6]. In the acute form, chills, fever, malaise, dry cough and dyspnea usually begin four to six hours after exposure to the causative organic dust. Physical findings include rapid respiration and crepitant rales, usually without wheezing. In the chronic form, due to relatively less intense and continuous exposure to the offending dust, cough and dyspnea may be progressive without acute episodes. Dyspnea on exertion, anorexia, weight loss and fatigue may occur without fever, and positive findings may be limited to fine basilar rales and, rarely, clubbing of the fingers. With the above history and clinical findings, we determined our patient had an acute and recurrent form of the disease.

No single clinical feature or laboratory test is diagnostic of the disease. Usually the diagnosis is based on a combination of characteristic symptoms, physical findings, chest x-ray abnormalities, pulmonary function, and immunologic tests[1,2,6]. The demonstration of precipitating antibodies to the pigeon antigens raises the suspicion of hypersensitivity pneumonitis, although it may not be diagnostic per se[7]. Reynaud et al.[8] found a high sensitivity (86%), specificity (93%) and accuracy (92%) of precipitins in the diagnosis of pigeon breeder's disease. In our patient, precipitating antibodies to avian antigen were demonstrated in the serum.

As in other allergic disorders, the therapy in pigeon breeder's disease is immediate removal of the bird from the environment and careful avoidance of re-exposure to causative antigens[1,4,6]. Steroids may be used for the treatment of acute and fulminating lung disease, as they may help subside the ongoing inflammation in the bronchioles and alveoli[2,6]. It is also reported that a fatal course may ensue in some cases presenting with an acute fulminant clinical picture, if steroids are withheld. Steroids are also believed to prevent the development of fibrosis, which is a late chronic complication in some cases. Our patient, who was admitted to the local hospital with severe dyspnea and heart failure, did not receive steroids, as her symptoms and signs were attributed to a nonspecific pulmonary infection rather than hypersensitivity pneumonitis. This is often the case in such children with the same disorder. Fortunately, she had a good recovery on both occasions, which may be attributed more to the removal of the offending antigens during her hospital stay rather than to the antibiotic regimen.

Considering the facts stated above, hypersensitivity pneumonitis in children should be borne in mind in the appropriate clinical setting, and the patient's history carefully checked, in order to prevent subsequent morbidity due to a delayed diagnosis.

REFERENCES

1. Fink JN. Hypersensitivity pneumonitis. J Allergy Clin Immunol 1984; 74: 1-10.
2. Experience and Reason-briefly recorded. Diagnosing Bird Fancier's disease in children. Pediatrics 1990; 85: 848-852.
3. Banham SW, McKenzie H, McSharry C, Lynch PP, Boyd G. Antibody against a pigeon bloom-extract: a further antigen in pigeon fanciers' lung. Clin Allergy 1982; 12: 173-178.
4. Barker PM, Warner JO. "Atypical pneumonia" due to parakeet sensitivity: bird fancier's lung in a 10-year-old girl. Br J Dis Chest 1984; 78: 404-407.
5. Reed CE, Sosman A, Barbee RA. Pigeon-breeder's lung. JAMA 1965; 193: 81-85.
6. Stiehm Er, Reed CE, Tooley WH. Pigeon breeder's lung in children. Pediatrics 1967; 39: 904-915.
7. Siersted HC, Hansen NC. Precipitating antibodies against pigeon bloom antigens as a specific and sensitive measure of pigeon exposure. Allergy 1990; 45: 293-297.
8. Reynaud C, Slosman DO, Polla BS. Precipitins in bird breeder's disease: how useful are they? Eur Respir J 1990; 3: 1155-1161.

The Turkish Journal of Pediatrics 1997; 39: 547-549

A CASE OF SPONDYLOCOSTAL DYSOSTOSIS WITH A FRA (5) (q32)*

Mehmet Satar MD, A. Kübra Temoçin MD*, Aytuğ Atıcı MD*
Osman Demirhan MD*

SUMMARY: Satar M, Temoçin AK, Atıcı A, Demirhan O. (Division of Neonatology, Department of Pediatrics, Çukurova University Faculty of Medicine, Adana, Turkey). A case of spondylocostal dysostosis with a fra (5) (q32). Turk J Pediatr 1997; 39: 547-549.

Spondylocostal dysostosis is a rare hereditary syndrome with various costal and vertebral deformities. No chromosomal abnormalities in connection with this syndrome were previously reported in literature. We report a case with spondylocostal dysostosis and chromosomal abnormality [fra (5) (q32)]. *Key words: spondylocostal dysostosis, chromosomal abnormality.*

Spondylocostal dysostosis is an uncommon heritable disease with multiple chondral and vertebral deformities (including cleft vertebrae, hemivertebrae and butterfly vertebrae) and bizarre costal features, such as variations in the size and thickness of the ribs and in the widths of the intercostal spaces, and partial rib fusion or missing ribs[1].

"Syndrome of bizarre vertebral anomalies", "syndrome of costovertebral segmentation anomalies" "spondylothoracic dysplasia" are some of the names given to this condition. There are two major subtypes of dysostoses with different survival rates, associated malformations, and inheritance patterns[1,2]. Chromosomal abnormalities in spondylocostal dysostosis and spondylothoracic dysostosis were not previously reported.

In this paper, a boy is presented with spondylocostal dysostosis and a fragile site at 5q32.

Case Report

The propositus was born in Balcalı Hospital, Obstetric and Gynecology Departments of Çukurova University. The male infant was the product of a full-term pregnancy with an uncomplicated delivery. He was admitted to the Neonatal Intensive Care Unit of the same hospital due to respiratory difficulty. His weight was 3510 g (50^{th}-90^{th} percentile), length was 46 cm (25^{th}-50^{th} percentile) and head circumference was 36 cm (50^{th}-90^{th} percentile). Clinical examination

* From the Division of Neonatology, Department of Pediatrics, Çukurova University Faculty of Medicine, Adana.

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

*** Assistant Professor of Medical Biology, Çukurova University Faculty of Medicine

**** Associate Professor of Pediatrics and Neonatologist, Çukurova University Faculty of Medicine.

revealed mild cyanosis, a short neck, a markedly short and wide chest, and a protruding abdomen (Fig. 1). Radiological findings included multiple vertebral deformities (hemivertebrae, cleft vertebrae and butterfly vertebrae) and marked costal anomalies (different widths of intercostal spaces and the absence of the 10^{th}, 11^{th} and 12^{th} ribs) (Fig. 2). Cranial and abdominal ultrasonographies were normal. Serum concentrations of amino acids and urinary excretion of mucopolysaccharides were normal. Chromosomal analysis of peripheral blood cells with G-banding and Giemsa stain revealed 46, XY, fra (5) (q32) (Fig. 3). This fragile site was seen in 20 metaphases. During the clinical course, the baby's respiratory difficulty gradually diminished and he was discharged on the 13^{th} day. At 14 months, the baby was healthy, but his length was 70 cm below the 3^{rd} percentile).

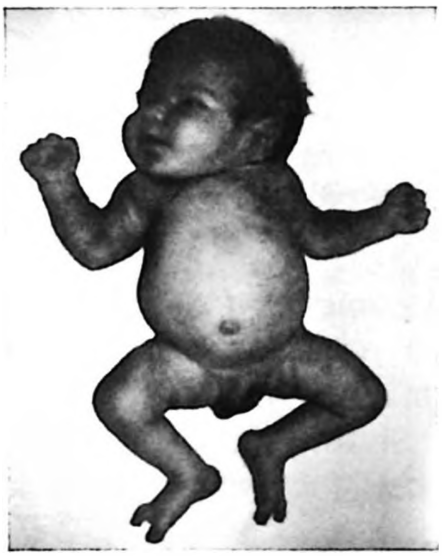


Fig. 1: General appearance of the case.

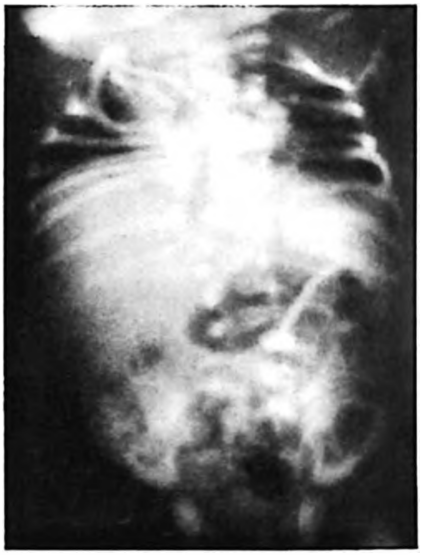


Fig. 2: Trunk anteroposterior roentgenogram of the case.

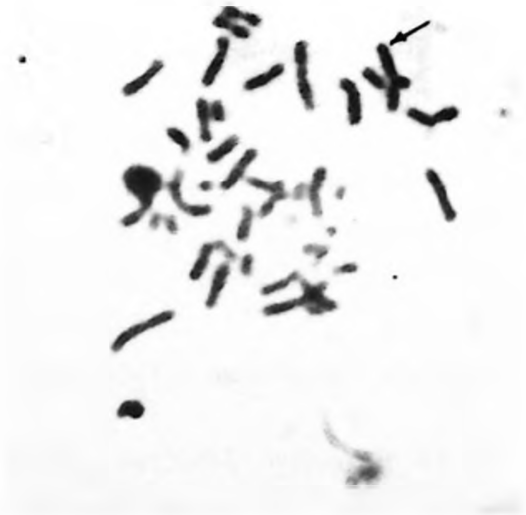


Fig. 3: Karyotype of the case (arrow indicates fragile site).

The father (33 years old) and the mother (28 years old), who were second cousins, were both healthy and their chromosomes were normal. The baby's two elder sisters were without deformities. However, the pedigree study did reveal a maternal uncle with chest deformities who died at 65 years of age.

Discussion

Chromosomal abnormalities in spondylocostal dysostosis were not previously reported. Some abnormalities on chromosome 5q were reported in certain skeletal dysplasia syndromes. Haastbacka et al.[3] concluded that the locus is probably in the 5q31-q34 area in diastrophic dysplasia. Cooke et al.[4] reported that a related gene may be located at 5q33.1 or 8q21.4 in camptomelic dysplasia. However, Maria et al.[5] observed a de novo paracentric inversion of 17q in an infant with camptomelic dysplasia. Chromosomal analysis revealed a fra (5) (q32) in this infant. We conclude that the gene related to spondylocostal dysostosis syndrome may be located on 5q32 or close to this region.

REFERENCES

1. Hall JG. Dysostoses. In: Emery AE, Rimoin DL (eds). Principles and Practice of Medical Genetics (2nd ed). London: Churchill Livingstone; 1990: 967-988.
2. Satar M, Kozanoğlu MN, Atilla E. Identical twins with an autosomal recessive form of spondylocostal dysostosis. Clin Genet 1992; 41: 290-292.
3. Hästbacka J, Sistonen P, Kaitila I, Weiffenbach B, Kidd KK, de la Chapella A. A linkage map spanning the locus for diastrophic dysplasia (DTD). Genomics 1991; 11: 968-973.
4. Cooke CT, Mulcahy MT, Cullity GJ, Watson M, Sragué P. Camptomelic dysplasia with sex reversal: morphological and cytogenetic studies of a case. Pathology 1985; 17: 526-529.
5. Maraia R, Saal HM, Wangsa D. A chromosome 17q de novo paracentric inversion in a patient with camptomelic dysplasia, case report and etiologic hypothesis. Clin Genet 1991; 39: 401-408.

The Turkish Journal of Pediatrics 1997; 39: 551-555

BUDD – CHIARI SYNDROME IN A CHILD SECONDARY TO MEMBRANOUS OBSTRUCTION OF THE HEPATIC VEIN TREATED BY PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY*

Report of A Case

*Buket Altuntaş MD***, Neşe Yaralı MD***, Semanur Kuyucu MD****

*Zafer Arslan MD*****, Ülker Ertan MD***, Ayşe Erden MD******

*Turan Cumhur MD*******, Tahsin Teziç MD********

SUMMARY: Altuntaş B, Yaralı N, Kuyucu S, Arslan Z, Ertan Ü, Erden A, Cumhur T, Teziç T. (Dr. Sami Ulus Children's Hospital and Department of Radiology, Yüksek İhtisas Hospital, Ankara, Turkey). Budd-Chiari syndrome in a child secondary to membranous obstruction of the hepatic vein treated by percutaneus transluminal angioplasty. Turk J Pediatr 1997; 39: 551-555.

Budd-Chiari syndrome (BCS) due to membranous obstruction of the hepatic vein and the inferior vena cava is rare in children. We report a child with BCS that had a membranous obstruction at the level of the hepatic veins. The web was successfully dilated percutaneously by balloon catheters. Symptoms and signs of obstruction improved without any complication. As percutaneous catheterization is an effective, safe and repatable procedure, we recommend this technique for treatment of children and adults with BCS due to membranous obstruction of the hepatic veins. *Key words: membranous obstruction, hepatic vein, children, Budd-Chiari syndrome, percutaneous transluminal angioplasty.*

Budd-Chiari syndrome (BCS) is a relatively uncommon group of disorders caused by occlusion of the major hepatic veins and/or the inferior vena cava (IVC), at or near the level of the hepatic vein ostia. Patients with BCS present with ascites, abdominal pain, hepatomegaly, edema, and occasionally jaundice. Depending on the rapidity and the extent of the obstruction of hepatic vein outflow, the course of the syndrome may be rapid or chronic. The majority of the cases are due to an obstruction of the hepatic veins or IVC caused by thrombi or tumors[1]. Very infrequently, BCS develops secondary to a membranous obstruction of the hepatic vein[2].

Interventional radiological procedures have gained wide acceptance in the treatment of patients with BCS. One such procedure is percutaneous transluminal angioplasty of occlusion or stenoses of the hepatic vein or IVC[3].

---

* From Dr. Sami Ulus Children's Hospital, and Department of Radiology, Yüksek İhtisas Hospital, Ankara.

** Pediatrician and Pediatric Gastroenterologist, Dr. Sami Ulus Children's Hospital.

*** Pediatrician, Dr. Sami Ulus Children's Hospital.

**** Associate Professor of Pediatrics and Pediatric Allergist, Dr Sami Ulus Children's Hospital.

***** Radiologist, Yüksek İhtisas Hospital.

****** Associate Professor of Radiology, Yüksek İhtisas Hospital.

******* Professor of Pediatrics and Pediatric Endocrinologist, Dr. Sami Ulus Children's Hospital.

In this case report, we report a child with BCS due to membranous obstruction of the hepatic vein who was treated by percutaneous hepatic vein angioplasty.

Case Report

A nine-year-old boy presented with a three-week history of abdominal distention and hematemesis. Physical examination revealed a thin boy, without jaundice, with a normal chest and heart. Slight dilatation of the superficial veins of the abdomen was noted. The liver edge was palpated 2 cm below the right costal margin and the spleen 2 cm below the left costal margin. There were marked ascites and edema of the lower extremities and scrotum. Paracentesis yielded a transudate with an albumin of 0.67 g/dl. Results of liver function tests were mildly elevated. Hemoglobin was 10.4 g/dl. Hypochrom microcytic anemia compatible with iron deficiency was present as demonstrated by low serum transferrin saturation. Serum ceruloplasmin, serum and urinary copper, α-1- antitrypsin and sweat electrolyte levels were normal. Hepatitis B surface antigen and anti hepatitis C antibody were negative. Portal system duplex Doppler ultrasonography demonstrated patent splenic and portal veins. There was an anastomosis between hepatic veins, and the caudate lobe was hypertrophic (Fig. 1). These findings are typical of Budd-Chiari syndrome. To assess the cause of thrombosis, serum levels of abnormal inhibitors of a hemostatic system, such as antithrombin III, protein C and protein S, were confirmed as normal, and antiphospholipid antibodies were negative. Patency of IVC was demonstrated by transfemoral inferior cavography. Membranous web between the hepatic vein and IVC was demonstrated by percutaneous transhepatic angiography (Fig. 2). Following transhepatic balloon dilatation of the membrane, ascites resolved completely and did not recur. An early follow-up ultrasonographic examination revealed a patent hepatic vein. Unfortunately, micronodular cirrhosis was diagnosed by liver biopsy (Fig. 3), and endoscopic examination of the esophagus revealed grade III varices.

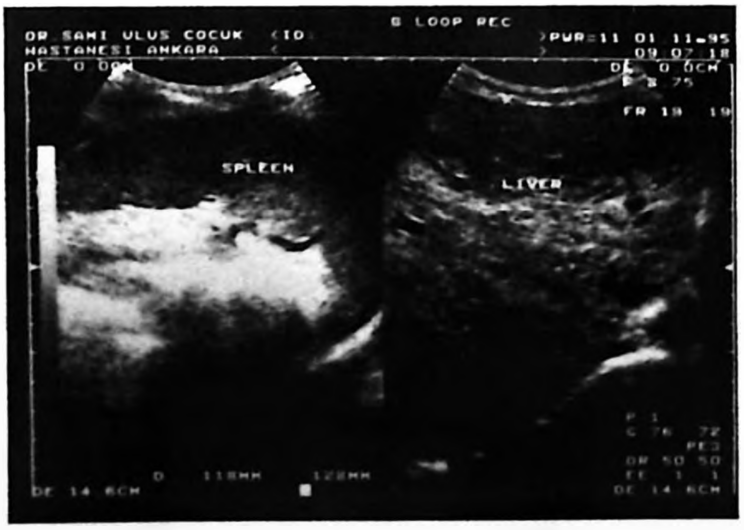


Fig. 1: Ultrasonography shows coarseness of the liver echogenicity. The hepatic veins are thin and irregular. Spleen is homogeneously enlarged.

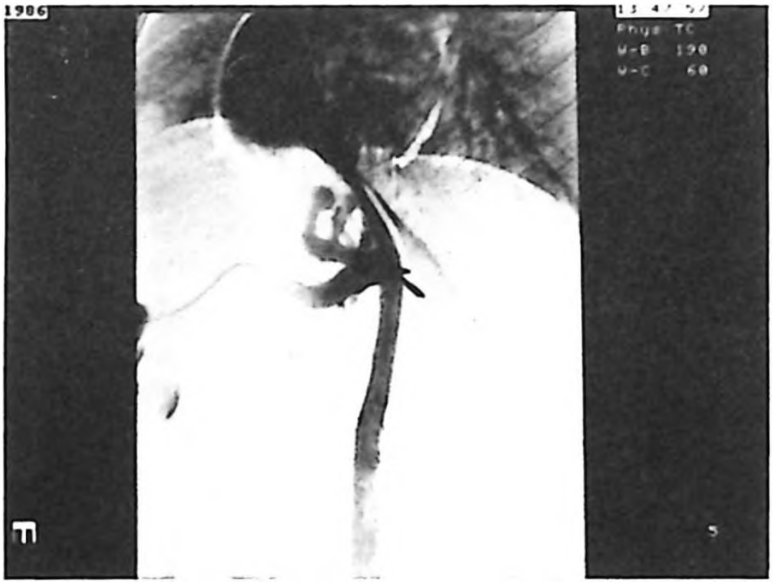


Fig. 2: Patent IVC at transfemoral inferior cavography; membranous web between the hepatic vein and IVC demonstrated by percutaneous transhepatic angiography.

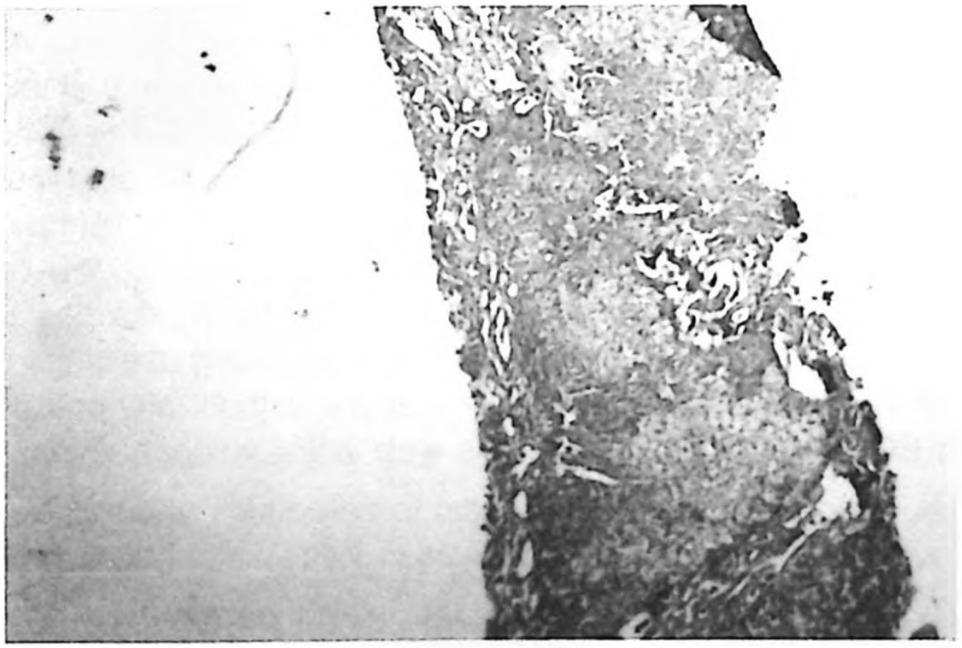


Fig. 3: Liver: Micronodular cirrhosis (HE x 400).

Discussion

Budd-Chiari syndrome is caused by an obstruction of the hepatic vein outflow. Possible causes of this syndrome are myeloproliferative disorders, thrombotic conditions, malignant neoplasm, and membranous obstruction of the hepatic vein and IVC. The latter is particularly infrequent, except in Orients, where it is a major cause of suprahepatic portal hypertension[4-6]. Chronic by nature, BCS can progress to liver cirrhosis if left untreated, resulting in a poor prognosis as in this case report[1].

In Turkish literature, we were unable to find information regarding the frequency of various causes of BCS. Okten et al.[7] reported four cases of membranous obstruction of IVC in adults. There is some controversy surrounding the etiology and pathogenesis of membranous obstruction. Some outhors accept the theory of thrombosis and organization, but others accept a congenital origin based on non-union or malunion during the developmental stages of the hepatic veins and IVC[8,9]. In our case, as the symptoms and the signs appeared at a very young age; the levels of antithrombin III, protein C and protein S (potential causes of thrombosis) were normal; and antiphospholipid antibodies were negative, we suggest that the membrane in the hepatic vein was congenital.

Treatment options include conservative treatment, interventional radiological techniques, a variety of surgical vascular by-passes, decompression procedures, and liver transplantation. Treatment method should be individualized according to the site of obstruction and the extent of liver failure. The commonly used surgical procedures include finger fracture, transcardiac membranotomy or bypass grafting. However, postoperative mortality rates with such procedures are high: 20 percent following transcardiac finger fracture and 40 percent following bypass grafting or inferior cavotomy[4]. Interventional radiological procedures have gained popularity in the treatment of BCS. These procedures include percutaneous transluminal angioplasty of occlusions or stenoses of the hepatic vein or IVC. Percutaneous transluminal angioplasty is a simple and safe procedure. It may be repeated if the initial attempt fails or the obstruction recurs[7,8]. Although there have been several reports on the use of balloon angioplasty in adults with BCS, there is little data regarding use of the procedure in children. We successfully used this procedure, without any complication, in a child with BCS due to a membranous web in the hepatic vein.

In conclusion, balloon angioplasty appears to be a safe and effective treatment for children with BCS due to membranous obstruction of the hepatic veins or IVC.

REFERENCES

1. Mitchell MC, Boitnott JK, Kaufman S, Cameron JL, Maddrey WC. Budd-Chiari syndrome: etiology, diagnosis and management. Medicine 1982; 61: 199-218.
2. Hoffman HP, Stockland B, Heyden U. Membranous obstruction of the inferior vena cava with Budd-Chiari syndrome in children: a report of nine cases. J Pediatr Gastroenterol Nutr 1987; 6: 878-884.
3. Martin LG, Henderson JM, Millikan WJ Jr, Casaerella WJ, Kaufman SL. Angioplasty for long-term treatment of patients with Budd-Chiari syndrome. Am J Roentgenol 1990; 154: 1007-1010.
4. Hirooka M, Kimura C. Membranous obstruction of the hepatic portion of the inferior vena cava. Arch Surg 1976; 100: 656-663.
5. Kimura C, Matsuda S, Koie H, Hirooka M. Membranous obstruction of the inferior vena cava: clinical study of nine cases. Surgery 1971; 74: 551-559.

6. Takeuchi J, Takada A, Hosumara Y, Matsuda Y, Ikegami F. Budd-Chiari syndrome with obstruction of the inferior vena cava. Am J Med 1971; 51: 11-20.
7. Okten A, Besisik F, Boztas G, et al. Vena cava inferiorun membranoz obstruksiyonu ve Budd-Chiari sendromu. Turk J Gastrohepatol 1993; 4: 295-298.
8. Terabayashi H, Okuda K, Nomura F, Ohnishi K, Wong P. Transformation of inferior vena caval thrombosis to membranous obstruction in a patient with the lupus anticoagulant. Gastroenterol 1986; 91: 219-224.
9. Kage M, Arakawa M, Kojiro M, Okuda K. Histopathology of membranous obstruction of the inferior vena cava in the Budd-Chiari syndrome. Gastroenterol 1992; 102: 2081-2090.
10. Sawada S, Oshima T, Kato T, Kubota Y, Tanaka Y. Budd-Chiari syndrome treated by percutaneous transluminal angioplasty. Acta Radiol Diagnosis 1985; 26: 25-27.
11. Yamada R, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Kobayashi N. Segmental obstruction of the hepatic inferior vena cava treated by transluminal angioplasty. Radiology 1983; 149: 91-96.

The Turkish Journal of Pediatrics 1997; 39: 557-560

NONIMMUNE HYDROPS FETALIS AND BILATERAL PULMONARY HYPOPLASIA IN A NEWBORN INFANT WITH NUCHAL VASCULAR HAMARTOMA*

*Hasan Özkan MD***, Hüseyin Gülen MD****, Namık Demir MD*****
*Erdener Özer MD******, Şebnem Haberal MD****, Derya Erçal MD***
*Necla T. Çevik MD*******

SUMMARY: Özkan H, Gülen H, Demir N, Özer E, Haberal Ş, Erçal D, Çevik NT. (Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Nonimmune hydrops fetalis and bilateral pulmonary hypoplasia in a newborn infant with nuchal vascular hamartoma. Turk J Pediatr 1997; 39: 557-560.

Nuchal vascular hamartoma was found in a newborn premature infant who presented with nonimmune hydrops fetalis, pulmonary hypoplasia due to bilateral pleural effusion and polyhydramnios in utero. The baby died 26 hours after birth despite maximal respiratory and circulatory support. Postmortem examination revealed a vascular hamartoma localized to the left posterolateral region of the neck. We suggest that nuchal vascular hamartoma may be associated with fetal hydrops, probably due to compromised lymph drainage. *Key words: vascular hamartoma, nonimmune hydrops fetalis, pulmonary hypoplasia.*

Nonimmune hydrops fetalis is a relatively rare and complex disorder that requires detailed investigation. A wide range of associations have been reported, but chromosomal abnormalities, cardiac anomalies, pulmonary abnormalities, infection and multiple births are the common causes of the disorder[1,2]. We describe a newborn infant with nuchal vascular hamartoma, who presented with nonimmune hydrops fetalis, bilateral pleural effusion, pulmonary hypoplasia and polyhydramnios in utero. The association between nuchal vascular hamartoma and pleural effusion and pathogenetic mechanisms leading to fetal hydrops are discussed.

Case Report

A 3400 g female infant was born to a 28-year-old multigravida at 36 weeks gestation by cesarean section. Pregnancy was complicated by polyhydramnios; bilateral pleural effusion and ascites were noted during ultrasound examination

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

at 33 weeks. Because of hydrops fetalis, a cordocentesis was performed for fetal karyotyping. The fetal blood hemoglobin was 17 g/dl. At birth, the baby was severely asphyxiated and showed nuchal mass and cervical edema, especially in the left posterolateral region of the neck (Fig. 1). She was immediately intubated and mechanically ventilated. Initial blood gases revealed severe respiratory acidosis. Chest x-ray showed bilateral pleural fluid, especially in the left hemithorax, and relatively good aeration of the right hemithorax (Fig. 2). Bilateral thoracocentesis was performed and a sero-hemorrhagic pleural fluid was obtained. Cell count of the pleural fluid revealed 820 leukocytes/ml, with a predominance of lymphocytes. The protein and glucose contents were 3.1 g/dl and 112 mg/dl, respectively. Triglyceride and cholesterol contents were 75 mg/dl and 57 mg/dl, respectively. Lactic dehydrogenase (LDH) content was 750 IU/L. Cultures of pleural fluid were negative as were serologic tests for toxoplasmosis, rubella, cytomegalovirus, and herpes simplex virus infections. Other laboratory investigations revealed normal urinalysis, complete blood count, blood urea nitrogen, creatinine, glucose, liver functions, electrolytes, total protein (4.1 g/dl), albumin (2.6 g/dl), cholesterol (58 mg/dl), triglyceride (24 mg/dl), and lactic dehydrogenase (1542 IU/L) levels. Chromosomal investigation revealed a normal karyotype. Despite maximal respiratory and circulatory support, severe hypoxemia and acidosis persisted, and the baby died 26 hours after birth.

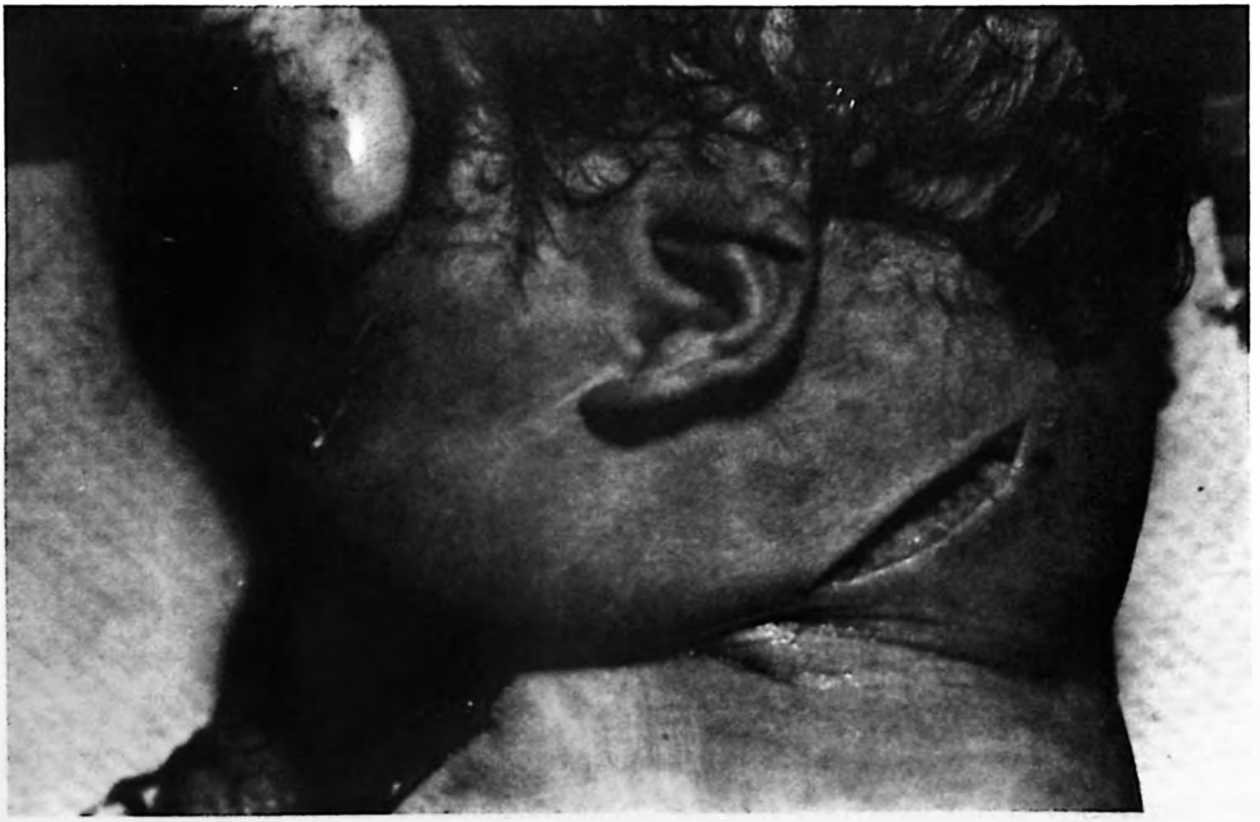


Fig. 1: Nuchal mass and cervical edema in the left posterolateral region (postmortem view).

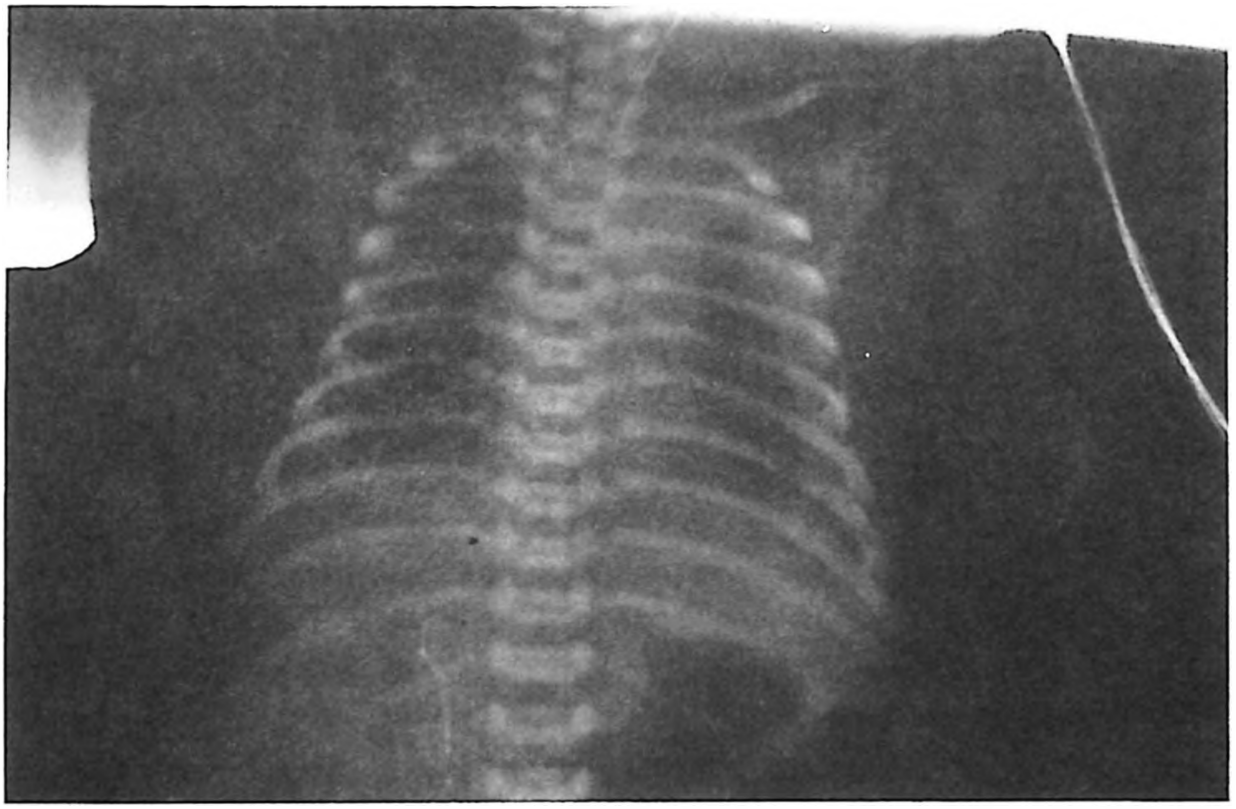


Fig. 2: Chest x-ray two hours after birth. Bilateral pleural fluid, especially in left hemithorax, and relatively good aeration of the right lung.

Upon postmortem examination, 25 ml sero-hemorrhagic pleural fluid was found. Both left and right lungs were hypoplastic, weighing 15 g each. In addition, external examination revealed a left cervical subcutaneous mass, 3.5 x 3 x 3 cm and firm, that was well-circumscribed and partially congested. Histologically, the lesion consisted of many vascular spaces lined by endothelial cells (Fig. 3). The lumen of some spaces contained red blood cells. A collection of lymphoid tissue was seen in the interstitial area. The histopathological diagnosis was vascular hamartoma (hemangio-lymphangioma).

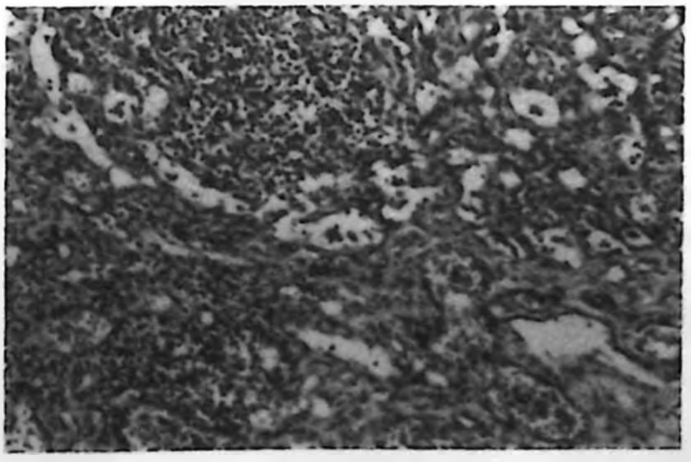


Fig. 3: Microscopic appearance of the nuchal mass. The lesion was composed of vascular spaces with a lymphoid stroma (x 100).

Discussion

Vascular hamartomas in the head and neck region are very unusual in infancy and childhood[3,6]. This case is unique because nuchal vascular hamartoma is associated with bilateral pleural effusion and hydrops fetalis. No report of this association was found in international literature for the past 20 years. There may be a coincidental association or it may be the cause of the hydrops.

The lymphatic channels in each uper quadrant drain into the jugular lymphatic sac. These jugular sacs eventually form communications with the venous system. We suggest that this communication and lymph drainage may be compromised by nuchal hemangio-lymphangioma in this case. Fetal pleural effusion and progression to hydrops is probably due to disturbed lymph drainage[2,7]. Based on the composition of pleural fluid before feeding, congenital chylothorax was considered to be the most likely diagnosis in our patient[8,9]. Pleural effusion may have caused pulmonary hypoplasia in our patient because significant pleural effusions before the alveolar phase of lung development irreversibly damage fetal lungs[10].

This report suggests that vascular hamartoma may be one of the many causes of non-immune hydrops fetalis.

REFERENCES

1. Stephenson T, Zuccollo J, Mohajer M. Diagnosis and management of non-immune hydrops in the newborn. Arch Dis Child 1994; 70: F 151-154.
2. McGillivray BC, Hall JG. Non-immune hydrops fetalis. Pediatr Rev 1987; 9: 197-202.
3. Samuel J, Fernandes CC. Hamartomas of the head and neck. A report of 4 cases. S Afr Med J 1985; 68: 265-267.
4. Arienzo R, Ricco CS, Romeo F. A very rare fetal malformation: the cutaneous widespread vascular hamartomatosis. Am J Obstet Gynecol 1987; 157: 1162-1163.
5. Willshaw HE, Deady JP. Vascular hamartomas in childhood. J Pediatr Surg 1987; 22: 281-283.
6. Mukherjee P. Vascular hamartomas in infancy and childhood. J Indian Med Assoc 1989; 87: 147-148.
7. Hagay Z, Rcece A, Roberts A, Hobbins JC. Isolated fetal pleural effusion: a prenatal management dilemma, Obstet Gynecol 1993; 81: 147-152.
8. Özkan H, Ay N, Özaksoy D, et al. Congenital chylothorax. Turk J Pediatr 1996; 38: 113-117.
9. Eddleman KA, Levine AB, Chitkara U, Berkowitz RL. Reliability of pleural fluid lymphocyte counts in the antenatal diagnosis of congenital chylothorax. Obstet Gynecol 1991; 78: 530-532.
10. Castillo RA, Devoe LD, Falls G, Holzman GB, Hadi HA, Fadel HE. Pelural effusions and pulmonary hypoplasia. Am J Obstet Gynecol 1987; 157: 1252-1255.

The Turkish Journal of Pediatrics 1997; 39: 561-564

SUPERIOR VENA CAVA SYNDROME AS A RESULT OF THROMBOSIS IN A CHILD WITH NEPHROTIC SYNDROME*

*Salim Çalışkan MD***, *Lale Sever MD****, *Ayşe Sarıoğlu MD*****
*Tayyar Sarıoğlu MD******, *Özgür Kasapçopur MD*******, *Nil Arısoy MD********

SUMMARY: Çalışkan S, Sever L, Sarıoğlu A, Sarıoğlu T, Kasapçopur Ö, Arısoy N. (Department of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine and Institute of Cardiology, İstanbul, Turkey). Superior vena cava syndrome as a result of thrombosis in a child with nephrotic syndrome. Turk J Pediatr 1997; 39: 561-564.

A three-year-old nephrotic girl is presented with vena cava superior syndrome. Angiography showed obliteration of the distal ends of both axillary veins and echocardiographic examination revealed a mobile mass in the right atrium. A thrombus originating from the vena cava superior with a stalk extending to the right atrium was surgically removed. To our knowledge, vena cava superior thrombosis in nephrotic syndrome has not been reported previously. *Key words: nephrotic syndrome, vena cava superior, thrombosis, thromboembolic complication.*

Increased tendency to hypercoagulability in nephrotic syndrome leads to thromboembolism, one of the syndrome's most serious complications. To date various localizations of venous and arterial thromboembolism have been reported. We present a nephrotic child with thromboembolism of the vena cava superior, a localization not previously reported, which led to vena cava superior syndrome.

Case Report

A three-year-old girl with nephrotic syndrome was admitted to our institution in September 1991. She was put on prednisolone (2 mg/kg/day, in divided doses) but there was no improvement after two months of treatment. The renal biopsy specimen showed segmental capillary wall collapse with sclerosis and adhesion to Bowman's capsule in two of nine glomeruli, which was consistent with focal segmental glomerulosclerosis. She was then treated with cyclosporine and low dose prednisolone for eight months, again with no response.

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

** Pediatric Nephrologist, İstanbul University Cerrahpaşa Faculty of Medicine.

*** Associate Professor of Pediatric Nephrology, İstanbul University Cerrahpaşa Faculty of Medicine.

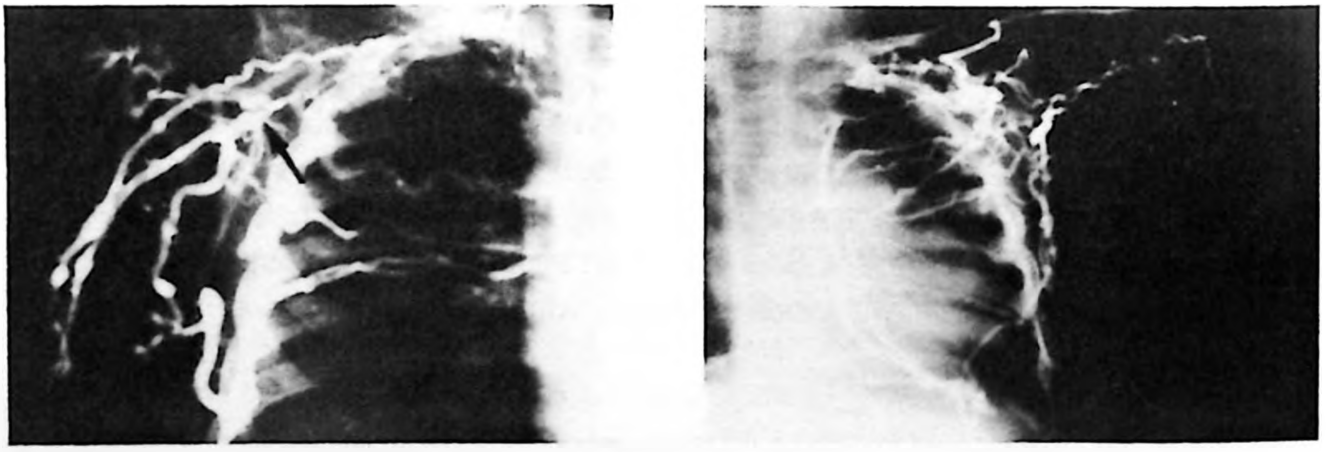
**** Associate Professor of Pediatric Cardiology, İstanbul University Institute of Cardiology.

***** Professor of Cardiovascular Surgery, Istanbul University Institute of Cardiology.

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

******* Professor of Pediatric Nephrology, İstanbul University Cerrahpaşa Faculty of Medicine

Having received prednisolone (5 mg/day) and cyclosporine (5 mg/day), the patient was readmitted in September 1992 with dyspnea and marked edema around the neck and shoulders. She did not receive diuretics and had no history of diarrhea. Collateral circulation was observed in the upper chest. Bilateral periorbital puffiness and pedal edema were also present. Blood pressure was 105/80 mm Hg, heart rate 120/min and respiration rate 34/min. Chest was dull to percussion on the left side with diminished breath sounds over the lower half of the same side; no rales were detected. Cardiac sounds and pulses were normal. A three centimeter hepatomegaly and minimal ascites were noted. Other clinical findings were within normal limits. Laboratory findings were as follows: serum protein 3.3 g/dl, albumin 1.7 g/dl, total lipid 2250 mg/dl, cholesterol 691 mg/dl, BUN 9 mg/dl, creatinine 0.45 mg/dl, sodium 143 mmol/L, potassium 3.6 mmol/L, Hb 11.5 g/dl, Hct 35%, WBC 12.000/mm^3, ESR 117 mm/h, GOT 26 IU/L, GPT 28 IU/L, PT 17" (53%), PTT 41". The urinary protein concentration was 0.8 g/dl (urinary protein/creatinine ratio 3.1). Chest x-ray revealed bilateral pleural effusion. On the second day of admission, the patient's right leg became swollen. It was painful and warm. Anti-aggregant therapy (salicylic acid 80 mg/day) was started. Echocardiographic examination revealed pericardial effusion but no other abnormality; however, the vena cava superior could not be detected. Thoracentesis yielded chylous fluid. Angiographic examination revealed extensive collateral circulation. The vena cava superior as well as both brachiocephalic and subclavian veins were not visible (Figs. 1a, 1b). A second echocardiographic examination, made 15 days after the



Figs. 1a, 1b: Angiographic frames showing obliteration of right axillary vein (arrow) and extensive collateral circulation. Note the vena cava superior, both brachiocephalic and subclavian veins, and left axillary vein were not visible.

first one, revealed a mobile mass (2.1 x 3.5 cm) in the right atrium. It was partly penetrating the right ventricle during diastole (Fig. 2). In the following days, a thrombus originating from the vena cava superior with a stalk extending to the right atrium was surgically removed. A thrombectomy as also performed on the

vena femoralis. Following initial anticoagulation with heparin (100 IU/kg/day) for three weeks, the patient was treated with Coumadin (1.25 mg/day). On the 17th post-operative day, fever and chest pain appeared; the physical findings were consistent with mediastinitis. Culture from the wound revealed plasma coagulase (-) staphylococcus. A surgical review was performed and the patient was treated with appropriate antibiotics. One month after thrombectomy, a control echocardiographic examination revealed no abnormality. She received a course of pulse methylprednisolone (30 mg/kg) weekly for three months, followed by alternate day prednisolone therapy (2 mg/kg/day) for two years. Proteinuria had disappeared completely by March 1994 and anti-coagulant and anti-aggregant therapies were discontinued.

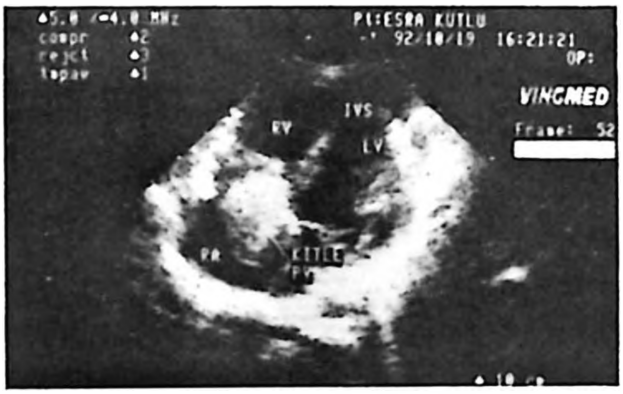


Fig. 2: Echocardiogram denoting the large thrombus in the right atrium partly penetrating the right ventricle.

At the time of her last visit in April 1995, the patient was doing well, and was active and healthy in appearance. Her physical examination was normal. Urinalysis and echocardiographic examination revealed no abnormality.

Discussion

Various factors, such as hypoalbuminemia; hemoconcentration; increased platelet aggregation; hepatic over-synthesis of fibrinogen, cofactor and lipoproteins; urinary loss of clotting inhibitors (especially anti-thrombin III); functional deficiency of protein C and protein S; and steroid and diuretic therapy, are responsible for hypercoagulability in nephrotic syndrome. It is assumed that this hypercoagulable state causes thromboembolic complications[1]. In spite of the low incidence of thromboembolic conditions in childhood[2], prospective studies showed a significant number of asymptomatic cases. This could contribute to an underestimation of the true incidence[3].

Deep vein thrombosis of the extremities and pulmonary embolism are most commonly encountered. A significant number of renal vein thromboses, with or without vena cava inferior thrombosis, have been reported[1]. Subclavian, axillary,

external jugular, splenoportal, mesenteric, hepatic and cerebral sinus veins and intracardiac spaces are uncommon sites for venous thromboembolism[4]. Arterial thrombosis is much less common than venous thrombosis and is observed mainly in children. Thrombosis of mesenteric, axillary, femoral, ophthalmic, carotid, cerebral, renal, pulmonary and coronary arteries has been reported[4]. To our knowledge, vena cava superior thrombosis in nephrotic syndrome has not been reported previously, but it has been encountered after interventions (venous catheterization, implantation of pacemaker and ventriculoatrial shunting), and in the course of systemic diseases (polycythemia, anti-phospholipid syndrome, Behçet's syndrome). It also occurs as the result of external compression (Hodgkin's and non Hodgkin's lymphomas)[5].

Another interesting point in this case is presentation of the thrombus as a right atrial mass in the second echocardiographic examination. The delay in diagnosis, due in part to technological inadequacy, may have caused the thrombus to grow and extend into the right atrium. Babcock et al.[6] reported the superiority of Doppler echocardiography of the superior vena cava, with its high frequency transducer for the diagnosis of thromboembolism, in which case an angiographic examination may be unnecessary.

Today, thromboembolic complications are successfully treated with thrombolytic agents like streptokinase and urokinase. In view of the stalkiness of the thrombus and the serious risks of pulmonary embolism, open-heart surgery was the preferred method of treatment for our patient.

In conclusion, vena cava superior thrombosis is a life-threathening complication that cannot be overlooked in nephrotic syndrome.

Acknowledgement

Careful review of the manuscript by Dr. Doli Yafet Aji is greatly appreciated.

REFERENCES

1. Llach F. Hypercoagulability, renal vein thrombosis, and other thrombotic complications of nephrotic syndrome. Kidney Int 1985; 28: 429-439.
2. Mehls O, Andrassy K, Koderisch J, Herzog U, Ritz E. Hemostasis and thromboembolism in children with nephrotic syndrome: differences from adults. J Pediatr 1986; 110: 862-867.
3. Hoyer PF, Gonda S, Barthels M, Krohn HP, Brodehl J. Thromboembolic complications in children with nephrotic syndrome. Rish and incidence. Acta Paediatr Scand 1986; 75: 804-810.
4. Cameron JS. Coagulation and thromboembolic complications in the nephrotic syndrome. Adv Nephrol 1984; 13: 75-114.
5. De Gennes C, Wechsler B, Godeau P. Thromboses cases. Rev Prat 1988; 38: 2068-2072.
6. Babcock DS. Sonographic evaluation of suspected pediatric vascular diseases. Pediatr Radiol 1991; 21: 486-489.

The Turkish Journal of Pediatrics 1997; 39: 565-571

COMBINATION OF STEROID WITH AZATHIOPRINE IN TREATMENT OF GIANT CELL AUTOIMMUNE HEPATITIS*

*Yasemen Eroğlu MD***, *Öznur Düzovalı MD****, *Salih Kavukçu MD*****
*Gülersu İrken MD******, *Benal Büyükgebiz MD*******

SUMMARY: Eroğlu Y, Düzovalı Ö, Kavukçu S, İrken G, Büyükgebiz B. (Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Combination of steroid and azathioprine in treatment of giant cell autoimmune hepatitis. Turk J Pediatr 1997; 39: 565-571.

Giant cell hepatitis is a rare disorder after the newborn period. Drugs, autoimmunity, and viruses (lately, paramyxovirus infection) have been implicated in its etiology. Without treatment, liver dysfunction is progressive and fatal. Immunosuppression with steroids and azathioprine has been demonstrated to sustain improvement in the disease. In this report, a one-year-old boy who has giant cell hepatitis with Coombs' positive hemolytic anemia and anti-smooth muscle antibodies is presented, and the course of the disease and the patient's response to treatment with steroid and azathioprine is reviewed. *Key words: giant cell hepatitis, autoimmune hemolytic anemia, steroid and azathioprine therapy.*

Although there is a frequent pattern of liver injury in neonates, giant cell hepatitis (GCH) is uncommon after infancy. Its association with drugs, viruses, and a autoimmunity has been reported previously[1-4]. Most recently, paramyxovirus infection has been implicated in its etiology[5]. Coombs' positive hemolytic anemia may occur concomitantly with GCH, though this is rare in young children[6,7]. GCH is known as a progressive and often fatal disease process. Since the liver disease was assumed to be immune-mediated, a combined immunosuppressive regimen with steroid and azathioprine has been introduced to patients, with sustained improvement in clinical, laboratory and pathological features[6,7].

This report presents a one-year-old boy who has GCH associated with Coombs' positive hemolytic anemia, and reviews the course of this disease under combination treatment of steroid and azathioprine.

Case Report

A one-year-old boy was referred to our clinic for jaundice, hepatosplenomegaly and elevated serum aminotransferases levels. One and a half months prior to his referral, he had upper respiratory tract infection and was treated with

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

trimethoprim-sulfamethoxazole. Otherwise, he had no history of toxin or drug exposure. There was nothing notable in his or his family's past history.

On physical examination, jaundice and mucosal pallor were strikingly apparent. Liver and spleen were palpable without pain 15 cm below the respective costal margins, and were smooth on surface.

On admission, the patient's laboratory findings were as follows: hemoglobin 2.6 g/dl, hematocrit 9%, erythrocyte count 590,000/mm^3, and reticulocyte count 50%. A peripheral blood smear revealed normoblastemia, anisocytosis, polikilocytosis, microspherocytosis and polychromasia. Prothrombin time was 17 seconds (N: 12-15) and activated partial thromboplastin time was 47 seconds (N: 35-45).

Blood chemistry was as follows: total bilirubin 30.5 mg/dl (N: < 1), conjuguted bilirubin 19.6 mg/dl (N: < 0.2), alanine aminotransferase (ALT) 685 U/L (N: < 45), aspartate aminotransferase (AST) 1000 U/L (N: < 55), gamma-glutamyl transferase (GGT) 127 U/L (N. 5-32), alkaline phosphatase (AP) 295 U/L (N: 145-200), total protein 6.7 g/dl (N: 6.1-7.9), albumin 3.5 g/dl (N: 3.9-5), alpha-1-antitrypsin 120 mg/dl (N: 50-200), ceruloplasmin 32 mg/dl (N: 27-47), NH_3 183 µg/dl (N: 20-120), and serum iron 110 µg/dl (N: 50-120). Copper excretion in urine was 96 µg/day (N: < 40).

Direct Coombs' test was strongly positive (IgG+ complement). Glucose-6-phosphate dehydrogenase activity, hemoglobin electrophoresis, serum and urine amino acid chromatography were normal.

Except for an immunoglobulin G antibody to hepatitis A, serologic tests for toxoplasmosis, rubella, cytomegalovirus, human immunodeficiency virus, and hepatitis A, B and C viruses were all negative.

C-reactive protein, rheumatoid factor, antinuclear antibody, antimitochondrial antibody, double-stranded DNA antibody, and anti-liver and kidney microsomal antibody were not detected. Serum C3 was 284 mg/dl (N: 80-150) and C4 was 27 mg/dl (N: 20-50). Test for anti-smooth muscle antibody was positive.

Abdominal ultrasonography was normal except for diffuse hepatosplenomegaly. Splenic vein diameter was 5.2 mm. There was no abnormality in the extrahepatic biliary system. Erythroid hyperplasia was prominent in bone marrow examination.

Prednisolone therapy (2 mg/kg/day) was initiated. Multiple transfusions were required due to severe hemolytic disease. Despite conventional steroid therapy, multiple hemolytic relapses were observed. On the 34^{th} day of admission, high dose methyl prednisolone (MP) was introduced. It was started at a dose of 30 mg/kg/day intravenously for the first three days, reduced to 20 mg/kg for five days, 10 mg/kg for one week and 5 finally to mg/kg/day. With this regimen, hemolysis was kept partially under control, and slight improvements in serum aminotransferases levels were observed. On several occasions, ofter tapering the steroid dosage, a relapse of hemolysis and hepatic disease followed, and the MP dose had to be increased again until remission was achieved.

In the second month of MP therapy, the patient had an encephalopathic episode with seizures. Arterial hypertension was detected (150/110 mmHg). Infectious and metabolic investigations, cerebrospinal fluid study and cerebral computed tomography were all negative. Steroid dose adjustments were made. The patient responded well to antihypertensive and anticonvulsant medication. No recurrence was observed during the rest of disease.

Because of the dependence on high dose MP, azathioprine (2 mg/kg/day) was added to the therapy on the 160th day of admission. Hemolysis could be controlled effectively with this combination and the patient's elevated transaminase levels showed improvement. During the full course of therapy, the direct Coombs' test was negative for only a very brief period. The hemolytic parameters and serum transaminase levels of the patient during the course of disease are shown in Figures 1 to 3.

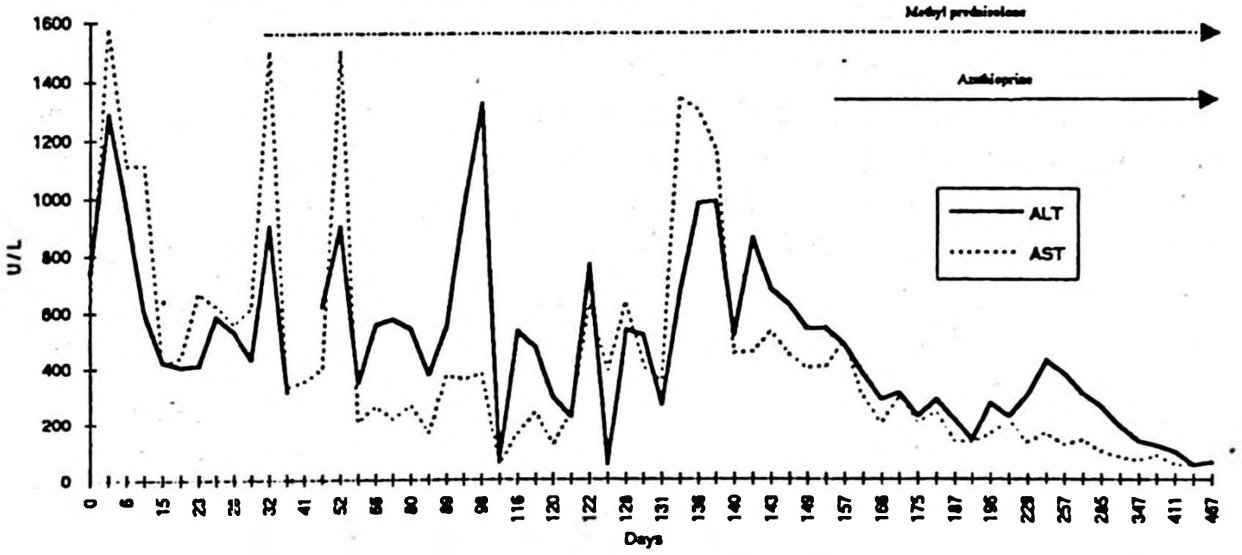
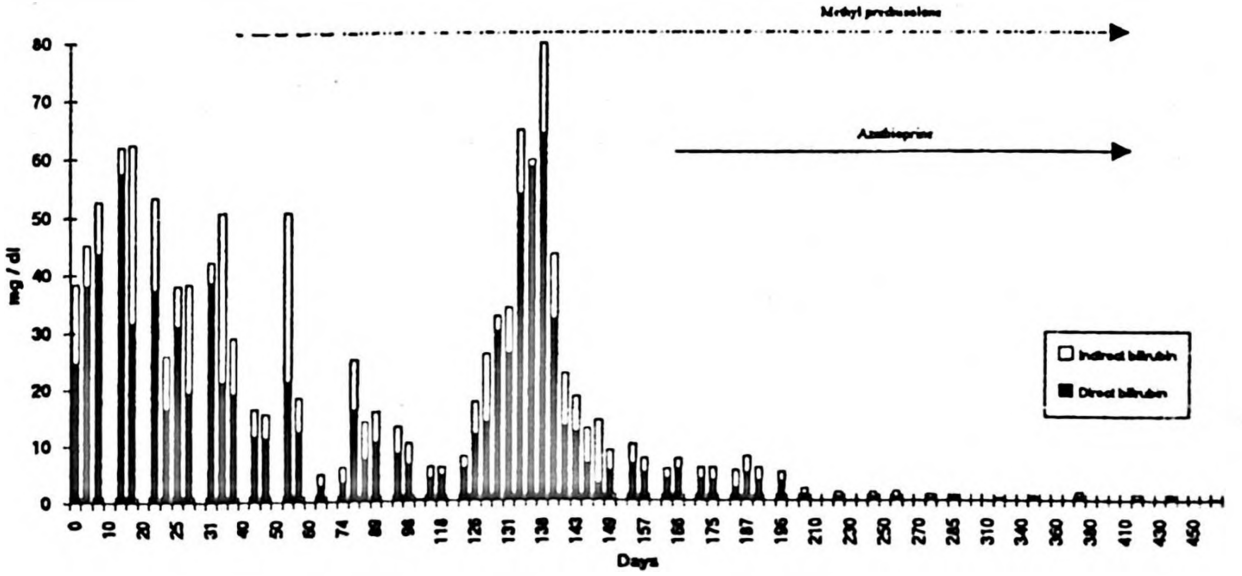
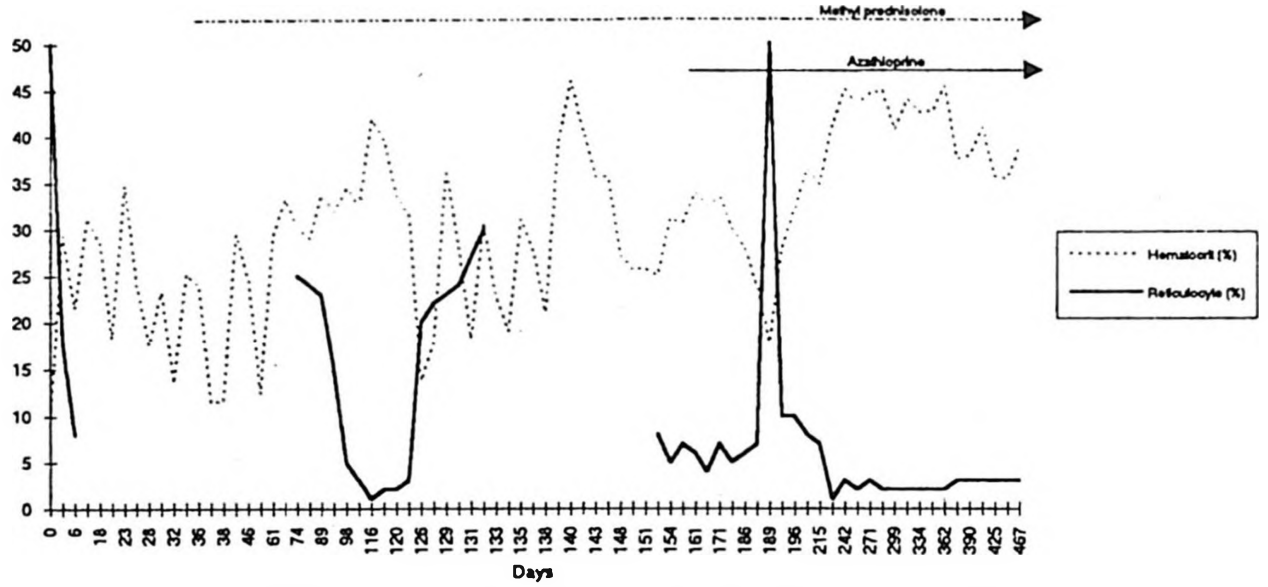


Fig. 1: Serum transaminase levels during follow-up.



Fg. 2: Serum bilirubin levels during follow-up



Fg. 3: Reticulocyte counts and hematocrit values during follow-up.

The addition of azathioprine to the treatment made it possible first to taper and then to terminate MP therapy in the 16th month of admission, without any exacerbation in the hemolytic and hepatic disease. Currently, at two and one-half years, the subject is doing quite well on 1 mg/kg/day azathioprine. He had only mild hepatosplenomegaly. His latest hemoglobin was 12.4 g/dl, Htc 35%, reticulocyte 3.6%, AST 54 U/L, ALT 33 U/L, AP 439 U/L, GGT 65 U/L, prothrombin time was 14.6 seconds and the direct Coombs' test was positive.

During the course of his illness, the patient had two percutaneous liver biopsies. The first biopsy was performed after two weeks of steroid therapy. The sections showed good cores of liver tissue with an apparently preserved, yet slightly distorted achitecture. The parenchyma demonstrated clarification of the cytoplasm of the hepatocytes, with a few multinucleated giant hepatocytes (Fig. 4). Overall,

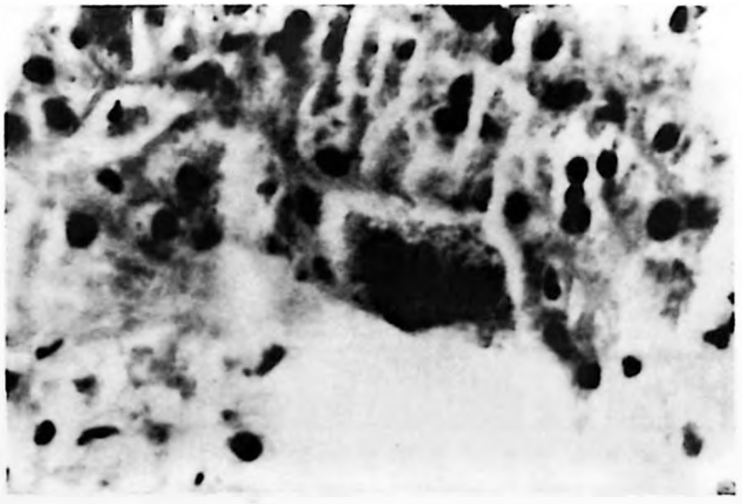


Fig. 4: Multinucleated giant hepatocyte in liver biopsy.

the inflammation was very mild. Iron-pigmented macrophages were present and there was mild to moderate cholestasis with canalicular bile plugs. A second biopsy was taken four and a half months later, while the patient was on MP and azathioprine therapy. The findings were basically similar, but multinucleated giant hepatocytes were more obvious than before and there was a grade I parenchymal siderosis and a more marked and diffuse reticuloendothelial iron load. Though inflammation was, overall, again minimal, the degree of fibrosis appeared to have progressed; the shape of the specimen suggested early parenchymal nodule formation. Liver tissue in both specimens showed GCH, with evidence of focal and bridging parenchymal necrosis.

Discussion

This one-year-old boy had severe and recurrent hemolytic anemia and hepatitis. Extensive investigation for virologic, metabolic and autoimmune causes was made. At first sight, a diagnosis of autoimmune hepatitis could have been considered because of the positive Coombs' test and the presence of anti-smooth muscle antibodies, a finding which was later confirmed. Type I autoimmune hepatitis is usually encountered in young women and associated with the antinuclear antibody and anti-smooth muscle antibody[8]. Type II is more common in children and associated with the anti-liver and kidney microsomal antibody. The very young age of our patient and seronegativity for the antinuclear antibody were not consistent with type I autoimmune hepatitis, while the seronegativity for the anti-liver and kidney microsomal antibody was inconsistent with the type II disease. In autoimmune hepatitis, the histological evaluation of liver tissue is essential, although a specific pathognomonic feature has not been identified. Moderate to severe periportal or periseptal piecemeal necrosis with a predominantly lymphoplasmocytic infiltrate (with or without portal-portal or central-portal bridging necrosis) and lobular hepatitis are more commonly found in autoimmune hepatitis[9, 10]. In our patient, the liver biopsy revealed giant cell hepatitis with focal and bridging necrosis. The minimal inflammatory reaction in both liver specimens was considered to be related to the steroid therapy. A lymphoplasmocytic infiltrate typical for autoimmune hepatitis was not identified. The liver histology together with the positive Combs' test and anti-smooth muscle antibodies in our patient led to the diagnosis of giant cell autoimmune hepatitis (GCAH).

GCH is a morphologic variant of a nonspecific expression of hepatocellular injury characterized by multinucleated giant hepatocytes. Drugs[1], sickle cell disease[11], hypoparathyroidism[12], hepatitis B virus[2], human immunodeficiency virus[3] and paramyxovirus infection[5] have been reported to be associated with the disease beyond the neonatal period. Autoimmune etiology has been implicated in 40% of the cases with giant cell hepatitis[4, 13]. In this subgroup

of the disease, elevated globulin levels, antinuclear antibodies, antimitochondrial antibodies, anti-smooth muscle antibodies, a positive direct Coombs' test, and circulating lupus anticoagulant have been noted[13, 14]. Our patient had both a positive Coombs' and seropositivity for the anti-smooth muscle antibody, indicating the autoimmune nature of GCH.

Steroid drugs have been used in the past with particular success in the treatment of GCAH. At present, a combination of steroid with azathioprine is the common treatment[7]. Usually, response to therapy is rapid and prolonged, though unresponsive fatal relapses have occurred when the drugs are tapered[6, 7, 13, 14]. During the clinical management, our patient's recurrent and severe hemolytic disease was more troublesome than his liver disease. There was no response to conventional steroid therapy, nor was the high dose MP treatment successful. With the addition of azathioprine, remission first in the hemolytic and then in the hepatic disease was achieved and sustained, so that weaning from the high dose steroid regimen was possible. The second liver biopsy of our patient, taken shortly after azathioprine had been started, demonstrated that histological findings were basically the same, but the degree of fibrosis had, in part, progressed. This might have been due to the partial therapeutic effect of high dose MP. Since a third biopsy had not been taken at the writing of this report, the complete effect of azathioprine on liver histology is not known.

Brichard et al.[7] described a progressive fatal encephalopathy with seizures in one of their patients, with no deterioration in the hematologic and hepatic diseases. The etiology remained unknown in spite of extensive investigations, including a brain biopsy. The encephalopathy was not an indication of brain involvement of an autoimmune multi-system process. During the high dose MP therapy, we observed a brief encephalopathic state with seizures in our patient. Hypertension, as the primary cause of encephalopathy, was probably steroid-induced. The patient responded well to medication. The neurological symptoms in these patients need further confirmation and should be carefully followed in other cases.

In conclusion, GCH is a descriptive pathologic diagnosis. After recognition, evidence should be searched for indication of viral and autoimmune disease. Idiopathic cases need to be looked at also for as-yet-unrecognized causes, since new etiologies and associated conditions are likely. Patients with autoimmune hemolytic anemia should also be examined for signs of liver disease. If present, a liver biopsy should follow, with investigation for other autoimmune markers. Since GCAH is a progressive and fatal hepatic disease, therapy with immunosuppressive drugs, preferably a combination of azathioprine and steroids, should be started as soon as possible.

Acknowledgement

We are indebted to Prof. Bernard Portmann for his excellent comments on liver histology and to Prof. Ali Küpelioğlu for providing the pictures of biopsy specimens.

REFERENCES

1. Pessare D, Degos F, Feldmann G, Degott C, Bernuau J, Benhamou JP. Chronic active hepatitis and giant multinucleated hepatocytes in adults treated with clometacin. Digestion 1981; 22: 66-72.
2. Shinozaki T, Saito K, Shiraki K, HBsAg-positive giant cell hepatitis with cirrhosis in a 10-month-old infant. Arch Dis Child 1981; 56: 64-76.
3. Witzleben CL, Marshall GS, Wenner W, Piccoli DA, Barbour SD. HIV as a cause of giant cell hepatitis. Hum Pathol 1988; 19: 603-605.
4. Thijs JC, Bosma A, Henzen-Logmans SC, Meuwissen SGM. Postinfantile giant cell hepatitis in a patient with multiple autoimmune features. Am J Gastroenterol 1985; 80: 294-297.
5. Philips MJ, Blendis LM, Poucell S, et al. Syncytial giant-cell hepatitis. Sporadic hepatitis with distinctive pathological features, a severe clinical course, and paramyxoviral features. N Engl J Med 1991; 324: 455-460.
6. Bernard O, Hadchouel M, Scotto J, Odièvre M, Alagille D. Severe giant cell hepatitis with autoimmune hemolytic anemia in early childhood. J Pediatr 1981; 99: 704-711.
7. Brichard B, Sokal E, Gosseye S, et al. Coombs-positive giant cell hepatitis of infancy: effect of steroids and azathioprine therapy. Eur J Pediatr 1991; 150: 314-317.
8. Czaja AJ. Autoimmune hepatitis. Evolving concepts and treatment strategies. Dig Dis Sci 1995; 40: 435-456.
9. Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992; 15: 572-577.
10. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. Hepatology 1993; 18: 998-1005.
11. Omata M, Johnson CS, Tong M, Tatter D. Pathological spectrum of liver disease in sickle cell disease. Dig Dis Sci 1986; 31: 247-256.
12. Gustafsson J, Holmberg I, Hardell LI, Foucard T. Hypoparathyroidism and liver disease evidence for a vitamin D hydroxylation defect. Acta Endocrinol 1984; 105: 211-214.
13. Devaney K, Goodman ZD, Ishak KG. Postinfantile giant cell transformation in hepatitis. Hepatology 1992; 16: 327-333.
14. Weinstein T, Valderrama E, Pettei M, Levine J. Early steroid therapy for the treatment of giant cell hepatitis with autoimmune hemolytic anemia. J Pediatr Gastroenterol Nutr 1993; 17: 313-316.

The Turkish Journal of Pediatrics 1997; 39: 573-577

VASOVAGAL SYNCOPE: ASYSTOLE PROVOKED BY HEAD-UP TILT TESTING UNDER SERTRALINE THERAPY*

Mustafa Koray Lenk MD, Dursun Alehan MD*, Şencan Özme MD***
*Alpay Çeliker MD***, Sema Özer MD****

SUMMARY: Lenk MK, Alehan D, Özme Ş, Çeliker A, Özer S. (Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Vasovagal syncope: asystole provoked by head-up tilt testing under sertraline therapy. Turk J Pediatr 1997; 39: 573-577.

Syncope is defined as a sudden transient loss of consciousness. Vasovagally mediated hypotension and bradycardia are believed common, yet difficult to diagnose, causes of syncope in healthy children and adolescents. These episodes are often both sudden and sporadic in nature and, if recurrent and severe (malignant vasovagal syncope), can be a source of morbidity and possibly mortality. Head-up tilt testing has emerged as a useful investigation in patients who are thought to have recurrent vasovagal syncope with systemic hypotension, bradycardia, or both, and it has been suggested as a potential method to test for vasovagal episodes.

Sertraline hydrochloride, a serotonin reuptake inhibitor, has been reported to be effective in preventing the vasovagal syncopal episodes in children and adults. Here, two cases of recurrent, unexplained syncope are presented. Both were under sertraline therapy and underwent provocative head-up tilt testing that resulted in asystole.
Key words: vasovagal syncope, head-up tilt test, childhood.

Recurrent unexplained syncope is a common and often frustrating clinical problem. Syncope annually accounts for up to three percent of all adult emergency room visits and six percent of all hospital admissions[1]. Although the exact incidence of syncope in children is unknown, it is thought to be similar to that in adults. As much as 15 percent of children will experience syncope prior to the end of adolescence[2]. With the introduction of the head-up tilt test in 1986, a significant number of these recurrent unexplained syncopal episodes were attributed to transient periods of vasovagally mediated hypotension and bradycardia[3], which was reported as the leading cause of syncope in children[4].

The purpose of this report is to present two patients with normal hearts who had unexplained syncopal episodes and prolonged duration of asystole with provocative head-up tilt test after having been treated with sertraline hydrochloride.

Case Reports

Case 1

A 16-year-old girl who was previously well had the onset of frequent syncopal episodes consisting of weakness, pallor, dizziness, nausea and losses of

* From the Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.
** Pediatrician and Fellow in Pediatric Cardiology, Hacettepe University Faculty of Medicine.
*** Professor of Pediatrics and Pediatric Cardiologist, Hacettepe University Faculty of Medicine

consciousness for three to four minutes. The episodes were not accompanied by palpitations or other cardiac signs, but myoclonic movements in her hands and urine incontinence were reported. The spells occurred while standing and occasionally appeared to be provoked by stress or pain. There was no history in the family of syncope, epilepsy, deafness or sudden death.

Neurologic and cardiovascular examinations, including electrocardiogram, ambulatory 24-hour Holter monitoring, echocardiogram, treadmill exercise testing, electroencephalogram, and laboratory tests (complete blood count, urinalysis, electrolytes, blood urea nitrogen and glucose), were all normal.

At the first head-up tilt test, the patient's blood pressure was 110/75 mm Hg and her heart rate was 95 beats per minute in the supine position. At the tenth minute of the 60° tilt, she had an extremely abnormal response of a fall in heart rate to 40 beats per minute and in blood pressure to 60/30 mm Hg with reproduction of symptoms and syncope. A mixed type syncope was diagnosed, and she was started on sertraline hydrochloride, 50 mg orally each day. Six weeks later, her response to the head-up tilt test was reevaluated under the same conditions using the same tilt protocol. In the first minute of the 60° tilt, her systolic blood pressure dropped to 60 mm Hg and her heart rate slowed to 50 beats per minute. As she was returned to the supine position she lost consciousness; the electrocardiogram demonstrated a 20-second interval of atrial and ventricular asystole (Fig. 1). With the intiation of external chest compression and intravenous atropine, the asystolic episode was terminated, resulting in a return to sinus rhythm with effective blood pressure and a return of consciousness. Cardioinhibitory syncope was diagnosed and the patient was started on atenolol (a beta-blocking agent), 1 mg/kg/day at once orally. At the writing of this report, she has been without symptoms for eight months.

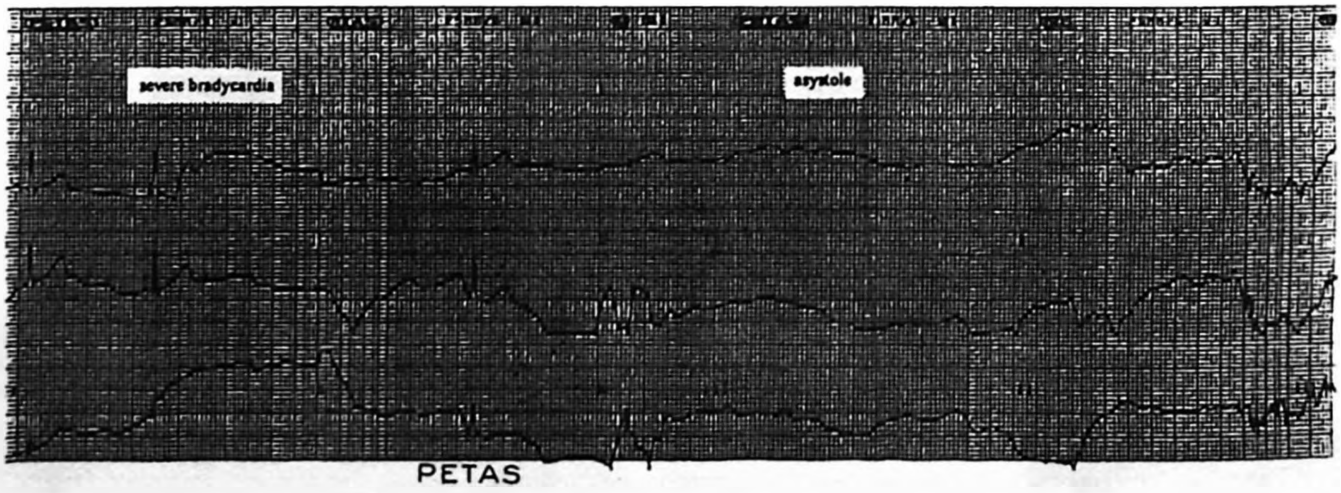


Fig. 1: Severe bradycardia and prolonged asystole during head-up tilt testing, showing a continuous tracing of electrocardiographic leads I, II and III.

Case 2

An 11-year-old girl had the onset of syncopal episodes by the age of seven consisting of pallor, weakness, dizziness and loss of consciousness. She had a total of six episodes. All occurred while standing, lasted for four to five minutes and were not related to situational events such as coughing, fever, defecation or severe pain. The episodes were accompanied by myoclonic movements in her hands and mild cyanosis in her lips. No family member had a history of syncope or sudden death; however, one of her uncles had epilepsy. Evaluations for these recurrent syncopal episodes, including neurologic and cardiovascular examinations, were all normal as in Case 1.

Prior to the initial head-up tilt test, the patient's blood pressure was 105/70 mm Hg and her heart rate was 90 beats per minute in the supine position. Mixed type syncope was diagnosed as her blood pressure and heart rate dropped to 50/30 mm Hg and 50 beats per minute, respectively, with reproduction of symptoms and syncope occurring in the fifteenth minute of the 60° tilt. She was given sertraline hydrochloride therapy, 50 mg orally each day, and six weeks later the response to the head-up tilt test was reevaluated. In the fifth minute of the 60° tilt, her blood pressure dropped to 55/20 mm Hg and an electrocardiogram demonstrated a nine-second interval of atrial and ventricular asystole. Cardioinhibitory syncope was diagnosed and the patient was started on disopyramide phosphate therapy, 150 mg three times daily. She also has been without symptoms, at the writing of this report, for seven months.

Discussion

Vasovagal syncope, also known as "the common faint", is a frequent cause of transient loss of consciousness[3, 4]. It is usually assumed to be a benign disorder with a benign prognosis; however, our two cases illustrate that it can cause major disability and possibly death.

Syncope is probably caused by activation of the Bezold-Jarisch reflex[5], provoked by a reduction in cardiac venous return and high concentrations of circulating catecholamines. The afferent limb of the reflex is transmitted to the A5 area of the medulla in the brain stem through vagal A and C fibers and may be activated by stimulation of ventricular mechanoreceptors caused by vigorous contraction of the relatively empty ventricles. Activation of the reflex causes central vagal stimulation and sympathetic withdrawal, resulting in arterial vasodilation, hypotension, and bradycardia. The head-up tilt test is a very useful diagnostic modality for use with patients with unexplained syncope because a significant number of these patients have neurocardiogenic or vasovagal syncope[2, 3, 6, 7]. In addition to the test's diagnostic value, results obtained during tilting can serve as a guide in assessing the efficacy of pharmacotherapies used to prevent

syncopal episodes[8]. The cause of syncopal episodes in our two cases was unclear despite thorough neurologic and cardiovascular examinations. This report supports the utility of a provocative head-up tilt test in diagnosing vasovagal syncope. The upright tilt table protocol we have used has been described previously[2, 8]. Although the methodological aspects vary[9, 10], the head-up tilt test results are generally characterized as vasodepressor, cardioinhibitory, or mixed, depending on the relative degree of hypotension and bradycardia. Mixed type syncope was diagnosed in our cases with the first head-up tilt test. Occasionally, the cardioinhibitory response may be profound, with asystole (an asystolic response is defined as the development of ventricular asystole of ≥5 seconds' duration). This type of response has been implicated in the occasional patient with neurocardiogenic syncope who has abrupt loss of consciousness during tilting[11]. We observed the same type of response in our patients during second tilting, the results of which have been applied in order to assess the efficacy of the pharmacotherapy given to prevent syncopal episodes.

To date, several pharmacotherapies have been used to prevent clinical and tilt-induced vasovagal syncopal episodes, such as transdermal scopolamine[12], theophyline[13], disopyramide[14], and beta-blockers[15]. Recently, the serotonin (5-hydroxytryptamine) reuptake inhibitor, sertraline hydrochloride, has been reported to be effective in treating up to 53 percent of young patients' neurocardiogenic syncope[16]. This was the only study performed in children. The serotonin pathogenesis in neurocardiogenic syncope is unclear. However, serotonin has been shown to be an important component of the responses seen as a result of acute blood loss, a mechanism essentially similar to that of neurocardiogenic syncope[17]. Abboud[18] recently reported that central intracerebrovascular serotonin induces hypotension, inhibition of renal sympathetic nerve activity and excitation of renal sympathetic activity. He further stated that serotonin acts at a central level to inhibit sympathetic activity and that serotonin antagonists are effective in the treatment of neurocardiogenic syncope. In order to evaluate its efficacy in preventing the syncopal episodes in our patients, we started treatment with sertraline hydrochloride (Lustral, Pfizer) and assessed the response by repeating the tilt test. Development of cardioinhibitory response led us to think the therapy with setraline hydrochloride was not effective in preventing tilt-induced syncope in our patients. Our two cases, the first treated with atenolol and the second with disopyramide, have been without symptoms. For eight and seven months, respectively.

Although the optimum treatment has not been defined for patients with cardioinhibitory vasovagal syncope, both drug treatment and permanent pacing have been used with some success[19, 20]. It seems reasonable to start treatment with pharmacotherapy. Permanent pacing, in which the choice is a dual chamber system, should be considered only if these measures fail.

REFERENCES

1. Kapoor WN. Diagnostic evaluation of syncope. Am J Med 1991; 90: 91-106.
2. Samoil D, Grubb BP, Kip K, Kosinski DJ. Head-upright tilt table testing in children with unexplained syncope. Pediatrics 1993; 92: 426-430.
3. Kenny RA, Ingram A, Bayliss J, Suton R. Head-up tilt: a useful test for investigating unexplained syncope. Lancet 1986; 2: 1352-1355.
4. Özme Ş, Alehan D, Yalaz K, Çakır S, Çeliker A, Özer S. Causes of syncope in children: a prospective study. Int J Cardiol 1993; 40: 111-114.
5. Mark AL. The Bezold-Jarisch reflex revisited: clinical implications of inhibitory reflexes originating in the heart. J Am Coll Cardiol 1983; 1: 90-102.
6. Sra JS, Anderson AJ, Sheikh SH, et al. Unexplained syncope evaluated by electrophysiologic studies and head-up tilt testing. Ann Intern Med 1991; 114: 1013-1019.
7. Alehan D, Çeliker A, Özme Ş. Head-up tilt test: a highly sensitive, specific test in children with unexplained syncope. Pediatr Cardiol 1996 (in press).
8. Grubb BP, Temesy-Armos P, Moore J, Wolfe D, Hahn H, Elliot L. The use of head upright tilt table testing in the evaluation and management of syncope in children and adolescents. Pacing Clin Electrophysiol 1992; 15: 742-748.
9. Almquist A, Goldenberg I, Milstein S, et al. Provocation of bradycardia and hypotension by isoproterenol and upright posture in patients with unexplained syncope. N Engl J Med 1989; 320: 346-351.
10. Fitzpatrick A, Theodorakis G, Vardas P, Sutton R. Methodology of head-up tilt testing in patients with unexplained syncope. J Am Coll Cardiol 1991; 17: 125-130.
11. Maloney JD, Jaeger FJ, Fouad-Tarazi FM, Morris HH. Malignant vasovagal syncope: prolonged asystole provoked by head-up tilt. Cleve Clin J Med 1988; 55: 542-548.
12. Abi-Samra F, Maloney JD, Fouad-Tarazi FM, Castle LW. The usefulness of head-up tilt testing and hemodynamic investigations in the workup of syncope of unknown origin. PACE 1988; 11: 1202-1214.
13. Nelson SD, Stanley M, Love CJ, Coyne KS, Schaal SF. The autonomic and hemodynamic effects of oral theophylline in patients with vasodepressor syncope. Arch Intern Med 1991; 151: 2425-2429.
14. Milstein S, Buetikofer J, Dunnigan A, Benditt DG, Gornick C, Reyes WJ. Usefulness of disopyramide for prevention of upright tilt-induced hypotension-bradycardia. Am J Cardiol 1990; 65: 1339-1344.
15. Sra JS, Murthy VS, Jazayeri MR, et al. Use of intravenous esmolol to predict efficacy of oral beta-adrenergic blocker therapy in patients with neurocardiogenic syncope. J Am Coll Cardiol 1992; 19: 402-408.
16. Grubb BP, Samoil D, Kosinski D, Kip K, Brewster P. Use of sertraline hydrochloride in the treatment of refractory neurocardiogenic syncope in children and adolescents. J Am Coll Cardiol 1994; 24: 490-494.
17. Schadt JC, Ludbrook J. Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. Am J Physiol 1991; 260: H305-318.
18. Abboud FM. Neurocardiogenic syncope. N Engl J Med 1993; 328: 1117-1120.
19. Sra JS, Jazayeri MR, Avitall B, et al. Comparison of cardiac pacing with drug therapy in the treatment of neurocardiogenic (vasovagal) syncope with bradycardia or asystole. N Engl J Med 1993; 328: 1085-1090.
20. Petersen ME, Chamberlain-Webber R, Fitzpatrick AP, Ingram A, Williams T, Sutton R. Permanent pacing for cardioinhibitory malignant vasovagal syndrome Br Heart J 1994; 71: 274-281.

The Turkish Journal of Pediatrics 1997; 39: 579-582

INTERRUPTION OF THE DISTAL LEFT PULMONARY ARTERY WITH PULMONARY ARTERIOVENOUS FISTULAS AND ATRIAL SEPTAL DEFECT*

*Nazlıhan Günal MD***, *Arman Bilgiç MD****, *Dursun Alehan MD***
*Mustafa Koray Lenk MD***

SUMMARY: Günal N, Bilgiç A, Alehan D, Lenk MK. (Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Interruption of the distal left pulmonary artery with pulmonary arteriovenous fistulas and atrial septal defect. Turk J Pediatr 1997; 39: 579-582.

A seven-year-old boy with interrupted left pulmonary artery, arteriovenous fistulas and atrial septal defect is presented. The anomalies were identified by echocardiography and angiography. On pulmonary artery angiograms, left pulmonary artery discontinuity (as though the distal segment had been interrupted), diffuse granulary opacification of right lung fields, and early visibility of the left atrium were observed. *Key words: pulmonary artery anomaly, interruption, arteriovenous fistula.*

Herein we present a seven-year-old boy who had interruption of the distal half of the left pulmonary artery, diffuse pulmonary arteriovenous fistulas, and atrial septal defect. Although anomalies of the pulmonary artery, such as pulmonary artery stenosis at different levels and unilateral absence of a pulmonary artery, have been well described[1-3], our case had a different kind of pulmonary artery anomaly, which can be described as an "interruption". To our knowledge, this is the first reported case in literature of this anomaly in association with pulmonary arteriovenous fistulas and atrial septal defect.

Case Report

A seven-year-old boy was admitted to our hospital with dyspnea, cyanosis and frequent lower respiratory tract infections of five years duration. On physical examination, diffuse cyanosis of a moderate degree, clubbing, and growth retardation were detected. The precordium was prominent with hyperactive right ventricular impulse. On auscultation, a mild ejection murmur was heard at the upper left sternal border, and the second heart sound was increased. Hemoglobin was 16 g/dl, and hematocrit was 54 percent. Chest x-ray showed a moderate degree of cardiomegaly, diffuse reticular infiltration and small rounded opacities in the right lung fields, and a prominent main pulmonary artery segment.

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

** Pediatrician and Fellow in Pediatric Cardiology, Hacettepe University Faculty of Medicine.

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

Electrocardiogram revealed right axis deviation and right ventricular hypertrophy. Two-dimensional echocardiography showed a secundum atrial septal defect and a mildly enlarged main pulmonary artery. Contrast echocardiography demonstrated rapid visibility of the left atrium, and also an atrial septal defect. On M-mode echocardiogram, the right ventricular PEP/VET ratio (pre-ejection period/ventricular ejection time) was greater than 0.3, which was a sign of pulmonary hypertensilon. Tricuspid regurgitation, with a velocity of 2.90 m/sec, was detected by Doppler echocardiography.

Right heart catheterization was performed through the femoral venous route. Systemic arterial oxygen saturation was 78 percent, which was detected in samples obtained from the pulmonary veins. Pressures were the same and increased both in the right and left pulmonary arteries with systolic 60 and diastolic 20 mm Hg. Right and left ventricular systolic pressures were 60 and 80 mm Hg, respectively.

Selective pulmonary angiograms showed multiple small, rounded opacifications disseminated on the right lung fields (Fig. 1). Early opacification of the left atrium was also seen. The angiography of the left pulmonary artery demonstrated an absence of its normal distal portion, as though it were interrupted with a sharp edge. Thin and collateral-like vessels were disseminating throughout the left lung fields from the interrupted left pulmonary artery (Fig. 2). A small atrial septal defect and left-to-right shunt were detected by left atrial angiography and oximetric data. The pulmonary to systemic flow ratio was 1.5/1, and pulmonary vascular resistance was 3 Wood units/m^2.

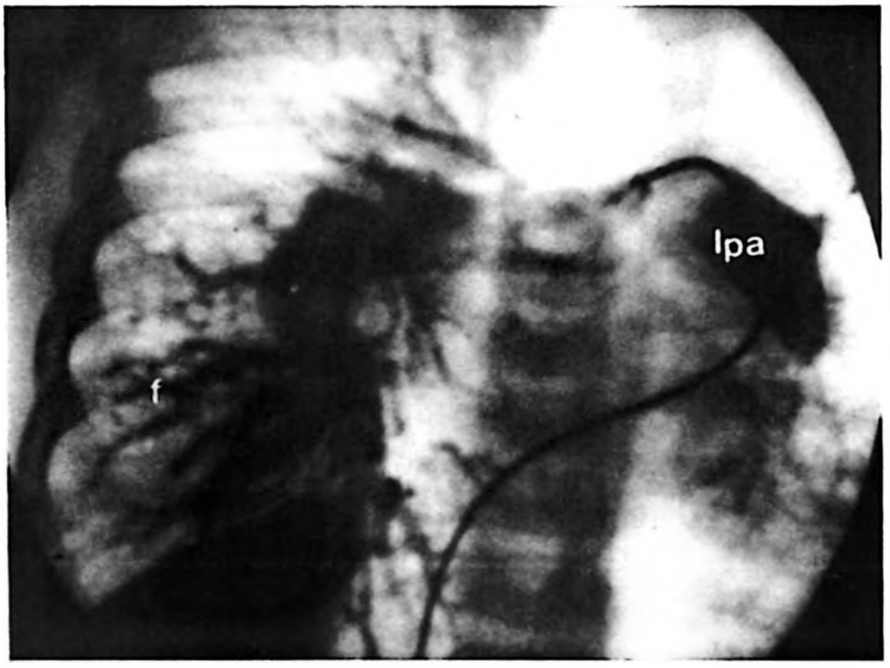


Fig. 1: Pulmonary arteriography in the anteroposterior projection. Multiple opacifications on the right lung fields due to arteriovenous fistulas and discontinuity of the left pulmonary artery are shown. f: arteriovenous fistulas, lpa: left pulmonary artery.

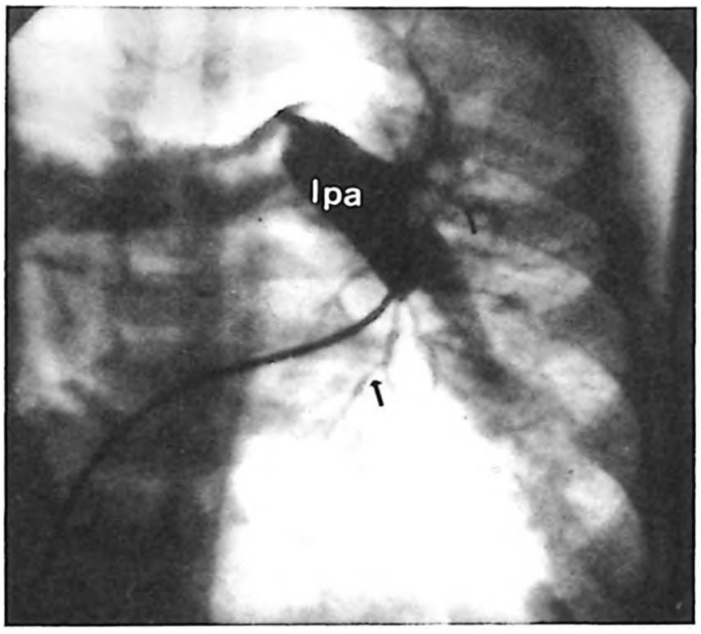


Fig. 2: The left pulmonary artery angiogram in the anteroposterior projection demonstrates the interrupted left pulmonary artery with thin collateral-like vessels (arrows) disseminating throughout the left lung fields. lpa: left pulmonary artery.

Discussion

Unilateral absence of one pulmonary artery, first described in 1868, is an uncommon congenital defect. Other cardiac defects, such as atrial septal defect, patent ductus arteriosus, and truncus arteriosus, have been described with this anomaly[5]. In our patient, the left pulmonary artery was not completely absent: the proximal half of the vessel was normally developed, with even some degree of enlargement, despite the absence of the distal part. Presbitero et al.[2] identified the previously mentioned absent pulmonary arteries on surgical examination and described than as interrupted pulmonary arteries. This also correlated with the pathologic findings of McKim and Wiglesworth[6]. Thus, we described this unilateral left pulmonary artery discontinutiy (or unilateral non-confluent left pulmonary artery with absence of distal part) as interrupted left pulmonary artery[7,8]. In our patient, diffuse pulmonary arteriovenous fistulas on the right lung and a secundum atrial septal defect were also associated with this pulmonary artery anomaly. Pulmonary hypertension was present, but its degree of severity was not correlated with the size of the atrial septal defect.

Pulmonary arteriovenous fistulas are almost always congenital in origin. The disease may also be acquired as a result of many conditions, such as schistosomiasis, juvenile cirrhosis, thyroid carcinoma, and trauma[9]. The majority of patients with pulmonary arteriovenous fistulas have an associated Rendu-Osler-Weber syndrome. Absence of the right lower lobe of the lung and

congenital heart diseases have been described in association with fistulas[10]. In this rare case of pulmonary arteriovenous fistulas with an absence of the distal left pulmonary artery, fistulas may either be congenital in origin, where a combination of these two disorders may be coincidental or, less likely, may be acquired due to hypoperfusion of the left lung.

It is well known that surgical resection is the preferred treatment for arteriovenous fistulas which are not too disseminated. Balloon embolization is an alternative therapy for patients at high risk surgically or whose lesions are too diffuse to resect[11]. New techniques have also been described for surgical repair of unilateral non-confluent pulmonary arteries[12]. Our patient was considered inoperable because of his pulmonary hypertension and diffuse arteriovenous malformations. Congenital heart diseases have been described in association with pulmonary fistulas. However, to our knowledge, this is the first case of a different form of pulmonary artery anomaly (such as an interruption of the left pulmonary artery) with pulmonary arteriovenous fistulas on the other lung, atrial septal defect, and pulmonary hypertension.

REFERENCES

1. Pfefferkorn JK, Löser H, Pech G, Toussaint R, Hilgenberg F. Absent pulmonary artery. A hint to its embryogenesis. Ped Cardiol 1982; 3: 283-286.
2. Presbitero P, Bull C, Haworh SG, de Leval MR. Absent or occult pulmonary artery. Br Heart J 1984; 52: 178-185.
3. Brassard JM, Johnson JE. Unilateral absence of a pulmonary artery. Data from cardiopulmonary exercise testing. Chest 1993; 103: 293-295.
4. Pool PE, Vogel JHK, Blount G. Congenital unilateral absence of a pulmonary artery: the importance of flow in pulmonary hypertension. Am J Cardiol 1962; 10: 706-732.
5. Shakibi JG, Rastan H, Nazarian I, Paydar M, Aryanpour I, Siassi B. Isolated unilateral absence of the pulmonary artery: review of the world literature and guidelines for surgical repair. Jpn Heart J 1978; 19: 439-451.
6. McKim JS, Wiglesworth FW. Absence of the left pulmonary artery: a report of six cases with autopsy findings in three. Am Heart J 1954; 47: 845-859.
7. Ellis K. Fleischner lecture. Developmental abnormalities in the systemic blood supply to the lungs. Am J Roentgenol 1991; 156: 669-679.
8. Morgan PW, Foley DW, Erickson SJ. Proximal interruption of a mainpulmonary artery with transpleural collateral vessels: CT and MR appearances. J Comput Assist Tomogr 1991; 15: 311-313.
9. Kerr A, Sauter D. Acquired traumatic pulmonary arteriovenous fistula: case report. J Trauma 1991; 4: 1301-1302.
10. Dines DE, Seward JB, Bernatz PE. Pulmonary arteriovenous fistula. Mayo Clin Proc 1983; 58: 176-181.
11. Puskas JD, Allen MS, Moncure AC, Wain JC Jr, et al. Pulmonary arteriovenous malformations: therapeutic options. Ann Thorac Surg 1993; 56: 253-258.
12. Cabral RJM. Unilateral absent pulmonary artery: surgical repair with a new technique. J Thorac Cardiovasc Surg 1991; 102: 463-465.

The Turkish Journal of Pediatrics 1997; 39: 583-585

Letter to Editor

PROTEIN C ANTIGEN LEVELS IN TUBERCULOSIS*

*Güler Kanra MD**, *İ. Zafer Ecevit MD****, *Gülten Seçmeer MD***

*Serdar Tuncer MD*****, *Mehmet Ceyhan MD***, *Neslihan Mutluay MD******

*Nihal Başay MD*******

Protein C is a vitamin K-dependent serine protease zymogen. Protein C functions in the vascular system as a potent anticoagulant, due largely to a rapid inactivation of the coagulation factors Va and VIIIa, the cofactors of the two rate-limiting steps of the coagulation cascade[1,2]. deficiencies of protein C, both inherited and acquired, have been described[3,4]. In the protein C deficiency, patients have developed cerebral thrombosis, ophthalmic thrombosis, disseminated intravascular coagulation and purpura fulminans[3-5].

Many investigators have studied protein C antigen levels and activity in various age groups and healthy populations[6,7]. It has been suggested that protein C antigen levels show variation related to age, sex, illness or medication[6-8]. Some of the infectious diseases can cause acquired protein C deficiency. For example, meningococcemia has been reported in association with acquired deficiency of protein C[10].

Because protein C levels in tuberculosis have not been studied to date, we investigated protein C antigen levels in relation to this disease.

In 46 cases of pulmonary tuberculosis, protein C antigen levels were measured by electroimmunodiffusion (EID) assay, according to Laurell[11]. There were no cases of diabetes mellitus, renal or liver disease, or of strokes with venous thrombosis among these patients, nor was any on anticoagulant therapy.

A total of 46 patients (35 male and 11 female) with pulmonary tuberculosis were included in the sutdy. The patients were between 20 and 35 years of age. For 13 normal adults, the mean level of protein C was 99.1 ± 11.8 percent. Individual values of protein C level ranged from 38 to 118 percent of pooled normal plasma. The mean level of protein C antigen was 70.3 ± 19.9 percent in all patients. The actual ranges and the means ± standard deviation (SD) ranges for male and female patients are shown in Table I.

* From the Pediatric Infectious Disease Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

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

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

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

***** Specialist of Chest Disease, Atatürk Chest Diseases Hospital, Ankara.

Table I: Mean ± SD of Protein C Levels in Female and Male Patients

Patients	Number of Patients	Protein C Level (%)	
		Range	Mean ± SD*
All patients	46	38-118	70.26 ± 19.90
Males	35	38-116	69.65 ± 18.75
Females	11	43-118	72.18 ± 24.11

* $p > 0.05$ (Mann-Whitney U test).

The mean protein C level for males was not significantly lower than that for females. There was not a statistically significant difference between male and female patients.

Protein C was significantly decreased (less than 70% of normal) in 23 (50%) of 46 patients. Eight (17%) patients had protein C levels less than 50 percent of normal. Only one patient had a protein C level under 40 percent of normal.

Since activated protein C plays an important regulatory role in blood coagulation, reduced levels of protein C result in thromboembolic disease[3-6]. Thrombophlebitis or recurring pulmonary emboli often appear later in adolescents or in young adults with protein C deficiency. In affected individuals, these findings are typical but not necessarily indications of protein C deficiency[3, 5, 7]. Congenital protein C deficiency occurs but at a low frequency and results in fatal neonatal thrombosis characterized by purpura fulminans[6]. A great variety of disease states resulting from alterations in protein C have been documented. Decreases in protein C are found to be associated with liver disease, disseminated intravascular coagulation, major surgery and use of oral anticoagulants, while increases in protein C are shown in nephrotic syndrome, in pregnancy and in individuals taking oral contraceptives[4, 9, 12]. Potential variation of protein C in relation to other diseases is limited.

This is the first study in literature about protein C levels and pumonary tuberculosis, in which mean values of protein C were normal in patients with tuberculosis but individual levels varied. In cases of acquired protein C deficiency, protein C antigen levels were usually less than 50 percent of normal. In this study, 17 percent of the patients had protein C levels less than 50 percent of normal. Although very low levels of protein C were not detectable, these findings suggest that patients with tuberculosis have low levels of protein C. Thus protein C levels can be evaluated in patients with tuberculosis, especially those with thromboembolic disease, and this study can be taken as a basis for future assessment.

REFERENCES

1. Esmon CT. The roles of protein C and thrombomodulin in the regulation of blood coagulation. J Biol Chem 1989; 264: 4743-4746.
2. Stenflo J. Structure and function of protein C. Semin Thromb Hemost 1994; 10: 109-121.
3. Broekmans AW, Veltkamp JJ, Bertina RM. Congenital protein C deficiency and venous thromboembolism. A study of three Dutch families. N Engl J Med 1983; 309: 340-344.

4. Griffin JH, Mosher DF, Zimmerman TS, Kleiss AJ. Protein C, an antithrombotic protein, is reduced in hospitalized patients with intravascular coagulation. Blood 1982; 60: 261-264.
5. Marlar RA, Montgomery RR, Broekmans AW. Diagnosis and treatment of homozygous protein C deficiency. Report of the Working Party on Homozygous Protein C Deficiency of the ICTH Subcommittee on Protein C and S. J Pediatr 1989; 114: 528-534.
6. Marlar RA, Montgomery RR, Broekmans AW. Report on the diagnosis and treatment of homozygous protein C deficiency. Thromb Haemost 1989; 61: 529-531.
7. Miletich J, Sherman L, Broze G Jr. Absence of thrombosis in subjects with heterozygous protein C deficiency. N Engl J Med 1987; 317. 991-996.
8. Tait RC, Walker ID, Islam SI, et al. Protein C activity in healthy volunteers-influence of age, sex, smoking and oral contraceptives. Thromb Haemost 1993; 70: 281-285.
9. Vigano-D'Angelo S, Comp PC, Esmon CT, D'Angelo A. Relationship between protein C antigen and anticoagulant activity during oral anticoagulation and in selected disease states. J Clin Invest 1986; 77: 416-425.
10. Fourrier F, Lestavel P, Chopin C, et al. Meningococcemia and purpura fulminans in adults: acute deficiencies of proteins C and S and early treatment with antithrombin III concentrates. Intensive Care Med 1990; 16: 121-124.
11. Laurell CB. Electroimmuno assay. Scand J Clin Lab Invest 1972; 29 (Suppl 124). 21-37.
12. Pabinger-Fasching I, Lechner K, Niessner H, Schmidt P, Balzar E, Mannhalter C. High levels of plasma protein C in nephrotic syndrome. Thromb Haemost 1985; 53: 5-7.

The Turkish Journal of Pediatrics 1997; 39: 587

To the Editor

ABOUT SALMONELLA GASTROENTERITIS

Şinasi Özsoylu MD[*]

I have read with interest Dr. Akan et al.'s paper entitled "Antibiotic susceptibilities....." which was published in the January-March 1997 issue of the Journal (1997; 39: 7-11).

Most of the Salmonella serotypes (289 of 309) were isolated from children with gastroenteritis. Since antibiotics are generally not recommended for salmonella gastroenteritis, as mentioned by the authors, with the exception of prematures, neonates, and malnourished and immunocompromised patients, why was antibiotic resistance studied in this form of the disease? Would it not give the impression to the practitioner that such studies are required? Since this study was done, may I ask the percentage of multi-resistance among the study group?

Salmonella typhi was isolated in 20 patients according to text but tabulated as 21 in Table II and III. I believe it needs clarification.

Would the authors agree with my conclusion that chloramphenicol (because of its effectiveness and cost) is still the drug of choice for enteric fever (without looking for antibiotic resistance)?

* Professor of Pediatrics and Hematology. Fatih University Faculty of Medicine, Ankara.

To the Editor

Özay Akan MD[*]

Thanks to Dr. Özsoylu for his interest in the paper about "Antibiotic susceptibilities....". Antibiotic susceptibility tests are not only performed for the treatment of infections but may also be performed for epidemiologic purposes or to learn the antibiotic resistance rates of a region in case of empirical antibiotic use[1]. In the discussion, it is clearly stated that antimicrobial therapy is not indicated in Salmonella gastroenteritis and is only limited to a special group of patients, so I do not believe that antibiotic susceptibility results presented in the study will give the wrong information to physicians. It may be helpful to choose the right antibiotic until the susceptibility results are available. In Tables II and III there is a print mistake; the number of S. typhi isolates should be 20 instead of 21. Thanks to Dr. Özsoylu for his special attention.

I think that the decision of whether or not to choose chloramphenicol in the treatment of enteric fever is far from the aim of this study, since only in vitro susceptibility results are presented. For the selection of antibiotics, other important factors should also be known, such as the clinical efficacy of antimicrobials, the effects on the duration of excretion of the pathogen, and relapse rates. I think with good cooperation between clinicians and clinical microbiology laboratories such protocols can be readily constructed among children in Turkey.

REFERENCES

1. Jorgensen JH, Sahm DF. Antimicrobial susceptibility testing. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds). Manual of Clinical Microbiology (8th ed). Washington: American Society for Microbiology; 1995: 1277.

* Associate Professor of Infectious Diseases and Clinical Microbiology

The Turkish Journal of Pediatrics 1997; 39: 591

To the Editor

ANTIBIOTIC SUSCEPTIBILITY OF ENTEROCOCCI

Şinasi Özsoylu MD[*]

I read with interest Dr. Akan et al.'s paper entitled "Antibiotic susceptibilities of enterococci isolated from Turkish children" in the January-March 1997 issue of the Journal (1997; 39: 13-17).

I wished that researchers from the infectious disease department would give us not only microbiologic results but also, at the same time, the clinical relevance of these important studies.

The authors stated in their paper, "For years enterococci have been considered as harmless inhabitants of the gastrointestinal tracts of human and animals", and presented their work on bacterias isolated from urine, wounds and blood. Does it mean, their initial phrase changed over the years?

If "enterococci do not possess the comon virulence factors", what does "intrinsic resistance factors" mean with regard to these bacteria?

I suppose 1954 on page 14 would be 1994.

* Professor of Pediatrics and Hematology, Fatih University Faculty of Medicine, Ankara.

To the Editor

*Özay Akan MD**

Thanks to Dr. Özsoylu for his interest in our study about the "Antibiotic susceptibilities of enterococci isolated from Turkish children". Enterococci are selected in the study since they are one of the pathogens which cause nosocomial infections at increasing rates[1]. Enterococci mostly cause urinary tract, wound and bloodstream infections (the clinical syndromes which are common in different bacteria). I believe that antibiotic susceptibility patterns of enterococci are more helpful than clinical data in the treatment of such infections, since one of the most important points about the enterococci is their antibiotic resistance which causes difficulties in the treatment.

Enterococci do not possess the common virulence factors like many other bacteria, and the intrinsic resistance to multiple antimicrobial agents (due to diminished affinity of PBPs or decreased permeability of the cell wall) is not a classical virulence factor[2]. As they are resistant to most of the common antibiotics, they survive and proliferate and cause endogenous and exogenous infections.

1954 on page 14 should be 1994.

REFERENCES

1. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. Am J Med 1991; 91 (Suppl 3B): 72.

2. Moellering RC. Enterococcus species, Streptococcus bovis and Leuconostoc species. In. Mandell GL, Bennett JE, Dolin R (eds). Principles and Practice of Infectious Diseases (4th ed). New York: Churchill Livingstone Inc; 1995: 1826.

* Associate Professor of Infectious Diseases and Clinical Microbiology.

ANNOUNCEMENTS

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

BURSA, 2-5 NOVEMBER 1997
VIIth NATIONAL ALERGY AND CLINICAL IMMUNOLOGY CONGRESS

Inquiries to: Prof.Dr. Nihat Sapan
PK: 9 Ulucami Şb.
Bursa
TURKEY
Phone : 0224 442 84 Ext. 1047
442 85 62
Fax : 0224 442 85 62
e-mail : nsapan@uu20.bim.uludag.edu.tr

İSTANBUL, 12-15 NOVEMBER 1997
XXVth NATIONAL HEMATOLOGY CONGRESS and
IIth POSTGRADUATE HEMATOLOGY COURSE

Inquiries to: Prof.Dr. Haluk Koç
Prof. Dr. Hamdi Akan
Ankara Üniversitesi
Tıp Fakültesi
İbni Sina Hastanesi
06100 Samanpazarı, Ankara
TURKEY
Phone : 0312 310 33 33 Ext. 2121, 2122, 2123
Fax : 0312 311 54 74

NICE, 20-22 NOVEMBER 1997
XXIVth CONGRESS OF UNION OF MIDDLE EASTERN AND MEDITERRANEAN PEDIATRIC SOCIETIES (UMEMPS)

Inquiries to: Alp Reyal Tourism and Trade Inc.
İzmir Cad. No: 36/4
06440 Kızılay, Ankara
TURKEY
Phone : 0312 425 71 91
Fax : 0312 418 32 14

ANKARA, 8-12 DECEMBER 1997

Ist NATIONAL ENVIRONMENTAL MEDICINE CONGRESS
NEW FRONTIERS IN ENVIRONMENTAL HEALTH

Inquiries to: Prof. Dr. Çağatay Güler
I. Ulusal Çevre Hekimliği Kongresi
Genel Sekreteri
Hacettepe Üniversitesi
Halksağlığı Anabilim Dalı
06100 Hacettepe, Ankara
TURKEY
Phone : 0312 324 39 75
0532 211 07 45
Fax : 0312 311 00 72

MADRID, 22-24 JANUARY 1998

VIIIth INTERNATIONAL SYMPOSIUM ON VIRAL HEPATITIS

Inquiries to: Dr. Inmaculada Castillo
Department of Hepatology
Fundacion Jimenez Diaz
Avda. Reyes Catolicos 2
E-28040 Madrid
SPAIN
Phone : +34 1 543 19 64
Fax : +34 1 544 92 28

AMSTERDAM, 9-14 AUGUST 1998

XXIInd INTERNATIONAL CONGRESS OF PEDIATRICS

Inquiries to: Congress Secretariat
XXII. International Congress of Pediatrics
Eurocongres Conference Management
Jan van Goyenkade 11
1075 HP Amsterdam
The NETHERLANDS
Phone : +31 20 679 3411
Fax : +31 20 673 7306
e-mail : eurocongres@rai.nl

CHA - AM, PETCHABURI 15-18 NOVEMBER 1998

IIIrd ASIA-PACIFIC REGIONAL MEETING OF THE INTERNATIONAL SOCIETY FOR NEONATAL SCREEN
NEONATAL SCREENING IN THE 21st CENTURY

Inquiries to: The Congress Secretariat
3rd 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

TRIBUTE TO REVIEWERS FOR VOLUME 39

Our journal depends on reviewers for their generous donations of time, effort and expertise. We thank them for their great contribution to maintaining the journal's high standards.

AKALAN Nejat, MD
AKOVA Murat, MD
AKYÜZ Canan, MD
ALTAY Çiğdem, MD
ARSLAN Serap, MD
AYSUN Sabiha, MD
BAKKALOĞLU Ayşın, MD
BALCI Sevim, MD
BALKANCI Ferhun, MD
BERKEL İzzet, MD
BEŞBAŞ Nesrin, MD
BÜYÜKPAMUKÇU Münevver, MD
CEYHAN Mehmet, MD
COŞKUN Turgay, MD
ÇELİKER Alpay, MD
DEMİRCİN Metin, MD
DOĞAN Rıza, MD
ECEVİT Zafer, MD
ERDEM Gülşen, MD
ERSOY Fügen, MD
GÖÇMEN Ayhan, MD
GÜMRÜK Fatma, MD
GÜRGEY Aytemiz, MD
HİÇSÖNMEZ Akgün, MD
İMAMOĞLU Ayten, MD
KALE Gülsev, MD
KALYONCU Fuat, MD
KANDEMİR Nurgün, MD
KİPER Nural, MD
KOÇAK Nurten, MD
KUTLUK Tezer, MD
MUŞDAL Yaser, MD
OĞUZ Aynur, MD
OLGUNTÜRK Rana, MD
ORAN Olcay, MD
ÖNERCİ Metin, MD
ÖZALP İmran, MD
ÖZCAN Osman Ekin, MD
ÖZÇELİK Tayfun, MD
ÖZÇELİK Uğur, MD
ÖZDEMİR Nasır, MD
ÖZDİRİM Emire, MD
ÖZEN Hasan, MD
ÖZEN Seza, MD
ÖZER Sema, MD
ÖZGÜÇ Meral, PhD
ÖZKUTLU Süheyla, MD
ÖZME Şencan, MD
ÖZSOYLU Şinasi, MD
SAATÇİ Ümit, MD
SANAL Özden, MD
SARAÇLAR Muhsin, MD
TEZCAN İlhan, MD
TOKATLI Ayşegül, MD
TOKGÖZOĞLU Mazhar, MD
TOPALOĞLU Rezzan, MD
TOPÇU Meral, MD
TUNCER Ayfer, MD
TUNCER Murat, MD
TUNÇBİLEK Ergül, MD
ÜNAL Serhat, MD
ÜNSAL Metin, MD
YETGİN Sevgi, MD
YÜCE Aysel, MD
YURDAKÖK Kadriye, MD
YURDAKÖK Murat, MD

AUTHOR INDEX TO VOLUME 39

ARTICLE INDEX TO VOLUME 39

KEY WORD INDEX TO VOLUME 39

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).