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LESS EQUAL THAN OTHERS

*Şinasi Özsoylu MD**

When I was reading "Letter from Bosnia" in the New England Journal of Medicine[1], I at once remembered an Editorial entitled" Less equal than others" published in the Lancet[2].

I used to believe in "human rights" for all people without consideration of their color, nationality, race, sex, religion, poorness or richness etc. However, I am almost losing my faith in human rights and the United Nations idea because of the indifference of the world towards people's lives, more specifically children's lives, in some parts of the world such as Bosnia Herzegovina, Caucasia, etc. The European champions of human rights used to draw attention to the condition of people in prison in developing countries, suggesting some standards according to European opinions[2]. However, I don't hear their voices being raised for the largest prison in Europe, Bosnia Herzegovina.

UNICEF being most sensitive about children's condition, also has not been active is saving children's lives on both sides of Bosnia Herzegovina. Poor and less organized communities cannot speak up adequately to gain attention from world organizations, but they are the people who require the most help. Thus we may soon lose our faith in these international organizations completely.

I am urging educated people, more specifically doctors, all over the world to raise their voices for the sake of poor and disorganized people, and in this way to show their sensitivity to human life, which is the most essential of human rights.

I am more concerned about medical people's reactions since they do their best every day to save lives even in hopeless situations. On this occasion, healthy people who cannot be held responsible for this senseless war are being killed in the middle of Europe. Are those people less equal than the rest?

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CHILDREN IN WAR

*Murat Yurdakök MD**

James P. Grant, Executive Director of UNICEF, mentioned the main problems of children in war in his 1994 Report. We can summarize the special section in this report as follows:

"In the 1990s, the world stands at a critical juncture for the cause of protecting children in war. In the last decade alone, an estimated 1.5 million children have been killed in armed conflicts. A further 4 million have become disabled. At least 5 million have become refugees, and 12 million more have been uprooted from their communities. Much larger numbers have seen their health, nutrition and education suffer as conflicts have destroyed crops, infrastructure, clinics, schools.

More recently, the rape of girls has been used as a systematic weapon of war in former Yugoslavia. And in many parts of the world, children have been tortured and forced to watch or participate in atrocities. Hundreds of thousands have been recruited into armies, given drugs and weapons.

In the face of these tragic events, for the protection of children in armed conflicts, a declaration signed by the world's political leaders at the 1990 World Summit for Children and after that the Convention on the Rights of Children has been ratified by some 150 governments. But progress in practice can only be maintained by a loud and insistent public demand for action".

We hear some good news for the protection of children in war from different parts of the world. Professor İhsan DOĞRAMACI, Observer Delegate from Turkey to the UNICEF Executive Board, made an important statement in New York in May 1994:

"Madame Chairperson, as mentioned in the Executive Director's report, Central and Eastern Europe continue to be areas of major concern, throughout 1993, and unfortunately, up to the present time, with the tragedy in the former Yugoslavia dominating world attention and creating Europe's biggest political, military and humanitarian crisis since the Second World War. The invasion of Gorazde is the most recent of the tragedies happening before our eyes.

From Afghanistan to Bosnia, we are facing outdated nationalism, irredentism and ethnic tensions fueling animosity and hatred among peoples. Xenophobic tendencies have emerged in Europe and elsewhere that run counter to the tolerance and understanding we are trying to instill in the world order. We witness

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expansionist movements motivated by sheer greed for land without regard for the misery and suffering of peoples.

The impact of this conflict on children has been devasting, with thousands killed, displaced or under siege. We ask the international community to be more active in stopping these tragedies and to bring justice for the benefit of the children, those who suffer most".

The number of war victims in Bosnia-Herzegovina is too high. Nearly 7% of the total population living in this country has killed or wounded. According to the official numbers provided by the Bosnia-Herzegovina Embassy in Ankara, 16,652 children have been killed, have died of undernourishment and cold or are missing (11.6% of 143,305 total deaths), 34,197 children have been wounded (20.7% of 164,884 total) and 18,279 children have been seriously wounded requiring hospital treatment (24.8% of 73,589 total) (Source: Health consequences of the aggression on the Republic of Bosnia-Herzegovina. The Bulletin of Committee for Health and Social Security, Republic Institute of Public Health No: 106, 2 April 1994).

Recently, the deans of medical faculties in Ankara prepared a declaration to give to the leaders of the world:

"A conflict which is trampling all the rules of the Geneva Convention is taking place in the heart of Europe for all to see before the eyes of the whole human race. An important part of this conflict has maimed, raped and stolen the most sacred of all human rights –the right to live– from hundreds of thousands of civilians, bombed hospitals, obstructed medical and social emergency assistance and forced people to leave their homes. No one shall escape from the moral and historic responsibility by remaining onlookers to this human drama any longer.

We the undersigned, as representatives of our institutions, call on all international organizations, and above all the United Nations, to bring this meaningless conflict to an end with all possible speed".

We hope to raise the voice of the medical authorities from different parts of the world without losing time to stop the tragedies of children in war.

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DEVELOPMENT AND GROWTH IN AN ATMOSPHERE OF PEACE

*Mithat Çoruh MD**

Our age is the age of globalism, where the fast development of communication systems has led to cooperation between nations, especially in the fields of commerce and industry. In parallel, regionalism has increased as neighboring countries are uniting in the struggle for economic influence over other countries. This has led to the marginalism of countries due to their political, racial or religious backgrounds, affecting these nations' inner peace and socioeconomic stability.

Children are the most perceptive group and they are deeply affected by the new situations which concern most of the countries in the world. They need a healthy environment in which to develop their knowledge and skills, and they need solidarity to produce solutions to problems.

In developed countries, most of the children's health problems have been solved, and they live and develop in an atmosphere of stable socioeconomic conditions; however, in less developed countries the children are faced with problems such as finding food, developing immunity and finding a warm house, while living in an atmosphere of fluctuating socioeconomic conditions during their development.

Since the only possible way to rectify the socioeconomic structure is to have a highly educated human force, the need to develop good communications with other nations cannot be denied. This should be made obligatory in the development and education of youth. Regardless of their social, religious, racial or economic backgrounds, the children of the world can be educated towards the same purpose in many fields, particularly environmental consciousness, social justice principles, independent world communities, living in peace and developing tolerance for other cultures.

In addition to the changes that should be made in schools' curricula, the youth groups, scouting institutions, career education institutions and associations should in their educational activities give importance to teaching the youth that the only route to healthy development is to discuss differences with each other and to try to understand each other instead of fighting.

The universities are a source of development because they produce, distribute and use knowledge. For this reason they can be used to make changes in the elementary and high school curricula, and they can develop new methods for

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effective education. Also, cooperation between universities will enable such projects to be carried out, developed and distributed.

Children are the sensitive group of a population who are deeply affected by socioeconomic struggles. They need special attention through programs and activities which can serve to protect them.

The people of the world can share similar perspectives, behaviors and attitudes only by knowing each other and by cooperating in shared situations. In order to secure the peaceful development of our world, UNICEF launched an education program called "Developmental Education" in 1990. Actually UNICEF had been applying a program designed for the children of developed countries in 1960 and these programs were run by the UNICEF National Committees in related countries. In 1989 a program called "Education For Peace" was launched in Lebanon where junior high, and high school students were put in summer camps, and with the use of activities like folklore and sports they were shown the possibility of living together in peace despite their racial, religious and denominational differences. Later similar programs were run in Sri Lanka and the Philippines with similar success. The globalism which has started in the fields of industry and commerce could be spread to social, cultural and political areas, and the founding hypothesis in this approach of economic growth and development of quality of life is education.

If people around the world gain access to issues such as social justice, environmental consciousness, human rights, rights of children, health, nutrition, education, conflicts and conflict resolution, the interdependence between nations and the necessity of living together in similar conceptual approaches, it will then become possible to engage in dialogues and develop solutions for peace. In order to attain these objectives, unity in Public Education programs is called for. The elementary and middle school education programs have to be revised in accordance with the postulate which, simply stated, is the fact that today's youth are tomorrow's administrators and that they should be prepared accordingly. In order to form tolerance with divergent cultural differences and to analyze human behavior among different cultures, workshops should be organized, thus leading to areas of discussion, enabling children of different cultures to contact and understand each other.

As generally known, every April Turkey hosts the children of various countries where cultural activities are performed. International associations around the world should encourage related activities and have the same consciousness when organizing international youth meetings.

Municipalities are good resources for bringing together youth to address issues related to the environment and citizenship. If the activities are well-organized and

offered on a regular basis, then children can learn democratical behaviors at an early age; also the new generation can develop the ability to work together, help and support each other and breed ideas.

Pediatricians are people whose ideas are given importance and respect by families. They can have great influence on social, psychological and intellectual development in addition to physical development of our children. They can play an important role as educators in the augmentation of the development of constructively participating, competitive risk-taking, determined and enterprising youth. For it seems that in the twenty-first century, the conficts will take place between people rather than nations. In an intensifying significance, the security of a person is gaining more importance than the security of nations. Since a more independent and democratic society can only be obtained through the determination and the consciousness of that society, there is a great deal of work for parents, teachers, pediatricians and administrators to do to prepare our youth accordingly.

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THE EPIDEMIOLOGY OF JUVENILE-ONSET INSULIN-DEPENDENT DIABETES MELLITUS IN TURKISH CHILDREN

A Retrospective Analysis of 477 Cases*

*Nurgün Kandemir MD**, Esin Açıkgöz MD***, Nurşen Yordam MD****

SUMMARY: Kandemir N, Açıkgöz E, Yordam N. (Endocrinology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). The epidemiology of juvenile-onset insulin-dependent diabetes mellitus in Turkish children. A retrospective analysis of 477 cases. Turk J Pediatr 1994; 36: 191-195.

The hospital records of 477 patients under 18 years of age with insulin-dependent diabetes mellitus (IDDM) who were followed in the Endocrinology Unit of Hacettepe University Children's Hospital between the years 1969 and 1991 were analyzed for age, sex, residence at time of diagnosis, date of onset of symptoms, date of diagnosis, family history of IDDM, and consanguinity between parents. The distribution of age at diagnosis showed a small peak between 4 and 6 years of age and a main peak between 12 and 14 years. In girls, the main peak appeared between 10 and 12 years, and in boys between 12 and 14. A significant difference was not seen between sexes (239 males and 236 females). The frequency of diagnosis showed seasonal variations the lowest in autumn and the highest in winter. Consanguinity between parents was 23.9 percent, and 10.3 percent of the patients had IDDM in first degree relatives. *Key words: diabetes, juvenile, epidemiology, Turkey.*

Diabetes mellitus is a common chronic disease in childhood. The etiology of this disease, which may lead to serious complications and early death, is still unknown. Epidemiologic and experimental evidence suggests that non-inheritable environmental factors may be causal in the genetically susceptible individual. Therefore, the epidemiology of insulin-dependent diabetes mellitus (IDDM) has been subjected to intense research world wide during the last 10 to 20 years.

In previous studies, it was reported that the onset of the disease occurred most frequently between 10 and 14 years of age, and a small peak of incidence could be seen between 5 and 7 years of age[1-4].

Investigations that have reported on the seasonal variation in the incidence of IDDM indicate that the greatest frequency occurs in autumn and winter, and the least in summer[4-7]. Ethnic differences and geographically diverse environments

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are likely to give rise to a spectrum of the diabetes mechanisms and manifestations. Consequently, the epidemiologic and familial characteristics of IDDM are described below in 477 patients under 18 years of age who were followed in the Endocrinology Unit of Hacettepe University Children's Hospital.

Material and Methods

All patients with IDDM followed in the Endocrinology Unit of Hacettepe University Children's Hospital between the years 1969 and 1991 were included in the study.

The study was limited to children under 18 years of age who required insulin. The small number of children presenting with abnormalities in glucose tolerance tests who could be managed with diet alone or diet plus oral hypoglycemic agents were not included in the study.

Date of birth, sex, residence at time of onset, date of onset of symptoms and diagnosis, associated infections, family history of IDDM and consanguinity between parents were determined from the hospital records of 477 children.

Results

The age at onset of IDDM among the patients ranged from 13 months to 18 years. The mean age was 9.52 ± 3.99 years.

The distribution of age at diagnosis (Fig. 1) showed a main peak between 12 and 14 years. The 12-14 year peak deviates significantly from the average ($p < 0.05$). In

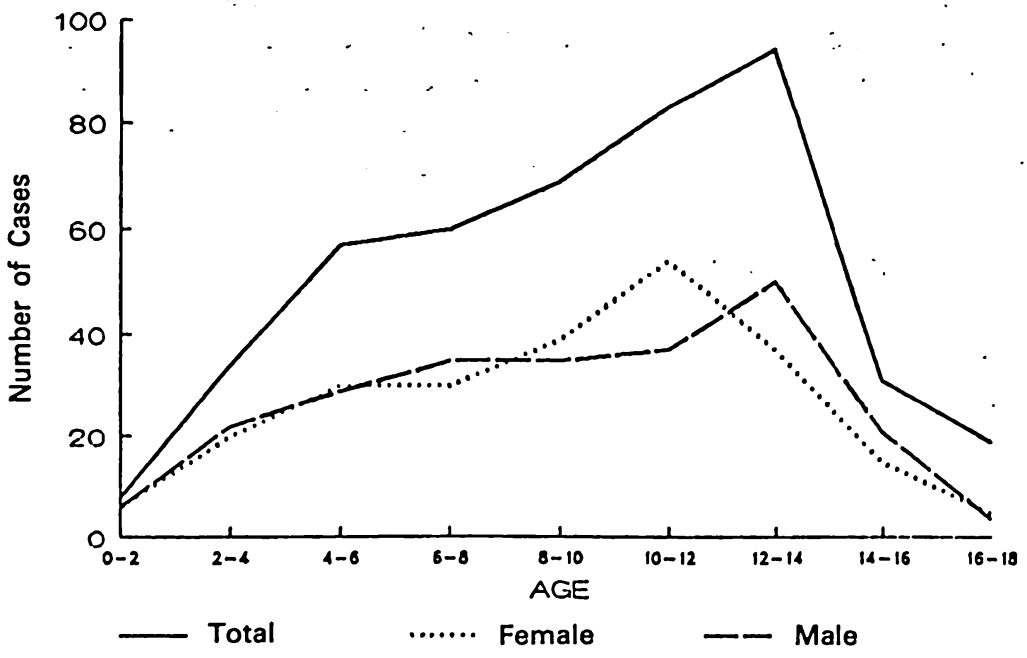

Fig. 1: Age at diagnosis of 477 juvenile-onset insulin-dependent diabetic patients (239 males and 236 females).

girls, the main peak appeared between 10 and 12 years, and in boys between 12 and 14. A significant difference in numbers of patients was not seen between sexes (239 males and 236 females).

In 70.2 percent of the patients (335 cases), the time period was one month from the beginning of symptoms until the diagnosis, and it was longer than one month in 29.8 percent (142 cases).

The data in the present study were plotted according to the month of diagnosis for evaluation of the seasonality of the disease (Fig. 2). The frequency of diagnosis showed seasonal variations, with the lowest frequency occurring in autumn (17.1%) and the highest in winter (40.1%) ($p<0.05$). In 44.9 percent of the patients, associated infections occurred at the time of diagnosis (19% upper respiratory infections, 8.8% urinary tract infections, 4% lower respiratory infections and 13.1% other infections). There was a higher incidence of associated infections in the 0 to 6 years age-group (51.3%) than in the 6 to 12 and 12 to 18 years age-groups (35.0% and 33.3%, respectively).

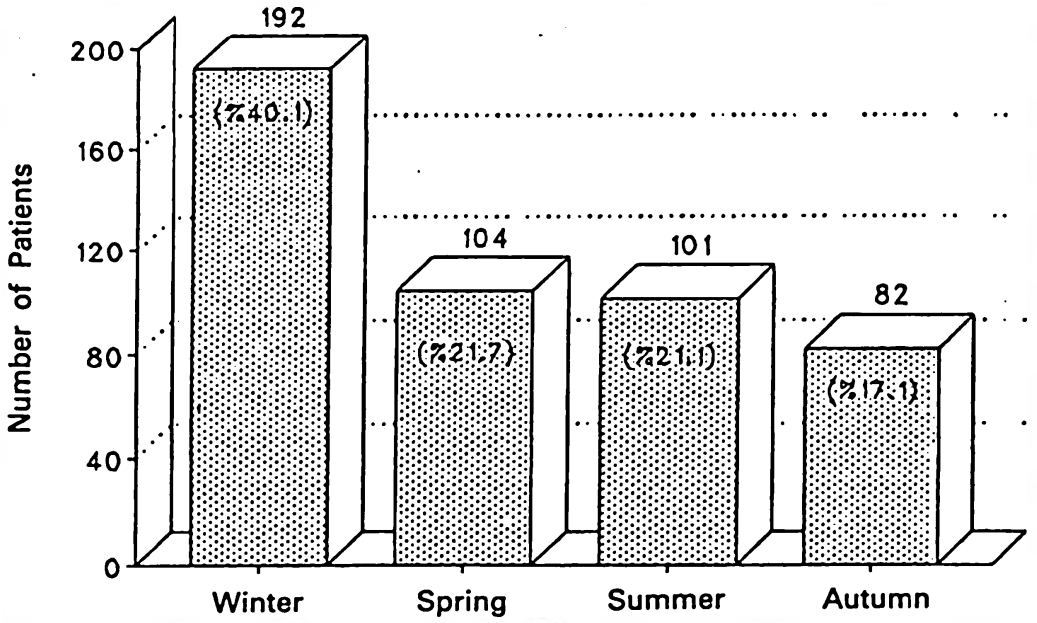

Fig. 2: Seasonal variation in the diagnosis of juvenile-onset insulin-dependent diabetes mellitus in Turkey (1969-1991).

Consanguinity between parents was present in 23.9% of the patients, and 10.3% of the patients had type I diabetes mellitus in first degree relatives.

The geographic distribution of patients was evaluated according to the seven geographic regions in Turkey. The patients were admitted mostly from the Central Anatolia region (66.2%), but all other regions were represented in small numbers: 11.0% Black Sea region, 5.9% Marmara region, 4.4% Aegean region, 7.0% Mediterranean region, 4.0% East Anatolia region and 1.5% Southeast Anatolia.

Discussion

This study was done retrospectively from a review of hospital records of insulin-dependent diabetics over a 22-year period including patients from all regions of Turkey.

The incidence of IDDM varies with age. Bloom[3] reported an initial slight peak in five-year-old children, suggesting the role of starting primary school for this age-group since major enviromental changes occur at this stage of childhood. In our country, the age for starting primary school is between 6 and 7 years; however, in this study a marked peak was not observed in this age-group, suggesting that there is probably no association between starting school and the onset of diabetes. Patients 0 to 6 years of age had a higher incidence of infections at the time of diagnosis. The influence of infections as an aggravating factor in early or established diabetes is conceivable, in that infection may act as a nonspecific stress that may precipitate clinical diabetes in an individual with latent or chemical diabetes.

The mean peak in incidence was between 12 and 14 years of age, and earlier for females than for males (10-12 and 12-14 years, respectively). A similar finding was reported from Denmark, Montreal and Chile, suggesting the role of puberty and the growth spurt[2,4,7]. However, since an objective assessment of pubertal status at the onset of symptoms is lacking in all studies to date, including this one, it cannot be completely ruled out that hormonal changes in the prepubertal years and psychological stresses are of importance in relation to the onset of diabetes.

The seasonality of the onset of disease has been shown in large surveys[4-7], with the highest frequency occurring in winter and the lowest in summer. In our study, while we found a similarly higher incidence in winter, we did not find a lower incidence in summer. The high incidence of viral infections such as gastroenteritis and hepatitis during the summer months in our country may play a role. Also, when considering the month of diagnosis rather than the month of onset of symptoms, small differences can be seen in the seasonality of the disease (Fig. 2).

The prevalence of juvenile-onset diabetes among families of diabetics is high. Oakley et al[8] found that 13 percent of children had a history of diabetes in a first-degree relative, a finding which is similar to Bloom et al's[3] estimation (11%). In our study, 10.3 percent of the patients had a first-degree relative receiving insulin treatment. Consanguinity between the parents of diabetics (23.9%) was not significantly different from that of the general population (21.6%)[9].

The results of this study can shed light on the epidemiological and familial characteristics of juvenile-onset IDDM in Turkish children, because of the limited data published to date on the Turkish population.

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SERUM IMMUNOGLOBULIN G SUBCLASS VALUES IN HEALTHY TURKISH CHILDREN AND ADULTS*

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SUMMARY: Berkel Aİ, Tezcan İ, Ersoy F, Sanal Ö. (Immunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Serum immunoglobulin G subclass values in healthy Turkish children and adults. Turk J Pediatr 1994; 36: 197-204.

Immunoglobulin G (IgG) subclass levels were determined in 393 serum samples of 329 healthy children from birth to 16 years of age, 24 healthy adults and 20 pairs of mother-cord blood by radial immunodiffusion. Age-normal percentile values for IgG1, IgG2 and IgG3 were calculated for age-groups up to 16 years and for adults. The broad range of IgG4 values in children did not permit calculation of reference values. *Key words: IgG subclasses, Immunoglobulins.*

Immunoglobulin G (IgG) subclass deficiencies have been associated with a variety of disorders including primary immunodeficiencies, recurrent respiratory tract infections and pyogenic infections, allergic disorders, autoimmune disorders, intractable epilepsy, acquired immunodeficiency syndrome and various isolated clinical conditions[1-8]. Deficiencies of one or more IgG subclasses with normal levels of serum IgG, IgM and IgA have been defined in children with recurrent infections, and some have been treated successfully with intravenous immunoglobulins[6,9,10]. IgG subclass deficiencies are the most common of humoral immunodeficiencies[11]. In recent years, since the availability of monoclonal antisera, the measurement of IgG subclasses has been included in diagnostic tests in clinical medicine. However, use of different laboratory techniques, variability of age-normal values and differences in IgG subclass values among populations have been the main concerns of physicians interpreting the results. Therefore, determination of age-normal values for different ethnic groups is considered mandatory in the evaluation of IgG subclass deficiencies.

In this study, IgG subclass levels were determined by radial immunodiffusion in healthy adults, pairs of mother and cord serum, and healthy children from birth to 16 years of age in order to establish age-normal reference values. The data obtained were used to construct age-normal percentile charts.

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Material and Methods

Serum specimens from 329 clinically healthy children (176 males, 153 females) from birth to 16 years of age with histories negative for allergy, recurrent infections and immunodeficiency in the family were examined. The patients had either been admitted to the hospital for minor surgery or had attended local health clinics. Informed consent was obtained from the parents. Twenty-four healthy adults (medical students or lab technicians) ranging in age from 18 to 34 years, the sera from 20 mothers and the cord blood of their babies were also included in the study.

Peripheral venous blood was drawn; serum was separated by centrifugation and then stored in aliquots at −20°C until use.

IgG subclass levels were determined by radial immunodiffusion using commercial kits from Binding Site Limited (University of Birmingham Research Institute, Birmingham, England). Samples to be tested were diluted 1:10 for IgG1 and IgG2; undiluted serum was used for IgG3 and IgG4. Plates were left at room temperature (22°C) for 72 hours and then read with an RID reader which can measure 0.1 mm fractions. Results were interpolated from a standard curve constructed from a pool of normal sera calibrated against WHO reference serum (67/97). When necessary, the subclass determination was repeated using low-level plates.

Total IgG, IgM and IgA levels were measured by turbitometry using the Behring Turbitimer (Behringwerke AG, Marburg, Germany). Only sera with normal levels of IgG, IgM and IgA for age were included in this study.

Statistical analysis: different statistical approaches were used to analyze the data of the children and adults.

For children, the second-order polynomial equation method was applied to the log 10 transformed data[12]. This method places the data on an age-normal subclass level curve excluding the first two months. Mean and ±3 SD curves were constructed for IgG1, IgG2 and IgG3. IgG4 values were not included because of the wide variations observed.

In the adults' data, there was no variation with age for any of the four subclasses.

Results

The numbers included in each age-group, including geometric means and ranges of the IgG1, IgG2 and IgG3 levels, are reported in Table I. Individual data, mean and ±3 SD curves for the three IgG subclasses are shown in Fig. 1. IgG subclass values for mothers and cord blood are shown in Fig. 2. Serum IgG1, IgG2 and IgG3 levels all tended to increase with age. However, the pattern of the curves differed for each subclass.

Table I: Serum IgG Subclass Values (g/L)
(Ranges for the Age-Groups are Given in Parenthesis)

Age	Number of Subjects	IgG1	IgG2	IgG3
0-30 days	12	9.25 (7.42-12.60)	1.83 (0.80-2.93)	0.57 (0.42-0.75)
31-60 days	12	3.05 (1.85-4.21)	1.11 (0.45-2.16)	0.32 (0.21-0.53)
3-5 months	12	3.42 (2.20-7.15)	0.62 (0.40-1.40)	0.44 (0.17-0.80)
6-8 months	12	3.81 (1.51-7.69)	0.62 (0.25-1.20)	0.48 (0.20-1.44)
9-12 months	16	4.02 (2.44-5.50)	0.46 (0.19-0.90)	0.46 (0.28-0.56)
13-24 months	23	6.45 (4.66-11.30)	0.93 (0.40-1.70)	0.60 (0.33-1.01)
25-36 months	24	7.16 (4.50-11.10)	1.10 (0.43-2.41)	0.55 (0.37-1.20)
37-48 months	24	7.97 (5.13-15.50)	1.37 (0.43-2.70)	0.60 (0.26-1.14)
49-72 months	40	7.40 (4.21-13.60)	1.61 (0.80-3.60)	0.56 (0.21-1.14)
7-8 years	40	7.68 (4.10-11.80)	1.91 (0.80-5.70)	0.78 (0.33-1.83)
9-10 years	30	8.81 (5.13-19.80)	2.38 (1.40-4.30)	0.86 (0.37-1.53)
11-12 years	24	10.26 (6.36-19.40)	2.16 (0.95-3.30)	0.89 (0.26-2.07)
13-14 years	30	8.00 (4.66-14.00)	2.39 (1.00-5.20)	0.86 (0.49-2.00)
15-16 years	30	8.98 (5.90-14.00)	2.33 (0.77-5.20)	0.93 (0.36-2.00)
Adults	24	.6.62 (4.10-12.30)	3.77 (2.16-5.90)	0.70 (0.16-1.38)
Cord serum	20	10.12 (6.10-14.00)	2.53 (1.26-4.39)	0.91 (0.39-1.70)
Mothers' serum	20	7.10 (4.66-9.72)	2.83 (1.26-6.26)	1.05 (0.58-1.82)

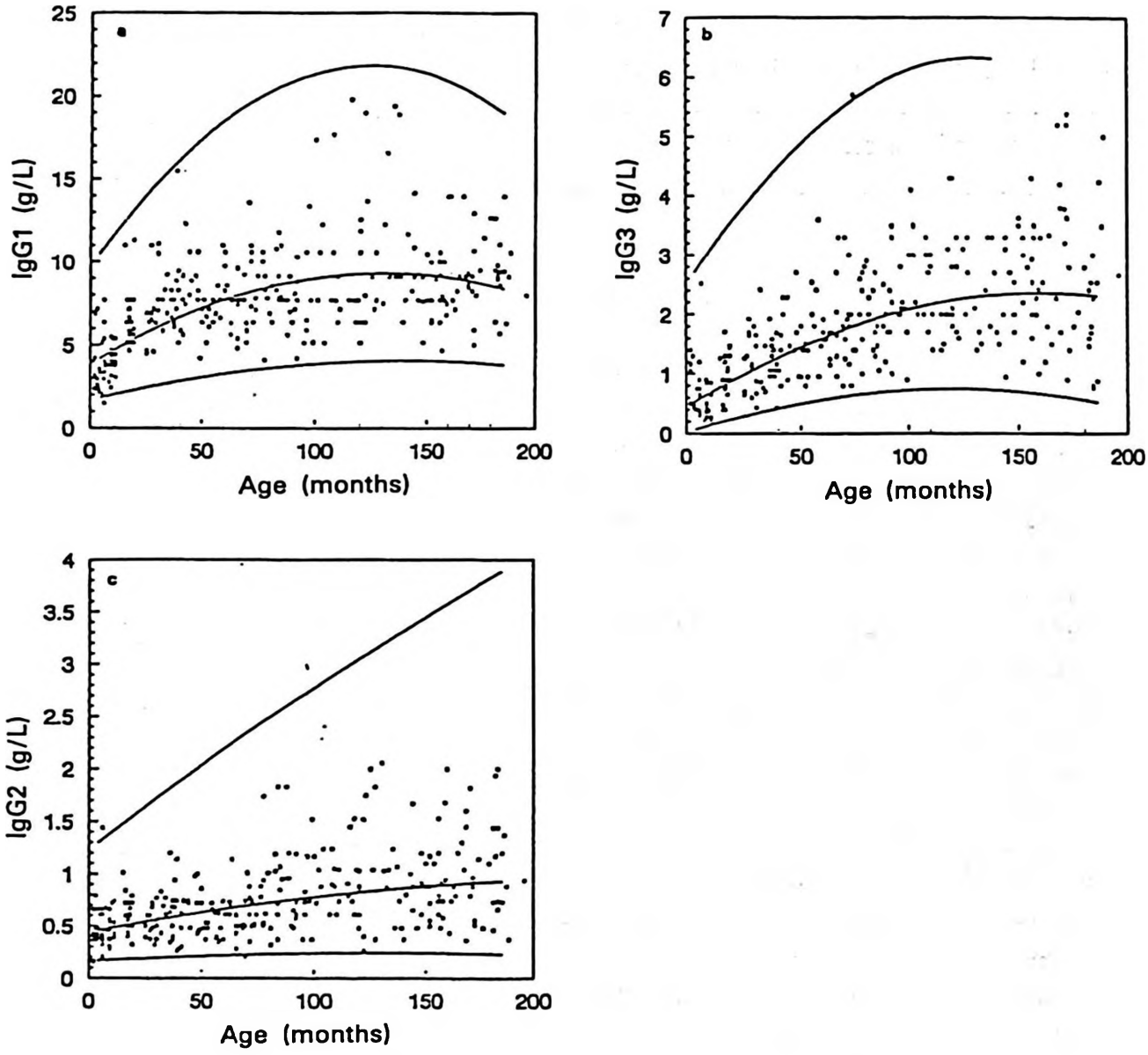

Fig. 1: Scatter diagrams of IgG subclass levels (Mean and ± 3 SD curves)
a) IgG1. b) IgG2. c) IgG3.

IgG1 levels increased gradually, peaked at approximately 12 years of age and decreased slightly thereafter. The values for adolescents were higher than those for adults. IgG1 levels in cord sera were higher than those of the mothers. IgG2 levels also increased with age, peaking at ten years of age, but reached only 67 percent of the adult levels at the age of 16. IgG2 levels of cord sera were the same or slightly lower than the mothers' serum levels. IgG3 levels increased until 12 years of age and reached a plateau thereafter. Adults had lower IgG3 levels than did adolescents. IgG3 levels were similar in cord and mothers' sera. IgG4 levels showed a non-homogeneous distribution until the age of 16 years;

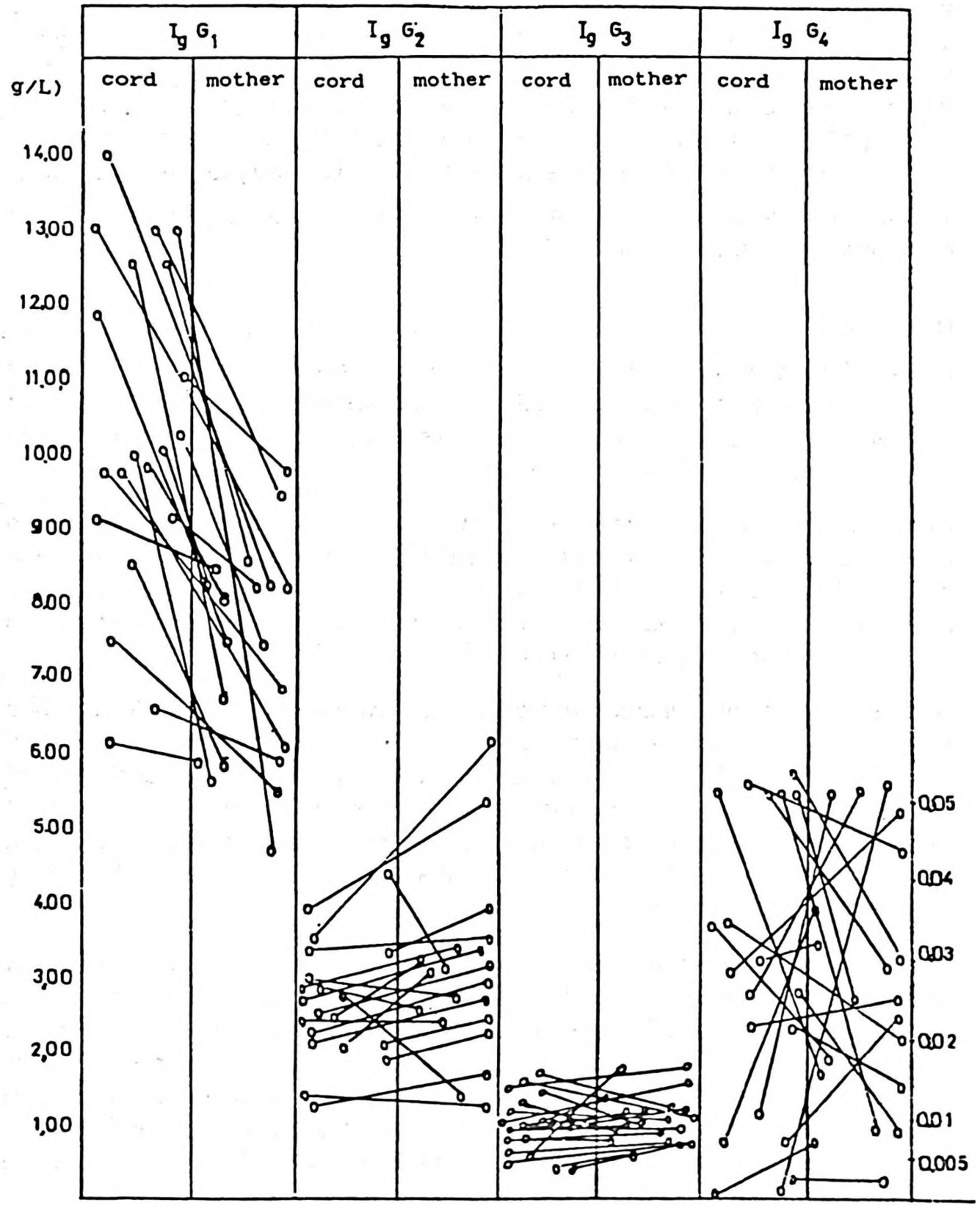

Fig. 2: IgG subclass values in cord and mother sera.

therefore, normal ranges for IgG4 could not be established. IgG4 values were very low (undetectable or <0.005 g/L) in 38 percent, greater than 0.05 g/L (above the upper reading range of IgG4 plate) in 24 percent, and between 0.005-0.05 g/L in the remaining 38 percent of tested sera. Twenty percent of adults had very low values (<0.005 g/L). In half of the cord sera, the IgG4 levels were higher than those of the mother, and in three samples the level was very low (<0.005 g/L).

The sum of the concentrations of the four subclasses corresponded with the concentration of total IgG (r: 0.7131).

Discussion

IgG subclass levels in healthy children and adults have been studied by various investigators. In some studies monoclonal antibodies were used[13-17], while other studies utilized polyclonal antisera[18-22]. In this study, IgG1, IgG2 and IgG3 levels were assessed using polyclonal antibodies.

During early childhood, serum IgG1 and IgG3 concentrations are the first to reach adult levels, with IgG2 and IgG4 maturation lagging behind[17,19,20]. We found higher IgG1, IgG2 and IgG3 (mean and-3 SD) values than were reported previously, except for IgG2 levels which were lower than many reported series in patients under the age of one year[7,9,15,17,19,20,23,24].

All IgG subclass levels increased progressively with age. IgG1 and IgG3 levels peaked at 12 years of age, while IgG2 peaked at approximately 16 years of age. However, IgG2 levels were approximately two-thirds of adult levels at the age of 16 years. IgG4 levels varied so widely that age-normal reference ranges could not be established. This finding is in accord with other studies where IgG4 levels were low in 10-20 percent of healthy adults and 8-21 percent of healthy children[14,16,17,19].

The mechanisms that regulate IgG subclass ontogeny are not completely understood. Regulation of IgG subclass concentrations may occur at many levels, such as B-cell subsets, T-cell subsets, lymphokine production or lymphokine receptor expression[13]. The correlation of IgG subclass levels with Gm factors supports the role of genetic factors[23,25,26]. Other potential factors for IgG subclass levels are differences in socioeconomic status, nutrition and environmental exposures in different populations or ethnic groups[13]. Constant exposure to antigens may result in higher IgG subclass levels[13,27]. We feel that the higher IgG1, IgG2 and IgG3 levels observed in our study may be a result of early or constant exposure to antigens in our society. The results of our study show that normal values are widely scattered in childhood, and the use of well-defined age-normal limits is crucial for the correct definition of true subclass deficiencies.

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EFFECT OF COPPER ON LIVER AND BONE METABOLISM IN MALNUTRITION*

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SUMMARY: Güler AH, Sapan N, Ediz B, Genç Z, Özkan K. (Departments of Biochemistry, Pediatrics and Medical Biology, Uludağ University Faculty of Medicine, Görükle, Bursa, Turkey) Effect of copper on liver and bone metabolism in malnutrition. Turk J Pediatr 1994; 36: 205-213.

This study was planned to investigate the effects of copper (Cu) deficiency on liver and bone metabolism in malnourished children. Serum total calcium (Ca), inorganic phosphorus (P), Ca/P, Cu/Ca, Cu/P ratios and alkaline phosphatase (ALP) activity values were analyzed. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) enzyme activities and the ALT/AST (De Ritis) ratio as well as their correlations with Cu were tested to determine liver function.

The results of the study showed that Cu deficiency directly affects the organic matrix formation, and by the suppression of ALP activity, indirectly causes decalcification. In the liver, however, no direct effect of Cu deficiency was seen. Deterioration in liver function and Cu deficiency increased parallel with the severity of malnutrition. Thus we concluded that a correlation exists between Cu and the parameters that indicate liver function. *Key words: malnutrition, bone, liver, Cu-deficiency.*

Copper (Cu) is an essential trace element found in the structure of numerous enzymes[1]. In addition, Cu can affect many enzymes' activities and related metabolic processes indirectly, even if it is not a structural component. Therefore, Cu deficiency causes deterioration of many metabolic processes and results in numerous clinical symptoms. In protein-energy malnutrition (PEM), Cu deficiency is seen almost characteristically[2,3]. Thus, in this study we investigated mild and moderate malnutrition cases and evaluated in particular the effects of Cu

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deficiency on liver and bone metabolism as follows: a) determination of serum Cu, calcium (Ca) and phosphorus (P) levels, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phoshatase (ALP) and gamma glutamyl transferase (GGT) activities, and comparison of them with normal, healthy children's values; b) investigation of the correlation between these parameters and serum Cu; c) establishment of the alterations in the Cu/Ca, Ca/P, Cu/P and ALT/AST (De Ritis) ratios in the patient groups; and d) correlations of these ratios with serum Cu levels.

Material and Methods

This study was performed on malnourished children classified into two groups according to Gomez's criteria[4]: the first group comprised cases of mild or first degree malnutrition; the second group comprised children with moderate or second degree malnutrition; and the control groups for each comprised 25 children. The children's serum Cu was measured with an atomic absorption spectrophotometry (AAS) apparatus with graphite tube[5,6]. Ca was measured with the classical complexometry, and P determination was made with Biotrol/A 02478, phosphorus UV kit. AST, ALT and GGT activities were measured with Biotrol A 03011 AST/TGO, Biotrol A 03021 ALT/TGP and Biotrol A 03026 monoreactive kits, respectively. In the determination of ALP activity, coulter's optimized kinetic method (Reagent Ref. No. 9866357) was applied. Statistical analyses of all obtained data were done on an IBM PS/2 Model 30 (UK) computer.

Results

Serum mean Ca and Cu levels were significantly ($p<0.001$) lower while P was higher ($p<0.001$) in the patient groups than in controls (Table I). Although they

Table I: Serum Ca, P, Cu Levels in the Malnutrition Groups and Comparison with the Control Group's Means

	Groups					
	Control (n=25)	1. Group (n=25)		2. Group (n=25)		
Measured Parameters	$\bar{X}\pm$SEM	$\bar{X}\pm$SEM	P	$\bar{X}\pm$SEM	P	P'
Ca (mg/dl)	10.43±0.08	9.42±0.06	<0.001	8.92±0.07	<0.001	<0.001
P (mg/dl)	3.89±0.10	4.45±0.05	<0.001	5.28±0.05	<0.001	<0.001
Cu (µg/dl)	118.35±0.97	71.84±0.70	<0.001	64.84±0.81	<0.001	<0.001

P: Statistical significance of the difference between the patient and control groups.
P': Statistical significance of the difference between the first and second groups.

were in the normal ranges, the patient groups' ALT and AST values were higher then those of controls and GGT was also increased, particulary in group 2 ($p<0.001$). ALP activities were significantly ($p<0.001$) lower in the patient groups than in controls. However, we could not establish any significant difference between the patient groups (Table II). Besides the "De Ritis" ratio, the other ratios (Cu/Ca, Ca/P and Cu/P) decreased in the first and second groups, with the lower ratios found in group 2. On the other hand, the ALT/AST ratio was higher in the second group (Table III).

Table II: Serum Mean ALT, AST, GGT and ALP Activities in the Patient Groups and Comparison with the Control Group's Means

	Groups					
	Control (n=25)	1. Group (n=25)		2. Group (n=25)		
Measured Parameters	$\bar{X}\pm$SEM	$\bar{X}\pm$SEM	P	$\bar{X}\pm$SEM	P	P'
ALT (U/L)	17.40±0.97	29.30±0.24	<0.001	38.44±0.46	<0.001	<0.001
AST (U/L)	22.12±0.92	31.72±0.34	<0.001	39.36±0.36	<0.001	<0.001
GGT (U/L)	12.28±0.56	13.66±0.25	<0.05	20.48±0.56	<0.001	<0.001
ALP (U/L)	476.24±2.75	385.92±11.31	<0.001	380.16±2.41	<0.001	>0.05

Table III: The Mean Ratios Established in the Patient and Control Groups and Their Comparisons with Each Other

	Groups					
	Control (n=25)	1. Group (n=25)		2. Group (n=25)		
Ratios	$\bar{X}\pm$SEM	$\bar{X}\pm$SEM	P	$\bar{X}\pm$SEM	P	P'
Cu/Ca	11.36±0.12	7.64±0.09	<0.001	7.28±0.11	<0.001	<0.001
Ca/p	2.72±0.07	2.13±0.03	<0.001	1.69±0.03	<0.001	<0.001
Cu/p	30.82±0.72	16.22±0.29	<0.001	12.28±0.13	<0.001	<0.001
De Ritis (ALT/AST)	0.79±0.04	093.92±0.01	<0.01	0.98±0.02	<0.001	<0.05

We could not establish any correlation between Cu and Ca in any of the groups; however, the correlation between P and Cu was negative, and this inverse relation increased gradually in the first and second groups, respectively. The relation between Cu and ALP became negative in the first group and was lost in

the second group, while the correlations of ALT and GGT with Cu increased inversely only in the second group (r= −0.24 and −0.40 respectively). The relation between AST and Cu was found to be negative as well, but only in the first group (Table IV).

Table IV: Linear Regression Equations and the Correlation Coefficients (r) Established Between Cu, Ca, P and the Enzymes

Parameters Compared with Cu	Groups					
	Control		1. Group		2. Group	
	Linear Regression Equations	P	Linear Regression Equations	P	Linear Regression Equations	P
Ca	y=110.14+0.78x r=0.07	N.S.	y=60.63+1.191x r=0.10	N.S.	y=71.431−0.739x r=−0.07	N.S.
P	y=106.941+2.931x r=0.30	N.S.	y=96.88−5.629x r=−0.41	<0.05	y=19.201−8.644x r=−0.53	<0.01
ALT	y=117.937+0.024x r=0.02	N.S.	y=77.351−0.188x r=−0.07	N.S.	y=81.014−0.421x r=−0.24	N.S.
AST	y=117.162+0.054x r=0.05	N.S.	y=98.191−0.831x r=−0.40	<0.05	y=47.914+0.43x r=0.19	N.S.
GGT	y=116.096+0.183x r=0.11	N.S.	y=65.245+0.483x r=0.17	N.S.	y=75.076−0.05x r=−0.40	<0.05
ALP	y=80.431+0.080x r=0.23	N.S.	y=78.344−0.017x r=−0.27	N.S.	y=71.981−0.019x r=−0.06	N.S.

P : Statistical significance of the correlation coefficients.
N.S. : Non-significant.

Correlations between Cu and Cu/Ca ratios were significantly ($p<0.001$) higher in the patient groups and somewhat increased compared to controls. Again, the negative correlation between Cu and Ca/P ratios was slightly increased in the first and second groups. There was no correlation between Cu and Cu/P in the control group, but this relation increased significantly in the patient groups. Cu and "De Ritis" were inversely related, while a direct relation existed between GGT and ALT/AST ($p<0.05$) in these groups (r=0.4 and 0.5 respectively) (Table V).

Discussion

One of the characteristic findings of PEM is Cu deficiency[2,3]. In malnourished children, bone defects, depigmentation, connective tissue defects and neurological disorders can be seen as a result of Cu deficiency[7,8]. After observing the

Table V: Linear Regression Equations and the Correlation Coefficients Between Cu and the Ratios (Cu/Ca, Ca/P, Cu/P and ALT/AST) in the groups

	Groups					
	Control		1. Group		2. Group	
Parameters Compared with Cu	Linear Regression Equations	P	Linear Regression Equations	P	Linear Regression Equations	P
Cu/Ca	y=54.26+5.64x r=0.71	<0.001	y=20.40+6.73x r=0.82	<0.001	y=20.56+6.08x r=0.84	<0.001
Cu/P	y=130.41−4.43x r=−0.32	N.S.	y=55.25+7.80x r=0.36	N.S.	y=86.87−13.00x r=−0.40	<0.05
Cu/P	y=118.34+34.0x r=0.00	N.S.	y=41.08+1.89x r=0.80	N.S.	y=14.06+4.13x r=0.70	<0.001
ALT/AST	y=118.49−0.18x r=−0.01	N.S.	y=56.61+16.44x r=0.29	N.S.	y=78.91−14.37x r=−0.28	N.S.
GGT ALT/AST	y=11.27+1.26x r=0.1	N.S.	y=6.49+7.74x r=0.40	<0.05	y=4.82+15.99x r=0.5	<0.05

occurrence of spontaneous fractures in domestic animals consuming forage low in Cu, investigators are exploring the effects of Cu on bone metabolism.

Bone defects have been observed experimentally in rabbits, pigs, chickens and dogs. In these experiments, total ash as well as calcium and phosphorus remain within normal limits in bones, despite Cu deficiency. In conclusion, the biochemical defect of Cu deficiency is believed to reside only in the organic matrix[1]. In an another experiment, Cu-deficient chick bone was found to contain a higher-than-normal proportion of soluble collagen (immature collagen)[9,10]. This shows that the reactions with lysyl oxidase (a cuproenzyme) and the formation of allysin (an aldehyde form of lysin) are lesser than normal. The implication is that collagen cross-linking in bones is impaired by Cu deficiency. Failure of collagen maturation in the organic matrix of bone can account for the greater fragility of bone and associated abnormalities seen in PEM cases.

The second effect of Cu deficiency on bones is related to zinc (Zn) and ALP. Zn is an essential trace element for carbonic anhydrase and ALP enzyme activities[11]. These enzymes play important roles in both organic matrix formation and calcification processes (especially ALP in the latter)[12,13]. Most of the body Zn is found in the bones. In Cu deficiency, to maintain the equilibrium between Zn and Cu, Zn enters into the plasma in larger than normal quantities and in time, total

body Zn may be decreased. However, as long as the plasma Zn concentration is high, Cu absorption by the intestine is depressed. In the intestine, Zn and Cu are absorbed after binding to metallothionein (MT), and they compete for this binding process[14]. The relation between Zn and Cu is very similar to the relations between Ca-P and Na-K. High dietary Zn intake can depress Cu absorption and may result in Cu deficiency. Although adequate Zn is found in the initial phases of malnutrition, Zn deficiency may also supervene gradually as a result of inadequate diet and other causes of malnutrition (diarrhea, etc.). Secondary to Zn deficiency, ALP activity decreases and thus calcification slows down and may be depressed completely.

In PEM there is another factor which causes decalcification, independent of Cu deficiency. In mild and moderate malnutrition cases, ALP activity is suppressed secondary to the increase in cortisol levels[15,16]. In this condition, the organism's first aim is to maintain the plasma Ca concentration in the normal range. Therefore, as a result of mobilization of Ca from the bones (i.e., decalcification), osteoporosis develops. In this period, formation of bone and osteoblastic activity are also suppressed, and growth retardation occurs. As malnutrition progresses, growth hormone (GH) compensates by increasing parallel to the severity of the disease, which in turn can activate the bone ALP isoenzyme and increase the Ca and P absorption by the intestines.

In the present study, serum Ca and P levels were found to be in the normal ranges. However, Ca levels were lower and P higher as compared to controls' parallel to the severity of the disease. We can say that these changes were an attempt to maintain the equilibrium between Ca and P in the blood. ALP activities were also in the normal ranges in the first and second groups, but they were lower than controls. This finding may be accepted as a suppressive effect of cortisol on ALP activity in addition to probable Zn deficiency. In the first group, the negative correlation found between Cu and ALP acvitiy may also support the latter finding. In the second group, we have found this relation to be lacking. Therefore we believe that GH has entered into the cycle.

ALT, AST and GGT activities are specific indicators of liver function. In mild cases, as a response to an organism's stress, cortisol increases in plasma. In stressful conditions, an organism primarily needs energy and cortisol to supply energy and tries to activate gluconeogenesis. If there is inadequate glucose in the diet, the tissues and primarily the muscle (proteins) degrade into amino acids. These amino acids then lose their amino groups by transamination reactions and convert to glucose via gluconeogenesis[17]. Thus, cortisol-activating transaminases stimulate glucose formation.

GGT (gamma glutamyl-peptide: amino acid gamma glutamyl transferase) is a peptidase[18] that can also be used as an indicator of malnutrition. AST and ALT

activities were found to be increased compared to controls in every stage of malnutrition, although the causes of the elevations differ. For example, in mild malnutrion cases, the increase of cortisol causes AST and ALT to increase, while in the severe cases, AST and ALT increases may be seen secondary to the development of fatty liver. In other words, GGT increases in proportion to the degree of fatty degeneration of the liver. The correlation between GGT and the De Ritis ratio supports the latter idea as well. In various conditions affecting the liver, ALT is characteristically as high as or higher than AST, and the ALT/AST (De Ritis) ratio, which is normally less than 1.0, becomes greater than unity. In our study, De Ritis ratios were also found to be less than 1.0 in all groups. They were higher in the patient groups than in controls, and highest in the second group. This finding may support the idea that the De Ritis ratio can show the severity of liver pathology (Table III). In our work, the gradual increase in ALT and AST values in the patient groups and the fact that GGT was significantly higher in the second group than in the controls is consistent with the above results. The inverse relation between Cu and GGT in the second group again shows the severity of PEM (Table IV).

Cu is a trace element transported 95 percent by ceruloplasmin and about 5 percent by albumin in the plasma. Ceruloplasmin, besides its many other functions, is also an acute phase reactant. Especially in acute infections, trauma and in any type of acute disease, ceruloplasmin levels increase in serum. Secondary to the leukocyte endogenous mediator (LEM)'s acute phase action, initially serum ceruloplasmin and therefore the serum Cu level rises[19]. The finding of Cu and ceruloplasmin levels generally low in chronic and degenerative diseases supports these observatious[20-22]. In malnutrition the phenomenon is rather complex, and can vary with the severity of disease. In the early periods of malnutrition, the first aim is to maintain the organism's integrity. Generally PEM is a chronically progressing disease. Therefore, even in the beginning of PEM, neither ceruloplasmin nor Cu is increased in serum as an acute response. Ceruloplasmin synthesis continues as long as there is adequate substrate (protein and amino acids) and the liver functions are normal in the organism. As the disease progresses, because of protein inadequacy and breaking down of liver function[23], ceruloplasmin synthesis gradually decreases. As a result, Cu cannot be transported to the places where it is needed in the body, and serum Cu decreases. In malnutrition, Cu absorption can be lessened as a result of MT synthesis suppression secondary to protein inadequacy, in addition to local intestinal disorders such as diarrhea. The third reason which can cause hypocupremia in PEM is the inadequacy of dietary Cu. In such cases, Cu deficiency is not a cause but a result of PEM, and the severity of Cu deficiency is proportionate to the severity of PEM.

We conclude that Cu deficiency directly affects the organic matrix formation in bones and, by the suppression of ALP activity, indirectly causes decalcification. In the liver, on the other hand no direct effect of Cu deficiency was seen. However, the breaking down of liver functions and the amount of the Cu deficiency increases parallel with the severity of malnutrition. Therefore we believe that some correlations exist between Cu and the parameters that indicate liver function.

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A STUDY ON ENZYME ACTIVITIES OF SOME SPHINGOLIPIDOSES*

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SUMMARY: Özkara HA, Çevik Arıkan M, Topçu M, Emre S, Renda Y. (Department of Biochemistry, Hacettepe University Faculty of Medicine, Ankara, Turkey). A study on enzyme activities of some sphingolipidoses. Turk J Pediatr 1994; 36: 215-221.

Enzyme activities were determined in fibroblast cell cultures of eight patients suspected of having a type of sphingolipidosis. The patients were 0 to 4 years of age; four were female and four were male. Thirteen age-matched controls were also included in the study.

In one of the cases, hexosaminidase A activity was found to be 0% (43-82%), while in two other cases β-galactosidase activity was found to be 5 nmol/h/mg protein (100-1035 nmol/h/mg protein) and arylsulfatase activity was found to be 12 nmol/h/mg protein (106-990 nmol/h/mg protein), respectively. Two more enzymes, α-galactosidase (11-39 nmol/h/mg protein) and cerebroside β-galactosidase (3.7-6.9 nmol/h/mg protein), were also evaluated but were found to be in the normal ranges in these patients. Therefore, these patients were considered to have Tay-Sachs disease, GM1 gangliosidosis and metachromatic leukodystrophy, respectively.

The remaining five patients were normal in respect to the five enzyme activities determined.

For the prenatal diagnosis of metachromatic leukodystrophy, arylsulfatase A activity was determined in one amniotic cell culture. The activity found in this case was lower than normal (34 nmol/h/mg protein versus 387 nmol/h/mg protein found in three control amniotic cell cultures). *Key words: sphingolipidoses, lysosomal enzymes, prenatal diagnosis, postnatal diagnosis.*

The sphingolipidoses are defined as disorders caused by genetic defects in catabolism of sphingosine-containing lipids. Underlying genetic defects are mutations in lysosomal hydrolases or activator proteins[1]. Major sphingolipidoses are Farber disease, Niemann-Pick disease, Krabbe disease, metachromatic leukodystrophy, Gaucher disease, Fabry disease, Tay-Sachs disease, GM1 gangliosidosis and Sandhoff disease (Table I)[1].

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Table I: Major Sphingolipidoses

Disease	Storage Material	Deficient Enzyme
1. Farber	Ceramide	Acid ceramidase
2. Niemann-Pick	Sphingomyeline	Sphingomyelinase
3. Krabbe	Galactosylceramide, Galactosylsphingosine	Cerebroside β-galactosidase
4. Metachromatic leukodystrophy	Sulphatide	Arylsulphatase A
5. Gaucher	Glucosylceramide, Glycosylsphingosine	Glucosylceramidase
6. Fabry	Trihexosylceramide	α-galactosidase A
7. GM1 gangliosidoses	GM1 ganglioside, Glycoproteins	β-galactosidase
8. Tay-Sachs	GM2 ganglioside	β-hexosaminidase A
9. Sandhoff	GM2 ganglioside, Asialoganglioside GM2	β-hexosaminidase A and B

The disorders are generally classified according to the age of onset as infantile, late infantile, juvenile and adult cases. Infantile patients have virtually no detectable enzyme activity[2]. Patients affected by the sphingolipidoses can be diagnosed definitively by appropriate enzyme assays, and heterozygous carriers can be identified in most instances with reasonable reliability in serum, urine, leukocytes and fibroblasts. Affected fetuses can be diagnosed during pregnancy either with cultured amniotic fluid cells or with biopsied chorionic villi[3-9].

In Turkey, the incidence of consanguineous marriages is relatively high (21.2%)[10]. Therefore, we may expect a high incidence of autosomal recessive disorders in the Turkish populatilon. The aim of this study was to diagnose sphingolipidoses either prenatally or postnatally by related enzyme activity determinations.

Material and Methods

The patients were from Hacettepe University Children's Hospital Pediatric Neurology Unit and were suspected of having a type of sphingolipidosis. The study group comprised four female and four male patients between the ages of 0 and 4 years. Enzyme activity was determined in only one amniotic fluid cell culture for metachromatic leukodystrophy. Thirteen age-matched controls (skin fibroblast

culture) and three amniotic fluid cell culture controls were also included in the study.

Until the time of enzyme activity determination, cells from tissue cultures were frozen at −50°C and investigated within one month.

Case Reports

Case 1

E.Y. was a 10-month-old boy admitted to Hacettepe Children's Hospital with complaints of hypotonicity, motor and mental deterioration which started at five months of age. He was the second child of nonconsanguineous parents. On physical examination his height was 75 cm, weight was 12 kg and head circumference was 45 cm. He had prominent hypotonicity and hyperacusia. On eyeground examination cherry-red spots were seen bilaterally. There was no organomegaly. Deep tendon reflexes were hypoactive bilaterally. On laboratory examination, hexosaminidase A (Hex. A) activity was found to be 0% in cultured skin fibroblasts. The patient was diagnosed with Tay-Sachs disease.

Case 2

İ.M. was a 16-month-old boy followed up at Hacettepe Children's Hospital with motor and mental deterioration, recurrent pulmonary infections and convulsions. He was the first child of nonconsanguineous parents. Signs of deterioration first appeared at six months of age. On physical examination his height was 72 cm, weight was 9 kg and head circumference was 45 cm. He had coarse facial features and was unable to support his head. The liver was palpable six cm below the right costal margin. On eyeground examination, the optic discs were pale bilaterally. On laboratory examination, urine mucopolysaccharides were found to be less than 3 mg/dl (normal: <3 mg/dl), bone marrow aspiration was normal: X-rays of his extremities and skull displayed changes compatible with mucopolysaccharidosis, while computerized brain tomography displayed a leukodystrophic appearance. In the postmortem skin fibroblast cell cultures, β-galactosidase activity was found to have 4.78 nmol/h/mg protein. The patient was diagnosed with GM1 gangliosidosis.

Case 3

S.T. was a stillbirth from a 32-week pregnancy at risk for metachromatic leukodystrophy. The family also had a child with metachromatic leukodystrophy who had died at three years of age. The mother did not come for amniotic fluid examination during the early months of pregnancy. The patient was born spontaneously at 32 weeks gestation. Arylsulphatase A activity was found to be

severely deficient (12 nmoles/h/mg protein) in postmortem fibroblast cell cultures, and the patient was diagnosed with metachromatic leukodystrophy.

Skin Fibroblast Culture Procedure

After skin disinfection and local anesthesia, a skin sample of 3 mm by 3 mm by 1 mm deep was taken from the medial aspect of the forearm. The skin fragment was then transferred to a sterile bottle containing Ham's F10 culture medium with antibiotic-antimycotic solution (10.000 µ penicillin/ml, 10 µg streptomycine/ml, 2.5 µg amphotericin B/ml).

The sample was then divided into three to five pieces and transferred to one or two 25 cm^2 culture flasks which had previously been moistened with 2-3 ml of culture media. To facilitate adherence of the skin fragments, the flasks were inverted and incubated at 37°C in an environment consisting of 5 percent CO_2 and 95 percent air. After two to three hours, the flasks were turned right-side-up and supplemented with 5 ml of fresh growth medium (Ham's F10, Sigma, USA) containing 20 percent fetal calf serum (Seromed, Germany), 100 U/ml penicillin and 100 µg/ml streptomycine (Flow, England). When confluency was attained, the cells were trypsinized with trypsin-EDTA (0.05%), 0.02%, Sigma, USA). This subcultivation (p1 passage) was continued until sufficient cells were available, and samples were kept at −50°C until the time of investigation.

Amniotic Fluid Cell Culture Procedure

A sample of 10-20 ml of amniotic fluid was removed by transabdominal puncture at 15-16 weeks gestation. The amniotic fluid and cells were separated by centrifugation, and the cells were placed in a culture medium (Ham's F12) (Seromed, Germany) supplemented with 20 percent fetal calf serum. Penicillin (100 U/ml), streptomycine (100 µg/ml) and Hepes (100 u/ml) were added. The primary cultures of all the amniotic cells underwent one passage before the enzyme analysis.

Enzyme Activity Determination

Galactosylceramide sulphate, p-nitrocatecole sulphate, trinitrophenylaminolauryl-galactocerebroside, 4-methyl-umbelliferyl ∝-galactoside, 4-methylumbelliferyl β-galactoside, 4-methylumbelliferyl N-Acetyl β-glucosaminide and p-nitrocatecole were purchased from Sigma, USA; 4-methyl-umbelliferone, bovine serum albumin were purchased from Serva, Germany.

Fibroblast and amnion fluid cell cultures were homogenized with freezing and thawing three times before protein determination. The protein concentration was measured using the method of Lowry et al[11] with bovine serum albumin as standard.

Hexosaminidase A, β-galactosidase, arylsulphatase A and α-galactosidase were assayed using 5-15 µg, 20-50 µg, 100-400 µg and 50-200 µg of cellular proteins, respectively, according to Suzuki[1]. Cerebroside β-galactosidase was assayed using 100-400 µg of cellular protein according to Besley and Gatt[12]. All the assays were performed in duplicate.

Enzyme activities were expressed as nanomoles of substrate hydrolysed per milligram of protein per hour.

Results

Normal values for enzyme activities are given in Table II. In one patient, Hex A was totally inactive (0%). In another patient, β-galactosidase activity was found to be significantly low (4 nmol/h/mg protein). Arylsulphatase A activity was found to be lower than normal controls (12 nmol/h/mg protein) in a third patient. In another case, arylsulphatase A activity was determined as 33 nmol/h/mg of protein in cultured amniotic cells, which led to a prenatal diagnosis of metachromatic leukodystrophy.

Table II: Enzyme Activity Levels in Control Skin Fibroblasts and Amniotic Cell Cultures

	Skin Fibroblast Culture		Amnion Cell Culture	
Enzyme	Sample (n)	Mean±SD	Sample (n)	Mean±SD
Hexosaminidase A (%)	14	63.65±12.63 (43–82.6)*	4	76.68±5.24 (74.38±86.2)
β-galactosidase (nmol/h/mg protein)	14	417.65±224.54 (100-1035)	3	838.9±625.7 (235.8-1485)
α-galactosidase (nmol/h/mg protein)	9	24.89±9.71 (11.1±39.2)	–	–
Arylsulphatase A (nmol/h/mg protein)	10	442.7±348.37 (106-990)	1	387.16
Cerebroside β-galactosidase (nmol/h/mg protein)	7	5.3±1.33 (3.69±6.87)	–	–

* Numbers in parentheses indicate lowest and highest values.

Discussion

Sphingolipidoses, which are a group of metabolic and inherited diseases causing mental retardation, can be diagnosed prenatally by measuring the activities of

some specific enzymes in amniotic cells[1]. The correct diagnosis of affected siblings is an important prerequisite for prenatal testing of the risk groups[13].

The normal values of the enzyme activities reported here correlated closely with the results of the following reports. O'Brien et al[14] reported Hex A levels of 62-79 percent and 50-60 percent as normal values for amniotic cell cultures and fibroblast cell cultures, respectively. In two other studies, normal levels were reported as 62-80 percent for amniotic cells[15] and 46-47 percent for fibroblasts[16]. Our patient with undetectable Hex A activity had a clinical diagnosis of Tay-Sachs disease. The activity of arylsulphatase A increases during fetal development and postnatal life, a fact which must be taken into consideration in the evaluation of the test results[5]. Similar studies revealed normal levels of 146-760 nmol/h/mg protein[17], and 181-576 nmol/h/mg protein[15] for amniotic cells, and 263-981 nmol/h/mg protein[18] for skin fibroblasts. Our patient with 4 nmol/h/mg protein β-galactosidase activity had nonspecific clinical signs, and the diagnosis was established as GM1 gangliosidosis only after enzyme activity determination. α-galactosidase activity was 35-90 nmol/h/mg protein and 25-60 nmol/h/mg protein and 25-60 nmol/h/mg protein for normal fibroblast and amniotic cell cultures, respectively[7]. Normal cerebroside β-galactosidase activity was reported to be 2.2-3.6 nmol/h/mg protein for amniotic cells in another study[8].

Although we had no patients with the diagnosis of Fabry's or Krabbe's disease, we tried to establish normal levels of α-galactosidase and cerebroside β-galactosidase enzyme levels.

In conclusion, our study is a preliminary attempt to establish a prenatal and postnatal diagnostic set of assays for evaluating groups at risk for sphingolipidoses in our country.

Acknowledgements

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SPECIAL TECHNIQUES IN DIAGNOSTIC MYOPATHOLOGY*

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While muscle biopsy has been standardized since the introduction of electron microscopy, histochemistry, and morphometry into diagnostic myopathology, additional diagnostic techniques have recently been added. Immunomorphologic methods-firmly established in oncology long ago-are beginning to be applied in diagnostic myopathology. These methods concern the evidence of intermediate filaments in regenerating and denervated fibers as well as in certain myopathies marked by intermediate filament-related inclusions such as cytoplasmic bodies, spheroid bodies, or Mallory body-like inclusions. The connotation of the immunomorphologic approach is further emphasized by the discovery of dystrophin, a normal protein within muscle fibers which is deficient in Duchenne's and Becker's muscular dystrophies resulting in the now-standard examination of dystrophin in muscle biopsy specimens. It is to be expected that immunomorphologic techniques will rapidly develop further, depending on the isolation of muscle-and disease-specific proteins or defective proteins and the availability of antibodies against these proteins. It is conceivable that immunohistological diagnostic methods in myopathology may, one day, render currently established techniques obsolete. *Key words: myopathology, morphologic diagnosis, electron microscopy, histochemistry, morphometry, immunomorphology, dystrophin, intermediate filaments.*

Muscle biopsy is a diagnostic procedure, not a curative one, as removal of tissue alone does not represent therapy but only may occasionally lead to treatment, e.g., in the case of inflammatory myopathy. On the other hand, postmortem myopathologic studies have become extremely rare and are usually confined to employing old-fashioned techniques, i.e., fixation of muscle tissue and subsequent embedding in paraffin for a very limited number of histological stains.

Contrary to and in accordance with the diagnostic importance of a muscle biopsy, a spectacular armamentarium of morphologic techniques have been developed over the past 20 years to examine a biopsied muscle specimen as thoroughly as possible. As a matter of fact, it may safely be stated that no other tissue obtained by biopsy is or can be subjected to such a large variety of morphologic methods as muscle tissue. These methods comprise regular histology; histochemistry to

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detect amounts of fat, glycogen, calcium or iron; enzyme histochemical preparations to determine fiber types and structural abnormalities as well as specific enzyme defects; morphometry; immunohistochemistry; and finally, electron microscopy. For all methods, muscle tissue must be prepared at the time of biopsy. Part of the tissue must be left unfixed for frozen preparations, while another part must be fixed for light microscopy, i.e; semithin plastic embedded sections and ultrathin sections for electron microscopy. Histological studies may be amplified when abundant tissue is available, by fixing the tissue in formalin and subsequently embedding it in paraffin to examine inflammatory infiltrates and vessel walls, including step and/or serial sections.

The basic procedure involves freezing the unfixed muscle tissue in liquid nitrogen for enzyme histochemical preparations that may also reveal fiber types as well as for morphometric studies, assuming that muscle tissue in the unfixed frozen state most closely resembles in vivo tissue in terms of the size and contours of myofibers. These must be mounted for cross-sectioning rather than longitudinally.

Unfixed frozen muscle tissue is also necessary for a number of immunohistological techniques, foremost of which is the determination of dystrophin. Furthermore, if metabolic myopathy is suspected, unfixed frozen tissue, retrieved separately or from the tissue block, may be utilized for biochemical studies.

Finally, electron microscopy is a technique integrated into the so-called "muscle biopsy program" requiring fixation and embedding of muscle tissue from each patient biopsied, even if subsequent ultrastructural examination is not warranted.

Beginning in the early sixties[1], these numerous myopathologic techniques led to reliable and reproducible standardized results of high quality, now documented in an impressive number of references and textbooks[2,3].
In view of the muscle tissue being affected (primarily or in association with other conditions) in well over 600 nosological entities, the spectrum of myopathologic lesions is rather limited. Therefore, a myopathologic diagnosis must always be incorporated into a final nosological diagnosis. On the other hand, in spite of the numerous techniques employed in a standardized "muscle biopsy program", new ways are consistently being explored to enhance diagnostic interpretation of diseased human muscle tissue. A few such technical aspects and trends are described below.

Conventional Histology: The regular histological stains comprise hematoxylin-eosin, the modified trichrome preparation[2], the standard method for routine examination of unfixed frozen muscle, and Elastica van Gieson to determine the amount of connective tissue and the state of the intramuscular vessel walls. Other stains used in general or neuropathology have been rarely employed.

Conventional techniques to ascertain myelinated and unmyelinated nerve fibers may be added.

The Azur B stain reveals regenerating fibers staining ribonucleic acids engaged in protein synthesis. In particular, groups of regenerating fibers, frequent in Duchenne's, Becker's and autosomal recessive (the "Arabic" type) muscular dystrophies are conspicuous. Disseminated regenerating fibers following bouts of rhabdomyolysis may sometimes be the only evidence of preceding muscle fiber necrosis.

While lysosomal enzymes (the acid phosphatase technique) may reveal enzyme activities, the autofluorescent nature of certain abnormal lysosomes attests to their lipopigment character, as seen in regular lipofuscin or accretion of abnormal amounts and abnormally structured lipopigments in vitamin E deficiency[4] and in childhood form of neuronal ceroid-lipofuscinosis. The amount of lipofuscin bodies within muscle fibers may vary considerably among individuals, even of the same age; lipofuscin appears increased in adult denervated muscle fibers, while denervated muscle fibers in childhood display small amounts of lipopigments.

Histochemistry: A muscle specimen should be checked regularly for the amount of lipid droplets and PAS-positive material, usually glycogen. PAS-positive inclusions may further be defined, characterized and differentiated by enzyme digestion, particularly amylase/diastase, but also by others such as glucosidase, mannosidase or even trypsin, e.g., when countering polyglucosan bodies.

Calcium may be revealed by several techniques, e.g., Alizarin red. Calcium is usually transported into the muscle fiber through plasmalemmal defects from the systemic circulation. Calcification of necrotic fibers may occur other wise, but seems to be a rare event. Likewise, iron found within a muscle specimen, though rare, usually indicates an earlier hemorrhage, and iron may be seen in interstitial macrophages as well as in muscle fibers.

Enzyme Histochemical Preparations serve three purposes:

1. Typing of fibers which is usually based on the ATPase techniques after alkaline and acid preincubations revealing types I, IIA, IIB, and IIC. The oxidative enzyme preparations also illustrate fiber types, even subtypes IA and IB[5].
2. Revealing of abnormalities, such as "cores" or "target" phenomena.
3. Revealing of metabolic abnormalities such as deficiencies of amylophosphorylase, phosphofructokinase, myadenylate deaminase, cytochrome c oxidase, or structural abnormalities indicating metabolic lesions, such as the enzyme histochemical equivalent of "ragged red fibers", abnormal mitochondria.

In the wake of studying enhanced enzyme activities in mitochondrial myopathies, additional enzyme histochemical preparations have become available, such as

cytochrome c oxidase or mitochondrial ATPase. The importance of the latter enzyme histochemical preparations is that they may reveal biochemical defects that can subsequently be confirmed or detailed by biochemical analysis of the same muscle tissue.

While the spectrum of oxidative enzymes may be further enhanced by adding succinate dehydrogenase, lactate dehydrogenase or malate dehydrogenase to the standard program, rarer enzyme histochemical preparations allow the disclosure of carboanhydrase or triosephosphate isomerase activities.

Acid phosphatase activity may be enhanced in disorders with activated lysosomal metabolism such as vitamin E deficiency[4], in the neuronal ceroid-lipofuscinoses[6], mucolipidosis IV[7], and foremost in the different clinical forms of acid maltase deficiency. Macrophages within nerotic muscle fibers as well as within the interstitium display acid phosphatase and non-specific esterase activities. Preparations of the latter enzyme further supported by that of acetylcholine esterase may demonstrate the presence, density and distribution of motor endplates.

Alkaline phosphatase activity may outline endothelial cells, but not consistently, and may be histochemically absent in lethal hypophosphatasia[8]. Degenerating-regenerating fibers may also have alkaline phosphatase activity.

The actual number of fruitfully employed enzyme histochemical preparations in diagnostic myopathology may disclose additional diagnostic features. However, this area has not been explored systematically as to muscle morphology and diagnostic myopathology. Lectins may be applied to muscle tissue, especially in the case of inclusions within muscle fibers, such as polglucosan bodies which stain with concanavalin A (Fig. 1) and PSA (Pisum sativum) (Fig. 2). On the contrary, endogenous neoglycoproteins as receptors for certain sugars, so-called endogenous lectins, may also be revealed by respective antibodies[9]. This recent technique may at least differentiate fiber types to a certain degree.

Morphometry : Morphometric analysis in diagnostic myopathology has utilized the smaller diameter technique[10] or the square area method to determine subtle deviations in muscle fiber size from the normal range of age and sex-dependent muscle fiber diameters. Certain pathomorphometric patterns correspond to certain groups of neuromuscular conditions such as the double-peak pattern in neurogenic atrophy. The congenital fiber-type disproportion is one such condition that is based on morphometric analysis. Capillary density, usually determined in plastic-embedded 1 µm-thick sections, has been studied in neuromuscular disorders such as inflammatory myopathies or muscular dystrophies. An increased vascular density is also revealed, particularly in dermatomyositis, in ATPase preparations after acid (pH 3.9) preincubation.

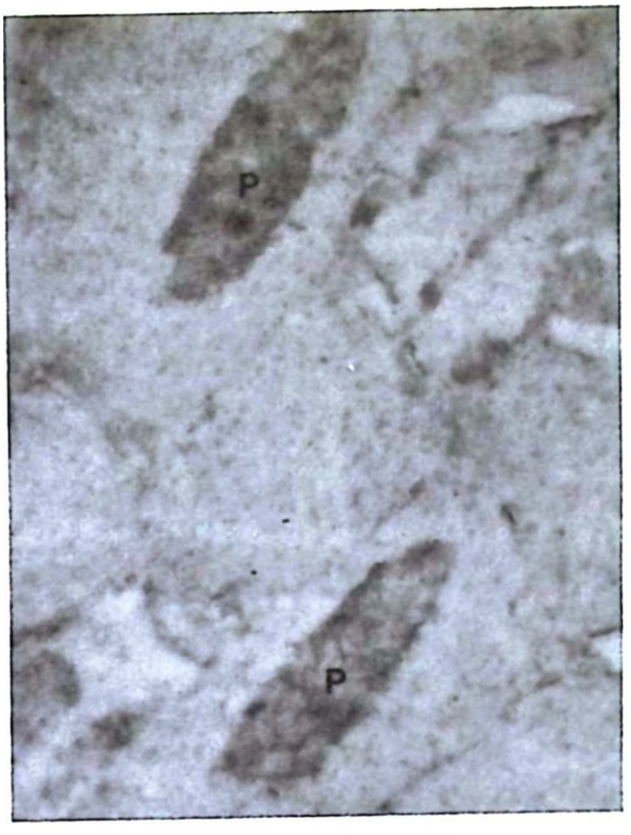

Fig. 1: Polyglucosan (P) bodies react with the lectin concanavalin A, X 220.

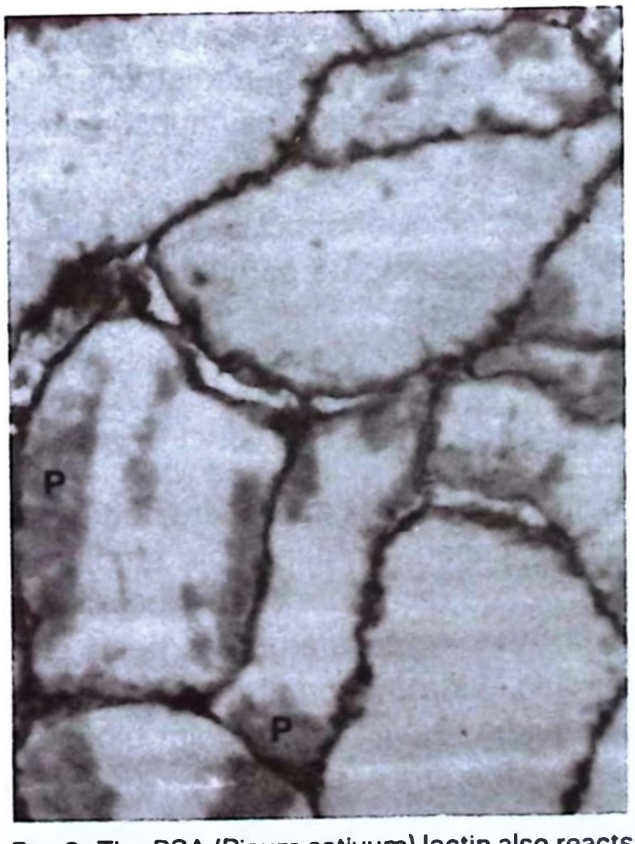

Fig. 2: The PSA (Pisum sativum) lectin also reacts with polyglucosan (P) bodies, X 228.

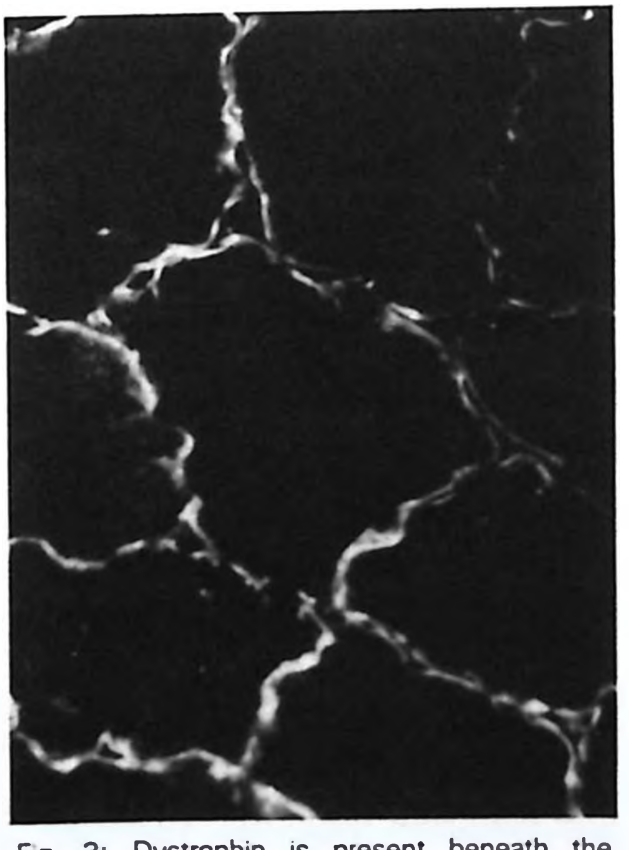

Fig. 3: Dystrophin is present beneath the plasmalemma of each muscle fiber in spite of ice crystal artifacts. Biopsy specimen was retrieved 6 years prior to dystrophin preparation, X 416.

An extremely cumbersome technique combines morphometry and electron microscopy, i.e., ultrastructural morphometry of organelles and other cellular constituents. Nuclear size, mitochondrial volume and histograms of mitochondria may reveal subtle respective defects such as mitochondrial lesions, provided age, sex, muscle-specific and possibly type and state of activity-related control data are available. As this is usually not the case, due to the time-consuming aspect of ultrastructural morphometry, only few such studies have been undertaken.

Whether automatized image analysis will improve the situation and reduce the time spent in morphometric electron microscopy remains to be tested.

Immunomorphology: During the past decade, immunohistological techniques have been firmly ensconced in the diagnostic histopathology of tumors. The presence of viral antigens in tissues may also be more easily detected by using antibodies that visualize viral infections. As regards diagnostic myopathology, the application of immunomorphological methods is still in its infancy. Such an immunohistochemical spectrum may entail several categories of neuromuscular disorders. As many of the immunomorphological methods are performed on praffin-embedded material it is also conceivable that, with the future spread and advance of immunohistochemical techniques in diagnostic pathology, the currently somewhat restrictive procedure of not fixing muscle tissue aside from electron microscopic examination may be altered or expanded by regularly incorporating old-fashioned formalin fixation for eventual immunohistochemical studies. On the other hand, primarily unfixed frozen sections may subsequently be fixed for immunomorphology.

When studying inflammatory myopathies, immunological subclassification of B and T-lymphocytes as well as other cytological components participating in the inflammatory process has repeatedly been performed[11-16]. While this immunological cell-typing largely concerns polymyositis, in dermatomyositis, which is now considered an inflammatory vascular disease[17], immunoglobulins and complement may be demonstrated in diseased vessel walls[18,19].

The myasthenic syndromes, the foremost being myasthenia gravis, are defined as conditios in which the motor endplates are diseased. Immunologic studies of such abnormal motor endplates as to acetylcholine receptors or the localization and identification of antibodies and other immunologic parameters been published in the literature[20,21]. However, systematic incorporation of motor endplate examination into diagnostic myopathology requires regular motor-point biopsies. Identification of the motor point prior to biopsy, therefore, should be included in the complete diagnostic evaluation of a patient's neuromuscular condition.

Detection and analysis of the gene for Duchenne's muscular dystrophy (DMD)[22] have created a completely new approach to diagnostic morphology. The protein

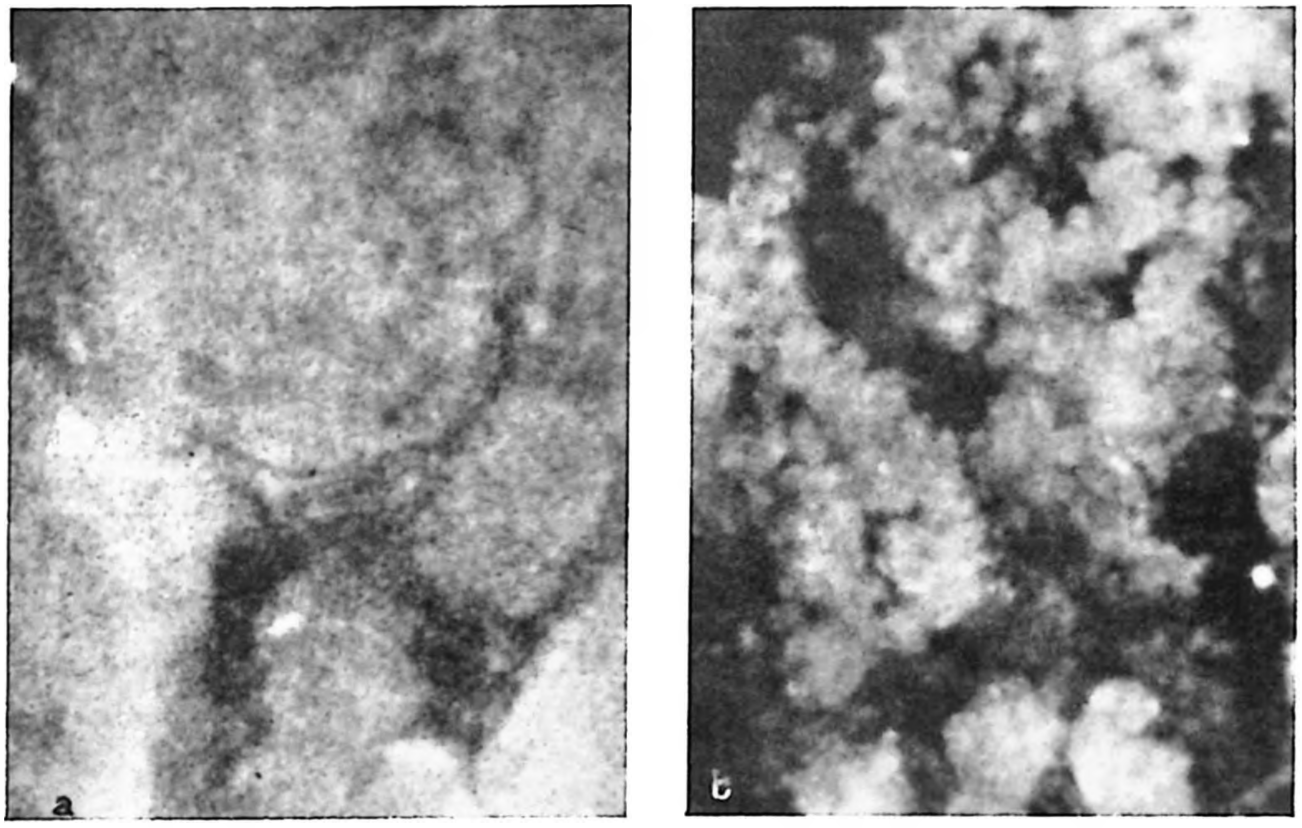

Fig. 4: Absence of dystrophin from muscle fibers reveals background staining, but no subsarcolemmal dystrophin.
a) Boy with Duchenne muscular dystrophy (DMD), X 228.
b) Fetal muscle at 22 weeks gestation from a DMD carrier, X 800.

dystrophin is the coded product of the respective normal gene and is located beneath the plasma membrane of the muscle fiber (Fig. 3). In DMD, dystrophin is either completely absent (Figs. 4 a, b) or present in only trace amounts. In Becker's muscular dystrophy (BMD), dystrophin is segmentally deficient, due to either too little formation or production of a molecularly abnormal dystrophin. In carriers of DMD and BMD, only disseminated muscle fibers may show respective deficiencies of dystrophin (Fig. 5)[23]. Thus, immunohistology and the Western blot technique for analysis of dystrophin now represent standard procedures[24] in evaluating DMD and BMD as well as respective biopsied tissues from carriers. As a result, diagnostic myopathology has closed ranks with other molecular biological techniques. All this may be just the beginning of a trend to revolutionize diagnostic myopathology of hereditary neuromuscular diseases by identifying immunological defects as they are discovered. As genetic and immunologic analyses of DMD and BMD have modified the nosological and clinical spectra of these disorders, examination of muscle tissue for dystrophin now goes well beyond the DMD and BMD muscle tissues (Table I). Conservation of unfixed frozen tissue also enables retrospective studies and there by renewed genetic counselling. Furthermore,

Table I: Diagnostic Examination of Dystrophin

– Confirmation or elimination of DMD
– Confirmation or elimination of BMD
– Carriers for DMD or BMD
– Fetal muscle tissue
– Pathological male muscle tissue specimens without any disease-specific pathology
– Pathological female muscle tissue specimens without any disease-specific pathology
– Congenital muscular dystrophy
– Severe myopathology

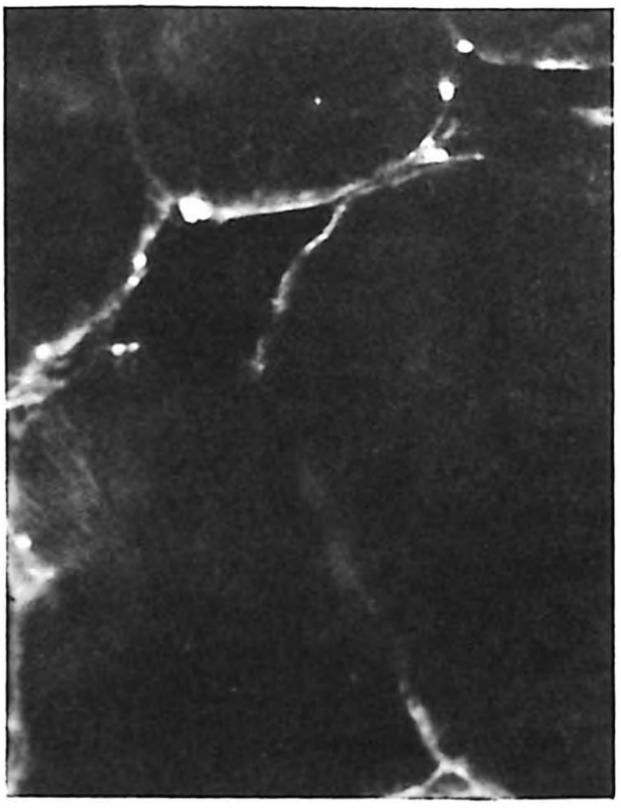

Fig. 5: There are several segments within muscle fibers free of dystrophin; mother of the patient with Becker muscular dystrophy, X 528.

modification of the current immunofluorescent technique of demonstrating dystrophin and application to paraffin-embedded tissue may even enable examination of muscle tissues retrieved by biopsy or at autopsy many years or decades ago. In this context, it would be tempting, as a medico-historical vignette, to reexamine still-available blocks of original patients studied and described by Duchenne himself.

As the number of proteins detected and defined in skeletal muscle fibers increases, their immunological evaluation as regards diagnostic myopathology of neuromuscular diseases will also grow. Nebulin[25], titin[26], alpha actinin[27] and certain myofilamentous proteins such as the myosins[28,29] and actin have already been studied, but not in a very systematic fashion concerning the entirety of neuromuscular diseases.

Among the skeletal muscle proteins, the intermediate filament proteins of muscle, the foremost of which is desmin, and vimentin in undifferentiated muscle cells, play an increasing diagnostic role. In regenerating fibers, coexpression of desmin and vimentin may occur within the same muscle cell (Figs. 6 a, b). Furthermore, atrophic fibers in neurogenic processes or in myotonic dystrophy may similarly coexpress desmin and vimentin (Figs. 7 a, b). Whether these fibers are actually atrophic fibers undergoing reinnervation remains to be seen.

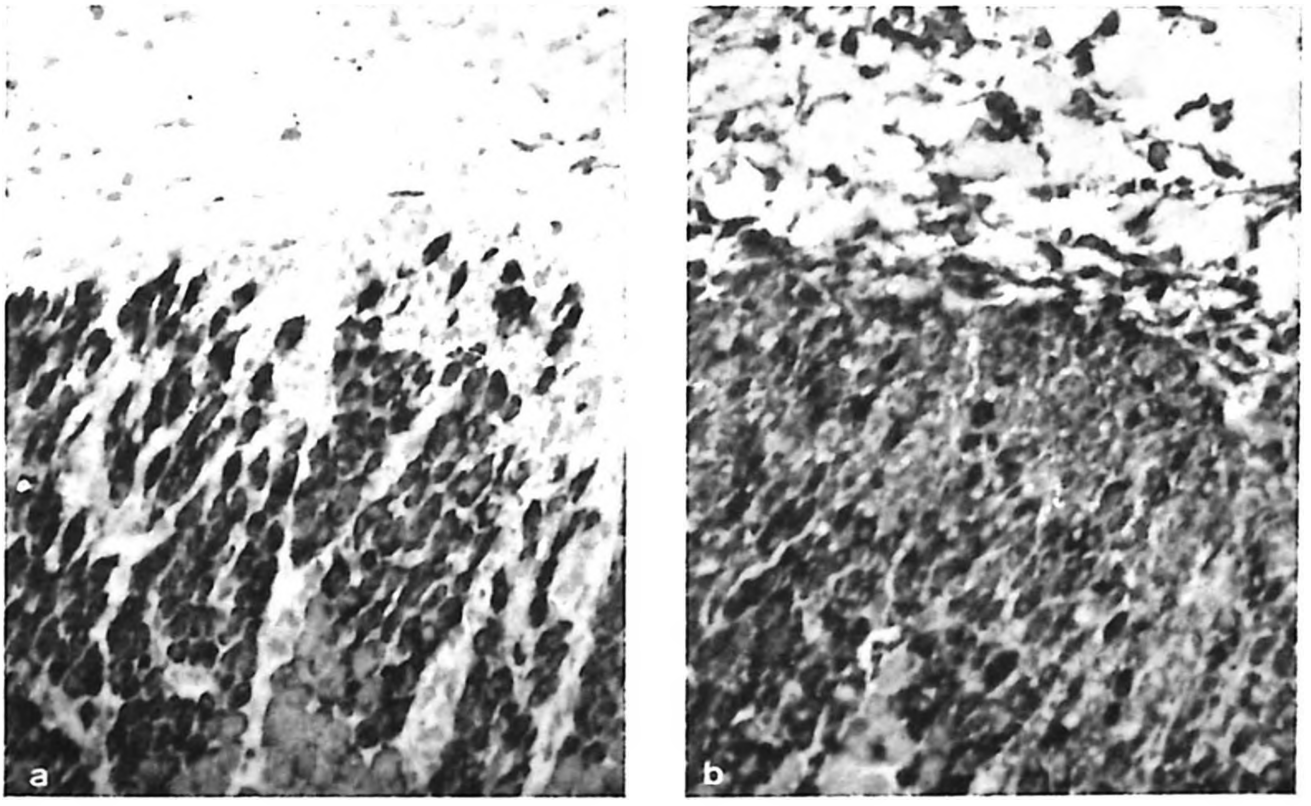

Fig. 6: Degenerating-regenerating fibers in the periphery of muscle fascicle express: a) desmin, X 140, b) vimentin, X 208, within the same muscle fibers.

On the other hand, desmin may be abnormally aggregated in certain inclusions within muscle fibers, such as cytoplasmic bodies[30], spheroid-cytoplasmic bodies, or Mallory body-like inclusions[31]. A number of patients with muscle tissues marked by abnormal aggregates of desmin intermediate filaments have now been described, and even abnormal aggregates of desmin have been seen in cardiac myofibers in certain cardiomyopathies[32,33].

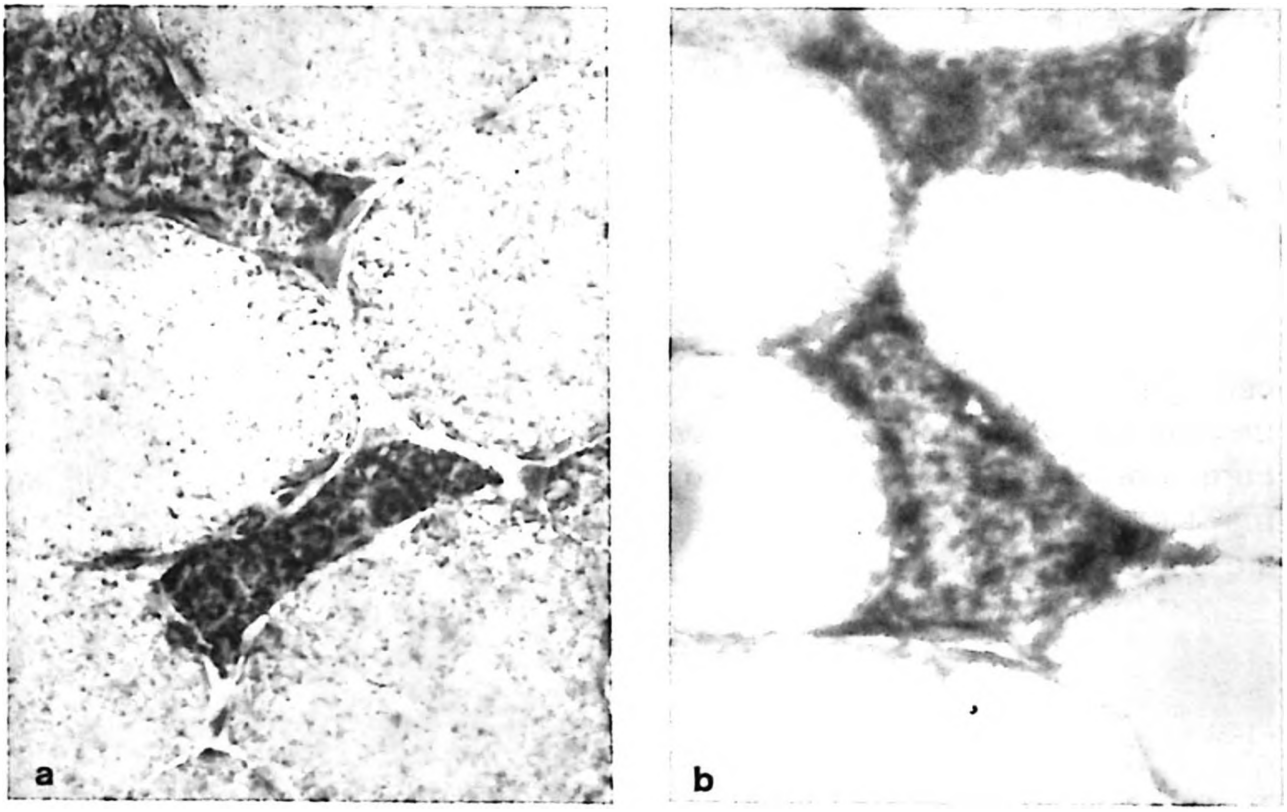

Fig. 7: Coexpression of a) desmin, X 400, and b) vimentin, X 360, within two denervated muscle fibers in neurogenic atrophy.

Denervated muscle fibers are marked by atrophy and are present within the respective muscle tissue in either scattered or grouped arrangements. As denervation in man if not traumatic, is a chronic process of varying length, the dynamics of which within the motor neuron, at the motor endplate and within the respective muscle fiber are largely unknown, the spectrum of denervated muscle fibers is not uniform, as the period of denervation is of different lengths within a group of denervated muscle fibers in both spinal muscular atrophies and neuropathies. The neural cell adhesion molecule (N-CAM) is expressed in denervated fibers[34] (Fig. 8 a), but not every denervated fiber expresses N-CAM. Characterization of N-CAM-positive fibers is still incomplete. Likewise, denervation results in resurfacing of extrajunctional acetycholine receptors during a certain period after denervation, and the presence and extent of extrajunctional acetylcholine receptors may follow different patterns in different forms of neurogenic atrophy. It is further conceivable, as denervation in chronic neurogenic disorders most likely is not a temporal event of very brief duration, that meticulous examination of the motor endplate regions of such patients' muscle tissues may also be rewarding when a) motor point biopsies are regularly performed and b) more detailed morphologic parameters of diseased motor endplates may become known. N-CAM is also expressed in regenerating muscle fibers (Fig. 8 b)[35].

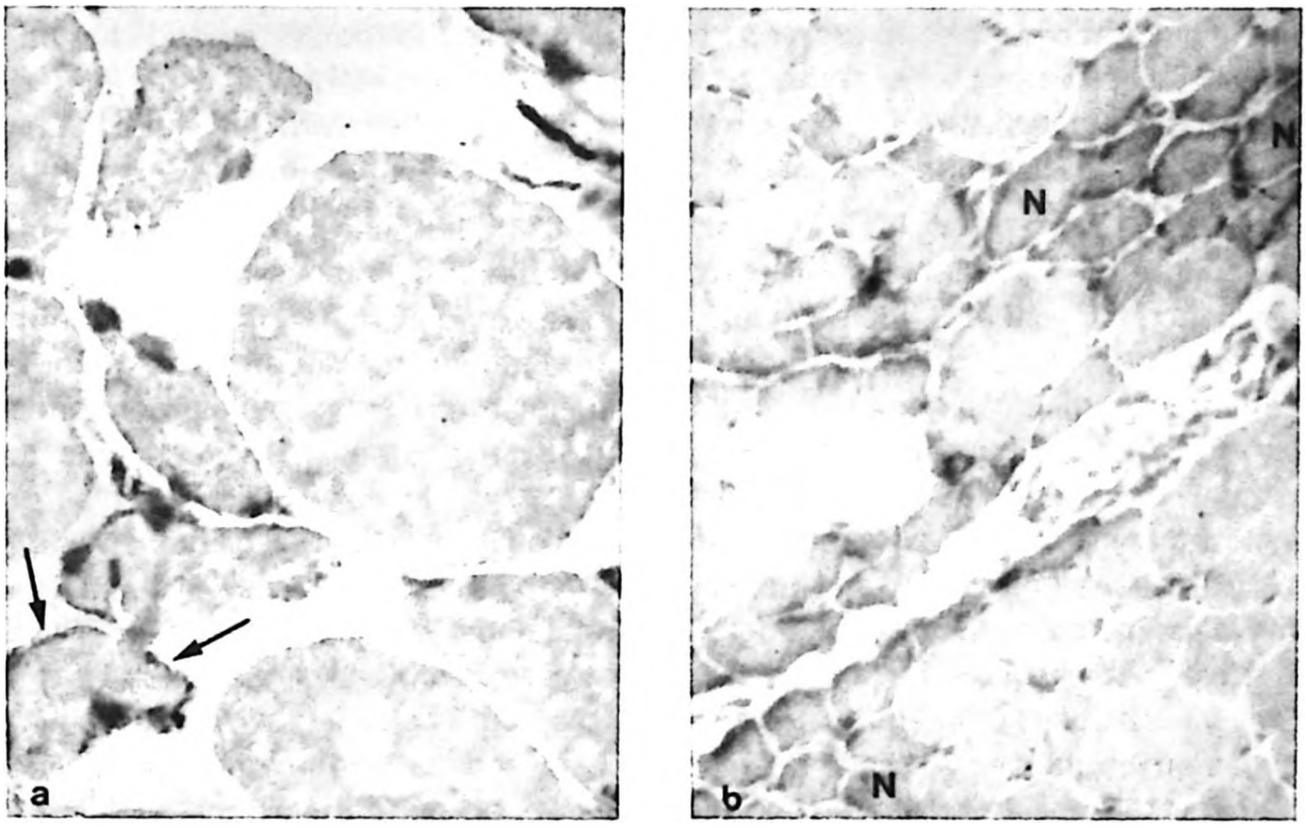

Fig. 8: Presence of the neural cell adhesion molecule (N-CAM), evidenced by antibodies against Leu-19:
a) Superficial location of N-CAM (arrows) in denervated fibers, X 530.
b) Diffuse presence of N-CAM (N) in regenerating myofibers arranged in groups, X 220.

Although the era of immunohistochemical myopathology is rapidly expanding, the full scope of this new field is far from being defined. However, one may speculate that it is this area within diagnostic myopathology, immunomorphology, which may modify, alter or even revolutionize our myopathological diagnostic approach in future years and thereby even render current techniques, such as those employed in enzyme histochemistry, somewhat obsolete.

Molecular Pathology : In situ hybridization is currently applied to tissues affected by viral diseases. However, it is certainly conceivable that morphologic demonstration within tissues, even using the polymerase chain reaction for amplification, may reveal chromosomal deletions in DMD and BMD myofibers and respective muscle fibers in carriers. Localization of abnormal genes in other neuromuscular diseases, such as infantile spinal muscular atrophy, and demonstration of chromosomal deletions in other neuromuscular conditions already portend an additional new era in diagnostic myopathology.

Electron Microscopy : Since the beginning of the modern time of diagnostic myopathology, and even before the introduction of enzyme histochemical

techniques, the electron microscope has been applied to the systematic study of neuromuscular conditions. Actually, the group of congenital myopathies largely emerged as a separate group of neuromuscular entities with the advent and introduction of electron microscopy into diagnostic myopathology.

Apart from systematic ultrastructural studies of neuromuscular disorders so far described in small numbers or even newly defined conditions as well as the evaluation of hitherto unrecognized lesions, employment of the electron microscope in myopathologic examination henceforth will probably involve combined techniques, i.e. morphometry and electron microscopy, as mentioned above; electron microscopy and histochemistry, e.g., the Thiéry technique to identify glycogen at the ultrastructural level such as in polyglucosan bodies or inclusions in Lafora disease; electron microscopy and enzyme histochemistry, e.g., ultrastructural activity and localization of cytochrome c oxidase[36]; or electron microscopy and immunomorphology, e.g., localizing dystrophin or desmin. These combined techniques, however, will further complicate the organization of the "muscle biopsy program", because at the time of biopsy, sophisticated cytochemical studies will have to be set up in advance in order to utilize the tissue within a reasonable time after the biopsy has been performed, thus avoiding a second biopsy of the same patient for such intricate studies when a preoperative diagnosis is not yet known or strongly suspected.

Conclusion

Unlike neoplastic diseases, which are defined by the histological diagnosis of the respective tumors, i.e., are nosologically detailed in the individual patient by the histopathologist, neuromuscular diseases are nosologically diagnosed by the concerted effort of the clinician (often including the geneticist), the electrophysiologist, the clinical chemist, and the myopathologist, and often the biochemist, among whom importance of the individual contribution by each of these experts rests upon the type of the individual neuromuscular condition. To ascertain a congenital myopathy or an inflammatory myopathy, myopathologic examination of a biopsied muscle specimen is essential. The myotonias are the domain of the electrophysiologist, while the clinician and geneticist identify the different hereditary forms of muscular dystrophy. However, each of the participating diagnosticians should be aware of the findings of the others within the concerted diagnostic work-up. As the muscle biopsy usually occurs at the end of the diagnostic regimen, it is the myopathologist who initiates and executes all necessary studies to be performed on the biopsied tissue is responsible for seeing that all essential techniques are readily and optimally applied to the tissue. However, the results of his studies may require additional endeavors by his diagnostic colleagues, such as family studies or further follow-ups if a diagnosis cannot be achieved at the time of the muscle biopsy. Therefore, more frequently

and more intensely than in other concerted enterprises, close cooperation and mutual information among the participants in the diagnostic program of a patient's neuromuscular condition appear mandatory. As the overwhelming majority of recently detected neuromuscular conditions have been delineated by biopsy rather than by postmortem studies, a muscle biopsy, always intended to aid in the diagnosis, should also leave room for additional scientific studies, among which sophisticated studies such as mitochondrial and nuclear DNA analyses of mitochondrial and dystrophic myopathies, respectively, only loom on the diagnostic horizon.

Acknowledgements

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The Turkish Journal of Pediatrics 36: 239-242 1994

AASE–SMITH SYNDROME: REPORT OF A NEW CASE WITH UNUSUAL FEATURES*

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*Turgay Coşkun MD*****, Burhan Say MD******

SUMMARY: Yetgin S, Balcı S, İrken G, Coşkun T, Say B. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Aase-Smith syndrome: report of a new case with unusual features. Turk J Pediatr 1994; 36: 239-242.

A new case of Aase-Smith syndrome has ben reported with additional findings, including microcephaly, axial rotation of the kidneys, preaxial polydactyly and leukopenia. The, patient had almost complete clinical remission with corticosteroid treatment. The findings in this case suggest that it is very difficult to differentiate Aase-Smith syndrome from Blackfan-Diamond and Fanconi's anemias as well as from those cases reported by Murphy and Lubin, and Jones and Thompson. *Key words: Aase-Smith syndrome, Blackfan-Diamond anemia, Fanconi's anemia.*

In 1969 the association of congenital hypoplastic anemia (CHA) and triphalangeal thumbs was described by Aase and Smith as an entity different from both Fanconi pancytopenia and the radial aplasia-thrombocytopenia (TAR) syndrome[1].

The purpose of this report is to present another case who also had unusual findings including microcephaly, axial rotation of the kidneys, preaxial polydactyly, leukopenia and chronic steatorrhea. An almost complete clinical remission upon corticosteroid treatment was observed.

Case Report

A 15-month-old female infant was seen because of pallor and growth retardation. The history showed that she was the second child born to a couple who were first cousins. The delivery was by breech presentation at term, but the baby was small for gestational age. At birth, a preaxial polydactyly of the right hand was noted. Soon after birth the infant was put on a diet of cow's milk. She had chronic steatorrhea and recurrent bronchopneumonia. At the age of 24 days, the anemia was severe enough to require a blood transfusion. There was no family history of

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hematological disease or congenital anomalies, except that the mother had a white forelock but normal auditory function. The patient's six-year-old brother was in good health. The patient had growth retardation, with a height of 68 cm, weight 5700 g, and head circumference 43 cm all being below the 3rd percentile. She also had micrognathia, a short neck, a strawberry hemangioma 2 cm in diameter on the left scapula, and bilateral proximally located triphalangeal thumbs (Fig. 1).

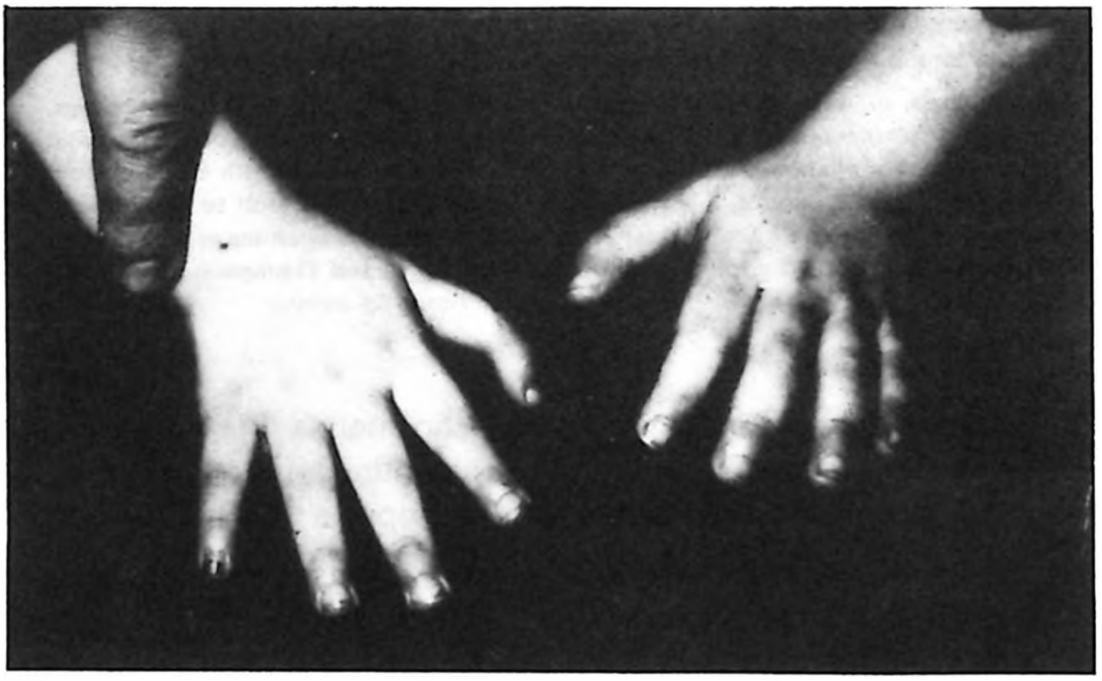

Fig. 1: Bilateral triphalangeal thumbs.

Hemoglobin was 4.87 g/dl, and white cell count was 2.8×10^9/L with 48% neutrophils, 50% lymphocytes and 2% monocytes. Reticulocyte count was 3% mean corpuscular volume (MCV) 99 fl and platelets 320×10^9/L. Red blood cells showed mild hypochromia. Bone marrow displayed normal cellularity with megaloblastoid changes, occasional fatty spaces, and increased mast cells. Chromosome analysis on cultured lymphocytes exhibited normal 46, XX karyotype. Sister chromatid exchange (SCE) and diepoxybutane (DEB) test were also normal.

Levels of serum iron and total iron-binding capacity were 11.2 and 63 µmol/L, respectively. Folic acid level was 1.9 µg/L; vit B_{12} 600 ng/L Mg 1.76 mg/dl; Zn 35 µg/dl. Total protein was 6 g/dl and albumin 3.7 g/dl. glucose-6-phosphate-dehydrogenase activity, acid ham, sugar-water test, C_3, C_4 and growth hormone levels were all within the normal limits. Duodenal fluid tryptic activity was normal, and direct Coombs tests were negative. Sweat chloride was found to be increased on four separate measurements: 61, 95, 53 and 63 mEq/L (upper normal limit 45 mEq/L). Radiologic studies showed bilateral triphalangeal thumbs and hypoplasia of carpal bones. There was axial rotation of the kidneys,

specifically over the lower pole of the left kidney, suggesting horseshoe kidney malformation. The connection was a fibrous band.

The patient was treated with iron, folic acid, multivitamins, zinc and pancreatic enzymes as well as a diet enriched with medium-chain fatty acids due to the presumptive diagnosis of cystic fibrosis. There was no satisfactory response to this therapeutic regimen. The restricted diet was discontinued, but blood transfusions were administered for three years at 3-4 month intervals. At the age of 4.5 years, the diagnostic possibility of Aase-Smith syndrome was raised on the basis of congenital hypoplastic anemia and triphalangeal thumb associated with normal SCE, DEB test and chromosomal analysis. Corticosteroid treatment (2 mg/kg po) was started, after which a significant response in Hb level (12.5 g/dl) was noted but not in WBC ($3x10^9$/L) within two months. Corticosteroid therapy was tapered to 1 mg/kg and continued for the next two months and then tapered again for one year at 5 mg daily doses. Subsequent examination revealed improved physical growth (height 93 cm, weight 15 kg), and the findings such as diarrhea and lower respiratory tract infections had more or less disappeared. Thus, the earlier findings suggestive of cystic fibrosis were considered to have been no more than a reaction to cow's milk, responding as expected to steroid therapy.

At last follow-up, the patient was 7.5 years old, 102 cm in height and 17.5 kg in weight, with a head circumference of 49 cm all below the 3rd percentile. Hemoglobin levels were consistently in the range of 10-12 g/dl. Macrocytosis had completely disappeared, but WBC counts showed no significant changes ($2\text{-}3.9x10^9$/L).

Discussion

To the best of our knowledge, only ten well-documented cases of Aase-Smith syndrome have been reported in the literature since 1969[1-11] although two other probable cases have been documented[12-13]. Our patient had characteristic findings of the syndrome, such as CHA, triphalangeal thumbs and normal SCE and chromosomal analysis. Additionally, she had short stature, a short neck, microcephaly, proximal polydactyly, urinary system abnormality and decreased WBC count, which would suggest the diagnosis of Fanconi's anemia. None of the reported patients with Aase-Smith syndrome have had similar findings. The mother's having a white forelock but normal hearing also pointed to the possibility of Fanconi's anemia. Fanconi's anemia heterozygotes are known to have some increased skin pigmentation and findings similar to those mentioned above. A few other patients are on record with multiple physical abnormalities and leukothrombocytopenia, suggesting Fanconi's anemia[2,3]. While our patient had persistent leukopenia, the normal cytogenetic findings and early onset of anemia seem to exclude Fanconi's anemia as a diagnostic possibility. Also, such a good response

to corticosteroid treatment is not usually expected in Fanconi's anemia cases, giving further support to the diagnosis of Aase-Smith syndrome.

In patients with CHA as well as Aase-Smith syndrome, recovery has been obtained by administration of corticosteroids in most cases. Spontaneous remission has also occurred in both groups of patients. Although triphalangeal thumbs is one of the cardinal findings of Aase-Smith syndrome, in 1978 Alter pointed out that triphalangeal thumbs occurred in 6 of 133 cases of CHA[14]. In 45 of these cases (34%), there was some type of hand abnormality. For that reason, Alter suggested that this syndrome is not an entity distinct from the Blackfan-Diamond syndrome[12]. It is clearly very difficult to differentiate with certainty Aase-Smith syndrome from Blackfan-Diamond and Fanconi syndromes as well as from entities described by Murphy and Lubin[2], and Jones and Thompson[3]. The etiology of Aase-Smith syndrome is still unknown; however, the occurrence in siblings and in both sexes makes autosomal recessive inheritance most likely. We believe further cases need to be studied to resolve these questions.

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VIRGINAL HYPERTROPHY*
Case Report

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SUMMARY: Küçükaydın M, Kurtoğlu S, Okur H, Patıroğlu TE, Zorlu M, Gündüz Z, Kazez A. (Departments of Pediatric Surgery, Pediatrics and Pathology, Erciyes University Faculty of Medicine, Kayseri, Turkey). Virginal Hypertrophy: a case report. Turk J Pediatr 1994; 36: 243-248.

A 13-year-old girl with virginal hypertrophy (bilateral extensive juvenile hypertrophy) of the breasts is presented. Her breasts began to grow rapidly after puberty and reached an enormous size within a year. On examination, both breasts were greatly enlarged. Routine blood chemistry and the endocrinological investigations were normal. The computerized tomography scan of the sella was unremarkable. A bilateral reduction mammaplasty was performed, and histological analysis of the breast tissue revealed the diagnosis of virginal hypertrophy. After four months her breasts began to grow again, and a second mammaplasty was performed. After this operation, tamoxifen citrate was given to prevent recurrence for four months, and during the follow-up period of 20 months, no recurrence was noted. *Key words: virginal hypertrophy, mammaplasty, tamoxifen.*

Virginal hypertrophy (infantile breast hyperplasia, juvenile hypertrophy) is an uncommon condition of massive breast hypertrophy. Histologically, it is characterised by an enormous increase in the fibrous connective tissue of the breast, and there is only moderate ductal proliferation[1]. Virginal hypertrophy is not associated with hormonal abnormalities or remote tumors and is not a premalignant lesion. Reduction mammaplasty is technically difficult because of the large size of the breast and may be followed by recurrence. In severe cases, subcutaneous mastectomy, free nipple transplant and insertion of prosthesis has been suggested[2,3]. A review of the literature revealed only a few cases in which girls required a breast reduction plasty at age thirteen. For this reason we want to present this case and stress the importance of tamoxifen to prevent recurrence.

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Case Report

A 13-year-old girl was admitted to the Pediatric Surgery Department because of extensive bilateral juvenile hypertrophy of the breasts. The patient's menarche was at age 12 with irregular periods. Her breasts began to grow rapidly after puberty and continued to grow. The patient complained of increased back pain when sitting for a long time. Socially the child was completely integrated into the family but suffered greatly because of her physical appearance and had become depressed. She was the second child in the family and had a brother and two sisters. There was no evidence of drug or medication use.

On examination, the girl weighed 46 kg (25^{th} percentile) and was 155 cm tall (25^{th} percentile). Her breasts were greatly enlarged and extended to the umbilicus (Fig. 1). Capraro's technique was used when measuring the breasts. The right breast was 40x32 cm and 1280 Capraro units; the left breast was 38x31 cm and 1178 Capraro units. (Breast measurements in a normal 13 year-old-girl according to Capraro's original technique are 15x11 cm and 165 units on each side). The diameter of the areola was 9 cm on the right and 8 cm on the left side. The skin was very taut and slightly red with many prominent superficial veins. Neither galactorrhea nor a mass was found on physical examination[4]. Pubescent development was stage V according to Tanner scales. On general physical examination, there were no abnormal findings except for kyphosis.

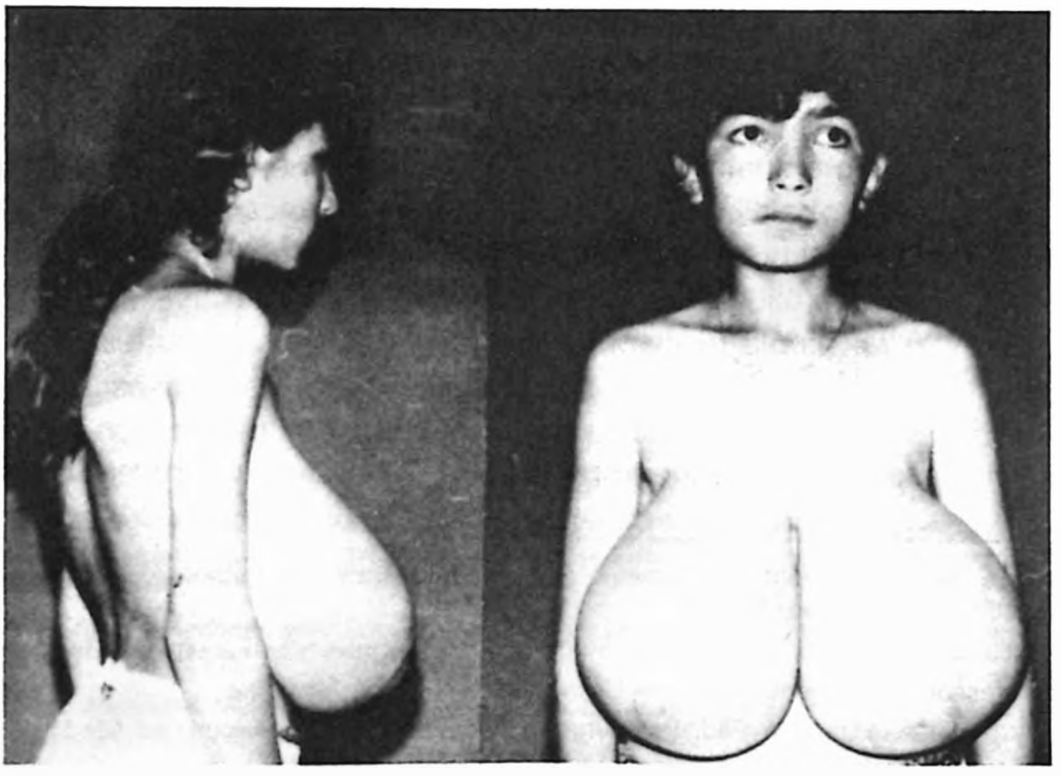


Fig. 1: 13-year-old girl. Both breasts were greatly enlarged.

Routine blood chemistry, complete blood count and urinalysis were normal. The endocrinological investigations, including baseline and thyrotropin releasing hormone-stimulated prolactin levels, baseline luteinizing hormone, follicule stimulating hormone and luteinizing hormone releasing hormone stimulated levels, were normal. Estrogen and progesterone levels were also normal. The computerized tomography scan of the sella was unremarkable. Lateral chest x-ray showed considerable kyphosis of the vertebral column which could have been caused by the weight of the breasts.

A bilateral reduction mammaplasty was performed as described by Blomquist[5], resulting in the removal of 2400 g tissue from the left breast and 2600 g from the right. Macroscopically the tissue consisted of mainly lobular parenchyma with little fat.

Histological analysis revealed a breast parenchyma with little fat infiltration. Embedded were numerous lactiferous ductules with verruciform proliferating epithelia without atypia. In a few places, the periductal stroma was myxoid (Fig. 2). These features confirmed the diagnosis of virginal hypertrophy. The patient had an uneventful recovery and was discharged from the hospital on the 24th day.

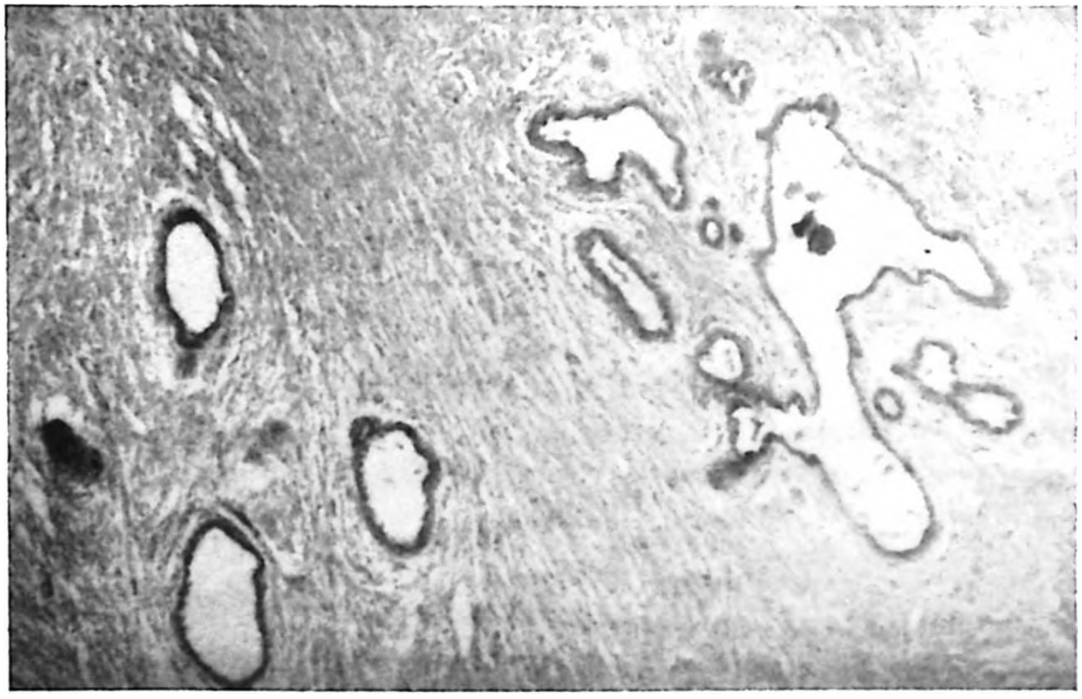
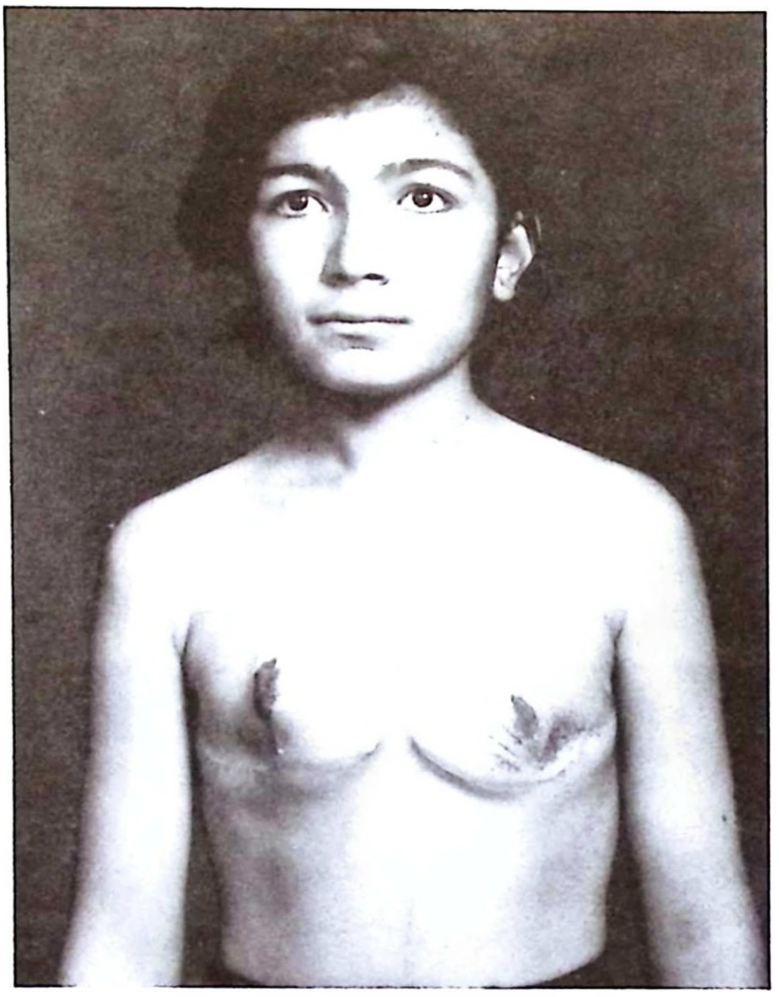


Fig. 2: Histology of excised breast tissue showing a parenchyma with numerous lactiferous ductules without atypia (H and E x 63).

Four months after the operation, her breasts started to grow again. After seven months they had enlarged significantly, and a second reduction mammaplasty was done. A total of 950 g tissue from the right and 850 g tissue from the left was resected. At that time, tamoxifen citrate 20 mg/day was given for four months.

Twenty months after the second operation, there had been no further breast growth (Fig. 3).



Fg. 3: Result 20 months after surgery.

Discussion

Virginal hypertrophy of the breast is a disease of unknown etiology. It is caharacterized by massive growth of both breasts, usually at the time of puberty. The breasts may become very pendulous and heavy. Even moderate hypertrophy can cause emotional symptoms similar to those in patients with hypoplasia. The breasts occasionally become gigantic, either at puberty or, more often, in later life in association with pregnancy. A plastic reduction in breast size is indicated whenever the breasts are large enough to cause real distress to the patient[4].

In 1974 Mayl[6] described an 11-year-old girl with virginal hypertrophy who initially underwent a reduction mammaplasty but had to undergo a complete mastectomy because of recurrence at twelve years of age. Fisher and Smith[7] reported a ten-year-old girl who needed surgery for virginal hypertrophy, and in this case, a

postoperative recurrence occurred as well. In 1977, Gardoso de Castro[8] published a case of a 12-year-old girl who underwent a breast reduction. In this case, repeated recurrence occurred and subsequently subdermal mastectomy was unavoidable. In 1991, Piza-Katzer and Umbricht-Sprüngli[9] presented an 11-year-old girl who required surgery for infantile breast hyperplasia. Postoperative recurrence did not occur.

The decision of surgery ultimately depends on the degree of suffering and the individual symptoms of the patient. Frequent complaints are: heaviness of breasts, grooves and scars on the shoulders from the pressure of bra straps, shoulder pain, back pain and deformities which may even cause kyphosis, breast tension, orthopnea, restriction of mobility, dermatoses, and headaches which often are augmented premenstrually[10]. Nearly all of the complaints mentioned above, especially kyphoses and shoulder pain, were noted in our case. In addition to somatic complaints, psychosocial symptoms were present, including poor social relations with friends, nervousness, and feelings of inferiority and insecurity. When the symptoms are so advanced, surgery is usually unavoidable in spite of the risk of recurrence.

To prevent recurrence, medications are often advised. The administration of dihydrogesterone or tamoxifen citrate has been used with some success[11]. Bilateral recurrence occurred in our patient, and after the second mammaplasty tamoxifen citrate was administered to prevent recurrence.

In less acute cases, one should not be operated on until the breast is completely developed. The initial examination should involve mammagraphy. Carcinomas are rarely found in this age-group. Cystosarcoma phylloides is found more often, and can only be verified histologically[12].

It is currently being discussed whether hormonal imbalances as well as hypersensitivity of estrogen receptors to estrogen may be the cause of infantile hypertrophy of the breasts. A frequent argument against extensive reduction plasty is that such a procedure would inhibit proper functioning of the breasts during lactation. It must be mentioned that hypertrophic breasts rarely function during lactation[3,11,13].

This report shows the importance of the use of tamoxifen citrate to prevent recurrence after surgical reduction of the breasts in virginal hypertrophy cases.

Acknowledgment

I am grateful to Mr. D.M. Burge, Consultant and Pediatric and Neonatal Surgeon, Wessex Regional Centre for Paediatric Surgery, U.K, for helping me in the detailed revision of the paper.

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The Turkish Journal of Pediatrics 36: 249-253 1994

ABSENT PULMONARY VALVE SYNDROME WITH AGENESIS OF THE LEFT PULMONARY ARTERY*

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*Süheyla Özkutlu MD****

SUMMARY: Paç A, Özme Ş, Çeliker A, Özkutlu S. (Cardiology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara Turkey). Absent pulmonary valve syndrome with agenesis of the left pulmonary artery. Turk J Pediatr 1994; 36: 249-253.

Absent pulmonary valve syndrome is represented by rudimentary nodules without identifiable leaflets, and it is commonly associated with a ventricular septal defect and tetralogy of Fallot. Unilateral absence of a pulmonary artery is also a rare congenital anomaly. The following is a case report of a one-day-old newborn with cyanosis. The physical examination revealed a to-and-fro murmur and no expansion of the left hemithorax. Echocardiography revealed tetralogy of Fallot, absent pulmonary valve, and enlarged main and right pulmonary arteries. The catheterization and angiography confirmed the diagnosis and absence of the left pulmonary artery. *Key words: absent pulmonary valve syndrome, tetralogy of Fallot, pulmonary artery agenesis.*

Absent pulmonary valve syndrome is represented by a complete absence of pulmonary valve leaflets or an uneven rim of rudimentary tissue found in the area of the pulmonary annulus. There is also stenosis of the pulmonary annulus. The most often recognized characteristic of the syndrome is aneurysmal dilatation of the pulmonary trunk and one or both of its branches[1].

This syndrome can occur as an isolated lesion and is also commonly associated with ventricular septal defect and tetralogy of Fallot[1-4]. Other malformations such as atrial septal defect, double-outlet right ventricle, patent ductus arteriosus, endocardial cushion defect, transposition of the great arteries and other complex cardiac malformations have occasionally been described in association with absent pulmonary valve syndrome[1,4,5].

On the other hand, congenital unilateral absence of a pulmonary artery is also a rare anomaly which is frequently associated with other cardiovascular abnormalities such as tetralogy of Fallot[6].

These two congenital anomalies detected together are presented because of their very rare occurrence.

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Case Report

A one-day-old female newborn was admitted to the hospital in December 1991 with the complaint of cyanosis. Birth weight was 3300 g. A marked left parasternal impulse and to-and-fro systolic and diastolic murmurs were noted along the left upper sternal border. The second heart sound was single and increased in intensity in the second left intercostal space. No expansion was seen in the left hemithorax. Auscultation revealed no sound in the left lung. The electrocardiogram showed sinus rhythm, right axis deviation and right ventricular hypertrophy. The chest radiograph revealed a homogeneously dense left hemithorax (Fig. 1). The echocardiography showed an overriding aorta with a subaortic ventricular septal defect and a markedly dilated main pulmonary artery and right pulmonary artery. Pulmonary annulus was notably narrow, and a thickened rim of tissue was found in the region of the pulmonary valve annulus (Fig. 2).

Doppler echocardiography demonstrated a 45 mm Hg systolic gradient and diastolic retrograde flow at the pulmonary valve level. The left pulmonary artery could not be detected by two-dimensional echocardiography. The patient

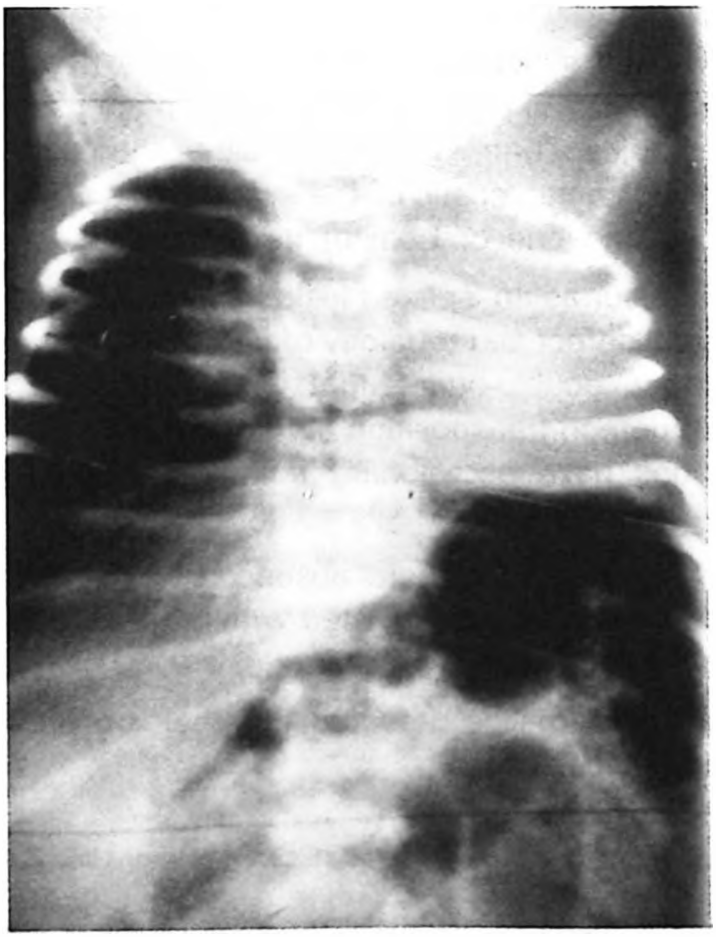

Fig. 1: The chest roentgenogram of the patient showing dense left hemithorax.

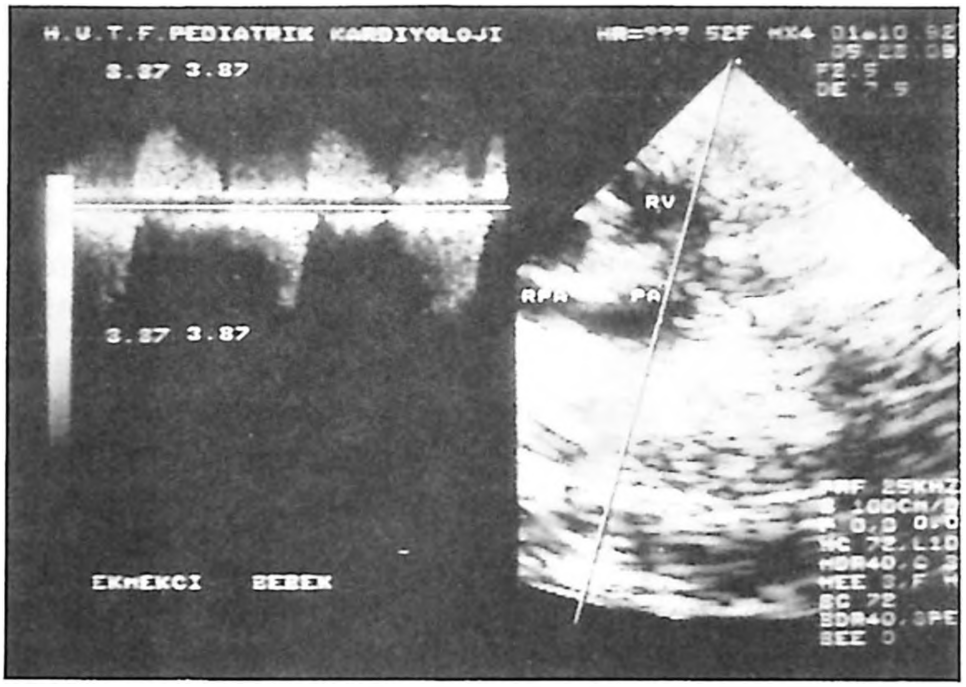

Fig. 2: The echocardiographic appearance of the patient. It shows enlarged main and right pulmonary arteries and absence of the left pulmonary artery. Doppler echocardiography demonstrates systolic flow with an important gradient across the right ventricular outflow tract and diastolic flow.

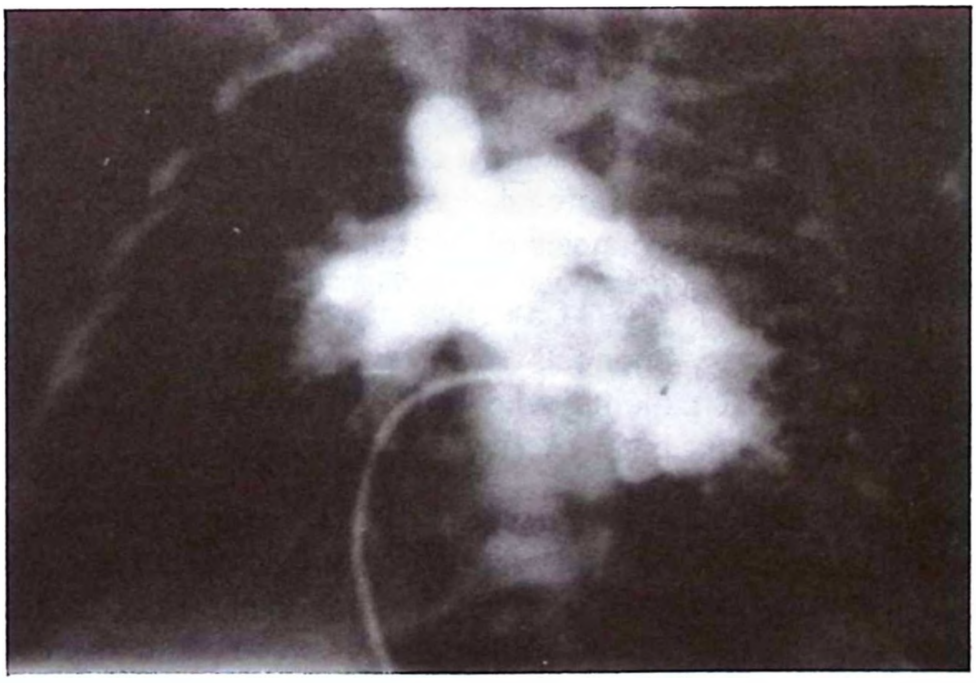

Fig. 3: Angiography of the right ventricle, revealing overriding aorta, right-sided aortic arch, dilated main and right pulmonary arteries, and absence of the left pulmonary artery.

underwent cardiac catheterization, which confirmed the diagnosis of absent pulmonary valve syndrome and detected the absence of the left pulmonary artery.

Hemodynamic findings of right-side cardiac catheterization were as follows: right ventricular systolic pressure 45 mm Hg, and main pulmonary artery systolic/diastolic pressure 16/2 mm Hg. Angiography revealed a ventricular septal defect, overriding aorta, enlarged main and right pulmonary arteries, right-sided aortic arch, and absence of the left pulmonary artery. Regurgitant flow was detected at the pulmonary valve level (Fig. 3).

Respiratory distress was not seen during the patient's ten-day hospital stay, and she was discharged. She is still being followed by the Cardiology Department.

Discussion

Absent pulmonary valve syndrome is seen in two well-defined groups. The first group comprises infants with severe respiratory symptoms in the neonatal period. Unfortunately, most infants who are symptomatic die as a result of heart failure or bronchial compression[7]. Morbidity and mortality in this group are high[7-9]. The second group comprises older children whose symptoms are less severe. Although our patient was a one-day-old newborn, she had no respiratory distress. Respiratory failure is most often due to tracheobronchial compression by the massively enlarged pulmonary arteries, and treatment has to date focused on reduction of the pulmonary arterial size[7].

Absence of the pulmonary valve is usually associated with a ventricular septal defect, significant pulmonic stenosis and a absent ductus arteriosus[7]. It has been postulated that closure of the arterial duct in early intrauterine life plays an important etiologic role in the genesis of this syndrome[10,11]. In our case as well, the arterial duct was closed.

Unilateral pulmonary artery agenesis is an anomaly uncommonly seen in the general population. Most patients who have pulmonary artery agenesis have abnormal chest x-ray films but lack respiratory symptoms.

Absent pulmonary valve syndrome can be diagnosed accurately with two-dimensional echocardiography[1,12]. There are, however, certain limitations to this technique, and evaluation of peripheral and intraparenchymal pulmonary arteries is one of them. We diagnosed absent pulmonary valve syndrome in our case by echocardiography, but the left pulmonary artery could not be seen. Therefore, the differential diagnosis was made by cardiac catheterization. We performed cardiac catheterization and confirmed the absent pulmonary valve syndrome. The left pulmonary artery was absent, and no vascular imaging was seen in the left hemithorax. Abnormalities of hilar branches of the pulmonary artery in absent

pulmonary valve syndrome were demonstrated on postmortem pulmonary angiography by Rabinovitch et al[13].

Unilateral absence of a pulmonary artery is a rare congenital anomaly that affects the right pulmonary artery more commonly than the left[6]. Typically the absent artery is on the side opposite the aortic arch, as seen in our patient. Tetralogy of Fallot is the most common underlying cardiac anomaly in patients with absence of the left pulmonary artery.

This is the first reported case of left pulmonary artery and parenchymal agenesis in absent pulmonary valve syndrome according to our knowledge of the literature in the last ten years.

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The Turkish Journal of Pediatrics 36: 255-258 1994

VACUOLATED WHITE BLOOD CELLS IN THALASSEMIA MAJOR*

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SUMMARY: Duru F, Gümrük F, Gürgey A. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Vacuolated white blood cells in thalassemia major. Turk J Pediatr 1994; 36: 255-258.

We report a case of thalassemia major in which severe cytoplasmic vacuolization was seen in white blood cells as well as in their precursors. Cytochemical examination of the vacuoles revealed Sudan Black staining. Consequently, we believe that our patient had Jordans' anomaly coexisting with thalassemia major. *Key words: vacuolated white blood cells, thalassemia major.*

Vacuolization of polymorphonuclear leukocytes (PNLs) has been described in various conditions, but is not known to be associated with thalassemia major. Here we report the occurrence of vacuolization of PNLs in a case of thalassemia major.

Case Report

A ten-year-old boy, the product of a marriage between cousins, was diagnosed with thalassemia major at the age of three years. In September 1990, when the patient was nine years old, he was referred to our institution because of an increased transfusion requirement and an extremely enlarged spleen. On admission, he looked pale with a typical thalassemic face. He was 24 kgs in weight and 120 cms in height with no signs of infection (hematological findings are shown in Table I). The differential leukocyte count revealed 72% PNLs, 5% eosinophils and 23% lymphocytes. Three to twelve round, regular, cytoplasmic vacuoles were noted in 70% of the PNLs on peripheral blood smear, a feature which had persisted since he was first diagnosed. Bone marrow aspiration smear revealed similar vacuoles in most myeloid precursors (Fig. 1). Vacuoles were stained only with Sudan Black (SB) but not with Periodic Acid Schiff (PAS) (Fig. 2). Among all of the patient's family members, scant vacuoles in a few leukocytes were detected only on his mother's peripheral blood smear.

Except for a slight increase in transaminases (SGOT 60 IU, SGPT 66 IU), all blood biochemical determinations were found to be normal, with a vitamin B_{12} level of 539 pg/ml and folic acid of 9.8 ng/ml.

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Table I: Some Hematologic Data of the Patient and His Parents (on Admission)

	Hb g/dl	WBC /mm^3	MCV fl	RBC 10^{12}/L	HbF %	HbA_2 %	Spleen in cm	Liver in cm
Patient	6.4	6500	63	2.4	17.4	7.8	10	6
Mother	10.2	8000	74	5.6	2.0	5.1	–	–
Father	10.5	9200	76	5.9	2.4	5.3	–	–

Hb : Hemoglobin.
WBC : White blood cell count.
MCV : Mean corpuscular volume.
RBC : Red blood cell count.
HbF : Hemoglobin F.
HbA_2 : Hemoglobin A_2

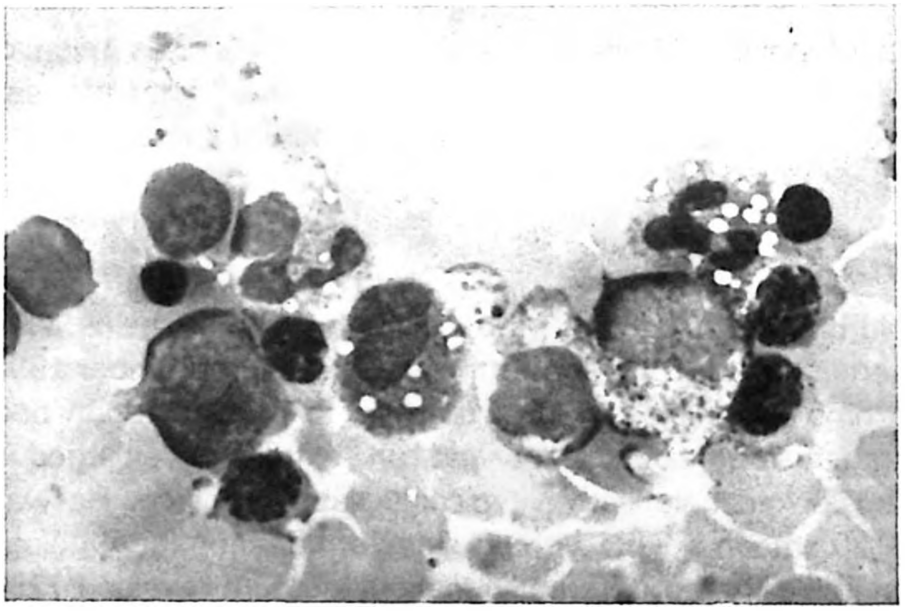

Fig. 1: Patient's bone marrow smear stained with Wright's stain.

Since vacuolization of white blood cells has been described in riboflavin and pyridoxine deficiencies, a therapeutic course of riboflavin (15 mg/day) and pyridoxine (25 mg/day) was given for fifteen days. Vacuolization in PNLs persisted following splenectomy.

Currently, the patient receives red blood cell transfusions monthly along with daily folic acid and penicillin prophylaxis.

Discussion

Vacuolization of blood cells may be found in various conditions, such as chloramphenicol administration[1], phenylketonuria[2], riboflavin[3] or copper

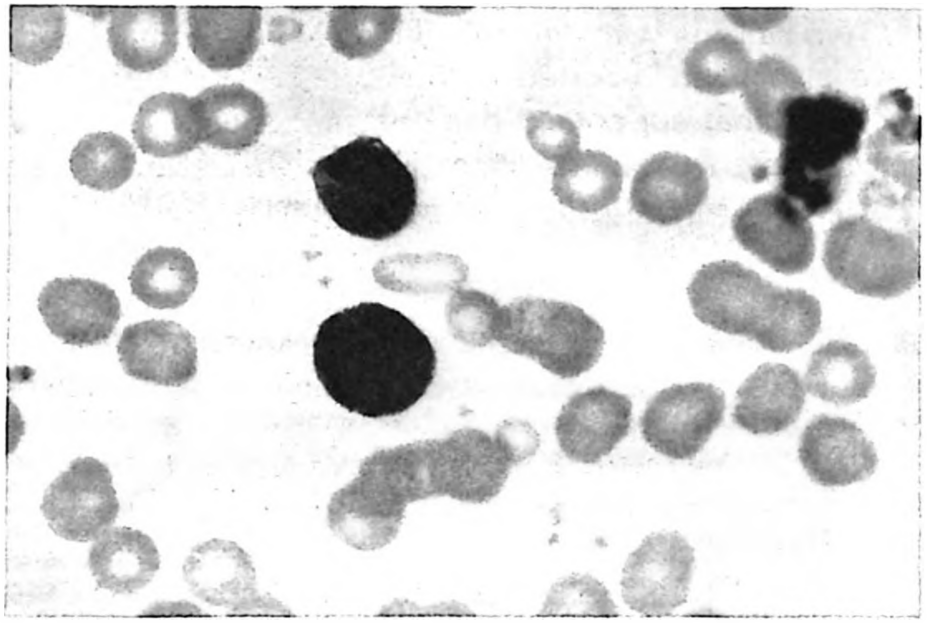

Fig. 2: Peripheral blood smear stained with Sudan Black. Note stained vacuoles in white blood cells.

deficiency[4], pyrazinamide toxicity[5], alcohol consumption[6], severe megaloblastic anemia[7] and infections[8].

In the present case, there was no history of chloramphenicol or pyrazinamide administration; the patient's alcohol usage, vitamin B_{12} and folic acid levels were normal, and no megaloblastic changes were observed on bone marrow aspiration smear. Since we were not able to determine riboflavin and pyridoxine levels he was given a therapeutic trial of these vitamins with no response. Actually, vacuolization in the above-mentioned conditions has generally been described as occurring in the erythroid cell line, whereas it was strikingly in the myeloid cell line in our case.

Vacuolization of lymphocytes has been described in association with lipid metabolism diseases[9], but in our patient's case the cholesterol and lipid levels were normal. Vacuolization in the myeloid cell line has been defined in Shwachman-Diamond syndrome[10], Pearson's bone marrow and pancreatic insufficiency syndrome[11,12]. Our patient, however, had no evidence of malabsorption or leukopenia, as is described in those syndromes. He also suffered from no severe or frequent infections, despite being diagnosed with thalassemia major. Therefore, an immunodeficiency or functional abnormality in his vacuolated leukocytes was not suspected.

In 1953, Jordans described a syndrome with the presence of fat-containing vacuoles in the white blood cells of two brothers with progressive muscular dystrophy[13]. Shortly thereafter, a similar anomaly of white blood cells coexisting with ichthyosis was reported in several families with autosomal recessive

inheritance[14]. Two siblings with Jordans' anomaly but no muscular dystrophy or ichthyosis have also been reported in Turkey[15].
We strongly believe that our patient has Jordans' anomaly and that his case is unique in the coexistence of this anomaly with thalassemia major.

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The Turkish Journal of Pediatrics 36: 259-262 1994

ACUTE HEMOLYSIS IN ASSOCIATION WITH HEPATITIS B INFECTION IN A CHILD WITH BETA–THALASSEMIA TRAIT*

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*Nurten Koçak MD***

SUMMARY: Gürgey A, Yüce A, Özbek N, Koçak N. (Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Acute hemolysis in association with hepatitis B infection in a child with beta-thalassemia trait. Turk J Pediatr 1994; 36: 259-262.

Autoimmune hemolytic anemia may occur in the course of some viral diseases such as Coxsackie virus, cytomegalovirus, Epstein Barr virus, Influenza A, herpes simplex virus, and rarely hepatitis B virus infection. The role of being heterozygous for beta-thalassemia in hemolysis during acute viral hepatitis is not known.

In this report, we present an eight-year-old boy with jaundice and anemia. The diagnoses of hepatitis B virus infection and hemolytic anemia were made on the basis of physical and laboratory findings. A hemoglobin electrophoresis revealed that the child was heterozygous for beta-thalassemia. No specific etiology could be found for hemolytic anemia. It remained unclear whether hemolytic anemia in this patient was merely a coincidental finding or whether hepatitis B virus infection and beta-thalassemia trait had played a role in causing hemolysis. *Key words: hemolysis, hepatitis B infection, thalassemia trait.*

Shortening of the erythrocyte life span occurs in the course of some viral diseases such as Coxsackie virus, cytomegalovirus, Epstein Barr virus, Influenza A and herpes simplex virus[1]. Autoimmune hemolytic anemia in the course of acute hepatitis B virus (HBV) infection has been reported rarely[2-4]. In this report, we present a child with acute, self-limited hemolysis during hepatitis B infection.

Case Report

An 8-year-old boy was brought to Hacettepe Children's Hospital for evaluation of severe anemia and hepatitis. Twenty days prior to admission he had abdominal pain, nausea, vomiting, jaundice accompanied by pallor, and passing of dark urine. Physical examination revealed a well-developed boy (75^{th} percentile for height and weight) with yellow sclera. The liver was palpable 2 cm below the right costal margin. Kayser-Fleischer ring was absent and other physical findings were unremarkable.

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The laboratory examination demonstrated a severe anemia (hemoglobin 4.04 g/dl, hematocrit 13%) and elevated reticulocyte count (18%). The peripheral blood smear showed polychromasia, anisocytosis and basophilic stippling, as well as hypochromia in some of the red cells. Total serum bilirubin was 3.1 mg/dl with a direct bilirubin of 1.6 mg/dl. Glucose-6-phosphate dehydrogenase (G_6PD) was 250 U (normal: 80-100 units). Hemoglobin electrophoresis at pH 8.6 showed an elevated Hb A_2 band. Hb A_2 was quantitated by microcolumn chromatography and was present at 6.7%. The patient's father also had beta thalassemia trait (HbA_2 5.2%). Serum copper, ceruloplasmin, direct, indirect and complement-mediated Coombs, pyruvate kinase, erythrocyte osmotic resistance, autohemolysis, cold agglutinin, methemoglobin, unstable hemoglobin, erythrocyte, urine and fecal porphyrin levels, acidified serum lysis and the sucrose hemolysis test were all normal. Hepatitis A virus antibody IgM (HAVAb IgM) was negative. Hepatitis B surface antigen (HBsAg) was positive, while anti-HBs was negative. Other laboratory tests done during the follow-up period are displayed in Table I.

The patient's hemolysis was self-limited, thus required no blood transfusion. Levamisole was given as a nonspecific immunostimulant agent for two months for the hepatitis B infection. Hemolytic anemia and hepatitis resolved over the following two months. The patient was then completely healthy. liver function tests were normal and HBsAg was negative.

Discussion

Aplastic anemia, thrombocytopenia and autoimmune hemolytic anemia may occur during or following acute hepatitis B virus infection[1,5,6]. The presented patient's clinical course, serologic findings of hepatitis B [HBsAg (+), anti-HBsAb (−)] and the disappearance of HBsAg in the fourth month of the disorder were consistent with an acute hepatitis B infection. Previously, autoimmune hemolytic anemia has been reported in three patients with viral hepatitis[2-4]. Autoimmune hemolytic anemia was not considered because of the patient's self-limited hemolysis and absence of markers for autoimmune hemolytic anemia, including Coombs tests and cold agglutinin. Autoimmune hemolytic anemia associated with viral hepatitis usually requires emergency measures including blood transfusion, steroid therapy and plasmapheresis[2-4], but none of these was given to the patient. Only levamisole, a nonspecific immunostimulant, was administered, and may have accounted for the patient becoming HBsAg negative[7]. The presence of the beta thalassemia trait in the patient is interesting. It is not known whether or not heterozygosity of beta thalassemia can play a role in hemolysis during acute viral hepatitis.

Table I: Selected Laboratory Findings

Day	Hb g/dl	Ret %	Normoblast %	MCV fl	Plasma Hb mg/dl	Haptoglobin mg/dl	AST U/L	ALT U/L	Alk.phos U/L	T.bil mg/dl	HBsAg	Anti-HBs	Anti-HBc IgM	Anti-HBc IgG	HBeAg	Anti-HBe
1	4.64	18.8	3	86	13.9	0	366	598	176	3.1	+	–				
4	6.28	19.2	2	–	–	–	–	–	–	–						
7	6.86	15.2	3	–	–	–	–	–	–	–						
14	8.68	10.4	1	–	–	–	–	–	–	–						
26	10.0	2.2	0	–	8.0	0	342	445	260	2.5						
55	11.8	0.2	0	–	–	63	46	27	316	0.6	+					
65	12.4	0.4	0	62	0.0	70	32	25	–	–	+					
131	11.9	0.4	0	62	–	–	–	–	–	–	–	–	–	+	–	+

Hb : Hemoglobin.
Ret : Reticulocyte.
MCV : Mean corpuscular volume.
AST : Aspartate amino transferase.
ALT : Alanine amino transferase.
Alk.Phos. : Alkaline phosphatase.
T.bil : Total billirubin.
HBsAg : Hepatitis B surface antigen.
Anti-HBs : Hepatitis B surface antibody.
Anti-HBc : Hepatitis B core antibody.
HBeAg : Hepatitis B e antigen.
Anti-HBe : Hepatitis B e antibody.

Possible mechanisms for HBV-associated hemolytic anemia in the patient may be immune-mediated hemolysis that could not be demonstrated, or direct destruction of red cells due to HBV. Another possibility is that the simultaneous occurrence of hemolytic anemia and viral hepatitis was a coincidental finding.

In conclusion, we recommend that screening for HBV in addition to G_6PD be done in children with hemolytic anemia during viral hepatitis.

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IS BIRTH TRAUMA RESPONSIBLE FOR IDIOPATHIC PERFORATION OF THE BILIARY TRACT IN INFANCY?*

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SUMMARY: Topuzlu Tekant G, Yiğit Ü, Bulut M. (Department of Pediatric Surgery, Şişli Children's Hospital, İstanbul, Turkey). Is birth trauma responsible for idiopathic perforation of the biliary tract in infancy? Turk J Pediatr 1994; 36: 263-266.

This case report details the clinical presentation and surgical management of a neonate with idiopathic perforation of the biliary tract. A three-day-old baby girl presented with a right-upper-quadrant mass and signs of peritonitis following a prolonged, difficult vaginal delivery. At surgery, she was found to have a perforation at the junction of the cystic and common bile duct. Simple drainage of the right upper quadrant was performed, and the patient recovered uneventfully. Early presentation and the nature of delivery suggests the possibility of birth trauma as an etiological factor in this condition. *Key words: biliary tract perforation, billary peritonitis, bile ascites, hepatobiliary imaging.*

Idiopathic perforation of the biliary tract is a relatively rare condition but is the second most common surgical cause of jaundice in infancy[1]. The first reported case was by Dijkstra in 1932 and involved a three-month-old baby girl who died following five weeks of progressive jaundice and abdominal distension. Postmortem study demonstrated a perforation at the junction of the common hepatic and cystic ducts[1].

The mechanism of perforation still remains ill-understood due to the variability of the age of presentation and symptomatology[2]. The chief aim of this report was to draw attention to birth trauma as a possible etiological factor responsible for perforation of the biliary tract in infancy.

Case Report

A three-day-old white baby girl was transferred to the Department of Pediatric Surgery of Şişli Children's Hospital due to abdominal distension, dyspnea and a delay in passage of meconium. She was a full-term 3600 g neonate, the product of a prolonged, difficult vaginal delivery, with Apgar scores of four and six at one and five minutes, respectively. She had perioral cyanosis and dyspnea that improved with administration of nasal oxygen in the neonatal unit. The following day she developed marked abdominal distension and had a delay in passage of meconium.

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Physical examination on presentation revealed a pale, ill-appearing neonate in respiratory distress with rapid and shallow respirations. Her abdomen was grossly distended with decreased bowel sounds, and a right upper quadrant mass 3 cm x 3 cm in diameter was palpated. There was a small amount of meconium passage following rectal examination.

Laboratory findings were as follows: Hematocrit 42%, white cell blood cell count 26,600/mm^3, serum bilirubin 2.8 mg/dl (conjugated 1.8 mg/dl), SGOT 20 U/L, SGPT 13 U/L, and alkaline phosphate 145 U/L. Urine analysis was normal. Radiological examination of the abdomen showed dilated loops of bowel, and the patient was transferred to our department with an initial diagnosis of intestinal obstruction. Abdominal ultrasound suggested a cystic mass occupying the upper abdomen, closely related to the intestines. The gallbladder and extrahepatic bile ducts were not visualized.

An initial diagnosis of a duplication cyst leading to partial intestinal obstruction was made. At laparotomy through an upper transvers incision, 100 ml bile mixed with blood was evacuated from the cavity behind the right lobe of the liver, and further dissection revealed a perforation of the anterior aspect of the common bile duct at the junction with the cystic duct. The gallbladder was found to be distended with blood, and manual examination resulted in drainage of the contents from the site of perforation. The peritoneal cavity was drained with a Penrose drain through a separate incision. The patient received seven days of total parenteral nutrition (TPN) and broad-spectrum antibiotics post-operatively, followed by breast feeding. Initially small amounts of bile drained from the Penrose drain, and it was removed on the 10^{th} day when the drainage had ceased. The hepatobiliary scan using Tc-99m disofenin at two weeks demonstrated a normal gallbladder and normal excretion of radioisotope into the duodenum. The patient was discharged two weeks after surgery.

The patient was readmitted two weeks after her discharge due to bilious vomiting and abdominal distension. On laparotomy, multiple adhesions leading to intestinal obstruction were lysed. The previous site of perforation was found to have healed completely.

One year later, the patient was healthy and gaining weight.

Discussion

The clinical manifestations of idiopathic biliary tract perforation may be divided into two groups[3-5]. The acute form presents with a sudden onset of shock, generalized peritonitis, vomiting, abdominal distension and leukocytosis[6]. With leakage of bile into the peritoneal cavity, jaundice, biliary ascites and acholic stools appear. The child may die due to cardiorespiratory collapse or septic shock at this stage. However, 80 percent of cases have a subacute or chronic form of

presentation[5], where jaundice may be unnoticed within the first months of life until the clinical picture is dominated by persistent jaundice, biliary ascites and abdominal distension. Our case presented clinically in the acute form, with abdominal distension that developed rapidly after birth.

The acute cases are more difficult to diagnose preoperatively, but usually have surgery earlier due to symptoms of an acute abdomen[2,6,7]. In chronic cases, diagnosis is made on the basis of clinical presentation[8,9], abdominal paracentesis[6,10] or nuclear hepatobiliary imaging[7,11]. In our case, which presented acutely, the condition was not suspected preoperatively due to the lack of more specific symptoms (i.e., jaundice, ascites) and the suspicion of a duplication cyst on ultrasound examination.

Different etiological factors for idopathic biliary tract perforation have been proposed, including trauma[5], biliary lithiasis[12], inspissated bile[13], anomalies of the biliary tract[14], congenital weakness and vascular impairment[1,15]. Johnston[8] suggested the existence of a localized developmental weakness of the wall of the common bile duct. In that case, a sudden increase in intra-abdominal pressure during labor could lead to perforation of the area of mural weakness. Whatever the cause of spontaneous perforation, the resulting bile leak leads to the formation of a biliary pseudocyst, which in turn may leak into the peritoneal cavity[11]. The early presentation of this infant with symptoms starting from birth and the history of a prolonged, difficult vaginal delivery suggest the possibility of birth trauma as an etiological factor. In addition, the intraoperative findings in this case correlate well with the early stage of pseudocyst formation. It has been proposed that not all ruptures may occur immediately at the time of injury, and the devitalized area or clot may slough in subsequent days, with a delay in the onset of symptoms[9]. This may explain the wide variation in time intervals between trauma and the onset of symptoms.

Treatment of perforation of the biliary tract is surgical. In a recent review of 70 cases, the five that were medically treated all died. On the other hand, of the 65 surgical cases, 55 have survived[2]. However, there is controversy regarding the surgical treatment options. Some surgeons prefer invasive surgical techniques such as inserting T-tube choledochal drains, or decompressing the biliary tract by cholecystostomy[1,4,5,14,16] or by draining it into the jejunum through a Roux en "Y" anastomosis[1,4,17,18]. But due to the small size and fragile nature of an infant's common bile duct, such procedures have led to further complications of perforation, stricture formation and obstructive jaundice[5,14]. Successful results have been obtained by a more conservative surgical approach, with local drainage of the right upper quadrant with or without external decompression of the gall bladder[2,4,6,7,19,20]. In the presence of choledocholithiasis, a cholecystostomy is advocated for decompression and radiological access to the biliary tree in the

postoperative period[16]. On the other hand, nuclear imaging studies are sufficient in evaluating the patency of the biliary tract following simple drainage procedures[11].

Successful postoperative management depends on TPN, complete gastrointestinal rest and broad-spectrum antibiotics with a high biliary concentration[10,16]. It is important to realize that this condition has a high curative rate with early diagnosis and appropriate surgical intervention.

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