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IgG SUBCLASSES IN SYMPTOMATIC IgA DEFICIENCY*

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SUMMARY: Tezcan İ, Ersoy F, Sanal Ö. (Immunology Unit, Hacettepe University Institute of Child Health, Ankara, Turkey). IgG subclasses in symptomatic IgA deficiency. Turk J Pediatr 34:1-4, 1992.

We evaluated IgG subclass levels in 11 symptomatic patients (ages between 3 and 22; mean 7.5 years) with IgA deficiency, seven with selective IgA deficiency, and four with low IgA levels. All patients had experienced three or more episodes of sinopulmonary infections a year. Combined IgG2-IgG4 deficiency was detected in two patients, IgG2 deficiency in one patient and IgG4 deficiency in two patients. Elevated IgG1 and IgG3 levels were detected in most of the IgG subclass deficient and sufficient patients. It is known that gammaglobulin replacement therapy reduces the frequency of infections significantly in IgG subclass deficiency. Although immunization against IgA is a risk in IgA deficiency, these patients can be treated with gammaglobulins containing low IgA. *Key words: IgG subclass, IgA deficiency, recurrent infections.*

IgA deficiency is the most common immunodeficiency disorder. The incidence has been estimated to be between 1 in 500 to 1 in 700 in the normal population[1]. Individuals with IgA deficiency have different clinical presentations; some have recurrent infections and allergic or autoimmune disorders, while others are asymptomatic.

While the factors which play a role in frequent infections are unclear, IgG subclass abnormalities have been demonstrated in some symptomatic patients with IgA deficiency[2-6].

The aim of this study is to evaluate IgG subclass levels in symptomatic IgA deficient patients and plan long-term treatment for the disorder.

Material and Methods

We evaluated 11 patients, 4 females and 7 males with IgA deficiency whose ages ranged between 3 and 22 years (mean 7.5 years). All patients had frequent episodes of sinopulmonary infections which occurred three or more times a year. Serum IgA levels were undetectable in seven patients who were defined as

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selective IgA deficiency. Four patients, whose serum IgA levels were two standard deviations below the age-matched control values, were classified as patients with low IgA levels.

Serum immunoglobulin levels (IgG, IgM, IgA) were measured by nephelometry (Behring, Marburg). IgG subclasses were determined by enzyme-linked immunosorbent assay (ELISA-Serotec, England). IgG subclass levels were compared with the levels of the age-matched healthy controls reported by Oxelius[7]. The values that were two standard deviations below or above the age-matched control levels were considered abnormal.

Results

IgG1 levels were highly elevated in nine out of 11 patients (Table I). Normal values were observed in two patients, one (IA) with selective IgA deficiency and the other (EK) with a low IgA level.

TABLE I: IgG Subclass and IgM Levels in Patients with Symptomatic IgA Deficiency (g/L)

	Age (Yrs)	IgG1	IgG2	IgG3	IgG4	IgM
1. BK	3	13.4[a]	2.8	0.50	0.4	2.0[a]
2. EB	15	>20.8[a]	3.8	1.25	0.5	2.5[a]
3. FA	3	>20.8[a]	0.6	1.35[a]	<0.01[b]	3.3[a]
4. OI	10	>20.8[a]	6.7[a]	2.75[a]	0.09	1.6
5. ES	5	15.6[a]	2.6	1.65[a]	0.04	0.7
6. GD	9	17.5[a]	1.05	1.35[a]	<0.01[b]	1.6
7. IA	12	7.4	<0.42[b]	1.30	0.04	0.6
8. SU*	22	>20.8[a]	<0.42[b]	0.33	<0.01[b]	1.6
9. EY*	3	13.7[a]	0.1[b]	1.80[a]	<0.01[b]	1.2
10. BY*	5	14.2[a]	2.7	0.75	0.7	0.9
11. EK*	5	5.8	5.5[a]	1.75[a]	0.17	1.8

* patients with low IgA levels
a: two standard deviations above the age-matched mean
b: two standard deviations below the age-matched mean

Low IgG2 levels were observed in three patients, one (IA) with selective IgA deficiency and two (SU, EY) with low IgA levels. High levels were detected in two patients (OI, EK).

IgG3 levels were found to be elevated in six patients, while normal levels were found in five patients including two with IgG2 deficiency (SU, IA).

Four patients (SU, EY, FA, GD) had low IgG4 levels, two of whom (SU, EY) also had IgG2 deficiency. The IgG4 level was elevated in one patient (BK).

IgM levels were found to be elevated in three selective IgA deficient patients, one of whom also had IgG4 deficiency.

Discussion

In this study, we found several IgG subclass abnormalities in symptomatic IgA deficient patients. Combined IgG2-IgG4 deficiency was determined in two patients, selective IgG2 deficiency in one patient and IgG4 deficiency in another two patients.

The association of IgG subclass deficiency and recurrent infections has been described in several studies[5,8-10]. Most of the protein antigens induce IgG1 antibodies whereas the majority of antibodies against polysaccharide antigens are in the IgG2 subclass. IgG2 antibody deficiency is one of the factors responsible for increased susceptibility to infections caused by *H. influenza,* pneumococci and other encapsulated microorganisms. IgG subclass abnormalities have been reported in IgA deficient patients[2,3,5]. The incidence of subclass deficiencies in IgA deficient patients with recurrent infections is reported as 20-35 percent, and IgG4, IgG2 and IgG2-IgG4 deficiencies have been found to be the most common deficiency patterns. The incidence of IgG2 subclass deficiency found to be 27 percent in our patients, is consistent with results reported in the literature[3,6]. Several studies on IgG subclass concentrations in healthy children and adults have been reported[7,11,12]. Due to the variability of the age-normal range of immunoglobulins in different populations, a description of age-normal IgG subclass limits is needed in our population in order to determine the real incidence of IgG subclass deficiency[13].

IgG3 deficiency has been reported in a few patients with IgA deficiency[5]. IgG3 deficiency was not detected in our patients. The IgG3 level is the most often conserved, and experimental studies suggest that the expression of this subclass is less T dependent[14].

IgG1 and IgG3 levels were found to be elevated in most of our patients with deficient and normal IgG subclass levels. Increased levels might be related to frequent infections, as a compensatory mechanism. High levels of IgG1 and IgG3 do not always correlate with low IgG2 values in IgA deficiency[2,3,5].

A few reports indicate that panhypogammaglobulinemia may develop in some IgA-IgG subclass deficient patients during the follow-up period[5,6]. Thus, the immunoglobulin levels of patients should be reevaluated periodically.

IgG subclass deficiency is not the only factor responsible for frequent infections in IgA deficiency since recurrent infections also occur in patients with normal IgG subclass values. In recent years, IgA deficient patients with a defective response to polysaccharide antigens, in spite of normal IgG subclass values, have been reported[15].

IgG subclass deficient patients generally respond well to gammaglobulin therapy[4,8,16]. Although immunization against IgA is a risk in IgA deficiency, these patients can be treated with gammaglobulins containing low IgA.

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IMMUNOFLUORESCENCE STUDY OF CHILDHOOD RENAL AMYLOIDOSIS*

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SUMMARY: Tınaztepe K, Güçer KŞ (Nephropathology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Immunofluorescence study of childhood renal amyloidosis. Turk J Pediatr 34:5-14, 1992.

In this paper, the findings of immunofluorescence (IF) studies of 57 patients with childhood biopsy-proven renal amyloidosis are presented. All specimens were investigated by the direct IF technique and the simultaneous use of antisera to human IgG, IgM, IgA, fibrinogen and C3. Antisera to C1q, C4, HbsAg, IgE (in each of ten cases), kappa and lambda light chains of immunoglobulins (Igs) and albumin (in each of five cases) were also used. AA type amyloidosis was determined in all patients by Wright's potassium permanganate reaction. In thirty-four of these patients (60%), Familial Mediterranean Fever (FMF)was found to be the underlying disease for renal amyloidosis.

In 39 cases (68.5%), renal biopsy showed positive fluorescence staining while in 18 cases (31.5%), fluorescence staining was negative. The immunofluorescence pattern of glomerular deposits was neither granular nor linear but large isolated or confluent masses which were located in the mesangium and in the capillary walls, and were similar in all cases whatever the antisera used. The areas showing immunofluorescence staining almost corresponded to the locations of amyloid deposits. Immunoreactants showed various combinations of deposition with the exception of IgE, HbsAg and albumin antisera which yielded continuously negative reactions. C3 was the immunoreactant most commonly encountered. Kappa and lambda light chains of Igs were demonstrated in one of five biopsy specimens tested. Although it was not diagnostic, this IF pattern was found to be rather characteristic.

Demonstration of immunoglobulins and other components of the humoral immune system is not a rare occurrence in renal amyloidosis, and passive absorption of plasma proteins does not simply explain these immunohistologic findings. *Key words: immunofluorescence, immunoglobulins, renal amyloidosis, childhood.*

Amyloidosis is a disorder of protein metabolism characterized by the accumulation of abnormal extracellular deposition of an eosinophilic and hyalin material which is recognized on light microscopy by its distinctive staining properties and its fibrillary structure[1]. Despite great advances in the characterization of the chemical nature of amyloid fibrils, the precise mechanism of amyloid fibril formation and deposition remains unsettled. In recent years, two predominant types of amyloidosis, AA and AL, have been histochemically recognized. Immunoglobulin light chain-related deposits (AL) have been found to be the major

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components of amyloid fibrils obtained from patients with primary amyloidosis whereas the presence of amyloid protein A has been found to be associated with amyloid fibrils of the AA type in secondary amyloidosis[2-4].

Several authors have reported the presence of immunoglobulins, complement components and fibrinogen in amyloid deposits, and these proteins have been postulated to play a role in the pathogenesis of amyloidosis[5-9]. However, little systematic data are available in the literature regarding immunofluorescence findings and immunohistologic patterns in human renal amyloidosis.

This study describes direct immunofluorescence findings in childhood renal amyloidosis using fluorescein-labeled antisera specific for various human immunoglobulins, complement components and other serum proteins.

Material and Methods

The study population consisted of 57 patients with tissue-diagnosed renal amyloidosis. There were 33 males and 24 females, with a male/female ratio of 1.4:1. The ages of the patients ranged between five and eighteen years. Significant clinical data of these patients are listed in Tables I and II. Fifty-five needle and two open-biopsy specimens of 57 cases were studied.

Renal tissue samples were processed for conventional light and immunofluorescence microscopic studies. Representative sections contained at least three glomeruli. Specimens were fixed in Duboscq-Brasils solution and embedded in paraffin for light microscopic examination. Sections cut at 2-4 microns were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), Jones Silver, Masson's trichrome and trichrome-L stains. The tissues were sectioned at 6 microns and stained with the Buchtler modification of the Congo red stain. The presence of amyloid was confirmed in the Congo red stained sections by demonstration of an apple-green birefringence under polarized light.

The differentiation between AA and AL type amyloid was made by Wright's potassium permanganate reaction[10] and evaluating the presence or absence of any recognized associated disease.

For study by immunofluorescence microscopy, biopsy material obtained from all patients was rapidly frozen in a mixture of CO_2 and acetone and sectioned at 2 microns in a Tissue-TEK II Cryostat at −20 °C, and incubated with fluorescein-conjugated antisera specific for human IgM, IgA, IgG, fibrinogen and C3 (Behringwerke AG Laboratories, Marburg). Antisera to C1q, C4, HbsAg and IgE (in each of ten cases) were also used. Staining with antisera for kappa and lambda light chain of Igs and albumin was accomplished in each of five cases. Sections were immediately examined under an ortho-lux Leitz microscope with an epifluorescent lamp.

Results

All specimens demonstrated evidence of amyloid deposition both histochemically and histopathologically. They showed green birefringence of Congo red positive tissue sections when examined by polarized microscopy (Figs. 1, 2). The secondary (AA) type of amyloidosis was determined in all cases by the use of the potassium permanganate ($KMnO_4$) reaction. The glomeruli mostly contained amyloid deposits whereas the other elements of the nephron such as the tubular basement membranes and vessels were rarely affected (Table III). In general, Familial Mediterranean Fever (FMF) was the condition most commonly associated with renal amyloidosis in the cases we studied (60%) (Tables I, II).

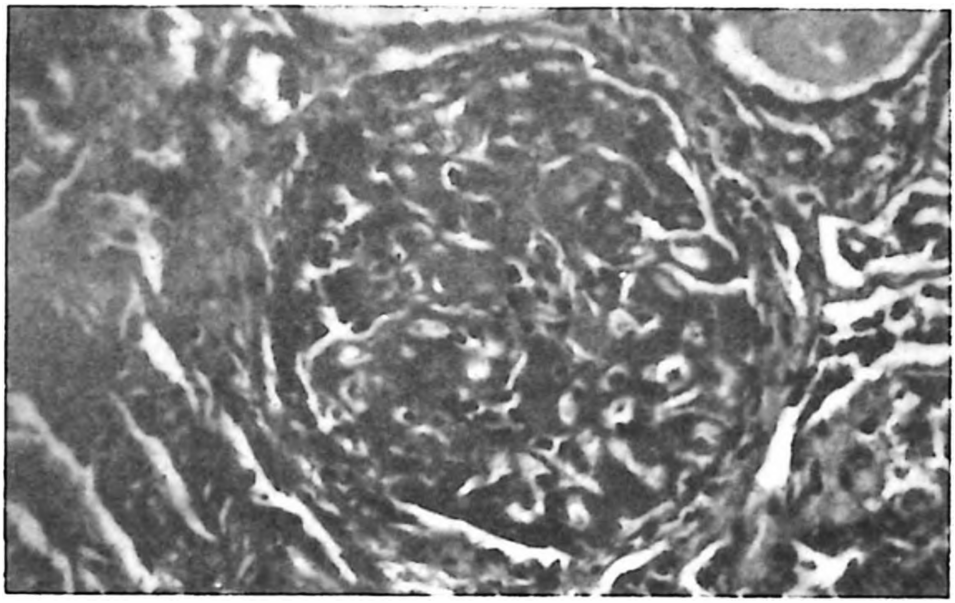

Fig. 1: Diffuse amyloid involvement of glomerular capillary wall (lower left) (Congo red × 450).

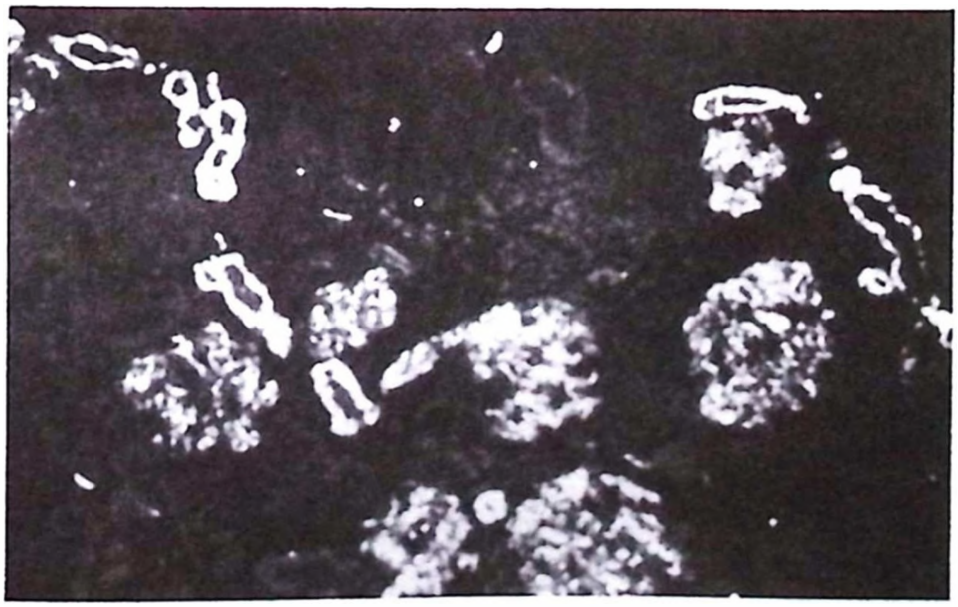

Fig. 2: Diffuse amyloid involvement of glomeruli tubuli and vessels under polarized light (Congo red × 90).

TABLE I: A Summary of Clinical Data of 39 Patients with Renal Amyloidosis Showing Positive Staining With Immunoreactants

No. of Patient	Age / Sex (yrs)		Associated Condition	Serum Creatinine (mg/dl)	Proteinuria (g/24 hr)
1	6	F	UD	0.4	4.6
2	5	F	FMF	0.6	2.0
3	14	M	MPGN	1.8	4.0
4	12	M	FMF	0.6	4.4
5	13	F	FMF	0.6	4.4
6	12	M	FMF	0.4	4.2
7	12	F	FMF	0.6	4.0
8	14	F	UD	6.0	2.6
9	16	F	FMF	0.4	3.3
10	13	F	FMF	0.8	0.5
11	14	F	FMF	0.8	4.4
12	5	F	FMF	0.6	2.6
13	9	M	Hodgkin's L.	0.6	0.1
14	12	M	UD	0.6	3.6
15	13	M	FMF	0.5	1.0
16	11	M	FMF	0.5	2.4
17	12	M	FMF	0.8	0.4
18	18	M	FMF	0.5	2.5
19	11	M	FMF	0.6	1.0
20	13	F	Tuberculosis	0.3	3.0
21	15	M	UD	0.6	2.6
22	13	M	FMF	0.6	1.3
23	10	M	FMF	0.5	1.7
24	15	M	FMF	0.3	6.0
25	15	F	Hodgkin's L.	0.5	1.0
26	11	M	Bronchial Asthma	0.5	2.7
27	14	M	FMF	1.0	4.9
28	11	M	FMF	0.3	3.3
29	14	F	UD	1.8	2.1
30	15	M	UD	1.0	3.2
31	12	M	FMF	0.8	11.0
32	18	M	UD	1.0	1.6
33	9	F	JRA	0.4	5.0
34	15	F	UD	0.8	–
35	13	M	UD	0.8	5.0
36	14	M	Tuberculosis	0.8	3.9
37	11	M	FMF	0.5	10.0
38	7	F	FMF	0.5	4.8
39	12	M	FMF	0.5	5.4

UD : Undetermined.
FMF : Familial Mediterranean fever.
MPGN: Membranoproliferative glomerulonephritis.
JRA : Juvenile Rheumatoid Arthritis.

TABLE II: A Summary of Clinical Data of 18 Patients with Renal Amyloidosis Demonstrating Negative Immunofluorescence

No. of Patient	Age / Sex (yrs)		Associated Condition	Serum Creatinine (mg/dl)	Proteinuria (g/24 hr)
1	12	F	Cr.Pyelonephritis	–	–
2	9	M	UD	–	–
3	13	M	FMF	1.0	4.0
4	8	M	FMF	1.2	2.5
5	13	F	UD	0.8	4.5
6	13	F	FMF	1.0	2.0
7	12	F	FMF	0.6	4.0
8	11	F	FMF	0.6	3.5
9	12	F	FMF	0.6	3.8
10	12	M	FMF	0.8	6.7
11	17	M	FMF	1.2	2.5
12	15	M	FMF	3.8	–
13	15	M	JRA	0.6	5.0
14	13	M	UD	0.2	4.1
15	8	F	FMF	0.6	2.9
16	13	F	FMF	7.0	4.8
17	7	M	JRA	0.4	2.5
18	11	F	UD	0.6	3.1

UD : Undetermined.
FMF: Familial Mediterranean fever.
JRA : Juvenile Rheumatoid Arthritis.

TABLE III: Localization of Immune Reactants in the Glomeruli of 39 Patients With Renal Amyloidosis

Glomerular Location	Number of Patients
Mesangium only	16
Mesangium + GBM	9
Mesangium + GBM + TBM	6
Mesangium + TBM	4
Mesangium + Vessels	2
Mesangium + GBM + TBM + Vessels	2

GBM: Glomerular basement membrane.
TBM : Tubular basement membrane.

On immunofluorescence microscopy, there was no staining in the specimens of 18 of 57 patients (31.5%). All biopsy material was classified into two groups. Group I consisted of the biopsies of patients performed before 1981 and studied with monospecific antisera to IgG, IgA, IgM C3 and fibrinogen. The specimens of 42 patients were included in this group, and 16 (35%) of them showed no staining. Group II consisted of the specimens of 15 patients which were studied with additional specific antisera. Tests for immunofluorescent staining of HbsAg, IgE and albumin were all negative. Positive fluorescence was demonstrated in one of five cases tested with kappa and lambda light chain of Igs. Both groups showed that C3 was the immunoreactant most commonly encountered in the amyloid deposits except for C1q in the second group (Tables IV, V). Of the staining patterns, C3, IgM and fibrinogen were the most common combination patterns.

TABLE IV: Distribution of Deposition of Immunoreactants in Biopsy Specimens of Group I Patients (42)

Immunoreactant	No. of Cases Studied	No. of Positive Stainings
C3	42	21 (50.0%)
Fibrinogen	42	20 (47.4%)
IgM	42	18 (42.8%)
IgA	42	9 (21.4%)
IgG	42	7 (16.6%)

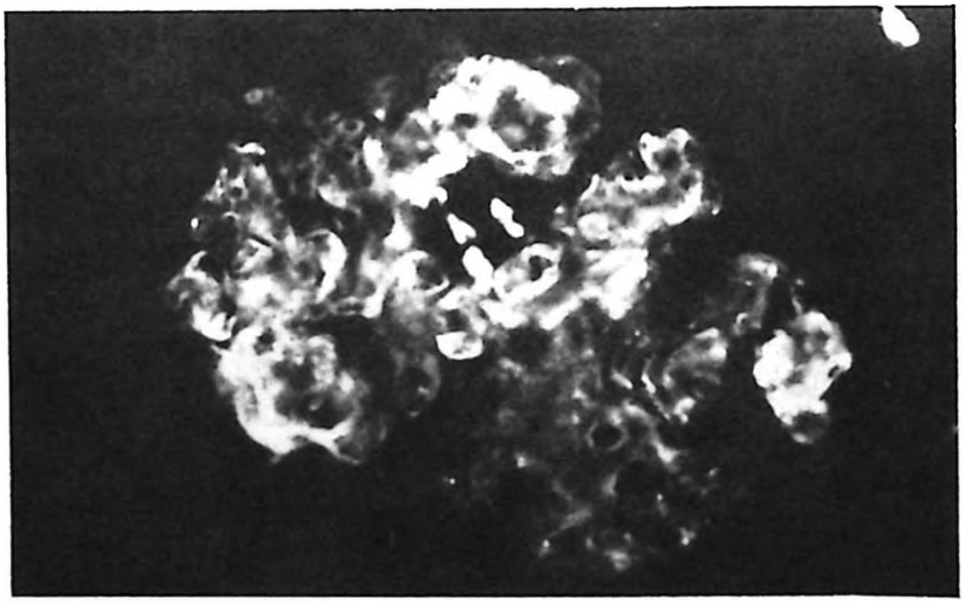

Fig. 3: Large, confluent mass of IgM in the mesangium; typical pattern of fluorescent staining in renal amyloidosis (fluorescence microscopy × 450).

TABLE V: Distribution of Deposition of Immunoreactants in Biopsy Specimens of Group II Patients (15)

Immunoreactant	No. of Cases Studied	No. of Positive Stainings
C3	15	10 (66.6%)
IgM	15	8 (53.3%)
Fibrinogen	15	8 (53.3%)
IgG	15	5 (33.3%)
IgA	15	2 (13.3%)
C1q	10	8 (80.0%)
C4	10	3 (30.0%)
HbsAg	10	0 (00.0%)
IgE	10	0 (00.0%)
Lambda light chain	5	1 (20.0%)
Kappa light chain	5	1 (20.0%)
Albumin	5	0 (00.0%)

Immunofluorescence of amyloid deposits was rather characteristic whatever the antisera used. Positive fluorescence in the specimens was mainly in the mesangium of the glomeruli (Figs. 3, 4). Glomerular depositions formed characteristically large isolated or confluent round masses which were localized to

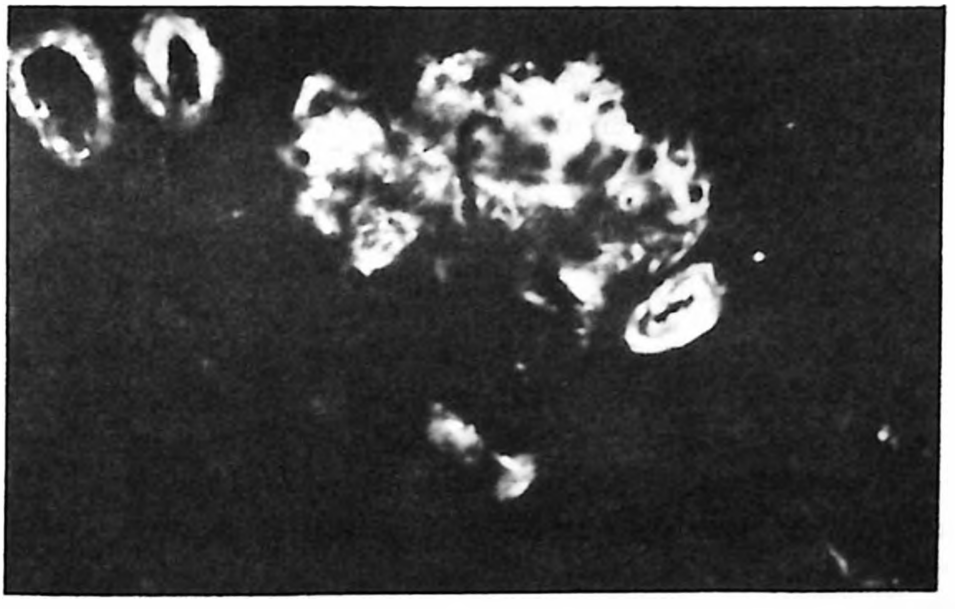

Fig. 4: Diffuse and homogenous deposition with C1q in the glomerulus. There is also staining of preglomerular arterioles (fluorescence microscopy × 350).

mesangial areas and capillary walls (Figs. 4, 5). A few specimens showed staining along the glomerular and tubular basement membranes. There was also staining of tubular casts and arterial walls (Fig. 4).

The localization of immunoreactants and amyloid deposits observed by light microscopy evidenced good conformity.

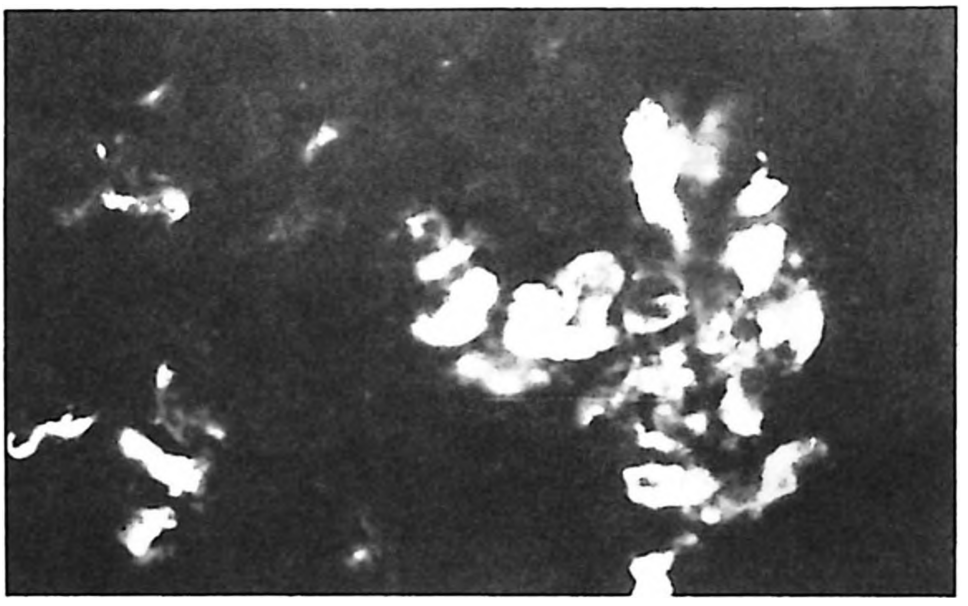

Fig. 5: Nodular deposition of C3 in the mesangium in a glomerulus (fluorescence microscopy × 450).

Discussion

In this study, it was found that FMF was the underlying desease in the majority of renal amyloidosis cases studied. This result correlated well with Turkey's geographic location. With the advances in treatment of infectious diseases, FMF has recently replaced suppurative conditions as a cause of amyloidosis in our country[7,11-13] (Tables I, II). Of 57 cases in this study, 13 (22.8%) showed neither clinical nor laboratory findings of any recognizable underlying disease of renal amyloidosis. These patients were thought to be phenotype II, because AA type renal amyloidosis might have preceded the acute attacks and was possibly the sole manifestation of FMF[14].

The presence of immune complexes in glomerular capillary walls provides substantial evidence for an immunological mechanism in renal disease[15]. In several studies, deposits of immunoglobulins and other components of the humoral immune system have been demonstrated immunohistologically in both primary and secondary amyloidosis[5,9-16]. In many of these reports, amyloid deposits have been demonstrated to localize primarily in mesangial areas. Our study confirmed previous studies. The other elements of the nephron were also demonstrated to have been involved in a number of our patients (Table III). The

distribution of mesangial amyloid fluorescent deposits may resemble immune-complex glomerulonephritis but differs morphologically. The fluorescence pattern in amyloidosis is nongranular, nonlinear and homogenous, which is quite different from the granular pattern of immune complex diseases and the linear pattern of anti-glomerular basement membrane antibody disease (Fig. 4).

Glenner et al[17] reported that a relationship existed between amyloid and light chains of Igs and showed that it was possible to create amyloid fibrils from certain Bence-Jones proteins by proteolytic cleavage in vitro. The major protein found in some amyloid fibrils is a portion of an immunoglobulin (Ig) which might point to its being causally involved in renal amyloidogenesis[7].

In recent years, it has been determined that the formation and depositions of amyloid fibrils depend on the presence of an autologous protein precursor which is either a protein circulating or produced locally with proteolytic cleavage by macrophages, endothelial cells, etc[2-4]. This could be a circumstantial, immunological role played by glomerular epithelial cells in renal amyloidogenesis. On the other hand, staining with antisera to IgE, albumin and transferrin has been reported to be consistently negative[6-8], and immune complexes have been demonstrated in some AA and immunocytic amyloidosis cases[9-13]. Due to these results, passive nonselective absorption of plasma proteins cannot account for the immunofluorescence findings observed in a few cases of FMF[9-13].

In this study, we observed positive fluorescence in 68.5 percent of all cases, which is in accordance with the study of Jerath et al[8] and Orfila et al[9]. In most of our cases, C3 was found with variable amounts of IgG, IgA and IgM (Tables IV, V). C3, IgM and fibrinogen were the most commonly encountered immunoreactants in the group I specimens we studied. The biopsies of group II, of which additional C1q stainings were applied, yielded about the same results, but C1q (80%) was observed more frequently than C3 (66%), IgM (53.3%), IgG (33.3%) and others (20.0%-13.3%) (Table V). A positive reaction for IgG was noted in 12/57 (21.0%) of overall cases. This value differs slightly from that reported by Müftüoğlu et al[7], Jerath et al[8] and Orfila et al[9].

The staining patterns were homogeneous, non-granular and nonlinear (Figs. 4, 5). The immunofluorescent deposits were observed to form round and confluent masses, mostly in the mesangium. They were also observed to be present along the glomerular and tubular basement membranes, on the vascular walls and in the tubular casts, similar to results mentioned in the literature[7-9] (Table III). The immunofluorescence patterns were rather characteristic and similar in all patients except for minor differences related to primary disorders. The lambda and kappa light-chains of Igs were found in 1/5 (20%) of our patients but albumin and IgE were consistently absent in the tested cases. In view of these biologic data, it is possible that these immunoreactants may be involved in the development of renal

lesions in amyloidosis though their exact role is not clear. It is, therefore, necessary to perform further detailed investigations to elucidate the true mechanism with regard to the presence of humoral components of the immune system and the roles they play in renal amyloidogenesis.

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EVALUATION OF THE HYPERCOAGULABLE STATE BY MEASURING PROTEIN C AND ANTITHROMBIN III LEVELS IN NEPHROTIC SYNDROME AND IN FAMILIAL MEDITERRANEAN FEVER-RELATED AMYLOIDOSIS*

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SUMMARY: Topaloğlu R, Saatçi Ü, Bakkaloğlu A, Beşbaş N, Başsoy Y. (Pediatric Nephrology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Evaluation of the hypercoagulable state by measuring protein C and antithrombin III levels in nephrotic syndrome and in Familial Mediterranean fever-related amyloidosis. Turk J Pediatr 34:15-20, 1992.

The levels of protein C (PC) and antithrombin III (AT III) antigens (ag) were measured in the plasma of 39 patients with various histologic types of primary nephrotic syndrome (NS) and in 12 patients with amyloidosis secondary to familial Mediterranean fever (FMF). The controls comprised 15 healthy children. Normal or elevated PC levels were observed in primary NS patients (mean 64%, range 36-98%) and in amyloidosis patients (mean 58%, range 48-70%).

There was no difference found between PC ag levels in primary NS and in amyloidosis patients. In addition, no correlation existed between protein selectivity and the PC ag levels in the primary NS patients. Normal and decreased levels of AT III were observed (mean 29 mg/dl, range 11.1-39 mg/dl) in the patients with primary NS and amyloidosis (mean 31 mg/dl range, 21-39 mg/dl). The AT III ag levels of these two groups did not differ and no correlation was found between protein selectivity and AT III levels in primary NS patients.

These results suggest that in patients with primary NS, or amyloidosis secondary to FMF, hypercoagulability is not related to a deficiency in PC ag levels due to a dynamic balance between urinary losses, increased rate of hepatic synthesis, catabolism and the distribution of PC in the body compartments. Patients with low AT III levels may be more susceptible to thromboembolic complications than patients with normal levels. *Key words: primary nephrotic syndrome, amyloidosis, protein C, antithrombin III.*

Protein C (PC) is a vitamin K dependent major anticoagulant which upon activation in vivo by thrombin binds to endothelial thrombomodulin and rapidly cleaves and destroys the procoagulant functions of activated factor V and factor VIII and also stimulates fibrinolysis[1,2]. Antithrombin III (AT III), an alpha$_2$ globulin synthesized in the liver, is the major inhibitor of thrombin and several other serine proteases such

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as activated factor IX and X. Both inherited and acquired deficiencies of AT III or PC are the major causes of recurrent venous or arterial thrombosis[1,3].

Patients with nephrotic syndrome (NS) are at high risk of venous thromboembolism. This fact has been attributed to abnormalities in the concentration of almost every coagulation factor and inhibitor as well as the platelets and fibrinolytic enzyme system[4].

Activity and antigen levels of PC and AT III have also been investigated as one of the possible causes of thromboembolic tendencies in nephrotic patients. But conflicting data have been published on changes in AT III and PC levels of NS patients[5-11].

In view of this controversy, the present study was undertaken to examine PC and AT III antigen levels in primary NS patients and also in a group of FMF-related amyloidosis patients. This is a preliminary report about PC and AT III antigen levels in patients with amyloidosis secondary to FMF.

Material and Methods

The study population consisted of 39 patients with primary NS (18 males and 21 females) aged between 2 to 17 years, and 12 patients with amyloidosis secondary to FMF (8 males and 4 females) aged between 6 and 19 years. The controls comprised 15 healthy children.

Proteinuria exceeding 2 g/m^2/day and hypoalbuminemia of a value of 2.5 g/dl or lower were considered the criteria for NS.

Protein selectivity was determined by the ratio of IgG clearance to albumin clearance. A ratio of < 0.1 indicated selective proteinuria while a ratio of > 0.1 indicated non-selective proteinuria.

In FMF patients, the diagnosis of amyloidosis was confirmed by renal biopsy.

All patients had normal creatinine clearance, SGOT, SGPT and PTT levels.

For PC and AT III antigen levels, blood samples were added to one ninth volume of 3.8% trisodium citrate anticoagulation solution. The plasma was separated by centrifugation for 10 min at 3000 rpm. Plasma samples were frozen immediately and stored at −20 °C. The AT III ag concentrations were measured using the Behring nor-partigen AT III kit which is based on the radial immunodiffusion method. Normal values were expressed as a percentage or g/dl of normal (normal 73-130% or 0.22-0.39 g/dl). PC concentrations were measured using the Asserachrom Protein C kit and the ELISA method. All values were expressed as a percentage of a normal plasma pool. Our laboratory normal ranged between 25-57% ($\bar{x} = 41\% \pm 16$). The Fischer's exact probability test and the Chi-square test were used for statistical analysis.

Results

No reduction was observed in the PC ag levels in the plasma of the primary NS patients and the amyloidosis patients when compared with the controls. The mean PC level was 64 percent (range 36-98%) in primary NS patients and 58 percent (range 48-70%) in amyloidosis patients (Fig. 1). This result was statistically insignificant ($p > 0.05$; Fig. 1).

Both normal and elevated PC ag levels were noted in primary NS patients and their relation to protein selectivity was studied. No correlation was found between PC levels and protein selectivity ($p > 0.05$; Table I).

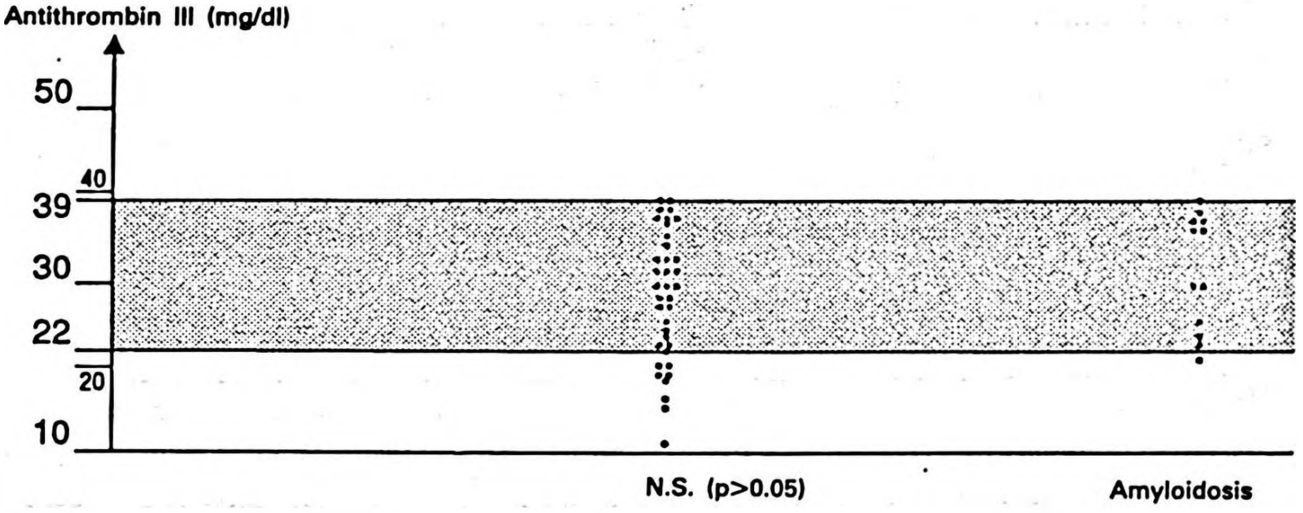

Fig. 1: Antithrombin III antigens levels in nephrotic syndrome and amyloidosis.

TABLE I: Protein C Antigen Levels and Protein Selectivity in Nephrotic Syndrome

	Protein C	
	Normal	Elevated
Nephrotic syndrome with selective proteinuria	11	13
Nephrotic syndrome with nonselective proteinuria	5	10

$p > 0.05$

AT III ag levels were either decreased or normal in primary NS patients (mean 29 mg/dl, range 11.1-39 mg/dl) while these levels were normal in all amyloidosis patients (mean 31 mg/dl, range 21-39 mg/dl) except for one. AT III levels were not significantly different in primary NS patients and amyloidosis patients ($p > 0.05$; Fig. 2). The AT III ag level was not correlated with protein selectivity ($p > 0.05$; Table II).

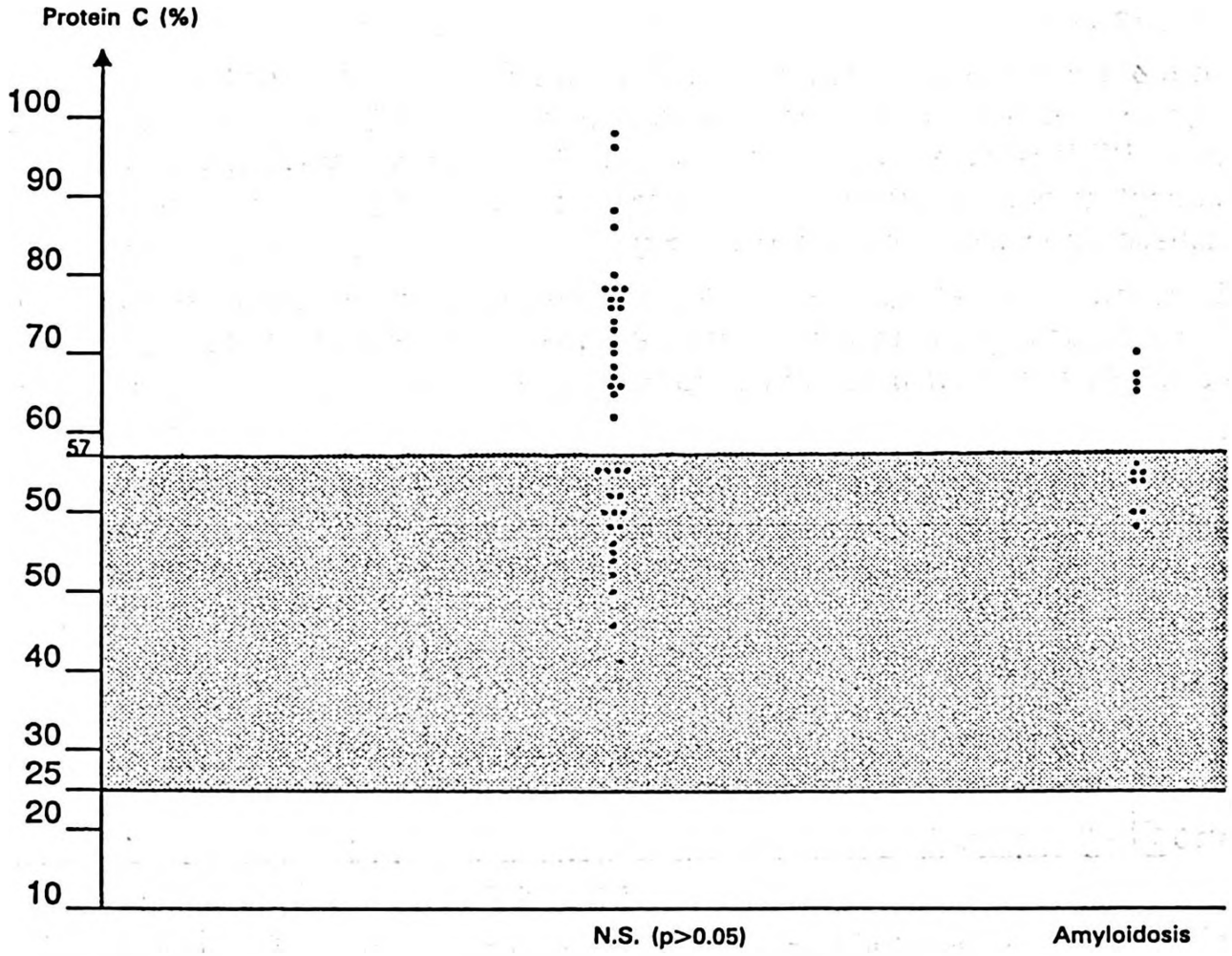

Fig. 2: Protein C antigen levels in nephrotic syndrome and amyloidosis.

TABLE II: Antithrombin III Antigen Levels and Protein Selectivity in Nephrotic Syndrome

	Antithrombin III	
	Normal	Low
Nephrotic syndrome with selective proteinuria	17	5
Nephrotic syndrome with nonselective proteinuria	12	3

$p > 0.05$

Discussion

PC ag levels were found to be high and/or normal in primary NS patients with normal renal function, PT and PTT levels and the absence of thrombotic symptoms. Pabinger-Fasching[5], Soff[6], and Mannucci[7] reported high and/or normal plasma PC ag levels in idiopathic NS. These investigators demonstrated

that PC ag is detectable in the urine of primary NS. It is concluded that patients compensate for PC losses in the urine by increased synthesis or by some other mechanism[6,7]. We have not studied urinary PC loss but PC has a very similar molecular weight to albumin (~ 66 000 daltons), and the negative charge is presumed to be lost in the urine. Due to conflicting data in the literature, we decided to study PC ag levels in patients with primary NS having selective or non-selective proteinuria and in patients with amyloidosis secondary to FMF. No statistically significant difference was found between the PC ag levels of NS patients with selective or non-selective proteinuria.

FMF induced amyloidosis is one of the major causes of secondary nephrotic syndrome in the Mediterranean region. There is an accumulation of amyloid fibrils in various tissues as well as in the kidneys[12], and bleeding, fibrinolysis, intravascular coagulation and vitamin K deficiency have also been reported[11-14]. We studied PC and AT III ag levels in patients with amyloidosis secondary to FMF to see if the levels of these two potent anticoagulants differed from patients with primary NS. No difference was observed between these two groups. AT III ag levels studied in patients with primary amyloidosis have been found to be low in reports in the literature. However, with the exception of one case, we found these levels to be normal. As to our knowledge, reports describing PC and AT III levels in patients with amyloidosis secondary to FMF have appeared only once in the literature.

In primary NS, the data for AT III ag varies. Some authors have observed decreased levels, while others have reported normal levels which are consistent with our findings[5-8,10,15,16]. In our study, conflicting AT III levels were not found to be related to protein selectivity.

In conclusion, PC and AT III ag levels are not only altered by urinary loss but are also affected by the synthesis, degradation and distribution rate as well. PC ag levels seem not to be related to hypercoagulability but low AT III levels may be related to it. In short, the AT III level should be determined in every nephrotic patient.

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EFFECTS OF CORTISOL AND GROWTH HORMONE ON THE METABOLISM OF LIVER AND BONE IN CHILDREN WITH MALNUTRITION*

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SUMMARY: Güler A, Sapan N, Salantur E. (Department of Biochemistry, Uludağ University Faculty of Medicine, Bursa, Turkey). Effects of cortisol and growth hormone on the metabolism of liver and bone in children with malnutrition. Turk J Pediatr 34:21-29, 1992.

A protein-energy deficit produces stress in the organism affecting all systems. Proportional to the degree of disease, cortisol and GH are mostly responsible for some of these effects. To investigate the effects on liver and bone, cortisol, GH, AST, ALT, ALP activities and Ca_T and P_I in serum were measured in 21 marasmus, nine kwashiorkor and 34 control children. In the marasmus group, we found a positive correlation between cortisol and AST, ALT and Ca_T and a negative correlation between cortisol and ALP. In the kwashiorkor group there were positive correlations between the same parameters, although, they were of a lesser degree. Furthermore, in the kwashiorkor group we established a positive correlation between GH and ALP. Cortisol stimulates transaminases directly and suppresses ALP activity, thus indirectly increasing Ca_T, whereas GH has no direct effect on these enzymes. As the disease progresses and as liver functions deteriorate, AST, ALT and ALP increase in serum. *Key words: malnutrition, cortisol, growth hormone, stress.*

Malnutrition is a condition that affects almost all the systems of an organism. A group of clinical findings are observed in the malnourished as a result of the disturbances of some systems; the most important being growth retardation[1]. Whatever the etiology, an inadequate diet and stress are the predominant causes for these changes[2]. In response to stress, some hormone levels of the endocrine system decrease, while others increase. Although some differences are seen during the course of the disease, the most marked difference is an increase in cortisol and growth hormone (GH) levels[2,3]. Even though GH levels are found to be elevated in malnourished children, growth retardation is present. The reason for this paradox is the breaking down of the metabolic integrity and the protein of the organism. The disturbances exhibit themselves through various clinical and laboratory findings. For example, as a result of liver failure, carbohydrate, lipid and protein metabolisms are affected[4,5]. In addition, secondary to an inadequate diet, some changes are also seen in bone metabolism.

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The purpose of this study was to evaluate the fore-mentioned changes, and investigate the effects of cortisol and GH on liver and bone metabolism. It was therefore necessary to measure the cortisol, GH, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities, and total calcium (Ca_T) and inorganic phosphorus (P_i) levels in the serum of children suffering from malnutrition and to investigate correlations of these hormones with the biochemical parameters mentioned in patient groups.

Material and Methods

The study population consisted of 35 subjects chosen at random from children with protein-energy malnutrition (PEM) who had been seen at the Pediatric Polyclinic of Uludağ University Faculty of Medicine. Using the Wellcome Classification[6], marasmus was diagnosed in 21 children, kwashiorkor in nine children, marasmic kwashiorkor in two children and three children were underweight. The latter five children were excluded from the study because their number was insufficient to comprise a group for statistical evaluation. The control group consisted of 34 healthy children whose ages approximately matched those of the subjects.

The patients fasted nearly ten hours before venous blood was drawn from the forearm. On the same day, serum concentrations of AST and ALT using the Biotrol A 03011 AST/TGO monoreactive kit and the Biotrol A 03021 ALT/TGP monoreactive kit, respectively, were determined. These kits were based upon the continuous monitoring principle[7].

The optimized kinetic method devised by the Coulter firm (Reagent Ref. No. 9866357) was used to determine ALP activity. P-nitrophenyl phosphate was the substrate used. The Biotrol / A 02478 phosphorus U.V. kit was used to measure P_1 levels and the classical "Complexometry method"[8] was used to measure Ca_T levels.

Cortisol levels were determined by using the Quanticoat Cortisol kit (Cat. No. 825, Kallestad Laboratories Inc., USA) and GH levels were arrived at by using the Quantitope^R HGH kit (Cat. No. 850, Kallestad Diagnostics, USA). These kits were based on the RIA principle.

Statistically significant differences between patient groups and correlations of cortisol and GH levels with enzyme activities and Ca_T and P_i levels were calculated using the Student's *t* test.

Results

The mean values of AST, ALT and ALP were within normal ranges in the patient groups, but when compared with the control group, highly significant differences ($p<0.001$) were found (Table I).

TABLE I: Evaluation of AST, ALT and ALP Mean Values in Children With Marasmus and Kwashiorkor Compared to the Control Group

Parameters	Control n = 34 $\bar{X} \pm SE$	Groups: Marasmus n = 21 $\bar{X} \pm SE$	p	Groups: Kwashiorkor n = 9 $\bar{X} \pm SE$	p	p*
AST (U/L)	24 ± 2.3	31.7 ± 1.9	<0.001	38.2 ± 2.9	<0.001	<0.02
ALT (U/L)	12.4 ± 1.8	28.4 ± 3.1	<0.001	37.1 ± 4.0	<0.001	<0.02
ALP (U/L)	173.5 ± 7.0	328.5 ± 22.3	<0.001	375.2 ± 31.7	<0.001	N.S.

N.S.: Non-significant.

p* : Statistically significant differences between patient groups.

In the marasmus and kwashiorkor groups, serum Ca_T levels were found to be significantly lower ($p<0.01$ and $p<0.001$, respectively) than in the control group. However, only in the kwashiorkor group were serum P_i levels significantly higher ($p<0.01$) than in the control group (Table II).

When AST and ALT were compared between the two patient groups, the mean values of the kwashiorkor group were found to be higher ($p<0.02$) than the marasmus group (Table I). When the mean values of the two groups were compared with each other, Ca_T levels were observed to be higher in the marasmus group ($p<0.01$) while P_i levels were found to be significantly higher in the kwashiorkor group ($p<0.01$) (Table II).

TABLE II: Investigation of Serum Ca_T and P_i Levels

Parameters	Control n = 34 $\bar{X} \pm SE$	Groups: Marasmus n = 21 $\bar{X} \pm SE$	p	Groups: Kwashiorkor n = 9 $\bar{X} \pm SE$	p	p*
Ca_T (%mg)	10.2 ± 0.1	9.9 ± 0.1	<0.01	9.1 ± 0.3	<0.001	<0.01
P_i (%mg)	4.1 ± 0.2	4.3 ± 0.3	N.S.	5.3 ± 0.3	<0.01	<0.01

N.S.: Non-significant.

p* : Statistically significant differences between patient groups.

Serum ALP, GH and cortisol levels were not significantly different in the two patient groups (Tables I, III). However, in comparison with the control group, the GH and cortisol values in the marasmus group were seen to be significantly higher ($p<0.01$ and $p<0.001$, respectively). In the kwashiorkor group, the serum GH

level was noted to be significantly elevated ($p<0.001$) while the cortisol value was slightly elevated ($p<0.05$) (Table III).

In the marasmus group, we found a positive correlation between cortisol values and AST, ALT and Ca_T and a negative correlation between cortisol and ALP (Table IV). However, in the kwashiorkor group, the most marked positive correlation was between GH and ALP activity ($r = 0.92$) (Table V).

TABLE III: Serum Cortisol and GH Compared to Control Group

		Groups				
	Controls n = 34	Marasmus n = 21		Kwashiorkor n = 9		
Parameters	$\bar{X} \pm SE$	$\bar{X} \pm SE$	p	$\bar{X} \pm SE$	p	p*
GH (ng/ml)	6.7 ± 1.7	11.8 ± 1.7	<0.01	14.7 ± 1.5	<0.001	N.S.
Cortisol (ng/ml)	184.0 ± 8.6	285.6 ± 23.5	<0.001	244.7 ± 24.9	<0.05	N.S.

N.S.: Non-significant.
p* : Statistically significant differences between patient groups.

TABLE IV: Correlations of Cortisol and GH With Various Parameters in the Marasmus Group

	Cortisol		GH	
AST	$y = 1.8x + 210$	$r = 0.94$	$y = 0.24x + 6.9$	$r = 0.17$
ALT	$y = 1.5x + 265$	$r = 0.91$	$y = 0.30x + 14.7$	$r = -0.15$
ALP	$y = -0.5x + 390$	$r = -0.85$	$y = 0.09x + 17$	$r = -0.04$
Ca_T	$y = 59x + 478$	$r = 0.79$	$y = 6.84x + 79.5$	$r = 0.30$
P_i	$y = -45x + 414$	$r = -0.28$	$y = -4.28x + 29$	$r = 0.21$

TABLE V: Correlations of Cortisol and GH With Various Parameters in the Kwashiorkor Group

	Cortisol		GH	
AST	$y = 1.2x + 260$	$r = 0.75$	$y = 0.07x + 11$	$r = 0.06$
ALT	$y = 0.4x + 285$	$r = 0.79$	$y = -0.04x + 17$	$r = -0.3$
ALP	$y = -0.54x + 410$	$r = -0.50$	$y = 0.08x + 3.7$	$r = 0.92$
Ca_T	$y = 93x + (-659)$	$r = 0.40$	$y = 1.3x + 1.9$	$r = 0.04$
P_i	$y = 44x + 109$	$r = 0.27$	$y = 0.13x + 16$	$r = -0.03$

Discussion

Former opinions to the effect that serum enzyme levels in malnourished children, were reduced because of protein deficiency are no longer accepted[9]. Pancreatic enzymes are found to be reduced only in PEM and the decrease is parallel to the severity of the disease. The reason for this is probably decreased external stimulation of pancreatic enzyme secondary to a low diet intake and probable decrease of the "growth hormone-releasing factor" which stimulates exocrine pancreatic enzyme secretion[10]. As a result, malabsorption occurs which worsens the child's nutritional status[11].

In the liver, inadequate protein intake in particular causes liver functions to become abnormal. Although, the histological changes in the liver cannot explain and do not show a correlation with the biochemical changes in serum, both show an increase proportional to the severity of PEM[12].

As a result of inadequate lipoprotein synthesis in the liver, lipids cannot form lipoproteins and their release into the circulation becomes limited. Therefore, fat, primarily as triglyceride, accumulates in the liver parenchyma[13]. The individual liver parenchymal cells bloat and appear to block the narrow sinusoids giving rise to the theory of an intrahepatic obstructive cause for the presenting jaundice[14]. The liver cells, though distended, rarely undergo necrosis. In epidemiological studies, no direct relation has been found between PEM and cirrhosis or other chronic liver diseases[15]. Kwashiorkor is found to play a role in the etiology of cirrhosis because malnourished children are more sensitive to toxic or infectious agents but viral hepatitis is believed to be the responsible factor in cirrhosis occurring in these cases[12]. The increased immunosuppressive effect of cortisol in addition to protein inadequacy, reduces the resistance of an organism, causing a greater risk of infection.

It is observed in PEM that alterations in the concentration of serum amino acids are correlated with changes in hormonal balance[16]. In normal children, serum insulin concentrations tend to be high, while cortisol and GH values are normal or subnormal. It is postulated that relatively high insulin levels could reduce the plasma concentration of essential amino acids by stimulating their movement out of the plasma into the muscle. When total food intake is curtailed, the metabolic response becomes similar to that of a wasting situation, that is, serum cortisol concentrations become high, and insulin correspondingly low. The main metabolic difference between this phase in the development of kwashiorkor and that found in uncomplicated marasmus is a concomitant rise in serum GH concentration. The high level of GH, even in the presence of elevated cortisol concentrations, might be the major reason for the even more dramatic fall in amino acid concentrations. The effects of high concentrations of serum cortisol, coupled with low insulin levels, would normally have replenished plasma aminoacid concentrations by

reducing amino acid utilization for protein synthesis in muscle in addition to increased proteolysis. Although the actions of cortisol and GH are complementary in maintaining blood glucose concentrations, they are antagonistic in their effects on amino acid metabolism. In contrast to cortisol, GH stimulates the passage of amino acids out of the serum into the tissues as part of its anabolic action.

In malnutrition another factor which might further decrease the concentration of serum amino acids is the increased activity of the transaminases in muscle[17]. Transaminases are activated by cortisol. This action of cortisol actually increases amino acid catabolism. To meet energy requirements, first transamination then the oxidation of alpha-ketoacids increase. As the severity of the disease increases, especially in severe kwashiorkor cases, serum amino acid levels drop. Alanine is also one of these amino acids, however its marked elevation can be found in pre-kwashiorkor cases. It is suggested that where protein is deficient and energy is adequate in the diet (as in the pre-kwashiorkor states), the alanine level rises, since it is neither utilized for gluconeogenesis nor metabolized to urea[18-19]. In marasmus there is an inadequate caloric intake due to insufficiency in the diet[1] and, therefore, in these children, alanine and aspartate are used for gluconeogenesis[18].

Increased cortisol in marasmus affects protein metabolism in three ways: increases protein catabolism, increases the mobilization of amino acids from muscle and stimulates the activity of transaminases. In kwashiorkor, although there is adequate energy, the utilization of these amino acids in gluconeogenesis is decreased by the effect of GH. As a result of protein inadequacy, fatty liver occurs. Structural and functional disturbances of the liver cause these enzymes to seep into plasma. Although the increasing mechanisms of transaminases in serum are different in PEM, they can be used as an index for establishing the stage of the disease. For example, in addition to the increase in hormone levels, the presence of hepatomegaly and elevated bilirubin and transaminase (AST, ALT) levels indicate that there is liver insufficiency, and a diagnosis of kwashiorkor can be made. A rise only in serum transaminase levels can be interpreted solely as a compensation or adaptation effect of cortisol and this increase is seen predominantly in marasmus patients.

In our study, we also found AST and ALT mean values to be significantly higher in both patient groups than in the control group (Table I). Although the mean values of both groups were within the normal ranges, when compared with each other, the mean levels of these enzymes in serum were found to be higher in kwashiorkor patients. As a result of our correlations, we found a more marked, positive correlation of cortisol with AST and ALT in the marasmus group ($r = 0.94$, $r = 0.91$) when (Table IV) compared with the kwashiorkor group ($r = 0.75$, $r = 0.79$) (Table V), respectively.

Even though ALP levels were found to be within normal ranges, they were significantly higher than the mean of the control group. However, when the mean values of the two patient groups were compared with each other, the mean value of the kwashiorkor group was found to be higher (Table I). ALP, a membrane-bound enzyme found in the liver, forms the cell and biliary canalicula, and is located on the sinusoidal border of the parenchymal membrane[21]. In any of the obstructive liver states, as a result of cholestasis, ALP regurgitates into the plasma and its level is found to be high. In bone, ALP catalyzes the breakdown of inorganic pyrophosphates, and thus, plays an important role in the deposition of calcium salts, i.e., calcification. An increase of ALP in serum is generally proportional to an increase of osteoblastic activity. These processes are complex in malnutrition. In order to maintain blood Ca_T at normal levels, as a result of nutritional inadequacy, decalcification is generally seen in PEM. Ca_T levels in malnourished children are generally found to be around 8 mg/dl[9]. These values are lower than those normally found in children, but, if a correction is made for the reduced serum protein, the ionic calcium values are found to be within normal limits. In our study Ca_T and P_i values were established to be at normal levels. However, when compared with the mean values of the controls, we found that there were very significant differences between them (Table II).

Since we did not perform ALP isoenzyme electrophoresis, we could not ascertain whether or not the increase in ALP was from the liver or bone. However, when transaminase levels are considered, it may be argued that in kwashiorkor, in addition to the effect of GH, an obstruction secondary to a mild degenerative process in the liver explains the relative increase of this enzyme observed in the plasma. GH increases the intestinal absorption of calcium and the renal retention of phosphorus, which in a way increases the metabolic activity in bone, so that ALP in bone is also found to be activated. However, in marasmus, as a result of dietary inadequacy, and to compensate and maintain blood calcium levels within normal ranges, mineral depletion occurs in bone, leading to suppression of osteoblastic activity. Only after initiation of treatment, are signs of rickets and an increase in ALP observed.

In conclusion, it is suggested that this enzyme level is normally expected to decrease in marasmus and increase in kwashiorkor. We believe that the reason for the relatively high enzyme level observed in the marasmic patients we studied was either because the pathology of these cases was more severe than it had appeared in reference to the clinical picture or that treatment had been initiated in some patients. However, we tend to give more weight to the former explanation.

We found a negative correlation between cortisol and ALP ($r = -0.85$) in the marasmus group, suggesting ALP is relatively suppressed. There was somewhat of a negative correlation between the same parameters in the kwashiorkor group

(Tables IV, V). The positive correlation established between GH and ALP in the kwashiorkor group supports the afore-mentioned conjectures. In the marasmus group, the positive correlation found between cortisol and Ca_T indicates that cortisol attempts at maintaining metabolic integrity and at keeping serum calcium at normal levels (Table IV).

As a result of this study, it may be said that in marasmus, an increase in cortisol stimulates transaminase activity and gluconeogenesis. In bone, it increases calcium mobilization and prevents blood Ca_T from falling below normal levels, but this compensation effect causes osteoporosis. Whereas in kwashiorkor, the accumulation of lipid in the liver probably causes enzyme levels to increase in the plasma. Although GH is found to be elevated in these patients, and attempts at displaying its anabolic effect, GH cannot be useful in both calcium deposition in bone and in maintenance of normal protein metabolism in the liver as long as dietary inadequacy continues. It is known that when GH is found to be predominantly increased in PEM patients, it signifies the terminal stage of the disease, and that dysadaptation has begun.

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STANDARD AND LOW BIRTH WEIGHT FORMULAS COMPARED FOR EFFECTS ON GROWTH OF PRETERM INFANTS*

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SUMMARY: Yeşilipek M.A. (Maternity and Children's Hospital, Samsun, Turkey). Standard and low birth weight formulas compared for effects on growth of preterm infants. Turk J Pediatr 34:31-36, 1992.

The study population consisted of 22 infants hospitalized for low birth weight or prematurity at the Maternity and Children's Hospital in Samsun. Eleven infants were fed the low birth weight formula and the rest were fed the standard formula. Weight gain, increases in length and occipitofrontal circumference were higher in the low birth weight formula group than in the standard formula group.

In cases which reguire it, a low birth weight formula should be given instead of the standard formula. *Key words: preterm infants, low birth weight formula, growth.*

It is a known fact that breast milk is the most suitable food for newborns. Its immunological properties may help prevent infection and reduce the incidence of necrotizing enterocolitis[1,2]. However, sometimes breast milk may be insufficient or undesirable, due to maternal conditions (illness, drug use, etc.), particularly in the early postpartum period.

Since brain growth occurs mainly between the second half of the third trimester of pregnancy and the second year of life, some authors stipulate that for normal neurological development, adequate growth of preterm and low birth weight infants is necessary[3].

Recently, there have been a few studies concerning newly modified formulas for preterm infants that are higher in protein, minerals and calories than standard formulas[1,4-7]. Preterm and low birth weight infant growth has been demonstrated to be better with these new formulas.

The present study was designed to compare the newly modified formula with the standard formula to determine if the former had any effect on the growth of preterm infants when used as a supplement to breast milk, and also to evaluate if it could be used as a replacement for breast milk.

Material and Methods

The study population consisted of 22 preterm infants who had been hospitalized at the Maternity and Children's Hospital in Samsun between January-March 1989.

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All infants with a birth weight under 2000 g were randomly divided into two groups. The infants were healthy at the time of the study and none had congenital malformations, infections or respiratory distress syndrome (RDS). The gestational age of the infants was determined from the obstetric history and from Dubowitz scoring[8]. Infants in group I were fed SMA-26 (Wyeth Lab) and the infants in group II SMA-Low Birth Weight Formula SMA-S-26 (Wyeth Lab) (Table I). All infants were fed 60 ml/kg of their respective formulas during the first day. This amount was gradually increased to 150 ml/kg/day over a period of five days. The study ended when each baby reached 2500 g.

TABLE I: Nutrient Content of Formulas
(per 100 ml)

	SMA-26	SMA-S-26 LBW***	Breast Milk
Energy (kcal)	66.66	81.00	64.00*
Fat (g)	3.60	4.32	3.50*
Protein (g)	1.50	1.92	1.46*
Carbohydrates (g)	7.20	8.40	7.30**
Sodium (mg)	15.00	32.00	29.00**
Calcium (mg)	42.00	80.00	28.00**
Phosphorus (mg)	28.00	40.00	12.00**

* Ref. 9
** Ref. 1
*** LBW: low birth weight.

Weight, length and occipitofrontal circumference (OFC) were measured weekly. The infants were weighed on a scale to the nearest 10 g, the crown-heel length and OFC were measured to the nearest mm using a disposable paper tape measure. All measurements were taken by the same observer.

The compositions of the two formulas were supplied by the respective companies. Serum electrolytes, urea, calcium, total protein and glucose were measured one week after the start of the study using a flame photometer (Lange M-7D) for electrolytes and a spectrophotometer (Shimadzu) for the other measurements. Osmolality was calculated by the following formula: Osmolality = $(Na^+ + K^+)$ 2+ (Glucose/18) + (Urea/3).

For statistical analysis, the groups were compared using *Student's t* test.

Results

Pertinent data regarding the infants are presented in Table II. No statistical differences were found between the two groups with regard to gestational age or birth weight (Table II).

TABLE II: Data Pertaining to the Infants

	Group I (SMA-26) (n = 11)	Group II (SMA-S-26) (n = 11)	
Gestation (wk)	31.73 ± 2.53*	31.45 ± 2.54	p>0.05
Birth weight (g)	1622.72 ± 368.35	1677.27 ± 378.39	p>0.05
M/F**	5/6	5/6	

* Mean ± SD
** M: male, F: female

Weight gain, increases in length and OFC gain were greater in Group II when compared to Group I. The differences between the two groups were statistically significant (Table III).

Mean serum sodium, potassium, total protein concentrations and serum osmolalities were similar in the two groups and no significant difference was observed statistically. However, there were significant statistical differences found with regard to the serum urea, calcium and glucose levels (Table IV).

TABLE III: Average Growth Rate of the Two Groups

	SMA-26 Group I	SMA-S-26 Group II	
Weight gain (g/day)	25.75 ± 1.46*	42.88 ± 2.41	p<0.001
Length increase (cm/wk)	1.09 ± 0.11	1.63 ± 0.16	p<0.05
OFC increase (cm/wk)	0.80 ± 0.09	1.19 ± 0.11	p<0.05

* Mean ± SD

Discussion

Nutrition is an important problem in premature and low birth weight infants. Although protein and energy requirements of low birth weight infants are still

TABLE IV: Blood Chemistry of the Groups

	SMA-26 Group I	SMA-S-26 Group II	
Sodium (mEq/L)	135.55 ± 2.58*	136.09 ± 1.81	p>0.05
Potassium (mEq/L)	3.39 ± 0.23	3.45 ± 0.38	p>0.05
Urea (mg/dl)	15.00 ± 3.60	11.64 ± 1.69	p<0.05
Total protein (g/dl)	6.86 ± 0.74	7.24 ± 0.46	p>0.05
Calcium (g/dl)	9.04 ± 0.35	9.42 ± 0.41	p<0.05
Glucose (mg/dl)	73.63 ± 8.05	64.54 ± 3.22	p<0.05
Osmolality (mosm/kg)	285.89 ± 4.53	286.72 ± 3.70	p>0.05

* Mean ± SD

controversial, some authors have recommended that intakes should be higher than in infants born at term to promote intrauterine growth performance and prevent hypoproteinemia[4]. In order to provide the energy requirements of most preterm and low birth weight infants, an intake of 110-165 kcl/kg/day is sufficient for a growth rate to be similar to that occurring in utero[10]. There have been several reports of differences in protein content between the milk of mothers who have delivered full term infants and those who have delivered preterm infants. This observation has been cited in support of preterm milk as an evolutionary adaptation to the greater protein requirements for growth of preterm infants[11-13].

Until now, breast feeding was accepted as the best method of nutrition for the premature infant[1]. Although the daily protein requirement of small preterm infants is not exactly known, breast milk is believed to be insufficent[1]. In recent years, newly modified milk formulas with higher protein, mineral and calorie content have been produced for the low birth weight infant[1,4-7]. When the new formulas were compared with breast milk and with standard milk formulas, the growth of preterm infants was found to be better with the new formula. Furthermore, low birth weight formulas have been shown to be metabolically safe for smallest infants[5]. Although optimum weight gain of the preterm infant has not been clearly defined, according to some authors, the optimal growth rate criteria should be taken as the intrauterine growth rate of the fetus in the third trimester of pregnancy. Thus a fetus of 32-36 week gestational age should grow at a rate of 30.7 g/day[1]. In this study, weight gain of infants fed the SMA-S-26 formula was above this value while those fed the standard formula (SMA) was below (Table III). There were statistically significant differences between the two groups ($p<0.001$).

Some authors suggest that the higher sodium content of preterm mother's milk may provide sufficient sodium for growth and reduce the incidence of late

hyponatremia. Therefore, they recommend the regular monitoring of plasma sodium concentrations and sodium supplements, if necessary[10]. However, in some reports, it is noted that higher sodium contents may have been responsible for the gain in weight. But these infants usually have higher serum sodium levels and their gain in weight is not accompained by increases in length or OFC[1]. In this study, sodium intakes were higher in group II infants than in group I infants. However, the sodium content of this formula is similar to that of breast milk. In addition, we found no cases evidencing hypernatremia, and although the differences in serum urea, calcium and glucose levels were found to be statistically significant, there were no significant differences between serum osmolality values of the two groups (Table IV). In addition the gain in weight was accompanied by increases in length and OFC in group II infants. There were statistically significant differences between the two groups regarding increases in length and in OFC ($p<0.05$).

Preterm and very low birth weight infants fed human milk are at some risk of developing phosphate depletion syndrome and bone disease[10]. The European Society of Pediatric Gastroenterology and Nutrition has recommended that formulas for these infants should be high in calcium and phosphorus and that mineral supplements should be given in a proportionate amount to maintain the calcium/phosphorus ratio in the range of 1.4-2.0. The calcium/phosphorus ratio of the low birth weight formula given our subjects was in accordance with this recommendation.

As in many other reports, the results of this study proved that the low birth weight formula is suitable for feeding preterm infants. However, some pediatricians continue to advise mothers to breast feed their premature infants. Lewis and Smith[14] have shown that high volume breast milk achieved optimum growth in the preterm infant. In their retrospective survey, they found no evidence of complications secondary to the use of high volume feeding. Therefore, they suggested that high volume feeding is a satisfactory alternative to the low birth weight formula, particularly for infants above 30 weeks gestation and of a birth weight of 1000 g.

In conclusion, breast milk may be preferred particularly for the initial feeding of the ill preterm infant. However, the low birth weight formula should be used instead of the standard formula if breast milk is insufficient or undesirable for feeding preterm infants. In addition, pediatricians should pay special attention to the sodium and protein contents of these formulas.

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A RARE ENTITY AMONG CHILDHOOD MALIGNANT LYMPHOMAS*

(Large Anaplastic T-cell Ki-1 +)

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SUMMARY: Ulukutlu L, Hitzig W, Maurer R. (Department of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey). A rare entity among childhood malignant lymphomas (large anaplastic T-cell ki-1 +). Turk J Pediatr 34:37-41, 1992.

A case of peripheral type T-cell lymphoma is presented to underline the difficulty in distinguishing the initial clinical findings of an inflammatory neoplastic disorder since the diagnosis could only be arrived at after several repeated lymph node biopsies.

An 11 10/12-year-old boy admitted to the hospital with inguinal lymph node enlargement was diagnosed as having adenitis and periadenitis. The disease had progressed and the patient had remittent fever rising to 39 °C, and another biopsy was taken. Cervical lymphadenomegaly was present, A diagnosis of chronic lymphadenitis with lymphocyte loss and fibrosis was established.

The diagnosis could only be made from biopsy material taken from the deep cervical (jugularis interna) and axillary lymph nodes one year later, which showed Ki-1 antigen positive large T-cell lymphoma.

The disease showed continuous activity in spite of chemotherapy and the child survived only 17 months. *Key words: lymphoma, large cell KI-1 + lymphoma, peripheral T-cell lymphoma*

The diagnosis of non-Hodgkin's lymphoma, the most frequent malignancy occurring in childhood after leukemia, can be established by the histopathological findings of a lymph node biopsy. However, the differential diagnosis between a malignancy and an infectious process may be difficult during late childhood, as in the case presented. We present this rare entity with the aim of underlining the importance of subtypes in the prognosis and treatment and possible insufficiency of a histopathological diagnosis in addition to emphasizing the cooperation between different centers which has become essential in view of the diagnostic importance of cell markers and monoclonal antibodies[1,2].

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Case Report

An 11 10/12-year-old boy, the elder of two brothers, was referred to the Pediatric Oncology Department of the Cerrahpaşa Faculty of Medicine at the end of January 1988 for evaluation of fever and enlarged cervical and inguinal lymph nodes. Past history of the patient and family history were unremarkable. Immunizations including BCG were given at the appropriate intervals.

Lymph node enlargement in the left inguinal region was first recognized in November 1987, and was biopsied and diagnosed as acute adenitis and periadenitis. A repeat lymph node biopsy of the left cervical and inguinal regions was performed in January 1988, but this time an evaluation of chronic lymphadenitis with lymphocyte loss and fibrosis was made. Ampicillin, gentamicin and penicillin were administered for a pharyngeal infection. The fever dropped in the evenings with daily elevations ranging from 37 °C to 39.2 °C.

Physical examination revealed a boy with normal somatic development. Multiple pigmented nevi of various sizes were present on the back. Biopsy incision sites were visible on the left inguinal and cervical regions, the latter still showing fresh granulation tissue. A 3 × 4 cm, firm, fixed and painless swelling (lymph node packet) was palpable in the left cervical region. The tonsils were hyperemic and hypertrophic. Other systemic findings were normal.

Laboratory findings revealed a hemoglobin of 13 g/dl, hematocrit 40 percent, white blood cells 23 × 10^8/L, a differential count with 5 percent band forms, 58 percent polymorphonuclear leukocytes (with coarse cytoplasmic granulation), 2 percent eosinophils, 3 percent monocytes and 32 percent lymphocytes. Reticulocytes were 2.3 percent and platelet count 270 × 10^8/L. The erythrocyte sedimentation rate was 65 mm/hour.

Serum copper concentration was 210 µg/dl, fibrinogen 592 mg/dl, alkaline phosphatase activity 150 U/L, lactic dehydrogenase activity 300 U/L, IgG 1900 mg/dl, IgA 263 mg/dl, IgM 261 mg/dl with decreased alpha-2 and gamma globulin at protein electrophoresis.

Endotoxin stimulated nitroblue tetrazolium (NBT) was 74 percent and the tuberculin test was negative. The bone marrow examination was consistent with anemia and infection. Few white blood cells were detected in the urine sediment. The chest X-ray was normal. Computerized tomographic examination of the thorax and abdomen showed thymic hyperplasia, hepatomegaly and enlargement of the extrarenal pelvis.

On the seventh day of admission, red, tender masses appeared in front of and behind the cervical swelling and additional bean-sized lymph nodes were palpable bilaterally in the cervical and axillary regions. Cefazolin was administered intravenously. No material could be obtained upon incision of the fluctuant

appearing left cervical biopsy site and no response could be obtained after the administration of various antibiotics.

After contact with Zurich Children's Hospital, it was decided that the patient be reevaluated for chronic granulomatous disease and referred for a repeat biopsy, if necessary. On examination at Zurich Children's Hospital in February 1988, the patient was found to be mildly obese and extremely pale with enlarged right cervical, axillary, occipital and bilateral inguinal lymph nodes and also presented with left supraclavicular and cervical multiple masses. The apple-sized swelling with an incomplete biopsy scar was fixed to the underlying tissue. The liver and spleen were palpable 5 and 2 cm, respectively below the costal margin. The chest X-ray showed a normal mediastinum and ultrasonographic examination of the abdomen indicated splenomegaly and only two lymph nodes at the origin of the arteria mesenterica superior. Deep cervical (jugularis interna) and axillary lymph node biopsy specimens were diagnosed as being large cell mesenchymal, histiocytic, lymphoblastic tumor (T-cell lymphoma).

Immunohistochemical examination of the tumor tissue with antibodies against Ki-1 (activated T and B lymphocytes) showed evident positivity in the tumor tissue and in the intact subcapsular and peritrabecular sinus areas of the lymph node. Due to mild positivity with Uchl (T-cell marker) and the negativity with MB2 (B-cell marker) in the tumor tissue, the tumor was most probably a large cell anaplastic T-cell lymphoma. Leukocyte common antigen was positive in the lymph node tissue and slightly positive in the plasma cells in the tumor tissue. Factor 8 rAg was negative in the tumor.

When the histological diagnosis was almost certain, chemotherapy was initiated and the fever dropped promptly. The induction course was completed with four weeks of a modified ACOP regimen (adriamycin, cyclophosphamide, vincristine and prednisolone). Since leukopenia developed following the initiation of chemotherapy, this treatment had to be interrupted for short periods during which time mild fever, cervical, axillary and inguinal lymphadenomegaly, a maculopapular rash and hepatomegaly 1.5 cm below the right costal margin were observed on physical examination. During maintenance therapy, consisting of three-week-cycles, the patient had a relapse. Bone marrow examination revealed myeloid hyperplasia and erythroid hypoactivity. The hili were prominent and increased left paravertebral density was seen on the chest X-ray. Abdominal ultrasonographic examination revealed multiple small lymph nodes under the liver, at the hili of the liver and spleen and mild splenomegaly. Due to histiocytic predominance, the Pro-MACE scheme including VP-16 (adriamycin, cyclophosphamide and prednisolone) was administered. As a result of this treatment, the fever and the rash disappeared and the lymph node enlargement regressed considerably.

In spite of the chemotherapy, the disease is probably still active. In view of recent reports concerning Ki-1 antigen positive large T-cell lymphoma[3,4], the patient's treatment was reevaluated at relapse when typical, locally recurring manifestations were observed in February 1989. Since the disease has been reported to have responded favorably to the regimens used in treating acute lymphoblastic leukemia (ALL), the ALL protocol of the International Society of Pediatric Oncology (SIOP) 79/84 (cyclophosphamide, vincristine, prednisolone, methotrexate, L-asparaginase, cytosine arabinoside and thioguanine) was administered. Manifestations of the disease disappeared. However, bilateral cervical and right axillary lymphadenomegaly, rash and fever recurred soon after completion of induction. The occurrence of lymphomatous activity following the administration of L-asparaginase suggested a resistance to the drug. Hepatomegaly, initially indirect and later direct hyperbilirubinemia, splenomegaly and ascites appeared. The use of high-dose steriods and later additional cytosine, arabinoside and thioguanine were not able to prevent the patient's death. The duration of survival was 17 months.

Discussion

Large cell T-cell lymphoma of the anaplastic type is a new entity, which is now differentiated from early histiocytic malignant lymphomas. The involvement of peripheral lymph nodes without associated mediastinal involvement is typical. This type of lymphoma might present as either an infectious or a neoplastic process.

Although rare, recent reports point to the existence of peripheral T-cell lymphoma in childhood[3,4]. The monoclonal antibody Ki-1 shows a selective reactivity to the Reed-Sternberg cells seen in Hodgkin's disease. In differentiating the anaplastic type of diffuse large cell lymphoma (peripheral T-cell lymphoma) from other histiocytic lymphomas, the presence of Ki-1 antigen is clearly diagnostic. Large cell anaplastic Ki-1 positive lymphoma in childhood clinically differs from malignant histiocytic lymphoma with initial peripheral lymph node or extra-nodal involvement and no central nervous system or bone marrow invasion. It has been suggested that a translocation at the q35 site of the fifth chromosome could be specifically associated with Ki-1 positive lymphoma and has a causal role aside from these histopathological characteristics[5].

In a study carried out on 15 children (age range 9-15 years) between 1975-1986, Gasparini et al[3] reported that most of these patients, in whom immunohistochemical examination of paraffin sections showed Ki-1 antigen positivity, had been febrile with dermatological manifestations. In 1989 Leake et al[4] also reported large cell anaplastic Ki-1 positive tumors in three of six children (median age 9.5 years) with peripheral T-cell lymphoma. The disease was extranodal in two of

these patients. Our patient showed a similar clinical picture to the patients reported in the literature[3,4]. These patients were initially treated with the CHOP (cyclophosphamide, hydroxylurea, oncovin, prednisolone) regime which was also applied to our patient, and they later used the UKALLx regime (daunorubicin, cytosine arabinoside, thioguanine, VP-16) protocols.

The case presented clearly illustrates that it is at times difficult to differentiate peripheral type T-cell lymphoma from an inflammatory neoplastic disorder. Histopathological examination of lymph nodes coupled with the use of cell markers and monoclonal antibodies are of importance in the establishment of a specific final diagnosis.

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BETA-KETOTHIOLASE DEFICIENCY*
A Case Report

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SUMMARY: Altıntaş B, Teziç T, Coşkun T, Özalp İ, Kükner Ş, Kaya A. (Dr. Sami Ulus Children's Hospital, and the Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Beta-ketothiolase deficiency: a case report. Turk J Pediatr 34:43-46, 1992.

A four-month-old boy with beta-ketothiolase deficiency is described in this report. Presenting symptoms and signs were vomiting, irritability and acidotic respiration. Laboratory investigations revealed hyperglycinemia, metabolic acidosis and ketosis. Subsequent urinary GC-MS analysis of the patient's urine sample showed the typical pattern of beta-ketothiolase deficiency. Our experience with this case indicates that accurate diagnosis and early treatment of inborn errors might be lifesaving.
Key words: ketotic hyperglycinemia, beta-ketothiolase deficiency.

Beta-ketothiolase deficiency is a rare metabolic disorder of branch chain amino acids, which is characterized by an increased plasma glycine level, metabolic acidosis and ketosis. The differential diagnosis of beta-ketothiolase deficiency should include propionic acidemia, methylmalonic acidemia and isovaleric acidemia[1].

This report describes a four-month-old boy with beta-ketothiolase deficiency. To the best of our knowledge, this is the first case of beta-ketothiolase deficiency reported from Turkey.

Case Report

A four-month-old boy was admitted to Dr. Sami Ulus Children's Hospital with the chief complaints of irritability, crying and vomiting of two days' duration. The prenatal period and delivery were uneventful. The parents were unrelated. Past history disclosed normal physical and motor development. The patient had a two-year-old healthy sister.

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Physical examination revealed a feverish child (body temperature 40 °C) with a pulse rate of 180 beats/min and a respiratory rate of 72/min. The patient appeared critically ill, was irritable and had acidotic respiration. The liver was 2 cm palpable below the right costal margin. He weighed 4700 g (above the 5th percentile) and measured 54 cm in length (above the 5th percentile) and 39.5 cm in head circumference (above the 5th percentile).

Laboratory investigations revealed a hemoglobin of 10.6 g/dl, white blood cells 16100/mm^3 with 82% polymorphonuclear leukocytes, 14% lymphocytes and 4% monocytes. The platelet count was normal. Urinalysis yielded 2+ ketonuria. The blood pH was 7.1 and HCO_3– 7.9 mEq/L. Paper chromatography of the blood and urine samples demonstrated increased concentrations of glycine. Urinary organic acid analysis by gas chromatography and mass spectrometry (GC-MS) showed a pattern consistent with the diagnosis of beta-ketothiolase deficiency (Table I).

TABLE I: Urinary and Serum Organic Acid Pattern of the Patient

Organic Acid	Urine (µmol/mol creatinine)	Serum (µmol/L)
Lactic acid	110	3600
2-OH-butyric acid		120
3-OH-butyric acid	980	310
Adipic acid	70	
3-OH-2-methylbutyric acid	300	820
4-OH-phenylacetic acid	90	
4-OH-phenyllactic acid	60	
Hippuric acid	220	

Following intravenous alkali replacement therapy and the initiation of a protein restricted diet, the patient's condition improved. The child was subsequently discharged on a low-protein diet. Within two weeks, he became symptom-free and his psychomotor status became normal.

Discussion

Since the first description of a beta-ketothiolase disease by Daum et al[2,3], Keating et al[4], Hillman et al[5,6] and Gompertz et al[7] attributed the disease to an inherited disorder of isoleucine catabolism. Later, it was discovered that the disease was not simply a defect of isoleucine degradation, but that the deficient enzyme was the K^+ dependent short-chain mitochondrial thiolase which also plays a major role in ketone body metabolism[8]. Deficient activity of mitochondrial acetoacetyl CoA thiolase in fibroblasts of patients with beta-ketothiolase deficiency has been reported[9,10].

Beta-ketothiolase deficiency presents with vomiting, acidosis, lethargy and coma usually triggered by intercurrent infections during infancy and late childhood. Our patient presented with vomiting, irritability and acidotic respiration during a febrile illness[1].

The "ketotic hyperglycinemia syndrome" was first described by Childs et al[11] in 1961. Elevated plasma glycine levels may occur in a number of inborn errors including propionic acidemia, methylmalonic acidemia and isovaleric acidemia[1]. In all these disorders, acidosis and ketosis are in association with increased plasma and urinary concentrations of glycine, as in the case presented. GC-MS analysis of a urine sample is, therefore, needed to make a distinction between these metabolic disorders having similar clinical and biochemical findings. Beta-ketothiolase enzyme converts alpha-methyl acetoacetyl CoA to acetyl CoA and propionyl CoA during the degradation of isoleucine. Patients with this disorder excrete large amounts of 3-OH-2-butyric acid, 2-methylacetoacetate and tiglylglycine[1,12]. We detected increased excretion of 3-OH-2-butyric acid in our patient (Table I). Although neutropenia and thrombocytopenia were not present in our patient, hematologic abnormalities have been reported in some cases[12].

The control of an acute metabolic crisis by intravenous fluid and alkali therapy, and the implementation of a protein restricted diet, as in our case, demonstrated that most of the inborn errors of metabolism can be managed successfully if a correct diagnosis is made and treatment is initiated early in the course of the illness. Establishment of a correct diagnosis not only increases the chance of controlling the disorder but also affords the parents an opportunity of having a healthy child in subsequent pregnancies by making use of prenatal diagnosis.

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ECHOCARDIOGRAPHIC FINDINGS IN ENDOMYOCARDIAL FIBROSIS*

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SUMMARY: Saraçlar M, Özer S, Öztunç F, Çeliker A. (Department of Pediatric Cardiology, Hacettepe University Institute of Child Health, Ankara, Turkey). Echocardiographic findings in endomyocardial fibrosis. Turk J Pediatr 34:47-53, 1992.

An 18-month-old infant diagnosed as having endomyocardial fibrosis by echocardiography is presented. Most patients with endomyocardial fibrosis reported in the literature are either older children or adults. To our knowledge, our patient was the youngest ever to have been reported. Echocardiographic studies showed obliteration of the left ventricular apex and increased echo reflectance at the left ventricular endocardium and subendocardium. The left atrium and right ventricle were significantly enlarged. Doppler echocardiography showed minimal mitral, but significant tricuspid regurgitation. In regard to the contribution of echocardiography in the diagnosis, we recommend this method for suspected cases. Contrary to the other patients reported, there was no thickening of the atrioventricular valves. Mitral valve insufficiency was related to the restriction of the ventricular filling rather than to valve involvement occurring with the disease. *Key words: endomyocardial fibrosis, echocardiography.*

Restrictive cardiomyopathy may be arbitrarily divided into two major categories: 1) endomyocardial fibrosis including Löffler's fibroblastic endocarditis, and 2) infiltrative myocardial diseases, which include amyloid, sarcoid, hemochromatosis, and Pompe's and Fabry's diseases[1].

Endomyocardial fibrosis (EMF) was first described in Europe by Löffler in 1936. Later, a restrictive variant was reported from black Africa in 1948. Subsequent reports of the disease came essentially from tropical black Africa (Uganda, Nigeria). Since 1950, EMF has been observed in South America (Brazil, Venezuela, etc.)[2], but it has also been seen in temperate zones in the U.S.A., France and Japan. However epidemiological studies have shown the disease to be more prevalent in the tropics than in temperate areas[2,3].

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Until recently, procedures other than echocardiography have been used in the diagnosis of EMF. In this report, we describe an 18-month-old infant in whom a diagnosis of EMF was established using echocardiography. As to our knowledge, this is the youngest case of EMF ever to have been described in the literature and the first report from Turkey in the pediatric age-group.

Case Report

An 18-month-old boy was admitted to the hospital for evaluation of tiredness and malaise. Physical examination revealed an infant with a height of 74 cm and weight 9250 g. The arterial blood pressure was 110/70 mm Hg. At the xyphoid area there was a grade III/VI blowing holosystolic murmur. The second heart sound at the pulmonary area was single and loud. The liver edge was palpable 4 cm below the right costal margin, while the spleen was enlarged 2 cm. No cyanosis was noted.

The ECG showed a northwest QRS axis in the frontal plane and right ventricular hypertrophy. The telecardiogram revealed global cardiomegaly. The hemoglobin was 10.50 g/dl, hematocrit 33%, white blood cells 7800/mm^3, mean corpuscular volume 77fl, reticulocytes 0.2%, erythrocyte sedimentation rate 29 mm/h, ASO titer 625 units, CRP positive. The latex test was negative. Total eosinophil count 100 cells/mm^3, total protein/albumin 6.9/3.8 g/dl, alkaline phosphatase 98 BU, uric acid 4.5 mg/dl, AST (aspartate aminotransferase) 25 U, ALT (alanine aminotransferase) 8 U, and fasting blood glucose 83 mg/dl. Bone marrow and urinalysis were normal. There were no parasite ova or cysts in the stool. Echocardiographic studies were carried out using a Toshiba Sonolayer-S SSH 60 echocardiograph. A standard 2½-MHz as well as a 5-MHz transducer were utilized.

Two-dimensional echocardiography illustrated obliteration of the left ventricular apex in the apical four-chamber and parasternal long axis views (Figs. 1, 2). However, left ventricular functions were within normal limits (ejection fraction 53%, shortening fraction 26%). There was increased echo reflectance at the left ventricular endocardium and subendocardium, however, this could only be detected in some parts on the right ventricle. The structure of the mitral and tricuspid valves was normal echocardiographically. The left atrium and right ventricle were significantly enlarged. The left atrium was 20 mm. Pulmonary arteries and veins were dilated. Doppler echocardiography showed minimal mitral regurgitation, but significant tricuspid regurgitation. The systolic pressure gradiants were 16 mm Hg in the mitral and 80 mm Hg in the tricuspid valve.

Right and left heart catheterization and angiocardiography were performed from the right femoral region by the percutaneous technique. The catheter entered the right atrium, right ventricle, and pulmonary arteries via the inferior vena cava. The left heart catheter entered the left ventricle and atrium from the ascending aorta.

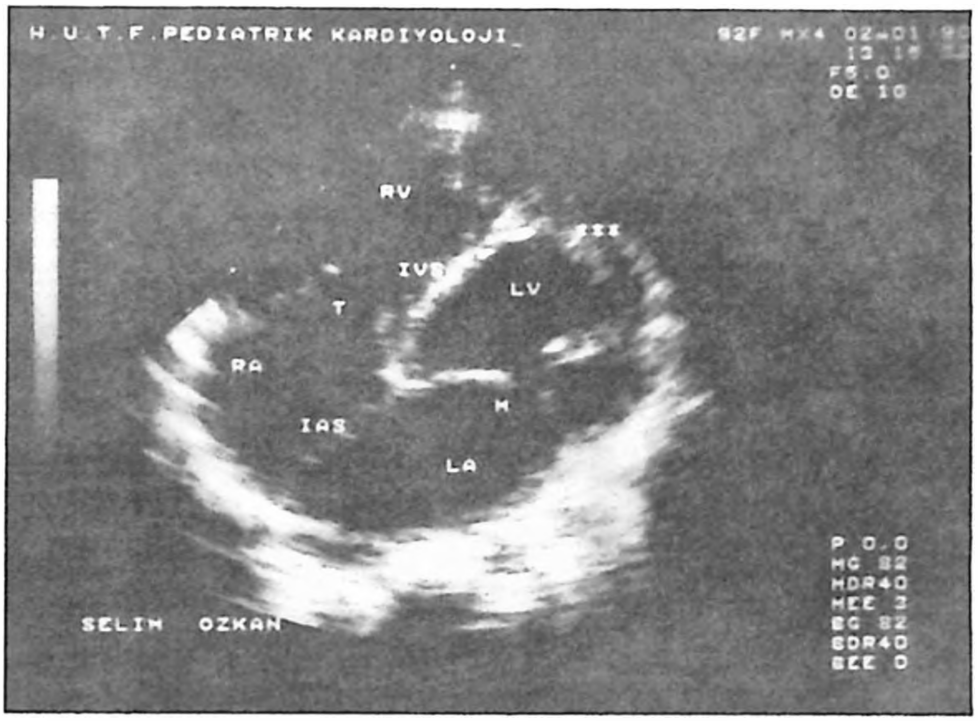

Fig. 1: Apical-four chamber view echocardiogram. Obliteration of left ventricular apex is seen (xxx). RV: right ventricle, T: tricuspid valve, RA: right atrium, LV: left ventricle, M: mitral valve, LA: left atrium, IVS: interventricular septum, IAS: interatrial septum.

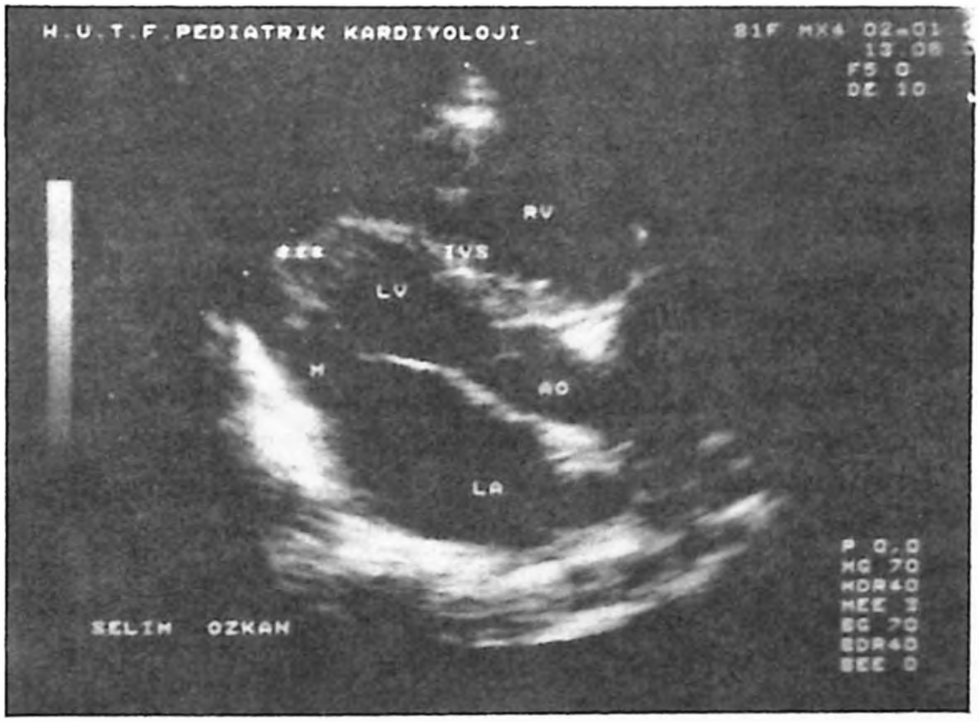

Fig. 2: Parasternal long axis view echocardiogram. Obliteration of left ventricular apex is seen (xxx).

There was no significant change in oxygen saturation. Pressures were pulmonary wedge, 20 mm Hg (mean); main pulmonary artery, 80/25 mm Hg (mean 64); right ventricle, 80/2-13 mm Hg; right atrium, 10 mm Hg (mean); left atrium, 22 mm Hg (mean); left ventricle, 90/3-16 mm Hg, and aorta, 90/54 mm Hg (mean 75). The

right ventricular angiocardiogram showed a dilated right ventricle, large main pulmonary artery and tricuspid insufficiency. Left ventricular angiocardiograms showed severe amputation of the left ventricular cavity resembling an "apple stalk" (Fig. 3). Moderate mitral insufficiency was observed. Left atrial angiography revealed that the left atrium and pulmonary veins were enlarged.

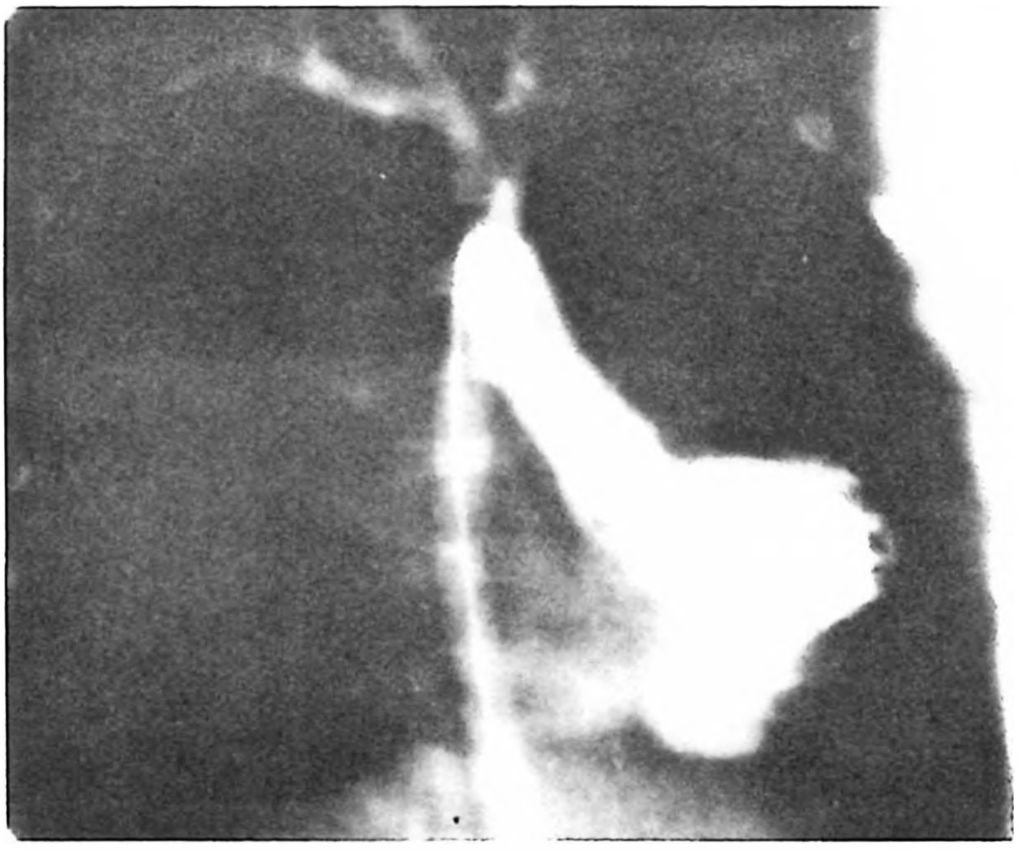

Fig. 3: Left ventricular cineangiogram in the right anterior-oblique projection: severe amputation of the left ventricular cavity (arrows) and mitral insufficiency are illustrated.

Bioptome was inserted through the right femoral vein and four specimens were taken from the right ventricular outflow tract, which were studied by light microscopy. Endomyocardial biopsy material showed moderate thickening of the endocardium and hypertrophy in some heart muscle fibers along with edema of the connective tissue of the myocardium.

After studying the clinical and laboratory findings, the patient was diagnosed as having EMF. He will be closely followed and measures will be taken to prevent congestive heart failure, in addition to his being considered a candidate for endocardiectomy in the future.

Discussion

Most patients with EMF reported in the literature are either older children or adults. Metras et al[4] reported 20 patients whose mean age was 13.3 years, the youngest being six years old. Gottdiener et al[5] reported 21 patients between the

ages of 16 and 61 while Vijayaraghavan et al[6] reported the ages to be between 5 and 48. Martines et al[7] studied 15 consecutive patients with an age-range of 11 to 56 years. In a larger series, Mady et al[8], reported 110 patients with EMF, the youngest, aged five. The patients studied by Acquatella et al[9] were all adults. None of the patients described by these workers was as young as our patient, 18 months of age.

There was a male predominance in the patients reported in the literature. Our patient was also a male.

EMF may present with or without eosinophilia. However, pathologic series suggest that both forms represent a spectrum of a single entity[9]. There was no eosinophilia observed in our patient.

In the past, this disease was mainly diagnosed in endemic areas by its clinical picture, by the characteristic obliteration of the ventricular apex or at necropsy[9]. However, recent reports indicate that echocardiography alone is sufficient to arrive at a reliable diagnosis of EMF. At the same time, this technique may be used to assess the extent and progression of the disease. During life, early isolation of the activity of the disease may also be evaluated by echocardiography[6]. In our patient, we were able to diagnose the disease first by echocardiography. Then, we confirmed the diagnosis with angiocardiography and biopsy.

EMF may only involve the right or left ventricle. However, in some patients both ventricles are affected[2]. In the majority of EMF patients, sheets of fibrous tissue of varying thickness develop in the subendocardium, initially involving the apices of the ventricles and extending into the inflow tracts of the right and the left ventricles. In addition an intracavitary thrombus appears as a filling defect in the apices of one or both ventricles[1]. In the case presented, echocardiographic findings showed that the left ventricular apex was involved (Figs. 1, 2). However the right ventricular apex was not apparently involved. The left ventricular apical intracavitary amputation was also detected by angiocardiography (Fig. 3).

The distribution of endocardial fibrosis may be either patchy or confluent. St John Sutton[1] reported that increased echo reflectance of the subendocardial surface is consistent with fibrous replacement. The spectrum of left ventricular geometry in restrictive cardiomyopathy is more varied than in either hypertrophic cardiomyopathy or dilated cardiomyopathy. Although left ventricular chamber size is almost always normal, wall thickness may be decreased, normal or increased, and these changes may occur throughout ventricles or be localized to specific regions within the ventricles[1]. In our patient, the left ventricular endocardium was involved extensively and the left ventricular functions determined were within normal limits echocardiographically. However, the right ventricle showed increased echo

reflectance of the subendocardial surface in some areas, which suggested patchy infiltration of fibrous tissue.

Since the left ventricular cavity was decreased in size, the left ventricular diastolic pressure was elevated. This results in left atrial enlargement[1]. In our patient, we detected only minimal mitral regurgitation but significant enlargement of the left atrium, demonstrated by Doppler, two-dimensional echocardiography or by angiocardiography. Left atrial mean pressure and pulmonary artery wedge pressure were elevated because of the situation described above. This results in pulmonary hypertension and elevation of right ventricular pressure, as detected in our patient. We related significant tricuspid regurgitation to the elevation of right ventricular pressure, as described by St John Sutton[1].

It has been reported that in most cases evaluated by echocardiography, thickening of the mitral and/or tricuspid posterior valves is one of the characteristic findings of EMF[6,9]. However, in our patient, the leaflets of the atrioventricular valves did not illustrate echocardiographic thickening. Therefore, we related atrioventricular valve incompetence to restriction of ventricular filling rather than to valve involvement, as suggested by Cherian et al[10] and Metras et al[4].

Strong echoes were recorded from the left ventricular endocardium, which were interpreted as fibrotic changes in the endocardium or subendocardium. At the same time, the left ventricular apex was obliterated and reflected dense echoes. This occurred because of endomyocardial scarring of the left ventricular apex. We could not detect any significant evidence of fibrosis in the right ventricle, but there were increased echoes in some parts of the endocardial surface of the right ventricle. Schmaltz et al[11] has demonstrated that an endomyocardial biopsy performed during childhood was a diagnostic tool which could add useful information to the etiology or pathogenesis of an underlying myocardial disease. They found that biopsies were diagnostic in 11.7 percent of patients, helpful in 71.7 percent of patients, and were of no help in 16.6 percent of patients. In our patient, the biopsy taken from the right ventricle supported the diagnosis. If the biopsy had been obtained from the left ventricle, where the lesion was very significant, it would have probably been diagnostic.

Surgical treatment of. EMF consists basically of endomyocardiectomy and valvular replacement[4,7,8,10]. Therefore, we decided to follow up our patient and to perform an endocardiectomy in the future.

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HLA AND LEUKEMIA: Is it a Simple Allelic Association?*

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SUMMARY: Dorak MT, Chalmers EA. (Department of Internal Medicine, Akdeniz University Faculty of Medicine, Antalya, Turkey, and the Royal Infirmary Leukaemia Research Laboratory, Glasgow, United Kingdom). HLA and leukemia: is it a simple allelic association? Turk J Pediatr 34:55-59, 1992.

The first association between a disease and MHC antigens was established for viral leukemogenesis in mice. Since then, numerous studies in humans have been carried out and the data obtained suggest that Cw3 and Cw4 may be markers for leukemia susceptibility genes. Given the relatively weak relevance of HLA-C locus antigens in immune response, unrecognized properties of Cw3 and Cw4 should be considered to explain this association. Family studies have consistently revealed limited heterogeneity of HLA antigens in those families, increased parental HLA antigen sharing resulting in increased homozygosity among offspring and high frequency of recombinations within the MHC. Furthermore, the HLA genotype of leukemic patients was found more frequently among their siblings than the anticipated 25 percent. Another significant deviation from the Mendelian expectation was increased HLA-DR identity of offspring to that of their mother in those families. These and other observations imply that in leukemia families unknown MHC-linked recessive factors linked to Cw3 and Cw4 alleles may be susceptibility genes which also cause segregation distortion of HLA genes and probably developmental errors. *Key words: HLA antigens, acute lymphoblastic leukemia, segregation, family, chromosome 6.*

The existence of a major histocompatibility complex (MHC) linked susceptibility factor has long been postulated from the well-known role of H-2 haplotypes and virus-induced leukemogenesis in mice[1]. The resistance loci for the Friend, Gross and Moloney murine leukemia viruses have all been mapped in the H-2 region[2-4]. To date, the strongest associations have been established in humans between HLA-Cw3, Cw4, A30, B17, DPw2 and DPw5 and acute leukemias whereas HLA-Aw19, B14, DR7 and DPw1 have been found to be decreased in leukemia patients[5-12]. Most other antigens reported to be increased in leukemia patients are indeed in strong linkage disequilibrium with Cw3 and Cw4[13]. The HLA-Cw3, Cw4 and HLA-DP association is most significant in acute lymphoblastic leukemia (ALL) patients[5-9]. In a family study, two of four sibs with ALL had the HLA haplotype "Aw31, Cw3, Bw61, DR5"[14]. In a subgroup of adult ALL patients of whom 45.5 percent had at least one close relative with familial leukemia, HLA-Cw3 was consistently positive along with high transcortin levels[7]. A strong association of the acute type of adult T-cell leukemia with "HLA-A26, Bw62,

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Cw3" haplotype has recently been reported[15]. Survival following bone marrow transplantation is good for individuals bearing a DRw52 haplotype and poor for those bearing a DRw53 haplotype[13]. The strong positive association of Cw3 and Cw4 with leukemia supports the possibility that the genes encoding them are, or are closely linked with, putative leukemia susceptibility gene(s)[6].

In a survey of 194 families of patients with acute leukemia, Chan et al[16] found that a genotypically HLA-identical sibling was available for 35 percent of leukemic patients although 25 percent had been anticipated. In a different series of ALL patients, more than 25 percent HLA identity was reported in sibs of patients[17]. A striking excess of identical antigens (especially HLA-DR) among the parents of leukemic patients has been reported by a number of authors[6,16,18,19]. Limited heterogeneity of HLA antigens has also been reported in parents of children with Down's syndrome which is associated with an increased risk of acute leukemia[20].

In the study of Carpentier et al[18], the HLA genotype was determined in 73 unrelated patients with acute myeloblastic leukemia (AML) and also in their first degree relatives. A significant finding was the presence of a clear genetic distortion favoring HLA homozygosity in the affected offspring. In offspring with leukemia of such families, there is a significant deviation from the Mendelian expectation of the number of patients homozygous for DR alleles shared by parents but not in healthy sibships. Von Vliedner et al[19] reported a two-fold increase in the number of homozygotes among patients compared to the expected value in families where parents shared a B or DR antigen, and significantly more heterozygotes for the shared antigens among the healthy siblings. In the same patient population of Carpentier et al[18]. The distribution of DR antigens shared by parents markedly favored phenotypic HLA identity of patients with their mothers as compared with their fathers. Obviously, this is possible only if the parental shared haplotype has a high transmission ratio as compared to the homologous haplotype (Fig. 1).

On the other hand, in a very large data set of non-diseased families, parental sharing of one or two HLA class-I antigens did not result in altered allele segregation among the 2569 healthy offspring tested[21]. In the same study, homozygous children of HLA-sharing couples and children histocompatible with their mothers were observed with frequencies close to that envisaged. In another comprehensive survey, no evidence was obtained for segregation distortion of MHC in non-diseased families[22].

The possible implications of these studies are: the segment of chromosome 6 carrying the HLA genes also carry the recessive gene(s) influencing leukemogenesis and thus the HLA alleles per se are not the critical genes; haplotypes including shared antigens should also bear a gene or genes which confer(s) the ability of preferential (male-specific) gametic transmission to those haplotypes, and if there

is a leukemia susceptibility locus on chromosome 6, it is likely linked to the MHC, as in the case of mice[1].

As mentioned above, in general, more unaffected sibs than anticipated are found to have the same HLA genotypes as their sibs with ALL, which conforms with segregation distortion. However, the subgroup of ALL patients with both HLA-Cw3 and high transcortin levels and familial tendency reacts in the opposite way. Instead of more HLA-identical sibs, fewer are found, suggesting fetal wastage of the latter combination[23].

If one can explain the association of certain HLA antigens with leukemia along with segregation distortion and relevance of homozygosity to expression of leukemic phenotype, this may well be a major step towards understanding the mysteries of leukemogenesis. The same explanation should clarify the typical maternal reproductive history of childhood leukemia seen with recurrent abortions or fetal wastage[24], and how paternal but not maternal preconceptional accumulated doses of radiation may confer a risk for leukemia in offspring[25]. Another observation which is yet unexplained is the high recombination frequency within MHC in leukemic families[26,27].

It is a well-known phenomenon that couples experiencing recurrent abortions share HLA antigens more frequently than control couples[28]. Not only recurrent abortions and leukemia but also aplastic anemia[29], gestational trophoblastic tumors[30] and anencephaly[31] seem to be associated with parental sharing of HLA antigens. The properties of the HLA system are not sufficient to rationalize these associations, but some HLA-linked factors influencing embryogenesis, as well as carcinogenesis may be responsible. Previously, these associations have been interpreted from the point of view that increased maternal-fetal HLA compatibility may somehow cause defects in embryogenesis and recurrent abortions. However, in cases of HLA antigen sharing between parents, repetitive maternal-fetal HLA compatibility is possible only if male transmission distortion exists.

The oncogene pim-1 (located at 6p21 adjacent to the MHC)[32] and the myb oncogene (located at "6q22-q24)[33] have been reported to be over-expressed in acute leukemias. There are even more oncogenes at or beyond 6q21, such as mas-1, fyn, syn, and ros-1[34]. A Friend murine leukemia virus integration site (fim-1) was recently mapped onto the human chromosome 6p23[35]. This retroviral integration region is known to be involved in mouse myeloblastic leukemias. Furthermore, there are six fragile sites on chromosome 6, three of which are located within 6p22.2-p25.1[36]. Various translocations involving 6p23 or 6q27, and more frequently (particularly in ALL) deletions at 6q15, 6q21 and 6q23 have been reported at considerable frequencies in acute leukemias[34]. Accumulation of oncogenes and fragile sites at two regions on the same chromosome with MHC may have some implications regarding the relevance of chromosome 6 in leukemogenesis in humans.

Considering the current hypothesis[37] regarding the development of childhood ALL, the first developmental error initiating a preleukemic clone in utero might be homozygosity of putative lethal factors linked to the MHC, and rearrangements on chromosome 6, such as deletions in the long arm may fit this hypothesis as a subsequent genetic error. In conclusion, investigators attempting to establish an association between HLA and leukemia should consider the entire chromosome 6 along with the genetic evolution of this particular region of the human genome.

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