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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

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PERSISTENT DIFFUSE PERITONITIS IN CHILDREN*

*Güngör Karagüzel MD***, Feridun Cahit Tanyel MD****

*Mehmet Emin Şenocak MD****, Nebil Büyükpamukçu MD****

*Akgün Hiçsönmez MD****

SUMMARY: Karagüzel G, Tanyel FC, Şenocak ME, Büyükpamukçu N, Hiçsönmez A. (Department of Pediatric Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey). Persistent diffuse peritonitis in children. Turk J Pediatr 1998; 40: 151-158.

In adults, persistent diffuse peritonitis (PDP) having a special microbiologic spectrum is now defined as a distinct intraabdominal infection because of its aggressive clinical course. Considering also other types of peritonitis, this study was performed to determine characteristics of childhood PDP in regard to clinical picture, microbiologic features, treatment and outcome. Classification of 175 patients with peritonitis showed that nine patients had primary peritonitis and 121, 37 and eight patients had secondary peritonitis, PDP, and intraabdominal abscess, respectively. rates of host defense affecting disease, extra-appendicular origin, and mortality were markedly higher in the PDP group. However, polymicrobic and anaerobic infection rates were lower in the PDP group than those of the secondary peritonitis group. While 26 of 37 patients with PDP underwent surgical interventilon, the remaining 11 patients were managed by conservative measures. In the PDP group, mortality was 18 percent for conservatively and 23 percent for surgically treated patients. Being of young age, presence of an accompanying disease and fungal growth increased the mortality. The results indicate that PDP is a distinct type of peritonitis in children as well as in adults. In addition, accurate identification of these patients may prevent some unnecessary operations and improve survival by the choic of more conservative treatment plans. *Key words: peritonitis, intraabdominal infection, conservative surgery.*

Because of its high morbidity and mortality, peritonitis is still one of the most important therapeutic problems in medical practice. In recent years, to achieve more effective treatment with low morbidity and mortality, new classification and stratification systems for intraabdominal infections have been recommended[1-3].

From these studies, peritonitis is suggested to progress into persistent diffuse peritonitis (PDP) in some patients who are unable to control an infection either because of poor host defense mechanisms or highly virulent pathogens[4, 5]. PDP, also known as tertiary peritonitis, has a different microbiological spectrum and its treatment is a controversial issue, although more aggressive management has been advocated in adults[6, 7].

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

** Pediatric Surgeon, Hacettepe University Faculty of Medicine.

*** Professor of Pediatric Surgery, Hacettepe University Faculty of Medicine.

In English literature, we could not find any clinical study on childhood PDP. Our impression is that the definition and characteristics of childhood PDP, and the aspects differentiating it from other types of peritonitis, are not clear. The present study was planned in order to elucidate these unclear points of childhood peritonitis.

Material and Methods

One hundred and seventy-five patients diagnosed with peritonitis from 1988 to 1993 were retrospectively evaluated in this study. Diagnosis of peritonitis was established with the presence of at least two of three criteria including clinical background, microbiological studies, and operative findings. Fever, abdominal pain, vomiting, failure to pass stool and findings of acute abdomen on physical examination constituted clinical background of the diagnosis. Microbiological data included aerobic, anaerobic, and fungal cultures. During laparotmy, presence of purulent fluid or fibrin plaques in the peritoneal cavity together with an edematous and fragile peritoneal surface were regarded as operative diagnostic features. According to the following criteria modified from Wittmann's classification[3], the patients were grouped as primary peritonitis, secondary peritonitis, PDP, and intraabdominal abscess.

The patients who had peritonitis originating from a suspected extraperitoneal source (intraperitoneal origin was excluded by laparotomy) were included in the primary peritonitis group. The secondary peritonitis group was established from the patients who had peritonitis resulting from an apparent intraabdominal source proven operatively, such as perforation of the gastrointestinal tract, leakage of an anastomosis of penetrating abdominal trauma. The patients with prolonged peritonitis (beyond 15 days) who were under conservative treatment (intestinal resting, intravenous nutrition and antibiotics) constituted the PDP group. Patients with intraabdominal abscess confirmed by ultrasonography and/or computed tomography were regarded as having localized peritonitis and included in the classification as a distinct group.

All charts of the patients were further reviewed to find clinical characteristics, type of management, clinical course, and outcome. Accompanying diseases, anatomical sources of peritonitis and causative microorganisms were also noted and compared. Statistical analyses were performed using chi-square and Fischer's exact tests (p value of less than 0.05 was considered statistically significant).

Results

While nine (5%) of the 175 patients fulfilled the criteria to be included in the primary peritonitis group, 121 (69%), 37 (21%), and eight (5%) patients fulfilled the criteria for the secondary peritonitis, PDP and intraabdominal abscess groups, respectively (Table I).

Table I: Age (mean ± SD) and Sex Distribution of 175 Patients with Peritonitis (F: Female, M: Male)

	1-28 Days		29 Days-12 Months		13 Months and Up	
Groups	n	Age (Day)	n	Age (Month)	n	Age (Year)
Primary peritonitis (n = 9, F = 4, M = 5)	–	–	–	–	9	10.0 ± 3.71
Secondary (n = 121, F = 70, M = 51)	7	4.43 ± 3.15	12	5.83 ± 3.04	102	9.69 ± 3.81
PDP (n = 37, F = 20, M = 17)	6	20.17 ± 4.88	11	5.18 ± 3.09	20	9.60 ± 4.62
Intraabdominal abscess (n = 8, F = 4, M = 4)	–	–	1	5	7	5.71 ± 3.55

The ratio of boys to girls was 1.3 and the average age was seven years at the time of diagnosis, with a range of one day to 19 years. There was no significant difference among the groups for sex distribution ($p > 0.05$). Primary peritonitis was not seen in the newborns and infants. The majority of the patients with secondary peritonitis (84.3%) and intraabdominal abscess (87.5) were older than one year of age. However, about half (46%) of the patients with PDP were younger than one year of age and this difference was statistically significant ($p < 0.001$). In primary peritonitis, microbiological results revealed no growth in four patients, E.coli in three patients, and Streptococcus in two patients. Major pathogens were E. coli, Klebsiella, Proteus, Bacteriodes and Fusobacterium in secondary peritonitis, with the rate of 18.2 percent of no growth. Pseudomonas and Staphylococcus were the main pathogens responsible for PDP (Table II).

Table II: Subgroups of Patients with PDP According to Microbiological Data

Subgroups	No. of Patients	%
PDP with low-grade pathogenic bacteria*	(20)	(54)
Pseudomonas	10	
Staphylococcus	6	
Enterococcus	3	
Other**	4	
PDP with fungi	(3)	(8)
Candida	3	
PDP without pathogen	(14)	(38)
Total	37	100

* 23 microorganisms were identified in 20 patients.
** 2 streptococci, one E. coli Propionibacterium.

Polymicrobial and anaerobic infection rates in the PDP group were 8.1 and 5.4 percent respectively. However, these rates were 37.2 and 19 percent, respectively in the secondary peritonitis group, and 12.5 and 25 percent, respectively, in the intraabdominal abscess group. Both differences were statistically significant ($p < 0.001$). There was no polymicrobial or anaerobic growth in patients with primary peritonitis, and the number of sterile culture results was significantly lower in secondary peritonitis than in the primary peritonitis, PDP and intraabdominal abscess groups ($p < 0.05$).

Besides microbiological data, anatomical sources also showed differences between secondary peritonitis and PDP. While appendicular origin was the most common in the secondary peritonitis group, PDP most commonly originated from the colon (Table III). Furthermore, an accompanying disease was more common in primary peritonitis (44.4%) and PDP (27%) than in secondary peritonitis (7.4%) and intraabdominal abscess (0%) (Table IV). These differences were statistically significant ($p < 0.005$).

Table III: Anatomical Sources of Secondary Peritonitis and PDP

	Secondary Peritonitis		PDP	
Sources	n	%	n	%
Stomach and duodenum	4	3.3	1	2.7
Jejunum and ileum	11	9.1	10	27.0
Appendix	92	76.0	4	10.8
Colon	10	8.3	12	32.5
Other*	4	3.3	3	8.1
Unknown**	–	–	7	18.9
Total	121	100.0	37	100.0

* Biliary and pancreatic origins.
** Non-operated patients.

Table IV: Accompanying Diseases in Patients with Primary Peritonitis and PDP

Accompanying Disease	No. of Patients	%
Primary peritonitis (n: 9)	(4)	(44.4)
nephrotic syndrome	2	22.2
liver pathology	1	11.1
urinary infection	1	11.1
Persistent diffuse peritonitis (n: 37)	(10)	(27.0)
non-Hodgkin's lymphoma	4	10.8
leukemia	3	8.1
IgM deficiency	1	2.7
Henoch-Schonlein purpura	1	2.7
chronic renal failure	1	2.7

Analysis of the charts indicated that conservative treatment (parenteral nutrition, nasogastric decompression and combined antibiotic therapy-a penicillin derivative, an aminoglycoside and one of ornidazole, metronidazole or clindamycin) was initially tried in all patients with PDP. Antibiotics were changed according to the clinical status and sensitivity reports. If fever, leukocytosis, abdominal tenderness or findings of intestinal obstruction persisted or worsened during conservative treatment, patients underwent surgical intervention.

Twenty-six (70%) patients with PDP underwent operations to overcome the diffuse peritoneal inflammation. In these patients, peritoneal drainage, lavage or debridement was done in various combinations. Peritoneal drainage was employed in 23 patients and it was maintained postoperatively through Penrose, sump or hemovac drains. Peritoneal lavage using isotonic saline was performed only intraoperatively in 21 patients. Peritoneal debridement was applied to 10 patients in a limited manner to remove gross debris, necrotic tissues, and fibrin plaques by minor surgical trauma. The remaining 11 (30%) patients with PDP were managed by conservative measures. Four of them had persistent diffuse postoperative peritonitis and the remaining seven patients had PDP of unknown origin (4 with malignant disease and 3 newborns with necrotizing enterocolitis). Presence of a high risk for anesthesia or a life-threatening extraabdominal pathology contributed to the selection of conservative management in these patients.

Mortality rates were 0.0 percent in primary peritonitis, 9.5 percent in secondary peritonitis, 22 percent in PDP, and 12.5 percent in intraabdominal abscess groups. The difference between mortality rates of secondary peritonitis and PDP was significant ($p < 0.05$). Eight patients died in the PDP group and all had sepsis in conjunction with generalized failure of host defence or multiple system-organ dysfunction consisting of renal, hepatic, pulmonary or cardiovascular failure. The highest mortality was seen in the newborn period (50%) and followed by existence of an accompanying disease affecting host defense (40%). Mortality of fungal PDP was 33 percent, PDP of infancy 27 percent and low-grade pathogen-originated PDP 25 percent. Survival was the best in older children (10%) and aseptic PDP (14%). Additionally, although the difference was not significant ($p > 0.05$), the patients treated with a non-operative approach had a lower mortality (18%) than those surgically treated (23%).

Discusion

When the above-mentioned results were considered, it could be proposed that PDP has different clinical characteristics and outcomes than other types of peritonitis. Age distribution, accompanying diseases and anatomical sources were considerably different in patients fulfilling the criteria of PDP and mortality was also higher in the PDP group. Therefore, as have some authors dealing

with adulthood peritonitis, we also claimed that PDP constitutes a specific group of childhood peritonitis. Critical reviews of the topic support that secondary peritonitis is the most common type of intraabdominal infections, followed by primary peritonitis[5, 8]. From our paper, PDP replaces primary peritonitis and becomes the second most common type of peritonitis. This is an important finding from nosologic and epidemiologic perspectives. However, we are unable to compare our data because there has been no previous study concerning this subject in children.

It is generally possible to differentiate primary peritonitis from secondary through typical clinical, diagnostic and operative findings. However, the border between secondary peritonitis and PDP is not always obvious in some of the patients. Besides the microbiological data presented herein, persisting clinical findings of peritoneal inflammation is the basic feature for PDP[4, 9]. While the microbiological criteria of PDP are defined well in adult patients, definition of the persistence is not clear. Our experiences on peritonitis support that the criterion of 15 days is an acceptable period for persistence. In such cases, the correct and early prediction of PDP is important because failure to diagnose carries an increased morbidity and mortality. Furthermore, some patients with PDP may not initially require an urgent operation as do those with secondary peritonitis, and conservative treatment may be sufficient in these critically ill children. Consequently, a correct diagnosis may prevent some unnecessary operations for PDP.

Supporting our findings, microbiological data pertaining to the patients with secondary peritonitis show high anaerobic and polymicrobial infection rates[5, 10, 11]. On the other hand, PDP has a different microbiologic spectrum. S.epidermidis and antibiotic-resistant Gram-negative microorganisms (Pseudomonas, Enterococcus, etc.), rather than E. coli and B. fragilis which are typical in the early phase of the disease, have been identified from adult patients with PDP[4, 5]. Nevertheless, the microbiological spectrum of PDP has not yet been clearly documented in children. Because we used the criteria of adulthood PDP, our study has a limited contribution on the microbiology of PDP. Therefore, it would be better if microbiological characteristics of childhood PDP were clarified by prospective studies.

Candida is infrequently identified as a primary pathogen of intraabdominal infections, but it has become recognized as a prominent pathogen under broad spectrum antibiotic and immune depressant therapy[5, 8]. Additionally, fungal peritonitis has a morbid or mortal course[12]. In our series, although the number of patients with fungal peritonitis was relatively small, fungi were responsible for the highest mortality when the causative microorganism was considered. In contrast, PDP without pathogens had a better survival rate than the other two subgroups. In this regard, a more competent immune system may prevent the growth of pathogens and may play a role in the better survival rate of these patients.

Surgical management of PDP is another controversial topic. Some aggressive therapeutic alternatives, including continuous peritoneal drainage and lavage, radical peritoneal debridement, open abdomen and planned abdominal reexplorations, have been advocated to treat patients with PDP[13-15]. In addition to the surgical procedures, broad spectrum antimicrobial therapy that is effective on all possible microorganisms has been recommended in the management of PDP[16, 17]. While our policy on antibiotherapy agreed with this conventional practice, our surgical choices remained more conservative than recommended ones. Despite this conservative approach, our results related with mortality were somewhat better than in adults who had been subjected to more aggressive therapeutic modalities. Recently, some reports in favor of more conservative surgery have been published[18, 19]. Such equivocal results require further studies to determine the ideal treatment method of childhood PDP.

To evaluate both severity of peritonitis and prognosis, various scales such as Peritonitis Index Altona, Acute Physiology Score and especially Acute Physiology and Chronic Health Evaluation Score (APACHE II or III) have been used in adult patients[1, 5, 20, 21]. Considering these scales, establishment of a modified system for children may provide a more rational approach to childhood peritonitis. Although we did not use a scoring system, our study supported that mortality risk of PDP is higher in younger infants, in the existence of an additional disease affecting host defense and in fungal origin. Current experiences with neonatal infection suggest that survival is now significantly better as a result of skillful neonatal intensive care[22]. However, poor prognosis has been reported for neonatal peritonitis, and PDP still carries a high mortality risk in younger infants because of the underdeveloped immune system[23].

Since it has a special clinical picture, PDP also looks like a different type of peritonitis in children, such as it is defined in adults. Identification of these cases from the patients with prolonged peritonitis and use of therapeutic modalities including more conservative measures may decrease mortality risk. Finally, we hope that our preliminary experience presented here will call to mind some overlooked points about childhood peritonitis and stimulate prospective clinical studies on the subject.

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DEMOGRAPHIC FEATURES AND PSYCHOSOCIAL PROBLEMS IN SINGLE – PARENT FAMILIES: 1985 TO 1995*

*Ferhunde Öktem PhD***

SUMMARY: Öktem F. (Department of Child and Adolescent Psychiatry, Hacettepe University Faculty of Medicine, Ankara, Turkey). Demographic features and psychosocial problems in single-parent families: 1985 to 1995. Turk J Pediatr 1998; 40: 159-166.

Single-parent families and children seen in Hacettepe University's Department of Child and Adolescent Psychiatry in 1985 and 1995 were evaluated. Divorce was the most frequent reason for single parenthood. Children of divorced families experienced a higher number and severity of symptoms, and a longer delay before applying for medical help. Oppositional defiant behavior was at equal frequency in all groups, but suicide attempts, school failure due to psychological causes, conduct disorder, and somatoform disorders were more frequent in divorced families. Divorced mothers were more likely to have a higher level of education and were more likely to work. From 1985 to 1995, mothers' age and educational level increased; the number and age of children decreased. Studies on such families are indicated to determine risk factors for psychosocial problems and to develop preventive measures. *Key words: single parent, divorce, separation.*

Most studies on children living with a single parent concentrate on divorced families. However, many single-parent situations also result from death or separation due to various causes, including work. Loss of a parent, due to any reason, is traumatic for a child[1].

Many factors affect the adjustment of the child to the new situation and his/her coping with this stress: relationship with the missing and remaining parents, interaction and harmony between parents, and financial situation[1,2]. The presence of a stepparent as a factor is controversial: some reports indicate a detrimental effect on the child-parent relationship while others suggest that the formation of a healthy couple might benefit the child's identification problem and also biring financial security[2,3]. In divorce or separation, the frequency of visits with the other parent also has an impact on the adjustment of the child.

Children raised by a single parent are more likely to show psychological problems in the future[4]. They are less successful at school than children with two parents[5]. Children of divorced families have lower self-esteem and weaker skills for self-sufficiency and coping[6].

* From the Department of Child and Adolescent Psychiatry, Hacettepe University Faculty of Medicine, Ankara.

** Associate Professor of Clinical Psychology, Hacettepe University Faculty of Medicine.

The reason for the single-parent status seems important in determining the nature of the problems. Single parenthood resulting from separation or divorce creates important psychosoial problems[7,8]. Although symptoms may disappear after the status is over[1], they increase progressively in divorced families where interparental agreement is lacking[9].

Turkey is a country of rapid scoial changes due to various reasons, among them: migratilon, moving to other countries for work, military service, and the very high rate of traffic accidents. We planned to investigate changes in the demographic profiles of children and families seen in the Department of Child and Adolescent Psychiatry of Hacettepe University over a ten-year period. The present study concentrates on the features and symptoms of single-parent families.

Material and Methods

Patients: All records of patients seen in the Department of Child and Adolescent Psychiatry of Hacettepe University for their first visit in 1985 (1155 children) and 1995 (1556 children) were examined for single parenthood. The study group consisted of 89 children (47 male, 42 female) from 1985 and 66 children (32 male, 34 female) from 1995, all living with only one parent. The distribution of the study group according to the reason for single parenthood is shown in Table I.

Table I: Sex Ratios According to Years and Reasons of Single Parenthood

	1985		1995	
Reason of Single Parenthood	F	M	F	M
Separation	3	12	11	5
Divorce	29	21	18	19
Death	10	14	5	8

Method: The following features were recorded for each patient: age; sex; parents' ages, educational status and profession; number and order of siblings; the parent with whom the patient is living; visiting frequency of the other parent; duration of the present family status; and any symptoms reported.

Results

Results are examined from two aspects: time trends according to the year of evaluation and cause of single parenthood.

I. Time Trends

The rate of single parenthood among our patients' families was 7.7 percent in 1985 and 4.24 percent in 1995. Patients' sex ratio was similar in both years, with close to equal sex distribution ($p > 0.05$, Table I).

Mothers' education level changed significantly in time (p = 0.00058). In particular, fewer mothers with primary education and more with higher education were seen in 1995 (Table II). A similar but less pronounced trend was observed in fathers' education level (Table II). Mothers' working status also changed in time: while 58.4 percent of mothers were homemakers in 1985, this rate was 30.3 percent in 1995 (p = .01).

Table II: Mothers' and Fathers' Education According to Years (Percentage)

	Years	Illiterate	Literate	Primary	Middle	High School	Faculty	Missing Value
Mothers	1985	11.24	4.49	37.08	12.36	20.22	13.48	1.13
	1995	1.5	1.5	15.15	10.61	37.88	30.3	3.06
p = .00058								
Fathers	1985	4.49	1.12	30.34	14.61	15.73	33.71	0
	1995	1.52	–	12.12	6.06	33.33	43.94	3.03
p = .00048								

Mean parental age in 1985 was 31.2 for mothers, 32.5 for fathers; in 1995, 35.1 and 37.0, respectively. On the other hand, childrens' mean age decreased from 119.1 months in 1985 to 108.4 months in 1995. The interval between the beginning of single parenthood and the first hospital visit was 61.4 months in 1985 and 45.5 months in 1995.

The ratio of single children in this group changed over time: 22.2 percent in 1985 to 45.5 percent in 1995 (p = 0.05). The majority of children brought to hospital were the first or the only child of the family. The family size decreased over time: average 3.25 children in 1985 and 1.85 in 1995 (p = 0.05). In both years, the parent in charge was also the parent bringing the child to hospital, and was the mother in 70 percent of the cases.

The average frequency of symptoms, calculated by dividing the sum of the symptoms by the number of children, was not different in the two years: 1.21 in 1985 and 1.24 in 1995.

II. Findings Related to the Cause of Single Parenthood

Demographic features of the families with a single parent due to divorce, separation, or death were examined. Divorced mothers tended to have a higher education; however, the difference was not statistically significant (Table III). The ratio of mothers who were homemakers was 65 percent in the separated or widowed group, while it was only 31.4 percent among divorced mothers. More mothers in the divorced group worked as civil servants: 44.1 percent compared to the widowed (23.6%) or separated (19.3%) groups (p = 0.028).

Table III: Mothers' Education According to Reason of Single Parenthood

	Illiterate	Literate	Primary	Middle	High School	Faculty	Missing Value
Separation	6.45	6.45	35.48	9.67	19.35	19.35	3.25
Divorce	2.3	2.3	22.98	11.49	34.48	24.14	2.31
Death	18.92	2.7	32.43	13.51	18.92	13.51	0

Mothers' average age was 27.2 in separated, 33.7 in divorced, and 37.9 in widowed group ($p = 0.00004$), and fathers', 34.9, 38.5, and 36.4, respectively ($p = 0.000$).

Mean number of children was highest in widowed families with an average of 3.24 children. Divorced families had 1.93 and separated families 2.26 ($p = 0.05$) children on average. It was more common for divorced parents to have only one child: 40.2 percent when compared to separated (33.3%) or widowed families (13.1%) ($p = 0.05$). In all three groups, the majority of children who were brought to the clinic were the first children.

The cause of single parenthood appeared to influence the delay of the hospital visit: a higher number of separated or widowed parents and fewer divorced parents brought their child within the first six months of the event. Intervals longer than three years were more frequent in the divorced group (Table IV).

Table IV: Duration of Single Parenthood

	Up to 6 Months	6 Months-2 Years	3 Years and More	Missing Value
Separation	29	22.58	32.26	16.16
Divorce	6.9	18.39	71.26	3.45
Death	21.62	18.92	59.46	

The frequency of visits with the non-residing parent was evaluated in the divorced and separated groups. Although the ratio of parents having regular and frequent visits was similar in both groups (17% and 16%), 12.6 percent of divorced parents were never seeing their children. The difference between the frequency of the visits was significant ($p = 0.00$, Table V).

Table V: Frequency of Visits

	Regular		Irregular						
	Frequent	Weekly	Monthly	Once Every Few Months	Twice a Year	Once a Year	No Attach for a Long Period	Unclassified	Never
Separation	4	1	–	2	1	2	1	20	–
	12.9	3.23	–	6.45	3.23	6.45	3.23	64.52	–
Divorce	6	9	4	10	3	8	7	29	11
	6.9	10.34	4.6	11.49	3.45	9.19	8.05	33.33	12.64

A stepmother was present in 22 percent of the divorced families and a stepfather in 14 percent. These ratios, especially that of a stepfather, were lower in widowed families: 15.7 and 5.2 percent, respectively ($p = 0.0029$).

Table VI summarizes the distrubiton of the symptoms. Children of divorced parents were more often brought to the clinic and had more symptoms. The mean number of symptoms was 1.13 in separation, 1.32 in divorce, and 1.05 in death ($p = 0.0023$). Regarding the nature of the symptoms, mood disorders and depression were common among divorced parents' children (13.9%). Depression was also frequent in the death group (15%). Oppositional defiant behavior was common in all three groups. Somatoform disorders, adjustment disorders, and suicide attempts were seen in children of divorced families.

Table VI: Symptoms According to Reason of Single Parenthood

DSM-4* Classification		Separation		Divorce		Death	
		N	%	N	%	N	%
Mental Motor Retardation		3	8.57	2	1.74		
Learning Disability	School Failure			4	3.48		
Language and	Articulation Disorder	2	5.71				
Speech Disorders	Stuttering	1	2.86	2	1.74	4	10
Disruptive	Attention Deficit Hyperactive Disorder	2	5.71	3	2.61	2	5
Behavior	Hyperactivity			2	1.74		
Disorder	Oppositional Defiant Behavior	5	14.29	15	13.04	4	10
	Conduct Disorder			16	13.91		
Eating	Eating Disorder	1	2.86				
Disorder	Anorexia Nervosa			1	0.87		
Tic Disorder	Tic Disorder			2	1.74		
Elimination	Enuresis	2	5.71	10	8.7	5	12.5
Disorders	Encopresis			3	2.61		
	Delayed Control			1	0.87		
Dissociactive Disorder	Dissociative Disorder			1	0.87		
Somatoform Disorder	Pain Disorder			4	3.48		
	Conversion Disorder			2	1.74	3	7.5
Anxiety	Fears			1	0.87		
Disorders	Obsessive-Compulsive Disorder	1	2.86	1	0.87		
	Anxiety	2	5.71	4	3.48	1	2.5
Mood Disorders	Depression			16	13.91	6	15
	Nightmare	1	2.86	1	0.87	1	2.5
Sleep Disorders	Night Terror			2	1.74		
	Somnambulism					1	2.5
Personality Disorders	Personality Disorder	1	2.86			1	2.5
Adjustment Disorders				3	2.61		
Habit	Biting Nails	1	2.86	1	0.87		
Disorders	Masturbation	2	5.71	3	2.61		
Suicide				4	3.48		
Reaction to Parental Loss		5	14.29	6	5.22	7	17.5
Others		6	17.14	5	4.35	5	12.5

* Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV).

Discussion

The demographic changes of families observed over 10 years should be evaluated in the context of social structure. An interesting observation was that the number of hospital visits from divorced families decreased in later years while the rate of divorce went up in our country.

We think mothers' characteristics are important since children frequently live with their mother[10]. Many reports point to the hardship on a mother who is the householder: she has to work, and psychosocial problems are frequent[11]. A strong relationship exists between the presence of financial problems and the frequency of psychopathology[2, 11]. The great majority of children in our study were also living with their mother, and more divorced mothers were working compared to separated or widowed ones. However, the educational level of these mothers was also higher compared to the two other groups and to the literature, and this may have directed them into professional life. The amelioration in parents' education level in time may be taken as a beneficial sign. However, this may also indicate a bias due to changes in our hospital population and needs to be studied in more detail.

Most separations in this study were due to working abroad, especially in 1985. Military service was also a frequent reason. Therefore, the separation is seen as an obligatory but also favorable status, although the extension of the separation and the establishment of new relationships abroad sometimes complicate the situation. In the literature, less acceptable causes are encountered for single parenthood: abandonment, being in jail, going to war, or drug dependency, which may explain differences among results of studies[1, 8].

Important observations are made on the quality and the quantity of complaints bringing the child to the clinic. Symptoms were most frequent in the divorced group, with a markedly higher conduct disorder and oppositional defiant behavior. These disorders may be a sign of depression[12]. Interparental conflict continues in most divorced families, and the child is conceived as a hostage of the battle. Both parents tend not to limit the child as a bribe to obtain the child's love. The child who has difficulty with belonging and possessing is more likely to show behavioral problems. Depression and suicide attempts observed in this group point to the seriousness of this situation. In our study, 12.6 percent of divorced parents were never seeing their children. Children more readily accept the death of a parent rather than the existence of a parent who deserted them[12].

Attachment figures help to develop attachment behavior which is then generalized. Being separated from these figures causes anxiety and hostility. To reduce these negative feelings attempts are made to get together with the attachment figure. Hampering these attempts increases the hostility further[3]. On

the other hand, a parent's loss due to death, in the beginning, produces symptoms of deprivation, anger and feelings such as blaming that parent and fear of losing the remaining one. Insecurity, anxiety and later, acceptance and depression, develop. This process is longer and more intense in children of divorced families[9]. The finding that oppositional defiant behavior was one of the most frequent disorders in all three groups points to a serious discipline problem in single-parent families. In fact, many reports emphasize the need to work on reinforcing mothers' coping strategies[11, 13].

Children of divorced families are at risk for school failure[14]. In our study, all children with behavior problems had school failure. In addition, school failure due purely to psychological causes was also observed.

Poznanski et al.[15] suggest that children who live with a depressed parent fluctuate between aggression and depression, which makes their adjustment harder. Raising a child as a single parent and going through deprivation is difficult for adults too. Therefore, emphasis should be placed on parents' mental health in order to decrease risk factors.

We planned to, but could not, evaluate the role of other relatives of single-parent families, due to incomplete data in patients' charts. A traditional family style is a protective factor for children living with one parent. The involvement of grandparents and other close relatives helps alleviate the problems considerably[16]. However, disciplinary issues need to be approached with caution: returning to parents' home after divorce results in the interaction of three generations where autonomy, respective roles, and parental skills are questioned.

The role of siblings in single-parent families has not been evaluated in detail, in general, children brought to our unit are the first child of the family, followed by the second, last, and only children[17]. The present study also reflected this pattern, although the rate of single children was higher in the divorced group. Bayrakal and Kope[18] emphasize the importance of siblings in single-parent families, and also the sex of the siblings as well as the parent's. Future studies will concentrate on these factors.

In conclusion, research is needed on the risk factors and preventive measures for children living in single-parent families, particularly in countries going through transition periods.

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BRUCE TREADMILL TEST IN HEALTHY TURKISH CHILDREN: ENDURANCE TIME, HEART RATE, BLOOD PRESSURE AND ELECTROCARDIOGRAPHIC CHANGES*

*Mustafa Koray Lenk MD***, Dursun Alehan MD****, Alpay Çeliker MD*****
*Faruk Alpay MD******, Ümit Sarıcı MD*******

SUMMARY: Lenk MK, Alehan D, Çeliker A, Alpay F, Sarıcı Ü. (Department of Pediatrics, Gülhane Military Medical Academy and Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Bruce treadmill test in healthy Turkish children: endurance time, heart rate, blood pressure and electrocardiographic changes. Turk J Pediatr 1998; 40: 167-175.

Heart rate, systolic and diastolic blood pressure and electrocardiographic changes were studied in 80 healthy children during treadmill exercise with Bruce protocol. Mean duration of exercise in boys increased from 10.01 ± 1.61 minutes at age four to six years with a mean body surface area of 0.75 ± 0.06 m^2, to 17.86 ± 2.66 minutes at age 13 to 15 years with a mean body surface area of 1.48 ± 0.10 m^2. Mean endurance time in girls increased from 11.06 ± 1.92 minutes at age four to six years with a mean body surface area of 0.76 ± 0.08 m^2, to 15.69 ± 2.23 minutes at age 13 to 15 years with a mean body surface area of 1.41 ± 0.07 m^2. Mean maximal heart rate was 193.38 ± 10.89 beats/minute in boys and 196.78 ± 10.99 beats/minute in girls. The maximum level of systolic blood pressure attained at peak exercise was lower in girls. Although data of healthy Turkish children including mean endurance time, heart rate and blood pressure responses to exercise were consistent with the results from several countries using the Bruce protocol, mean maximal heart rates for all groups were slightly higher than those obtained with the Balke protocol. The data obtained from this study offers age- and size-appropriate normal data in both sexes in healthy Turkish children and may be used as reference values during treadmill exercise. *Key words: treadmill exercise test, healthy children.*

Exercise, a common physiological stress, can elicit cardiovascular abnormalities not present at rest and can be used to determine the adequacy of cardiac function. Hemodynamic and cardiopulmonary responses of children with congenital heart lesions to exercise have been published for several years[1-6]. Exercise testing was also used as a principal method for exposing an arrhythmia,

* From the Department of Pediatrics, Gülhane Military Medical Academy and Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Assistant Professor of Pediatrics and Pediatric Cardiologist, Gülhane Military Medical Academy.

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

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

***** Associate Professor of Pediatrics, Gülhane Military Medical Academy.

****** Research Assistant in Pediatrics, Gülhane Military Medical Academy.

for identifying the patient at risk for a serious tachyarrhythmia and for evaluating the effect of an antiarrhythmic drug[7]. Many of these evaluations were accomplished by using either a bicycle ergometer or a treadmill.

The Bruce treadmill test has been commonly used to assess exercise capacity and the electrocardiographic response to exercise stress in adults[8]. To determine the normal values for heart rate, blood pressure and endurance time, numerous investigations have been done in various countries[9, 10]. This study is performed to obtain normal data for healthy Turkish children on the treadmill test using the Bruce protocol. ,

Material and Methods

Study Groups: 40 boys and 40 girls, a total of 80 children, with a normal cardiovascular system were selected for the study. For data analysis the subjects were classified into four groups according to their ages, body surface area and sexuality as follows: group I- four to six years old (0.64 to 0.86 m^2) 10 boys and 10 girls; group II- seven to nine years old (0.87 to 1.05 m^2) 10 boys and 10 girls; gorup III- 10 to 12 years old (1.06 to 1.35 m^2) 10 boys and 10 girls and group IV-13 to 15 years old (1.36 to 1.61 m^2) with 10 boys and 10 girls. As responses to exercise vary with age, size and sexuality we preferred such grouping as mentioned.

Treadmill Protocol: All children exercised on a Quinton 4000-Q55 model treadmill. The protocol was programmed as outlined by Bruce et al.[8], using stages at which the speed and grade were progressively increased at three minute intervals from 1.7 through six miles per hour (mph) and from 10 through 22 percent, respectively. Heart rates, blood pressures and electrocardiograms were monitored and 12-lead electrocardiographic strep was recorded before the test while the child was standing and at the end of each stage during exercise. Finally, last strips were recorded after the exercise, at the end of each minute for five minutes. Blood pressures were obtained from the left arm with a system connected to the treadmill's monitor (Model 410 Quinton Blood Pressure Recorder). Blood pressure values were obtained using cuff sizes of 9.5 and 12.5 cm, the cuff covering at least two thirds of the upper arm length.

After written consent was obtained, each child was given a general explanation about the equipment and the test procedure. The exercise test was continued for the maximal time allowed (21 minutes) or until severe fatigue prevented further exercise. After peak effort was reached, a walkdown period of one to two minutes was allowed with a very slow treadmill speed to prevent vagal responses. The remaining heart rate and blood pressure determinations in the postexercise recovery period were obtained in a supine position on a bed.

At the end of the test a tabular final report of speed (mph), grade (%), heart rate (beats/minute), blood pressure (mm Hg), endurance time (minutes) and electrocardiographic ST levels were given in all the stages, minute to minute, throughout the test. In the analysis and interpretation of ST segment changes on exercise electrocardiography, a line is drawn from the beginning of one QRS to the beginning of the next, which is known as the PQ-PQ isoelectric line method[11].

Continuous values were expressed as mean ± standard deviation. Student's t test was used for direct comparison of mean values of the patient population. A probability (p) value of < 0.05 was considered statistically significant.

Results

Duration of Exercise (Endurance Time): Table I shows the mean duration of exercise for each of the four groups based on age, sex and body surface area. There was an increase in exercise duration from the younger to the older boys which was not so in girls. Girls in group II had a longer duration of exercise than girls in group III ($p < 0.05$). Girls had a longer duration of exercise than boys in groups I and II and boys had longer endurance times than girls in groups III and IV, but the differences were not statistically significant ($p > 0.05$). The average duration of exercise was 13.86 ± 3.59 minutes in boys; 13.44 ± 2.5 minutes in girls ($p > 0.05$) and 13.63 ± 3.08 minutes in all groups. In general there was an increase in endurance time from the younger ages to the older ages.

Table I: Mean Duration of Exercise in Healthy Turkish Children (Minutes ± SD)

	Group I	Group II	Group III	Group IV
Boys	10.01 ± 1.61	12.67 ± 2.32	14.91 ± 1.98	17.86 ± 2.66
Girls	11.06 ± 1.92	14.15 ± 1.79	12.73 ± 1.42	15.69 ± 2.23
Total	10.53 ± 1.80	13.41 ± 2.15	13.82 ± 2.01	16.78 ± 2.64

Heart Rate Responses: Table II shows the heart rates before exercise, at one minute of exercise and at peak exercise. Although girls had slower heart rates than boys before exercise in groups I and II, they had faster heart rates in groups III and IV, but the differences were not statistically significant ($p > 0.05$). At peak exercise, girls had faster heart rates than boys in groups I, II and IV ($p > 0.05$). Figure 1 shows the minute to minute mean heart rates for all groups during and after exercise. All groups showed a similar trend, with an abrupt increase in heart rate during the first minute of exercise and a more gradual increase thereafter. The younger children had faster heart rates during exercise than the older children. The mean maximal heart rate was 193.38 ± 10.89 beats/minute (median 193) in boys; 196.78 ± 10.99 (median 199) in girls and 195.07 ± 11.00

(median 196) in all groups. The difference in the mean maximal heart rate was not statistically significant between boys and girls ($p > 0.05$). All groups had an abrupt decline in heart rate after exercise.

Table II: Mean Heart Rates of Both Sexes During Different Stages of Exercise (Beats/Minute ± SD)

	Group I	Group II	Group III	Group IV
Boys				
Pre exercise	113.3 ± 12.6	101.9 ± 15.8	97.2 ± 10.4	75.4 ± 10.9
1 min exercise	142.7 ± 15.6	131.0 ± 13.9	129.5 ± 13.0	115.0 ± 12.0
Peak exercise	187.9 ± 14.7	184.3 ± 18.5	190.0 ± 9.0	183.6 ± 13.2
Girls				
Pre exercise	106.3 ± 15.9	97.7 ± 9.9	101.0 ± 8.0	86.8 ± 16.7
1 min exercise	140.6 ± 15.4	134.0 ± 7.8	140.2 ± 14.0	114.2 ± 18.4
Peak exercise	188.1 ± 10.1	194.8 ± 12.1	188.9 ± 19.5	189.4 ± 5.6

1 min exercise: 1 minute of exercise.

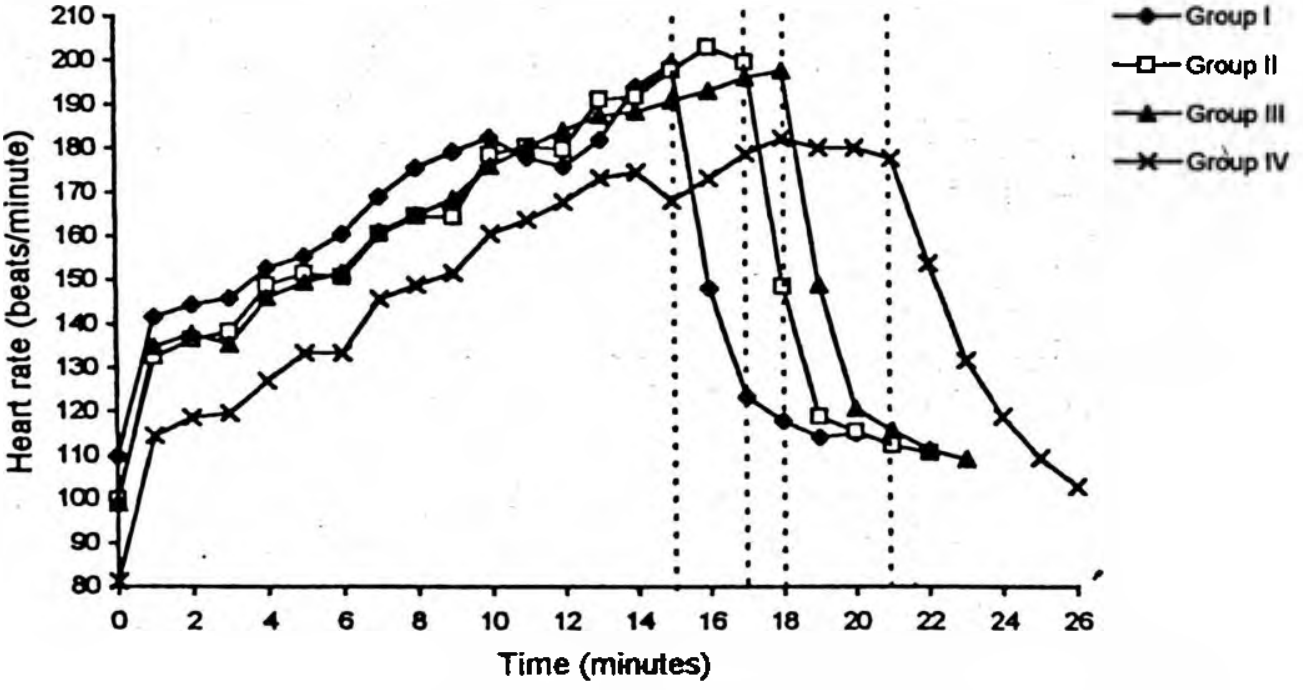

Fig. 1: Minute to minute mean heart rate responses during exercise in four groups (the peak mean heart rate values are shown as vertical thin lines and the values after lines indicate the postexercise values).

Blood Pressure Responses: The mean minute to minute systolic and diastolic blood pressure responses of the children are shown in Figures 2, 3, 4 and 5. A gradual, but not an abrupt, increase in systolic blood pressure occurred in all age groups. Diastolic blood pressures remained relatively constant and even tended to decrease in the older children, resulting in an increased pulse pressure

during exercise. All groups had an abrupt decline in systolic blood pressure after exercise. The rate of increase in systolic pressure during exercise and the maximum level attained at peak exercise were greater in older ages with a larger body surface area (Fig. 6).

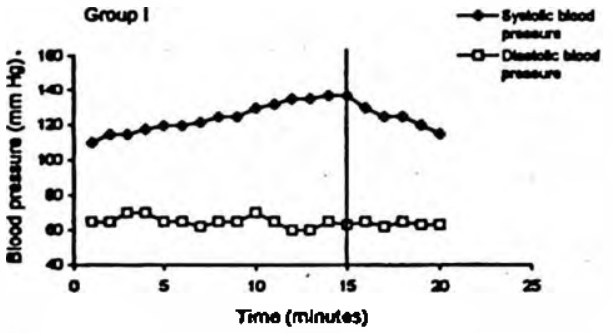

Fig. 2: Minute to minute mean systolic and diastolic blood pressure responses during exercise in group I (the values after the vertical line indicate the postexercise values).

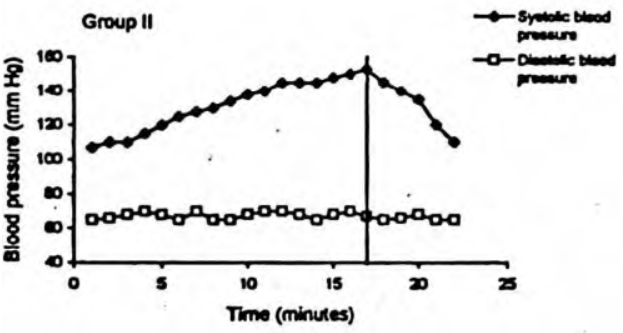

Fig. 3: Minute to minute mean systolic and diastolic blood pressure responses during exercise in group II (the values after the vertical line indicate the postexercise values).

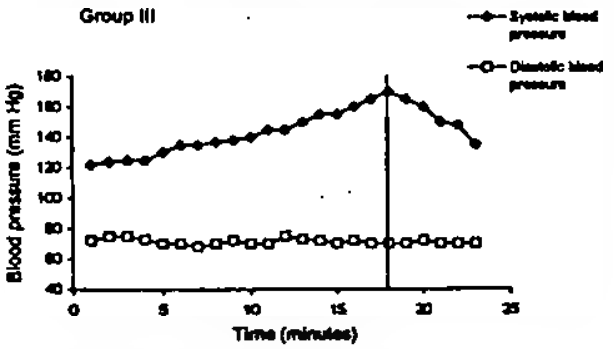

Fig. 4: Minute to minute mean systolic and diastolic blood pressure responses during exercise in group III (the values after the vertical line indicate the postexercise values).

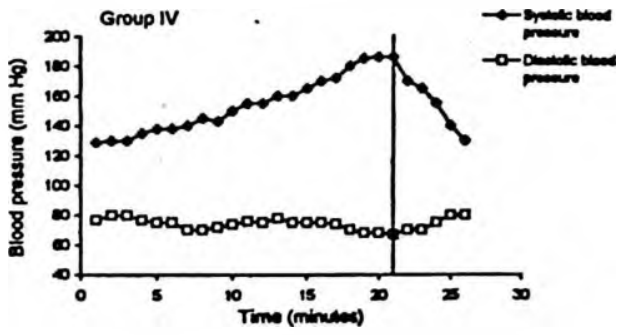

Fig. 5: Minute to minute mean systolic and diastolic blood pressure responses during exercise in group IV (the values after the vertical line indicate the postexercise values).

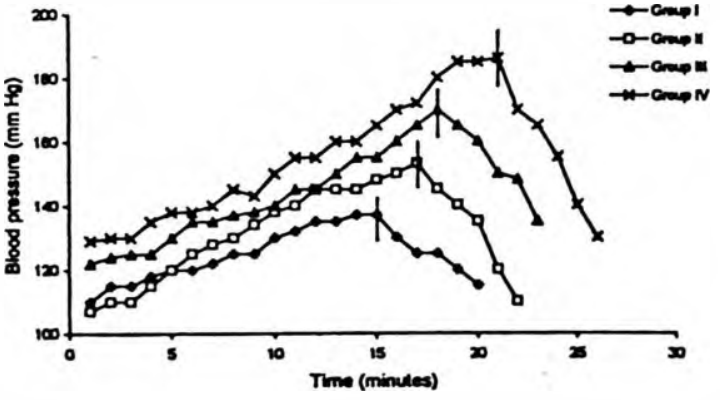

Fig. 6: Minute to minute mean systolic blood pressure responses during exercise in four groups (the peak mean systolic blood pressure values are shown as vertical lines and the values after lines indicate the postexercise values).

Tables III and IV list the systolic and diastolic blood pressure values before exercise, at one minute of exercise and at peak exercise for all the groups. Differences in systolic pressures of boys and girls of each group and of all groups before exercise were not statistically significant ($p > 0.05$). The maximum levels attained at peak exercise were lower in girls ($p > 0.05$).

Table III: Mean Systolic Blood Pressure of Both Sexes During Different Stages of Exercise (mm Hg ± SD)

	Group I	Group II	Group III	Group IV
Boys				
Pre exercise	105.2 ± 4.2	107.2 ± 4.0	113.0 ± 3.7	119.6 ± 2.6
1 min exercise	107.7 ± 16.0	112.2 ± 9.2	132.0 ± 11.0	127.3 ± 5.9
Peak exercise	160.6 ± 32.5	163.7 ± 6.2	179.2 ± 22.3	176.4 ± 5.5
Girls				
Pre exercise	105.0 ± 4.3	106.8 ± 3.7	112.1 ± 4.0	117.9 ± 2.1
1 min exercise	104.4 ± 7.0	111.5 ± 7.6	123.8 ± 3.7	120.0 ± 4.7
Peak exercise	146.1 ± 4.9	153.2 ± 7.2	175.3 ± 26.5	166.5 ± 5.8

1 min exercise: 1 minute of exercise.

Table IV: Mean Diastolic Blood Pressure of Both Sexes During Different Stages of Exercise (mm Hg ± SD)

	Group I	Group II	Group III	Group IV
Boys				
Pre exercise	63.4 ± 3.2	65.6 ± 3.5	71.2 ± 2.2	76.1 ± 2.9
1 min exercise	59.7 ± 6.9	62.9 ± 12.2	61.6 ± 3.9	62.6 ± 3.5
Peak exercise	57.8 ± 11.9	60.3 ± 5.6	64.1 ± 8.0	49.8 ± 3.4
Girls				
Pre exercise	61.9 ± 2.2	64.9 ± 3.3	70.8 ± 3.2	75.3 ± 2.5
1 min exercise	58.3 ± 5.3	62.9 ± 4.7	62.1 ± 3.3	62.0 ± 2.6
Peak exercise	58.0 ± 5.1	61.0 ± 3.7	59.3 ± 9.3	47.7 ± 3.4

1 min exercise: 1 minute of exercise.

Electrocardiographic Changes: All children were in sinus rhythm before exercise and maintained sinus rhythm throughout the test.

T Wave: The mean T wave amplitude for all groups increased from 4.2 ± 2.8 mm before exercise to 6.8 ± 2.4 mm at peak exercise and returned to baseline values during the recovery period.

J-point Depression: Parallel to increase in heart rates, a J-point depression of ≤ 1 mm occurred in all groups.

ST Segment Changes: The ST segment was normal in all children before exercise. Parallel to increase in heart rates during exercise, the ST segment tended to have an upward slope in all groups. No child had a flat or downsloping ST segment depression during the exercise.

Discussion

Exercise testing evaluates work performance and identifies mechanisms that limit the work performance of children with cardiac and other problems. Several protocols using the cycle ergometer or treadmill have been used in children[12]. As the Bruce treadmill test is a commonly used exercise test to assess exercise capacity we used the same treadmill protocol in our study.

Exercise Endurance Time: The mean endurance time for all children in the study groups was 13.63 ± 3.08 minutes (median 13.45). Endurance times using the Bruce protocol for children in similar age groups averaged between 12 and 13 minutes[9]. In another study using the Balke protocol, the average duration of exercise for all groups was reported to be 10.0 ± 1.3 minutes[10]. In our series, older boys had longer endurance times than girls whereas younger girls had longer endurance times than boys. In general, there was an increase in endurance time from the younger to the older ages. These findings were similar to those obtained in previous studies[9, 10].

Heart Rate Responses: The immediate response of the cardiovascular system to exercise in our study groups was an increase in heart rate due to a decrease in vagal tone. Initially, there was a rapid increase in heart rate during the first minute of exercise, and then a linear increase in heart rate occurred until peak exercise was reached. Although Cumming et al.[9] (using the Bruce exercise protocol) reported no differences in maximal heart rate between boys and girls, we found slightly faster heart rates in girls. The younger children had faster heart rates during exercise than the older children as they compensated for a smaller heart and lower stroke volume by an increased heart rate at a given rate of work, thus attaining higher maximal heart rates than the older children. The mean maximal heart rate for all groups in our study was 195.07 ± 11.00 beats/minute (median 196) which is similar to results obtained with the Bruce protocol[9] and slightly higher than those obtained with the Balke protocol[10]. It has been reported that maximal oxygen consumption was approached when the heart rate was 180 beats/minute or greater[13]; therefore, the mean heart rate of 195.07 ± 11.00 beats/minute attained by our subjects should reflect the achievement of a level of exercise at or near maximal oxygen consumption.

The mean heart rates returned to preexercise levels in the recovery period within five minutes after exercise was stopped. As the decrease in resting heart rate is characteristic of conditioning, our children lying down during the recovery period might have played a role in the abrupt decrease in the heart rate.

Blood Pressure Responses: Current guidelines for exercise testing[14] indicate the importance of obtaining blood pressures during the test, as blood pressure recordings during exercise were reported to be important both in adults with coronary artery disease[15] and in children with congenital heart defects[14].

A gradual, but not an abrupt, increase in systolic blood pressure occurred in all age groups in our study throughout the exercise test. This finding was not in accordance with the observations of the previous study by Riopel et al.[10]. There was a progressively higher rise in systolic pressures with age. As males have a higher maximal stroke volume than females, boys in our study had a higher systolic blood pressure response. Diastolic blood pressures tended to remain constant or to increase or decrease slightly during exercise. These circulatory changes can be explained by the dilation of local vascular beds in skin and muscle during exercise. Systolic blood pressure increases as the cardiac output increases to a greater degree than the reduction of resistance. Dilation of the vascular beds, however, causes the diastolic blood pressure to decrease slightly or remain constant.

It was reported that a maximal exercise systolic blood pressure above 220 mm Hg in adults was considered an excessive rise, and it rarely exceeded 200 mm Hg in children[14]. Maximal systolic pressures in our study groups were below 200 mm Hg.

Electrocardiographic Changes: The increase in T wave amplitude during exercise is a well known entity in normal subjects[15]. We observed the same findings in T wave amplitude during and at peak exercise, which then normalized in the recovery period. Changes in ST segment during exercise have been well described in adults[16, 17] and in children[11]. None of the children in our study met the criteria for significant ST segment J-point depression (a J-point depression of ≥ 2 mm and an ST depression of > 1 mm with a flat or downsloping ST segment at 60 milliseconds). These electrocardiographic changes during ventricular repolarization were reported to be used to identify a mismatch of myocardial oxygen demand and supply[14]. Parallel to increase in heart rates, we observed normal physiological J-point depression and the ST segment tended to have an upward slope in all groups.

Although our data, including mean endurance time, heart rate and blood pressure responses to exercise, were similar with the findings from several countries using the Bruce protocol, mean maximal heart rates for all groups in our study were slightly higher than those obtained with the Balke protocol.

In conclusion, the data obtained from this study offers age- and size- appropriate normal data in both sexes in healthy Turkish children and may be used as reference values during treadmill exercise.

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SERUM MALONDIALDEHYDE CONCENTRATION AS A MEASURE OF OXYGEN FREE RADICAL DAMAGE IN PRETERM INFANTS*

*Şule Yiğit MD**, Murat Yurdakök MD***, Kamer Kılınç MD*****

*Olcay Oran MD***, Gülşen Erdem MD***, Gülsevin Tekinalp MD****

SUMMARY: Yiğit Ş, Yurdakök M, Kılınç K, Oran O, Erdem G, Tekinalp G. (Neonatology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Serum malondialdehyde concentration as a measure of oxygen free radical damage in preterm infants. Turk J Pediatr 1998; 40: 177-183.

Lipid peroxidation was measured in 35 preterm infants by determining serum malondialdehyde (MDA) levels during the fist week of life. Serum concentrations of MDA were measured at one hour, 24 hours, 48 hours and one week of age. There were no correlations between gestational age, birth weight, and serum malondialdehyde levels at one, 24, or 48 hours, or on day seven. Serum MDA levels were higher in infants requiring mechanical ventilation compared to those breathing spontaneously, but the difference was not statistically significant. There were no correlations between MDA levels and corresponding fraction of inspired oxygen (FIO_2) levels and arterial oxygen tension. No significant difference in serum MDA concentration was seen in relation to intravenous feeding with lipid emulsions. Serum MDA concentrations of infants with sepsis were not different at hours one, 24 and 48, but were significantly higher on day seven, when compared with infants without sepsis. MDA levels in the first and second serum samples were significantly higher in infants who were born after spontaneous vaginal delivery, compared to those born by cesarean section. *Key words: malondialdehyde, oxygen free radical damage, preterm infants.*

Free radical reactions are part of many biological processes. Molecular oxygen, present in all aerobic organisms, readily accepts an electron, resulting in the formation of oxygen free radicals[1]. Radicals are known to injure membranes, mainly by lipid peroxidation, which leads to structural alterations in protein and DNA. Oxygen free radicals are implicated as pathologic mediators in many disease processes. It has been suggested that bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), intra ventricular hemorrhage (IVH), and patent ductus arteriosus (PDA) can be grouped together as oxygen radical diseases in newborn infants[1-4].

Oxygen radicals, of which a number of different species are known such as the superoxide and hydroxyl radicals), can be produced from different sources: normal energy metabolism, by hypoxanthine-xanthine oxidase, metabolism of

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

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

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

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

catecholamine and arachidonic acids, and so fort[1]. The body has defense mechanisms for protection against the toxicity of oxygen free radicals: enzymatic (e.g. superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic (e.g. several antioxidant vitamins)[5-7]. Furthermore, a series of compounds and metabolites, such as mannitol, uric acid, bilirubin and others, act as oxygen radical scavengers[6, 8, 9].

As shown by studies[10-16], the concentration of most antioxyenzymes increases during pregnancy. Phylactos et al.[17] have demonstrated that preterm babies have lower erythrocyte superoxide dismutase activitiy than term babies. Therefore, a premature infant may be at increased risk of radical damage.

It has been shown that malondialdehyde (MDA) is a highly reactive metabolite of free radical-induced lipid peroxidation[18]. Studies have shown that MDA levels are high in infants who develop BPD[19, 20]. The results of these studies[19, 20], concerning factors that influence MDA levels (i.e., birth weight, oxygen treatment, intravenous nutrition), are conflicting. This study to evaluate factors involved in free radical damage in preterm infants was, therefore, conducted by determination of MDA levels.

Material and Methods

The study group consisted of 35 preterm neonates (27-35 weeks gestation) who were born in Hacettepe University Hospital. All infants under 2500 g and of less than 36 weeks gestation were eligible for inclusion. Full details of the pregnancy, birth history, Apgar scores and neonatal course, including details of nutrition, bilirubin levels at the time of sampling, oxygen supplementation, and ventilatory support, were collected prospectively. Infants with severe congenital malformation or birth asphyxia were excluded from the study. Sepsis was diagnosed in infants by positive blood culture, in addition to clinical findings. Parenteral nutrition with glucose and amino acids was started on day three and lipid emulsions were started on day five for patients in whom sufficient enteral feeding had not been established.

Serum concentrations of MDA were measured at one hour, 24 hours, 48 hours and one week of age. Blood sampless were centrifuged at 3000 rpm for three minutes within 15 minutes of collection. Serum samples were stored at -20 °C and analyzed within two months.

Serum malondialdehyde levels were measured by the thiobarbituric acid (TBA) test, as described by Wade and van Rij[21]. Although the reactivity of TBA is not limited to malondialdehyde, this method is one of the most common used for detecting oxidative modification of lipids, and hence, oxidative stress. For detecting thiobarbituric acid reacting substances (TBARS), 0.2 ml of trichloroacetic acid (TCA, 25 gr TCA in 10 ml distilled water) was added into 1 ml serum, with vigorous shaking. After centrifugation at 1000 g for 10 minutes,

the supernatant was neutralized with 4 M NaOH. One ml of neutralized supernatant was added to 1 ml TBA (0.67%) and the mixture was then heated at 100 °C for 30 minutes. The absorption of cooled samples was measured at 532 nm. Tetramethoxy propane was used as the standard. MDA levels (measured as TBARS) were calculated as μmol/L[21].

Results are given as mean ± standard deviation (SD). Differences among groups were tested by Student's t test and chi-square analysis. Correlation analysis was used for determination of relationship between variables. Differences between two samples were tested by paired t-test.

Results

Some characteristics of the study group are shown in Table I. Artificial ventilation had been performed at least 24 hours by intermittent mandatory ventilation on 25 of 35 infants. There were no correlations between gestational age, birth weight, and MDA levels at one, 24 or 48 hours or on day seven ($r = 0.03$, 0.17, 0.11 and 0.07).

Table I: Study Group (n = 35)

Male/Female	17/18
Gestational age (wk)	31.9 ± 2.1 (27-35)
Birth weight (g)	1653 ± 508 (702-2460)
Delivery route CS/spontaneous vaginal	26/9
Maternal age (year)	28.3 ± 5.1 (20-38)
No. of infants ventilated more than 24 hours	25 (71.6%)
No. of infants who received parenteral nutrition	7 (20%)

Mean ± standard deviation (range).

Three groups were formed according to birth weight: low-birth-weight infants (1500-2500 g), very low-birth-weight infants (1000-1500 g) and extremely-low-birth-weight infants (< 1000 g). MDA levels were higher in extremely-low-birth-weight infants, but no statistically significant difference was shown in the other groups ($p > 0.05$). (Table II).

Malondialdehyde levels in the first and second serum samples were significantly higher in infants who were born after spontaneous vaginal delivery, compared to those born by cesarean section ($p < 0.05$) and $p < 0.05$) respectively. This difference was slight between samples at 48 hours and there was no difference between samples on day seven ($p > 0.05$) (Table II).

Apgar scores did not correlate with MDA levels ($p > 0.05$). MDA levels were not different in infants who were resuscitated at birth.

Table II: MDA Levels According to Some Factors

	MDA Levels (mmol/L)*			
	1 Hour	24 Hours	48 Hours	7 Days
Male (n: 17)	3.9 ± 1.0	5.1 ± 1.3	5.9 ± 2.1	5.9 ± 1.9
Female (n: 18)	3.9 ± 1.1	4.7 ± 1.2	6.2 ± 2.7	4.9 ± 1.7
Gestational age				
< 32 weeks (n: 12)	3.9 ± 1.3	5.1 ± 1.1	6.3 ± 2.8	6.2 ± 1.8
> 32 weeks (n: 23)	3.9 ± 1.0	4.8 ± 1.3	5.9 ± 2.3	5.0 ± 1.8
Birth weight				
< 1000 g (n: 3)	4.3 ± 0.7	5.1 ± 1.3	7.7 ± 5.5	5.7 ± 2.5
1000-1500 g (n: 12)	3.9 ± 1.0	5.0 ± 1.1	6.1 ± 1.8	5.2 ± 2.0
1501-2500 g (n: 20)	3.8 ± 1.2	4.8 ± 1.3	5.8 ± 2.2	5.4 ± 1.8
Delivery route				
Spontaneous vaginal (n: 9)	4.6 ± 1.0[a]	5.7 ± 1.3[b]	7.2 ± 2.7	5.9 ± 2.1
Cesarean section (n: 26)	3.7 ± 1.0[a]	4.6 ± 1.1[b]	5.6 ± 2.2	5.2 ± 1.7
Resuscitation at birth				
Yes (n: 15)	3.7 ± 0.8	4.8 ± 1.3	5.9 ± 2.5	5.1 ± 1.7
No (n: 20)	4.0 ± 1.2	4.9 ± 1.2	6.1 ± 2.4	5.6 ± 2.0
Ventilation > 24 hours				
Yes (n: 25)	4.0 ± 1.1	5.0 ± 1.3	6.3 ± 3.5	5.7 ± 1.7
No (n: 10)	3.6 ± 0.8	4.8 ± 1.2	5.9 ± 1.7	5.2 ± 2.3
FiO_2 requirement at the time of sampling				
< 40%	3.7 ± 1.1 (n: 14)	4.9 ± 1.2 (n: 23)	5.9 ± 1.6 (n: 25)	5.4 ± 1.9 (n: 33)
> 40%	4.0 ± 1.1 (n: 21)	4.9 ± 1.2 (n: 12)	6.4 ± 3.9 (n: 10)	4.5 ± 1.5 (n: 2)
Sepsis				
Present (n: 5)	3.9 ± 1.4	5.6 ± 1.1	7.5 ± 3.7	8.1 ± 0.7[c]
Absent (n: 30)	3.9 ± 1.0	4.8 ± 1.2	5.8 ± 2.1	5.0 ± 1.7[c]
Intravenous lipid				
given (n: 7)	–	–	–	6.4 ± 1.6
not given (n: 28)	–	–	–	4.9 ± 2.0

* Mean ± standard deviation, a: $p < 0.05$, b: $p < 0.05$, c: $p < 0.01$.
Abbreviations: MDA malondialdehyde, FiO_2 fraction of inspired oxygen.

Serum MDA levels were higher in infants requiring mechanical ventilation compared to those breathing spontaneously, but the difference was not statistically significant. There was no correlation between MDA levels and corresponding FiO_2 levels or arterial oxygen tension ($p > 0.05$) (Table II).

Parenteral feeding with lipid emulsions created no significant change in serum MDA concentration ($p > 0.05$).

Sepsis was diagnosed on the third day of life in two infants, on the fourth day of life in one infant, and on the tenth day of life in two infants. Serum MDA concentrations of infants with sepsis were not different at hours one, 24 and 48, but were significantly higher on day seven when compared with infants without sepsis ($p < 0.01$) (Table II). Because only one patient had been diagnosed as having BPD, no statistical analysis could be performed.

Discussion

The antioxidant status of the premature infant has been shown to be poorly developed[13-16]. In the present study, although MDA levels were highest in extremely-low-birth-weight infants, MDA concentrations could not be related directly to immaturity.

The concentration of MDA in cord blood samples of the premature infants who developed chronic lung disease, was found to be higher than that in the others without chronic lung disease[19]. Gladstone and Levine[22] have shown that protein oxidation is enhanced in bronchoalveolar lavage samples in babies exposed to high oxygen concentrations compared with samples from babies with low oxygen supplementation.

No difference could be found in this study, between MDA levels in infants requiring assisted ventilation and those breathing spontaneously. Furthermore, there was no correlation between serum MDA levels and breathing oxygen concentrations. This might be due to the fact that serum MDA levels might not reflect oxidation of lipids in the lung. Similar to the results of our study, no correlation was shown between urinary MDA levels and mean airway pressure, arterial oxygen tension, or ventilatory index in the study of Schlenzig et al.[20].

It has been demonstrated that a lipid emulsion used in parenteral nutrition undergoes peroxidation that causes free-radical-mediated damage to human cells in vitro[23]. In contrast, it has also been shown that the amount of MDA administered to an infant, when infused with 0.5-1.5 g/kg/day of a lipid emulsion, is considered insignificant[20]. In accordance with this latter finding, MDA levels were not higher in infants infused with intravenous lipid than the levels in the others in our study who were not infused.

Serum MDA concentrations of infants with sepsis were significantly higher on day seven when compared with concentrations in infants without sepsis. The activated phagocyte might contribute to tissue injury by an excess production of oxygeen radicals as part of the defense against microorganisms[24, 25]. This might explain why MDA levels were found higher in infants with sepsis than in the others.

Malondialdehyde levels in the first and second serum samples were significantly higher in infants who were born after spontaneous vaginal delivery, compared to levels in those born by cesarean section. We could not find any factor which could affect serum MDA levels in infants who were born after spontaneous delivery. A more detailed study has been planned to investigate this issue.

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CITRULLINEMIA*

Clinical Experience with 23 Cases

*Ayşegül Tokatlı MD**, Turgay Coşkun MD***, İmran Özalp MD****

SUMMARY: Tokatlı A, Coşkun T, Özalp İ. (Metabolism and Nutrition Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Citrullinemia: clinical experience with 23 cases. Turk J Pediatr 1998; 40: 185-193.

A retrospective study was performed on the clinical outcome of 23 patients with citrullinemia diagnosed during the last 20 years in our clinic. The study group consisted of 13 patients with the neonatal form of the disease, four patients with the subacute form, five patients with the late-onset form and one with the asymptomatic form. All patients were treated with natural protein restriction, sodium benzoate and arginine administration. Almost all of the neonatal-onset patients were treated with exchange transfusions and/or peritoneal dialysis. Fourteen patients died: 11 with the neonatal form, one with the subacute form, and two with the late-onset form. The general neurological outcome of the patients who were alive was not satisfactory. Despite these results, it was concluded that the prognosis and quality of life of patients with citrullinemia might be improved with early diagnosis and appropriate therapy.
Key words: citrullinemia, neonatal, subacute, late-onset, asymptomatic.

Ammonia, coming from protein and amino acid metabolism and from decomposition of urea and other nitrogenous compounds by microorganisms in the intestinal tract, is eliminated from the body through the urea cycle. The major route of ammonia detoxification consists of five steps, each catalyzed by a different enzyme: carbamoyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC), argininosuccinic acid synthetase (AS), argininosuccinic acid lyase (AL) and arginase. A sixth enzyme, N-acetylglutamate synthetase, is also required for synthesis of N-acetylglutamate, which is an activator of the CPS enzyme. Inborn errors are known to occur at each step in the urea cycle[1]. AS catalyzes the production of argininosuccinic acid from citrulline and aspartate. AS deficiency leads to hyperammonemia and the accumulation of citrulline in the blood and spinal fluid[2].

The spectrum of clinical presentation in citrullinemia is so wide that some of the cases may run a rapidly downhill course in the neonatal period while others may show normal or near normal development[3, 4].

In this paper, we describe 23 patients with citrullinemia diagnosed at our clinic so far to draw attention to the variable expression of the disorder.

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* From the Metabolism and Nutrition Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

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

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

Material and Methods

Twenty-three patients who had been diagnosed as having ASS deficiency (citrullinemia) at Hacettepe University Children's Hospital Metabloic Unit over the past 20 years were included in this study. Clinical and laboratory data of patients were obtained from hospital records. Plasma ammonia concentration was determined by colorimetric method[5]. One- and two-dimensional paper chromatography with Ehrlich's stain was used when necessary for serum and urinary amino acid patterns in some patients[6], whereas plasma and urine amino acids were measured by ionexchange chromatography in the rest (Biotronic and LKB amino acid analyzers).

Results

Of the cases with citrullinemia, 13 were male (56.5%) and 10 female (43.5%). Most of the cases (14/23, 60.6%) were in the newborn period, four patients (17.4%) were between one month and two years of age, and five cases (21.8%) were above two years when the diagnosis was made. Some details of patients are shown in Table I and the patients who illustrate the typical manifestation of the different types of citrullinemia are reported.

Table I: Some Clinical Characteristics of Cases with Citrullinemia

					Laboratory Findings			
Case	Age Sex	Consan-guinity	Family History of Sibling Deaths	Outcome	NH_3 (µg/dl)	BUN (mg/dl)	Serum Amino Acid (µmol/L)	Urine Orotic Acid (mmol/mol Cr)
1-Baby Y	3 Days Male	+	+	died at 4th day of life	425	7	citrulline↑↑* glutamine↑	N.D.
2-SB	1 day female	+	+	diet at 1.5 months of age	268	12	citrulline↑ glutamine↑	N.D.
3-BK†	1 day male	+	+	died at 2.5 years	318	9	citrulline↑ glutamine↑	N.D.
4-Baby Ö	3 days female	+	+	diet at 5th day of life	2000	7	citrulline↑↑↑ glutamine↑↑	N.D.
5-EA	2 days male	–	+	died at 7th day of life	600	8	citrulline (15864) glutamine (11729)	↑↑
6-DK	5 days female	+	+	died at 6th day of life	1077	14	citrulline (2333) glutamine (2315)	500↑**
7-Baby Ç	1 day female	+	+	died at 5th day of life	1153	12	citrulline (3544) glutamine (534)	↑↑
8-SÖ	2 days male	+	+	diet at 4th day of life	1913	6	citrulline (1262) glutamine (2284)	53.7
9-TK†	22 days female	+	+	alive MMR‡	180	5	citrulline↑ glutamine↑	N.D.
10-AD	1 day male	–	+	lost to follow-up at 5.5 y	345	8	citrulline↑↑ glutamine↑	N.D.

Table I (Continued)

Case	Age Sex	Consan-Guinity	Family History of Sibling Deaths	Outcome	Laboratory Findings			
					NH_3 (µg/dl)	BUN (mg/dl)	Serum Amino Acid (µmol/L)	Urine Orotic Acid (mmol/mol Cr)
11-Baby U	3 days male	+	+	died at 4th day of life	1020	5	citrulline (3580) glutamine (1570)	500↑
12-EÖ	5 days female	+	+	died at 8th day of life	340	11	citrulline↑↑ glutamine↑	↑
13-Baby Y	1 day female	+	+	died at 5th day of life	1141	9	citrulline↑↑↑ glutamine↑↑	500↑
14-MA	6 mos female	+	+	alive MMR	268	3	citrulline (4916) glutamine (1003)	↑↑
15-YB	22 mos male	+	+	died at 22 months of age	254	8	citrulline↑ glutamine↑	↑↑
16-HY	7 mos male	+	–	alive MMR	275	12	citrulline (2304) glutamine (534)	↑↑
17-CE	2 years male	+	–	lost to follow-up	187	9	citrulline↑↑ glutamine↑↑	N.D.
18-İA	9 years male	+	+	died during the acute attack at 9 years of age	278	10	citrulline↑↑ glutamine↑	N.D.
19-PS	7 years female	+	–	alive	180	7	citrulline (6100) glutamine (1050)	500↑
20-RD	6 years male	–	–	lost to follow-up	473	12	citrulline (10359) glutamine (880)	500↑
21-KT	9 years male	–	–	lost to folow-up	366	9	citrulline↑ glutamine↑	N.D.
22-İZ	15 years male	–	–	died during the first attack	950	24	citrulline (2283) glutamine (1912)	500↑
23-AK	15 days male	+	–	alive 16 year old	65	18	citrulline↑ glutamine↑	N.D.

† siblings.
* by paper chromatography with Ehrlich reagent.
** measurement is out of range.
ND: not done.
‡ MMR: mental-motor retardation.

The Neonatal-Onset Citrullinemia

Thirteen patients with the neonatal form of citrullinemia were admitted to the hospital between the first and twenty-second days of life. Each of them was the healthy product of a term pregnancy with normal anthropometric measurements. All had a positive family history retrospectively; only five of them were symptom free when admitted for further evaluation because of previous sibling deaths. Vomiting, poor feeding, grunting, altered consciousness, convulsion and irregular respiration were common presenting symptoms in this group. Within two to three days, the babies who were symptom free but identified as being at risk on the

basis of sibling deaths became increasingly lethargic and had difficulty in feeding. They were put on a protein-restricted diet along with oral sodium benzoate and arginine supplements. The lethargy progressed to coma. Diagnosis was based on elevated plasma ammonia levels (ranging from 270-2000 µg/dl) and increased plasma citrulline levels. Treatment included peritoneal dialysis and exchange transfusions in addition to sodium benzoate and arginine supplements in symptomatic patients. Nine babies had respiratory arrest requiring mechanical respiration. Ten patients died in the neonatal period and two cases died at 2.5 and 5.5 years of age respectively. Only one patient remained alive. She has been hospitalized several times with hyperammonemic coma and is now three-and-one-half years old and has severe mental retardation.

Case 6 illustrates the typical manifestation of the neonatal-onset citrullinemia.

Case 6 (DK): A five-day-old female infant who weighed 3800 g at birth was the third child of consanguineous healthy parents. The pregnancy was uneventful. The infant was normal at birth. During the first two days of life, the infant seemed to develop normally and was sucking well. She became less alert on the third day and sucking became poor. She became drowsy and hypotonic on the fifth day vomiting and with intermittent depressed breathing and was admitted to hospital, comatose. Physical examination revealed a deeply encephalopathic infant unresponsive to any stimuli. The Moro and sucking reflexes were absent. Her respiration was depressed. The blood pH was 7.02, HCO_3: 12.1 mmol/L, serum urea nitrogen 14 mg/dl, and NH_3: 1077 µg/dl (N: < 120 mg/dl). The serum amino acid analysis showed greatly increased citrulline (2333 µmol/L) and glutamine (2315 µmol/L) levels. The respiration arrest necessitated intubation and artificial ventilation. Nevertheless, her general condition deteriorated rapidly and the infant died on the sixth day of life. There was a family history suggestive of inborn errors of metabolism. The two siblings of the proband had been normal at birth, but had become lethargic on the fourth day of life and died.

The Subacute Form of Citrullinemia

Four patients with citrullinemia in this series had been presented in infancy. Two of them were referred to our hospital for further investigation for developmental delay, while the other two cases were admitted to hospital with hyperammonemic coma. Besides altered consciousness, vomiting, convulsion and developmental delay were presenting symptoms in these patients. In this group, three patients were alive and on a low-protein diet with medication, but they were severely mentally retarded. One died during hyperammonemic coma.

Case 16 shows the typical clinical presentation of the subacute form of citrullinemia.

Case 16 (HY): A seven-month-old boy was referred to our hospital for evaluation of his developmental delay. He was the second child of first degree relative

parents and the product of an uneventful 40-week pregnancy and delivery (birth weight 3170 g). His family history was said to be negative for metabolic disease. At five months of age, he was examined at a local hospital for fever, vomiting, lethargy and irritability, but a specific diagnosis could not be made. He was breast-fed and his body weight did not increase. On admission, his body weight was 6100 g (< 5th percentile) and length was 68 cm (50-75th percentile). He was unable to hold his head up or sit. The postprandial blood ammonia concentration was 275 µg/dl (N: < 120 µg/dl). Blood count and acid-base status were normal. Quantitative estimation of amino acids in plasma demonstrated increased citrulline (2304 µmol/L) and glutamine (534 µmol/L) concentrations. Blood ammonia concentration was reduced by restricting protein intake to 1.5 g/kg/day using a special formula (Milupa UCD-1), and by administration of sodium benzoate 250 mg/kg/day, and L-arginine (4 mmol/kg/day). On this regimen, moderate growth was achieved and blood ammonia levels remained within the normal range. At nine months of age he was able to hold his head up, at 10 months he could sit without aid, and at two years of age he could walk with aid. This case promptly responded to the therapy, but he had permanent neurological sequelae.

The Late-Onset Citrullinemia

Five cases with citrullinemia presented at different ages in late childhood. They were between six and 15 years. Only two patients had positive history for sibling deaths. The presenting symptoms and clinical findings were similar in all but case 19 (PS). Details about this case are given below. Altered consciousness, vomiting and convulsion were presenting symptoms. Protein avoidance and developmental delay were reported retrospectively. Two boys died during the first hyperammonemic attack despite peritoneal dialysis and medication. The other three patients were put on a low-protein diet, and sodium benzoate and arginine were prescribed. Only case 19 (PS) is doing well; the other two cases were lost to follow-up.

Case 19 exemplifies an acute presentation of late-onset citrullinemia as a bizarre behavior.

Case 19 (PS): A seven-year-old girl was brought to the emergency department with the chief complaints of agitation and crying. She was the full-term product of an uneventful pregnancy and delivery and had normal developmental milestones. Her parents were first degree relatives and her younger sister was healthy. At 1.5 and 2.5 years of age, she had been taken to other hospitals with the same symptoms, but no specific diagnosis was reached. The last time, eight days previously, she awoke from a nap and was found to be combative and had no interpretable speech. She spontaneously recovered within 24 hours. On admission, physical examination revealed normal vital signs and a hyperactive confused child with ataxia and no coherent speech. It is significant that she had a dietary protein avoidance, and thus was at the 3rd per centile for height for

which an endocrine evaluation was unrevealing. Routine laboratory findings were all within normal limits. Electroencephalography showed generalized irregularity compatible with a metabolic disease. The first impression was an underlying metabolic disease. Measurements of blood ammonia and amino acids were recommended. Laboratory studies revealed an only slightly high ammonia level, 180 μg/dl (N: < 120 μg/dl); however, her plasma amino acids revealed high citrulline (6100 μmol/L) and glutamine (1050 μmol/L) levels. The orotic acid level in her urine highly increased, the measurement being out of range. She was put on a protein-restricted diet and given sodium benzoate orally. For the last two years, her blood ammonia level has remained within normal limits.

The Asymptomatic Citrullinemia

Case 23 was an example of the asymptomatic variety of citrullinemia. He showed biochemical disturbances of a milder degree without hyperammonemia or any signs of the disease.

Case 23 (AK): A male infant, the first offspring of consanguineous healthy parents, was born after an uncomplicated pregnancy of 40 weeks with a normal birthweight. He was screened for metabolic disease by paper chromatography in the neonatal period. His serum and urinary amino acid patterns showed a high citrulline level, but his repeated serum ammonia levels were within normal limits. His developmental milestones were normal during childhood and he has never experienced hyperammonemia during intercurrent illnesses. At the present time, he is 16 years old and his neurological and psychometric evaluation are normal. His school performance is good. Until now he is asymptomatic, but he was advised to avoid protein loading.

Discussion

Citrullinemia is one of five urea cycle defects and caused by argininosuccinate synthetase deficiency: conversion of citrulline to argininosuccinate is blocked. There is considerable phenotypic variability: 1. a disease which runs a rapid and fatal neonatal course (neonatal form), 2. onset in infancy with clinical symptoms of periodic vomiting, seizure and mental retardation (subacute form), 3. a variant which has a lesser elevation of serum citrulline without clinical symptoms during infancy (late onset form), 4. a variant detected by routine screening and with biochemical disturbances of a milder degree without any signs of disease (asymptomatic form)[7-9].

The neonatal form presents a fatal course with vomiting, feeding difficulties, tachypnea, grunting, irritability proceeding to rigidity (sometimes with opisthotonus), apnea, convulsion, stupor and coma in the first days of life, generally after institution of protein feedings[10, 11]. With aggressive management,

which consists of protein restriction; administration of arginine, sodium benzoate and sodium phenylacetate; and hemodialysis, the acutely ill infant can be rescued, but frequently has permanent neurological sequelae because of the toxic effects of ammonemia. Cases 1-13 can be categorized as having the acute neonatal form of the disorder.

Four patients in whom the subacute form of the disorder was diagnosed presented during infancy. The presentation of this form of citrullinemia may not be so obvious and is characterized by developmental delay, vomiting and failure to thrive. Alternatively, there may be episodic symptoms with mild or severe neurological manifestations[12, 4].

Diagnosis of the late-onset type of citrullinemia was made in five patients. The most characteristic feature of this variant is the late onset of serious and continuing symptomatology, starting from childhood to adulthood. Patients with late-onset urea cycle defects present with episodes of acute metabolic encephalopathy although they may also have chronic symptoms. Usually there is an obvious trigger such as infection or protein loading. Sometimes they present with agitation or irritability or confusion, with or without any apparent cause. Vomiting, headaches, ataxia, fluctuating levels of consciousness, fits, focal neurological findings, learning difficulties and mental retardation are common symptoms and signs. In this series, a 15-year-old boy (Case 22) who was mentally normal died during the first hyperammonemic coma. A seven-year-old girl (Case 19) who was admitted to hospital three times with agitation and crying was also mentally normal. Her verbal and performance IQ were 98 and 105, respectively. The other three cases were retarded. Two patients died under observation, two were lost to follow-up, and one is doing well (Case 19). In this form of citrullinemia, some patients voluntarily restrict their protein intake[2]. This behavior was reported in three patients in this series. Delayed physical development occurred in some. Case 19 (PS) had been evaluated for growth retardation, but her endocrine evaluation was unrevealing. It is noteworthy that growth failure is attributable to a metabolic disease besides endocrinopathy.

There are some cases in literature in whom citrullinemia was detected by routine hospital screening and whose growth and development were normal. In this variety of citrullinemia, citrulline plasma concentrations were always high whether compared with normal controls or with heterozygote relatives of the cases, though for lower than levels in patients with the other types of citrullinemia[13-15]. Case 23 (AK) was detected by newborn screening in Ankara Maternity Hospital in 1980. His development and growth were normal and he never had hyperammonemia despite the constant high citrulline level in his plasma. Therefore, we considered him as having the fourth type of the disorder.

Citrullinemia is reported to be a rare urea cycle disorder[4], but AS deficiency was the most commonly encountered enzymatic defect in Turkey[16], probably due to high rate of consanguineous marriages. The parental consanguinity rate was 78 percent among the cases reported in this paper.

If hyperammonemia is thought to be due to a urea cycle defect in a patient, the enzymatic defect should be localized. Serum and urinary amino acid analysis is of value in this context. In AS deficiency, the plasma citrulline level is found to be markedly increased.

The diagnosis can be confirmed by enzyme activity in the liver[2, 17]. In this study, the diagnoses were based on serum and urinary amino acid patterns except in Case 5 (EA). AS activity in the liver was 11 percent of the control activity in this case; however, enzyme assays were not possible in all patients due to technical limitations.

Recent developments in treatment improved the survival rate of citrullinemic patients on the condition that the effective treatment is commenced before irreversible changes occur in the central nervous system[18]. In addition to a low-protein diet and supplementation with essential amino acids or their nitrogen-free analogues, use of the alternative pathways of waste nitrogen excretion is essential. Peritoneal dialysis and exchange transfusion are utilized to remove the accumulated ammonia from the body[4].

In this series, fourteen cases died during hyperammonemic attack. Ten neonatal cases died despite vigorous therapy; some of them had been admitted to hospital after severe brain damage had occurred. Four patients were lost to follow-up. Five patients, including the asymptomatic case, are alive, but only two are doing well; three of them are mentally retarded. It seems that our experience with infants affected with citrullinemia is discouraging, particularly in some of the neonatal cases in whom treatment was started on the first day of life. But some catabolic processes such as sepsis frequently overwhelm any residual enzyme activity and patients die despite appropriate therapy.

The cases presented here with different ages and symptoms of presentation illustrate the genetic heterogeneity of citrullinemia. Variability in the clinical course of our patients cannot be explained without genetic evaluation.

The authors think that this series is one of the largest studies reported on this subject and is useful in alerting clinicians to the possibility of urea cycle disorders in neonates who present with vomiting and seizure lapsing into lethargy, stupor or coma, especially if there is positive family history of sibling deaths and parental consanguinity. The same holds true for older children who have periodic vomiting, seizure and developmental delay of unknown etiology or who show these signs and symptoms after ingestion of a protein-rich diet or during an intercurrent illness. Awareness of this possibility should lead to a complete diagnostic evaluation and thus early diagnosis which is essential for the success of therapy.

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INTRAVENTRICULAR HEMORRHAGE IN PRETERM NEWBORNS*

*Barbaros Ilıkkan MD**, Mehmet Vural MD**, Demet Yardımcı MD****
*Sibel Özbek MD**, Yıldız Perk MD****, Özdemir İlter MD******

SUMMARY: Ilıkkan B, Vural M, Yardımcı D, Özbek S, Perk Y, İlter Ö. (Department of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey). Intraventricular hemorrhage in preterm newborns. Turk J Pediatr 1998; 40: 195-200.

Intraventricular hemorrhage (IVH) originating from germinal matrix is the major brain injury of the premature. We studied IVH incidence and risk factors which are implied in this pathology in newborns, hospitalized in the neonatal intensive care unit (NICU) of İstanbul University Cerrahpaşa Faculty of Medicine. Ninety-seven premature newborns with a birth weight of less than 2500 g were examined systematically with cranial ultrasonography (CU). Mean birthweight was 1540 ± 430 g (720-2450) and gestational age 31.6 ± 2.4 weeks (26-37). IVH was diagnosed in 40 cases (41%). The significant risk factors are prematurity (gestational age < 33 weeks), artifical ventilation and infusion of fresh frozen plasma. *Key words: intraventricular, hemorrhage, newborn, risk factors.*

Intraventricular hemorrhage (IVH) originating from germinal matrix is the major brain injury of the premature. Even though screening procedures are becoming more efficient, contrary to expectation, its incidence is decreased. This is obviously directly related to the advancements in perinatology and neonatology. While IVH incidence ranged from 34 to 49 percent in prematures in the early 1980's[1,2], it decreased to about 20 percent in the late 1980's[3]. Perlman and Volpe[4] classified IVH according to birth weight and found that it was seen with an incidence of 25 percent between 700-1500 g and 62 percent in weights of less than 700 g. In another study, 50 percent of IVH was found in the first, 25 percent in the second and 15 percent in the third 24 hours. Many factors, such as birth weight, gestational age, Apgar score, base deficit in umbilical cord blood, low mean arterial pressure, infusion of volume expanders, tracheal intubation, surfactant application and presence of symptomatic patent ductus arteriosus (PDA), are implied in the pathogenesis of IVH[5].

In the study of Hope et al.[6], results of cranial ultrasonography assessment were compared with autopsy findings in the brains of 56 infants with a gestational age of less than 33 weeks, to determine the diagnostic accuracy of ultrasonography in detecting IVH. Ultrasound identified IVH with a sensitivity of 91 percent and a specificity of 81 percent intraparenchymal hemorrhage with

* Pediatrician, İstanbul University Cerrahpaşa Faculty of Medicine.
** Research Assistant in Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine.
*** Associate Professor of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine.
**** Professor of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine.

a sensitivity of 82 percent and a specificity of 97 percent, and germinal matrix hemorrhage with a sensitivity of 61 percent and a specificity of 78 percent.

The aim of this retrospective study is to show IVH incidence in our neonatal intensive care unit (NICU) and risk factors which are implied in this pathology.

Material and Methods

Ninety-seven premature newborns, hospitalized in our NICU between January 1995 and January 1997 with a birth weight of less than 2500 g were examined systematically with cranial ultrasonography (CU). Standard coronal and parasagittal images were performed with Pie Medical (Holland) equipment with 5 MHz transducer. Intracranial hemorrhage is recognized by one or more of the following features: echogenic intra-parenchymal mass, unilateral or bilateral ventriculomegaly with intraventricular hyperechongenic clot and periventricular germinal matrix hyperechogenicity. χ^2 test was used to show the relation between IVH and risk factors like low birth weight, intrauterine growth retardation, low gestational age, type of delivery, artificial ventilation, necrotizing enterocolitis, patent ductus arteriosus, sepsis, thrombocytopenia ($< 50,000/mm^3$), hypoglycemia, (< 40 mg/dl), hypercarbia ($pCO2 < 60$ torr), fresh frozen plasma (FFP) infusion, hypotension (< 50 mmHg in term; < 40 mmHg in preterm newborns), and hypertension (> 90 mmHg in term; > 80 mmHg in preterm newborns). Values are expressed as mean ± standard deviation and $p < 0.05$ denotes a statistically significant difference between groups.

Results

Mean birth weight of this population was 1540 ± 430 g (720-2450), gestational age 31.6 ± 2.4 weeks (26-37), first minute Apgar score 5 ± 2 (0-9). Most of the cerebral ultrasonographic scans (64%) are done in the first seven days and mean age at the first CU is 7.9 days. In this population of 97 newborns, IVH is diagnosed in 40 of them (41%) and its classification as a function of severity is shown in Table I. Incidence of IVH as a function of birth weight is listed in Table II and statistical relations between IVH and risk factors are shown in Table III. Incidence of IVH is statistically more significant in newborns with a gestational age of less than 33 weeks, having artifical ventilation and infusion of FFP.

Table I: Classification of IVH in Function of its Severity

	NICU of Cerrahpaşa University Hospital		Volpe[5]
Grade I	6	15%	35%
Grade II	20	50%	40%
Grade III	14	35%	25%
IVH + periventricular hemorrhagic infarction	7	17%	15%
Total	40		

Table II: IVH Cases Classified in Function of Birth Weight

	IVH +	IVH –	Total
500-1000 g	6 (50%)	6 (50%)	12
1001-1500 g	19 (54%)	16 (46%)	35
1501-2000 g	10 (30%)	23 (70%)	33
2001-2500 g	5 (29%)	12 (71%)	17

Table III: Risk Factors and IVH Incidence

		Presence of Intraventricular Hemorrhage		
Risk Factors		(+)	(–)	P
Intrauterine growth	SGA	8 (50%)	8 (50%)	NS
	SGA	32 (40%)	49 (60%)	
Delivery	vaginal	20 (43%)	26 (57%)	NS
	cesarean section	20 (39%)	31 (61%)	
Gestational age	< 33 weeks	35 (55%)	29 (45%)	< 0.0005
	≥ 33 weeks	5 (15%)	28 (85%)	
Birth weight	≤ 1500 g	25 (53%)	22 (47%)	< 0.05
	> 1500 g	15 (30%)	35 (70%)	
Surfactant application	+	9 (64%)	5 (36%)	NS
	–	31 (37%)	52 (63%)	
Artifical ventilation	+	32 (52%)	29 (48%)	< 0.01
	–	8 (22%)	28 (78%)	
Sepsis	+	18 (51%)	17 (49%)	NS
	–	22 (35%)	40 (65%)	
Clinical PDA	+	4 (33%)	8 (67%)	NS
	–	36 (42%)	49 (58%)	
NEC	+	1 (100%)	0	NS
	–	39 (41%)	57 (59%)	
Thrombocytopenia	+	3 (50%)	3 (50%)	NS
	–	37 (41%)	54 (59%)	
Hypoglycemia	+	11 (55%)	9 (45%)	NS
	–	29 (38%)	48 (62%)	
Hypercarbia	+	10 (45%)	12 (55%)	NS
	–	30 (40%)	45 (60%)	
Infusion of FFP	+	28 (52%)	26 (48%)	< 0.05
	–	12 (28%)	31 (72%)	
Hypotension	+	9 (47%)	10 (53%)	NS
	–	31 (40%)	47 (60%)	
Hypertension	+	13 (57%)	10 (43%)	NS
	–	27 (36%)	47 (64%)	

SGA : small for gestational age. AGA : appropriate for gestational age.
PDA : patent ductus arteriosus. NEC : necrotizing enterocolitis.
FFP : fresh frozen plasma.

Discussion

In the NICU of the University Hospital of Cerrahpaşa, IVH incidence was found to be 41 percent. This number is similar to those presented in the literature in the late 1970's and early 1980's. Distribution of our newborns with IVH in function of severity (Table I) is different from those of Volpe[5]; in particular, the incidences of IVH with grade II and III are higher than the results of Volpe. When we compare our IVH incidence in function of birth weight (Table II) with the results of Perlman[4], we find that they are 50 and 51 percent, respectively, between 500-1000 g; 54 and 17 percent, respectively, between 1001-1500 g; and 30 and 15 percent, respectively, between 1501-2000 g. Although these differences can be explained by the small number of our population, it is also related to the pre- and postnatal conditions of the two populations. Technological advances, better understanding of neonatal problems, and increased numbers of trained medical staff decreased the incidence of IVH to 20 percent in the early 1990's. We think that we also will be able to decrease our IVH incidence with better pre- and postnatal care.

As already shown in anatomic studies, the capillary bed becomes more mature and the subependymal germinal matrix, which is the most susceptible region to IVH, undergoes a progressive decrease in size from a width of 2.5 mm at 24 weeks to 1.4 mm at 32 weeks[7]. It was not suprising for us to find a statistically significant difference of IVH incidence between infants born before and after 33 weeks of gestational age. On the other hand, when only birth weight is considered, there is no statistically significant difference.

Kuban et al.[8] showed in their study that premature small-for-gestational-age (SGA) infants of toxemic mothers had a reduced incidence of germinal matrix hemorrhage because these infants exhibit an accelerated maturation of the brain. In our population, the risk of IVH was not different between SGA and appropriate-for-gestational-age (AGA) infants.

It has been widely discussed whether abdominal delivery reduces the risk of IVH. Leviton et al.[9] showed that infants delivered vaginally were more likely to develop IVH than those delivered abdominally. We could not show a statistically significant relation between the mode of delivery and IVH, but we did find that 43 percent of newborns with vaginal delivery presented an IVH in contrast to only 39 percent of cases with abdominal delivery.

The role of coagulopathy as a cause of IVH is not very clear. Though administration of FFP is shown to decrease the incidence of IVH[10] without any amelioration of coagulation parameters, in our population, the risk of IVH is higher for infants who have received it. In a study where an experimental group receiving two doses of coagulation factor concentrate was compared to a control group,

IVH was more frequent in treated infants[11]. These results, which are similar to ours, may be explained by the increased blood flow of germinal matrix secondary to increased arterial blood pressure after FFP administration.

Artifical ventilation along with high inspiratory positive pressure, tracheal aspiration and complications increase cerebral venous pressure or cause fluctuating cerebral blood flow velocity, both of which are known to be important factors of IVH[12]. Another study[13] showed that there was a significant decrease in fluctuating cerebral blood flow from the first day of life to 72 hours of age between ventilated infants paralyzed by pancuronium bromide and those that are nonparalyzed. Our results are consistent with those of Perlman et al.[13] because we also found higher IVH incidence in ventilated premature infants. None of our patients was paralyzed.

It is now almost evident that surfactant treatment reduces mortality and pulmonary complications in preterm infants with respiratory distress syndrome. On the other hand, Horbar et al.[14] showed in a large multicenter study that infants receiving bovine surfactant had an increased number of severe periventricular hemorrhages compared with the control group. Cowan et al.[15] tried to show physiological changes after surfactant administration and found a 15 percent fall in mean airway pressure and a 36 percent fall in cerebral blood flow velocity. They concluded that these acute changes may increase the risk of IVH. Even though the difference was not significant, we also found that infants receiving surfactant had a greater incidence of IVH (9/14) than the ones who were not treated (31/83).

The role of hypoglycemia in the pathogenesis of IVH is not clear, but Pryds et al.[16] showed that it increased cerebral blood flow which might contribute to IVH. In our group of patients, hypoglycemia was not a significant risk factor of IVH.

Hypercarbia is also considered as a risk factor in IVH. Greisen et al.[17] showed that in a group of ventilated preterm infants an important reactivity of cerebral blood flow to changes in $PaCO_2$ existed. In another large study[18], hypercarbia, defined as $PaCO_2$ greater than 60 mmHg, showed a positive relation with IVH. We could not find a significant difference for IVH between infants with or without a hypercarbia episode.

Arterial hypertension is shown to have a potential role in the subquent occurrence of IVH[19]. In our study, presence of hypertension increased the risk of IVH, but this was not statistically significant probably because of the small number of the population.

We believe that we will be able to decrease our IVH rate with improvements in our pre- and postnatal care of newborns.

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LEFT VENTRICULAR DIASTOLIC ABNORMALITIES IN CHILDREN WITH β-THALASSEMIA MAJOR: A DOOPLER ECHOCARDIOGRAPHIC STUDY*

*Işın Yaprak MD**, Sadık Akşit MD***, Can Öztürk MD****, Ali R. Bakiler MD**
*Coşkun Dorak MD****, Meral Türker MD****

SUMMARY: Yaprak I, Akşit S, Öztürk C, Bakiler AR, Dorak C, Türker M. (Department of Pediatrics, Social Security Tepecik Teaching Hospital, İzmir, Turkey). Left ventricular diastolic abnormalities in children with β-thalassemia major: a Doopler echocardiographic study. Turk J Pediatr 1998; 40: 201-209.

Left ventricular filling patterns were assessed by Doppler echocardiography in 63 β-thalassemia major patients, aged for to 21 years, with no clinical evidence of congestive heart failure and 63 age- and sex-matched normal controls. The patients with β-thalassemia major were divided into three age groups, namely four to nine years (6.8 ± 1.5 years), 10-15 years (12.1 ± 1.6 years) and older than 15 years (17.3 ± 1.7 years). They were compared with age- and sex-matched normal controls in respects of Doopler diastolic indices. The ratio between the early and late (atrial) peaks of flow velocity was higher and peak flow velocity in late diastole was significantly lower in patients with β-thalassemia major as compared to controls in all three age groups ($p < .001$). As compared with the controls, peak early diastolic flow velocity was also significantly higher in the thalassemics aged 10 to 15 years (92 ± 16 vs 80 ± 12 cm/s, $P < .01$) and in those older than 15 years (95 ± 16 vs 79 ± 13 cm/s, $p < .001$). Restrictive left ventricular diastolic abnormalities were detected in a total of 34 (54%) patients with β-thalassemia major, whereas left ventricular systolic abnormalities were identified only eight (13%) of them. None of the patients without left ventricular diastolic abnormalities showed left ventricular systolic abnormalities. There was not any significant correlation between the hematologic parameters, such as mean serum ferritin, maximum serum ferritin and the number of blood units transfused, and left ventricular Doopler diastolic indices ($p > .05$). From the data presented here, we therefore conclude that left ventricular diastolic abnormalities develop in patients with β-thalassemia major in the early phase of the disease and before the apearance of systolic abnormalities, when clinical symptoms of congestive heart failure are absent.
Key words: β-thalassemia major, Doopler, echocardiography, left ventricular filling.

Congestive heart failure is the most frequent cause of death in patients with β-thalassemia major[1,2]. It has been reported in many studies that initial cardiac dysfunction can be determined in β-thalassemia major patients without clinical manifestations of heart failure by using the conventional echocardiographic/Doppler technique[3-9].

* From the Department of Pediatrics, Social Security Tepecik Teaching Hospital, İzmir.
** Associate Professor of Pediatrics, Social Security Tepecik Teaching Hospital.
*** Pediatrician, Ege University Faculty of Medicine, Bornova, İzmir.
**** Pediatrician, Social Security Tepecik Teaching Hospital.

Doppler echocardiography has been extensively used to assess left ventricular diastolic filling in patients with β-thalassemia major; however, these studies are rather conflicting[10-14]. Although some studies reported that left ventricular diastolic function was not altered in thalassemic patients[10, 11], more recent studies demonstrated that diastolic left ventricular filling abnormalities developed in the early phase of the disease in β-thalassemia major patients[12-14]. However, most of these studies were performed in adult thalassemics and, to our knowledge, there is no detailed study in the literature assessing left ventricular filling in thalassemic children in their first decade of life.

In the present study, our purpose was to investigate the left ventricular filling pattern, using Doppler echocardiography, in three different age groups of patients with β-thalassemia major who had no clinical evidence of heart failure.

Material and Methods

Sixty-three patients with β-thalassemia major without clinical signs of cardiac failure were studied by echocardiography. The patients were in the range of four to 21 years (mean, 12 ± 4 years), and 30 of them (48%) were male. None of the patients was treated with cardioactive medication. Each patient was receiving a transfusion every three to four weeks to maintain hemoglobin levels greater than 10 g/dl. Transfusion therapy had been started before the age of two years in all patients. The mean (± SD) onset of iron chelation therapy was 5.3 ± 2.5 years. For the last five years, all patients in our clinic with thalassemia major have been receiving subcutaneous deforaxime five days a week. Deferoxamine was given to each patient by subcutaneous infusion with an infusion pump for eight to 12 hours during the night at a dose of 25-50 mg/kg body weight. Prior to the last five years, the patients had received intramuscular deferoxamine three to five days a week with variable compliance.

The mean serum ferritin value in each patient was derived as the mean of six values obtained at two-month intervals during the last year. Maximum ferritin value was obtained in each patient as the highest value since the onset of transfusion therapy. Cardiac evaluation was performed 48 hours after the last transfusion, and hemoglobin levels were determined before and after transfusion in all patients.

Sixty-three age- and sex-matched normal subjects with no clinical, electrocardiographic, or echocardiographic evidence of cardiovascular disease were selected as the control group.

A Hewlett-Packard Sonos 1000 ultrasound sytem was used for the study. Two dimensional, M-mode, and Doopler echocardiographic assessments were performed using mechanical and phased-array sector scanners with 2.5-, 3.75- or 5.0-MHz transducers. Long-axis and parasternal short-axis views at the

midventricular level were used to derive the following M-mode measurements according to the criteria of the American Society of Echocardiography: left ventricular end-systolic (LVDS) and end-diastolic (LVDD) dimensions, left atrial systolic dimension (LAD), and end-systolic and end-diastolic thicknesses of interventricular septum (IVSs and IVSd) and left ventricular posterior wall (LVPWs and LVPWd)[15]. Left ventricular percentage of fractional shortening (FS) was calculated as follows: [(LVDD-LVDS)/LVDD] x 100.

A complete pulsed-wave, continuous-wave, and Doppler examinations were performed. The lowest wall filter settings were used to record flow velocities. Doppler signal, ECG lead II, and phonocardiogram were recorded on videotape simultaneously with speeds of 25, 50, and 100 mm/second.

The Doppler transmitral flow velocity profile was obtained from the apical four chamber view. In each subject, mean values were obtained by averaging at least two beats during inspiration and two beats during expiration for four to six cardiac cycles.

Peak flow velocities of the left ventricular inflow in early diastole (E) and late diastole with atrial contraction (A) were measured from the baseline to the maximum flow velocity. An E/A flow velocity ratio was calculated from each cardiac cycle. Deceleration time (DT) was measured as the distance (time) between the projection of the peak E velocity on the baseline and the point where the EF slope encounters the baseline, and the rate of deceleration of flow velocity (DR) in early diastole as the EF slope. Isovolumetric relaxation time (IVRT) was measured as the time from the onset of the aortic component of the second heart sound to the onset of diastolic flow velocity[8, 16].

Each patient and control underwent a conventional two-dimensional and transmitral Doppler flow evaluation on the same day. The echocardiographic study was performed by the same person (A.R.B.) in all patients, and Doppler tracings of patients and controls were coded. The measurements were obtained by another person (C.Ö.), who had no knowledge of the identity of the subjects. Arterial blood pressure was measured in all patients and controls at the time of the echocardiographic examination, and was within the normal range in all subjects.

Data were given as mean ± SD. Differences between study and control groups were compared using Student's t and Mann-Whitney U tests as appropriate. The relationship between hematologic data and echocardiographic findings was investigated with correlation analysis. Plus/minus two standard deviation values were calculated for echocardiographic/Doppler indices form the measurements of age- and sex-matched normal controls. If one or more Doppler variable exceeded two SD limits, the left ventricular filling pattern was accepted to be abnormal. A FS value of less than 28 percent was accepted to be evidence of left ventricular systolic abnormality. A P value of less than 0.05 was considered statistically significant.

Results

Clinical Findings

Twenty-six patients were four to nine years of age (6.8 ± 1.5 years), 23 patients 10 to 15 years (12.1 ± 1.6 years), and 14 patients older than 15 years (17.3 ± 1.7). The mean (SD) age of all the patients and controls was 12 ± 4 years. None of the patients with β-thalassemia major had clinical symptoms or signs of congestive heart failure. Thirty-three (52%) patients had a splenectomy. The hematologic profile of patients is shown in Table I. At the time of the examination, hemoglobin levels ranged from 11 to 14 g/dl (mean, 12.6 ± 0.7 g/dl). Patient and control groups were comparable with respect to systolic and diastolic blood pressure and heart rate.

Table I: Hematologic Data in Patients with β-Thalassemia Major in Three Different Age Groups*

	Age Groups (yr)			
	4-9 (n = 26)	10-15 (n = 23)	> 15 (n = 14)	Total (n = 63)
Transfusion, units/yr	22 ± 5 (14-35)	28 ± 6 (15-40)	26 ± 6 (16-36)	25 ± 6 (14-40)
Mean serum ferritin, ng/ml	3376 ± 1048 (1680-4700)	4277 ± 1468 (2340-7230)	4248 ± 1585 (2154-9340)	4884 ± 1825 (1680-9340)
Maximum serum ferritin, ng/ml	3878 ± 1226 (1845-6874)	5223 ± 1869 (2800-9340)	6195 ± 1732 (3600-8580)	4884 ± 1825 (1845-9340)
Pretransfusion hemoglobin, g/dl	9.3 ± 0.7 (8.1-10.6)	9.3 ± 0.6 (8.2-10.7)	9.6 ± 0.5 (8.7-10.6)	9.4 ± 0.6 (8.1-10.7)
Posttransfusion hemoglobin, g/dl	13.3 ± 0.8 (12.1-15.4)	12.9 ± 0.5 (12.2-14.0)	12.6 ± 0.6 (11.7-13.8)	13.0 ± 0.7 (11.7-15.4)

* The values are mean ± SD (range).

Doppler/Echo Findings

The echocardiographic measurements concerning left ventricular end-systolic and end-diastolic diameters, left atrial dimension, and the thickness of left ventricular posterior wall and interventricular septum were not different in thalassemic patients and controls. However, left ventricular FS in patients with β-thalassemia major was significantly less than that in the controls in the age groups of 10-15 years (34 ± 7% vs 39 ± 5%, $p < 0.05$) and older than 15 years (34 ± 4% vs 40 ± 5%, $p < .05$). In a total of eight (13%) patients with β-thalassemia major, fractional shortening of the left ventricle was less than 28 percent.

Left ventricular filling patterns differed substantially in the patients and controls (Tables II, III and IV). While peak flow velocity in early diastole (E) was not different in the thalassemics and controls aged four to nine years (94 ± 14 vs 92 ± 13 cm/s, $p > 0.05$), it was significantly higher in the thalassemic patients aged 10

to 15 years and older than 15 years than those in the control groups (92 ± 16 vs 80 ± 12 cm/s, $p < .01$ and 95 ± 16 vs 79 ± 13 cm/s, $p < .001$, respectively). Also, peak late diastolic flow velocity (A) was significantly lower ($p < .001$), and the ratio between the early and late peaks of flow velocity (E/A) was significantly higher ($p < .001$) in patients with β-thalassemia major than in the controls in all three age groups. Although the duration of isovolumetric relaxation time was shorter and the rate of deceleration of flow velocity after the early diastolic peak was higher in patients as compared with controls, they did not reach a statistical significance in any of the three age groups ($p > .05$). Flow velocity deceleration time was also comparable in thalassemic patients and controls ($p > .05$). Of the 63 patients with β-thalassemia major, 34 (54%) had left ventricular diastolic abnormalities, while only eight (13%) showed systolic abnormality (Table V). None of the thalassemic patients with a normal left ventricular diastolic filling pattern had a left ventricular systolic abnormality.

Table II: Echocardiographic/Doppler Data in 26 Patients with β-Thalassemia Major Aged 4-9 Years and 26 Age- and Sex-Matched Normal Controls (Mean ± SD)

Echo/Doppler Indices	Patients	Controls	P Value	Patients with Abnormal Indices, n (%)
M-mode				
LVDD (mm)	34 ± 5	33 ± 4	NS	1 (4)
LVDS (mm)	21 ± 3	20 ± 3	NS	1 (4)
FS (5)	38 ± 7	39 ± 6	NS	0 (0)
IVSs thickness (mm)	10 ± 2	11 ± 2	NS	1 (4)
IVSd thickness (mm)	6 ± 1	6 ± 1	NS	0 (0)
LVPWs thickness (mm)	10 ± 2	11 ± 2	NS	0 (0)
LVPWd thickness (mm)	6 ± 1	6 ± 1	NS	0 (0)
Doppler				
E (cm/s)	94 ± 14	92 ± 13	NS	1 (4)
A (cm/s)	43 ± 11	56 ± 9	< .001	8 (31)
E/A	2.20 ± 0.67	1.64 ± 0.21	< .001	10 (38)
DT (miliseconds)	116 ± 42	117 ± 22	NS	3 (12)
DR (cm/s^2)	643 ± 166	622 ± 164	NS	3 (12)
IVRT (miliseconds)	23 ± 12	28 ± 13	NS	2 (8)

LVDD : left ventriculardiastolic diameter.
LVDS : left ventricular systolic diameter.
FS : fractional shortening.
IVSs : interventricular septum systolic thickness.
VSd : interventricular septum diastolic thickness.
LVPWs : left ventricular posterior wall systolic thickness.
LVPWd : left ventricular posterior wall diastolic thickness.
LAD : left atrial systolic diameter.
E : peak early diastolic flow velocity.
A : peak late diastolic flow velocity.
E/A : ratio of peak flow velocity in early diastole to peak flow velocity in late diastole.
DT : flow velocity deceleration time.
DR : rate of deceleration.
IVRT : isovolumetric relaxation time.

Table III: Echocardiographic/Doppler Data in 23 Patients with β-Thalassemia Major Agend 10-15 Years and 23 Age- and Sex-Matched Normal Controls (Mean ± SD)

Echo/Doppler Indices	Patients	Controls	P Value	Patients with Abnormal Indices, n (%)
M-mode				
LVDD (mm)	39 ± 5	39 ± 4	NS	1 (4)
LVDS (mm)	26 ± 5	NS	2 (9)	
FS (%)	34 ± 7	39 ± 5	< .05	7 (30)
IVSs thickness (mm)	11 ± 2	12 ± 3	NS	1 (4)
IVSd thickness (mm)	7 ± 2	7 ± 1	NS	1 (4)
LVPWs thickness (mm)	12 ± 2	12 ± 3	NS	0 (0)
LVPWd thickness (mm)	7 ± 2	7 ± 1	NS	0 (0)
Doppler				
E (cm/s)	92 ± 16	80 ± 12	< .01	6 (26)
A (cm/s)	38 ± 9	48 ± 9	< .001	3 (13)
E/A	2.42 ± 0.63	1.67 ± 0.30	< .001	14 (61)
DT (miliseconds)	131 ± 41	134 ± 29	NS	4 (17)
DR (cm/s^2)	632 ± 179	573 ± 167	NS	5 (21)
IVRT (miliseconds)	35 ± 16	44 ± 18	NS	2 (9)

Abbreviations: See Table II.

Table IV: Echocardiographic/Doppler Data in 14 Patients with β-Thalassemia Major Older Than 15 Years and 14 Age- and Sex-Matched Normal Controls (Mean ± SD)

Echo/Doppler Indices	Patients	Controls	P Value	Patients with Abnormal Indices, n (%)
M-mode				
LVDD (mm)	44 ± 4	43 ± 2	NS	1 (7)
LVDS (mm)	29 ± 4	26 ± 4	NS	1 (7)
FS (%)	34 ± 4	40 ± 5	< .05	1 (7)
IVSs thickness (mm)	14 ± 3	14 ± 3	NS	0 (0)
IVDd thickness (mm)	8 ± 2	8 ± 1	NS	1 (7)
LVPWs thickness (mm)	14 ± 3	13 ± 2	NS	1 (7)
LVPWd thickness (mm)	8 ± 2	8 ± 1	NS	1 (7)
Doppler				
E (cm/s)	95 ± 16	79 ± 13	< .001	5 (36)
A (cm/s)	35 ± 8	46 ± 11	< .001	2 (14)
E/A	2.70 ± 0.76	1.72 ± 0.33	< .001	8 (57)
DT (miliseconds)	135 ± 34	138 ± 33	NS	1 (7)
DR (cm/s^2)	613 ± 142	558 ± 182	NS	3 (21)
IVRT (miliseconds)	38 ± 14	45 ± 11	NS	4 (29)

Abbreviations: See Table II.

Table V: Comparison of Systolic and Diastolic Indices of Left Ventricle in Patients with β-Thalassemia Major*

	Diastolic Indices		
Systolic Indices	Normal	Abnormal	Total
Normal	29 (46)	26 (41)	55 (87)
Abnormal	–	8 (13)	8 (13)
Total	29 (46)	34 (54)	63 (100)

* The values express number (%) of the patients.

There was no statistically significant correlation between hematologic data (mean serum ferritin, maximum serum ferritin, pretransfusion hemoglobin, number of blood units received) and left ventricular systolic (FS%) or diastolic (E, A, E/A, DT, DR, IVRT) indices ($p > .05$).

Discussion

Most of the previous authors investigated systolic and diastolic functions of the left ventricle in adult thalassemics. To our knowledge, there is no detailed study in the literature assessing both systolic and diastolic left ventricular functions in children with β-thalassemia major in their first decade of life.

Normal values are available for cardiac chamber dimensions measured from the two dimensional echocardiogram in adults. However, well-established normal data for cardiac chamber dimensions and Doppler diastolic indices from infancy to adulthood are not yet available. To minimize the influence of age on the Doppler diastolic indices, we divided thalassemic patients into three age groups, and compared them with age- and sex-matched normal controls.

Our study demonstrated that, in normal controls, peak early and late diastolic flow velocities and rate of deceleration of flow velocity after the early diastolic peak progressively decreased and flow velocity deceleration time and isovolumetric relaxation time progressively increased as the age of the child increased. However, while peak early diastolic flow velocity remained high in all three age groups of thalassemic children, the rate of deceleration increased with an increasing age as compared with controls. Peak late diastolic flow velocity was lower in the thalassemics than in the controls in all three age groups. This led to, in combination with higher peak early diastolic flow velocity in the thalassemics compared with controls, a greater E/A ratio in patients.

The Doppler data in our patients suggest a left ventricular restrictive abnormality characterized by increased filling in early diastole and impaired filling in late diastole, with an abnormal Doppler waveform of steeper slope of the early diastolic flow-velocity peak and decreased height of the late peak. These results are in accordance with Spirito et al.[12] who also detected restrictive diastolic abnormalities of the left ventricle in patients with β-thalassemia major, even in the early stage of the disease when iron overload had not yet caused systolic dysfunction.

When individual patient analysis was performed, in a total of 34 (54%) patients, one or more Doppler diastolic indices were beyond ± two SD limits obtained from the normal controls. The most common diastolic abnormalities were the increased ratio between early and late peaks of flow velocity and decreased late flow velocity. The E/A ratio was found to be abnormal even at an earlier stage of the disease [in 10 (38%) patients aged 4 to 9 years].

Our study also demonstrated that left ventricular systolic function in patients with β-thalassemia major was well-preserved, and became abnormal only after diastolic functions deteriorated. These results are also in accordance with the recent reports[12-14]. There were a total of eight (13%) patients with a FS value of less than 28 percent in our study.

Left ventricular and atrial dimensions and the thicknesses of IVS and LVPW were not different in thalassemic patients and controls. These findings are also in accordance with the previous studies[12-14]. Kremastinos et al.[11] also reported that these parameters become abnormal in thalassemic patients only after the clinical symptoms of congestive heart failure appear.

The pathogenesis of cardiac complications in β-thalassemia major is not yet clear. Many investigators blamed iron as the cause of cardiac abnormalities and reported that early and appropriate treatment with deferoxamine delayed cardiac complications[17-19]. Lewis et al.[6] did not determine any differences in left ventricular systolic performance between patients with β-thalassemia major and intermedia, and reported that clinical cardiac failure was the consequence of volume overload and abnormal cardiac compliance. Özbarlas et al.[20] also reported that restrictive diastolic abnormalities of the left ventricle developed in thalassemic patients when systolic functions were normal and that systolic and diastolic functions of the left ventricle were found to be comparable in the patients with thalassemia major and intermedia.

In the present study, there were not any significant correlations between hematologic data and left ventricular systolic and diastolic indices. This is in accordance with the studies of Kremastinos et al.[10, 11, 13] in which they also reported that iron overload is not the causative factor in the development of congestive heart failure but possibly only a predisposing factor in combination with others.

In conclusion, left ventricular diastolic abnormalities develop in patients with β-thalassemia major in an early stage of the disease when symptoms of congestive heart failure are absent and systolic functions are normal.

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A SEX – SPECIFIC DIFFERENCE IN HOMOZYGOSITY FOR HLA – DR53 IN NEWBORNS*

*Asuman Sunguroğlu PhD**, Dicle Güç MD PhD***, Tevfik U. Sipahi MD**** M. Tevfik Dorak MD******

SUMMARY: Sunguroğlu A, Güç D, Sipahi TU, Dorak MT. (Department of Hematology, University of Wales College of Medicine, Cardiff, U.K.) A sex-specific difference in homozygosity for HLA-DR53 in newborns. Turk J Pediatr 1998; 40: 211-216.

Homozygosity for HLA-DR53 confers increased susceptibility to major forms of leukemia. In childhood leukemia, this influence is male-specific. Two separate studies have shown a male-specific increase in the hemozoygosity rate for HLA-DR53 in healthy adults. This finding was attributed to possible preferential transmission of HLA-DR53 towards male offspring. If this is the case, the consequences of such a prenatal event should be evident in the newborn population. The present study investigated HLA-B and -DQA1 genotype frequencies in a sample of 134 newborns (73 boys, 61 girls) in Turkey. Restriction fragment length polymorphism (RFLP) analysis showed a homozygosity rate of 8.2 percent for HLA-DR53. Nine of 11 homozygotes were boys and the sex-specific rates were 12.3 percent vs 3.3 percent in boys and girls, respectively (p = 0.05). The DR53 homozygosity rate in males was higher than the expected rate (p = 0.02). These findigns suggested a prenatal mechanism behind the excess of DR53 homozygotes in the male population. To maintain equilibrium, this excess seems to be eliminated postnatally. This model also explains how a deleterious genotype escapes natural selection. *Key words: HLA-DR53 homozygosity, sex factors, genetics, newborn.*

The major histocompatibility complex (MHC) encodes for polymorphic transplantation antigens (H-2 in mice, HLA in humans) and a number of other genes with immunological or non-immunological functions[1]. Among those, there seem to be genes influencing the development of malignancies. The first diseases associated with the MHC were leukemia in mice[2] and Hodgkin's disease in humans[3]. The mouse-leukemia association is with homozygosity for the H-2^k haplotype, whereas several molecular studies have shown that its counterpart in humans is HLA-DR53[4-7]. This is not surprising, as these two speificities are serologically cross-reactive[8].

It was originally postulated that an HLA specificity conferring increased susceptibility to a lethal disease should have a portective mechanism to propagate itself over many generations, i.e., escape natural selection[9]. It appears

* From the Department of Hematology, University of Wales College of Medicine, Cardiff.

** Assistant Professor of Medical Biology, Ankara University Faculty of Medicine, Ankara.

*** Assistant Professor of Basic Oncology, Hacettepe University Institute of Oncology, Ankara.

**** Obstetrician, Zübeyde Hanım Maternity Hospital, Ankara.

***** Lecturer in Hematology, University of Wales College of Medicine.

that homozygosity for HLA-DR53 is increased in healthy males[10, 11]. This finding suggests an increased transmission ratio of HLA-DR53 from parents to offspring as the protective mechanism[10]. This model fits in with the original postulate that the susceptibility haplotype should behave like a mouse t-haplotype, which is a recessive lethal and protected against natural selection by its preferential transmission from heterozygotes[12, 13]. Interestingly, the mouse MHC is embedded within the t-complex and intermingled with several recessive lethals[14, 15].

The present study investigated the HLA-DR53 homozygosity rate in a group of newborn babies to gain further insight into the male-specific increase and to see whether a prenatal (pre- or post-zygotic) mechanism may be responsible for it. It was expected that a newborn population which has not been subject to any postnatal selection process would be ideal for this purpose.

Material and Methods

Anonymous cord blood samples and sex of the babies were obtained from the Zübeyde Hanım Maternity Hospital in Ankara, Turkey. Altogether 134 samples were collected in a period of three months with no selection. All the samples were from Turkish babies born to families living in the Ankara province. DNA was extracted using standard methods from cord blood samples anti-coagulated with sodium EDTA.

HLA-DQA1 and HLA-B RFLP analyses were done as described elsewhere using the restriction endonuclease TaqI and the cDNA probes 10-8 and pB250, respectively[16]. These RFLP typing systems yield five alleles at each locus. One allele of each locus is strictly associated with a common serological HLA specificity. These are the 5.8 kb DQA1 allele and HLA-DR53, and 2.6b HLA-B allele and HLA-B7 (in addition, 3.2kb HLA-B corresponds to HLA-B57, but this is extremely rare in the Turkish population). Therefore, it was possible to estimate gene frequencies and homozygosity rates for these alleles by direct counting.

Statistical evaluation of the differences was made using the Fisher's exact test when a number was less than 5 in a 2 x 2 table as well as the Z-test for two proportions from independent groups. In confirmation of the previously shown male-specific higher homozygosity rate, a one-tailed p value was used. Because the direction of the difference was specified in the hypothesis, a one-tailed test was deemed to be appropriate. The observed rates were compared to expected rates using the Z-test for sample proportion versus hypothesized value.

Results

The total and sex-specific allele frequencies and homozygosity rates are presented in Tables I and II. They only significant difference was noted between male- and female-specific homozygosity rates for the 5.8kb RFLP band of the

DQA1 locus which corresponds to HLA-DR53 ($p = 0.05$ by Fisher's exact test; $p = 0.03$ by Z-test). The HLA-DR53 allele frequency was not significantly different between the two groups (Table III). No other genotype of either locus showed a similar trend.

Table I: HLA-B Allele Frequencies (AF) and Homozygosity Rates (HR) (%)

		Total n = 134	Male n = 73	Female n = 61
Allele A	AF	36.9	35.6	38.5
9.0kb	HR	14.9	12.3	18.0
Allele B	AF	22.4	21.0	23.0
4.2kb	HR	4.5	2.7	6.6
Allele C	AF	35.1	8.4	31.1
3.4kb	HR	15.7	17.8	6.6
Allele D	AF	0.0	0.0	0.0
3.2kb/HLA-B57	HR	0.0	0.0	0.0
Allele E	AF	5.2	4.1	6.6
2.6kb/HLA-B7	HR	0.0	0.0	0.0

The nomenclature for alleles is arbitrary (see ref. 16).
Allele D and homozygosity for allele E (HLA-B7) were not observed at all.

Table II: HLA-DQA1 Allele Frequencies (AF) and Homozygosity Rates (HR) (%)

		Total n = 134	Male n = 73	Female n = 61
Allele A	AF	11.6	14.4	8.2
2.7kb	HR	1.5	2.7	0.0
Allele 1B	AF	16.8	13.0	21.3
6.7kb	HR	0.7	1.4	0.0
Allele 1C	AF	15.3	15.8	14.8
7.4kb	HR	3.7	5.8	1.6
Allele 2	AF	27.2	26.0	31.5
4.8kb	HR	9.0	9.6	8.2
Allele 3	AF	29.1	30.8	21.3
5.8kb/HLA-DR63	HR	8.2	12.3	3.3

The nomenclature for alleles is from Ref. 17.

Table III: Sex-Specific Gene Frequencies (GF) and Homozygosity Rates (HR) of HLA-DR53 and Homozygosity Rates for a Supertypic DR53 Haplotype (%)

			Newborns		Adults
		Total n = 134	Boys n = 73	Girls n = 61	(Female) n = 65
HLA-DR53	GF	29.1	30.3[a]	27.0	25.4[b]
HLA-DR53	HR	8.2	12.3[c]	3.3	4.6

a: The difference between boys and girls is not significant and originates from the difference in the homozygosity rates.
b: Yields an expected homozygosity rate of 6.45% calculated by the formula hr = gz.
c: For comparison to the homozygosity rate in girls p = 0.05.

Discussion

The present study aimed to distinguish a prenatal mechanism from a postnatal one causing the male-specific increase in the HLA-DR53 homozygosity rate. Although the numbers are relatively small for a population study, the mechanism of increased homozygosity for HLA-DR53 in healthy adult males reported previously seemed to be a prenatal one as the same increase was noted in newborns. This sex-specific difference in the homozygosity rates may be due to an increase in males or a decrease in females, or both.

In the other two British studies, the homozygosity rate for DR53 in adult males was higher than the expected rate calculated from the gene frequency of the same population. As a control group for an ongoing young-onset breast cancer study, 65 females from Ankara were typed at the DQA1 locus also by RFLP analysis. In that group, the HLA-DR53 gene frequency was 25.4 percent (Table III). This frequency yielded an expected homozygosity rate of 6.45 percent. The observed rate of 12.33 percent was significantly higher (p = 0.02, Z-test), in line with the findings of the previous larger studies. The general excess of males in the newborn population is well/recognized; this excess gradually disappears later in life[18]. The sex-differential in the homozygosity rate for HLA-DR53 seems to correlate to the overall male excess among live births.

On the other hand, the female-specific homozygosity rate in newborns is non-significantly lower than the excepted rate, probably due to small numbers (3.28% vs 6.45%). If this trend persists and reaches statistical significance in a larger group, it will have important implications. This might turn out to be the immunogenetic basis for a higher abortion rate of female fetuses[19, 20].

A plausible model would be that homozygosity for HLA-DR53 is increased due to preferential transmission of HLA-DR53 at the pre-zygotic level, with the excess being eliminated during the prenatal period in female fetuses and postnatally in males. In line with the increased abortion rate in female fetuses, there is a male predominance in childhood malignancies[21]. The male-specificity of HLA-

DR53 association in the most common childhood malignancy, leukemia, supports this model[5]. The long-suspected link in the genetics of spontaneous abortions and cancer[9, 22], and even more strikingly, an increased leukemia risk among the survivors of threatened abortions[23, 24], imply that abortions and childhood malignancies may serve the same evolutionary purpose. In light of the currently available data, we suggest that HLA-DR53 homozygosity may be a factor in the common background of abortions and leukemia susceptibility.

The results of the present study lead the way to further studies to follow up the findings which have been shown in three different populations to date. Considering the possible implications of these findings in genetics, predictive epidemiology and preventive medicine, we find it would be worthwhile to take further steps, such as increasing the size of the sample analyzed in this study and extending it to healthy adults to examine the role of HLA-DR53 in longevity. The DNA samples collected can also be used for a comprehensive (class I, II, III) HLA polymorphism study at the DNA level for the first time in the Turkish population.

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THE STATUS OF CHILD HEALTH AND CHILD SURVIVAL AND DEVELOPMENT PROGRAMS IN TURKEY*

*Hilal Özcebe MD***

SUMMARY: Özcebe H. (Department of Public Health, Hacettepe University Faculty of Medicine, Ankara, Turkey). The status of child health and child survival and development programs in Turkey. Turk J Pediatr 1998; 40: 217-230.

"Child Survival Activities in Turkey" are: growth monitoring programs, expanded programs of immunization (elimination of neonatal tetanus, reducing morbidity and mortality of measles, eradication of polio), control of diarrheal diseases (oral rehydration therapy), control of deaths from preumonia (ARI), baby-friendly hospitals initiative and promotion of breast-feeding, salt iodization programs, elimination of vitamin A deficiency, safe motherhood projects, and phenylketonuria screening programs. Furthermore, family planning, nutrition and education of the mother were among the subjects covered because of their role in child health. The activities, aims and strategies related to these programs are taken up separately. The status of child health and some of the child survival and development programs (growth monitoring program, expanded program of immunization, control of diarrheal diseases, control of deaths from pneumonia, baby-friendly hospitals initiative and promotion of breast-feeding) are discussed in the article. *Key words: child survival programs, Turkey.*

The concept of "Child Survival" started gaining momentum with analysis of figures of infant deaths at the beginning of the 1980s. In 981-82, 40,000 children were dying each day, 100 million children were hungry every day, and 10 million children were disabled in mind or body. Analysis clearly showed that deaths were largely due to diarrheal disease and consequent dehydration; vaccine preventable diseases, especially measles; and malnutrition. The idea of a revolution then gathered strength from the fact that effective techniques were already available. These were growth monitoring, oral rehydration therapy, breast-feeding and immunization (GOBI). In 1984, UNICEF declared that world-wide support had been gathering behind the idea of a revolution which could save the lives of seven million children each year, protect the health and growth of millions more and help slow population growth. The strategy of a limited number of potentially vertical programs was made as it was felt to be cutting across the Primary Health Care integrated approach, even though the Expanded Program of Immunization (EPI), Control of Diarrheal Diseases (CDD) and Maternal Child Health/Family Planning (MCH/FP) are not as integrated as was hoped. To increase the development as well as the survival elements and broaden the approach, three F's were added-food supplementation (which soon gave way

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

** Associate Professor of Public Health, Hacettepe University Faculty of Medicine.

to the better aim of food security), birth spacing, and female education, although these were recognized as more costly and more difficult. The recent consensus view on components in child survival includes: immunization, growth monitoring, oral rehydration therapy, breast-feeding, female education, family planning, food security, drinking water, control of acute respiratory infections, AIDS control, control of malaria, vitamin A supplementation, and prevention of iodine deficiency disorders[1].

The Status of Child Health in Turkey

The initial phase of any child survival program should be a detailed situation analysis of the mortality and morbidity of the mothers and children. Infant mortality, the probability of dying in the first year of life, is a sensitive indicator of social welfare to assess where the greatest need may be. Another indicator is the under-five mortality rate: the number of deaths of children under five years of age per 1,000. This is the probability of dying between birth and exactly five years of age[2]. Today, in many countries of the world, governments are more concerned about where they are ranked among nations on infant and under-five mortality than on gross national product (GNP) per capita. Developing countries are in a process of rapid change in child survival; many register very significant improvements, others are losing momentum. The infant mortality rate shows the general health and socio-economic status of the population and is particularly sensitive to changes in environmental and social conditions. In most developing countries, there are no reliable or continuous records for estimating childhood mortality.

There are an insufficient number of doctors and health personnel in provincial and district centers outside the major cities in Turkey. For this reason, and because of the low priority given to collecting statistics in the outlying provinces, information flow from these centers is not complete. It is required, by law, to obtain a "Permission Form for Burial" for a dead person. However, this is neglected in most of the provincial and district centers. Thus, the reported rate of death is lower than the real rate of death calculated by researchers. Female deaths, especially in child mortality, are irregularly recorded in the burial permission registrations and the life expectancy of females is generally higher than that of males. Distribution of deaths by age group indicates that deaths under one year of age make up a large proportion of total deaths due to the high infant mortality rate in Turkey. The proportion of infant deaths among total deaths is approximately 15 percent in the country[3].

The infant mortality rate was 154 per 1,000 for the period 1967-1968 in the survey conducted by the Ministry of Health, but there was no estimation of neonatal and postneonatal mortality rates. Basic information on the infant mortality rate was collected from Turkish Demographic and Health Surveys conducted by the Hacettepe Institute of Population Studies since 1972. According to these studies,

the infant mortality rate was estimated as 134 per 1,000 for 1972-1977, 95 per 1,000 for 1978-1983, 77.7 per 1,000 for 1985-1987, and 52.6 per 1,000 for 1988-1993[4,5] (Table I). The estimated infant mortality rate for 1997 is 42.2 per thousand[6]. Although the present rate of infant mortality is still high, it has decreased over the years simultaneously with improved social and economic development. In rural areas, infant mortality is 65.4 per thousand, that is, higher than the national average, while the rate in urban areas (44.0 per thousand) is lower than the national average. That again could be due to more health facilities, more opportunity to use them and higher socio-economic levels in urban areas. The infant mortality rate of western Anatolia is lowest among the regions (42.7 per 1,000) while that of eastern Anatolia is the highest (60.0 per 1,000). There are inadequate health conditions and a lower socio-economic level in eastern Anatolia than in the other regions[4,5].

Table I: Infant, Neonatal, Postneonatal and Under-Five Mortality Rates (per 1,000) Between 1967-1993 in Turkey

Years	Infant Mortality Rate	Neonatal Mortality Rate	Postneonatal Mortality Rate	Under-Five Mortality Rate
1967-68	154	–	–	–
1972-77	134	60	74	–
1978-83	95	42	53	–
1985-87	77.7	35.5	42.2	97.5
1988-93	52.6	29.2	23.4	60.9
Declining Rate (%)	60.7 (1967-93)	51.3 (1972-93)	68.9 (1972-93)	37.5 (1985-93)

Source: Bertan M, Özcebe H. Infant mortality trends in Turkey. Acta Reproductiva Turcica 1992: 14: 27-35.

Ministry of Health (Turkey), Hacettepe University Institute of Population Studies, and Macro International Inc. Turkish Demographic and Health Survey 1993; 1994.

The infant mortality rate is usually examined as neonatal and postneonatal mortality, the former covering deaths at one to four weeks, and the latter at five to 52 weeks of age. The neonatal mortality rate was 60 per 1,000 for 1972-1977 and 29.2 per 1,000 for 1988-1993. The declining rate was found to be 51.3 percent between these two periods. On the other hand, the postneonatal mortality rate was estimated at 74 per 1,000 for 1972-1977 and 23.4 per 1,000 for 1988-1993, and its declining rate was 68.9 percent in this period[4,5] (Table I). According to the 1993 Demographic and Health Survey, the neonatal mortality rate is 29.2 per 1,000, while the postneonatal death rate is 23.4 per 1,000. The neonatal death rate is 29.9 1,000 in urban and 28.1 per 1,000 in rural areas. Postneonatal rates, on the other hand, are 14.1 per thousand in urban and 37.4 per 1,000 in rural areas[5]. There is no data for perinatal mortality rate for all of Turkey.

The under-five mortality rate has been designated by UNICEF as the most important indicator of the state of a nation's children. It is estimated that for the country overall the under-five mortality rate for 1988-1993 is 60.9 per 1,000. This is undoubtedly very high, implying that overall for every 100 births, almost six children did not reach their fifth birthday. There are some differences between regions and place of residence: 50.5 per 1,000 in urban areas, 76.4 per 1,000 in rural areas. The eastern part of Turkey has the highest rate among all regions, 70.4 per 1,000. Rates for the other parts of Turkey are: western Turkey 48.0 per 1,000 southern Turkey 62.8 per 1,000, central Turkey 69.2 per 1,000 and northern Turkey 49.5 per 1,000[5].

The causes of child mortality are not known accurately for the country. Regarding causes of death, data is available for only towns and cities. Pneumonia and enteritis were the main causes between 1967-1975.

According to the State Institute of Statistics, the main causes of child mortality in Turkey are birth injury, difficult labor, and other causes of perinatal mortality. These causes make up about half of child mortality cases occurring in the first month of life. Therefore, causes which are mainly related to genetic makeup, pregnancy, and labor are the main reasons for child mortality. Among the other causes, pneumonia is the second and heart diseases the third most common causes. Causes of child deaths are given in Table II.

Table II: Percentage of Child Mortality, Under Five Years of Age, by Main Causes of Deaths in 1993

Causes of Deaths for Children	%
Other causes of perinatal mortality	36.30
Birth injury, difficult labor, anoxia and other anoxic and hypoxic conditions	16.13
Pneumonia	10.42
Heart diseases	8.64
Meningococcal infections	6.05
Enteritis and other diarrheal diseases	5.63
Congenital anomalies	2.99
Symptoms and ill-defined conditions	1.68
All other diseases and accidents	12.16

* These causes are only from cities and towns.

Source: State Institute of Statistics, Under Prime Minister Republic of Turkey. Death Statistics From Provincial and District Centres 1993. State Institute of Statistics, Printing Division. Publ. No. 1836; 1995: IX-XIII.

Some indicators of child health in Turkey (1977) can be seen in Table III, some factors affecting infant mortality in Turkey can be summarized as follows[5, 8, 9]:

Table III: Some Indicators of Child Health in Turkey (1977)

Mortality and health	
Infant mortality rate (per 1,000)	42
Under-five mortality rate (per 1,000)	50
Children who are breastfed (%)	
0-1 months	99
4-5 months	80
Children 12-23 months	
Given BCG (%)	69
Given DPT (3 doses) (%)	84
Given Polio DPT (3 doses (%)	83
Given measles vaccination (%)	84
had diarrhea in the last 2 weeks (%)	25
Had acute respiratory infections in the last 2 weeks (%)	12
Chronically undernourished (stunted) (%)	19
Acutely undernourished (wasted) (%)	3

Source: Republic of Turkey Ministry of Health. Turkey: Demographic and Health Indicators; Ankara: 1997.

- The mothers's level of education. Educated mothers tend to provide better quality care for their children, thus securing their health. Also, the educational level of the father has a bearing on the infant mortality rate. Children of mothers with no education (those who have never attended school or did not complete the primary level) experience over 1.6 times the level of infant and under-five mortality as children of mothers who have at least completed primary school.
- Maternal age at birth (under 20 and over 35 years have an adverse effect on infant mortality). The highest mortality rates are associated with children whose mothers were younger than 20 years of age or were 30-39 years, respectively. The strongest effect of the mother's age on childhood mortality occurs in the case of neonatal mortality. Children of mothers who were younger than 20 at the time of birth experienced 88 percent higher mortality risks during the first months of life than children of mothers who were aged 20-29 at the time they gave birth.
- An interval of less than two years between births is also an important factor contributing to high mortality. On the other hand, infant mortality is relatively high for women over 30 years and whose parity is four or more. The infant mortality rate for birth orders of seven and more ise 2.5 times higher than that of birth orders of two to three, and the differential in the neonatal mortality rate is even greater.
- Utilization of health facilities and the daily observance of hygienic practices both work to decrease infant mortality. Prenatal care, identification of high-risk pregnancies and some economic conditions of families are also related to

infant mortality. Under-five mortality rate is 55 percent higher (77 per 1,000) among children born to women who received neither antenatal care nor delivery care from a trained health professional, compared to that among children whose mothers received either or both of these services (50 per 1,000).

Child Survival and Development Activities in Turkey

The Government of Turkey identified its health targets in the fifth five-year Development Plan and special emphasis was given to improvements in maternal and child health. One key goal is the reduction of infant and childhood mortality to less than 50 per 1,000 live births. Causes of deaths of infants and toddlers are mainly pneumonia, measles, diarrhea, birth trauma and injuries. The infant mortality rate was 95 per 1,000 live births in 1983. Total infant deaths every year are about 150,000 infants. Twenty-nine thousand children die from vaccine preventable diseases alone and more than half that number are permanently handicapped. The infant mortality rate in 1988 was 64 per 1.000. It is apparent that, to reach the WHO European Regional Office target infant mortality rate set for its own region, of 20 per 1,000 by the year 2000, is rather difficult. To meet these targets requires systematic, insistent, planned and effective management and supervision. The child and maternal health programs initiated as a result of the situation are a step towards the realization of the contents of the "Revolution of Child Survival and Development" supported by UNICEF. As a result of these consultations, a tripartite framework for a Program of Cooperation for Child Survival and Development within the context of Primary Health Care 1985-1989 was signed between the Government of Turkey, UNICEF and WHO[10, 11].

The program consists of immunization against the most frequent diseases seen (diphtheria, pertussis, tetanus (DPT), polio, measles and tuberculosis), encouragement of breast-feeding, provision of sufficient nutrition, growth and development monitoring, intervention in diarrheal diseases through early application of oral rehydration therapy, and controlling lower respiratory tract infections. Furthermore, family planning, nutrition and education of the mother were among the subjects covered because of their role in child health. The activities, aims and strategies related to these programs are taken up separately[10, 11].

The world's leaders who attended the historic children's summit agreed on a remarkable package of strategies and goals that represent what the world's leading experts and development agencies believe can be accomplished for children and women in 1990s. At the 1990 World Summit for Children, 149 countries formally committed themselves to establishing a national program for achieving the year 2000 goals adopted by the summit. Turkey's National Program of Action was finalized in December 1993 and submitted to the UN Secretary General[12]. In Table IV, the defined targets of the National Plan of Actions, Child Health by the Year 2000 is given (these targets are based on the 1990s rates)[13].

Table IV: Defined Targets of the National Plan of Actions, Child Health by the Year 2000

- Reducing infant mortality rate by one-third,
- Reducing the mortality rate of children under the age of five years by 50 percent,
- Reducing the mortality rate of children under the age of five years due to diarrheal diseases by 50 percent, and the incidence of diarrheal diseases by 25 percent,
- Increasing oral rehydration therapy to 80 percent,
- Reducing the mortality rate of children under the age of five years due to ARI*, particularly pneumonia,
- Reducing the severe and moderate malnutrition of children under five years of age by 50 percent,
- Achieving 95 percent immunization coverage for each vaccine in the under one age group,
- Eradicating polio,
- Eliminating neonatal tetanus,
- Reducing measles incidence by 90 percent and the mortality rate due to measles by 95 percent.

ARI: acute respiratory infections.

Source: Akin A, Biliker MA, Benli N. Reproductive Health Programs in Turkey (1977). Ministry of Health, General Directorate of Maternal Child Health Family Planning.

The Ministry of Health has "Child Survival and Development Activities" to meet these targets[7]. In the text, some of these programs will be discussed. These programs are:

- Growth monitoring program.
- Expanded program of immunization.
- Elimination of neonatal tetanus.
- Reducing morbidity and mortality of measles.
- Eradication of polio.
- Control of diarrheal diseases (oral rehydration therapy.
- Control of deaths from pneumonia (ARI).
- Baby-friendly hospitals initiative and promotion of breast-feeding.
- Salt iodization program.
- Virtual elimination of vitamin A deficiency.
- Safe motherhood projects.
- Phenylketonuria screening program.

Growth Monitoring Program

Growth monitoring and promotion have been extensively evaluated and assessed from the point of view of the instruments (availability and suitability of scales and growth cards), of the process (the involvement, education, understanding,

behavioral change of mothers, and incorporation of routine child care), and of the impact on nutritional status. The program is also used for growth surveillance of a community or district. The activities of growth monitoring must begin early before any faltering or malnutrition develops. Growth monitoring activity is a matter of prevention and education, rather than being curative or rehabilitative.

The aim of growth monitoring is to indicate malnutrition before it causes serious damage to a child's health. At this early stage, a nurse or a midwife can educate a family/mother on ways to improve a child's nutritional status, even when food and money are scarce. Growth monitoring is done using a chart usually coupled with an immunization record. A child is followed three times in the first two months and five times until one year. From one to three years of age the child is followed every three months and once every year from four to six years of age[14].

The height-for-age index is an important indicator of chronic undernutrition. A period of at least 12 months or even 24 months is necessary to see the outcome chronic nutritional problems. According to the 1993 Demographic and Health Survey, the youngest children (under 6 months old) show no evidence of malnutrition. Among the children 24-59 months of age, 25 percent are classified stunted (weighted average of the percentages in the 24-59 months age group). According to the survey, by age five nearly one-fifth of the children are chronically undernourished and about 10 percent are severely stunted. These patterns reflect inadequate feeding practice and the presence of recurrent and chronic illness[14, 15].

Expanded Program of Immunization

Many infectious diseases stimulate the production of productive antibodies that usually confer long-lasting, even lifelong, protection against reinfection. Vaccines and toxoids stimulate production of these antibodies without causing disease[20].

Until 1985, the Expanded Program of Immunization carried out through primary health care had not reached a desired level of coverage of the target child population. In order to accelerate coverage to reach universal child immunization by 1990, the Ministry of Health, as the main governmental body responsible for the provision of health services, planned a national immunization campaign in 1985. Preparations started in 1984 included a series of high-level meetings and a preparatory workshop with participation from the Ministry of Health, other sectors as well as UNICEF and WHO. Intensive immunization against the five diseases (DPT, polio, measles) where protection can be achieved with vaccination was begun with the Ministry of Health campaign in 1985: 4.3 million children under five years of age were vaccinated against DPT, polio and measles. Thus, it was estimated that approximately 25,000 deaths from measles, 10,000 deaths from pertussis and 14,000 cases of polio were averted[21].

Hacettepe University's Institute of Population Studies, with samples chosen before and after the immunization campaign, showed that the ratio of three doses of DPT and polio vaccine application had risen from 37 percent to 76 percent in all children below five years of age, and immunization against measles had risen from 37 percent to 83 percent. The percentage of vaccination for measles in infants less than one year old was 12 percent before and 72 percent after the campaign. In 1986, this level was not maintained due to a lack of inter-sectoral coordination and proper emphasis of training strategies. In 1987, the Immunization Program was integrated into the routine services of primary health care, while in places without a health unit, the service was provided through mobile teams. The 0 age group BCG vaccination was also integrated into routine health services in 1990[14].

A "Vaccination Coverage Survey" carried out in 67 provinces during 1987 showed that 67 percent of children aged 12-23 months were vaccinated with BCG, 86 percent with DPT3/polio3 and 82 percent with measles vaccines. Total immunization rate for this age group was 60 percent[14].

According to the 1993 Demographic and Health Survey, among children aged 12-23 months, the coverage rate of BCG was 89.1 percent, the coverage rate of DPT3 was 77.1 percent and the coverage of measles was 77.8 percent. The results indicate that 65 percent of the children had received all of their immunizations at some time before the survey. Only three percent had not received any vaccination at all. The remaining 32 percent were partially vaccinated. The percentage of children who were fully immunized by 12 months of age was 59 percent[18].

In Turkey, the routine vaccination program at present covers six diseases: diphtheria, pertussis, tetanus, tuberculosis, polio, and measles. The EPI program in Turkey is well established, and its impact apparent from the reduction in disease incidence.

Since neonatal tetanus is an important health problem in some areas, tetanus immunization has special importance in pregnancy. The elimination of neonatal tetanus program was started in the middle of 1994, and evaluation of this program has been ongoing. According to the Turkish vaccination schedule, two doses tetanus toxoid (TT) during pregnancy are necessary for full immunization of an unvaccinated woman. However, if a woman has been vaccinated during a previous pregnancy in five years, she might only require one dose for her current pregnancy. With regard to TT coverage during pregnancy for all births in the five years preceding the survey, 16 percent had one dose, and 26 percent had two or more doses. According to the 1988 Turkish Population and Health Survey, these figures were eight percent and three percent for the last pregnancy, respectively[18, 19]. The high drop between TT1 and TT2 was probably due to incorrect interpretation of the immunization calendar. There is not a recording card system for women, therefore the tetanus vaccination rate cannot be known among women[10].

The program assessment carried out in 1988, within the context of Turkish Government/UNICEF cooperation, suggested that vaccination delivery should go beyond a mere "vaccination" program and start with the disease eradication and elimination phase. Thus, a preparatory plan for polio eradication was started in 1989, and provincial-based flaccid paralysis monitoring practices have been initiated and polio eradication activities are being implemented. "National Vaccination Days" have been hold since 1995. The coverage rate of poliomyelitis was 81 percent among the children under one year old, and there were 23 polio cases in 1994[7].

The Control of Diarrheal Diseases (CDD)

Diarrhea is a major public health problem worldwide. The prevalence and incidence of diarrhea vary from area to area, depending upon climatic and environmental factors. The incidence also differs from time to time according to seasonal variations. These factors lead to fluctuation of diarrhea incidence from one year to another[16].

Diarrheal diseases are the leading cause of morbidity and death among children in Turkey. It is estimated that in general a Turkish child under five experiences an average of 2.02 episodes of diarrhea per year[17]. The National Control of Diarrheal Diseases Program was implemented in 1986. The main objective of the program was prevention of deaths by prevention of dehydration. For this reason, oral rehydration therapy (ORT) has been taught actively since the 1980s. It is also a fact that dehydration associated with diarrhea can be prevented by appropriate hydration therapy. Oral rehydration is one of the most effective, inexpensive and widely applicable forms of therapy[7, 14].

In Ministry of Health (MOH) organized training seminars for health providers oral rehydration solution (ORS) treatment has been widely practiced both in health centers, Mother and Child Health-Family Planning (MCH-FP) units and hospital outpatient departments. Some hospitals established special ORS units for daily care. An orally-taken salt and sugar solution (ORS) is only recommended for cases of dehydration. ORS use was also promoted by pharmacists[7, 14].

Overall, one-quarter of the children under five years had experienced diarrhea at some time in the two weeks preceding the 1993 Demographic and health Survey, and 11 percent were still having an episode of diarrhea at the time of the survey. Since the prevalence of diarrhea varies seasonally, the results do not represent the average prevalence of diarrhea throughout the year in Turkey. In the 1988 Turkish Population and Health Survey (TPHS), the two-week prevalence of diarrhea for the same period (August-September) was 24 percent. This finding suggests that measures designated to prevent diarrhea, which were introduced following the 1988 survey, did not result in any change in diarrhea prevalence over the next five years[18].

The prevalence of diarrhea appears to be slightly higher among rural children (28.0%), children in the east (33.3%), and children whose mothers are without any education (28.2%) than among other children. With regard to treatment practices, 24 percent of children with diarrhea were not given any treatment; one-fourth of the children were taken to health facilities. Fluids made using packets of oral rehydration salts (ORS) were used in treating the diarrhea in 11.4 percent of cases, and 4.9 percent were given recommended home fulids. In 57.0 percent of the cases, fluids were increased[18].

On the other hand, statistics of MOH hospitals reveal a definite reduction in diarrhea-related deaths. Case fatality rates also decreased by 56 percent from 4.41 in 1982 to 1.96 percent in 1988[14].

Control of Acute Respiratory Infections (ARI)

Acute respiratory infections are responsible for high morbidity in children all over the world. Mortality due to ARI is unacceptably high in developing countries where the figure may reach 1,000 or more per 100,000 livebirths, compared to 30-40 per 100,000 livebirths in industrial nations. Poverty, malnutrition, ignorance, lack of and underutilization of health facilities, and predominance of bacterial pneumonia are considered responsible for this difference[22].

Respiratory infections, particularly pneumonia, were the serious diseases causing mortality both within the first year of life and for those under five. Acute respiratory infections are the most prevalent diseases among children, mostly during winter months, with pneumonia ranking as the first cause of child mortality. Furthermore, both urban air pollution (which reaches high levels in cities) and indoor pollution (due to heating and smoking) contribute to the increasing number of acute respiratory infections[22].

The Ministry of Health also launched a program for control of acute respiratory infections in 1986. In 1987, Çankırı was selected as a pilot province and the project was later extended to 22 provinces. By 1994, the program was extended to 11 provinces with first priority for development and 17 provinces with second priority for development and 13 additional provinces in 1994. Thus, in total, program has been extended to 63 provinces. The program covered 72 percent of the total population. The main activities of the project consisted of collection of baseline data about the knowledge and attitudes of mothers and health personnel on ARI. Training was conducted on ARI and medicine distributed. Health personnel working in primary health care were trained about ARI and other health personnel were trained on criteria for diagnosing ARI cases and the rules of treatment. Another intervention of the ARI program distribution of co-trimaxazole and penicillin was implemented in some high risk provinces. A decrease in ARI-related deaths is expected as a result of increasing immunization rates for measles and whooping cough and improvements in the health infrastructure[7, 14].

In the 1993 Demographic and Health Survey, the prevalence of ARI was estimated by asking mothers if their children had experienced coughing, accompanied by short, rapid breathing, in the two weeks preceding the survey. At some time preceding the survey, carried out during summer and early fall when ARI levels would be lower than in the winter, concerning treatment patterns, 37.3 percent of children with ARI were taken to a health facility, 29.6 percent were reported to have received antibiotic treatment including injections, and 43.6 percent received other medicines[18].

Baby-Friendly Hospitals Initiative and Promotion of Breast-Feeding

Breast milk alone, without supplements, is the optimal choice for feeding full-term infants for the first six months. Mother's milk contains an ideal balance of nutrients, enzymes, immunoglobulins, antiinfective and antiinflammatory substance, hormones, and growth factors that protect the infant and encourage growth. Breast milk changes over time to match the changing needs of the infant. For the mother, breast-feeding facilitates the physiological return to the pre-pregnant state and offers hygienic and economic benefits over bottle-feeding. For both infant and mother, breast-feeding confers an intense opportunity for bonding and interaction[22]. WHO and UNICEF have issued a joint statement entitled "Ten Steps to Successful Breast-feeding" in the program of promotion and support of breast-feeding[23]. The idea is to persuade all hospitals to follow the "Ten Steps to Successful Breast-feeding" which include informing all mothers of the advantages of breast-milk, keeping newborn babies in the same room as their mothers, rejecting the use of feeding bottles, and helping mothers with any problems they may have with breast-feeding in the beginning. Hospitals which follow the 10 steps are designated "baby friendly".

The Baby-Friendly Hospital Initiative was implemented in Turkey in 1989 and University Hospitals, MOH and Social Security (SSK) Hospitals still encourage the ten rules of breast-feeding. A meeting was held in Ankara with baby food companies. An official paper that supports the national health policy on breast-feeding was prepared by participants which secured their written commitment to the Government that they would also promote breast-feeding and would not distribute free breast-milk substitutes to hospitals. The necessary action for implementation, the international code of marketing for breast-milk substitutes, was also taken. In 1995, 56 hospitals were declared as "baby-friendly hospitals"; the target for 2000 is that all hospitals will be baby-friendly hospitals[7].

Early initiation of breast-feeding is of benefit to both mother and infant. Only one-fifth of lastborn children were started with breast-feeding as early as within one hour of birth. As regards the subgroups, there is almost no variation in the initiation to breast-feeding with respects to sex of the child, residence, educational level of mother, and utilization of health services during delivery. The median duration of breast-feeding is 11.9 months.

Breast-feeding is very popular among Turkish mothers, but the proper time for introducing supplementary food to the diet of infants is not widely known. Only 18.9 percent of children were exclusively breastfed (receiving only breast milk) in the first month of life. One-third of the children (32.9 percent) are being given supplementary food as early as one month of age. The percentage of children receiving supplements rapidly increases to 53.3 percent among children two to three months of age. The median duration of exclusive breast-feeding is one-and-a-half months and the median duration of full breast-feeding (either exclusively breastfed or receving plain water only in addition to breast-feeding) is 2.7 months. Early introduction of supplementary food to infant nutrition increases the risk of gastrointestinal infection, which is one of the leading causes of infant mortality in Turkey[15].

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BLOCKADE OF ACUTE GRADE IV SKIN AND EYE GRAFT – VERSUS – HOST DISEASE BY ANTI – INTERLEUKIN – 2 RECEPTOR MONOCLONAL ANTIBODY IN GENOIDENTICAL BONE MARROW TRANSPLANTATION SETTING*

*Hülya Özşahin MD**, Peter Tuchschmid MD**, Roger Lauener MD***
*Katharina Waldvogel MD**, David Nadal MD***, Reinhard A. Seger MD*****

SUMMARY: Özşahin H, Tuchschmid P, Lauener R, Waldvogel K, Nadal D, Seger RA. (Divisions of Immunology/Hematology and Intensive Care, University Children's Hospital, Zürich, Switzerland). Blockade of acute grade IV skin and eye graft-versus-host disease by anti-interleukin-2 receptor monoclonal antibody in genoidentical bone marrow transplantation setting. Turk J Pediatr 1998; 40: 231-235.

A six-year-old boy with homozygous β-thalassemia in the favorable class 1 risk group received a bone marrow transplant, from his histocompatible sister. He developed grade IV skin and eye graft-versus-host disease (GVHD) following varicella zoster reactivation. Despite the appropriate prophylactic use of cyclosporin A (CsA), methotrexate (MTX), and prompt treatment with high-dose steroids, GVHD progressed resulting in total body epidermal necrolysis. Anti-IL-2 receptor monoclonal antibodies (anti-IL-2R moAb) in combination with steroids were administered to selectively block the activated T cells. After 27 days of daily administration, followed by 17 doses of alternate-day therapy with anti-IL-2R moAb, the severe skin and eye GVHD resolved. The patient, at two years post-transplant, has full engraftment and immune reconstitution without chronic GVHD (cGVHD). In conclusion, we suggest that in the HLA-genoidentical bone marrow transplantation setting, very severe and steroid-resistant GVHD can be controlled through the use of anti-IL-2 receptor antibodies which specifically block the activated IL-2 receptor expressing T cells. *Key words: acute GVHD, skin, eye, anti-IL-2R, monoclonal antibodies.*

Acute grade IV graft-versus-host disease (aGVHD) is not commonly observed in the younger low-risk (class I) patients with homozygous β-thalassemia following genotypically identical sibling bone marrow transplantation (BMT) compared to the higher-risk groups[1]. Class 1 patients have a very high probability of cure, with low early and late morbidity and mortality rates. The prognosis of acute grade IV GVHD in any BMT setting despite prompt and aggressive treatment with steroids and cyclosporin A (CsA) is very poor[2,3]. Severe aGVHD is usually steroid-resistant. In this report, we present a six-year-old boy with homozygous β-thalassemia in the low-risk group who, despite CsA and methotrexate (MTX)

* From the Divisions of Immunology/Hematology and the Intensive Care Unit, University Children's Hospital, Zurich.

** Pediatrician, Immunology/Hematology, Intensive Care Unit, University Children's Hospital.

*** Assistant Professor of Pediatrics, University Children's Hospital.

**** Associate Professor of Pediatrics, University Children's Hospital.

prophylaxis, developed skin and eye GVHD following varicella zoster reactivation after an HLA-genotypically identical sibling BMT. Under high-dose steroids GVHD progressed, including the conjunctivae. Under the additional use of anti-IL-2 receptor monoclonal antibodies (anti-IL-2R moAb)[4,5] the GVHD resolved after two months with no relapse at two years post BMT.

Case Report

A six-year-old boy with homozygous β-thalassemia, under a well-controlled transfusion and chelation regimen without portal fibrosis or hepatomegaly (class 1 risk group)[1], was bone-marrow transplanted from his genotypically identical sister. The HLA types of the recipient and the donor were as follows: A2/3, B18/Bw6, DRB1.1301-1.11/DRB3.0101-DRB 3.02. Because of the genoidentity, cytotoxic T-lymphocyte precursor assay and mixed lymphocyte cultures were omitted. The patient was prepared for BMT with busulfan (14 mg/kg total dose) and cyclophosphamide (200 mg/kg total dose). GVHD prophylaxis consisted of CsA (10 mg/kg on day 0 and thereafter adjusted to obtain serum levels of 400-600 µg/L in the polyclonal assay) and MTX (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6 and 11). He received 2.4×10^8 nucleated cells/kg body weight. The first 15 days post BMT were unremarkable with hematopoietic recovery (> 0.5×10^9 granulocytes/L on day 15. On day 16, vesicular eruptions appeared on the trunk for which acyclovir (1500 mg/m^2) was immediately started. Electron microscopic examination of the vesicular fluid showed presence of herpesviruses. The lesions began to regress on day 25. A maculopapular rash appeared on day 29, covering all of the trunk and extremities, spreading to the face. Skin biopsy on day 30 showed no evidence of virus. The patient, still under acyclovir, was started on steroids at first at a dose of 2 mg/kg/day and subsequently at 5 mg/kg/day i.v. Between days 31 and 34 (Table I), there was continuous bullae formation covering the whole body, including ears, hands, feet, penis and mucosa of the eyelids. The liver and the gastrointestinal tract were not involved. On day 36 there was complete involvement of the skin with bullae, desquamation and rupture along the epidermal-dermal border resembling toxic epidermal necrolysis. The eye became extremely photosensitive. On this day, anti-IL-2R moAb (BB-10, BT 563, Leucotac, Biotest, Othmarsingen, Switzerland) were started at 0.4 mg/kg/day i.v.[4,5]. On day 40, as the skin manifestations seemed to be stabilized, the eye findings reached their summit with edema and shedding of the mucosa of the upper and lower eyelids with ulcerations. There were no signs of other organ involvement. On day 55 the lesions started to regress with the beginning of epithelial regeneration. Anti-IL-2R moAb treatment was discontinued after 19 days of daily administration. Steroids were continued at 2 mg/kg/day i.v. Three days after the moAb were stopped, the rash reappeared. The soluble IL-2 receptor levels are shown in

Table I. Anti-IL-2R moAb were restarted at 0.25 mg/kg/day and steroids increased to 4 mg/kg/day i.v. On day 64, skin manifestations improved. However, conjunctivae were once again severely involved. After eight days of daily administration, anti-IL-2R moAb were continued every other day at the same dose together with 2 mg/kg steroid administration. On day 69, the patient developed septic shock, due to Acinetobacter baumannii, with anuria, disseminated intravascular coagulopathy, liver failure; he was intubated and needed intesive care for eight days. He was treated with ceftazidime and ciprofloxacin. during this period, GVHD treatment was continued. Thereafter, the skin and eye manifestations regressed continuously with complete recovery at day 91. The soluble IL-2-receptor level was 82 U/ml. The anti-IL-2R moAb were then stopped. Under anti-IL-2 moAb treatment, a slow but consistent increase in the CD4 and CD8 cell counts could be observed, so that at the time of discharge, the absolute CD4 cell count was 0.32×10^9/L with a normal poliferation to phytohemagglutinin stimulation. The patient was discharged on day 116 with no signs of acute or chronic GVHD and no scar formation. He had received a total of 27 doses daily and 17 doses on alternating days of anti-IL-2 moAb. At present, two years post BMT, the patient is well with full engraftment (as shown by variable number of tandem repeat analyses of bone marrow cells), immune reconstitution and no signs of cGVHD.

Table I: Evolution of the T-lymphocyte Subsets and Soluble IL-2 Receptor Levels During the Course of Graft-Versus-Host Disease Following Bone Marrow Transplantation

Days post-BMT	31-34	40	55	62	69	91	116
Clinical Signs of GVHD	Grade IV Skin GVHD	Grade IV Skin and Eye GVHD	Repression of Skin and Eye Findings		Grade II Skin and Eye GVHD; Septicemia	Complete Recovery	Discharge from Hospital
CD4*	80	70	70	110	140	250	320
CD4 + DR*	30	20	20	30	20	150	90
CD8*	120	70	90	150	140	170	100
CD8 + DR*	40	20	30	60	20	70	30
CD25*	100	1	2	10	1	0	230
sIL-2R**	1821	ND	ND	27	438	82	ND
Treatment							
Steroids	+	+	+	+	+	+	–
anti-IL-2R moAb	–	+	–	+	–	+	–

BMT : bone marrow transplantation; GVHD : graft-versus-host disease; CD4 : helper T-lymphocytes; DR : stimulated (HLA-DR expressing) lymphocytes; CD8 : cytotoxic T-lymphocytes; CD25 : IL-2 receptor expressing lymphocytes; sIL-2R : soluble IL-2 receptor (normal range = 0-477 U/ml); anti-IL-2R moAb : anti-IL-2 receptor monoclonal antibody.

* number/µl.

** U/ml.

Discussion

Our patient was in the low-risk thalassemia group in terms of morbidity related to HLA-genoidentical BMT[1]. The evolution of the symptoms suggests that the varicella zoster infection was probably responsible for the development of severe GVHD, although the causal relationship is usually inverse, i.e., the aGVHD precedes the reactivation of varicella zoster infection[6]. The prognosis of grade IV aGVHD, which is mostly steroid-resistant, is usually very poor with an almost 100 percent mortality rate[2,3]. Anti-thymocyte immunoglobulins or various anti-pan-T moAb have been used as second-line therapy[7,8]. We decided not to use anti-thymocyte immunoglobulins because of their nonselective elimination of all T cells leading to a significant delay in immune reconstitution. Since the binding of IL-2 to its receptor stimulates clonal expansion of T cells reactive with specific antigens, the anti-IL-2R moAb are theoretically able to inhibit this response by blocking the transplanted T-helper cells directing the immune reaction against recipient tissue antigens and the cytotoxic cells mediating the tissue damage during the early phase of aGVHD[4,5]. Anti-IL-2R moAb have successfully been used with good tolerance of long-term administration[4,9]. The anti-IL2 moAb do not seem to influence the overall cellular immune reconstitution, as evidenced by our case, in which a steady increase was seen in the mature T-cell population with a normal proliferation to mitogen stimulation. Racadot et al.[10] recently reported a combined treatment directed against IL2 and TNFα. The sequential combination of moAb seems to be more effective compared to anti-IL-2R moAb alone. However, the recovery in our patient with severe but probably limited GVHD was due to the genoidentical nature of the transplantation, and thus, anti-IL-2R moAb alone sufficed to specifically block the activated T cells. To our knowledge, there are no published reports regarding the evolution of severe eye involvement. In conclusion, this case suggests that steroid-resistant acute grade IV skin and severe eye GVHD in HLA-genoidentical BMT can be effectively treated using anti-IL-2R moAb without relapse and with full immune reconstitution.

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FATAL OUTCOME OF INFANTILE OXALOSIS: CASE REPORTS FROM THREE FAMILIES AND A REVIEW OF LITERATURE*

*Ayşe Öner MD**, Gülay Demircin MD***, Keriman Tınaztepe MD*****
*Mehmet Bülbül MD***, Tahsin Teziç MD******

SUMMARY: Öner A, Demircin G, Tınaztepe K, Bülbül M, Teziç T. (Pediatric Nephrology Unit, Dr. Sami Ulus Children's Hospital and Nephropathology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Fatal outcome of infantile oxalosis: case reports from three families and a review of literature. Turk J Pediatr 1998; 40: 237-243.

Infantile oxalosis is a rare, autosomal recessive disorder. We present three unrelated cases of infantile oxalosis and their families, emphasizing its place as a cause of acute renal failure in infancy, and showing the clinical heterogeneity of the disease within the same family. The affected infants (two males, one female) were 2.5, 3.5, and five months old. Two families had first degree parental consanguinity; two revealed a history of nephrolithiasis; and one of these two had a member who received liver and kidney transplants because of primary hyperoxaluria type I. All the patients presented with the symptoms and findings of acute renal failure. Their hemoglobin levels were between 6.8-9.6 g/dl, urinalysis revealed (+) to (++++) proteinuria and microscopic hematuria. All had metabolic acidosis with BUN levels 67-113 mg/dl and creatinine 3.5-7.7 mg/dl. The abdominal ultrasonographies revealed normal sized hyperechogenic kidneys with the loss of corticomedullary junctions. Calcium oxalate crystals were demonstrated in retina and bone marrow of two patients, and in renal parenchyma of all the patients. The patients were treated with peritoneal dialysis. Renal functions continued to be abnormal (BUN: 47-168 mg/dl, creatinine: 2.8-11 mg/dl) after dialysis, and the outcome was fatal in all. In the presented families, because of the variation of the clinical presentation and the fatal outcome, presence of the multiple genetic loci appeared to be most likely. Further molecular studies will clerify the heterogeneity of this disorder.
Key words: infantile oxalosis, fatal outcome, renal failure, clinical heterogeneity.

Oxalosis in infancy is a rare condition, most frequently caused by a fulminant form of primary hyperoxaluria type I (PHI) resulting from a deficiency of the enzyme alanine-glyoxylate aminotransferase (AGAT)[1, 2]. There is considerable clinical variety within the disease, paralleled by enzymatic heterogeneity[3]. Although it is inherited most commonly as an autosomal recessive trait, other types of genetic inheritance, including an autosomal dominant pattern, have also been reported[4, 5].

* From the Pediatric Nephrology Unit, Dr. Sami Ulus Children's Hospital and the Nephropathology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Associate Professor of Pediatric Nephrology, Dr. Sami Ulus Children's Hospital.

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

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

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

This report was presented at the 28th annual meeting of the European Society for Pediatric Nephrology, September 25-28, 1994 in Amsterdam, The Netherlands.

We present three unrelated cases of infantile oxalosis and their families to emphasize this disease as a potential cause of renal failure during infancy, to show the clinical heterogeneity of the disease within the family of the first case, and to present a review of the literature.

Case Reports

Case 1

A 2.5-month-old boy was admitted to the hospital with the complaint of vomiting since birth. He was the first child of unrelated parents, and his family history revealed some cousins with urolithiasis. One cousin (born from the marriage of his aunt and uncle) had received at the age of five combined renal and hepatic transplants because of PHI (Fig. 1). The patient's weight was 4600 g (below the 10^{th} percentile) and length 54 cm (3^{rd} percentile). He was pale, and retinal crystal deposition was detected on funduscopic examination. The laboratory tests showed: hemoglobin (Hb): 9.6 g/dl, (+) proteinuria, and microscopic hematuria. He was in renal failure with levels of blood urea nitrogen (BUN): 67 mg/dl, creatinine: 3.5 mg/dl, uric acid: 12.3 mg/dl, calcium: 5 mg/dl, phosphorus: 11 mg/dl, alkaline phosphatae (AP): 62 IU, Na: 140 mEq/L, K: 3.4 mEq/L, total protein: 5.4 g/dl, and albumin: 3.9 g/dl. The creatinine clearance was 7 ml/min/1.73 m^2; blood gases showed metabolic acidosis. Bilateral nephrocalcinosis was shown on plain abdominal radiography (Fig. 2). Abdominal ultrasonography revealed normal sized and diffusely hyperechogenic kidneys with a loss of corticomedullary differentiation (Fig. 3). Skeletal survey was normal. Urinary oxalate excretion rate was 60 µmol/day (N. 140-420 µmol/day). Peritoneal dialysis was done, and after the acute symptoms subsided, percutaneous renal needle biopsy was performed which showed calcium oxalate crystals located in the tubules, and tubular epithelial cells extending into the interstitial tissue (Figs. 4, 5). Calcium oxalate crystals were also detected in the examination of bone marrow aspiration. The patient was discharged with supportive therapy and his renal functions continued to deteriorate (BUN: 47 mg/dl, creatinine: 2.8 mg/dl) with metabolic acidosis. He died after 2.5 months of follow-up.

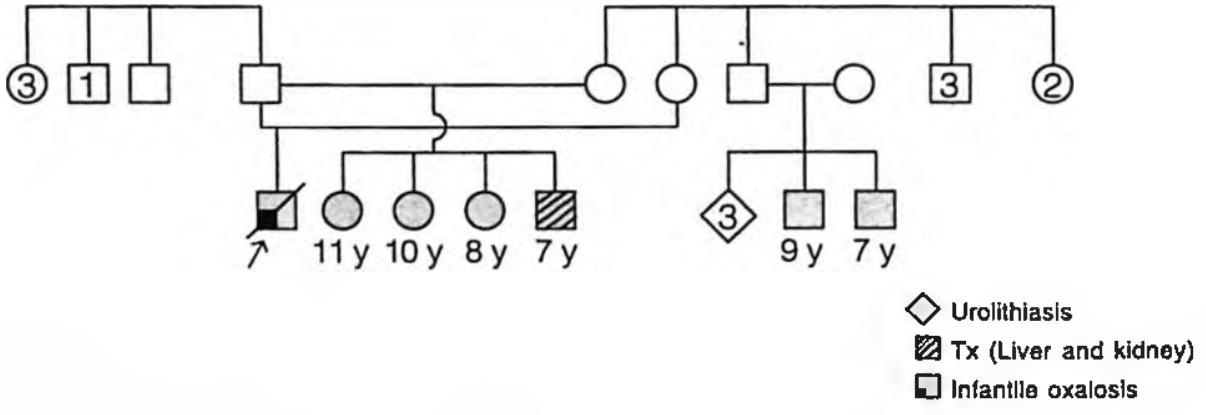

Fig. 1: Pedigree illustrating the family of Case 1 and the variable clinical expression of enzyme activity.

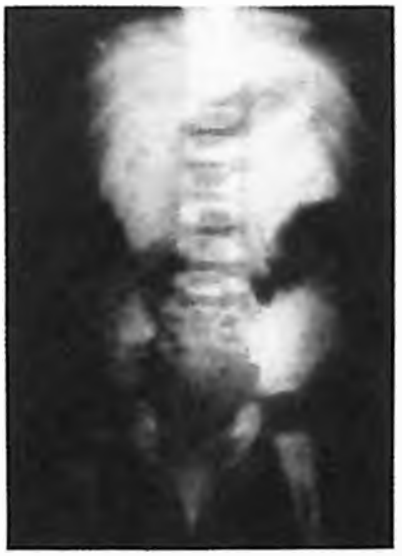

Fig. 2: Plain abdominal roentgenogram of Case 1 showing nephrocalcinosis.

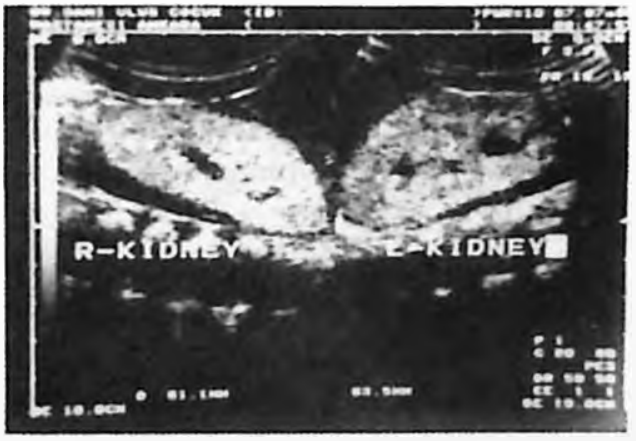

Fig. 3: Abdominal ultrasonography of Case 1 demonstrating normal sized kidneys with diffuse and intensive hyperechogenity and loss of corticomedullary differentiation.

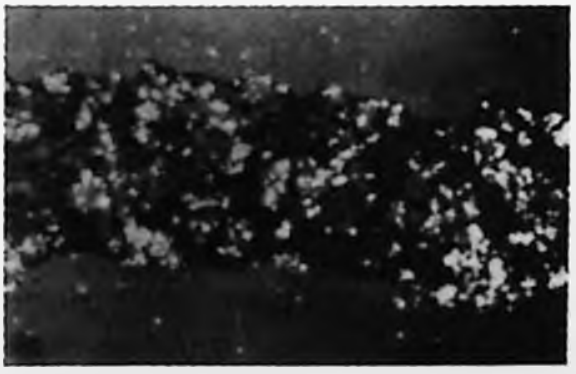

Fig. 4: Renal biopsy of Case 1 under lower magnification with half-polarized light showing masses of calcium oxalate crystals through the renal parenchyma (H+E stain).

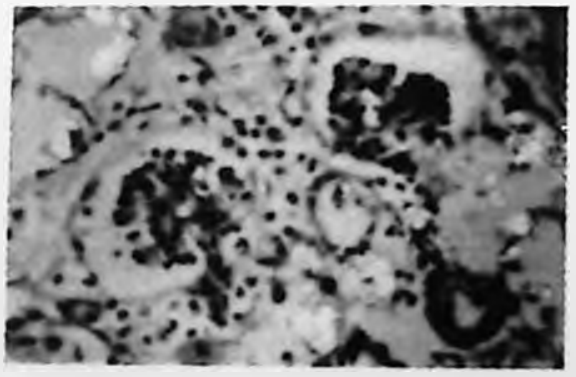

Fig. 5 Renal biopsy of Case 1 under the higher magnification with partially polarized light showing crystals located in the tubules, tubular epithelial cells extending into the interstitial tissue (H+E stain).

Case 2

A 3.5-month-old boy was admitted because of vomiting since birth and convulsions lasting for one week. His parents were first degree relatives and the family history revealed no significant disease. On physical examination he weighed 4800 g (below the 25th percentile) was 60 cm (below the 50th percentile) in length, and presented with hypertension and pretibial and periorbital edema. Funduscopic examination showed retinal crystal deposits. The laboratory examination revealed: Hb: 9.6 g/dl, (+) proteinuria and microscopic hematuria. He had renal failure with BUN: 113 mg/dl, creatinine: 7.7 mg/dl, uric acid: 16 mg/dl, calcium: 6.3 mg/dl, phosphorus: 11.1 mg/dl, A.P: 267 IU, Na: 138 mEq/L, K: 3.8 mEq/L, total protein: 5 g/dl, albumin: 3.7 g/dl. Creatinine clearance was 3.5 ml/min/1.73 m^2 and metabolic acidosis was detected. Urinary oxalate level could not be measured. Plain abdominal radiography showed bilateral nephrocalcinosis and bone roentgenograms were normal. Abdominal ultrasonography demonstrated normal sized kidneys that were diffusely and intensively hyperechogenic with the loss of corticomedullary differentiation. After therapy with peritoneal dialysis, percutaneous renal needle biopsy was performed and calcium oxalate crystals were shown through the renal parenchyma. After being discharged with supportive therapy, the patient was followed up for one month before his death, occurred with levels of BUN: 98.9 mg/dl, creatinine: 11 mg/dl, and metabolic acidosis.

Case 3

A five-month-old girl was admitted to the hospital because of vomiting and irritability since birth. Her parents were first degree relatives. Her 12-year-old sister had an operation for urolithiasis, and her grandmother had a nephrectomy, etiology unknown. Her weight was 5100 g (below 3rd percentile) and length 59 cm (below 10th percentile). On physical examination, she had pallor, a functional murmur, and hepatomegaly of 4 cm below the costal margin. On laboratory investigation Hb was 6.8 g/dl, (++++) proteinuria and microscopic hematuria were present, BUN: 77 mg/dl, creatinine: 6 mg/dl, uric acid: 2.8 mg/dl, Ca: 7.5 mg/dl, phosphorus: 18 mg/dl, A.P.: 113 IU, Na: 128 mEq/L, K: 8.2 mEq/L, total protein: 8.1 g/dl, and albumin: 4 g/dl. She had metabolic acidosis. Creatinine clearance was 5.5 ml/min/1.73 m^2. Urinary oxalate level measured 368 Umol/day (N: 140-420 Umol/day). The plain abdominal ultrasonography were similar to Cases 1 and 2, revealing bilateral nephrocalcinosis. Skeletal survey revealed no pathological finding. Peritoneal dialysis was performed. After dialysis, her BUN level was 168 mg/dl and creatinine 7.06 mg/dl. Calcium oxalate crystals were detected in a bone marrow aspiration. She died within 15 days. Postmortem renal necropsy showed calcium oxalate crystals in renal parenchyma.

Clinical data and laboratory findings of the patients are shown in Table I and II.

Table I: Clinical Data of the Patients

	Case 1	Case 2	Case 3
Age	2.5 months	3.5 months	5 months
Sex	Male	Male	Female
Complaints	Vomiting	Vomiting, convulsion	Vomiting, irritability
Parental Consanguinity	Absent	First degree	First degree
Family History	– Urolithiasis – Combined liver and kidney transplantation	– Urolithiasis —	– Nephrectomy
Physical Examination	– Pallor – Renal crystal deposition	– Hypertension – Edema – Retinal crystal deposition	– Pallor – Hepatomegaly – Functional murmur

Table II: Laboratory Findings of the Patients

	Case 1	Case 2	Case 3
Hemoglobin	9.6 g/dl	9.6 g/dl	6.8 g/dl
Biochemical Tests			
BUN	67 mg/dl	113 mg/dl	77 mg/dl
Creatinine	3.5 mg/dl	7.7 mg/dl	6 mg/dl
Uric acid	12.3 mg/dl	16 mg/dl	2.8 mg/dl
Calcium	5 mg/dl	6.3 mg/dl	7.5 mg/dl
Phosphorus	11 mg/dl	11.1 mg/dl	18 mg/dl
Blood gases	Metabolic acidosis	Metabolic acidosis	Metabolic acidosis
Creatinine Clearance (ml/min/1.73 m²)	7	3.5	5.5
Urinary Examination			
Protein	+	+	++++
Microscopy	Hematuria	Hematuria	Hematuria

Discussion

Infantile oxalosis is a very rare disorder[6]. In 1984 Zegher et al.[5] reported two infants with oxalosis, presented a literature review citing 20 other patients reported since 1951, and demonstrated clinical picture, diagnosis and treatment of all the cases. At that time, to our knowledge, only two other cases were reported in English literature[7,8].

Although other types of genetic inheritance have been reported, the disease is generally inherited in autosomal recessive pattern, and according to the enzymatic heterogeneity, different clinical presentations can occur[3,4]. One-third of the patients in the literature showed parental consanguinity[5]. Two of our patients were also born to consanguineous parents, and Case 3 had a family history

of urolithiasis and nephrectomy of unknown etiology, suggesting the probable presentation of primary hyperoxaluria with urolithiasis. Case 1 had the most striking family history. As shown in Fig. 1, the children born from the marriage of Case 1's aunt and uncle were also affected by the disease. Three of them had only urolithiasis, while the fourth one had liver and kidney transplants because of oxalosis at age five. Moreover, two other cousins also had a history of urolithiasis. The occurrence of variable degrees of severity of the clinical disease in the several affected members of this child's family may reflect the clinical variability of the disease due to heterogeneic enzyme activity[3]. Although the two families were unrelated, they were from the same region; autosomal recessive inheritance is the most probable pattern in this family. However, an autosomal dominant inheritance with different expressions in different generations cannot be excluded. Although the disease is inherited with the same gene in these families, the occurrence of such different clinical presentations, varying from the benign form of urolithiasis to the most severe infantile form, needs to be clarified by genetic and enzymologic studies.

In the literature, most of the patients with infantile oxalosis presented within the first six months of life with the complaints of anorexia, vomiting, convulsion, and signs and symptoms of renal failure. Abdominal roentgenograms of the patients usually revealed normal sized kidneys with homogenously increased radiopacity, while ultrasonographical analysis of kidneys showed diffuse and excessively enhanced echoes which are the characteristic findings of nephrocalcinosis. The diagnosis of oxalosis was based on the histological demonstration of calcium oxalate crystals in the kidneys of all cases. The diagnosis of PHI was confirmed in seven of 24 patients with infantile oxalosis in the literature, showing the increased excretion of oxalate, glycolate and glyoxylate in urine samples, and the others were concluded to have this disease after other causes of oxalosis were excluded (2-5, 7-9). In some cases calcium oxalate crystals could be demonstrated in the tissues (bone, bone marrow, heart, thymus, brain, eyes and vascular walls)[8, 10-12]. Our patients presented primarily with the complaint of vomiting, and in renal failure with metabolic acidosis in early infancy, like the patients in the literature. The diagnosis of PHI was made on the basis of the clinical picture, characteristic findings of nephrocalcinosis in radiological examinations, and the histological appearance of the kidney biopsy specimens. Urinary oxalate levels were measured as low in Case 1 and normal in Case 3, the latter probably because of renal failure[1]. Unfortunately, confirmatory laboratory tests to show increased urinary excretion of glycolate and glyoxylate could not be performed in any of these children.

The main objective of treatment of PHI is good hydration and dietary restriction of oxalate-rich foods[1]. In cases with residual enzyme activity, pyridoxine was shown to reduce the production and excretion of oxalate[6]. Chronic dialysis and eventual renal transplantation, usually combined with hepatic transplantation,

are the other therapeutic procedures in the patients that progressed to end-stage renal failure[13, 14]. However, in infantile oxalosis, rapid progression toward terminal renal failure appears to be the rule, and dialysis and renal transplantation are generally not recommended[5, 6]. Most of the patients in the literature died within three months after onset of symptoms[5]. Our patients also died within 2.5 months after first presentation, confirming the poor prognosis of the disease.

We presented these three infants with oxalosis to stress not omitting this rare disorder in the differential diagnosis of renal failure in infancy, especially in countries like Turkey where parental consanguinity is high[15]. We also presented a current review of the literature, to demonstrate the characteristic findings of the disease once more. We also wanted to demonstrate the probable presentation of different clinical expressions of the disease even in the same family, despite inheritance of the same gene.

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The Turkish Journal of Pediatrics 1998; 40: 245-247

AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE: MAPPING TO CHROMOSOMAL REGION OF 6p21 – CEN IN A TURKISH CHILD*

*Nesrin Beşbaş MD**, Seza Özen MD***, Ümit Saatçi MD***

*Melda Çağlar MD****, Gabi Mucher MD*****, Klaus Zerres MD******

SUMMARY: Beşbaş N, Özen S, Saatçi Ü, Çağlar M, Mucher G, Zerres K. (Units of Nephrology and Rheumatology, and Pathology, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey and Institüt für Hemangenetik der Universitat, Bonn, Germany). Autosomal recessive polycystic kidney disease: mapping to chromosomal region of 6p21-cen in a Turkish child. Turk J Pediatr 1998; 40: 245-247.

Autosomal recessive polycystic kidney disease (ARPCD) is a congenital kidney disease with severe prognosis. We present a male infant who was diagnosed prenatally by ultrasonography. He died at two months of age in a septic stage. The genetic defect for ARPCD has been mapped to chromosomal region of 6p21-cen. This represents the first study from this region of the world. The linkage studies up to this date fail to show genetic heterogeneity. *Key words: autosomal recessive polycystic kidney disease, genetics.*

Autosomal recessive polycystic kidney disease (ARPCD) is a rare heritable disease affecting the kidneys and liver with a usually grave prognosis. Fusiform dilatations of the collecting tubules are noted and the classical hepatic involvement is in the form of congenital hepatic fibrosis[1,2]. There is great variation in the severity of the involvement of these organs, creating a heterogeneity in the clinical spectrum of the disease[3]. On the other hand, in spite of the wide variability in clinical presentation, only a single ARPCD locus has been reported[4,5].

We herein present a newborn with ARPCD with a fatal outcome.

Case Report

This male infant was diagnosed prenatally by ultrasonography to have polycystic kidney disease, thorax hypoplasia and oligohydramnios. Postnatal ultrasonography confirmed the diagnosis of ARPCD. The kidneys were markedly

* From the Nephrology and Rheumatology, and Pathology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

** Professor of Pediatric Nephrology and Rheumatology, Hacettepe University Faculty of Medicine.

*** Associate Professor of Pediatric Nephrology and Rheumatology, Hacettepe University Faculty of Medicine.

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

***** Research Assistant in Human Genetics, Institut für Humangenetik der Universitat, Bonn, Germany.

****** Professor of Human Genetics, Institut für Humangenetik der Universitat.

enlarged with increased echogenicity and medullary regions were more echogenic than the cortex. The mother and father had normal kidneys and no consanguinity. At birth he was hospitalized for respiratory distress. He had mild impairment of renal function at birth with a BUN level of 54 and serum creatinine of 1.4 mg/dl. At discharge he was put on antihypertensive therapy for his hypertension. He was readmitted two months later with a general deterioration in his condition. On admission he was ill-looking with marked edema and ascites. He had marked anemia and hematuria and proteinuria on urine examination. Results of renal function tests were as follows: BUN 45 mg/dl, serum creatinine 1.5 mg/dl, uric acid 5 mg/dl, serum albumin 2.9 g/dl, and normal liver function tests. E.coli was grown on urine culture. He was put on intravenous antibiotics and was transfused fresh blood. Nevertheless, he died on the 14th hour of hospitalization. Postmortem examination of the kidneys confirmed the diagnosis of ARPCD.

Linkage analysis was performed (in Dr. Klaus Zerres's laboratory) for the parents and the index case with appropriate markers (Fig. 1). The genetic defect previously defined for ARPCD, mapping to chromosomal region of 6p21-cen, was identified. Prenatal diagnosis has been planned for the next child of the family.

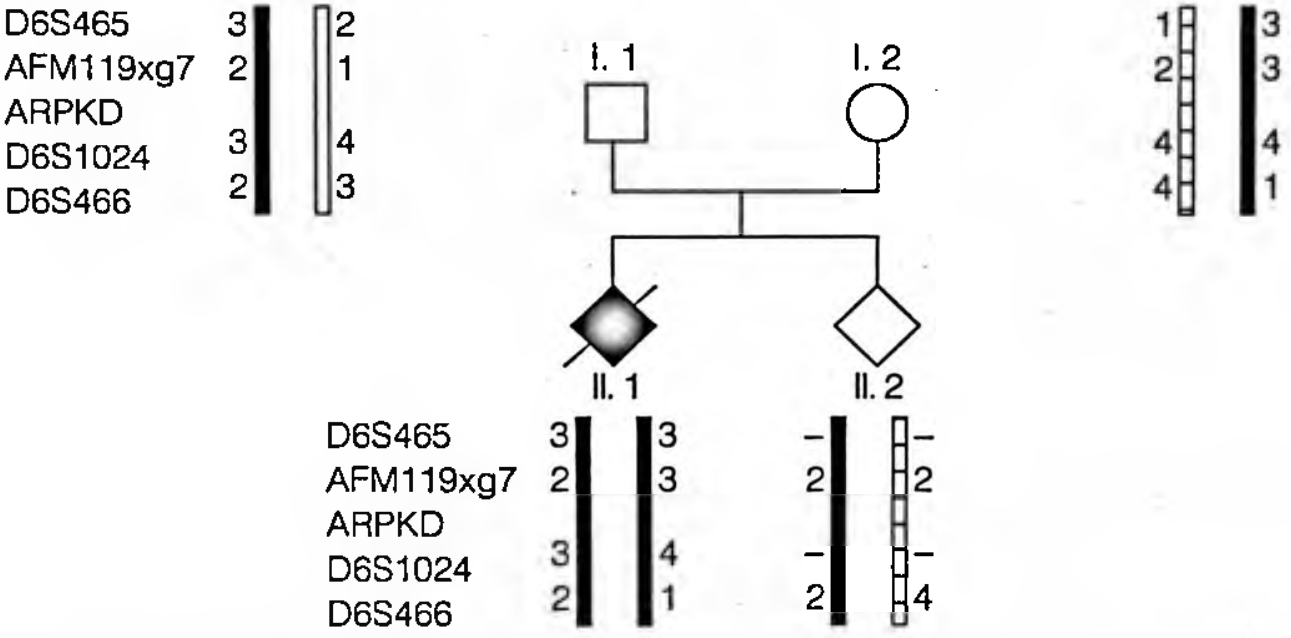

Fig. 1: Linkage analysis of the family members for the susceptible region on chromosome 6.

Discussion

Autosomal recessive polycystic kidney disease is a childhood disease with a varying clinical spectrum and age of presentation[1-3]. This child had pulmonary complications and a rapid fatal course probably complicated by ysepsis but without prominent liver involvement. Although ARPCD is usually accepted to have a grave prognosis, the southwest pediatric nephrology group has shown that chance of survival is not that poor and that aggressive medical management

is indicated[2]. In the largest series of ARPCD in children, Zerres et al.[3] reported that only 11 percent of their patients died and that urinary tract infections and hypertension were major aspects needing medical care.

Zerres et al.[4] first mapped the ARPCD locus to 6p21-cen with no evidence of genetic heterogeneity in the studied different clinical phenotypes. Guay-Woodford et al.[5] confirmed the existence of a single ARPCD locus in their patients with a varying spectrum of clinical presentation. The genetic defect has been mapped to the same locus in this Turkish patient, supporting the absence of genetic heterogeneity. On the other hand, the fact that there is only one ARPCD gene for a large spectrum of clinical features suggests that there are other complementing genetic factors in the disease manifestation.

This represents the first study on this subject from Turkey. Including our own case, all families tested thus far from different parts of the world show the same locus to be susceptible. The mutation-carrying chromosomes thus seem to descend from different ancestors. Prenatal diagnosis is quite feasible when the family members are available.

Addendum

Linkage analysis with chromosome 6p markers was performed during the second pregnancy. This study demonstrated that the fetus was heterozygous and not homozygous for the ARPCD gene. The mother has just delivered a healthy baby with normal ultrasonography findings.

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SCURVY*
A Case Report

*Sümer Yılmaz MD**, Selmin Karademir MD***, Ülker Ertan MD***
*Semanur Kuyucu MD**, Olgu Hallıoğlu MD****, Burhan Öcal MD***
*Nesrin Maviş MD******

SUMMARY: Yılmaz S, Karademir S, Ertan Ü, Kuyucu S, Hallıoğlu O, Ocal B, Mavlş N. (Departments of Pediatrics and Radiology, Dr. Sami Ulus Children's Hospital, Ankara, Turkey). Scurvy: a case report. Turk J Pediatr 1998; 40: 249-253.

An 18-month-old girl presented with irritability, epistaxis, spongy appearance of the gums perifollicular papules with follicular hyperkeratosis, ecchymosis, painful swollen knees and scorbutic rosary. Her diet consisted mainly of wheat flour. X-ray of the knees showed findings compatible with scurvy. Ascorbic acid level was below 0.003 g/L. Ascorbic acid therapy resulted in a dramatic clinical improvement. Scurvy is an uncommon disease in our society today. It is important to recognize the signs and symptoms of scurvy because it is easily treated with vitamin C replacement. *Key words: scurvy-vitamin C.*

Scurvy is now an uncommon disease in the world, but this condition is still a problem in developing countries, where general malnutrition prevails[1]. Most cases occur in elderly persons who live alone, the mentally ill, alcoholics and those who consume an inadequate diet[2]. It can also occur in children with peculiar dietary habits resulting from parental misguidance or neglect[3,4].

We report a case of scurvy in an 18-month-old female with an inadequate diet because of her mentally ill mother.

Case Report

An 18-month-old girl was admitted to our hospital with a four-week history of irritability, weakness, fever, repeated epistaxis, bleeding from the gums and swelling of the knees.

She was born to nonconsanguinous parents after an uncomplicated delivery. Her mother was mentally retarded. The parent was questioned about the child's nutritional history. There was no breast-feeding. She had been fed on too-diluted formula for the first three months and on a mixture of water and wheat flour later. No vitamin supplements had been given for the previous months.

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* From the Departments of Pediatrics and Radiology, Dr. Sami Ulus Children's Hospital, Ankara.
** Pediatrician, Dr. Sami Ulus Children's Hospital.
*** Associate Professor of Pediatrics, Dr. Sami Ulus Children's Hospital.
**** Research Assistant in Pediatrics, Dr. Sami Ulus Children's Hospital.
***** Radiologist, Dr. Sami Ulus Children's Hospital.

On physical examination the patient weighed 10 kg (25^{th}-50^{th} percentile) and was 80 cm in length (25^{th}-50^{th} percentile). Her head circumference was 44 cm (below third percentile). Rectal temperature was 39 °C, blood pressure was 90/50 mm Hg and her heart rate was 156/min. Her behavior was aggressive. Ecchymosis and perifollicular papules with follicular hyperkeratosis were present on her thighs. Her lips were dry and fissured. The gums were friable and eroded (Fig. 1). There was edematous swelling along the shaft of the legs. The knees were swollen and painful (Fig. 2). Rosary was observed at the costochondral junctions. Deep tendon reflexes were normal.

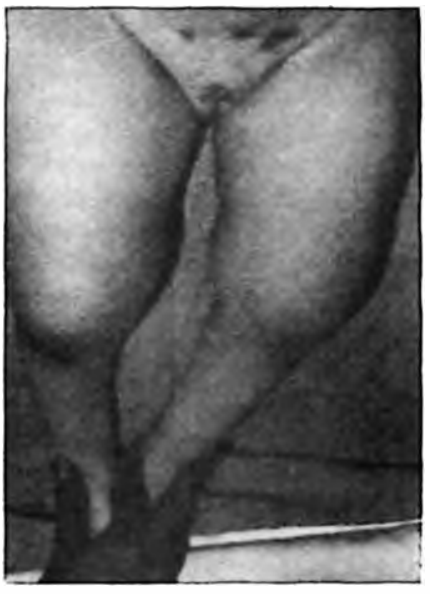


Fig. 1: The patient's gingivae show hemorrhage, edema and erosions.

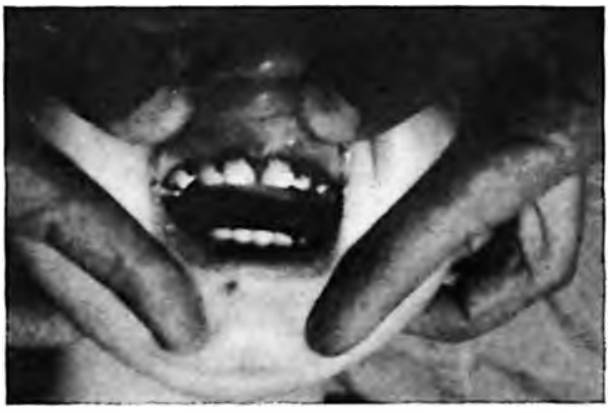
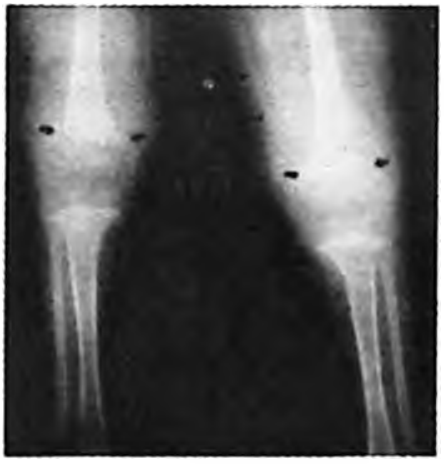


Fig. 2: Perifollicular papules with follicular hyperkeratosis, ecchymosis, and edematous swelling of the legs and knees are seen.

The patient's hemoglobin level was 70 g/L and her hematocrit was 28 percent with normocytic and normochronic indices. White cell and platelet counts were normal. Electrolytes, renal and liver function tests, prothrombin time, activated

partial thromboplastin time and bleeding time were found to be normal. Bone marrow examination revealed erythroid hyperactivity. The serum ascorbic acid level was less than 0.003 g/L (normal 0.005-0.018 g/L). Roentgenograms of both knees including femur showed features of acute scurvy (Fig. 3). The clinical and laboratory findings led to the diagnosis of scurvy. Our patient was treated with parenteral ascorbic acid, 300 mg daily. After 10 days, the patient showed dramatic clinical improvement and her serum ascorbic acid level rose to 0.01. Vitamin C was continued with a dose of 50 mg/day for three months. Follow-up x-ray of both knees showed signs of healing with calcification of subperiosteal hemorrhage (Fig. 4).



(A)

(B)

Fig. 3: X-ray of both knees including femur taken on admission shows peripheral metaphyseal defects (Pelkan's spur), osteoporosis, rings around the epiphyses of the femur and tibia (Wimberger ring), and subperiosteal hematoma (A). One week later, linear displacement of the epiphysis against the shaft is seen (B).

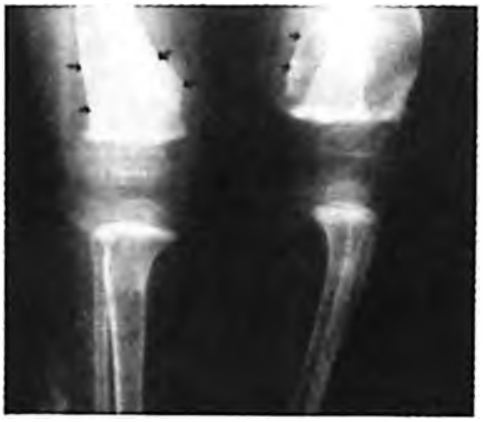


Fig. 4: X-ray of both knees after one month shows signs of healing with calcification of subperiosteal hemorrhage.

Discussion

Ascorbic acid (vitamin C) is an essential water soluble vitamin for human beings because of the absence of the microsomal enzyme L-Gulono-γ-lactone oxidase[5].

Vitamin C plays an important role in the formation of collagen; the synthesis of epinephrine, carnitine and steroids; folic acid metabolism; leukocyte function and iron absorption.

The infant is born with adequate stores of vitamin C if the mother's intake has been adequate. Breast-milk is an adequate source of vitamin C[1]. Infants fed with formula must also receive vitamin C supplements. Without ascorbic acid scurvy can occur. Today scurvy is a very rare disease. Nevertheless, cases of vitamin C deficiency are still being reported both in developed and developing countries[7,8]. Scurvy is also very rare in Turkey. Our patient's unusual eating habits due to her mother's mental retardation caused scurvy. Her mother did not know anything about the feeding of a child. Cases of scurvy have been previously published in patients with peculiar dietary habits as was observed in our patient.

The clinical findings of scurvy are generally first evidenced by petechial hemorrhages. These hemorrhages are usually perifollicular and occur in hyperkeratotic areas. Next to occur are ecchymosis and purpura, usually at sites of pressure. As the level of ascorbic acid continues to decrease, coiled and fractured hairs with hyperkeratosis develop. Gingivitis and bleeding from the gums may occur; these findings are important manifestations of scurvy. The manifestations of scurvy can progress to pseudoparalysis due to acutely painful subperiosteal hemorrhage in the long bones, lower extremity edema, muscle tenderness, conjunctival and intraocular hemorrhages, arthralgias, hemorthroses, gastrointestinal hemorrhage, Sjögen-like syndrome, poor wound healing and femoral neuropathy. Rarely, convulsions, hypotension and death can result.

Severe to moderate anemia secondary to hemorrhage may occur. Concurrent with these signs, the patient usually complains of general weakness, fatigue and weight loss[1]. The case illustrated here was presenting with aggressive behavior, depressed affect, irritability, perifollicular papules with follicular hyperkeratosis, ecchymosis, gingival erosions, painful swelling of knees and legs and rosary at the costochondral junction. There was also dietary history. Thus, the presumptive diagnosis of scurvy was made. The dignosis was confirmed by measuring the plasma ascorbic acid level, roentgenographic findings and clinical response to vitamin C.

The recommended daily allowance for vitamin C is 35 mg for infants, 45-50 mg for children and 60 mg for adults[1, 9-11]. Daily intake of 60 mg of ascorbic acid results in a 1500 mg pool. Clinical scurvy develops when the body's pool of vitamin C is depleted below 300 mg[10]. Replacement of the body store of vitamin C is the treatment for scurvy. Scorbutic children should be treated with 150-300 mg/day of vitamin C either intravenously or orally for a month, and 30-60 mg/day thereafter until complete recovery occurs[1, 10, 11]. Our patient was treated with 300 mg/day of vitamin C parenterally for 10 days, thereafter 50 mg daily for three months. A well-balanced diet containing fresh fruits and vegetables was recommended.

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KEARNS – SAYRE SYNDROME*
A Case Report

*Şakir Altunbaşak MD***, Gülbin Bingöl MD***, Nazan Özbarlas MD*****
*Zuhal Akçören MD******, Özlem Hergüner MD****

SUMMARY: Altunbaşak Ş, Bingöl G, Özbarlas N, Akçören Z, Hergüner Ö. (Department of Pediatric Neurology, Çukurova University Faculty of Medicine, Adana, Turkey). Kearns-Sayre syndrome: a case report. Turk J Pediatr 1998; 40: 255-259.

Kearns-Sayre syndrome (KSS) is a mitochondrial disorder. There is a large-scale mitochondrial DNA (mtDNA) deletion in most of the cases. In this article, a case of KSS who has progressive external ophthalmoplegia (PEO), retinitis pigmentosa (RP), complete heart block, encephalopathy attacks, type-I diabetes mellitus, ragged-red fiber (RRF) and lactic acidosis is presented and discussed in light of the literature available on this subjects. Diagnosis is confirmed by determination of mtDNA deletion.
Key words: mitochondrial myopathy, Kearns-Sayre syndrome.

Kearns and Sayre described a case with retinitis pigmentosa, progressive external ophthalmoplegia and complete heart block in 1958. Symptoms in KSS appear in childhood. Both sexes are affected equally. Although some familial cases were reported, it is usually sporadic. Large-scale mtDNA deletions have been shown in most cases[1,2].

We report the case of a 15-year-old boy with neuromuscular syndrome of the Kearns-Sayre type, type-I diabetes mellitus and complete heart block (a pacemaker was implanted).

Case Report

A 15-year-old boy was admitted to the hospital with ataxic gait and headache. Two and a half years previously, he had been admitted because of fever, unconsciousness and convulsions. Twenty days before the first admission he had complained of fever, headaches, vomiting and generalized tonic-clonic seizures. He had developed intellectual deterioration and his school performance had become poor during the previous six months. He had also suffered from hearing loss for one year. At that time, his physical examination revealed unconsciousness, hypertonicity of muscles with exaggerated deep tendon reflexes,

* From the Department of Pediatric Neurology, Çukurova University Faculty of Medicine, Adana.
** Associate Professor of Pediatrics, Çukurova University Faculty of Medicine.
*** Research Assistant in Pediatrics, Çukurova University Faculty of Medicine.
**** Associate Professor of Pediatric Cardiology, Çukurova University Faculty of Medicine.
***** Assistant Professor of Pediatrics, Hacettepe University Faculty of Medicine, Ankara.

loss of superficial abdominal reflexes, reduced anal sphincter tonus, and signs of meningeal irritation. Results of laboratory examination were: Htc 38.6 percent WBC 10300/mm^3 with 80 percent neutrophils and 20 percent lymphocyte, erythrocyte sedimentation rate (ESR) 43 mm/h, cerebrospinal fluid (CSF) pressure 120 mm H_2O, leukocyte count 40/mm^3, protein 202 mg/dl, glucose 85 mg/dl, and simultaneous serum glucose level 180 mg/dl. Computed tomography of brain (CT) was normal and electroencephalography showed diffuse slowing. He was given nonspecific antimicrobial therapy along with diphenylhydantoin (DPH) for seizures. On the seventh day of hospitalization, he had a complete A-V block and a premanent pacemaker was implanted. On the follow-up, glucose intolerance was determined. After discharge, he was twice admitted to the outpatient clinic of pediatric neurology. DPH was changed to valproic acid. Complaint of hearing loss was progressively increased and his visual performance was reduced. Six months previously he was hospitalized again because of diabetic ketoacidosis and insulin therapy was started. Thyroid hormone therapy was given for goiter. Two months previously he complained of leg pain and headache and of increasing ataxic gait. He was unable to walk without help. He was hospitalized again.

On physical examination, his weight and height were repeatedly below the third percentile. Vital signs were normal and stabile cardiac signs were normal and rhythmic with a permanent cardiac pacemaker. Pulmonary and abdominal examinations were normal. Thyroid was minimally enlarged. Secondary sex characters were poorly developed. On neurological examination, he was lethargic, and direct and indirect light reflexes were bilaterally positive. He had no signs of meningeal irritation. Oculocephalic reflexes were bilaterally poor. Muscle tonus was particularly increased at the lower extremities. He had quadripalsy. Babinski's sign and clonus were positive bilaterally. Sensory loss was absent. Cremasteric reflexes, superficial abdominal reflexes, and perianal reflex were weakly positive. Anal tonus was reduced. On the third day of hospitalization his mental status was improved and he became cooperative. His last neurological examination showed limited eye movements on all sides. He had bilateral ptosis (Fig. 1). He had no dysmetry and dysdiadokokinesis, but apparent ataxic gait. Gower's sign was positive. Fundoscopic examination revealed pigmentary retinopathy.

On laboratory examination. Htc 40.7 percent Hb 13.7 g/dl, WBC 13500/mm^3 with 68 percent neutrophils and 32 percent lymphocyte, ESR 57 mm/h. Blood gas analyses were normal. BUN: 21 mg/dl, Cr: 0.8 mg/dl, Na^+: 149 mEq/L, K^+: 4.4 mEq/L, SGPT: 38 mIU/L, SGOT: 32 mIU/L, LDH: 432 U/L, CPK: 98 U/L, Ca^{++}: 10.2 mg/dl, P: 4.6 mg/dl, ALP: 328 mIU/dl, serum lactic acid: 35 mg/dl (normal value: 10-20 mg/dl), serum pyruvic acid: 3.8 mg/dl (normal value: 0.5-2 mg/dl), parathormone: 20 pg/ml (normal: 1-43) aldosterone: 33 ng/dl (normal: 5-70). Routine examination and metabolic screening of urine, and aminoacid chromatography were

normal. CSF-protein: 220 mg/dl, glucose. 123 mg/dl with simultaneous serum glucose: 144 mg/dl and no pleocytosis were observed on microscopic examination. Cranial computed tomography (CT) was normal. Electromyography (EMG) showed minimal neurogenic pattern. Renal tubular functions were normal. T_3: 109 ng/dl (normal value: 52-176), T_4: 11.3 µg/dl (normal: 4.5-12.5), TSH: 3.5 µlU/ml (normal: 0.6-7.4) while receiving thyroxin replacement therapy. Brain stem auditory evoked response revealed bilateral involvement of eighth nerve. Common mtDNA deletion was determined in peripheral leukocytes (analysis was done in the Department of Medical Biology in Hacettepe University Medical School, Ankara, Turkey). Muscle biopsy showed scattered ragged red fibers; stained red with Gomori's trichrome and dark with oxidative enzyme stains, especially in the periphery. These findings were compatible with mitochondrial myopathy (Fig. 2).

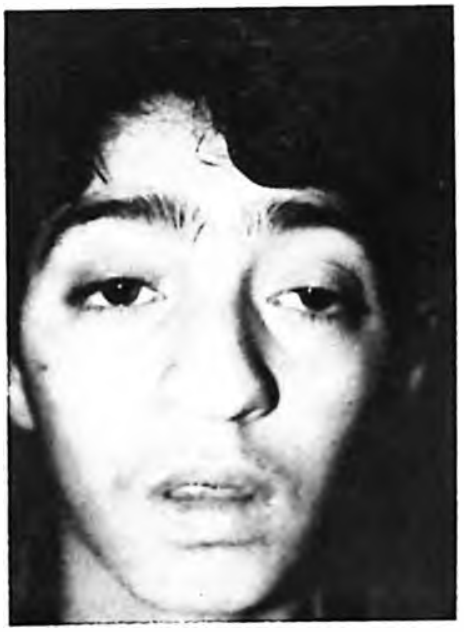

Fig. 1: Showing patient's face and bilateral ptosis.

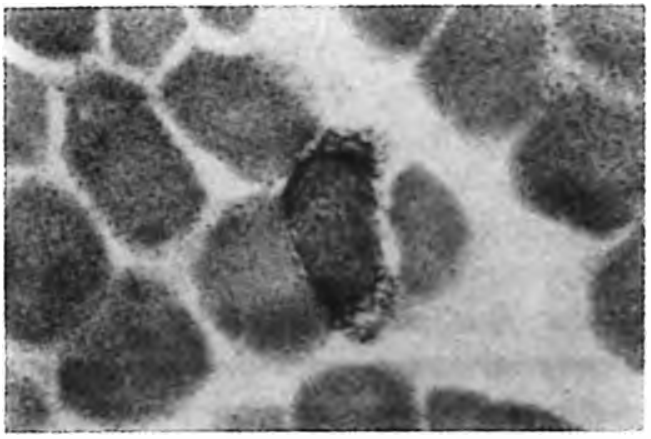

Fig. 2: Darkly stained muscle fiber especially in the periphery, "ragged red fiber" (x 132, succinic dehydrogenase).

Discussion

mtDNA is a small, circular, extra-nuclear chromosome encoding essential components of the respiratory chain. mtDNA-related syndromes can be divided into two groups: mitochondrial encephalomyopathies, characterized by the presence of raggedred fibers (RRF) as the morphological hallmark, or "pure" encephalopathies with no gross morphological abnormalities in the muscle. The first group includes myoclonic epilepsy with raggedred fibers (MERRF), mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO) and a new entity, maternally inherited myopathy and cardiomyopathy. The second group includes Leber's Hereditary Optic Neuropathy (LHON) and the newly described ataxia-retinitis pigmentosa-dementia complex[3]. Our case must be considered within the first group because of his having myopathy, encephalopathy attacks, and lactic acidosis.

Three kinds of molecular lesions have been identified: point mutations of protein-encoding mtDNA genes, point mutation of mtDNA-tRNA genes, and large-scale rearrangements of mtDNA. Point mutations are usually maternally inherited, while large-scale mtDNA rearrangements are either sporadic or are inherited as mendelian traits[3]. Large-scale rearrangements (deletions and insertions) and some point mutations are commonly associated with RRF and lactic acidosis as in KSS. The nuclear defects involving mitochondrial function are not generally associated with RRF[1]. Our case was found to have common mtDNA deletion.

KSS is a form of mitochondrial myopathy in which specific clinical features, namely progressive external ophthalmoplegia, pigmentary retinal degeneration and cardiac conduction defects occur[4]. Children with KSS usually appear normal at birth. Early development may be normal or mildly delayed. Ptosis and PEO are usually the first evidence of the disease. Later, mild visual loss with pigmentary retinopathy and sometimes optic atrophy may occur. The most common neurological feature is cerebellar syndrome which may become severe. Mental retardation or regression may be present. Usually, seizures do not occur unless there is concomitant hypoparathyroidism. Babinski's sign may be present[5]. Reported ECG abnormalities include first degree A-V block, fascicular blocks, sinus dysrhythmia and complete heart block as well as nonspecific S-T changes and T wave abnormalities[6]. Our case has most of these clinical features, such as ptosis, progressive external ophthalmoplegia, retinitis pigmentosa, myopathy, encephalopathy attacks, cerebellar dysfunction, pyramidal signs, and complete A-V block associated with implantation of a pacemaker. Although he does not have hypoparathyroidism, he had convulsions.

KSS has also been associated with a variety of endocrine and metabolic disorders, in particular short stature, gonadal failure, diabetes mellitus, thyroid disease, hyperaldosteronism, hypomagnesemia, and bone, tooth and calcification abnormalities. Short stature was not uncommon, being documented in 38 percent of cases. Gonadal dysfunction before and after puberty was also common (20%) of cases) and affected both sexes equally. Diabetes mellitus was recorded in 13 percent of cases, half of which required insulin. Thyroid disease, hyperaldosteronism, hypomagnesemia, and renal tubular dysfunction were uncommon[4]. Of these endocrinological abnormalties, our case has short stature, hypogonadism, insulin-dependent diabetes mellitus, and thyroid disease, but not hypoparathyroidism, renal tubular dysfunction, or hyperaldosteronism.

As shown in this case and cases in literature, KSS has variable clinical features due to differences of mtDNA deletion. However, progressive external ophthalmoplegia, pigmentary retinal degeneration and onset in childhood or adolescence are invariant features[5].

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AN ACUTE VASCULITIS RESEMBLING POLYARTERITIS NODOSA*

*Erbil Ünsal MD**, Ayşen Uğuz MD***, Necla Çevik MD*****

SUMMARY: Ünsal E, Uğuz A, Çevik N. (Division of Pediatric Rheumatology, Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). An acute vasculitis resembling polyarteritis nodosa. Turk J Pediatr 1998; 40: 261-265.

Peripheral vascular diseases are structural and functional abnormalities of the peripheral blood vessels that produce blood flow irregularities. The signs and symptoms relate either to ischemia or inflammation of the involved vessels, or to both. A previously healthy 4.5-year-old girl was referred to our hospital with bruises on fingers and toes. She had no history of environmental causes, including drug intake. Symptoms of abdominal pain, fever, and a swollen face preceded the symptoms of her extremities. On physical examination, her blood pressure, which could be obtained only on the thighs, was high. There were no pulses on the upper extremities or on either the a. dorsalis pedis or the a. tibialis anterior dextra. There was massive necrosis on fingers which led to dry gangrene. A rise in acute phase reactants accompanied the physical findings, and a segmental obstruction was found proximally to a. brachialas, and distally to a. radialis dextra and a. dorsalis pedis dextra on digital subtraction angiography (DSA). High-dose methylprednisolone, cyclophosphamide, salicylates and dipyridamole were given; as these medications did not relieve the symptoms, a thoracal sympathatectomy was performed. The peripheral circulation improved, but a demarcation zone developed on the fingertips, leading to amputation.

This patient is presented because she had an acute vascular spasm, of unknown etiology, resembling polyarteritis nodosa. *Key words: inflammatory vasculitides, polyarteritis nodosa, etiology, therapy.*

Inflammatory vasculitides are syndromes in which the various patterns depend on the size and location of the affected vessels. Vasculitis may be a primary disease or may be secondary to connective tissue disorder, infection, or other processes[1]. Peripheral vascular diseases are structural and functional abnormalities of the peripheral blood vessels that produce blood flow irregularities. The signs and symptoms relate to ischemia or inflammation of the involved vessels, or to both (changes in local temperature and skin color, as well as trophic changes that may be prone to ulceration, diminished arterial pulsations and local gangrene)[2]. When the injury is severe and prolonged enough that the cells cannot survive, various ultrastructural and histologic changes can be recognized. Tissue necrosis is obvious both histologically and clinically. There are two broad types of tissue necrosis, coagulative and liquefactive. Coagulative

* From the Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir.

** Instructor in Pediatrics, Dokuz Eylül University Faculty of Medicine.

*** Research Assistant in Pediatrics, Dokuz Eylül University Faculty of Medicine.

**** Professor of Pediatrics, Dokuz Eylül University Faculty of Medicine.

necrosis is the most common and is usually the result of arterial obstruction or inherent vascular disease that leads to sudden cessation of blood flow or ischemic infarction[3]. An attempt to classify vasculitides serves to emphasize the complexity of the subject and the heterogeneity of the clinical spectrum of these diseases[4]. In polyarteritis nodosa, (PAK), medium-sized arteries are the sites of inflammatilon. This necrotizing vasculitis affects all age groups, but is rare in childhood; males are affected more frequently than females. The cause is unknown, but the disease has been reported to follow specific drug exposure[1]. In this paper, a patient having an acute vascular spasm of unknown etiology resembling polyarteritis nodosa is presented.

Case Report

A previously healthy 4.5-year-old girl was referred to the pediatric rheumatology unit with bruises on fingers and toes. She had no history of environmental causes, including drug intake. Symptoms of abdominal pain, fever and a swollen face preceded the symptoms of her extremities. On physical examination, her hands were cold and edematous and the fingers were cyanotic. She had massive necrosis on fingertips with an infected wound on the left index finger, which led to dry gangrene (Fig. 1). Her feet were also cold and mildly cyanotic (Fig. 2).

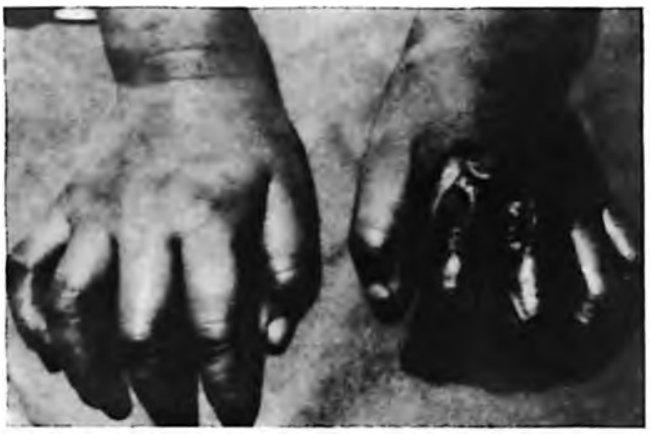

Fig. 1: There is massive necrosis on the fingertips and an infected wound on the left index finger.

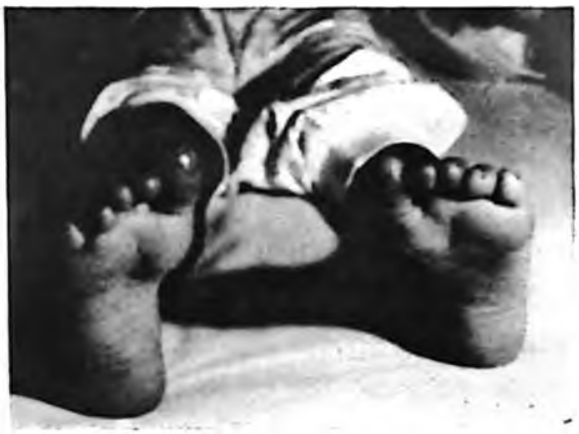

Fig. 2: There is mild cyanosis on the toes.

No pulses were detected on the upper extremities and the blood pressure, available only on the thighs, was 170/120 mmHg. Laboratory test results showed: leukocytosis (12.6×10^9 cells/L), C-reactive protein: 300 mg/L, erythrocyte sedimentation rate: 80 mm/hr, Hb: 8.5×10^9/L, Htc: 27%, and erythrocyte count: 3.2×10^9/L; the erythrocytes were hypochromic and microcytic. Renal function tests, including urine analysis, were normal. Prothrombin and partial thromboplastin time were within normal ranges. No hypocomplementemia was detected. Levels of antithrombin III (98%), protein C (2.20 mg/L), protein S

(13.8 mg/L), and anticardiolipin antibodies (ACA IgM: 1.4 MPL U/ml, ACA IgG: < 1.0 GPL U/ml) were within normal limits. Hepatitis B antigen was negative. On digital subtraction angiography, segmental obstruction was found proximally to a. brachiales and distally to a. radialis and a. ulnaris sinistra (Figs. 3-7). The

Fig. 3: Right brachial artery is obliterated in the proximal region and is refilled with the collateral arteries at the middle.

Fig. 4: Right brachial artery in the forearm is obliterated again just before the bifurcation and no filling is seen at the distal portion.

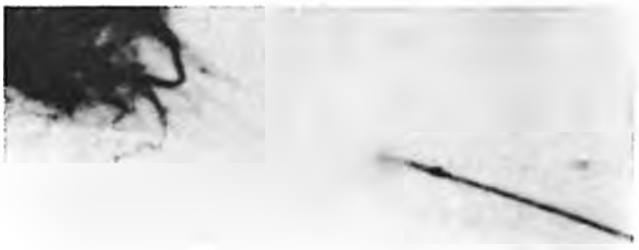

Fig. 5: Left brachial artery is obliterated in the proximal region, but it is refilled with larger collaterals.

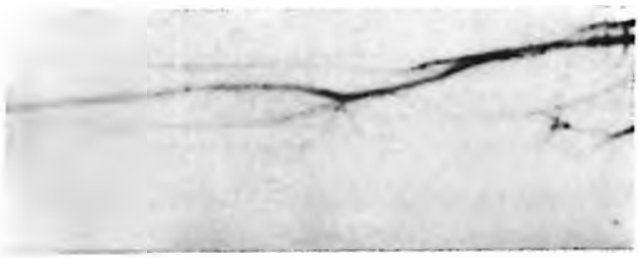

Fig. 6: Left brachial and radial artery can be seen in their entirety while ulnar and radial arteries cannot be filled at the level of the wrist.

patient was treated with high-dose methyl prednisolone (30 mg/kg/day) and cyclophosphamide (2 mg/kg/day) for immune suppression; acetyl salicylic acid (150 mg/day) and dipyridamole (5 mg/kg/day) as thrombolytic agents; nifedipine (2 mg/kg/day) for hypertension; piperacillin and amikacin for the infected lesion. Although she felt better and no signs of new skin necrosis on extremities were identified, the continued lack of pulses on the upper extremities necessitated a sympathatectomy, and the gangrenous fingers were amputated at the level of proximal interphalangeal joints (Fig. 8). Marked improvement was noted at the surgery sites, as well as in the general condition of the patient, within a week. She is being followed regularly since discharge.

Fig. 7: Left radial artery is refilled with the collaterals after being obliterated in the wrist line and forms the medial curve. The vascularizations of the third and fourth fingers are better than the others. The curve cannot be formed on the ulnar side.

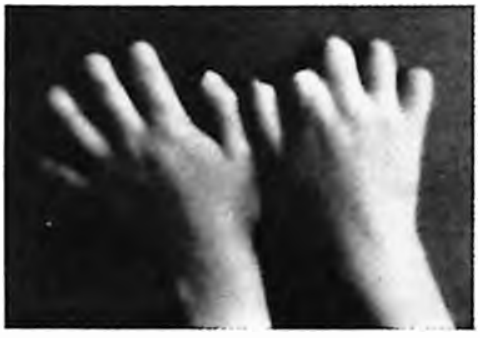

Fig. 8: The patient's hands after amputation. The thumbs are largely spared.

Discussion

The clinical features that suggest a vasculitic syndrome include weight loss, fever of unknown origin, abdominal pain, dermal necrosis, leukocytosis, and elevation of C-reactive protein and erythrocyte sedimentation rate[5,6]. This patient's symptoms of abdominal pain, fever, swollen face, hypertension, elevation of acute phase reactants, and gangrene of the fingers, strongly suggested an inflammatory vasculitis. Marked absence of pulses in a young girl should always bring to mind the most common giant cell arteritis of childhood, Takayasu's arteritis. The diagnosis of this disease is often delayed extensively because of the insidious onset, as well as the nonspecific nature of early manifestations.

The digital subtraction angiography of the entire aorta and its major branches showed no pathological changes, and helped to rule out this entity. The polyarteritis nodosa (PAN), though rare in a young pediatric age group, exhibits no single pattern of clinical presentation. However, abdominal pain, peripheral or central nervous system disease, arthritis and skin lesions occur in most children with PAN. Cutaneous involvement includes purpura, edema, peripheral gangrene, and nodular vasculitis. Although it can be suspected from a typical clinical presentation, diagnosis can be confirmed only by pathologic demonstration of characteristic lesions, or by radiologic documentation of aneurysms[7-9]. This patient had no typical lesions for a diagnostic biopsy. Digital subtraction angiography was done to determine the presence of abnormalities of the vascular system. There were obliterations in brachial arteries, but no aneurysm was detected, which is typical for polyarteritis nodosa. Ergotism is an acute or chronic poisoning caused by the ingestion of grain, or its products, infected by the ergot fungus, Clavicep purpurea. Patients may experience colic, coldness and cyanosis of the extremities, Raynaud's phenomenon, absence of peripheral pulsations, and gangrene of the digits[2]. As it is known that toxins are one of the factors triggering vasculitis, the symptoms and findings suggesting peripheral vasoconstriction in this patient could, presumably, be attributed to an ingestion of food or plant containing ergot or its derivatives, as the child's symptoms developed following three months spent in a village, where she might have been in close contact with the substances mentioned above.

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A DIFFUSE FORM OF NEUROFIBROMA OF BLADDER IN A CHILD WITH VON RECKLINGHAUSEN DISEASE*

*H. Aydanur Kargı MD***, *Tanju Aktuğ MD****, *Emek Özen MD*****
*Alp Kılıçalp MD******

SUMMARY: Kargı HA, Aktuğ T, Özen E, Kılıçalp A. (Departments of Pathology and Pediatric Surgery, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). A diffuse form of neurofibroma of bladder in a child with von Recklinghausen disease. Turk J Pediatr 1998; 40: 267-271.

Neurofibromatosis in infants is uncommon and involvement of the bladder is rare. The reported bladder lesions are rare, and in such patients include neurofibromas, neurofibrosarcomas and rhabdomyosarcomas. Neurofibromas can be of different histologic types. The histologic type of the about 20 reported bladder neurofibromas in children is not clarified or is stated to be of plexiform type. We describe herein an unusual case of a neurofibroma of bladder in a child with von Recklinghausen disease. The therapeutic management and the possible prognostic implication of the type of the bladder neurofibroma and bladder lesions other than neurofibroma are discussed. *Key words: diffuse neurofibroma, bladder, neurofibromatosis.*

Neurofibromatosis is a rare hamartomatous growth of neural crest derivation that indirectly affects supporting mesenchymal elements. The minimal criteria for the clinical diagnosis of neurofibromatosis are: 1. The presence of neurofibromas (NFs), or multiple cafe-au-lait spots with positive family story, or both; 2. At least six cafe-au-lait spots over 1.5 cm in diameter; 3. Multiple NFs[1, 2]. The involvement of the genitourinary tract or of the urinary bladder is rare. In fact, upon search of literature we have encountered about 60 cases of NFs of urinary bladder in patients with von Recklinghausen (vRH) disease. Only one-third of these cases are reported to occur in children[3-8]. The morphologic forms of these cases are either stated to be of plexiform type or are not clarified at all. We report a rare case of a diffuse form of NF involving the urinary bladder of a 17-month-old male infant with clinically diagnosed vRH disease.

Case Report

A 17-month-old white male infant was evaluated for motor retardation. His medical history included frequent upper respiratory and urinary tract infections.

* From the Departments of Pathology and Pediatric Surgery, Dokuz Eylül University Faculty of Medicine, İzmir.

** Associate Professor of Pathology, Dokuz Eylül University Faculty of Medicine.

*** Associate Professor of Pediatric Surgery, Dokuz Eylül University Faculty of Medicine.

**** Professor of Pathology, Dokuz Eylül University Faculty of Medicine.

***** Research Assistant in Pathology, Dokuz Eylül University Faculty of Medicine.

On physical examination, his height was at the tenth percentile (77 cm) and weight was at the fifth percentile (10.6 kg). Approximately 10 cafe-au-lait spots were present on his trunk, neck and extremities.

Laboratory studies revealed normal complete blood counts and serum chemistry. Urinalysis demonstrated positive protein,[8-10] neutrophils and few red blood cells per high power field. Urine culture was positive for Pseudomonas aeruginosa.

Further investigation of the patient included abdominal ultrasonography, intravenous urography (IVU), voiding cystourethrography (VCUG), abdominal computed tomography (CT) and TC-99m DMSA that demonstrated left-sided hydronephrosis and hydroureter, irregular thickening of the urinary bladder wall and a retrovesical mass compressing the urinary bladder posteriorly.

Exploratory laparatomy revealed multiple tumor masses involving the wall of urinary bladder which were more prominent in the posterior and superior walls. A partial cystectomy of the superior-posterior wall of the urinary bladder and a bilateral ureterocystostomy were performed. The pathologic examination showed the entire material to be composed of a diffuse form of NF. He was diagnosed as having vRH disease.

One month postoperatively, abdominal ultrasonography showed bilateral hydroureter and hydronephrosis. A cystoscopy at this time revealed complete obstruction of the orifices of both ureters. A bilateral percutaneous nephrostomy was performed. However, right-sided nephrostomy failed to function and approximately one month after this procedure, his serum BUN raised to 22 mg/dl, creatinine was 7 mg/dl and glomerular filtration rate was 46 ml/1.73/m^2. The blood electrolytes were within normal ranges. Abdominal computed tomography (CT) scan showed multiple tumoral masses involving the entire urinary bladder wall. A total cystectomy with ileocecal conduit was performed. The patient's postoperative course was uneventful.

The first partial cystectomy material consisted firm, grayish-white tissue, which appeared to be covered by mucosal tissue on one side, measuring 7 x 5 x 3 cm. The second cystectomy material measured 9 x 7 x 6 cm. The full thickness of almost the entire bladder wall was involved by grayish-white, firm tumor tissue which was bulging into the lumen as multiple, polypoid masses, ranging from 0.5 to 3.5 cm in greatest dimension (Fig. 1). The orifices of both ureters could not be probed. Light microscopic examination of both specimens revealed a diffuse form of neurofibroma, involving the entire thickness of the bladder wall except for the mucosa. The overlaying mucosa was attenuated. The tumor was composed of short, fusiform or round cells which were dispersed in a uniform matrix of fine fibrillary collagen. The tumor contained numerous, large clusters of Wagner-Meissner bodies (Fig. 2). In spite of extensive search, no cytologic atypia or mitotic activity was found.

Fig. 1: The cystectomy material showing multiple masses protruding into the lumen.

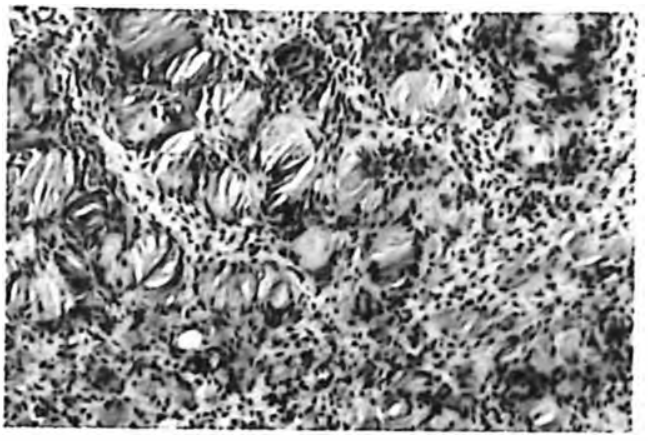

Fig. 2: An area showing diffuse sheets of Wagner-Meissner bodies (HE, 100X).

Discussion

Diffuse NF is a uncommon form of NF that occurs principally in children and young adults and is most common in the skin and subcutaneous tissues of the head and neck region. It is reported to occur in 10 percent of all neurofibromatosis cases. However, the literature on this form is scarce. The tumor has an ill-defined border with an infiltrative growth pattern. The histologic features of diffuse NFs are somewhat different from the plexiform or nonplexiform types of NFs. The tumor cells are short fusiform or round as opposed to the more elongated, wavy cells of the other forms. They are dispersed in a uniform matrix of fine fibrillary collagen. The characteristic feature of this lesion is the presence of tactoid (Wagner-Meissner) bodies[1].

Even though NFs can occur in any site in patients with vRH disease, visceral involvement is not frequent and when it does occur, the gastrointestinal tract is most often affected. Urinary tract involvement is rare and is reported to be of plexiform type in those cases in which the histologic type is clarified.

Although the treatment of bladder NFs is controversial, a conservative treatment with partial cystectomy is recommended for those localized bladder lesions. But some patients with concurrent ureteral, prostatic and urethral involvement require radical prostatectomy with urinary diversion[8]. However, the histologic type of NF may also be an important factor in deciding the extent and, therefore, the cohice of treatment. Even though the tumoral masses of the diffuse form of NF may appear as localized lesions, as was initially seen in our case, they are indeed ill-defined, infiltrative growths. Therefore, it is possible that the residual infiltrative lesion in the remaining bladder wall was responsible for the rapid progression of the tumor after the partial cystectomy in our case. This progressive feature of the illness was the cause of the obstruction of the neo-ureteral orifices.

Upon search of the literature, we found two cases of neurofibrosarcoma in the bladder and nine cases of rhabdomyosarcoma in the genitourinary tract of young children with neurofibromatosis[2, 9, 10]. The site of involvement was cited as the urinary bladder in four of nine cases and was not clarified in the remaining five cases. Since the total number of reported cases of bladder neurofibromas in children with neurofibromatosis does not exceed 22, it appears that sarcomas made up to second most common bladder tumor in those children. Because of the significant difference between the therapeutic management of those patients with bladder neurofibroma and sarcoma and the possible prognostic implication of the type of neurofibroma, it becomes important to obtain tissue confirmation of histologic findings before persuing or withholding any treatment.

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TWO SIBLINGS WITH FETAL HYDANTOIN SYNDROME*

*Ferda Özkınay MD**, Ayşe Yenigün MD**, Mehmet Kantar MD****
*Cihangir Özkinay MD****, Ali Avanoğlu MD******
*İbrahim Ulman MD******

SUMMARY: Özkınay F, Yenigün A, Kantar M, Özkınay C, Avanoğlu A, Ulman İ. (Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Two siblings with fetal hydantoin syndrome. Turk J Pediatr 1998; 40: 273-278.

An association between anticonvulsant drugs taken during pregnancy and congenital abnormalities was first identified by Meadow et al. in 1968. Manson and Frederic clarified teratogenic effects of hydantoin in their epidemiological studies in 1973. Varied malformations due to hydantoin intake during pregnancy include digit and nail hypoplasia, growth retardation, typical facial appearance, rib anomalies, abnormal palmar creases, hirsutism, and low hairlines. Ambiguous genitalia is rarely associated with this syndrome. We present two siblings, aged three years and three months, with fetal hydantoin syndrome (FHS). Both were born to an epileptic mother who was given diphenylhydantoin (DPH) and phenobarbital throughout her pregnanies. The patients showed many characteristics of FHS, and ambiguous genitalia. Clinical and laboratory examinations revealed that both have normal female internal genital organs and female karyotypes. *Key words: hydantoin, phenobarbital, pregnancy, craniofacial dysmorphism, genital anomaly.*

The association between anticonvulsant drugs taken during pregnancy and an increased incidence of congenital defects has been documented for nearly four decades. Among these anticonvulsant drugs, phenytoin (DPH), carbamazepine, valproic acid, and various combinations of these three drugs have been proven to have the most potential teratogenic effects[1-4].

The characteristic features of in utero exposure to fetal hydantoin syndrome (FHS) include typical craniofacial anomalies, prenatal and/or postnatal growth deficiency, mental retardation and increased risk of major malformations[5-7]. The risk of congenital malformations for infants exposed to DPH during their fetal lives is about 40 percent[6-9]. The most frequent morphological abnormalities seen in these children are hypoplastic toes or fingernails, while genital abnormalities are relatively rare[5-7].

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

Case Reports

The probands were the two siblings born to a 26-year-old woman with epilepsy whose medication included DPH (200 mg/d) and phenobarbital (100 mg/d) for eight years. The mother continued to take the same amount of medication throughout both pregnancies and did not have any seizures. There were no similar cases in the extended family other than the reported ones. The two siblings exhibited the same type of genital abnormality as well as the most common features of FHS.

Case 1

A four-year-old girl (the first child), with genital anomaly identified at birth, was referred to our hospital by a physician for corrective surgery. The delivery had been uncomplicated. At birth her weight was 2700 g and length was 48 cm. Moderate developmental delay was noted in early infancy: she sat at one year old and walked at 2.5 years old. She spoke only seven to eight words at the age of four.

On admission the patient's weight was 14.5 kg (10^{th}-25^{th} percentile), height 96 cm (25^{th} percentile), and head circumference 48 cm (10^{th} percentile). She had ocular hypertelorism; down-slanted palpebral fissures; a low anterior hairline; a broad, depressed nasal bridge; a short nose; long philtrum; broad alveolar ridge; short neck; low posterior hairline; and hirsutism (Fig. 1). Also noted were bilateral simian creases in both hands, broad first toes, and hypoplasia of the toenails, especially the last two toenails of both feet. Nipples were small and widely spaced. Genital examination showed fusion of the labia minora and a prominent clitoris (Fig. 2).

Fig. 1: Case 1. Note low anterior halirline, low and broad nasal bridge, long philtrum.

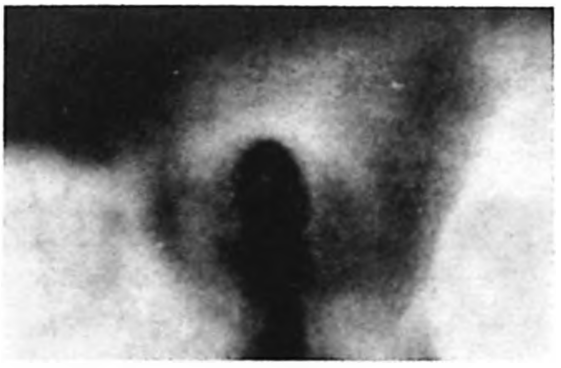

Fig. 2: Case 1, ambiguous genitalia with prominent clitoris and fused labia minora.

There was a single opening at the base of the clitoris. Laboratory tests revealed normal levels of hormones and blood electrolytes. On ultrasonographic examination, the uterus and two ovaries were detected. Cystoscopy showed urogenital sinus where the vagina and urethra opened, and a normal bladder. Roentgenograms revealed hypoplastic distal phalanges (Table I). Chromosomes were normal.

Table I: Clinical Features in Two Affected Siblings

Congenital Anomalies		Case 1	Case 2
Growth deficiency	Prenatal	+	–
	Postnatal	–	–
Developmental problems		+	+
Low anterior hairline		+	+
Low posterior hairline		+	+
Hypertelorism		+	+
Depressed nasal bridge		+	+
Short nose		+	+
Down-slanted palpebral fissures		+	+
Long philtrum		+	–
Broad alveolar ridge		+	–
Widely spaced nipples		+	+
Hypoplastic toenails		+	+
Hypoplastic fingernails		–	+
Broad first toe		+	+
Hypoplastic distal phalanges		+	+
Ambiguous genitalia		+	+

Case 2

A 7-month-old female, the sister of Case 1, was the product of the second pregnancy of the mother. Since, the etiology of the genital anomaly had not been recognized in the first child, the mother was unaware of the potential danger of DPH intake during pregnancy, and continued to take the medication throughout her second pregnancy. This delivery also was normal. Birth weight was 3000 g and length 50 cm. Ambiguous genitalia at birth was also identified in Case 2 and she was brought to hospital with her sister for correction of her genital anomaly.

On admission, her growth was at normal level with a weight of 7100 g (25^{th} percentile), height of 70 cm (50^{th} percentile), and a head circumference of 44 cm (75^{th} percentile). She could not sit without help. Physical examination revealed the same type of craniofacial and limb abnormalities as her sister's with the following exceptions: she had a bowed upper lip instead of the long

philtrum which was seen in Case 1, and the nail hyperplasia was more marked on her fingers (Figs. 3, 4). She also had the same type of genital abnormality (Fig. 5). Her laboratory tests and roentgenograms showed similar findings (Table I).

Fig. 3: Case 2, the sibling of Case 1. Note low anterior hairline, depressed and broad nasal bridge, bowed upper lip.

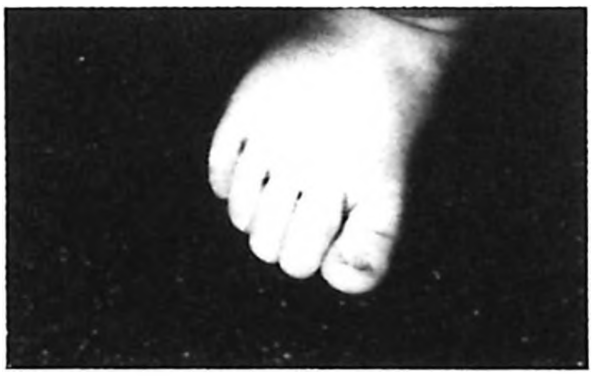

Fig. 4: Case 2, hypoplastic toenails.

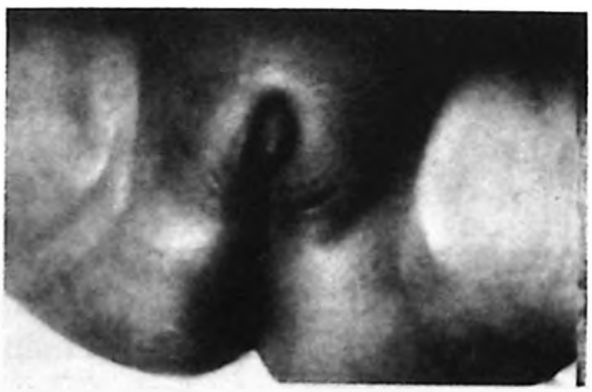

Fig. 5: Case 2, ambiguous genitalia with prominent clitoris and fused labia minora.

Discussion

The majority of minor morphological features seen in patients with FHS are not medically or cosmetically significant and tend to be overlooked unless a very thorough examination is made. Major defects recorded in the literature occur less frequently than minor defects. They include: cleft lip and/or palate, cardiovascular anomalies, renal defects, gastrointestinal anomalies and genital anomalies[9]. The alterations of growth and performance are as important as morphological anomalies in FHS, and are not commonly detected until later in childhood.

The patients presented here possess most of the characteristic features of FHS (Table I), and the genital anomalies which are rarely seen in the syndrome. Dyke et al.[6] recorded no patients with genital anomaly in their study of 62 families having at least two children exposed in utero to DPH. Hanson et al.[5] described only one patient with undescended testes among five patients showing FHS. In a series of 22 patients exposed in utero to DPH, one patient was recorded as having hypospadias[7].

Our cases had been investigated for adrenogenital syndrome before they were seen in our hospital. All laboratory tests for adrenogenital syndrome were found to be normal, as was expected.

Several hypotheses have been proposed to explain the DPH-induced teratogenesis. The most acceptable of these is that the toxic oxidative intermediary metabolites of DPH cannot be detoxified due to an enzyme deficiency in the metabolic pathway of DPH[10, 11]. Whether or not the toxic metabolites have an androgenic effect is not known. Since, all children born to mothers taking DPH during pregnancy do not show FHS, and the clinical findings of FHS differ from case to case, various enzymatic deficiencies may be responsible for causing the syndrome. Oxidative metabolites and their effects may differ in patients, depending on the molecular alterations in enzyme structure.

Unfortunately, the link between DPH intake during pregnancy and the anomalies found in Case 1 was not recognized before the conception of the second child, and genetic counseling was not given to the parents. Perhaps, if this link had been known by the family and doctors, DPH could have been changed to another anticonvulsant drug, or the family might have decided against having a second child.

Van Dyke et al.[6] studied 62 families with DPH exposure and found that, if the first child exposed to DPH in utero is affected, the risk for the second child will be much higher. Our cases support this finding and we would add that not only is the second child affected, but the abnormalities seen in the first child are replicated in the second child.

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SUBCUTANEOUS GRANULOMA ANNULARE AND IgA – IgG2 DEFICIENCY*

*Necil Kütükçüler MD***, *Sarenur Tütüncüoğlu MD***, *Deniz Yılmaz MD****
*Taner Akalın MD*****, *Gülşen Kandiloğlu MD******

SUMMARY: Kütükçüler N, Tütüncüoğlu S, Yılmaz D, Akalın T, Kandiloğlu G. (Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Subcutaneous granuloma annulare and IgA-IgG2 deficiency. Turk J Pediatr 1998; 40: 279-282.

Subcutaneous granuloma annulare (SGA) is a benign granulomatous disease occurring in childhood, with lesions most commonly located about the elbow, knee and scalp. The etiology of SGA remains obscure. We present a typical case with SGA also showing laboratory findings of IgA and IgG2 deficiency.

Histologic findings of the lesions on the scalp were characterized by multiple large foci of complete collagen degeneration with a peripheral palisade of histiocytes; the foci of degeneration was edematous basophilic. In contrast to current literature, an abnormality in the cellular immune system was not found. However, immune defects (IgA and IgG2 deficiency) related to the humoral immune system were observed.
Key words: subcutaneous granuloma annulare, IgA-IgG2 deficiency.

Subcutaneous granuloma annulare (SGA) is a benign inflammatory granulomatous disease occurring in childhood, with lesions most commonly located about the elbow, knee and scalp[1,2]. SGA is one of the four clinically distinct subtypes of granuloma annulare[1]. Although trauma and immune complex vasculitis have been considered, the etiology of SGA remains obscure and the disease is generally considered to be idiopathic[3]. In recent dermatology and pathology literature, there are some reports and letters noting the association of granuloma annulare and sarcoidosis[4], cervical adenocarcinoma[5], herpes zoster infection[6], and aberrant cell-mediated immune response[7]. An association between SGA and humoral immunity has not been reported. This case report relates two rare entities: SGA, and IgA (immunoglobulin A) and IgG2 deficiency occurring in the same patient.

Case Report

A four-year-old girl, with a one-year history of recurrent, rapidly growing, and spontaneously resolving painless masses on the scalp, had been presented to different physicians because of parental concerns of malignancy and lack of

* 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.
**** Pathologist, Ege University Faculty of Medicine.
***** Professor of Pathology, Ege University Faculty of Medicine.

specific and definitive diagnostic tests, except biopsy. Her past medical history was remarkable for recurrent upper respiratory tract infections. On admission, four lesions (firm, nontender and immobile, the largest 2 cm in diameter, located on the occipital region of the scalp) were found on physical examination. Cranial x-ray revealed a soft-tissue mass without direct osseous involvement. Complete blood count, erythrocyte sedimentation rate, renal and hepatic function tests, prothrombin time, and blood sugar were all normal.

Excisional biopsy was performed and the lesion was described as located in the subcutaneous tissue, attached to the periosteum over the bone. Histologic findings were characterized by multiple large foci of complete collagen degeneration with a peripheral palisade of histiocytes (Fig. 1). The foci of degeneration was edematous basophilic with hematoxylin-eosin that stained positively with Alcian blue. There were inflammatory cells, mostly lymphocytic, around the granulomatous areas (Fig. 1).

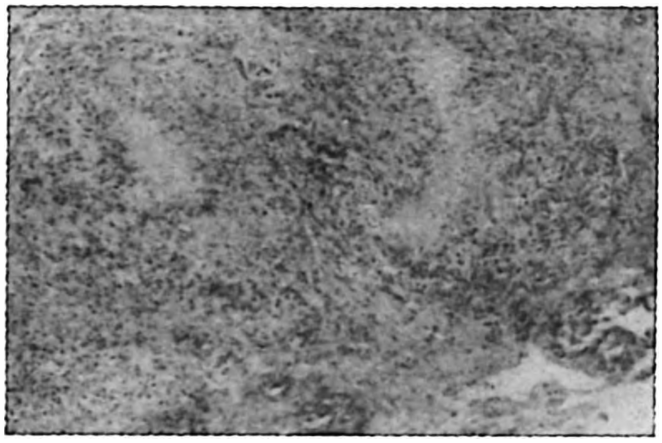

Fig. 1: The two granulamatous areas, with central complete collagen degeneration, which are surrounded by palisading histiocytes (H.E.x100).

After the diagnosis of SGA, some investigations related with autoimmune diseases, such as: antinuclear antibody (ANA), anti-ds-DNA, rheumatoid factor (RF), complement components (C3 and C4), C-reactive protein (CRP), serum haptoglobin, and ferritin and immunoglobulin (Ig) concentrations, were performed. All tests were within the normal reference intervals except the serum IgA and IgG2 levels. Serum immunoglobulins were investigated three times by nephelometry, and serum IgG subclass values by ELISA. Serum IgA and IgG2 values were found to be decreased when compared with values in healthy Turkish children[8] (IgG: 678-604-690 mg/dl, IgM: 122-97-141 mg/dl, IgA: 24.3-22.4-21.7 mg/dl (normal 66-120 mg/dl), IgG1: 5.0 g/L, IgG2: 0.2 g/L (normal 0.8-3.6 g/L), IgG3: 1.86 g/L, IgG4: 0.07 g/L). There was a slight decrease of gamma-globulin fraction (9.5%) (normal 12-22%) in protein electrophoresis. On the other

hand, the percentages and absolute counts of lymphocyte subsets by flow cytometry were normal when compared with normal age-related values[9]. Delayed-type skin reaction to purified protein derivative (PPD) due to previous vaccination with BCG (Bacillus-Calmette-Guerin) was observed.

The child was diagnosed with "subcutaneous granuloma annulare and IgA-IgG2 deficiency", and the lesions resolved spontaneously after three months. Only one recurrence has been observed during the follow-up period of one year.

Discussion

SGA is a benign inflammatory disorder that occurs in children. The mean age at initial presentation of lesions is 3.9 years[1]. Our case was three years old at initial presentation of SGA, and the lesions were located on the scalp, as mentioned in different series.

The etiology of SGA remains unclear. Early reports implicated tuberculosis, but this has never been confirmed[10]. More recently, it has been hypothesized that vasculitis and cellular immunity play a role in SGA. Direct immunofluorescence studies have shown blood vessel deposits of IgM, IgG and C3 complement, leading some investigators to question the significance of an immune complex vasculitis[11]. Immunologic studies of lymphocyte subsets support the hypothesis that SGA is the result of an aberrant cell-mediated immune response[7]. Despite years of investigation and application of the latest technology, the specific cause of SGA remains unknown.

In our case, the laboratory investigations related to the cellular immune system were all found to be normal and the patient had no other signs and symptoms of an immune complex disease.

Selective deficiency of the IgG2 subclass commonly accompanies IgA deficiency[12]. IgA deficiency and granuloma annulare are associated with many autoimmune diseases[1, 12]. However, our patient did not have any laboratory or clinical signs of an autoimmune disease.

No other case showing concurrent SGA and IgA-IgG2 deficiency has been found in the literature. Because of the questionability of immunologic abnormalities in the pathogenesis, this illustrative case is of interest.

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THE CHARGE ASSOCIATION IN A NEWBORN INFANT*

*Mete Akısü MD***, *Ferda Özkınay MD****, *Ruhi Özyürek MD***
*Abdullah Küçüktaş MD*****, *Nilgün Kültürsay MD******

SUMMARY: Akısü M, Özkınay F, Özyürek R, Küçüktaş A, Kültürsay N. (Department of Pediatrics, Ege University Faculty of Medicine, Bornova-İzmir, Turkey). The CHARGE association in a newborn infant. Turk J Pediatr 1998; 40: 283-287.

CHARGE association is the nonrandom association of congenital anomalies, including choanal atresia, coloboma, heart defects, retardation of growth and development, genital hypoplasia and ear abnormalities. We report a male newborn infant with CHARGE association. Other congenital abnormalities include micrognathia, high-arched palate, facial asymmetry, broad nasal bridge, hypertelorism, asymmetric eye size, and left microphthalmia. On radiologic examination, hemivertebrae were detected on the thoracal vertebrae. Although both autosomal and recessive transmission have been reported, most cases of CHARGE association have been sporadic (karyotype analysis is generally reported to be normal as in our patient). Transmission and recurrence risk of this association are not known. The presence of choanal atresia and/or coloboma must alert the clinician to search for other abnormalities for diagnosis of CHARGE association. *Key words: CHARGE association, choanal atresia, hemivertebra, microphthalmia, newborn, patent ductus arteriosus, vertebral anomaly.*

Hall[1] first reported a nonrandom association of congenital malformations occurring with choanal atresia, but CHARGE association (coloboma, heart defect, choanal atresia, growth and/or developmental retardation, genital hypoplasia, ear abnormalities) was described by Pagon et al.[2] in 1981. Additional features may include asymmetric face, micrognathia, cleft palate, facial weakness, difficulty in swallowing, hydronephrosis, and tracheoesophageal fistula[1,2]. These nonrandom anomalies were named as an "association" rather than as a "syndrome"[2].

Although autosomal dominant and recessive inheritances are also suggested by occurrence in some families, this association is mostly reported in sporadic cases[1-4]. CHARGE association may be a good example of genetic heterogeneity. A newborn with CHARGE association is reported here.

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

** Pediatrician, Ege University Faculty of Medicine.

*** 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.

Case Report

A male infant was born to a 28-year-old mother after 37 weeks of gestation at Ege University Medical School Hospital. No maternal drug exposure, infectious disease, alcohol or parental consanguinity was reported (Fig. 1). The mother had a phenotypically normal stillborn of 30 weeks' gestation one year previously. That pregnancy was complicated by oligohydramnios. One-minute and five-minute Apgar scores were 5 and 7, respectively.

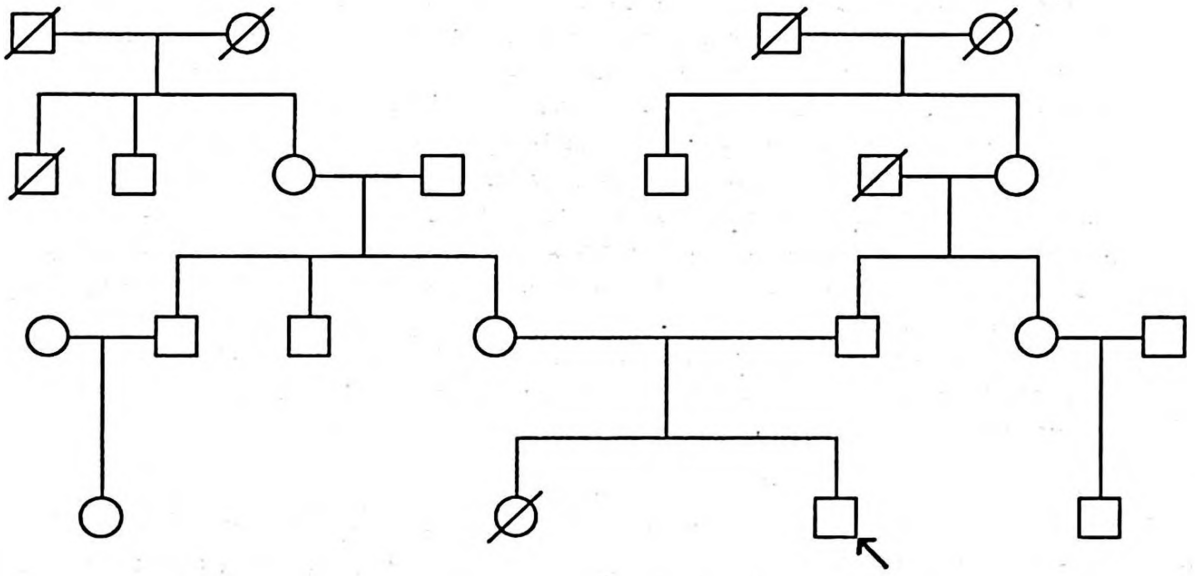

Fig. 1: The pedigree of the patient.

The infant had intrauterine growth retardation with a birth weight of 1530 g (below 10%) and height of 43 cm (below 10%). Micrognathia, high-arched palate, facial asymmetry, broad nasal bridge and hypertelorism were observed on physical examination. Asymmetric eye size and left microphthalmia, but no other ocular abnormality such as coloboma of iris and retina, were detected. The ears were small, cup-shaped and low-set but the external auditory canal was normal on otoscopy (Figs. 2 and 3). The infant showed respiratory distress and cyanosis. Bilateral choanal atresia was considered since a nasogastric feeding tube failed to be advanced through both nares. On cardiac auscultation a systolic murmur was heard. The infant was hypotonic and had weak and poorly organized neonatal reflexes. External genitalia were normal.

Complete blood count and other biochemical laboratory studies were normal. Blood gas analysis showed mild hypoxemia. Cytogenetic study showed normal male karyotype (46, XY).

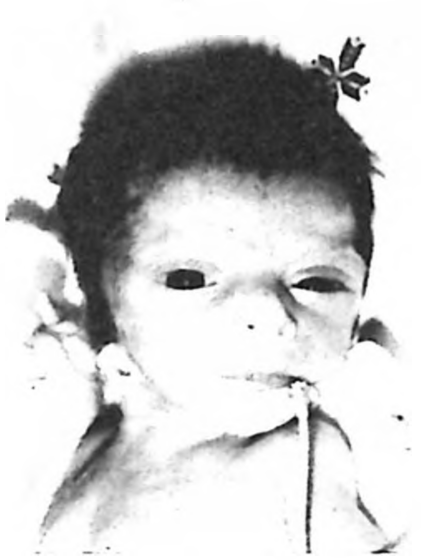

Fig. 2: Photograph of the case showing facial asymmetry, facial weakness, broad nasal bridge, hypertelorism, asymmetric eye size, left microphthalmia, and small, cup-shaped and low-set ears.

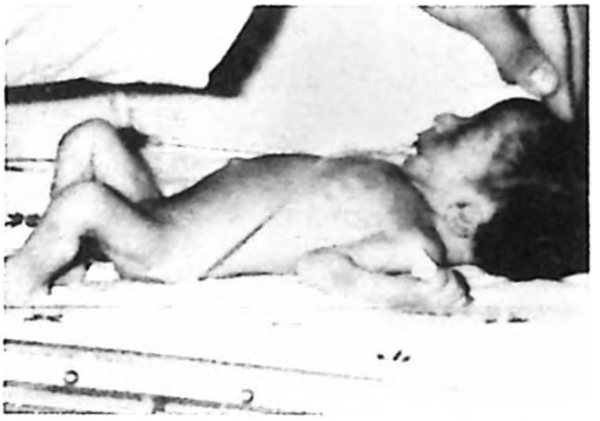

Fig. 3: Photograph of the case showing micrognathia, depressed nasal bridge, and small, cup-shaped and low-set ears.

Bilateral choanal atresia was visible on lateral skull x-ray with opaque medium given through nares and was also confirmed by fiberoptic rhinoscope (Fig. 4). Chest radiogram showed cardiomegaly and increased pulmonary circulation, in addition to hemivertebral appearance of 5^{th}, 7^{th}, and 9^{th} thoracal vertebrae (Fig. 5). Abdominal ultrasonography showed normal urogenital structure. Patent

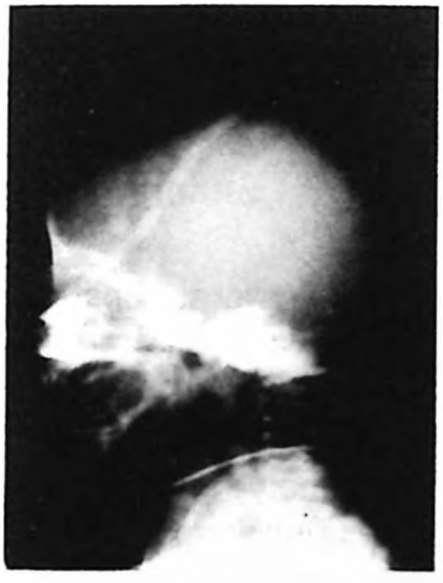

Fig. 4: Lateral skull x-ray with radioopaque material demonstrates choanal atresia.

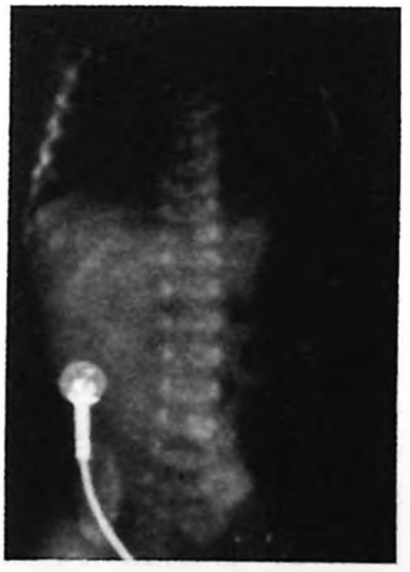

Fig. 5. Chest x-ray of the case showing cardiomegaly, increased pulmonary circulation and thoracal hemivertebrae.

ductus arteriosus (PDA) of 3 mm, patent foramen ovale of 3 mm and pulmonary hypertension were diagnosed on echocardiography.

The patient was diagnosed with CHARGE association. Any further intervention and investigation was refused by family because of the high expectancy of mental retardation in patients with CHARGE association.

Discussion

Major abnormalities in CHARGE association may be outlined as follows:

Ear Abnormalities: lop-or cup-shaped pinnae, low-set ears, small ears, deafness, and external ear abnormalities.

Choanal Atresia: bilateral or unilateral, membranous or bony obstruction.

Ocular Manifestation: coloboma of iris, coloboma of retina/choroid, asymmetric eye size, microphthalmia, nystagmus, strabismus, and refractive errors.

Genital Abnormalities: micropenis, undescended testes, and hypogonadism.

Heart Defects: PDA (very common, either alone or associated with more severe lesions), tetralogy of Fallot (very common), ventricular septal defect, atrioventricular canal, double-outlet right ventricle, and aortic arc anomalies.

Growth: (commonly due to the problems of feeding and swallowing) and developmental retardation (mental retardation is independent from the presence of microcephaly, and IQ scores range from 30 to 80)[1-7]: Blake et al.[3] reported that one-third of neonates with CHARGE association had intrauterine growth retardation and all subsequently continued to be growth retarded. The diagnosis of CHARGE association requires at least four of the six major diagnostic criteria (choanal atresia, heart defect, coloboma, growth and/or developmental retardation, genital hypoplasia and ear abnormalities)[3, 5, 8]. In addition to these major abnormalities, a number of anomalies were also described in this association, such as: micrognathia, facial weakness, asymmetric face, cleft palate, short neck, microcephaly, small mouth, difficulty in swallowing, hydronephrosis, tracheoesophageal fistula and skeletal abnormalities (hemivertebrae, scoliosis, syndactyly, clinodactyly)[1-3, 7].

Because our patient had choanal atresia, heart defects, ear abnormalities and intrauterine growth retardation, the diagnosis of CHARGE association was established. Microphthalmia and asymmetric eye size were detected as eye abnormalities. PDA, the most common cardiac anomaly in this association, was established, in addition to patent foramen ovale and pulmonary hypertension, external auditory canal was normal on otoscopy in spite of abnormal pinnae. Other minor abnormalities of the patient were micrognathia, high-arched palate, facial asymmetry, broad nasal bridge, and hypertelorism. X-ray study of thorax showed hemivertebrae, an uncommon abnormality in CHARGE association.

Although both autosomal and recessive transmission have been reported, most of the cases with CHARGE association have been sporadic. Transmission and recurrence risk of this association are not known. Karyotype analysis is generally reported as normal as in our patient[9]. The presence of choanal atresia must alert the clinician to search for other abnormalities for diagnosis of CHARGE association.

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The Turkish Journal of Pediatrics 1998; 40: 289-294

CONGENITAL CYSTIC ADENOMATOID MALFORMATION*

Report of Two Cases and Review of the Literature

*Bahar Kılıçarslan MD**, Müjgan Yaz MD**, Nadir Paksoy MD****

SUMMARY: Kılıçarslann B, Yaz M, Paksoy N. (Department of Pathoiogy, Akdeniz University Faculty of Medicine, Antalya, Turkey). Congenital cystic adenomatoid malformation: report of two cases and review of the literature. Turk J Pediatr 1998; 40: 289-294.

Congenital cystic adenomatoid malformation (CCAM) of the lung is a rare pulmonary lesion, characterized by an excessive overgrowth of the terminal respiratory bronchioles. The lesion is almost always unilateral and may occur in any lobe. We present two children with CCAM. The first case was a one-day-old female infant admitted with respiratory distress and cyanosis. The second case was a 19-month-old girl with a nine-month history of recurrent respiratory infections. Preoperative diagnosis of both cases was intrapulmonary mass. The histopathological examinations revealed CCAM.
Key words: congenital cystic adenomatoid malformation, pulmonary malformation, lung.

The congenital cystic adenomatoid malformation (CCAM) is an unusual lesion combining the features of dysplastic overgrowth, hamartoma and/or even neoplasia. CCAM is caused by an overgrowth of terminal bronchioles at the expense of saccular spaces, resulting in an adenomatoid proliferation of bronchiolar elements and cystic formations. CCAM is primarily found in newborns or very young children, although cases have been described in adults. Generalized edema, respiratory distress, cyanosis and inspiratory retraction are present in most instances, and these complications may be severe enough to cause death. No racial or genetic predisposition has been noted[1]. CCAM is seen more commonly in males. Antenatal diagnosis has been reported[2-4]. CCAM was first described as a separate entity by Ch'in and Tang[5]. In this study, we present the histopathologic findings in two cases of CCAM (type 3 and type 1) of the lungs in newborn infants.

Case Reports

Case 1

The case was a one-day-old female, the product of a full-term pregnancy, born to a 23-year-old mother. In her pregnancy, polyhydramnios had developed without evidence of hydrops. The patient was admitted to the University of Akdeniz Teaching Hospital shortly after birth because of severe respiratory distress and cyanosis. The baby was noted as normal at birth and no sign of

* From the Department of Pathology, Akdeniz University Faculty of Medicine, Antalya.

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

*** Professor of Pathology, Kocaeli University Faculty of Medicine, İzmit.

respiratory distress was present. Her Apgar score was nine. On examination, symmetrical chest wall movement was noted. Breath sounds were decreased over the superior segment of the left lower lobe. Fine rales were noted over the basal segment of the left lower lobe. Peripheric cyanosis was present. The remainder of the physical examination was normal. At the time of hospital admission, the white blood cell count was 29,200/mm^3, sedimentation rate was 18 mm/h. Hemoglobin was 17.8 g/dl; a hematocrit of 53 percent and a platelet count of 127,000/mm^3 were measured. Arterial blood gas on room air was not known. Her body temperature was 36.5 °C. The patient's diagnosis was thought to be a diaphragmatic hernia. Chest radiograph was taken for differential diagnosis. It showed a dense consolidation, round in shape, in the left lower lobe, with compression of heart and surrounding normal lung. The patient was sent to the pediatric surgery department for operation.

During thoracotomy a large mass (8 x 6 cm) was found over the superior segment of the left lower lobe and this was dissected form the normal lung tissue.

Grossly, the round mass was covered by a smooth-to-finely-wrinkled grayish-pink pleura. It weighed 110 g and measured 6 x 5 x 3.5 cm. The cross-section showed gray-pink spongy homogeneous tissue. The solid lesion was composed of multiple, curved, branched structures that resembled immature or dysplastic bronchioles (Fig. 1).

Fig. 1: Case 1, cut surface of the mass showing spongy homogeneous appearance with multiple cleft-like structures.

Microscopically, the tissue consisted of numerous bronchiolar-like cysts, less than 0.3 cm in diameter, with the exception of scattered, larger, bronchiolar-like structures. Irregular, satellite-shaped, bronchiolar-like structures, lined by cuboidal or low-columnar epithelium, were surrounded by alveolar ductules and saccules lined by cuboidal epithelium (Fig. 2). The thin walls contained loose connective tissue with few smooth muscle and elastic fibers, confirmed by Elastica van Gieson's stain.

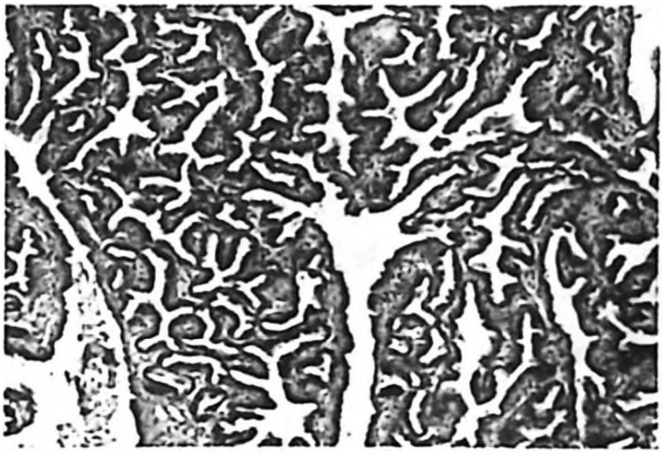

Fig. 2: Microphotograph from the same case showing irregular satellite-shaped, bronchiolar-like structures (hematoxylin and eosin; X10).

Case 2

Search of the files of our department revealed one other case of CCAM.

A 19-month-old girl was admitted to the University of Akdeniz Teaching Hospital with pneumonia. She had a nine-month history of recurrent respiratory infections. A chest x-ray showed a heterogeneous consolidation in the inferior segment of the left lung, with multiple tubular and linear lucencies and early pneumatocele formation. A thoracic computed tomography (CT) scan showed a multicystic lesion in the left lobe with compression of surrounding areas of the lung. Macrocystic lesions were composed of single or multiple cysts. The largest cyst measured 41 x 21 mm. During thoracotomy, an overinflated left lower lobe containing multiple cysts was found. A left lower lobectomy was performed.

Macroscopically, the mass was covered by a gray-to-pink pleura and measured 7.5 x 6 x 2 cm. Cross-sections showed larger cysts (1-3 cm in diameter) with smaller surrounding cysts. Microscopically, the larger cysts were lined with ciliated, pseudostratified columnar epithelium, and the smaller ones by cuboidal-to-columnar epithelium (Fig. 3). Mucus-

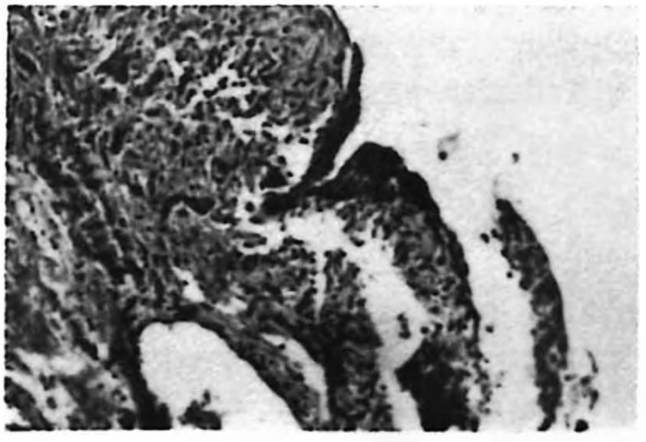

Fig. 3: Microphotograph from Case 2. The larger cyst is lined by pesudostratified columnar epithelium. It is surrounded by smaller cysts (hemotoxylin and eosin; X10)

producing cells were present in the epithelial lining of the larger cysts and in the bronchioles alveolar ductlike structures adjacent to the larger cysts. The walls of the cysts contained prominent smooth muscle, fibrous and elastic tissue.

Discussion

In 1993, Cloutier et al.[6] reviewed the literature and found 153 cases. Our updated search revealed an additional 42 cases of CCAM, excluding our two cases.

CCAM accounts for approximately 95 percent of congenital cystic lung diseases, and represents 25 percent of the congenital lung malformations[4,6]. This malformation is a cystic hamartomatous anomaly that may involve all or part of a lung. It consists of abnormal bronchiolar structures of varying size and distribution.

Embryologically, the origin of the conducting airways derived from endoderm, whereas the respiratory component stems from mesenchyme that concentrates around the tips of the bronchi[3].

Three major histological types of CCAM are described by Stocker et al.[7]. Histological criteria for its diagnosis include: Type 1 lesion is a large cystic or predominant cystic type. It accounts for nearly 65 percent of cases[1]. Single or multiple large cysts (3-10 cm in diameter) lined by ciliated pseudostratified columnar epithelium with numerous polypoid projections are surrounded by smaller cysts. The cysts' walls contain prominent smooth muscle, fibrous and elastic tissue and, occasionally, cartilage plates[1,6-9].

Type 2 lesion is a medium cystic type. It accounts for about 25 percent of cases[9]. Multiple distributed cysts (0.5-3 cm in diameter) are lined by cuboidal and columnar epithelium. The cysts are separated by thin, fibromuscular walls, elastic fibers, cartilage plates, and striated muscle fibers, but no mucogenic cells are expected[1,6-9].

Type 3 lesion is a small cystic or solid type, and occurs in 10 percent of cases[9]. Cysts are rarely over 0.2 cm in diameter, with the exception of scattered larger bronchiolar-like structures. They are composed of curved channels or cysts, lined by cuboidal epithelium. Their thin walls contain loose connective tissue with smooth muscle and elastic fibers[1,6-9].

Histological findings of our cases are consistent with type 3 CCAM and type 1 CCAM, respectively.

Prenatally, polyhydramnios is common. The etiology of polyhydramnios may be due to loss of protein from the lesion into the amniotic fluid[10]. Our first case manifested polyhydramnios. However, polyhydramnios is not a prognostic indicator[6], Fetal hydrops was caused by the malformation resulting in vena caval obstruction and cardiac compression. In our first case, the chest radiograph showed cardiac compression, which has been accepted as a prognostic indicator.

Clinically, CCAM is usually seen before the age of two years. CCAM occurs either in children less than six months of age with respiratory distress, including cyanosis and inspiratory retraction, or in older children with histories of chronic infectious respiratory distress. Our second case had recurrent lower repiratory infections. CCAM can occasionally be discovered by incidental chest radiograph[9]. If a CCAM is a huge lesion, it results in fetal cardiac compression, as in our first case who was admitted to hospital with respiratory distress and cardiac compression or fetal death.

Right and left lung are nearly equally involved and the lower lobes are affected in about 60 percent of the cases[9].

The diagnosis is often made from clinical and radiologic features. The definitive diagnosis is usually made by surgical resection of the mass and histological examination.

Clinically, differential diagnosis must be made extralobar sequestration, bronchogenic and enteric cysts, pulmonary abscesses, and mediastinal cystic teratoma[6,9]. However, these conditions do not cause acute problems in the newborn. Extralobar sequestration is discovered incidentally, or becomes a result of recurrent infections, especially in older patients. Presence of a CCAM or a sequestration is an indication for resection.

Type 1 lesion has a good prognosis, whereas type 2 lesion has a poorer outcome due to its more frequent association with other anomalies, which have been reported as predominately renal agenesis and cardiac anomalies. Anomalies are noted in 15 to 20 percent of all cases with CCAM[1,9]. Our cases had no other anomalies. Type 3 lesion has a high mortality due to its large size and association with maternal polyhydramnios and fetal anasarca. Our first patient's clinical condition was reported to be good after resection.

We think that the first case is interesting because CCAM does not usually present with respiratory distress in a one-day-old infant. In summary, CCAM is an uncommon congenital malformation. If infants less than six months of age present with respiratory distress, CCAM should be considered in differential diagnosis. In the differential diagnosis of recurrent respiratory infection, CCAM must be also considered.

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The Turkish Journal of Pediatrics 1998; 40: 295-297

LETTER to EDITOR

SOCIAL SCIENCE RESEARCHES AND COMMUNITY PEDIATRICS*

*Hilal Özcebe MD***

The population of Turkey is growing, people are moving from agrarian to industrial economies and everywhere social roles and social relationships are being redefined and transformed. To cope with these massive changes, we need to understand the social behavior that led to the new conditions and the implications of these changes for the future, especially in the field of health. Never has the need for the findings of social science been greater than in the past three decades.

The most important child health problems in the community are the ones which are the most prevalent, the most disabling and with the highest mortality. Some child health intervention programs must be carried out to decrease the prevalence of the most important diseases in the community. These intervention programs generally include primary prevention, secondary prevention and tertiary prevention. Primary prevention aims to prevent the diseases in the community, in other words primary prevention is conducted prior to the occurrence of diseases. Secondary prevention aims to diagnose the diseases before the clinical signs are seen and to provide early detection and treatment. Tertiary prevention includes therapy and rehabilitation programs. Information about the community, particularly about cultural structure necessary to carry out these child health prevention programs is necessary. Social behavior, social roles, and social relationships could show great variations even in a small community. Social science researches, which have great input in community pediatric interventions, could be used to learn details about the structure of the community.

Qualitative and quantitative researches are two different aproaches of social researches which can be conducted in the community by community pediatricians. The quantitative research that is carried out in the community mostly answers questions regarding the prevalence, the distribution, the cause and the resolution of the health problems. A questionnaire is usually designed based on the previous experience of the scientists and the questionnaires of the other researches in the community pediatrics. However, the questionnaire may not always give the real cause of the problem and sometimes the results of the research cannot be easily explained. Therefore, the recommendations drawn from the field survey to solve the problems may not be realistic at all times. For these reasons, it is recommended that community pediatricians use qualitative researches to describe the community prior to carrying out a quantitative survey[1,2].

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

** Associate Professor of Public Health, Hacettepe University Faculty of Medicine.

The type of data and the method of collecting the data of the qualitative researches are very diferent from that of the quantitative researches. In qualitative surveys, unstructured or semistructured interviewing can be used. Unstructured interviewing is the most widely used method of data collection in medical anthropology. The idea is to get individuals to open up and let them express themselves in their own terms, and at their own places. Semistructured interviewing is based on the use of an interview guide and has much of the freewheeling quality of unstructured interviewing[3, 4].

Focus group discussions and individual in-depth interviews are two leading qualitative research techniques. Focus groups capitalize on group dynamics and allow a small group of respondents to be guided by a skilled moderator into increasing levels of focus and depth on the key issues of the research topic. Individual in-depth interviews, like focus groups, are characterized by extensive probing and open-ended questions, but they are conducted on a one-to-one basis between the respondent and a highly skilled interviewer[3].

In pediatrics, there are several child health problems in the community. First of all, the most important one must be chosen to be solved. For example, diarrheal diseases are one of the important health problems in Turkey. Community pediatricians can study to learn the incidence of diarrhea, the distribution by age, sex or place, and the causes of the disease to conduct the prevention and control program in the community. They should also learn how much the mothers know about diarrhea and what they do when their children have diarrhea. They can prepare a questionnaire to learn the answers to these questions and analyze the results of the survey. However, they can design closed and open-ended questions in the questionnaire, and they can only analyze these results. Mothers may not answer the questions because they may feel that the questionnaire is examining their knowledge or because they cannot understand what the question is asking. If community pediatricians could conduct a qualitative research before their quantitative study, they will have many clues on the question studied. Focus groups can be helpful in the preliminary stages of survey planning by helping community pediatricians to ask the right questions or to word them in a more understandable manner. Focus groups can provide the reasons behind the response and the reasons related to the practices. Focus groups can also be used to probe in more depth about certain survey issues, for example, about why mothers who tried oral rehydration therapy (ORT) once are not reusing it or why mothers prefer not to breast-feed when their children have diarrhea.

In social scientist's thoughts, focus group discussions could be used as a preliminary step to aid in developing a quantitative study and a way to understand the results of a quantitative survey. There are so many social and cultural factors under child health diseases, it is essential to integrate qualitative researches in community pediatrics.

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BUDD – CHIARI SYNDROME

*Şinasi Özsoylu MD**

To the Editor

I read with enthusiasm Altuntaş et al.'s interesting case presentation entitled "Budd-Chiari Syndrome in a Child Secondary to Membranous Obstruction of the Hepatic Vein Treated by Percutaneous Transluminal Angiography" in the recent issue of the Journal (1997; 39: 551-555).

Since the authors could not find information on the subject in Turkish literature, I would like to bring to their attention that, a study involving one of the largest groups with Budd-Chiari syndrome was published by our group[1].

There were some interesting findings in the authors' case. Despite the most likely congenital nature of the membranous web between the hepatic vein and IVC, symptoms of the disease were noticed three weeks prior to the admission of this nine-year-old boy. The liver is usually enlarged in this syndrome but was found almost normal-sized, possibly due to the well-developed cirrhosis. Although intensive central venous congestion and hemorrhagic necrosis of the liver are generally expected in this syndrome, it was not found in the present case, again probably related to the late clinical presentation of the patient.

As a follow-up to the authors' case, it would also be interesting to observe whether the morphologic changes of the liver and the esophageal varices would improve in time, since the cause has been successfully eliminated.

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* Professor of Pediatrics and Hematology, Fatih University Faculty of Medicine, Ankara

REPLY

*Buket Altuntaş MD**

To the Editor

It was interesting to see the comments of Dr. Şinasi Özsoylu to our article on treatment of hepatic vein obstruction by balloon dilatation (Turk J Pediatr 1997; 39: 551-555). Dr. Özsoylu has drawn attention to his publication about Budd-Chiari Syndrome (BCS) in childhood. The noted article was not shown in the references of our article, as the main aim of our article was to treat BCS due to membranous obstruction of the hepatic vein by balloon angioplasty. We could not find any information on the treatment of BCS in childhood by balloon dilatation in the Turkish literature.

Although it is generally believed that membranous obstruction of the hepatic vein and inferior vena cava is caused by congenital malformation, the congenital anomaly theory cannot explain the late onset of the disease, thus inviting other theories (i.e. thrombosis and its sequelae)[1]. As the lesion is rarely observed symptomatically in children[2] and no thrombotic conditions were noticed in our patient, membranous obstruction was thought to be of congenital origin. It can be concluded that our patient may be asymptomatic until the collateral circulation becomes inadequate and decompensation takes place.

Ascites disappeared after the second day of the treatment and variceal size was reduced on follow-up. We did not need to plan a second liver biopsy because cirrhosis was well developed on the first biopsy.

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* Pediatrician and Pediatric Gastroenterologist, Gazi University Faculty of Medicine, Ankara

DEFECTIVE SERUM OPSONIZATION AND INFECTION

*Şinasi Özsoylu MD**

To the Editor

I read with interest Dr. İ. Tezcan et al.'s nice study entitled "Defective Serum Opsonization Activity in Children Aged 6-48 Months Having Acute Purulent Otitis Media" in the recent issue of the Journal (1997; 39: 453-457).

The authors showed defective serum opsonization activity in 13.7 percent of 51 patients with acute purulent otitis media compared to 2.9 percent in 103 healthy children. The authors neither mention the frequency of other infections in patients with AOM nor have another control with other bacterial infections. Thus, it is not easy to accept whether opsonization defect was the cause or the result of AOM. It is also hard to determine whether 5 in 23 children (which corresponds to 21.8% rather than 218% as written in the summary of the paper) have had recurrent otitis media due to defective opsonization or as a result of recurrences of infection, which were found decreased. If the defective opsonization was the result of recurrence of infection, "opsonic defect could not be considered as a contributing factor for recurrence of AOM".

Since opsonization defect was also shown in 20 percent of the patients with recurrent lower respiratory tract infection by Genç et al.[1], I would rather consider that it was most likely the result of the disease.

On this occasion may I draw attention to the method part of the paper. It was written, "In controls, 50 µl yeast particles made up to the same reaction volume of only saline were similarly incubated". To my understanding it should read, "In controls, 50 µl yeast particles made up to the same volume by 10 µl control serum (mixture of sera obtained from healthy people of AB blood group)...", instead of "only saline".

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* Professor of Pediatrics and Hematology, Fatih University Faculty of Medicine, Ankara.

REPLY

*İlhan Tezcan MD PhD**

To the Editor

Thanks to Dr. Özsoylu for his interest in our study entitled "Defective Serum Opsonization Activity in Children Aged 6-48 Months Having Acute Purulent Otitis Media" (Turk J Pediatr 1997; 39: 453-457).

This study consisted of the children presenting with the diagnosis of acute otitis media aged 6-48 months, when the antibody repertoire is relatively restricted. According to their medical histories, only two patients had recurrent urinary tract infections. The serum samples were obtained three weeks after the acute illness to exclude secondary opsonization defect. The results suggest that defective opsonization may play a role in susceptibility to recurrent otitis media. In baker's yeast opsonization assays, we use saline in the control tubes to count unphagocytosed yeast particles[1].

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

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