Acta Paediatrica Hungarica

VOLUME 32, NUMBER 1, 1992

Akadémiai Kiadó, Budapest

ACTA PAEDIATR HUNG HU ISSN 0231-441X

ACTA PAEDIATRICA

HUNGARICA

A Quarterly of the Hungarian Academy of Sciences

Editor

M. MILTÉNYI

Acta Paediatrica publishes original reports of studies in paediatrics and borderline fields.

Manuscripts and editorial correspondence should be addressed to

ACTA PAEDIATRICA HUNGARICA

Bókay J. u. 53.

H-1083 Budapest, Hungary

Subscription information

Orders should be addressed to

AKADÉMIAI KIADÓ

P.O. Box 245

H-1519 Budapest

Acta Paediatrica Hungarica is published in yearly volumes of four issues by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences

Prielle Kornélia u. 19—35.

H-1117 Budapest

Acta Paediatrica Hungarica is abstracted/indexed in Biological Abstract, Chemical Abstracts, Excerpta Medica, Index Medicus

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Acta Paediatrica Hungarica 32 (1), pp. 3-10 (1992)

A NEW CHROMOSOMAL TRANSLOCATION t/1;22/ /q25;q12/ IN A 17-MONTH-OLD GIRL

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Received 5 December 1990

A 17-month-old girl with multiple anomalies and a de novo translocation of chromosomes 1 and 22 (t/1;22/ /q25;q12/) is described.

INTRODUCTION

The symptoms in patients with deletion of chromosome 1q can be divided into three groups on the basis of the location of the deletion /10/, as follows: group 1: distal 1q /1q42-43→ 1qter/ deletion syndrome; group 2: interstitial 1q /1q24-25→ q32/ deletion syndrome; group 3: proximal 1q /1q21-22→q25/ deletion syndrome.

In 1988, Dasouki et al./1/ reported an eight-month-old girl with symmetrical bilateral oblique facial clefts, a calcaneovarus foot deformity, and a balanced chromosomal translocation t/1;22/ /q25;q12/.

In this report, we present a 17-month-old girl with a chromosomal translocation t/1;22/ /q25;q12/. Su-gui et al./9 earlier found a male patient with a chromosomal translocation t/1;15/ /q25;q24/ among couples associated with abortions or stillbirth. Unfortunately, the symptoms of their patient were not described.

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PATIENTS AND METHODS

Case report:

E.V. (date of birth: 01.03.1988), a 17-month-old girl, was examined clinically and cytogenetically, because of her craniofacial dysmorphism and wrinkled skin. She was born at term after a normal pregnancy, to a 35-year-old G1 P0-1 mother and a 28-year-old father: they were healthy. The patient reported here was resuscitated at birth. Her weight at 17 months (9.7 kg, 90 centile) was normal, but her movements and intellectual development were slightly retarded at the time of the investigation.

Cytogenic analyses were performed on conventional blood cultures /8/. GTG-banded slides were made according to the method of Seabright /7/. Forty mitoses of the patient and twenty mitoses of the parents were examined under the microscope. Karyotype analyses were carried out on ten mitoses of the patient and on five metaphases of the parents with the short version of ISCN /4/.

RESULTS

Table I shows the symptoms observed in the proximal 1q deletion syndrome /6, 10/, those in the intermediate 1q deletion syndrome /3, 5, 11/, those in a case with a t/1;22/ /q21;q12/ /1/, and those in our patient with a t/1;22/ /q25;q12/. It can be seen that all of the patients with the 1q deletion proximal to the 1q25 band had cleft lip and palate, in contrast with patients in whom the deletion 1q was in band q25 or distal to that.

Our patient exhibits craniofacial dysmorphism: rough features, not only a flat and broad nasal root, but also a flat and broad nose, low-set auricles, and a large mouth. She has very loose, wrinkled skin, which is very conspicuous on the neck and the hands, and a slight branchydactyly (Fig. 1a and 1b).

She displays retarded ossification of the bones; her bone-age is similar to that of a 3-6 month-old child. All of the results of laboratory investigations (serum electrolytes; hematocrit; hemoglobin, glucose, creatinin concentrations; white blood cell counts; immunoglobulins; etc.) were in the

TABLE I

Clinical symptoms in proximal 1q deletion syndrome, in intermediate del 1q syndrome, in a case of t/1;22//q21;q12/, and in our patient with a t/1;22//q25;q12/

Symptoms	Syndrome			
	1	2	3	4
Craniofacies				
microbrachycephaly	+			
microcephaly	+	+		+
cleft lip and palate	+	+	+	
round face	+			+
high forehead	+			
low-set ears	+	+		+
micrognathia	+	+		
upper epicanthic folds	+	+		
soarse, fine hair	+			
sparse eyebrows	+			
short nose	+			
microphthalmia		+		
rough features				+
flat and broad nose	+			+
large mouth				+
Brain and intelligence				
epilepsy	+			
profound motor and mental retardation	+			
normal brain parenchima			+	
severe motor and mental retardation		+		+
Abdomen				
hernias	+	+		
Genitalia				
genital anomalies	+	+		
Limbs				
small hands and feet	+			

Table I Cont.

Symptoms	Syndrome			
	1	2	3	4
clinodactyly of the fifth fingers	+	+		
brachydactyly	+	+		+
calcaneovarus foot deformity			+	
retarded ossification of the bones	+			
Other abnormalities				
exotropia	+			
anisocoria	+			
hypothyroidism		+		
growth hormone deficiency		+		
cardiac defects		+		
bilateral ocular hypoplasia			+	
loose, wrinkled skin				+

[1]proximal 1q deletion syndrome (del/1//q21-q23 q25/) /Schinzel and Schmid 1980; Taysi et al. 1982/

[2]intermediate 1q deletion syndrome (del/1//q24-q25 q32/) Turleau et al. 1974; Koivisto et al. 1976; Garver et al. 1976/

[3]t/1;22//q21;q12/ /Dasouki et al. 1988/

[4]t/1;22//q25;q12/ /our patient/

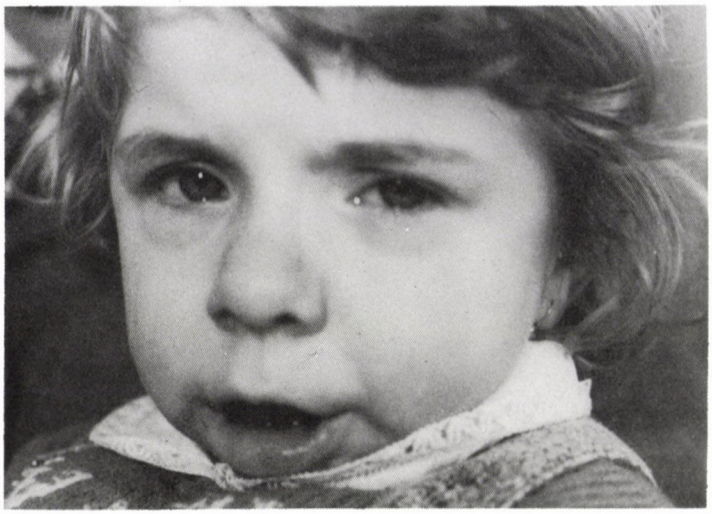


Fig. 1a. Our 17-month-old patient

Fig. 1b. The hands of the patient

normal range.

The partial karyotype (Fig. 2) demonstrates the translocation t/1;22/ /q25;q11/ observed in all the mitoses examined of the patients. The parents were cytogenetically normal.

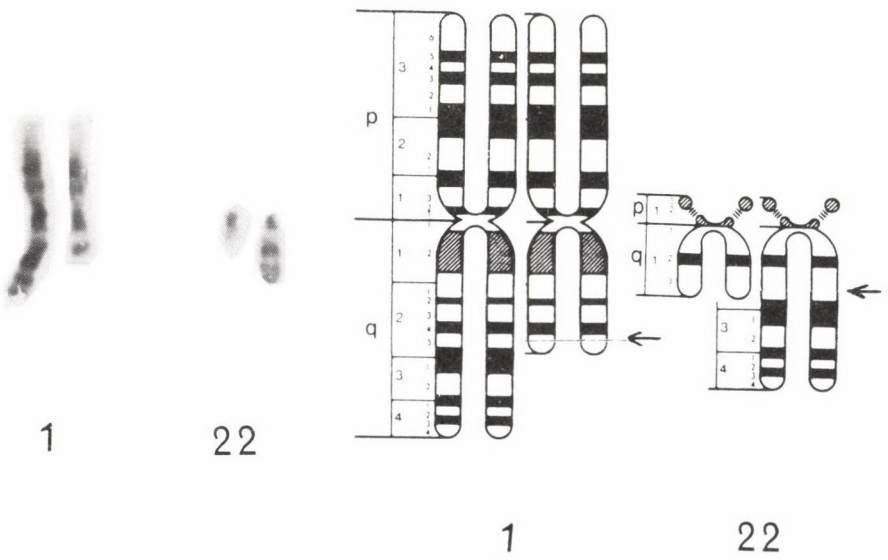
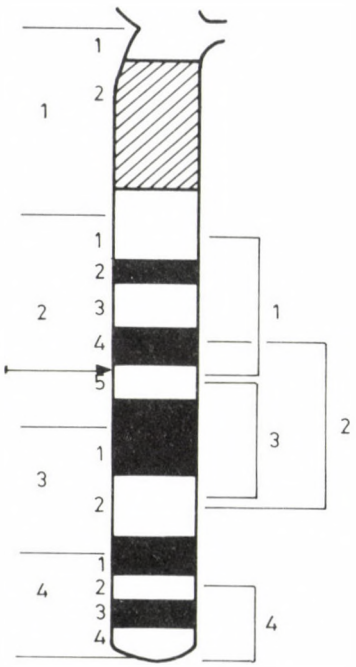


Fig. 2. The partial karyotype shows the balanced translocation t/1;22//q25;q12/ of our patient

DISCUSSION

Figure 3 shows the deleted segments in patients with proximal, intermediate, and distal deletions of 1q. It seems that the intermediate del 1q syndrome must be divided into two groups: into the proximal intermediate del 1q /q24→q32/ syndrome, and the distal intermediate del 1q /q25→q32/ syndrome. The patient with the proximal intermediate del 1q syndrome had cleft lip and palate /11/, in contrast with the patients with distal intermediate del 1q syndrome, who were without cleft lip and palate /3, 5 and our patient, if she has



[1]proximal 1q /q21-q22→q25/ deletion syndrome
[2]proximal intermediate 1q /q24→q32/ deletion syndrome
[3]distal intermediate 1q /q25→q32/ deletion syndrome
[4]distal 1q /q42-43→qter/ deletion syndrome
arrow: the breakpoint in our patient

Fig. 3. Schematic representations of the deleted segments in various 1q deletion syndromes

microdeletions in band 1q25).

In the present case, the "balanced translocation" is associated with an abnormal phenotype. Microdeletions at the breakpoints can cause abnormalities in the phenotype /1, 2/. In our patient, the possibility of microdeletions cannot be excluded, however the symptoms in the patient are rather unspecific.

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Acta Paediatrica Hungarica 32 (1), pp.11-30 (1992)

CHILDREN FATHERED BY MEN TREATED FOR TESTICULAR CANCER CONCEIVED BEFORE, DURING AND AFTER CHEMOTHERAPY - EXAMINATION FOR EVIDENCE OF CONGENITAL MALFORMATIONS, MALIGNANCIES AND IMMUNOLOGICAL DEFECTS

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Received 24 June 1991

Hundred children of 64 fathers with testicular tumour treated from 1979 on at the National Institute of Oncology, Budapest were studied. Three groups were formed on the basis of the time of conception. 59 children were born before the illness of the fathers, 19 during the 9 pretreatment months and 22 during or after combined chemotherapy.

Family anamnesis, perinatal and gestational data were listed, thereafter physical, laboratory, immunological, psychiatric, and, if required, radiological examinations were made.

No difference was detectable in the somatic and psychiatric status of the three groups, development was well balanced, corresponding to age. Protocols of the combined chemotherapy applied and incidence of anomalies, malformations, malignancies and other diseases were recorded. Their incidence was similar in all three groups though frequently this was higher than that of the normal population. Often cumulated incidence of severe congenital malformations was found in the group conceived after concluded therapy where twice as many girls were born as boys. The interval between conception and the end of therapy was established in the case of children conceived during and after therapy. This was shortest in the case of healthy children, the number of healthy children conceived during cytostatic treatment was also remarkable.

Further compilation of data and individual evaluation of case reports is recommended.

Akadémiai Kiadó, Budapest

Abbreviations:

Ca-e:	Embryonal cell carcinoma
Cc:	Choriocarcinoma
T:	Teratocarcinoma
T-a:	Teratoma adultum
S:	Seminoma
S+Ca-e:	Seminoma + Embryonal cell carcinoma
S+T:	Seminoma + Teratocarcinoma
VPB:	Vinblastine+Bleomycin+Cisplatin
VACAD:	Vincristin+Adriamycin+Cyclophosphamide+Actinomycin-D
AMV:	Adriamycin + Methothrexat + Vincristin
VAC:	Vincristin + Adriamycin + Cyclophosphamide

INTRODUCTION

The incidence of testicular tumours is greatly increased among males aged 20 to 35 years (6-7/10 0000/years) /41/. As a result of recent advances in the concept of combined therapy, based on histological-clinical parameters /4, 13, 52, 55/, their mortality rate was reduced /16/, and depending on the stage of their status, a major part of them might even be considered fully recovered /42/.

Now, beyond the improvement of therapeutic results, further aspects have to be considered, whether the presence of testicular tumours in fathers and their treatment with irradiation or chemotherapy constitutes a problem for advising them to have children.

In addition to the well-known short-term side effects of chemotherapy affecting the patient it may also impair the genetic material or may even trigger new malignant processes /27, 29/. Studies on long-term effects /45/ draw attention also to further pathological somatic alterations /12, 24/.

As most of the recovered patients are young, several papers were published about the procreative power /30/, sexual behaviour /53/ and parency /1/ of males as well as children conceived by these patients /11, 46/, though their deductions were highly variable.

The aim of the present study was to investigate whether congenital malformations are induced in children of fathers with testicular tumours submitted to combined chemotherapy and irradiation. The main accent was made on genetical disorders depending on children's conception time.

PATIENTS AND METHODS

Patients

This cohort represents data of 100 randomly selected children whose father had testicular tumour (Table I). This trial was performed on children in a blind study. The groups were classified depending on the time of conception as follows (see Table II).
In groups I and II 9 months were taken into consideration because of threefold termine time the maturation process of germ cells (53, 11). In total, there were 31 siblings, of them 15 had sibling before the treatment and was only one pair born after the treatment.

The fathers had histologically confirmed unilateral testicular tumour (Table I) and were treated between 1979 and 1988 at National Institute of Oncology Budapest managed after orchidectomy by VACAD, VACAD+irradiation (↯), VPB or exceptionally VPB+other combination.

Lymphocyte subpopulations of healthy children of the same sex and age (18 children) served as control in data processing.

Methods

Upon admission to the clinic the patients were informed about the aim of study. Ten patients (16 children = 13%) refused to participate, while 64 males were admitted with 100 children. Detailed family, pregnancy and perinatal anamneses were prepared. For examinations carried out on the children see Table III.

During the processing of test data the parameters of children born to healthy fathers (group I) were compared to those conceived immediately before the onset of the disease (group II) and to those born during or after chemotherapy (group III).

The rate of anomalies and disorders were presented as the incidence calculated in index population in comparison to national rate. At the evaluation of the time between termination of therapy and conception mathematical means were calculated. The investigation of lymphocyte subpopulations of children fathered by men treated for testicular cancer seems to be a very interesting, up to the present unclarified problem.

During the analysis of lymphocyte subpopulation ratios Student's "t" test was applied to determine the extrapolated range, in possession of the distribution of normal data /5/. The data of children conceived during and after therapy were demonstrated in this system.

TABLE I
Distribution of offspring fathered by men treated for testicular cancer

	Groups, examined depending on children's conception time			
	I More than 9 months before onset of treatment	II 9 months before the start of therapy	III During or after therapy	Total
A. Histological diagnosis of fathers				
S	9	1	1+1+	11+1+
S anaplast.	2	-	-	2
Ca-e	24+1+	5	8	37+1+
T	5	4	3	12
S+Ca-e	3+1+	4	-	7+1+
S+T	3	-	2	5
Ca-e+T	5	3	5	13
Ca-e+T+S	6	1	-	7
T+Cc	-	1	1	2
Rhabdomyosarcoma	-	-	1	1
B. Pregnancys sequence				
1st pregnancy	31+1+	9	8	48+1+
2nd pregnancy	13+1+	5	11+1+	29+2+
3rd pregnancy	7	4	1	12
4th pregnancy	3	1	-	4
5th pregnancy	-	-	-	-
6th pregnancy	1	-	-	1
no sign.	2	-	1	3
C. Mothers' age				
< 20 years	3	-	-	3
20-25 years	26+1+	7	11	44+1+
26-30 years	18+1+	7	6	31+1+
31-35 years	7	4	2+1+	13+1+
> 35 years	-	1	-	1
no sign.	3	-	2	5
D. Offspring age				
< 1 year	-	1	5+1+	6+1+
1- 3 years	14+1+	8	8	30+1+
4- 6 years	16+	9	6	31
7- 9 years	12+1+	1	1	14+1+
10-12 years	8	-	-	8
13-15 years	5	-	-	5
> 15 years	2	-	-	2
E. Offspring sex				
male	25+1+	9	6+1+	40+2+
female	32+1+	10	15	57+1+
	57+2+	19	21+1+	97+3+

+ dead child

TABLE II

Groups and number of children according to conception time

Groups of children	Number of children
I. More than 9 months before the initiation of therapy	59
II. 9 months before the initiation of therapy	19
III. During and after therapy	22

TABLE III

Examinations carried out on children and their number of offspring

Examinations carried out on children	Number of children
Physical examination	96
Bloodgroups for the identification of paternity	20 (in III)
Laboratory test	51
Radiological examination (bone-age)	21 (in III)
Abdominal ultrasound investigation	57
Immunological analyses (Behring, BMA)	18 (in III)
Wartegg-and anxiety test	62 (age 3 years)

No tests were performed on a single child in group III who died.

RESULTS

Age, diagnosis and treatment of fathers

Fig. 1, Table I show the age distribution and diagnosis of the fathers of the children studied. One third of the patients was 26 to 30 years old and a major part of them (35/64) had been submitted to VPB treatment at 4 to 6 occasions due to embryonic carcinoma. The paternity of recovered patients could not be excluded on the basis of blood tests.

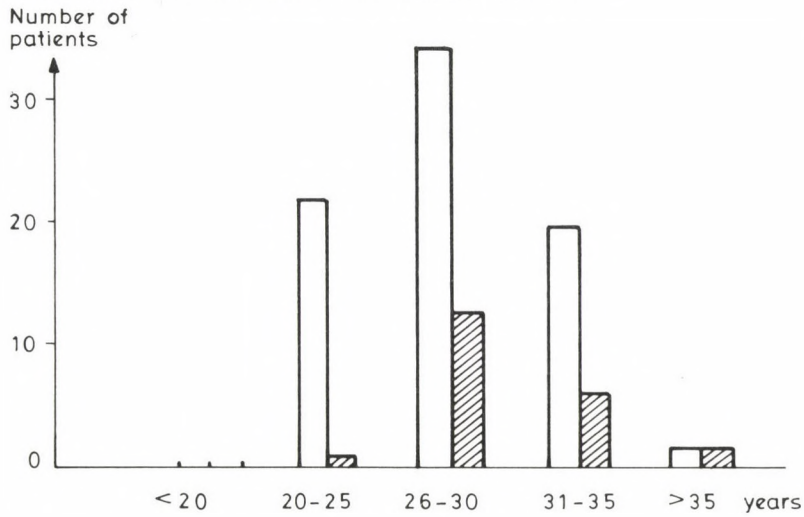

Fig. 1. Age of men treated for testicular cancer, either
▭: at the birth of offspring conceived before therapy,
▨: or at the birth of offspring conceived during and after chemotherapy

Gestational, perinatal anamnesis

Analyzing the pregnancy pattern of individual groups (Table I) in the first two groups the children were coming from the first pregnancy while most of those who were born after concluded therapy came from the second pregnancy. This observation was closely associated with the dramatic increase in the incidence of induced abortuses after the diagnoses became known (9/13) (Table IV) while earlier spontaneous abortuses had been the predominant pattern (5/8).

No difference was detectable between the 3 groups either as regards gestational or obstetric anamneses except for a single feature: premature delivery. Most of the premature infants (3/19) were conceived during the 9 months preceding therapy. In 89 of the 100 children studied the gestational period was in the range of 39 to 42 weeks.

Reviewing perinatal anamneses there was one feature deserving attention, the incidence of physiological jaundice; in each of the three groups (3/59, 2/19, 3/22) though with varying frequency. Symptoms suggesting cerebral haemorrhage or microcephaly were observed in two children born before castration. A newborn with persistant ductus Botalli was conceived 7 months before the onset of therapy while atrial septal defect and spine bifida/anencephaly were found in children conceived during the posttreatment period. If properly cared for, patients with valvular disease remained in normal condition. A single 6 weeks-old infant with neurological defect has died.

Mother's age and familiar anamnesis

Age of the mother, diseases and causes of deaths of grandparents as well as of brothers and sisters were recorded. The age of most mothers in all 3 groups was in the range of 20 to 25 years, there was a single mother who was older than 35 years at delivery (Table I). In the line of both parents malignancies had the highest incidence (28/100) followed by cardiovascular diseases (22/100) and diabetes of the elderly (16/100).

Sex, age and somatic development of children

The group of 100 children studied consisted of 58 girls. The incidence of girls conceived during and after therapy was 15/22 (Table I). It demonstrates the age distribution of children and percentual data of their development was observed, too. Compared to the balanced percentual weight and length curves of newborns at birth four fifth of the population studied was in the range of 10 to 90% in all three groups. 1/3 to 1/5 of them were born with a length higher than 90%, considered the limit of the normal range. Data recorded in 1989 show that 4/5 to 9/10 of the percentual weight and 3/4 to 9/10 of the percentual

TABLE IV

Loss of offspring due to different causes in families with testicular cancer

	Before onset of disease	Within months of start of therapy and the birth of offspring	After the birth of offspring	Total
Spontaneous abortion	5	1	2	8
Arteficial abortion	2	9	2	13
Still birth	0	0	0	0
Postnatal mortality (cause)	2 Wilms tumour neopl.of the brain	0	1 spina bifida and anencephaly	3

TABLE V

Perinatal data of children fathered by testicular cancer patients

Groups	Groups, examined depending on children's conception time: More than 9 months before treatment I	9 months before treatment II	During and after treatment III	Total
Total of children examined	59	19	22	100
Gestation time				
⩾ 39 weeks	54	15	20	89
37-38 weeks	4	1	1	6
< 37 weeks	1	3	1	5
Birthweight				
⩽ 2500 g	5	2	1	8
2600-3400 g	11	2	3	16
3500-4400 g	41	14	17	72
⩾ 4500 g	2	1	1	4

length of the children mostly aged 3-8 years and born before the illness of the father, or aged 2-5 years and born during the pretreatment months or aged 0.5-5 years and born during or after therapy were within the normal 10 to 90% range. There was no significant difference in the birthweight of children in groups I , II and III (Table V). The bone age of all children listed in the III. group was normal.

Congenital anomalies, malformations, malignancies and other acquired syndromes

The incidence of congenital anomalies was not higher in the population conceived during the pretreatment months, during and after therapy compared to those of the first group (Table VI).

However, as regards congenital malformations, the occurrence of heart diseases and neurological disorders should be mentioned in groups II and III.

At the assessment of minor anomalies (Table VII) and congenital malformations in two children, conceived during the 24th posttreatment month, the cumulated incidence of congenital malformations was observed.

Reviewing the incidence of malignancies (Table VI) two children, born when their fathers were not ill yet, died due to Wilms' and cerebral tumour at the age of 8.5 and 2.5 years, resp. There were also malignancies in the offsprings conceived after concluded therapy: the 4 - months - old child of a man treated for embryonic carcinoma had neuroblastoma but remission was attained by combined chemo- and radiotherapy. At present the child is 2 years old, untreated and symptom-free.

In the following the causal effect of the interval between conception and therapy as well as the effect of cytostatic agents was studied (Table VII).

Two fathers submitted to VACAD and VACAD+radiotherapy had children with anomalies. Analysing the incidence of congenital malformations and malignancies it was found that most of the patients received VPB therapy. Most of the fathers (12/14) whose children were declared healthy (14/22) have also been treated according to the VPB protocol (Table VII). The mathe-

TABLE VI

Occurrence of congenital anomalies and other disorders in children conceived by testicular cancer patients

Groups	Groups examined depending on children's conception time I.	II	III.	Total	National rate+ (control)
Total of children examined	59	19	22	100	1000
A. Occurence of minor anomalies					
hernia umbilical	1	-	-	1	0.98
nevus (over 1 cm)	2	-	-	2	2.74
alopecia (localised)	1	-	1	2	1.10
inner epicanthal folds	3	1	1x	5	2.35
clinodactily	2	-	1x	3	0.39
hypertelorism	2	1	1	4	4.41
flat occiput	1	-	-	1	0.88
prominent occiput	2	1	-	3	6.96
B. Occurence of congenital abnormalities					
atrial septal defect(ASD)	-	-	1xx	1	0.95
horseshoe kidney	-	-	1xx	1	0.40-0.8
patent ductus arteriosus	-	1	-	1	0.86
microcephaly	-	1	-	1	0.10
spina bifida+anencephaly	-	-	1	1	1.63-1.0
strabismus cong.	2	-	1	3	26.25
inguinal hernia cong.	3	1	-	4	12.10
dysplasia of hip	2	-	-	2	27.53
undescended testis	3	-	1	4	8.0
hydrocele testis	2	1	-	3	8.0
micrognathia	-	1	-	1	0.03
teleangiectasia nevus flammeus	4	1	-	5	30.0
C. Occurence of malignancies					
Wilms' tumour	1	-	-	1	$7/10^6$/year
neuroblastoma	-	-	1	1	$7/10^6$/year
neoplasm of the brain	1	-	-	1	$23.9/10^6$/year
D. Other disorders					
bronchitis obstr.	10	4	2	16	
malnutrition	5	2	2	9	
obesity	2	3	-	5	
kyphosis	4	3	2	5	
anemia (iron deficiency)	7	3	2	12	
atopic dermatitis	2	2	-	4	
lymphadenopathy (benign)	-	1	-	1	

\+ see references /7 ,8 ,54/

x the same child (epicanthal folds + clinodactily)

xx the same child (ASD + horseshoe kidney)

TABLE VII

Healthy children and offsprings fathered by men treated for testicular cancer

Disorders	Occur-rence	Sex	Fathers histology	Treatment	Time from completion of treatment to conception (month)	means (month)
A. Minor anomalies						
alopecia	1/22	F	Ca-e	VACAD+irr.	24	
epicanthal folds	1/22	F[x]	Ca-e+T	VACAD	24	22.75
clinodactily	1/22	F[x]	Ca-e+T	VACAD	24	
hypertelor.	1/22	F	T+Cc	6 VPB	19	
B. Congenital malformations						
ASD	1/22	F[xx]	Ca-e	7 VPB	24	
horseshoe kidney	1/22	F[xx]	Ca-e	7 VPB	24	
spina bifida+ anencephaly	1/22	M[xxx]	S	AMV+5 VPB	89	
strabismus congen.	1/22	F	Ca-e+T	6 VPB	24	32.0
undescended testis	1/22	M	Ca-e	6 VPB	22	
C. Malignancies						
neuroblastoma	1/22	F	Ca-e	4 VPB	9	
D. Healthy children		F	S+T	VACAD+4 VPB	18	
		F	Ca-e	4 VPB	30	
		M	T	4 VPB	55	
		F	Rhabd.sc	VAC	during therepy	
		F	Ca-e+T	4 VPB	32	
		M	Ca-e	4 VPB	16	
		F	Ca-e+T	5 VPB	15	21.0
		M	Ca-e	6 VPB	during therapy	
		F	Ca-e	6 VPB	30	
		F	T	6 VPB	18	
		F	T	6 VPB	33	
		F	S	AMV+5 VPB	23	
		M	S+T	6 VPB	24	
		M	Ca-e+T	6 VPB	6	

xxx dead child
x the same child (epicanthal folds+clinodactily)
xx the same child (ASD+horseshoe kidney)
irr.= irradiation
M= male
F= female

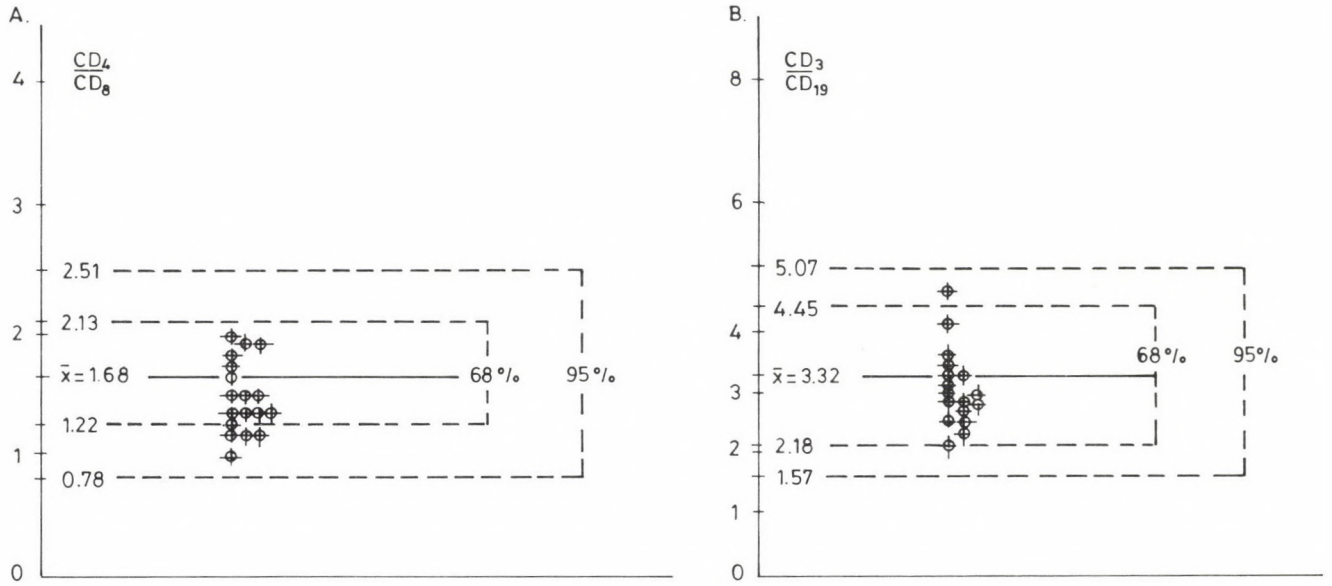

Fig. 2. T cell subsets.
A. CD_4/CD_8 ratio and
B. CD_3/CD_{19} ratio in children fathered during and after chemotherapy by men treated for testicular cancer
x = prospective mean of healthy control
%-interval = distribution of healthy control data

matical mean of the intervals between the end of therapy and conception was shorter (21.0 months) in the case of healthy children compared to the malformed ones (anomaly: 22.75 months, malformation+malignancy: 32 months). Even healthy children were conceived during therapy.

Immune status

The study could be evaluated in the case of 18 children of group III. Helper T/suppressor T or Pan T/Pan B ratios were plotted in the assumed scattering range of healthy controls (Fig. 2). The helper/suppressor ratio was beyond the 68% scattering range in the case of 4 children but only one was less than 1.0 (0.98). Only a few children (2/18) had Pan T/Pan B ratios in the range of 68 to 95%.

Psychic maturity

Psychic status was assessed by family patterns, Wartegg and anxiety tests applied differentially according to age. Two boys, one of them of group II, had adaptation difficulties and retarded development of speech. The major part of the offsprings (70/100) however, had an open, adaptive, creative, optimistic personality with emotional and mental maturity corresponding to their respective age. Only a single child was pathologically anxious, full of stress and uncertainty with strong mother fixation probably due to the rather disorderly relationship of its parents. Mother fixation was a dominant feature recurring in about 20% of the children (mother in the center in pantalons, rather muscular, with a hat or sometimes glass in hand).

DISCUSSION

The increased survival rate due to successful therapy is unfortunately accompanied by unexpected side effects, too /29/. This is especially the case in patients with testicular tumours. The increasing rate of remissions /16, 42/ with concomitant preservation of procreative ability /30/ may lead

to children with potential retardations, malformations /46/.

In agreement with the literature /16, 41, 42/ the data compiled in the field of testicular tumours demonstrate that young fertile males are the most affected population group, particularly the recovered 26-30 year old ones (Fig.1).

Though most of them had already a child before the diagnosis of the tumour, the chances of those born after successful therapy are most important.

Several papers review the pregnancy of mothers who suffered from malignancies /15, 17, 56/, the fate of their children /39/ and some even attempt to follow them up for prolonged periods of time /3, 44/. There are studies of pregnancy outcome after treatment during childhood or adolescence for neuroblastoma /6/, ALL /36, 20, 48/, Hodgkins' disease /19, 25/ and Wilms' tumor /31/. Case reports in infants of women treated for ALL have documented minor /37, 20/ and major congenital anomalies /36/, none has been diagnosed with any type of childhood cancer /37, 36, 20/. Beyond the articles mentioned in the introduction only two papers /28, 49/ report in detail about children born to fathers with testicular malignancies /25/. Several other studies /36, 20/ reported pregnancy outcome in men after treatment of patients for ALL. None has been diagnosed with congenital anomaly or childhood cancer. The current data do not suggest an increase in the spontaneous abortion and in the females/males ratio /20/. Some stress the high probability of abnormalities in the children of fathers with testicular tumours submitted to radiotherapy /46/ while others advise them after remission induced by chemotherapy to have children /49/. In fact the question is still open in the literature as well for the patients.

From the social point of view it has great importance that the number of artificial abortuses increases when the patient is informed about the diagnosed malignancy, since they are afraid of a potentially malformed child.

In agreement with literature data /56/ the number of premature births became higher in mothers who have learned about the condition of their husband during pregnancy /32/.

Similarly to the data of Senturia /49/ referring to the age

of mothers no difference was detectable between the groups born before castration and after treatment.

Comparing the observations on the growth and development of children born after combined cytostatic therapy with the respective literature data /22/ and with Hungarian growth standards they remained within the range of normal development. No skeletal retardations were recorded in children conceived during and after the chemotherapy of fathers with testicular tumour unlike children whose parents with Hodgkin's disease were submitted only to radiotherapy /25/.

The relationship between severe congenital malformations disease and therapy is reviewed by several authors /11, 22, 28, 46/. At the evaluation of data the incidence of malformations in the population /7, 8/ the in vitro and in vivo teratogenic effect of the cytostatic agents applied /26, 33/ as well as drug exposure and conception times /49/ were considered.

Investigating the relationship between therapy and congenital malformation in children conceived by fathers treated according to the VACAD protocol, primarily anomalies were found while after successful VPB therapy /4/ severe cumulated malformations (valvular disease, spina bifida, anencephaly, etc) were recorded in addition to the large number of healthy children (14/22) though congenital anomalies (17/59) and malformations (24/59) were also found among the offspring conceived before the illness.

The role of cytostatic agents in carcinogenesis is well known /40, 54/ but the relationship between combined chemotherapy and neuroblastoma /13, 50/ recorded in children conceived in the 9th posttreatment month may be subject to doubt if the Wilms' /9/ and cerebral tumour /2/ found in offspring born before the disease of fathers is considered.

Unlike the results of Senturia /49/ difference was found in the interval between concluded therapy and conception of progeny. The mathematical means of healthy children were lower.

Cytostatic therapy in pregnant mothers induced frequently chromosome aberration in the offspring /47/ while no chromosome analysis of children conceived during or after the treatment of fathers with testicular tumour was found in the literature

beyond our earlier paper /23/.

In our study chromosomal aberrations were recorded in children conceived during and after termination of the treatment, but no significant differences were found between control and "treated" offspring groups /23/.

ACKNOWLEDGEMENTS

Thanks are due to Head physician Ilona Zimonyi MD, Anikó Marosi MD, psychologist Ilona Jáki, and the Department of Radiological and Ultrasound, Urological Department and Laboratory of the Heim Pál Pediatric Hospital, Budapest for help in the health check of children.

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Acta Paediatrica Hungarica 32 (1), pp. 31-42 (1992)

SIMULTANEOUS OCCURRENCE OF SELECTIVE ACTH INSENSITIVITY, ACHALASIA AND ALACRIMIA ACCOMPANIED BY HYPERLIPOPROTEINAEMIA

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Received 22 March 1991

An extremely rare clinical syndrome on a 7-year-old-girl is presented. Besides isolated glucocorticoid insufficiency, achalasia and alacrimia disturbance of the lipid metabolism was also detected - being a special feature of this case. The details of the endocrine workup is discussed, providing clues for the possible pathomechanism.

The correct diagnosis and specific therapy is of utmost importance in the everyday life of the patient.

INTRODUCTION

Isolated glucocorticoid deficiency or selective ACTH insensitivity is a rare disease. Increased skin pigmentation and feeding difficulties may appear in early infancy, but the symptoms are manifested typically in the second year of life, as hypoglycaemia and recurrent attacks of vomiting. Spasms and coma may also occur. Low and non-stimulable serum cortisol values are characteristic together with elevated ACTH levels. There is no electrolyte aberration typical of hypadrenia; the aldosterone secretion is normal /2, 17, 25/. Familial occurrence has also been described /6, 21, 22/. The association of this disease with achalasia /16/ is very rare, but even the co-occurrence of isolated glucocorticoid deficiency of the adrenal, achalasia and alacrimia (3 A syndrome) has been observed /11, 18/. No publication has been found that describes an accompanying lipometabolic disorder.

Akadémiai Kiadó, Budapest

The report on our patient with isolated glucocorticoid deficiency, achalasia and alacrimia is considered interesting due to its rarity, the diagnostic difficulties and the observed shift in fat metabolism.

PATIENT AND METHODS

Case report:

T.B. a girl, was born in 1977 from a first, uneventful pregnancy, at term, as a mature newborn. Her development was problem-free for several months. Following weaning with solid food, she began to have episodes of vomiting with a severe oesophageal character, which from the age of about 3 years occurred at every meal. After infancy, the skin and later the oral mucosa became markedly pigmented. From babyhood on she was fatiguable, adynamic, often prostrate, extremely neuropathic, depressed and inactive. Her growth was in the range 75-90 percentile, but her weight was retarded, at 15-20 percentile.

At the age of 7 years, she was admitted to the Paediatric Department. Some days later, during the early morning hours, she had a severe attack of convulsions accompanied by loss of consciousness (a similar attack had not been experienced earlier), which proved to be of hypoglycaemic origin (blood sugar level: 1.7 mmol/l). Due to the appearance of the child, the possibility of Addison's disease had been suggested previously, but the normal serum electrolyte values and considerable hypopotassaemia observed in the ECG contradicted this. The low serum cortison level (1.2-3.4 µg/100 ml) and the high plasma ACTH value (291-330 pg/ml; normal: 20-80) obtained during the informative examinations supported, however, the possibility of an adrenocortical disease. At the same time, the renin and aldosterone levels were normal. On mobilization, the serum aldosterone level exhibited a 5-fold increase. On the administration of ACTH (2 µg/kg body weight) i.v., neither the serum cortisol nor the serum aldosterone level showed any elevation. For a further evaluation of the function of the adrenal cortex, a theophylline load was applied: 6 mg/kg body weight theophylline was administered i.v. for 20 minutes, and a dose of 1.2 mg/kg body weight/hour was then given in i.v. infusion for 2 hours. The serum cortisol and serum aldosterone levels were controlled hourly for 4 hours, but no elevation in their values was observed (Table I).

Gastrointestinal symptoms were checked by X-ray contrast examinations. These demonstrated a spasm in the cardia with a dilated oesophageal body (Fig. 1). The oesophageal sphincter pressure was normal (12-30 mmHg).

Other findings: serum cholesterol: 10.8 mmol/l, triglyceride: 0.68 mmol/l, HDL-cholesterol: 1.24 mmol/l, (values in pathological range found repeatedly) LE-cell: negative, latex: ++. Serum T_3 (2.0 nmol/l) and T_4 (126.9 nmol/l

TABLE I

Effect of a theophylline load in the 7-year-old patient

Sampling (hours)	Cortisol (μg/100 ml)	Aldosterone (ng/100 ml)
1	3.4	3.9
2	2.2	4.3
3	2.0	3.8
4	1.6	3.7

values characterizing the thyroid gland function were normal. Ophthalmological consultation revealed a decrease in lachrymation. (At the age of 11, there was a complete lack of lachrymation). During the diagnostic process, the life-endangering hypoglycaemia and the deficiency state due to nutritional disturbances necessitated the introduction of nasogastric nutrition, which resulted in a considerable improvement in the clinical state of the patient. To correct the glucocorticoid deficiency, substitution therapy with cortisone acetate (Adreson tablet) was started. Initially, this markedly improved the very severe symptoms of vomiting. The skin pigmentation also decreased. Later, however, the vomiting increased in severity again and a pneumatic dilation of the oesophagus became necessary; in accordance with recent literature recommendations /3/, surgery (Heller) was performed at the age of 10. As a symptomatological treatment of alacrimia, artificial tears have since been used regularly.

At the age of 11, the girl was still impuberal (height: 148 cm; weight: 32.5 kg). Glucocorticoid substitution therapy was interrupted for 3 days, and a more detailed analysis of adrenocortical steroid hormones in the blood serum was performed. Cortison was determined by fluorimetry, other steroid hormones, their precursors and plasma ACTH by our own specific radioimmunoassay methods, and the binding capacity of steroid binding transport proteins by the binding of ^{3}H-cortisol and ^{3}H-dihydrotestosterone /8, 9, 14, 23/.

Preliminary steroid studies (low cortisol and high ACTH levels) suggested the possibility that the glucocorticoid deficiency was caused by the defect of one of the enzymes involved in steroid hormone biosynthesis. Thus, in addition to aldosterone and cortisol, the serum concentrations of their precursors (pregnenolone, progesterone and 17-OH-progesterone) were also determined in the basal state and 1, 2 and 4 hours following in i.v. ACTH bolus. As shown in Fig.2, an upright

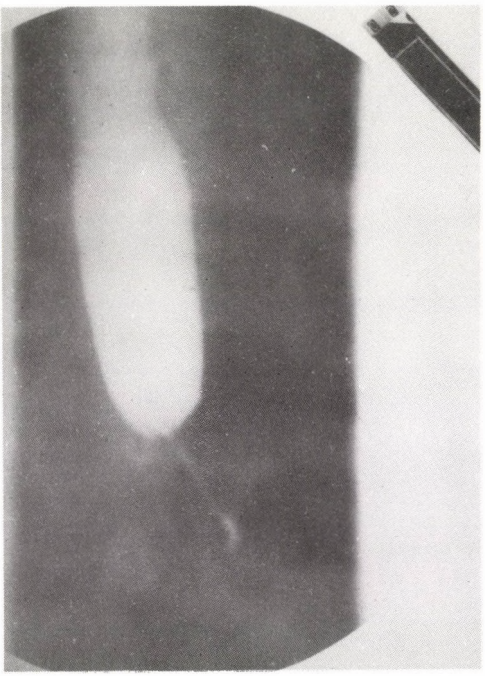

Fig. 1. Bariumswallow demonstrating typical finding of achalasia

position increased aldosterone secretion, but otherwise, the levels of cortisol and all investigated steroid precursors in the blood were extremely low. There was no increase in these or in aldosterone following the administration of ACTH. Likewise, pathologically low levels of testosterone and its precursors (dehydroepiandrosterone, dehydroepiandrosterone-sulphate, andostenedione) were detected. The serum oestradiol-17β level was in the normal prepuberal range. All these latter steroids were unresponsive to the administration of ACTH (Fig. 3). The binding capacities of serum steroid binding proteins ("Sex Hormone Binding Globulin = SHBG and "Corticosteroid Binding Globulin" = CBG) proved to be normal (Fig. 4).

A computer tomogram of the adrenal gland verified hypoplasia of the glands on both sides (National Institute of Vascular Surgery, Dr. K.Karlinger).

To prognosticate the sexual-steroid secreting capacity of the impuberal ovaries, choriongonadotropin (Choriogonin inj.) was administered in a 3000 U/day dose i.m. for 6 days. This led to an elevation of some precursor levels in the serum (17-OH-progesterone: from 0.5 ng/100 ml to 40 ng/100 ml; androstenedione

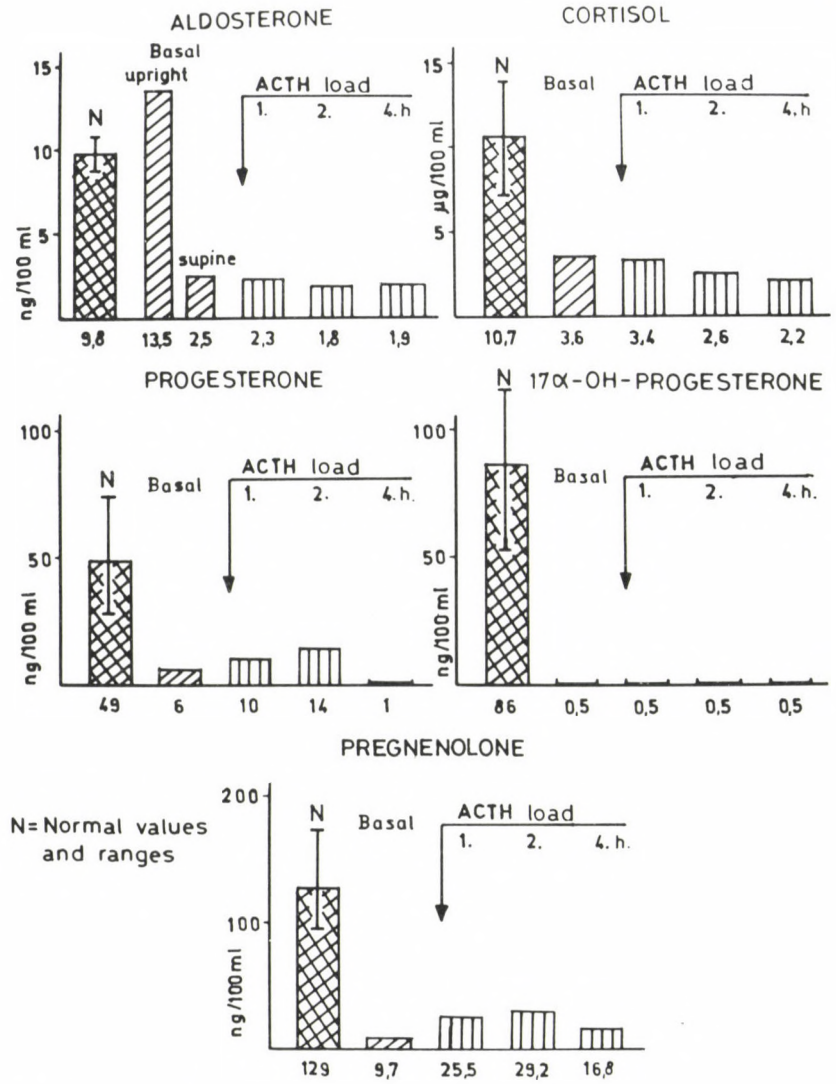

Fig. 2. Serum levels of aldosterone, cortisol and their precursors (T. B., 11 y., ♀)

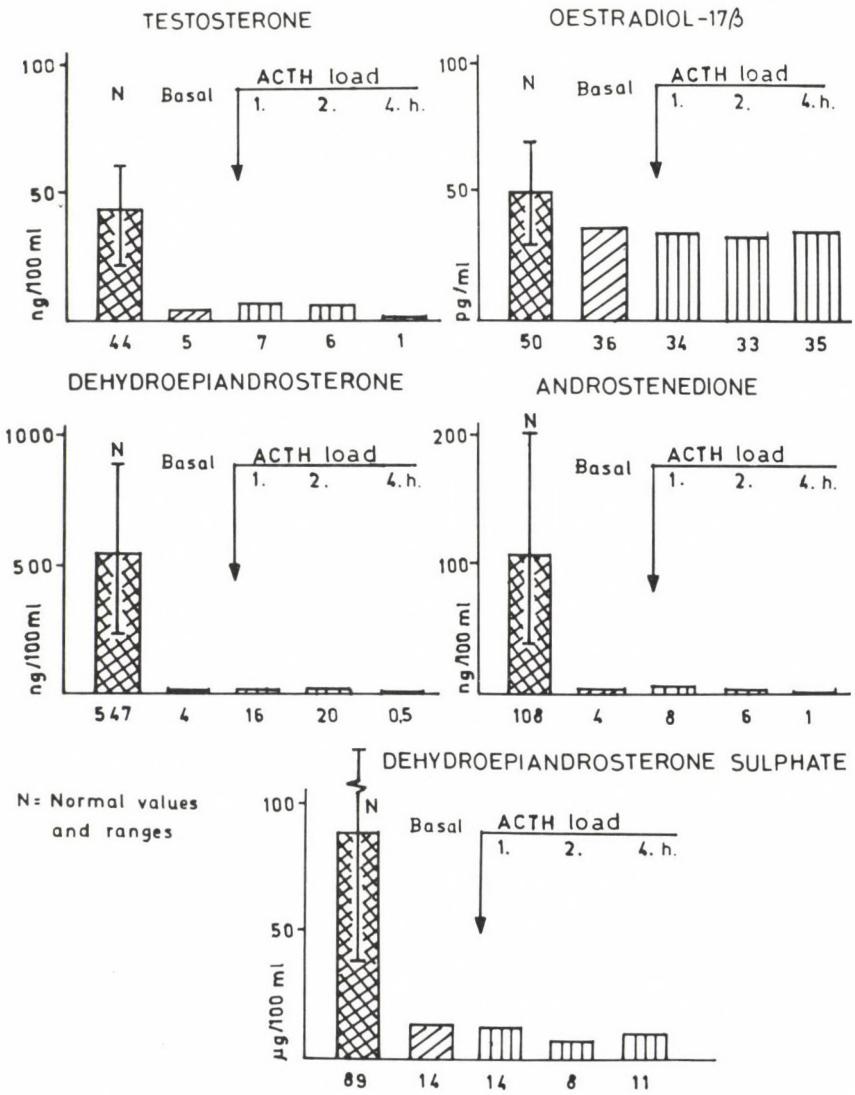

Fig.3. Serum levels of sex-steroids and their precursors (T. B., 11 y., ♀)

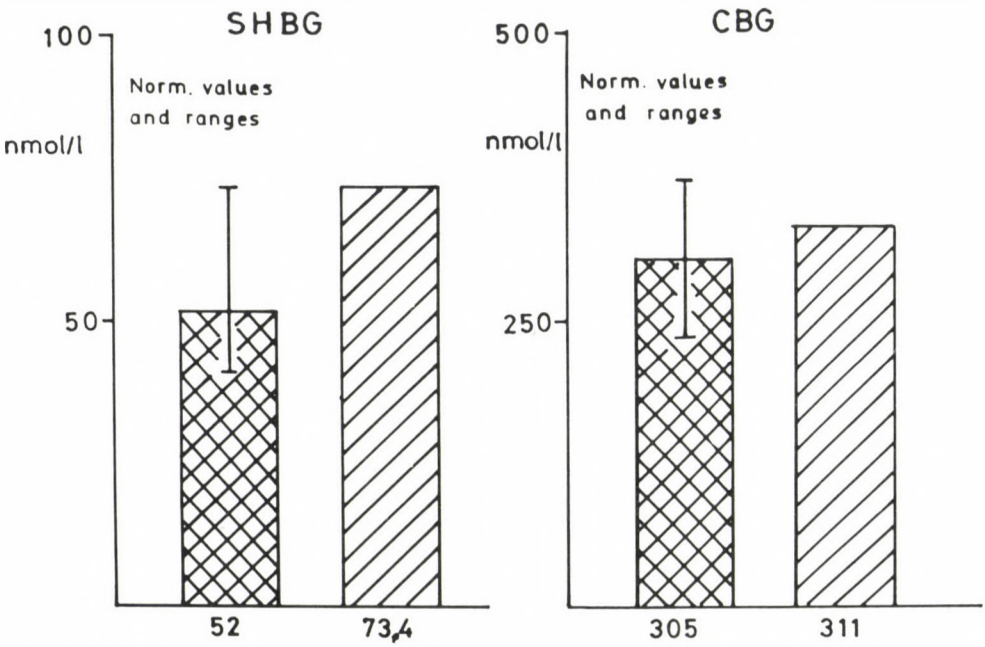

Fig. 4. Binding capacity of sex-hormone binding globulin and corticosteroid binding globulin in serum (T. B., 11 11., $\frac{O}{+}$)

from 4.0 ng/100 to 46 ng/100 ml), but no change was found in the levels of pregnenolone, progesterone, testosterone or oestradiol-17β.

As glucocorticoid substitution therapy, at present she receives 2x25 mg cortisone acetate orally daily. In addition, for normalization of her hypercholesterinaemia, an appropriate diet and the administration of cholestyramine have been introduced.

DISCUSSION

Isolated glucocorticoid deficiency or selective ACTH insensitivity is a very rare disease. It is considered to be a hereditary, progressive, degenerative disease or a primary insensitivity of the adrenal glands /18, 19, 22/. Its X-linked or autosomal recessive inheritance have been presumed /10/, but

the latter is more likely /25/. According to Werder et al, damage of the mineralcorticoid function /4, 25/ may also develop gradually. Some publications /16, 22/ stress the importance of a higher than average body length as a symptom, which was most conspicuous in our case because of the retarded increase in body weight.

Achalasia itself in children is very rare: its frequency is 1:10 000 in an average population /7, 20/ and less than 5% of the cases are observed under the age of 15 /3, 24/. It is a disease of unknown origin, characterized by a non-organic obstruction and an incapacity for relaxation of the distal segment of the oesophagus, and consequently dilation of the oesophagus develops. Its principal clinical symptoms are dysphagia, regurgitation of fluid and solid food, vomiting and a retarded increase in body weight. A disordered cholinergic innervation of the oesophagus is supposed. Its autosomal recessive hereditary predisposition is probable.

The first survey concerning the accumulated occurrence of glucocorticoid deficiency, achalasia and alacrimia was published in 1978 /18/ by Lanes et al /17/ who reported on its complication with a partial mineralcorticoid deficiency. Geffner et al /11/ detected the full syndrome in a patient aged 3 1/2. It has not been explained yet how these diseases are connected.

The ACTH insensitivity of the adrenals, the absence of ACTH receptors, or a steroid synthesis disorder may be assumed as causes of isolated glucocorticoid deficiency. Geffner et al /12/ suggested that a theophylline test may be useful in differentiating these possibilities. Theophylline acts by bypassing the ACTH receptors and inhibiting phosphodiesterase, which hydrolyzes cAMP. Theophylline therefore elevates the cAMP level directly and increases intracellular steroidogenesis. If theophylline elevates the steroid level, a receptor deficiency can be supposed. If theophylline is without any effect, an impaired intracellular synthetizing mechanism (as in our case) can by hypothesized.

The computer tomogram of the glands contradicts the possibility of any enzyme defect in the adrenal cortex: a deficincy of the enzymes involved in steroidogenesis would lead to adrenal hyperplasia. With such a clinical picture, well-controlled glucocorticoid supplementary therapy may decrease the size of the glands, but produces no hypoplasia as observed in our patient. Our detailed steroid investigations definitely exclude hypadrenia based on an enzyme insufficiency. High serum levels of steroid hormone precursors prior to the enzyme block would indicate this etiology. In the serum of our patient, however, all the biosynthetic intermediates (pregnenolone, progesterone, 17-OH-progesterone) were found in pathologically elevated concentrations. It may be presumed that the first step in the biosynthesis of steroid hormones (cholesterol ⟶ pregnenolone transformation), where the ACTH effect ought to prevail, is inhibited. The pathologically elevated ACTH level and the ineffectivity of ACTH therapy suggest a possible diagnosis of ACTH insensitivity. This may be due to a congenital lack of ACTH receptors or their insufficient function, or even to an unknown (possibly autoimmune) disease leading to atrophy of the zona fasciculate and the zona reticularis of the adrenal cortex, but not the zona glomerulosa. By stimulating the renin-angiotensin system, the erect posture of the patient may increase aldosterone secretion, as the function of this mechanism is independent of cAMP. Even a relatively small amount of precursor steroids may be enough to ensure as the serum aldosterone concentration is 1000 times lower than that of cortisol even in a healthy person /13, 15/. The above supposition might be confirmed by investigating the effect of i.v. angiotensin II, but we have had to forego this diagnostic method because of its potential danger /5/.

The normal levels of steroid binding proteins (SHBG and CBG) may be of interest because of the extremely low sexual steroid and glucocorticoid levels of our patient. Accordingly, the synthesis of the transport proteins in the blood is not regulated by the levels of steroid hormones alone (although a

very low testosterone concentration may play a role in the fact that the binding capacity of SHBG has attained the upper limit of the normal range).

The increase in the levels of sex hormone precursors following the administration of choriongonadotropin may promise that the ovaries will be able to synthetize their organ-specific hormones. More precise information could be obtained by applying FSH + LH sequentially, but we considered it unwise to provoke early puberty in a child who has not attained adult body height. Further follow-up observations of the patient will furnish more data on the endocrine function of the gonad.

During examinations for an absorption disorder as causal factor of the retardation in weight, we tested the serum cholesterol level and found it unexpectedly high. Hyperlipoproteinaemia of type IIa was diagnosed. In hypothyroidism, hyperlipidaemia of this type may occur secondarily /21/, but this etiological possibility was excluded. Associated with high-dose glucocorticoid therapy, hyperlipoproteinaemia of type IV may develop. At the beginning of observation, however, the patient was not given any steroid preparations and the later therapy consisted only of hormone substitution doses. Her mother was hyperlipoproteinaemia of type IIa, detected because of xanthelasma formation round her eyes. Similarly detailed steroid hormone tests were performed on the mother but the results were completely normal.

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Acta Paediatrica Hungarica 32 (1), pp.43-52 (1992)

PROGNOSTIC DATA OF THE SECOND FOLLOW-UP IN CHILDHOOD WHEEZY BRONCHITIS

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Received 18 April 1991

206 non-selected children who had wheezy bronchitis before two years of age were observed in the first follow-up (1985) about 9 years after their clinical wheezy episode. Among them 31 patients (15%) showed bronchial hyperreactivity (B.H.) after acetylcholine challenge and 9 children became asthmatic. In the second follow-up (1988) 28 children who had B.H. and randomly 17 children who did not have B.H. participated. The prognosis of wheezy bronchitis is good, there was no new asthmatic child at this time. The B.H. was found to diminish only in 7 cases. During the last 3 years wheezy episodes developed only in 5 asthmatic patients. The skin prick test (SPT) positivity did not change (26% versus 29%). The familial atopy in the group B.H. is higher (43%) than in the group non B.H. (23%) but the difference is not significant (X^2=1.72). No significant correlation was found between the familial atopy and SPT positivity. The familial smoking is higher in the group B.H. (78% versus 64%) but no significant influence on the B.H. (X^2=1.03) could be detected.

INTRODUCTION

Wheezing is common in infancy and early childhood, Brasher found about 10% under two years of age /1/ and in Hungary its frequency is similar /13/. Warner found wheezing in 10% of infants /10/. Clark and Godfrey mentioned that in spite of the very similar symptoms the wheezy bronchitis is not equal with bronchial asthma /2/. On the other hand, wheezy bronchitis is one of the both ends of "asthmatic scale" after Williams and McNicol's opinion /11/. Wheezing can develop in the case of

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different diseases (cystic fibrosis, bronchial anomalies, vascular ring, heart failure, foreign body, etc.). In the cases of repeated wheezy episodes attention should be paid to bronchial asthma.

In our study the following questions had to be answered:

1. What kind of prognosis do those children have who have had wheezy bronchitis before two years of age?
2. Whether bronchial hyperreactivity can be detected in them and how does it change?
3. What connections can be detected between prognosis and familial atopy, skin prick test positivity and familial smoking?

To answer these questions we carried out a two-step follow-up of children who were earlier treated with wheezy bronchitis at our Department. The criteria of wheezy bronchitis was: during a febrile respiratory tract infection taken place before two years of age wheezing with respiratory dyspnea could be detected. By clinical examination hyperinflated lung, wheezing and rhonchi were found. X-ray picture also showed hyperinflated lung.

MATERIALS AND METHODS

Patients material

In the first follow-up (1985) 206 children were examined, who were treated for wheezy bronchitis at our Department in the time period of 1974-76. The children were unselectedly invited some 9 years after their clinical treatment. The mean age was 11 years, 139 boys and 67 girls. In the second follow-up (1988 45 children participated:

1. Group B.H.: 28 children who had B.H. in the first follow-up, mean age 14.5 years,
2. Group non B.H.: 17 randomly selected children, who did not have B.H., mean age 14.1 years.

For the comparison of the acetylcholine challenge results data of 30 healthy children having had no atopy, no repeated lower respiratory tract diseases, and no other kinds of chronic diseases were used.

Method

The anamnestical data were registered on questionnaires which were planned for our follow-up. Physical examination of children was done.

Skin prick tests (SPT) were carried out on the forearms of children according to the prescription of manufacturer Bencard with the following allergens: Dermatophagoides pteronyssinus, cat, dog, ragweed, grass mix, tree mix, flower and shrub, moulds: A_{13}, M_{10}, M_{11}. After 15 minutes urtica 3-5 mm diameter as 3+, greater than 5 mm and with pseudopodium as 4+ was appreciated.

Bronchial challenge was carried out with 0.5% acetylcholine breathing for 2 minutes on tidal level from ultrasonic nebulizer (TUR-USI 50). The control was phys. saline solution. The output of the ultrasonic nebulizer reached a steady state after 8-10 minutes of working: 1.35 ml $\pm$ SD 0.11 ml/2 minutes, accordingly the challenge was started after 10 minutes of nebulising. The FEV_1 and PEF values were determined before and after 3 and 5 minutes of acetylcholine inhalation by SP_{21} spirograph. The degree of bronchospasm was expressed as the percentage fall of FEV_1 and PEF. The criteria of positive challenge - the bronchial hyperreactivity - was a decrease over 20% of FEV_1 or PEF.

The PC_{20} value was calculated from FEV_1 results. The challenge was done on symptomfree children, who had not respiratory tract infection in the last 4 weeks.

In the mathematical analysis we used the Pearson X^2 test and the level of significancy was at $p < 0.05$.

RESULTS

At the time of the first follow-up 9 of the 206 children proved to have asthma (4%), while at the second follow-up no new asthmatic was found. Wheezy episodes disappeared in 189 children (92%) by 7 years of age. At the second follow-up (1988) during the last 3 years wheezy episodes continued only in 5 of the asthmatic patients.

At the first follow-up the bronchial challenge showed a positive result in 31 (15%) of 206 children. At the second follow-up 7 out of 45 reacted positively upon bronchial challenge. In the control group no child had positive reaction on 0.5% acetylcholine solution. The change of FEV_1 and PEF mean values are demonstrated on Table I. The value of PC_{20} did not change in the asthmatics, however, it did increase in the Group B.H., as shown in Table II.

Skin prick test positivity was experienced in 35 (17%) of 206 children, while at the second follow-up 13 (28.8%) of 48 (in this group the previous SPT positivity was 26%). At the second follow-up there was no difference in SPT positivities of

TABLE I

Changes of PEF and FEV_1 values after 0.5 percent acetylcholine challenge

	mean decrease PEF%	mean decrease FEV_1%
Group B.H.		
1985	-27.3	-26.9
1988	-12.2	-14.9
Group non B.H.		
1985	-8.5	-6.1
1988	-6.2	-7.2
Control	-6.3	-4.9

TABLE II

Changes of PC_{20} values

	Control	Follow-up I.	Follow-up II.
All patients	2.01 (30)	1.20 (206)	0.79 (45)
Asthmatics	-	0.49 (9)	0.47 (8)
Non-asthmatics	-	1.26 (197)	0.92 (37)
Group B.H.	-	0.37 (28)	0.67 (28)
Group non B.H.	-	1.52 (17)	1.29 (17)

Group B.H. and Group non B.H. (28 vs 29%). By the end of the follow-ups the SPT remained negative in 28 children, while positive in 8 (Fig. 1): the SPT of 4 patients changed from positive to negative, while 5 changed inversely, from negative to positive. One of the last ones developed drug allergy, the others did not show allergic symptoms.

Family atopy (bronchial asthma, atopic eczema, allergic rhinitis) appeared as follows:

First follow-up (206 patients)	38%
Second follow-up (45 patients)	37.7%
Group B.H.	43%
Group non B.H.	23%

Familial atopy occurred more frequently in the Group B.H. than the Group non B.H., however the difference is not significant (X^2 = 1.72). No significant correlation could be found between the familial atopy and SPT positivity X^2=0.18 (Fig. 2).

At the second follow-up the effect of familial smoking upon bronchial hyperreactivity was also studied. Families of 45 children included smokers in 73%. The ratio of smokers in the Group B.H. was 78%, while in the Group non B.H. it was 64%, giving a non-significant difference X^2=1.03 (Fig. 3).

DISCUSSION

In the course of the two-step follow-up of non-selected wheezy bronchitis children the disease showed favourable prognosis. 92% of the children had no more wheezy episodes by the age of 7 years, similarly to Selander's results /9/ according to which 80% of the children became symptomfree by the age 6-11 years. Park and co-wokers /8/ investigating a large child population after wheezing disease prior to 5 years of age found 2% of the children to be asthmatic. At a follow-up 10 years after wheezing Foucard and Sjöberg /4/ detected 22 asthmatics from 80 wheezy children. In our patients the frequency of asthma was 4% and this ratio did not change by the

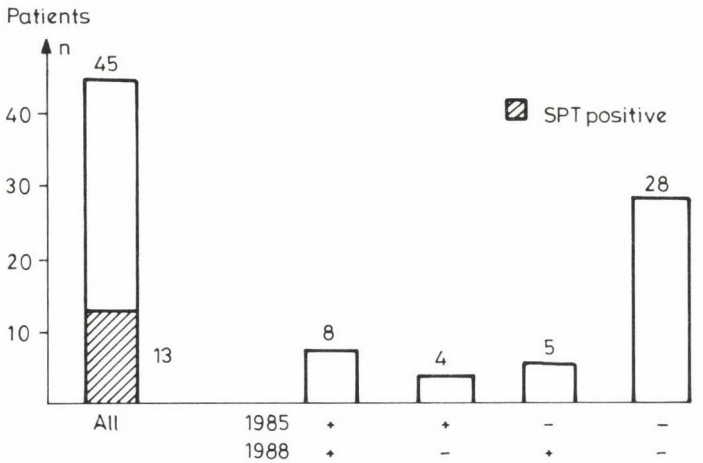

Fig. 1. The SPT positivity (3+ or 4+) and its change

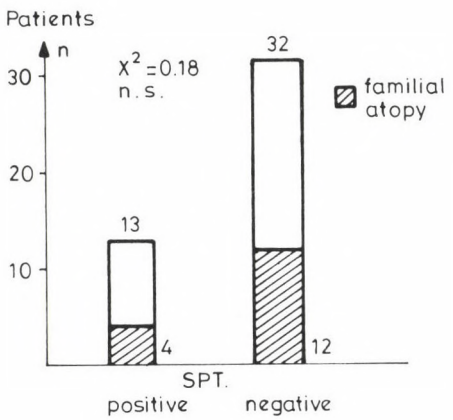

Fig. 2. The familial atopy and SPT positivity

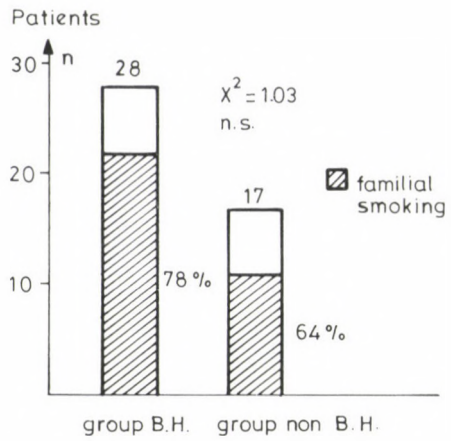

Fig. 3. Familial smoking and B.H.

time of the second follow-up, i.e. no new asthmatics could be discovered.

In the course of acetylcholine challenge 15% of the patients exhibited B.H. which is a higher frequency than that found in the healthy population /6/. In pubertal age however, the B.H. showed a decrease. At the second follow-up only 7 of 28 children had positive results indicated also by the increase of PC_{20} values which did not change in asthmatics. Asthma is known to improve or "heal" during puberty.

Godfrey and Griffiths detected SPT postitivity (3+ or 4+) in 33% of healthy children /5/, while Cserháti et al in Hungary only in 7% /3/. During the follow-up of wheezy bronchitis Foucard and Sjöberg registered 7% SPT positivity /4/. The SPT positivity found 17% in 206 children seems to be higher than that of the healthy population, and this ratio is even higher at the second follow-up (28.8%), but here all the asthmatic children were also present. The SPT positivity of children taking part in both follow-ups did not change as a whole. There were, however, individual changes, some of them changed from positive to negative, also inversely. SPT positivity showed no significant correlation with familial atopy.

Several data can be found on the harmful effect of smoking. Liard and co-wokers observed wheezy bronchitis in infants of smoking mothers significantly more frequently compared to non-smokers' infants /7/. At present we studied the effect of passive smoking on B.H. and found that parental smoking did not influence the frequency of their children's B.H.

Furthermore, in a two-step follow-up of non-selected wheezy bronchitis children we are going to confirm Jackson's statement "All that wheezes is not asthma" /12/.

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Acta Paediatrica Hungarica 32 (1), pp.53-70 (1992)

MONITORING THE THERAPY OF ACUTE LYMPHOID LEUKEMIA (ALL) IN CHILDREN - CHANGES INDUCED IN CLINICAL, CYTOLOGICAL AND IMMUNOLOGICAL PARAMETERS DURING TREATMENT OF HIGH MALIGNANCY ALL WITH ALL-BFM 88

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Received 2 January 1991

The ALL-BFM 88 RF > 1.7 treatment of high malignancy ALL patients in remission was monitored at the 4th week of Protocol I. The qualitative and quantitative processing of parameters indicating cellular transformations by light and electronmicroscopy was performed in lymphocytes separated from peripheral blood drawn at scheduled times of therapy.

The immunophenotype of sample lymphocytes was determined by indirect immunofluorescent techniques, by listing the percentual level of CD_2, CD_3, CD_4, CD_8, CD_{19} surface antigens.

The incidence of infections, confirmed clinically and bacteriologically, was recorded. The parameters of the patients were compared to those of healthy children.

In the group of patients with high malignancy in the intensive period of ALL-BFM 88 RF > 1.7 therapy the incidence of cells rich in organelles, dense granules and vacuoles was significantly ($p < 0.05$) higher compared to the control cells, poor in structures. During the weeks of induction and after the consolidating combination with VM-26+ARA-C the expression of surface antigens of the lymphocytes was reduced.

That means that up to the introduction of maintenance treatment the presence of surface antigens remained below the range of normal deviation.

These data obtained during monitoring revealed that the morphological and functional integrity of cells was damaged in patients with high malignancy ALL during intensive therapy, though only temporarily.

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INTRODUCTION

The favorable results obtained in ALL therapy of children /11, 21/ are due to the combined intensive chemotherapy and radiotherapy /23/ applied in patient groups differentiated on the basis of risk factors /2, 20, 21, 26, 28, 29/.

However, there is a group of patients with poor prognosis ALL-diagnosed on the basis of both qualitative and quantitative parameters /8, 12, 14, 16/ - who have a rather low life expectancy even today, because of early relapses and their nonresponsiveness to therapy /12, 30, 33/.

It was the aim of the study to investigate the cells separated from the blood samples of patients with high malignancy but presently in remission, i.e. to monitor the regeneration of normal lymphocytes appearing during combined cytostatic therapy and to observe the clinical pattern of changes occurring at cellular level. The present study focuses on the changes of morphology and immunophenotype of the lymphocytes, and of the associated clinical aspects of patients with high malignancy ALL induced by BFM-88 therapy.

PATIENTS AND METHODS

The analysis of morphological lymphocyte parameters (by light microscopical parameters) was drawn in peripheral blood samples from children with high malignancy during the ALL-BFM 88 RF > 1.7 protocol at scheduled treatment times (in weeks: 2, 9, 13, 21, 24, 29, 39, 44, 52). The electron microscopic analysis was performed from samples, fixed by Matutes' method /15/, 20 to 25 cells, chosen at random from each child on weeks 4 and 13, and evaluated on the electromicrograms with a semiautomatic planimeter, at 4000 to 12 000 fold magnification.

In addition to the routine hematological parameters (WBC), the immunophenotype of the lymphocytes was also determined /4, 7/. Applying indirect immunofluorescent techniques, the percentual incidence of surface antigens (CD_2, CD_3, CD_4, CD_8 CD_{19}) was determined after counting 100 to 200 cells per sample.

At the same scheduled treatment time the incidence of infections, bacterial resistance data /3, 17/ as well as the antibiotic therapy applied /27/ were registered.

During the study the blood samples of patients in remission were processed, therefore the monitoring of ALL patients was started in the 4th treatment week. The tests were always performed during the treatment-free period and at normal LDH levels /13/. The follow-up period was 52 weeks. In the Student's "t" test the expected control values ($\bar{x}$) as well as the standard deviation range of normal values were listed by processing the data of 20 healthy children.

In the present study the therapy of 7 children with high malignancy ALL was monitored. In Fig. 1/A sex, age, risk factors /21/ and FAB classification /2/ of the patients designated by code numbers are listed. Patient No 501 with a large submandibular tumour and patient No 507 with a breast tumour were also included in this high malignancy group. With regard to immunological characteristics /26/, 3 were CALLA positive, 2 T-lymphoma-like and one had T and B cell ALL.

The survival rate of patients is represented in Fig. 1/B. After a one year follow-up period, 3 of 7 patients are alive and their maintenance treatment is being continued. In agreement with the aim of the study only the data of patients in remission were processed which enabled the monitoring of the regeneration of healthy lymphocytes independently from the original diagnosis.

RESULTS

From the hematological parameters WBC remained at a lower level compared to the control throughout the treatment period (Table I).

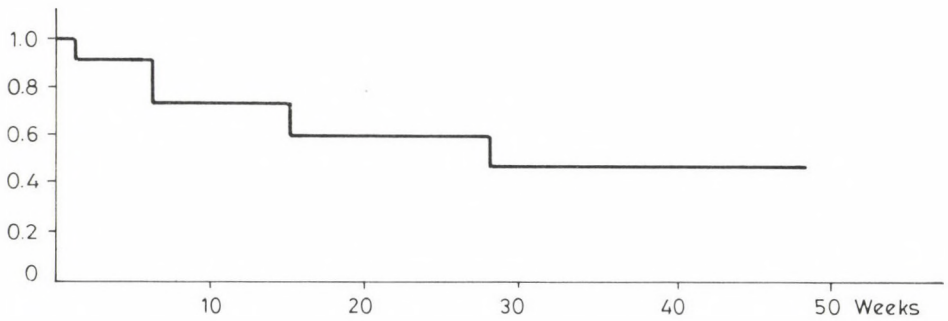
Of the parameters monitored by light microscope the ratio of nucleic diameters, rate of basophilia, presence of granulation and vacuoles gradually approached normal mean levels during and after inductive therapy. However, the regular bean or kidney shape, characteristic of normal lymphocyte nucleus, was retained only during maintenance therapy in the majority of cells (Table II).

Electron microscopic monitoring was performed before the remission and before application of consolidating therapy (Table III). At the 4th week of induction and at the start of consolidating therapy the incidence of cells rich in organelles was singificantly higher ($p < 0.05$) compared to the healthy control cells, poor in structure. Comparing data measured on weeks 4 and 13 respectively, significant differences ($p < 0.05$) were found in the ribosome count, in the presence of

A. Characterisation of the investigated patients

Code number	Sex	Age (years)	BFM (RF)	FAB (L)	Immunological characteristics
501	♂	7	0.82	L_1	C-ALL
502	♂	13	1.92	L_1	"T"
503	♂	12	1.48	L_1	T-lymphoma like
504	♂	3	1.75	L_1	C-ALL
505	♀	9	1.84	L_2	"B"
506	♂	1	1.70	L_1/L_2	C-ALL
507	♀	2	1.03	L_1	T-Lymphoma like

B. Survival of ALL (FR > 1.7) patients treated by "BFM-88 RF > 1.7" protocol



C. "ALL-88 RF > 1.7" treatment protocol

Protocol I.	Protocol M	V-C	Protocol II.	Maintenance

Fig.1. A: Sex and age distribution of patients with high malignancy ALL treated by "BFM-88 RF > 1.7" and indicated by codes, their BFM and FAB classification and immunological diagnosis

B: Survival rate of high malignancy ALL patients

C: Scheme of therapy applied for the treatment of high malignancy ALL patients ("BFM-88 RF > 1.7") with scheduled sampling time

TABLE I

Hematological parameter (WBC)
WBC of patients with high malignancy ALL during treatment

Weeks	4	9	13	21	24	29	39	44	Healthy control
Investigated parameters									
WBC	5.171	3.22	4.620	3.80	3.067	4.466	3.691	3.904	7.0429
(10^9/1)	±3.435	±1.434	±2.234	±0.163	±0.741	±0.591	±0.227	±0.097	±2.5127
No of cases	7	6	5	4	4	3	3	3	2

TABLE II

Parameters determined at scheduled time of therapy by light microscope using normal controls (ratio of nucleic diameters, presence of regular nucleic shape, rate of cytoplasma basophilicity, incidence of granulation, vacuoles)
Light microscopic findings in ALL (RF > 1.7) cases during treatment

Weeks	4	9	13	21	24	29	39	44	Healthy controls
Features									
Nuclear D_1/D_2	0.8729 ±0.0531	0.790 ±0.0629	0.828 ±0.0397	0.8367 ±0.033	0.730 ±0.0294	0.7967 ±0.0544	0.7963 ±0.0441	0.765 ±0.0946	0.7884 ±0.055
Basophilic cytoplasm (per cent)	60.43 ±14.31	59.0 ±19.73	44.60 ±17.33	0.220 ±0.0283	27.0 ±9.27	15.33 ±7.72	28.0 ±28.92	16.7 ±2.98	29.36 ±23.35
Azurophilic granules (per cent)	14.71 ±11.54	10.4 ±11.09	17.20 ±12.69	0.4633 ±0.264	8.33 ±6.24	8.00 ±4.32	20.53 ±19.80	18.54 ±11.34	19.7 ±12.25
Vacuoles (per cent)	0.771 ±0.0947	0.520 ±0.387	6.6 ±7.36	0.0033 ±0.0047	7.33 ±6.13	6.0 ±8.49	1.0 ±5.91	1.4 ±7.68	1.3 ±1.78
Regular nuclear outline (per cent)	37.57 ±27.16	55.60 ±15.87	53.80 ±24.5	46.0 ±25.14	35.0 ±9.2	32.33 ±29.85	32.0 ±34.43	47.53 ±27.75	63.87 ±16.74
No of cases	7	6	5	4	4	3	3	3	20

TABLE III

Change of ultrastructural indicators (nucleus-surface ratio, ribosome, endoplasmatic reticulum, mitochondrium, dense granulation, vacuoles, Golgi apparatus) and their incidence, determined in the lymphocytes of high malignancy ALL patients on weeks 4 and 13 compared to the respective data of healthy cells.
Transmission electron microscopic findings in ALL (RF > 1.7) cases

Weeks	4	13	Healthy control
Features			
N/C ratio +	0.8729 ± 0.0483	0.750 ± 0.066	0.0794 ± 0.1991
Ribosomes (per cent)	33.86 ± 8.04	14.2 ± 0.85	7.29 ± 1.819
Endoplasmic reticulum (per cent)	30.43 ± 14.61	13.0 ± 9.8	32.9 ± 8.32
Mitochondrium (per cent)	46.86 ± 15.72	49.2 ± 17.52	6.79 ± 16.92
Large/dense granules (per cent)	36.86 ± 20.87	28.0 ± 18.72	2.09 ± 5.32
Vacuoles (per cent)	21.29 ± 15.93	15.0 ± 15.87	0.16 ± 0.87
Active Golgi apparatus (per cent)	14.14 ± 6.998	6.6 ± 3.61	2.03 ± 5.29
No of cases (n)	7	5	11
No of cells (n)	7x25	5x25	11x25

endoplasmatic reticulum and Golgi apparatus. At the introduction of consolidating therapy Golgi apparatus and ribosomes were only rarely detectable compared to the cells with large nucleus rich in organelles, observed in the 4th week of the inductive treatment (Fig. 2/A) while the loose chromatin structure of the nucleus, the high incidence of mitochondria (Fig. 2 /B), dense granules and vacoules remained unchanged.

The expression of surface antigens of the lymphocytes, which are also characteristic phenotypes of healthy lymphocytes (9, 19), was monitored during therapy together with the absolute lymphocyte count and rate of cells forming rosettes with sheep red blood cells (Fig. 3 /A).

It appears that up to the start of maintenance treatment the presence of surface antigens remained below the range of normal deviation.

A similar curve was obtained during the monitoring of the CD_3 marker. It should be stressed that the level of both markers was reduced in the 4th to 9th week of induction and the lowest level was detectable after the consolidating combination treatment with VM-26 + Ara-C (Fig. 3/B).

The presence of the CD_4 antigen followed the trend of the two former markers, although it must be noted that a very low T-helper presence was observed beyond the inductive phase after the Protocol M (Fig. 4/A).

Values representing the rate of CD_8 marker bearing T-suppressors represented the exact "reciprocal curve" (Fig. 4/B) of the T-helpers while CD_4 and CD_8 both failed to attain the healthy mean value after the 44th week (Fig. 4/A+B).

The value expressing the ratio of the two former markers ($x=CD_4/CD_8$) became lower than the normal deviation range in the 22th week of consolidation, but by the end of Protocol II., or during the maintenance therapy it reached the normal levels again (Fig. 4/C).

The B cell specific CD_{19} surface antigen was hardly detectable in the second half of induction or after the VM-26+ARA-C Block of consolidation, on the 26th week (Fig. 5/A).

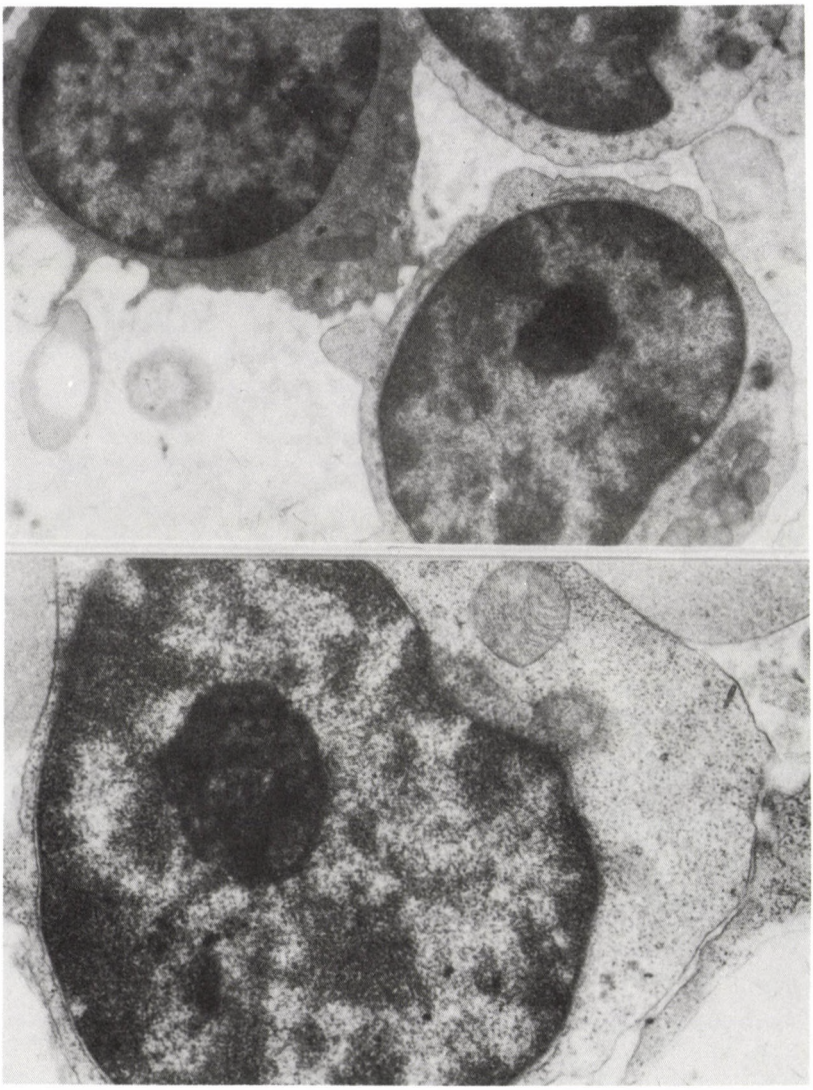


Fig. 2. Cells separated from the peripheral blood of a child with high malignancy
ALL submitted to "ALL-BFM 88 RF > 1.7" therapy.
A: During the inductive treatment (week 4)
B: During the first week of consolidation (week 13)

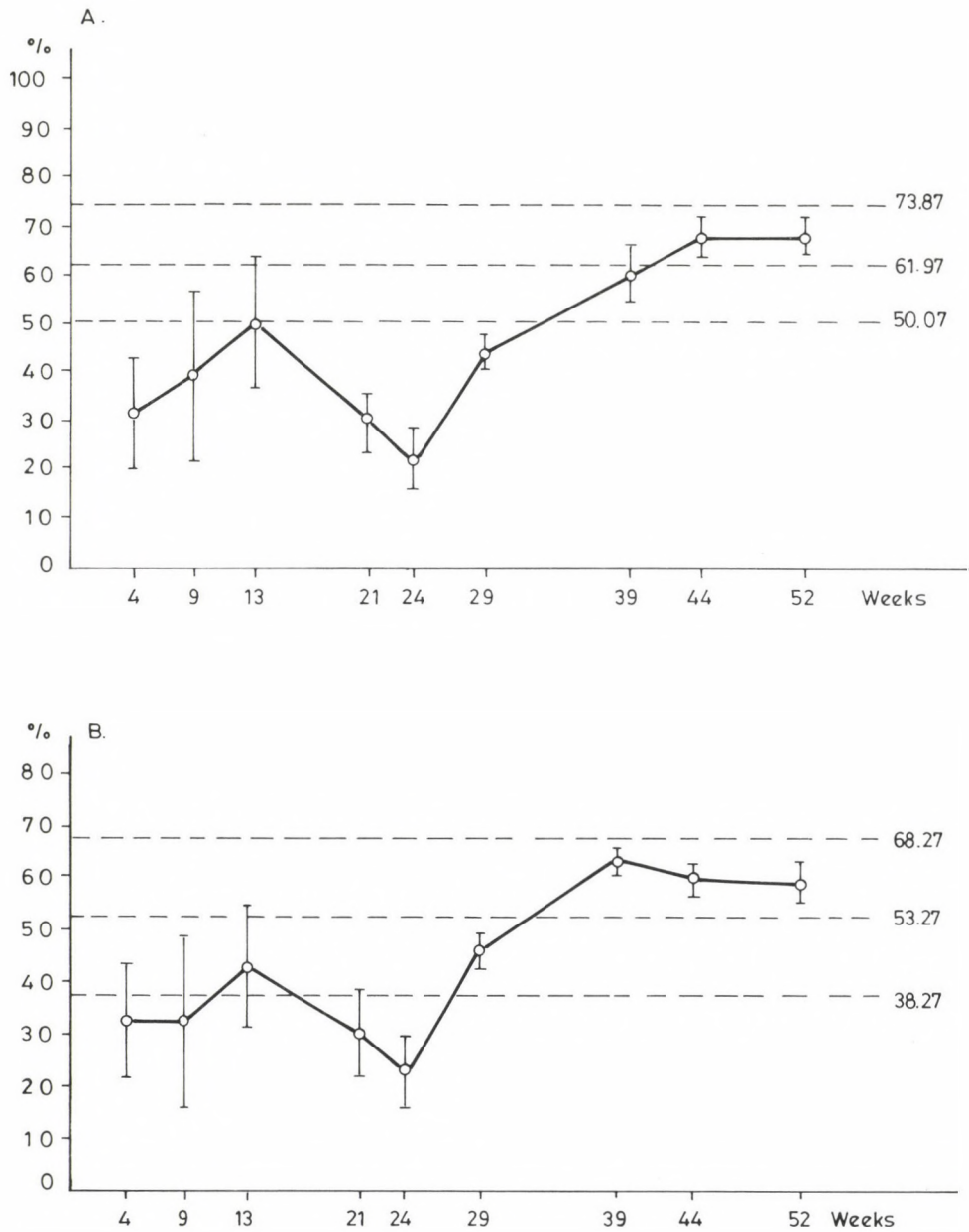


Fig. 3. A: CD_2 marker bearing cells forming rosettes with sheep RBC
B: Rate of cells with CD_3 surface antigen at specific time of therapy compared to controls (n = 20)

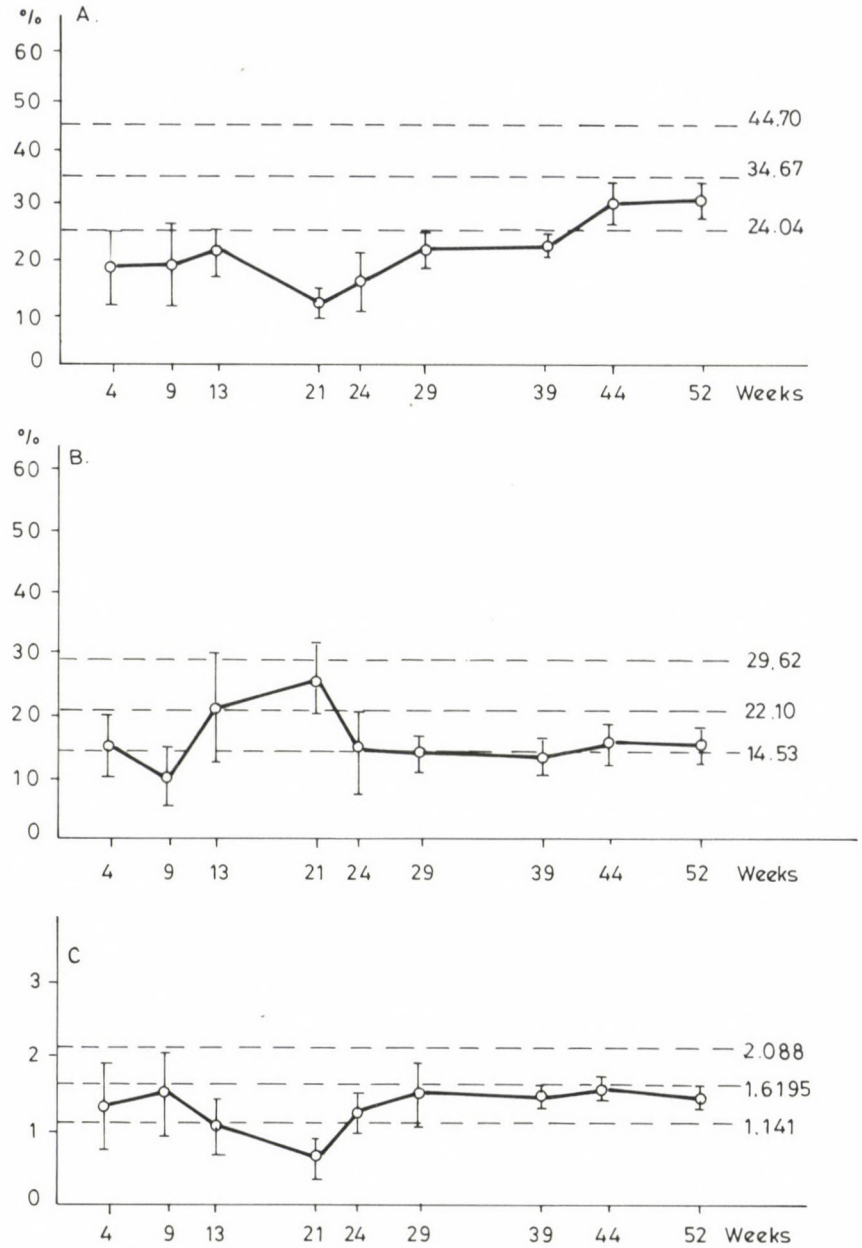


Fig. 4. A: T-helper cell levels with CD_4 marker
B: T-suppressor cell levels with CD_8 antigen expression
C: Ratio of the two markers compared to the entire range of deviation compared to controls (n = 20)

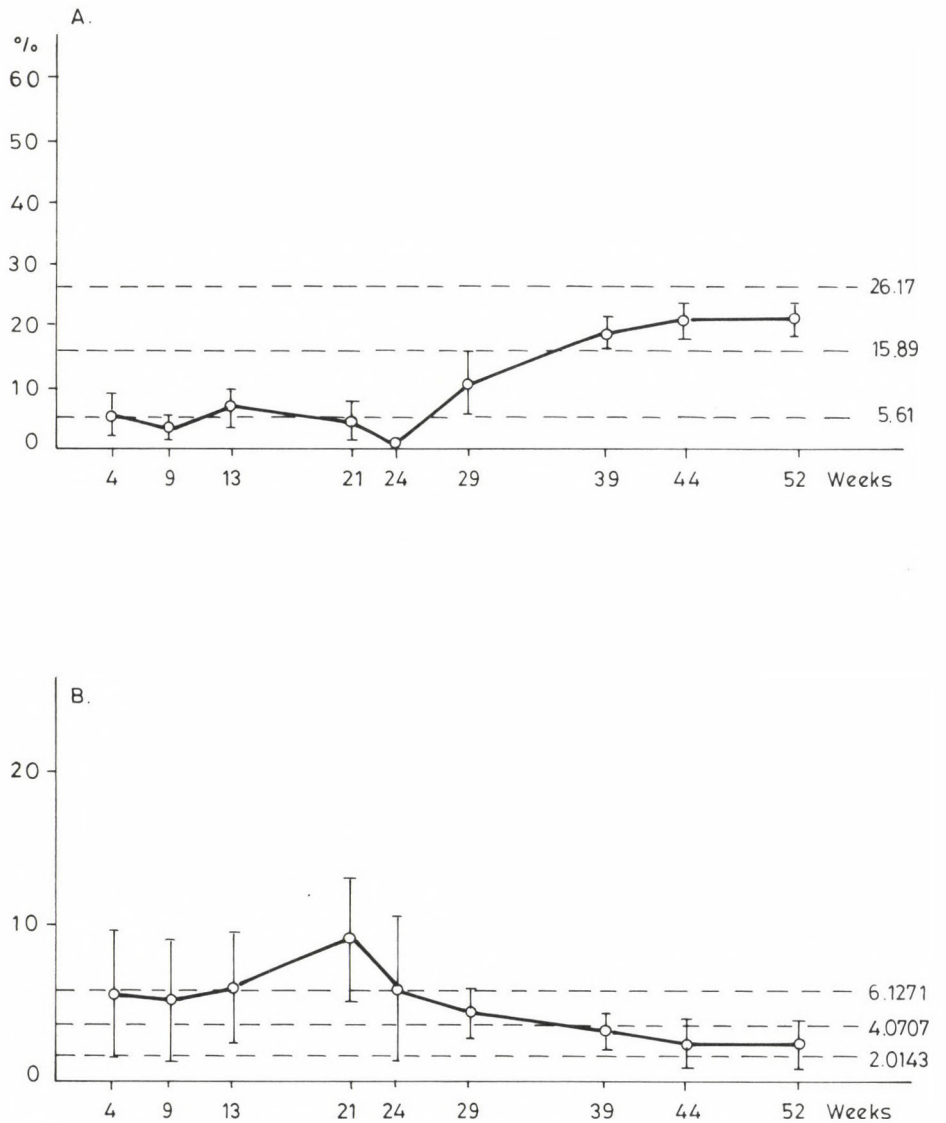


Fig. 5. A: Specific CD_{19} marker expression of the B cell during treatment
B: CD_3/CD_{19} values at scheduled time of treatment with the control data of healthy children (n = 20)

The change in the CD_{19} marker is indicated by the CD_3/CD_{19} ratio (Fig. 5/B).

Confirmed by bacterial or clinical tests, and cytopenia associated with febrile episodes /3, 27/ during treatment, revealed infections during the inductive phase, increasing repeatedly by the 22nd and 26th week at the low marker levels mentioned, though during this period of therapy fever complications were much less frequent (Table IV).

DISCUSSION

Intensive chemotherapy cures many children with ALL /5/. Encouraging results /30/ are reported in children with high-risk ALL /14, 16/ receiving modern chemotherapy /11, 12/.

As supported by recent data /22/ bone marrow transplants from twins in cases of high malignancy ALL proved to be successful in 60 per cent in first remission.

In the present study it was found that during the intense chemotherapy of patients with poor prognosis ALL, the parameters monitored by light microscope /6, 32/ reached the values of normal resting cells by the end of the induction, while electron microscopic investigation /6/ confirmed properties characteristic for still immature cells. In the same cells the surface antigen expression, corresponding to the immune phenotype of healthy lymphocytes /19/, became only detectable by the end of the consolidating treatment.

It was this high malignancy which justified the differentiation of patients /9, 14/ during intense, high dose chemotherapy /25/, by simultaneously interrelating cytological, clinical information with phenotypes /8/, the efficacy of treatments being determined, in addition to the correctly applied supportive therapy /1, 24/, by the physiological tolerance of tissues /25/ and the drug susceptibility of lymphocytes /31/.

TABLE IV

Incidence of infections, confirmed by bacteriological and clinical tests, and of febrile episodes associated with granulocytopenia during the first 52 weeks of "BFM-88 RF > 1.7" treatment
ALL-BFM 88 (RF > 1.7) Protocol

	Weeks 0	4	9	13	21	24	29	39	Total	Antimicrobial drugs: Penicillin	Oxacillin	Meticillin	Ceftazidim	Ampicillin	Clindamycin	Dala-C	Co-Trimoxazol
	Protocol I			Protoc.M.	V-C		Protocol II										
Granulocyte count (10^9/l)																	
< 0.5	+ + + +		+		+		+ +		8								
< 0.1																	
Granulocytopenic episodes																	
With fever	+ +	+	+				+		5								
Bacteriologically documented infection																	
Upper respiratory tract									1			+	+				
Non haem. strept.		+					+		1		+		+				
Urinary Tract																	
E.coli } Proteus }		+							1			+	+				
E.coli		+							1				+				
Clinically documented infection																	

Fever	+	++	++	++		++	++	+		+	+	+	+				16		+		++++		+		+	++	
Sinobronchitis									+		+						2	+		+			+				
Angina															+		1	+									
Tons.foll.											+		+				2	+		+							
Stomatitis												+		+			2										
Number of infection	2	2	2	3	1	2	2	1	1	1	3	1	2	2		2	27										
Number of drugs																		3	2	3	17	2		1	1	1	2

Papers reviewing the treatment results of high malignancy ALL recommend further controlled, prospective clinical studies /10/ to limit both the intensity and the duration of chemotherapy and improve relapse-free survival rates /18/. The results obtained justify continued extensive research in this area. Collecting further information /5/ on the lymphocytes of patients in remission during combined cytostatic therapy may furnish results which, later on, can be directly applied in the treatment of ALL patients.

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Acta Paediatrica Hungarica 32 (1), pp. 71-88 (1992)

DIAGNOSTIC VALUE OF CLINICAL FEATURES AND SUPPLEMENTARY INVESTIGATIONS IN TUBEROUS SCLEROSIS IN CHILDREN

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Received 18 July 1990

The author evaluates the usefulness of clinical features and some supplementary investigations in diagnosis of tuberous sclerosis in children. 52 children ranging in age from 1 month to 14 years took part in the study. Usually depigmented naevi were the first sign of tuberous sclerosis and were seen in 98% of affected children. Epileptic seizures occurred in 96% of patients, mostly during the first year of life. Axial computed tomography of brain seems to be very helpful in diagnosis of tuberous sclerosis in every age. Multiple periventricular calcifications were found in 98% of children. Two-dimensional echocardiography was especially useful in infants and children below 2 years of age. Retinal hamartomas were found in 25% of children with tuberous sclerosis. In some cases these changes were revealed during the first year of age. Abnormal EEGs were found in 90% of recordings. The EEGs tended to improve with increasing age. In 25% of children one of parents was affected by tuberous sclerosis. The careful skin examination of both parents can be especially helpful in children with doubtful diagnosis of tuberous sclerosis.

INTRODUCTION

Tuberous sclerosis (TS) is an autosomal dominant disease known for over a century and recognized by characteristic hamartomas or benign growths in one or more organs, notably the skin, the central nervous system including the retina, the kidneys and the heart. Other organs or tissues that may be affected are lungs, spleen, liver, pancreas and gastrointestinal tract /17/.

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The incidence was thought to be one in 100 000, but has been recently updated to suggest one in 10 000 /17/.

In 1908, Heinrich Vogt /28/ established the clinical triad of features for the diagnosis of TS consisting of seizures, mental retardation and facial "adenoma sebaceum". For years the disease was only suspected in those suffering from mental retardation and fits, but it is now known that the disorder may present with less than these classical findings as a "forme fruste".

In the experience of Gomez /10/ all the three features of Vogt's triad were found only in 29% of patients with TS. Notoriously 6% of the patients had none of the symptoms of the triad.

Diagnosis of tuberous sclerosis in children is especially difficult. Most symptoms and lesions are absent at birth and develop with age. Therefore this study was aimed to analyse the usefulness of clinical features and some investigations in the diagnosis of tuberous sclerosis in children, particularly in early age.

PATIENTS AND METHODS

52 children with tuberous sclerosis (27 males and 25 females) ranging in age from 1 month to 14 years were investigated in Department of Child Neurology in Memorial-Hospital Child's Health Centre in Warsaw in years 1984-1989. At admission there were 21 children below 2 years of age, 14 children aged 2-5 years, 9 children aged 5-9 years and 8 patients aged 9-14 years.

Only 18 children (35%) were referred to Child's Health Centre with the diagnosis of tuberous sclerosis. In the other children (65%) the main causes of hospitalization were drug-resistant epileptic seizures in 28 patients, cardiac defects in 3 patients, brain tumours in 2 patients, intraocular tumour in 1 child.

In each child thoroughful anamnesis was taken and particular stress was laid on the time of appearance of skin lesions and neurological features. Medical records concerning previous hospitalizations have been reviewed. Special attention was paid to familial anamnesis.

The health status of siblings, parents, grandparents, uncles and aunts has been questioned and particular attention was given to a history of mental retardation, seizures, brain or

cardiac tumours, kidney cysts, pulmonary failure, renal angiomyolipoma, skin lesions including depigmented naevi, facial angiofibroma and shagreen patches.

Full pediatric and neurological examinations were carried out for each child and the following investigations were performed: psychological examination in all 52 children, echocardiography in 29 children, computed tomography of the brain in 50 children, ultrasonography of the abdomen in 39 children, electroencephalography in 52 children and ophthalmologic examination in 51 children.

Psychological investigation was based on anamnesis and following tests: Psycho-Cattell test, Terman-Merrill test and Wechsler test.

Echocardiography was performed using apparatus Hewlett-Packard type 77020A. In retarded or agressive patients the examination was carried out after rectal administration of Thiopental Sodium.

Axial computed tomography of the brain was performed with apparatus Somatom 2 of Siemens.

Ultrasonography of abdomen was carried out with Echo-Camera SSD-280 LS of Aloka Co.ltd.

Electroencephalographic examination was performed mainly during sleep with apparatus Elema Mingograf of Siemens.

RESULTS

1. Dermatologic manifestations

Dermatologic features were an important part of tuberous sclerosis. Even in the neonatal period, lesions that were highly suspicious for TS could be recognized in some patients. With increasing age, additional lesions appeared and in certain cases the diagnosis of TS might be established even without performing any additional investigations.

Depigmented naevi were the most common skin lesions found in 51 children (98%) with TS. Usually, they were seen by parents in newborns (44 cases) or very small infants and were the first visible sign of TS. They were distributed in irregular way, mostly over the trunk and extremities and were rare on the face.

"Café au lait" spots were seen in 20 children (38%). In 19 patients the parents noticed them in neonatal period.

Pringle's tumours pathognomonic for TS were revealed in 35 children (67.3%). These lesions were identified in 29 children aged 3-5 years. They are very helpful in diagnosis of TS in

children above 5 years. In this age group Pringle's tumours were found in 27 of 30 children (90%). In 4 children Pringle's tumours appeared below 2 years of age. During the puberty the lesions usually became more numerous and prominent. An average age of appearance of Pringle's tumours was 3.89±2.10 years. In the group of 17 patients without Pringle's tumours, only 3 children have finished 5 years and 10 children were younger than 2 years of age.

Shagreen patches were seen in 19 patients (36.5%), but only in 1 child below 2 years of age. These changes commonly appeared in older patients. Multiple smooth papules (molluscum fibrosum pendulum) on the neck, axillae or near flexures of limbs were noticed in 10 patients (19%). Forehead fibrous plaques and Koenen's tumours were found in very small groups forehead fibrous plaques in 7 children (13.5%) and Koenen's tumours in one girl of 12 years of age.

The incidence of dermatologic features is shown in Table I.

2. Neurologic features

The most frequent neurologic feature, epilepsy, was observed in 50 patients (96%). Usually, the epileptic seizures appeared between 4 and 6 months of life. In 27 children (52%) onset of seizures appeared during the first six months and in 35 children (67.3%) during the first year of age. The most common type of first epileptic seizure was infantile spasms. Infantile spasms, as first type of seizures were observed in 24 of 27 children below 6 months of age, in 7 of 8 children aged 6 months-1 year and in 3 of 8 children between 1 year and 2 years of age.

Generalized hypotonia was seen in 17 children below 9 years of age. Spastic hemiparesis was observed in 11 children /21/.

3. Mental status

Mental retardation was one of the most frequent clinical features of patients with TS: 36 patients (70%) were known to be mentally retarded. Mental retardation was more severe in children in younger age groups. There was a clear correlation between the two most important neurologic symptoms of TS: the

TABLE I

Incidence of dermatologic features in children with TS

Skin lesion	No. of patients	% (52=100%)
1. Depigmented naevi	51	98
2. Pringle's tumors	35	67.3
3. "Cafe au lait" spots	20	38.5
4. Shagreen patches	19	36.5
5. Molluscum pendulum	10	19.2
6. Forehead fibrious plaque	7	13.5
7. Koenen's tumors	1	1.9

seizures and the mental subnormality. The age of seizure onset and the presence and severity of the mental subnormality were directly related. In the group of children whose epileptic seizures appeared in the first year of age mental development was more severely delayed than in the group of older patients ($p < 0.01$).

Almost all patients whose seizures began in the first six months of life, were mentally defective. Usually, the patients whose motor development was abnormal, were known to have seizures prior to failing to sit up, crawl or walk.

In 15% of children with infantile spasms some features of autistic behaviour were observed.

4. Ophthalmic findings

Retinal hamartomas were revealed in 12 children (23.5%). The most common type of retinal hamartoma was a relatively flat, smooth-surfaced, semitransparent lesion, which was seen in 9 patients. In 3 patients there was calcified mulberry-like tumour and in 1 patient a lesion had morphologic characteristics similar to both previously described lesions. In 3 children retinal hamartomas were observed during the first year of age.

5. Echocardiography

Cardiac tumours were identified in 10 (6 males and 4 females) of 29 children in echocardiographic examination. In 4 cases the tumours were multiple. Tumours were more common on the left side of the heart (5 children) than on the right (3 children), and were more frequent in interventricular septum (8 children) than in the ventricular wall (2 patients) ($p < 0.01$).

Detectability of these tumours diminished with increasing age. The highest detectability was in children below 2 years of age. Cardiac tumours were found in 4 of 6 children (66%) in this age group.

Echocardiography was repeated in 5 of 10 children with cardiac tumours and in 2 of them the size of tumours did not change, but in 3 patients tumours disappeared or their size significantly diminished.

6. Computed tomography of brain

Computed tomography revealed many of the gross pathologic processes of cerebral TS (Table II). Calcified subependymal nodules were the most diagnostic CT findings. They were found in 49 of 50 children (98%). Computed tomograhpy did not reveal these changes in three children below 5 years of age during the first examination. Next CT demonstrated calcified subependymal nodules along the lateral ventricules in two of them.

Focal areas of increased attenuation involving the cortex and/or white matter appeared on CT in 25 patients (50%). In 14 children (28%) hypodense tubers were seen. These lesions were observed in 38% of children below 2, and in 23% of children above 5 years of age.

Relatively often CT demonstrated a dilatation of the lateral ventricules (38% of children) and cortical atrophy (42%). These changes were seen more often in older children. In two patients CT showed brain tumours. Microscopically, subependymal giant cell astrocytomas were revealed.

7. Ultrasonography of abdomen

Ultrasonographic examination of abdomen was carried out in 39 children and in 5 of them (13%) bilateral, hyperechogenic nodules were revealed. Usually, they were not troublesome to the patients. Physical examination of patients with TS provided little evidence of their renal tumours. Hamartomas of the liver were seen in 5 children (13%).

8. Electroencephalography

EEG examination was carried out in all children. 13 of total number of 114 EEG recordings (11%) were normal (Table III).The most common abnormality - paroxysmal generalized activity was observed in 56 recordings (49%). There was exclusively generalized paroxysmal activity in 29 recordings and in others it was accompanied by focal or lateralized abnormalities. Hypsarrhythmia appeared only in 10 recordings, usually in patients below 2 years of age. In 35 EEGs (30%) non-paroxysmal activity was observed.

In general, the most severe abnormalities, particularly with

TABLE II

Cerebral findings revealed on computed tomography in children with tuberous sclerosis

Cerebral finding	No. of patients	% (50=100%)
1. Periventricular calcification	49	98
2. Other intracerebral calcifications	25	50
3. Cortical atrophy	21	42
4. Ventricular dilatation	19	38
5. Hypodense areas	14	28
6. Brain tumors	2	4

TABLE III

EEG findings in children with tuberous sclerosis

EEG finding	No. of EEG recordings	%
Normal	13	11
Hypsarrhythmia	10	9
Generalized paroxysmal activity	56	49
exclusively generalized	29	25.4
with focal or lateralized	27	23.6
discharges	27	23.6
Non-paroxysmal abnormalities	35	31
T o t a l :	114	100

regard to epileptiform abnormalities, were seen in young children with early onset of seizures. With increasing age the abnormalities tended to decrease in amount and degree. There was statistically significant difference ($p < 0.01$) in appearance of these changes in children below and after 5 years of age.

In the group of children below 2 years of age hypsarrhythmia and paroxysmal generalized activity were revealed in 24 recordings (73%). In children above 9 years of age, generalized paroxysmal activity appeared in 35% of EEGs.

There was a good correlation between the degree of EEG abnormality and the degree of mental retardation, with the most severe EEG abnormalities in patients with moderate or severe mental retardation. Children with infantile spasms and hypsarrhythmia showed a greater degree of mental retardation than children with other types of seizures or EEG abnormalities. Most of the patients with focal or generalized spike-and-wave discharges who had onset of seizures after 2 years of age were only mildly retarded or had normal intelligence.

Although epileptiform abnormalities were the most common type of abnormality, no one specific type of EEG pattern was seen in children with TS.

9. Family evaluation

In the group of 52 children with TS, each child had a thorough investigation of family history. The parents of 13 children from 10 families were found to have TS. There were 5 males and 5 females (Table IV)

Epilepsy was given in anamnesis in 6 parents. Thorough skin examination revealed depigmented naevi in 9 parents, facial angiofibroma in 7 parents, "café au lait" spots in 2 parents, shagreen patch in 1 and Koenen's tumours in 1 parent.

Computed tomography of brain was performed in 8 of 10 affected parents and in all of them the characteristic periventricular calcifications were seen. In 8 parents fundoscopic examination was performed and no ophthalmic lesions were found. There were two families with 2 and 3 siblings affected by TS.

TABLE IV

Familial cases of TS

No of pedi-gree	Sex of affectec parent	Skin lesions	Epilepsy	Computed tomography of the brain
1.	F	depigmented naevi facial angiofibroma „cafe au lait" spots	present	periventricular calcifications
2.	M	depigmented naevi facial angiofibroma	absent	periventricular calcifications
3.	M	depigmented naevi facial angiofibroma	present	not done
4.	F	depigmented naevi facial angiofibroma shagreen patch „cafe au lait" spots Koenen's tumors	absent	periventricular calcifications
5.	F	depigmented naevi facial angiofibroma	present	periventricular calcifications
6.	F	depigmented naevi	present	periventricular calcifications
7.	F	depigmented naevi	present	not done
8.	M	depigmented naevi	present	periventricular calcifications
9.	M	depigmented naevi facial angiofibroma	absent	periventricular calcifications
10.	M	facial angiofibroma	absent	periventricular calcifications

DISCUSSION

The cutaneous lesions are amongst the most important and most widely studied features of TS. Usually, depigmented naevi are the first sign of the disease and may be seen at birth or in first months of life. Few months later epileptic seizures develop. This coincidence of depigmented naevi, infantile spasms and mental retardation is very suggestive of TS.

The majority of patients with TS have depigmented naevi at the time of birth, although their presentation may be delayed by months or years. In my study parents noticed depigmented naevi in neonatal period in 44 of examined children.

The incidence of facial angiofibroma, the cutaneous lesion of great diagnostic significance is 47-100% according to different authors /3,10/. I found these lesions in 90% of the children more than 5 years old. Similar results were obtained by Sutterworth and Wilson /2/ and Nickel and Reed /21/.

Usually, facial angiofibroma appears between 3 and 5 years of age. It is rather seldom in children below 2 years. In one of the patients examined by Rogers /24/ the facial angiofibroma was present at birth.

The late appearance of facial angiofibroma needs very thoughtful verification. In the adolescent patient acne vulgaris may be very easily mistaken for angiofibroma, especially in the child with epilepsy.

Shagreen patches were noted in 36.5% of studied patients, but only in 1 child below 2 years of age. They become more common after the first decade of life /24/.

Forehead fibrous plaques and Koenen's tumours are regarded by many as pathognomonic for TS /10/. In my series these changes appeared in 13.5% of children respectively, and had only supportive value in diagnosis of TS.

There is a high frequency of epilepsy in TS patients. According to Gomez /10/ and Cendrowska et al /3/ epilepsy appears in 92% and 100% of patients respectively. In my study, 96% of children suffered from epilepsy and in all 50 patients epileptic seizures appeared below 5 years of age.

Seizures seldom begin in the first 8 weeks of life, but in most children the onset of seizures is between 4 and 6 months of life. 52% of patients in my series had first epileptic seizures during the first 6 months of life, and 67% of them during the first year of age. These results are similar to those found by Zaremba /31/ and Cendrowska et al /3/.

Epileptic manifestations appear with particular frequency in the form of infantile spasms. This kind of epileptic seizures was the presenting complaint in 89% of children below 6 months in my study and in 69% of the selected patients with TS reported by Pampiglione and Moynahan /22/ and in 68% of Hunt's patients /13/. During the course of TS the type of seizures changes.

The patients with onset of convulsions in early life are more likely to be mentally retarded. Almost all of the patients in my series, whose epilepsy appeared during the first six months of age were severely retarded. It confirms a strict correlation between epilepsy and mental subnormality. In the group of 160 patients examined in Mayo Clinic, Gomez /11/ found 20 patients without epilepsy. All of them had normal intelligence.

According to Fleury /7/ the incidence of ocular findings in TS is about 40%, but there are very few studies in children. In my study the charasteristic ophthalmological lesions (hamartomas) were found in 23.5% of children. This relatively low percentage of revealed lesions may be explained by young age of examined patients.

Ocular findings are of diagnostic value, particularly in "formes frustes" (as they are sometimes found in the absence of Pringle's tumours) and in some children below 1 year of age.

The frequent association of TS and cardiac rhabdomyoma has been recognized for many years. Most information on these tumours has of necessity been related to morbid anatomy /6,16/ and there are only very few papers on frequency of cardiac involvement in such patients during life /9,25/.

Cardiac rhabdomyoma, although benign histopatologically, may be life threatening especially during the first year of life /4,20/. In the Fenoglio et al /6/ series of 36 patients

with cardiac rhabdomyoma, 78% of the patients died during the first year of life and only 3 patients lived beyond age 5 years. Commonly asymptomatic, cardiac rhabdomyoma may cause fetal obstruction of the cardiac outflow tracts, cyanosis, arrhythmias, cardiogenic emboli and cardiac failure secondary to myocardial involvement.

Echocardiographically cardiac tumours were revealed in 35% of examined children. Smith et al. /25/ found them in 58% of children and 18% of adults affected with TS. Tumours seem to be especially frequent in neonatal and infantile period. I found cardiac tumours in 4 of 6 patients (66.6%) below 2 years of age.

The higher incidence of these tumours in young children may be explained both by frequent deaths reported in these patients and spontaneous regression of cardiac rhabdomyomas observed by Alkalay et al. /1/ and Smith et al. /25/. Also in my series in 3 children the cardiac tumours disappeared or diminished with increasing age. Because of high frequency of cardiac rhabdomyomas in small children, echocardiography seems to be extremely effective in diagnosis of TS in these patients.

Calcified subependymal nodules are the most frequent cerebral abnormality of TS. They were found on CT scan in 98% of children and in all children above 3 years of age in my series. In 3 younger children the first CT scan did not reveal any changes. Multiple subependymal nodules are regarded by many authors to be pathognomonic for TS /12/.

Intracerebral calcifications localized in white matter and cortex have been seen in 50% of children. They should be differentiated from those observed in toxoplasmosis and CMV infections.

Current study supports thesis proposed by Houser and Nixon /12/ that hypodense tubers may disappear with age. In my series they were observed in 38% of children below 2 and in 23% of patients above 5 years of age. The thesis that hypodense tubers change with age evaluating from hypodense through isodense to hyperdense tubers requires further CT follow up studies. Computed tomography should be periodically performed in all children with TS because of the danger of development of subependymal giant cell astrocytomas /14,15,19/.

Ultrasonography of abdomen may reveal lesions mainly in liver and kidneys. Relatively low frequency of changes disclosed in this study (13% in kidneys and 13% in liver) may be connected with young age of examined patients. Stillwell et al. /26/ observed angiomyolipomas of kidneys in 47% of patients and Van Baal et al. /27/ even in 60% of patients. Our recent follow up studies with applications of late generation ultrasonography revealed echogenic areas in kidneys suggestive of angiomyolipomas in about 40% of children /23/. Value of ultrasonographic examination in diagnosis of TS in children seems to be limited to "forme frustes" and cases in which the diagnosis is uncertain.

There is a high incidence of electroencephalographic abnormalities found in patients with TS. Only 10% of children in this study and 12% of patients reported by Westmoreland /29/ had normal EEGs. No characteristic EEG abnormality exists.

With increasing age, slow, but constant improvement of EEGs can be observed. In older patients there were less severe abnormalities than those seen in the young children /5/. It should be clear that the value of EEG lies in revealing latent epilepsy in "forme frustes" of TS or in allowing determination of the extent of cerebral involvement in diagnosed cases.

There was a good correlation between mental retardation and severity of abnormalities in EEGs.

The thorough examination of parents and siblings may be very helpful in establishing the diagnosis, especially in very young children with sporadic signs of TS /18,30/. Fryer /8/ suggested that careful examination of skin and ophthalmologic examination should be sufficient for diagnosis of TS in familial cases. In doubtful cases additional CT of brain should be offered.

Tuberous sclerosis, because of its variable expressivity may produce great diagnostic difficulties, especially in early age. The purpose of this communication is to evaluate the usefulness of some clinical features and laboratory investigations in the diagnosis of TS in children.

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PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

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Index: 26.021

Acta Paediatrica Hungarica

VOLUME 32, NUMBER 2, 1992

Akadémiai Kiadó, Budapest

ACTA PAEDIATR HUNG HU ISSN 0231-441X

ACTA PAEDIATRICA

HUNGARICA

A Quarterly of the Hungarian Academy of Sciences

Editor

M. MILTÉNYI

Acta Paediatrica publishes original reports of studies in paediatrics and borderline fields.

Manuscripts and editorial correspondence should be addressed to

ACTA PAEDIATRICA HUNGARICA

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H-1083 Budapest, Hungary

Subscription information

Orders should be addressed to

AKADÉMIAI KIADÓ

P.O. Box 245

H-1519 Budapest

Acta Paediatrica Hungarica is published in yearly volumes of four issuse by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences

Prielle Kornélia u. 19—35.

H-1117 Budapest

Acta Paediatrica Hungarica is abstracted/indexed in Biological Abstract, Chemical Abstracts, Excerpta Medica, Index Medicus

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PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

Acta Paediatrica Hungarica 32 (2), pp. 91-100 (1992)

MATERNAL WEIGHT GAIN AND BIRTH WEIGHT

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Received 12 November 1991

The data of first 1000 non-malformed, mature (≥ 2500 g) singletons of participants in the Hungarian Family Planning Programme were evaluated. The mean maternal weight gain during pregnancy was 13 kg which was modified by the body weight of women. Maternal weight gain exceeded 13 kg in 54% of pregnant women. There was a positive correlation between maternal weight gain and birth weight which was calculated as 26.6 g/kg.

INTRODUCTION

The results of previous paper /1/ suggested that a wider range of maternal weight gain than is currently recommended is associated with good pregnancy outcome. This finding is supported by a prospective Hungarian study /3/ complemented with an obvious positive correlation between maternal weight gain and birth weight.

PATIENTS AND METHODS

The Hungarian Family Planning Programme /3/ (HFPP) was established in 1984 for the reduction of unsuccessful pregnancy outcomes. The purpose of this feasibility study was to check the efficacy of the combination of the so-called qualitative family planning methods within the primary health care. Couples who satisfied all 5 of the following criteria were eligible for participation in the HFPP: (i) age between 18 and 35 in females, (ii) no previous unsuccessful pregnancies, (iii) no delayed conception and infertility, (iv) no pregnancy as yet and (v) voluntary participation and promise of compliance.

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The HFPP has 3 guiding principles. First, the checking-up of reproductive health which involves family history, case history of females including prepregnancy body weight and maternal height, exploration of psychosexual condition, examination of vaginal and cervical smears, sperm analysis, blood examination for serological status of rubella and HIV. Second, a 3 month preparation for conception: "protection of germ cells" (avoidance of smoking, alcohol, etc), stop of oral contraception, measurement of basal body temperature, periconceptional multivitamin supplementation, occupational history, check-up of dental status and the measurement of body weight. Third, protection of early pregnancy, i.e., after the diagnosis of pregnancy during the first missed menstrual period, the hazards (e.g., occupational) to the fetus are avoided. Nutrition education is provided by a written guideline. After the fourth, the so-called 'good-bye' meeting, these women are referred to regional prenatal care clinics. Female participants get a certificate form which is filled out and sent back by them confirmed by their obstetricians at the end of pregnancy. It involves the maternal body weight before delivery and birth weight. Eight months later mothers and their children are invited for a detailed check-up examination and that time, among others, previous maternal and newborn weights are checked. Some cases are lost to follow-up, but these couples are visited at home in order to clarify their pregnancy outcomes. At the end of 1989, 2,723 pregnancy outcomes were evaluated. The number of drop-out cases was 36 (1.3%).

The data of the first 1000 liveborn singletons with birth weight of $\leq$ 2500 g and without congenital abnormalities were used for this analysis. Three and 11 women had maternal diabetes or any hypertensive disorder, respectively. The number of cesarean delivery was 92. The correlation between gestational time and maternal weight gain and sex will be analysed separately.

RESULTS

Table I shows the distribution of pregnant women before their deliveries by maternal weight gain. The mean maternal weight gain was just 13 kg and 75.2% of cases had weight gain between 8 and 16 kg (Fig. 1). Only 18.6% of females had lower weight gain than 10 kg. The maternal weight gain distribution does not fit the Gaussian normal distribution mainly due to the skewness of tails.

The mean maternal body weights were 58 kg and 71 kg before conception and before delivery, respectively. It means 18.3%

TABLE I

Distribution of women and mean birth weight by maternal weight gain groups in 1000 women

Maternal weight gain (kg)	Pregnant women No.	Birth weight $\bar{x}$	S.D.
1-3	5	2650	354
4	6	3033	375
5	8	3200	541
6	20	3217	345
7	28	3104	400
8	51	3177	434
9	68	3190	416
10	76	3262	391
11	88	3274	430
12	109	3302	357
13	102	3372	452
14	94	3412	455
15	92	3364	526
16	72	3356	374
17	38	3523	411
18	36	3433	497
19	19	3536	431
20	26	3562	374
21	21	3584	414
22	10	3925	410
23	8	3463	415
24	3	3150	278
25	6	3743	372
26-29	5	3470	396
Total	1000	3340	442

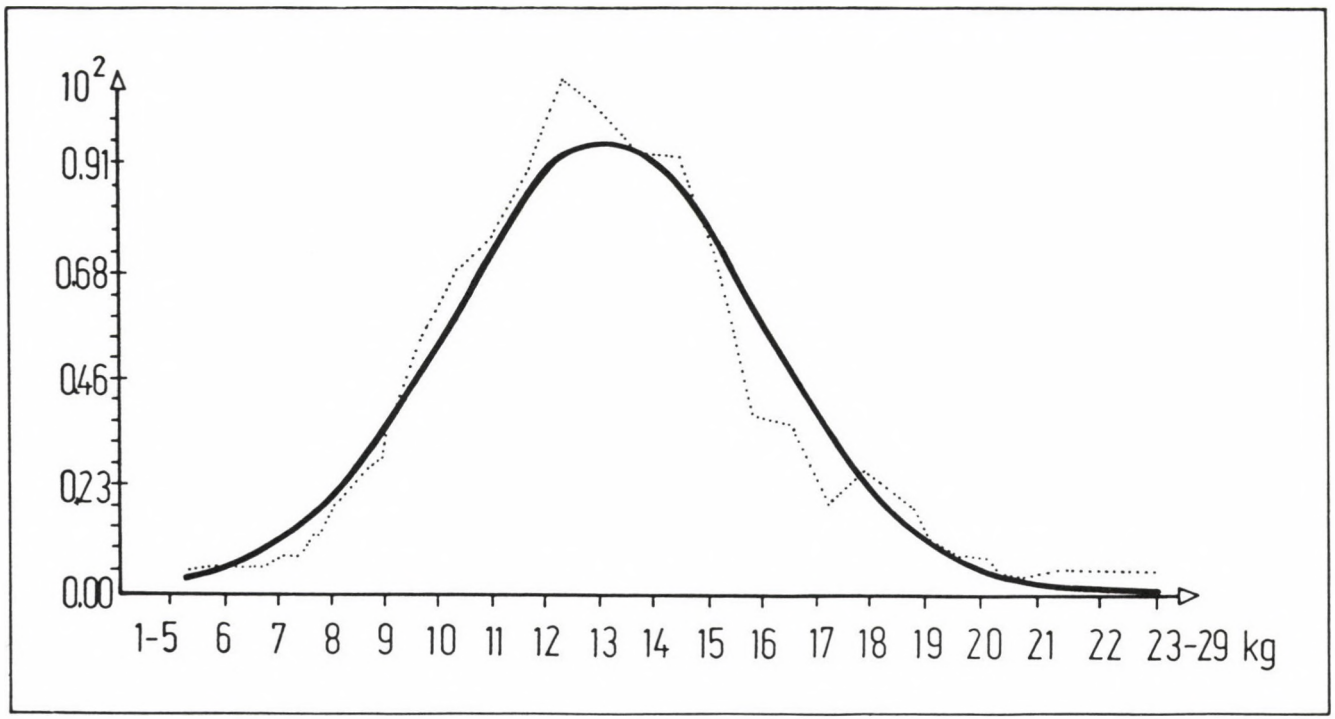

Fig. 1. Distribution of maternal weight gain

TABLE II

Maternal weight gain as a function of pregnancy body weight and maternal height. (The means are shown in brackets)

Body weight (kg) \ Height (cm)		-159 (156) No. $\bar{x}$	160-169 (164) No. $\bar{x}$	170- (172) No. $\bar{x}$	Total (165) No. $\bar{x}$	%
-49	(47)	53 12.5	44 12.6	0 -	97 12.5	21.0
50-59	(54)	78 11.7	394 13.0	75 13.3	547 12.8	19.2
60-69	(63)	14 12.3	149 13.3	117 13.8	280 13.5	17.6
70-	(75)	3 17.0	32 12.4	41 13.4	76 13.1	14.9
Total	(58)	148 12.1	619 13.0	233 13.6	1000 13.0	18.3

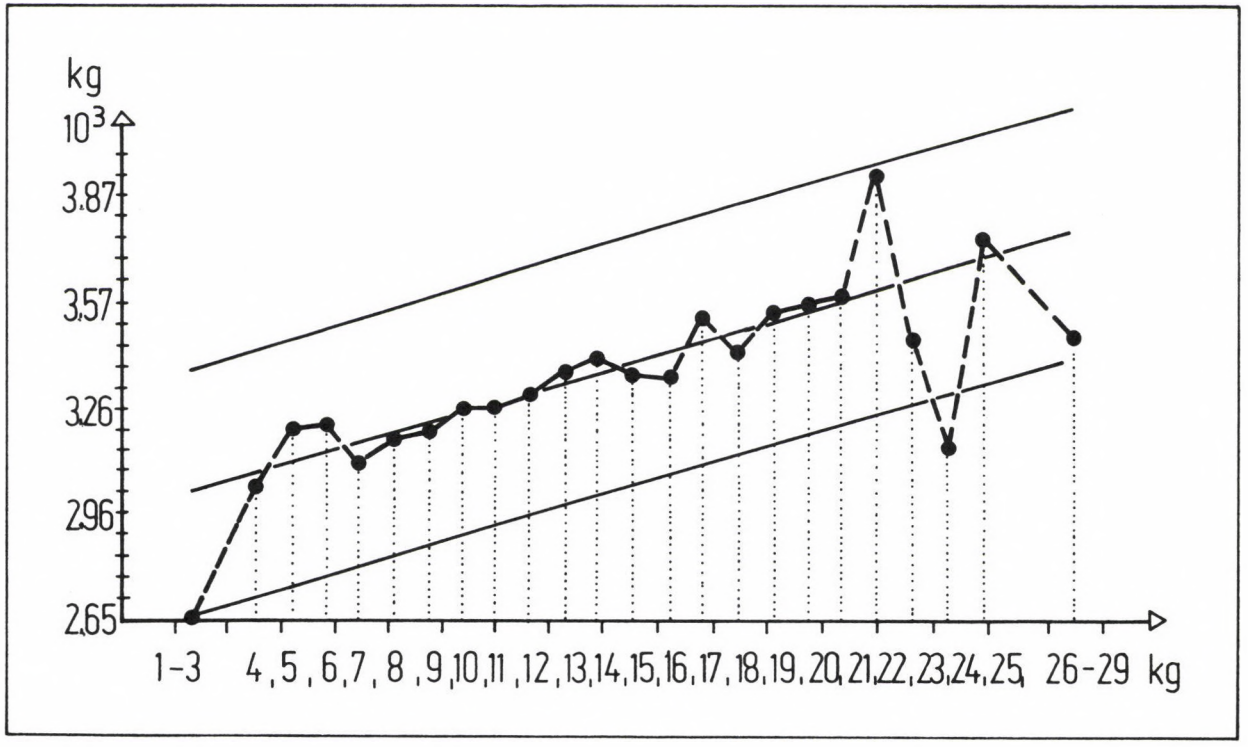

Fig. 2. Correlation between maternal weight gain and birth weight of newborns

increase in maternal weight during pregnancy (Table II). However, the maternal weight gain was modified by prepregnancy body weight. The relative maternal weight gain was 21.0% in females having less than 50 kg and 14.9% with body weight over 70 kg. The height indicated a less significant effect determined mainly by body weight. The maternal age did not modify significantly the maternal weight gain.

The distribution of mean birth weights as a function of maternal weight gain is also shown in Table I. There is an obvious correlation between these groups mainly after the exclusion of cases with very low (under 5 kg) and very high (over 21 kg) weight gains (r=0.87). However, this correlation is much lower at the analysis of all individual cases (r=0.25). The mean birth weight/maternal weight gain figure was 26.6 g/kg. After the reduction of birth weight from predelivery body weight the correlation was 0.14 between maternal weight gain and birth weight. The mean birth weight as a function of prepregnancy body weight and maternal height are shown in Table III and IV. The correlation between pregnancy body weight and birth weight is 0.30, while it was 0.05 between maternal height and birth weight.

DISCUSSION

The study of Abrams and Parker /1/ showed a wide variation in the weight actually gained by women with a good pregnancy outcome, i.e., a vaginal birth at term of a living healthy infant. Of the 4,674 women studied the mean maternal weight gain was 15 kg and nearly twice of cases exceeded the recommended maximum without any apparent ill effects. In Hungary the recommended maximum maternal weight gain is 11-12 kg, while the desirable average is advocated as 15% of prepregnancy body weight. In this Hungarian material, about half of female participants represented a higher socioeconomic status based on their education (12 or more classes of school)

TABLE III

Mean birth weight as function of maternal weight gain and prepregnancy body weight

Maternal weight gain (kg) \ Body weight (kg)	-49 No. $\bar{x}$	50-59 No. $\bar{x}$	60-69 No. $\bar{x}$	70- No. $\bar{x}$	Total No. $\bar{x}$
- 8	9 3119	65 3068	41 3194	12 3566	127 3156
9-12	43 3062	207 3252	76 3361	15 3507	341 3263
13-	45 3101	275 3346	163 3580	49 3693	532 3429
Total	97 3085	547 3279	280 3466	76 3638	1000 3340

TABLE IV

Mean birth weight as a function of maternal weight gain and maternal height

Maternal weight gain (kg) \ Height (cm)	-159 No. $\bar{x}$	160-169 No. $\bar{x}$	170- No. $\bar{x}$	Total No. $\bar{x}$
- 8	22	80	25	127
	2996	3207	3206	3170
9-12	62	211	68	341
	3172	3263	3347	3263
13-	64	328	140	532
	3310	3388	3580	3429
Total	148	619	233	1000
	3206	3322	3472	3340

and 93% of females were primiparous. The mean maternal weight gain was 13 kg, and 54.1% of women exceeded the recommended maximum (12 kg). Our results have also confirmed the finding that the relationship between maternal weight gain is lower in women with higher prepregnancy body weight /4, 1/.

The birth weight seems to be one of the most sensitive indicators of fetal development. The correlation between the maternal weight gain and bairth weight is a good example that the current medical advice concerning the maximum maternal weight gain have little scientific basis. In Hungary the rate of newborns with low birth weight is extremely high, the decreasing rates were between 10.2 and 9.2% in the eighties /2/. Thus the change of the above unreasonable advice concerning the maternal weight gain may help to reduce the high proportion of low birth weight. Further studies are needed to determine the longterm effect of larger maternal weight gain, e.g., for the recovery of body weight after puerperal period.

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Acta Paediatrica Hungarica 32 (2), pp. 101-113 (1992)

ALLELE FREQUENCIES OF CYSTIC FIBROSIS - LINKED MARKERS AND ΔF_{508} DELETION IN AFFECTED HUNGARIAN FAMILIES

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Received 10 July 1991

Linked marker haplotype analysis of 16 cystic fibrosis (CF)-affected children, 3 fetuses, 1 healthy child and their parents was performed by restriction fragment length polymorphism (RFLP) for J3.11, Met H, Met D, XV-2c, KM.19 markers. Polymerase chain reaction (PCR) to detect the main mutation of CF chromosome, a specific 3 base pair (bp) deletion (ΔF_{508}) was also performed in 17 CF patients.

Allelic frequencies on analysed CF chromosomes were: J3.11/Taq I 1.0, 0.0, J3.11/Msp I 0.44, 0.56, Met H/Taq I 0.73, 0.27, Met H/Msp I 0.80, 0.20, Met D/Taq I 0.75, 0.25, XV-2c/Taq I 0.85, 0.15, KM.19/Pst I 0.17, 0.83 for allele 1 and 2, respectively. Two markers, Met H and KM.19 were found to be in strong association with the CF mutation. The frequency of the ΔF_{508} mutation on all 34 CF chromosomes was 0.65 (of which 0.73 was homozygous and 0.27 heterozygous for this deletion).

INTRODUCTION

Cystic fibrosis (CF) is the most common recessive single gene lethal disorder, having 1 in 32 carrier frequency and 1 in 4000 newborn incidence in the Hungarian population. The disturbed function of exocrine gland epithelia is a common feature of the affected organs in CF patients. The inability of an epithelial chloride channel to respond to adrenergic stimuli and disturbances of its regulation is the basic defect of the disorder. Extensive genetic linkage analysis allowed mapping of the CF locus to the long arm of chromosome 7 (15, 27-29). This approach relied on the discovery that DNA is highly polymorphic

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/30/. The restriction endonucleases cleave DNA at specific sites producing DNA fragments with different lengths depending on the distance of the restriction sites. Variation in the position of these sites in DNA of different individuals or between DNA contained in homologous chromosomes will produce differences of the DNA fragments after digestion (restriction fragment length polymorphism = RFLP). The RFLPs are inherited in a stable Mendelian fashion and could be regarded as genetic loci. These sequence variants are detected primarily by Southern blot analysis in which recombinant DNA probe defines the region of interest. Before the discovery of the CF transmembrane conductance regulator (CFTR) gene in 1989 /13, 20, 21/ and the most frequent CF mutation (F_{508} deletion) inheritance of the disease could only be inferred via linkage analysis with nearby polymorphic DNA probes.

The CF gene resides in a region flanked by the following linked DNA markers: 7 cen ... ColIA1 - B79a - D7S18 - Met - XV-2c - CS.7 - KM.19 - Mp6d - CF - W32 - J3.11 - TCRB..7qter (1, 2, 4, 10, 23, 24, 29/.

These markers reveal genetic polymorphism useful in linkage and haplotype analysis and permit also the CF diagnosis of first - trimester chorionic villus biopsies, provided that material from an index affected child is available. Linkage analysis is dependent on marker haplotype information from proposite DNA and the results are valid within the same family.

When our laboratory initiated the project in Hungary, the CF locus has not been cloned yet, therefore we followed the "classic" RFLP analysis i. e. linkage studies with three closely linked DNA markers to the locus Met H, Met D, J3.11 /6/ and from the second CF family - examination, we completed the genetic analysis with two more tightly linked DNA markers (XV-2c and KM.19). We studied altogether 16 CF families who wanted to have another healthy child.

We examined also in 17 patients (all but two were new patients) the main CF mutation (ΔF_{508}) with polymerase chain reaction (PCR) which is a powerful new method to amplify specific DNA sequences several millionfold in a few hours. This

mutation means a specific 3 base pair (bp) deletion resulting in the loss of a phenylalanine at amino acid position 508 of the gene product.

MATERIALS AND METHODS

RFLP analysis: DNA was extracted from EDTA-anticoagulated venous blood of 16 CF affected children, their parents, one healthy brother and chorionic villi of three fetuses at 7-12 weeks of gestation, as previously described /14, 16/. 10 µg high molecular weight DNA was digested with 2 U/µg restriction enzymes: Taq 1, Msp I and Pst I, respectively. The samples were electrophoresed in 0.7% agarose gel at 25 V overnight and blotted into nitrocellulose filters as described by Southern /25/. The blots were hybridized with the appropriate DNA probes labelled with the random priming method /12, 16/, using an Amersham kit, and exposed to X-ray film with an intensifying screen for 3-6 days.

Hybridization probes: Probes were DNA sequencies, J3.11, XV-2c and KM.19 (kindly provided by Dr. R. Williamson, St. Mary's Hospital, London) and Met H and Met D (gifts of Dr. R. White, University of Utah, Salt Lake City).

Comparison of the allele frequencies on CF and normal chromosomes was made by use of Fisher's exact probability test.

PCR analysis for ΔF_{508} mutation:
The PCR for ΔF_{508} was performed as described by C.G. Matthew et al /17/. Reactions were subjected to 35 cycles (via PREM thermocycler) of denaturing at 94°C for 1 min, annealing at 55°C for 1.5 min and polymerase extension at 72°C for 1.5 min. The two opposite genotypes (50bp fragment of normal and a 47 bp by fragment of mutant genes) could be differentiated on 12% polyacrylamide gels. The fragments were photographed in ethidium bromide stained gels.

Clinical diagnosis: the CF diagnosis was always based on sweat test, the pancreatic and pulmonary function were assessed on the basis of clinical and laboratory findings.

RESULTS

Taq I and Msp I RFLP at J3.11 and Met H loci, Taq I RFLP at the Met D and XV-2c loci and Pst I RFLP at the KM.19 locus were used for allele analysis (Table I). The genotype of each individual was scored for the two alleles by using code 1 for the longer fragment and code 2 for the shorter fragment in the

TABLE I

DNA-analysis of CF families with linked probes

Family N°	probe	enzyme	father	mother	affected child	healthy child	fetus	informativity
1. S. Z.	J3.11	Taq I	1/2	1/1	1/1	–	–	partly inf.
	J3.11	Msp I	1/1	1/2	1/2	–	–	partly inf.
	Met H	Taq I	1/2	1/2	1/1	–	–	inf.
	Met H	Msp I	1/2	1/2	1/1	–	–	inf.
	Met D	Taq I	1/1	1/1	1/1	–	–	uninf.
2. Z. T.	J3.11	Taq I	1/1	1/1	1/1	–	–	uninf.
	Met H	Taq I	1/1	1/2	1/1	–	1/2	partly inf.
	XV 2c	Taq I	1/1	1/1	1/1	–	–	uninf.
	KM 19	Pst I	1/2	2/2	2/2	–	2/2	partly inf.
3. H. T.	J3.11	Msp I	1/1	1/2	1/2	–	–	partly inf.
	Met D	Taq I	1/1	1/1	1/1	–	–	uninf.
	XV 2c	Taq I	1/1	1/2	1/1	–	–	partly inf.
	KM 19	Pst I	1/2	1/1	1/2	–	–	partly inf.
4. A. H.	J3.11	Msp I	1/2	1/2	1/1	1/2	–	inf.
	XV 2c	Taq I	1/2	1/2	1/2	1/2	–	uninf.
	KM 19	Pst I	1/2	2/2	2/2	1/2	–	partly inf.
5. M. Sz.	Met H	Taq I	1/1	1/1	1/1	–	–	uninf.
	Met D	Taq I	2/2	2/2	2/2	–	–	uninf.
	XV 2c	Taq I	1/2	1/1	1/1	–	–	partly inf.
	KM 19	Pst I	1/2	2/2	2/2	–	–	partly inf.
6. J. Sz.	J3.11	Msp I	1/2	1/2	1/2	–	–	uninf.
	J3.11	Taq I	1/1	1/1	1/1	–	–	uninf.
	Met H	Taq I	1/1	1/1	1/1	–	–	uninf.
	Met D	Taq I	1/1	1/1	1/1	–	–	uninf.
	XV 2c	Taq I	1/1	1/2	1/1	–	–	partly inf.
	KM 19	Pst I	1/2	1/1	1/2	–	–	partly inf.
7. I. Cs.	J3.11	Taq I	1/1	1/1	1/1	–	–	uninf.
8. E. V.	J3.11	Taq I	1/1	1/1	1/1	–	–	uninf.
	KM 19	Pst I	1/2	1/2	2/2	–	–	inf.
9. T. M.	J3.11	Taq I	1/1	1/1	1/1	–	–	uninf.
	XV 2c	Taq I	1/2	1/2	1/1	–	–	inf.
	KM 19	Pst I	1/2	2/2	2/2	–	–	partly inf.
10. T. T.	XV 2c	Taq I	1/1	1/1	1/1	–	–	uninf.
	Met H	Taq I	2/2	1/2	1/2	–	–	partly inf.
11. P. L.	Met H	Taq I	2/2	2/2	2/2	–	–	uninf.
	Met H	Msp I	1/2	1/2	1/1	–	–	inf.
	J3.11	Msp I	1/2	1/2	1/2	–	–	uninf.
	XV 2c	Taq I	1/2	1/1	1/1	–	–	partly inf.

12. E.B.	XV 2c	Taq I	1/2	1/1	1/2	-	-	partly inf.
	Met H	Msp I	1/1	1/2	1/2	-	-	partly inf.
13. D. F.	Met H	Taq I	1/2	2/2	1/2	-	-	partly inf.
	XV 2c	Taq I	1/1	1/2	1/1	-	-	partly inf.
14. M.Gy.	J3.11	Msp I	1/2	1/2	1/2	-	-	uninf.
	Met H	Msp I	1/2	1/2	1/1	-	a - b	inf.
	Met H	Taq I	1/2	1/2	1/1	-	1/1 1/2	inf.
	XV 2c	Taq I	1/1	1/1	1/1	-	-	uninf.
	KM 19	Pst I	1/1	2/2	1/2		-	partly inf.
15. M. P.	Met H	Taq I	1/2	1/2	1/2	-	-	uninf.
	Met H	Msp I	2/2	1/2	1/2	-	-	partly inf.
	J3.11	Msp I	1/2	2/2	2/2	-	-	partly inf.
	XV 2c	Taq I	1/2	1/2	1/1	-	-	inf.
16. Sz. V.	KM 19	Pst I	1/2	1/2	2/2	-	-	inf.
	XV 2c	Taq I	1/2	1/2	2/2	-	-	inf.
	Met H	Taq I	1/2	1/2	1/2	-	-	uninf.

	Lengths of fragments 1 and 2			Allele frequencies for fragment 1 and 2 on CF and normal chromosomes	
1. J3.11	Taq I	1= 6 kb	2= 3.2 kb	1.0, 0.0	0.92, 0.08
2. J3.11	Msp I	1= 4.4 kb	2= 1.9 kb	0.44, 0.56	0.53, 0.47
3. [x]Met H	Taq I	1= 7 kb	2= 4.2 kb	0.73, 0.27	0.40, 0.60
4. [x]Met H	Msp I	1= 2.3 kb	2= 1.7 kb	0.80, 0.20	0.20, 0.80
5. Met D	Taq I	1= 5 kb	2= 4 kb	0.75, 0.25	0.75, 0.25
6. XV 2c	Taq I	1= 2.1 kb	2= 1.4 kb	0.85, 0.15	0.62, 0.38
7. [X]KM 19	Pst I	1= 7.8 kb	2= 6.6 kb	0.17, 0.83	0.67, 0.33

Probe with the closest association to the CF gene
[x] $p<0.05$, [X] $p<0.01$
informative: the probe shows unique picture for the CF child
partly informative: informative for only one parent
uninformative: the same picture for the CF affected child and its parents

absence or presence of the restriction sites. In cases where one or other probes were found to be informative in the family, we did not go on with another probe. 32 CF chromosomes of 16 affected children and 32 normal chromosomes of 32 compound heterozygote parents were surveyed for the linked haplotypes for parental CF chromosomes. Normal chromosomes of the parents were assigned by using the CF child genotype in each family. No significant difference was found in the frequencies of alleles 1 and 2 of J3.11 (Taq I 1.0, 0.0, Msp I 0.44, 0.56), Met D/0.75, 0.25) and XV-2c (0.85, 0.15) probes on the CF chromosomes as compared with those on the normal chromosomes of the parents. The number of CF allele associated with the longer fragment at the Met locus was nearly twice (Taq I) or four times (Msp I) as many as for the normal allele. The shorter fragment at the KM.19 locus was found to be two and a half times more frequent in the disease (CF) allele than in the normal allele (0.83 in CF versus 0.33 in normal $p < 0.01$).

Conversely: the other alleles associated more frequently with the normal chromosomes.

All but two CF families were fully informative by one probe or an appropriate combination of partly informative probes and polymorphisms. The informative probes presented unique allele picture for the CF affected child. The partly informative probes were informative for only one parent but not for the other one and the CF affected child. The uninformative probes presented the same allele picture for the CF affected child and its parents.

To the prenatal diagnosis each parent must carry a detectable CF allele. We performed 3 prenatal diagnosis on chorionic villus tissue (9-12 weeks after conception). Two fetuses were predicted by DNA analysis to be unaffected (although carriers) and one fetus to be affected. One of the two unaffected, but carrier children was born healthy, whereas the other and the affected one were twins and the parents decided on termination of the pregnancy.

The distribution of F_{508} deletion and other CF mutation on CF chromosomes in our 17 CF patients is found on Table II. Eight patients were homozygous for the F_{508} deletion, 6 heterozygous and 3 homozygous for absence of this deletion. The frequency of

Table II.

Distribution of CF chromosomes with F_{508} deletion and with other CF mutations in 17 CF patients

N°	Patients	Mutation of CF chromosomes	
		ΔF_{508}	other
1	M/34 (H.T.)	X	X
2	M/45 (M.Sz.)	X	X
3	M/50 (A.R.)	X	X.
4	M/157 (G.G.)	X	X
5	M/192 (G.F.)	X	X
6	M/199 (A.B.)	X	X
7	M/84 (E.V.)	X	–
8	M/87 (T.M.)	X	–
9	M/163 (G.G.)	X	–
10	M/164 (G.G.)	X	–
11	M/166 (N.Sz.)	X	–
12	M/172 (V.P.)	X	–
13	M/173 (A.H.)	X	–
14	M/185 (A.S.)	X	–
15	M/83 (F.B.)	–	X
16	M/148 (B.V.)	–	X
17	M/122 (A.T.)	–	X

The frequency of ΔF_{508} mutation on 34 CF chromosomes is 0.65 (of which 0.73 homozygous for this deletion).

ΔF_{508} mutation on 34 CF chromosomes of 17 CF affected child is 0.65.

As shown in Fig. 1 the PCR products of the mutant and normal alleles are clearly distinguished from each other in samples of heterozygotes or homozygotes for ΔF_{508} mutation. The first sample is homozygous for ΔF_{508} mutation shows single 47 bp long fragment. The second sample is heterozygous for the F_{508} deletion showing a 47 bp and a 50 bp long fragment and a

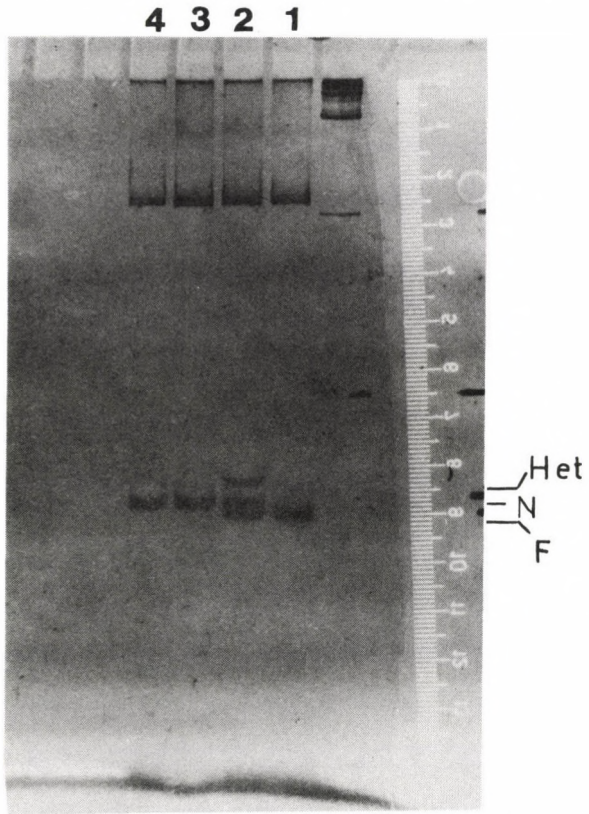

Fig. 1.

The PCR for ΔF_{508} was performed as described by Matthew et al. /17/. The two opposite genotypes 47 bp fragment of mutant /F/ and a 50 bp fragment of normal /N/ genes could be differentiated in 12% polyacrylamide gel stained ethidium bromide. A heteroduplex /Het/ with slower mobility in the gel originated from the normal and mutant sequences. The first sample is homozygous for the ΔF_{508} mutation, the second one heterozygous and the third and fourth samples have only normal sequences in this CF locus.

heteroduplex between the normal and mutant sequences with slower mobility in the gel. Those CF patients who carry the ΔF_{508} mutation only on one of their chromosome 7 and the other chromosome 7 carries a mutation elsewhere in the CF gene are indistinguishable by this simple analysis from the healthy compound heterozygotes for the ΔF_{508} mutation. The normal homozygotes (3^{rd} and 4^{th} sample) possess only 50 bp long fragments.

DISCUSSION

Our first analysis relied on an indirect approach that used DNA sequence polymorphisms and linkage analysis. The linkage disequilibrium means that a particular allele for the RFLP is found with a substantially increased frequency with the mutant gene than with the normal gene.

Our haplotype data show a strong allelic association between CF and allele 1 for the Met H locus and allele 2 for the KM.19 locus as compared with the normal chromosomes.

Conversely, the other alleles seemed to be associated more frequently with the normal chromosomes. There are differences in the CF and the normal haplotype distributions in the various geographic regions and ethnic backgrounds /3, 9, 11, 22, 27/. Our findings confirm the allele frequencies on CF chromosomes with J3.11/Msp I reported by Vitale et al (0.42, 0.58) /27/, with J3.11/ Taq I reported by Bates et al (0.92, 0.08) /3/, with Met H/Taq I reported by Bates et al (0.66, 0.34) /3/, Estivill et al (0.67, 0.33) /9/, but differ from data of Vitale et al (0.36, 0.64), Németi et al. /18/ Met H/Msp I allele frequencies of our CF patients confirm data of Bates et al (0.71, 0.29) but differ from results of Vitale et al (0.43, 0.57). The allele frequencies of our CF affected children with Met D/Taq I, XV-2c/Taq I are in agreement with those of Estivill et al (0.79, 0.21, 0.92, 0.08 respectively). The data with KM.19/Pst I confirm the ones of Santis et al (0.15, 0.85) /22/.

After the identification of linked DNA markers, a fundamental breakthrough in CF research was the localization of the CF gene. The cloning of the CF gene (approx. 250 kilobases (kb) long, its m-RNA is 6.5 kb) made possible the characterization of the mutations. It was found that a deletion of three nucleotides (CTT) in the central part of the first nucleotide (ATP) binding domain at position 508 results in omission of a phenylalanine from the cystic fibrosis transmembrane regulator (CFTR) protein (delta F_{508} mutation). The overall proportion of ΔF_{508} in a worldwide survey was 68% with a range of 40-80% /26/. In our patients 65% of CF chromosomes harbour this deletion.

At the diagnostic level analysis of the three base pair deletion will replace linkage analysis to a certain extent for appr. 50% of the couples when both parents are heterozygous for this deletion. In the remaining cases, the identification of the other mutations of CF gene and in some cases the closely linked markers would have role for marker information from proposites and parents.

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Acta Paediatrica Hungarica 32 (2), pp. 115-125 (1992)

IMMUNOGLOBULIN PROPHYLAXIS DURING INTENSIVE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

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Received 25 November 1991

60 children with acute lymphoblastic leukemia were sequentially randomized at the time of diagnosis: Immunoglobulin (Endobulin, Immuno) was administered intravenously to 30 patients at a dose 100 mg/kg/week during the first 3 months, followed by 2x200 mg/kg/month immunoglobulin during the 4., 5., 6. months. No immunoglobulin was administered to the control patients. We studied the effect of immunoglobulin prophylaxis on the number of days with fever, number of cases with bacteriologically proved infections, length and frequency of antibiotic therapy. Our data confirm the efficacy of immunoglobulin prophylaxis during the intensive phase of leukemia therapy in children.

INTRODUCTION

Intravenous immunoglobulin therapy has been proven to reduce the frequency of acute bacterial infections in patients with antibody deficiency diseases /1/, and recently it has been employed succesfully for the treatment of echovirus induced meningoencephalitis in an agammaglobulinemic patient /2/. Substitution therapy with IgG in patients with humoral immunodeficiency /3/ has been also attempted with promising results. In adults with CLL, immunoglobulin administration resulted in fewer bacterial infections in a randomized trial /4/. However, frequency and severity of acute or chronic infections do not seem to be always directly correlated with

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the extent and pattern of immunoglobulin deficiency in these patients /5/.

Due to the high morbidity and mortality caused by infections in leukemic children, prevention is of great importance. The aim of our study was to examine the effect of immunoglobulin prophylaxis in children with leukemia in a randomized trial.

PATIENTS AND METHODS

In our study 30-30 patients with acute lymphoblastic leukemia were subsequently randomized for prophylactic immunoglobulin administration. The patients in the experimental group received 100 mg/kg Endobulin i.v. per week for three months, followed by 2x200 mg/kg in the 4., 5., 6. months of treatment.

The age, sex and distribution for BFM risk factors were similar in the experimental group and in controls (Table I).

TABLE I

Patients' characteristics

	N°	Distribution of patients according to BFM		Sex		Age (year)
		Risk groups				(mean)
Experimental group	30	low	19	♀	11	6.3
		standard	6	♂	19	
		high	5			
Controls	30	low	18	♀	13	
		standard	8	♂	17	6.0

We examined the duration of initial aplastic phase during the treatment, the number of days with fever (higher than 38°C), the number of bacteriologically identified infections and the

number and duration of antibiotic treatments.

Student's T-test for paired samples was used for statistical comparison of the observations from the two groups of patients /6, 7/.

The immunoglobulin level in the blood was examined in 22 experimental and 21 control children by the method of Mancini /8/.

RESULTS

The duration of initial aplastic phase during the antileukemic treatment was similar in the experimental group and controls. The number of days with fever was only slightly less in the experimental group, however, the number of cacteriologically identified infections was significantly reduced (Table II). The number and duration of antibiotic treatments were lower in the experimental group during the immunoglobulin treatment (Table III).

The serum level of IgG increased in more patients and decreased in less children in the experimental group during the first 3 months of treatment. The change of the IgM and IgA serum level in the experimental and control group was not remarkably different (Table IV).

DISCUSSION

The great improvement in the survival in ALL is due to agressive multi-agent chemotherapy. However, treatment results are compromised by high occurrence of infections and related deaths during therapy. The high susceptibility of leukemic patients to different infections is caused partially by decreased immune response, due to the disease itself and to the intensive cytostatic/immunosuppressive treatment.

TABLE II

Infections and fever in patients with ALL during the first 12 months of therapy

	N°	Days with fever (mean/patient)	Microbiol. proved infections (mean/patient)	Unproved infections	Total N° of infections
Experimental group	30	11.1	0.53	1.63	2.16
Controls	30	12.0	1.13	1.73	2.86

TABLE III

Antibiotic treatment during the first 6 months of treatment

Endobulin	How frequently (mean/patient)	Duration of antibiotic treatment (days) (mean/patient)
Experimental group	3.6	27.2
Controls	4.16	31.63

Antibiotic treatment during the second 6 months of treatment

	How frequently (mean/patient)	Duration of antibiotic treatment (days) (mean/patient)
Experimental group	2.9	22.33
Controls	3.6	29.66

TABLE III/A

Distribution of diagnoses and aetiology of bacteriologically proved infections

	Experimental group (Endobulin treated)		Controls	
Infection	N°	Isolated pathogens	N°	Isolated pathogens
Septicemia	8	6 S. aureus 2 Klebsiella	12	7 Klebsiella 3 S. aureus 2 coagulose-negative staphylococci
Respiratory tract	5	5 S. aureus	14	5 S. aureus 2 coagulase-negative staphylococci 3 S. pneumoniae 3 Klebsiella 1 E. coli
Urinary tract	2	1 Enterobacter 1 P. mirabilis	7	3 E. coli 3 Klebsiella 1 P. aeruginosa
Skin	-	-	1	1 P. aeruginosa
Total	15		34	

TABLE III/B

Distribution of diagnoses of bacteriologically unproved infections

	Experimental group (Endobulin treated)	Controls
Infection	N°	N°
F.U.O.*	2	14
Respiratory tract	47	37
Abdominal	-	1
Total	49	52

* Fever of undetermined origin

TABLE IV

The change of immunoglobulin level during the first 3 months of ALL -treatment

	IgG		IgM		IgA	
	Controls	Experimental group	Controls	Experimental group	Controls	Experimental group
Increased	1	6	4	5	-	4
Unchanged	8	8	6	6	11	6
Decreased	13	7	12	10	11	11

N^{O} of Ig-untreated children: 22

N^{O} of Ig-treated children: 21

The more intensive therapy, the more prolonged are the agranulocytopenic intervals, which proved to correlate with the frequency of bacteriologically confirmed sepsis /9/. Several leukemia study groups administer pooled immunoglobulin preparations to decrease infections, however, its efficacy was not proved yet in children's ALL in randomised trials. In adult's chronic lymphatic leukemia, an American randomised cooperative study proved recently the efficacy of the immunoglobulin prophylaxis /4/.

Hartlapp and coworkers found also less infections in a randomised study of 56 adults with seminoma and 32 with bronchial carcinoma treated with immunoglobulin during chemotherapy /10/.

The studies described in children were done with unsufficient patient selection. Nishimura and Fujii /11/ found a good effect in septicaemia by the combination of antibiotics and immunoglobulin. The efficiency rate in their material was 71% against infections in children suffering from leukemia or a malignant tumor. The dose of immunoglobulin was 50-150 mg/kg 1-3x. However, it was not a randomised trial and the number of their patients was only 17. Matsumoto et al in leucopenic patients found a 50% efficacy rate, but it is also anecdotal, because the number of patients was only 12 /12/.

In our study the mean number of days with fever per patients and the mean number of bacteriologically unproved infections per patient showed no difference in the two groups, however,
the number of bacteriologically proved and total number of infections per patient was significantly lower in the immunoglobulin treated group. It is remarkable that control children got more frequently and more antibiotics, than the experimental group. The number of days with antibiotics during the first 6 months of treatment as well as during the 7.-12. months was slightly less in the group treated by immunoglobulin (27.2 versus 31.6; 22.3 versus 29.7).

The results of our randomised study demonstrate that the prophylactic use of immunoglobulin during the initial phase of antileukemic therapy in children results in a decrease in the number of infections.

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Acta Paediatrica Hungarica 32 (2), pp. 127-134 (1992)

SMITH-LEMLI-OPITZ SYNDROME IN SIBLINGS

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Received 31 October 1991

This paper presents two brothers with incomplete expression of the Smith-Lemli-Opitz syndrome.

A definite diagnosis, made after the birth of the second child, could only be reached when the clinical features of both patients were combined.

INTRODUCTION

The Smith-Lemli-Opitz (SLO) syndrome, first described in 1964 by Smith et al /12/ is an autosomal recessive condition characterized by a combination of severe psychomotor retardation and failure to thrive, microcephaly, hand and foot anomalies, typical facial appearance and genito-urinary abnormalities /5/.

In the absence of a specific marker, the diagnosis of this relatively common syndrome /8/, still entirely depends on its clinical manifestations /6, 10/.

Therefore, in the case of incomplete expression of the SLO-syndrome, the establishment of the correct diagnosis can become virtually impossible and hence genetic counseling hazardous. Here we report on two affected siblings with a diagnosis only becoming apparent after the birth of the second child.

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CLINICAL REPORTS

Patient 1. M.R., a 22-month-old boy was referred to evaluate his severe psychomotor retardation and for possible genetic counseling. The nonconsanguineous parents were healthy and under 30 years of age.

He resulted from his mother's second, uneventful pregnancy (the first pregnancy ending in a spontaneous abortion), terminated at 41. week with a Caesarian Section due to breech presentation. Weight at birth was 3100 gram. The infant needed intensive care in the perinatal unit because of severe neonatal apnea.

Feeding problems were still present after two weeks, when he was discharged.

At the age of nine months his condition was evaluated at several referral centres. An atrial septum defect (type II) was diagnosed, and further his discharge papers mention severe muscle hypotonia, a head circumference well below the tenth percentile (44 cm), a normal EEG, while an EMG and muscle biopsy showed signs of myopathy.

At the time of admission he was unable to sit or stand, there was a generalized muscle hypotonia, a left N. abducens paresis, normal reflexes difficult to elicit, without abnormal reflexes being detected.

Mild micrognathia, dyscrania, a retractile testis on the right side, with an inguinal testis on the other side were noted as well. A screening laboratory blood and urine work-up was negative, his karyotype appeared normal.

With no other anomalies detected his condition was designated cerebral palsy with psychomotor retardation due to perinatal hypoxia. Genetic advice to the parents was given accordingly.

Patient 2. M.B., the one-month-old brother of the propositus was referred to us for evaluation of his underdeveloped genitals. He was born after forcipal extraction, following an uneventful full-term gestation, weighing 3240 gram, Apgar score: 9/10. From birth on he had marked muscle hypotonia, feeding difficulties ensued and he had to be nourished by naso-gastric tube.

At the age of one month his height (53 cm) was on the 25th percentile, the weight (3650 gram) just under this percentile, and his head circumference (35 cm) below the 10th percentile. The skull was asymmetric, with a broad nasal bridge, open metopic suture and ridging of the sagittal suture. Anteversion of the nose, micrognathia, small shoulders, a sacral dimple and hypertelorism of the mamillae were noted.

On auscultation a 3/6 systolic murmur was heard, but an echocardiogram - made two days after birth - revealed no abnormalities. The liver extended 3 cm below the costal margin. Besides a conspicuously small penis (13 mm), there was bilateral cryptorchidism with a hypoplastic scrotum.

Routine urine examination revealed a urinary tract infection with E. Coli. Renal ultrasound disclosed no abnormalities.

TABLE I

Characteristics of patient 1 and 2, compared with reported frequency of clinical manifestation in SLO-syndromei.

	Pt.1	Pt.2	literature %
Intra-uterine			
decreased fetal movements	/	/	14*
birth weight less than 2500 g	-	-	33
prematurity less than 38 weeks	-	-	24*
breech presentation	+	-	13*
Post-natal			
feeding difficulties	+	+	90*
failure to thrive	+	+	92*
Head			
microcephaly	+	+	95
dyscrania	+	+	40
Facies			
Eyes			
epicanthic folds (1)	+	-	40
blepharoptosis (2)	+		64*
strabismus (3)	+	-	40
miscellaneous	-	-	10
Nose			
broad nasal bridge (4)	+	+	50
anteverted nares (5)	+	+	75
Mouth			
gothic palate (6)	-	-	55*
cleft palate or uvula (7)	+	-	40*
wide alveolar ridges (8)	-	-	60*
Ears			
low set or abnormal rotation (9)	-	-	33*
abnormal shape or size (10)	-	-	51*
Mandible			
Micrognathia (11)	+	+	80
Facies suggestive for SLO			
5 or more of the 11 facial manifestations	+	-	85*
Genito-urinary			
hypospadiasis	-	-	60*
cryptorchidism	+	+	50
microphallus	-	+	20

	Pt.1	Pt.2	literature %
Limbs			
post-axial polydactyly	-	-	25
soft-tissue syndactyly	-	-	65
Central Nervous System			
severe mental retardation	+	/	±100*
hypotonia/hypertonia	+/-	+/-	53/25*
EEG abnormalities	+	/	82*
Heart			
congenital heart disease	+	/	20
Chest			
narrow shoulders	+	+	occ.
inter-mammillary index ↑	-	+	occ.
Skeletal deformities			
coxa valga	+	-	occ
wedge shaped vertebrae	+	-	
Skin			
sacral dimple	+	+	occ.

1 frequency reported by R. Gorlin in 1990
* based on frequency reported in 1974 by V.P. Johnson
+ = present
- = absent
/ = not assessed

Repeated bloodtests showed transient elevation of liver-enzymes (SGOT 111 U/l, SGPT 79 U/l, γGT 81 U/l), total serum bilirubin 29.1 μmol/l, with a direct fraction of 23.8 μmol/l. WBC: 44.000 cells/cu mm, nucleated red cells: 8.800 cells/cu mm. FSH: 1.8 mE/ml, LH: 9,6 mE/ml. Testosterone 0.8 nmol/l, after HCG-stimulation 2.3 nmol/l. The result of a screen for viral antibodies (Herpes, CMV and Rubella) was negative. Karyotype was normal.

Since an unequivocal diagnosis could not be made, his then 4-year-old brother (patient 1) was also admitted. In Table I the above findings as well as newly found minor anomalies are summarized and compared with reported frequencies /4, 5/ of minor and major anomalies in the SLO-syndrome.

DISCUSSION

The diagnosis of multiple congenital anomalies syndromes that have no specific markers always carries some degree of subjectivity /11/, well illustrated by our, somewhat unusual, later detailed way of establishing the correct diagnosis.

When patient 1 was first seen, he showed three of the five characteristics of the Smith-Lemli-Opitz syndrome: microcephaly, severe psychomotor retardation with failure to thrive and mild genito-urinary abnormalities.

Some of the manifestations that now make his facial appearance typical for the SLO-syndrome had not yet completely developed (ptosis, epicanthic folds) or escaped clinical detection (uvula bifida, broad nasal bridge, anteverted nares).

However, at the age of 22-months his clinical presentation was certainly not enough for the diagnosis of the SLO-syndrome, with its far reaching consequences regarding genetic counseling.

Patient 2, with hypogonadism and floppyness as characteristics, could not fulfill the above mentioned criteria for the SLO-syndrome either. At the age of one month it was too early to form an opinion about his psychomotor development, and his facial appearance was at least not yet suggestive for the SLO-syndrome. This gave him only 2 out of the 5 possible criteria.

As none of these criteria is obligate or pathognomonic, the total clinical picture, resulting from a combination of these

criteria should be the basis for the diagnosis /5/.

By combining the abnormalities found in the two brothers, we overcome the hesitation in the interpretation of minor dysmorphic signs /9/ (their appearance in both brothers is much more significant) and at the same time we gain criteria for the diagnosis: we now have a clinical picture at hand that is very suggestive for the SLO-syndrome, with four of the five criteria being fulfilled. Considering the wide phenotypic variability of the SLO-syndrome /11/ we regard our approach as justifiable.

Furthermore, we can add one additional, hitherto unreported anomaly to the long list of reported anomalies: wedge shaped lumbar vertebrae in patient 1.

Although their clinical presentation much differed (severe mental retardation versus hypogonadism), the two brothers had a similar, albeit incomplete expression of the SLO syndrome gene. On the SLO severity score /1/, devised by Bialer et al, patient 1 would score 5, his brother (at this moment without complete evaluation of his cardiac condition) 2 points out of the maximum of 19 points.

This finding gives further support for the observation of consistency between affected sibs /1, 7/.

But if overall degree of severity is positively correlated with the degree of feminization of male genitals, polydactyly and cleft palate, as is suggested in the same article, our sibs cannot confirm this finding.

Patient 1 has a cleft uvula, patient 2 does not; Patient 2's genitals are far more feminized than the one's of patient 2.

The siblings presented in the article of Johnson /5/ (SLO score 3 resp. 2, with polydactyly causing the score difference) show marked familial concordance, while differing in polydactyly. Similar differences can be observed in the siblings reported by Blair et al /2/ and Curry et al /3/.

It would be interesting to know whether these three phenotypic correlations, established in the group of all 122 published patients, could also be found in the group of siblings, and whether the siblings were in general concordant for cleft

palate, polydactyly and degree of feminization of genitals, as would be expected, given the consistency between affected sibs. A syndrome with a diagnosis entirely depending on its clinical picture and without pathognomonic features will always be at risk of being too often identified, or in the words of Winter ".....this is a much overdiagnosed syndrome." /13/

Regarding current preference for small families, however, our siblings clearly illustrate that the risk of underdiagnosing the SLO-syndrome, with its autosomal recessive inheritance and wide phenotypic variability, is as present as ever.

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Acta Paediatrica Hungarica 32 (2), pp. 135-148 (1992)

SEVERE HYPOGLYCAEMIA AND CARDIOVASCULAR AUTONOMIC DYSFUNCTION IN TYPE I DIABETIC CHILDREN TREATED WITH INTENSIFIED CONVENTIONAL INSULIN THERAPY

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Received 18 July 1991

To assess the relationship between severe hypoglycaemias and autonomic dysfunction, five cardiovascular tests (resting heart rate, hyperventilatory arrhythmia, standing/lying heart rate ratio, orthostatic decrease in blood pressure, and increase in blood pressure during sustained handgrip) were performed in a 1-yr prospective study of 34 insulin-dependent diabetic children treated with intensified conventional insulin therapy (ICIT). There were twelve severe episodes in 7 diabetic children, and the remaining 27 patients had no severe hypoglycaemia. The hypoglycaemic group had a longer duration of diabetes than the nonhypoglycaemic group (5.4 SD 2.5 years vs. 2.8 SD 2.2 years, $p<0.02$). The hyperventilatory arrhythmia in the hypoglycaemic group in comparison with the nonhypoglycaemic group was significantly decreased (before ICIT: 16.1 SD 3/min vs. 24.4 SD 5/min, $p<0.01$; 1 yr thereafter: 17.3 SD 3/min vs. 26.0 SD 5/min, $p<0.01$). The hypoglycaemic group showed a pronounced orthostatic decrease in blood pressure compared to the nonhypoglycaemic group (before ICIT: 13.2 SD 4 mmHg vs. 6.0 SD 4 mmHg, $p<0.01$; 1 yr thereafter: 12.3 SD 4 mmHg vs. 5.6 SD 4 mmHg, $p<0.01$). Three or more abnormal cardiovascular test results were found in patients of the hypoglycaemic group who showed abnormal hyperventilatory arrhythmia and abnormal orthostatic decrease in blood pressure simultaneously, whereas such a coexistence was not found in the nonhypoglycaemic group. These observations may support the view that diabetic children and adolescents with autonomic dysfunction are susceptible to severe hypoglycaemia during ICIT.

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INTRODUCTION

Considerable evidence has accumulated that the frequency of complications of diabetes is associated with the degree of metabolic disturbance, primarily hyperglycaemia /1, 2, 3/. On the basis of this, during the treatment and care of diabetes the main purpose is to achieve the best metabolic control as well as the long-term assurance of normoglycaemia. It is well-known that conventional (twice a day) insulin therapy fails to restore in diabetic patients the finely regulated blood glucose levels of nondiabetic individuals. In the pediatric practice, out of recent applied new therapeutic insulin delivery systems, the intensified conventional insulin therapy (ICIT) is current for treatment of children with insulin-dependent diabetes mellitus. According to the majority of authors, the patients' metabolic condition using this method of treatment can be improved /4, 5, 6/.

With the continuing application of ICIT, the question has been raised about an increased risk of hypoglycaemic episodes as a result of the efforts to achieve normoglycaemia in diabetic children. The frequency and severity of hypoglycaemia seem to be increased in those patient populations who attempt to achieve metabolic normality /7/. One assumption is that impaired glucose counterregulation may be responsible for increased risk of hypoglycaemia (8, 9/. On the other hand, an association between impaired defense mechanisms against hypoglycaemia and dysfunction of autonomic system has been suggested /10, 11, 12, 13, 14/. However, in this respect, the clinical importance of disturbed autonomic function in childhood diabetes is not yet clear.

The aim of our present study was to investigate the relationship between serious hypoglycaemias and autonomic nervous system dysfunction in diabetic children treated with ICIT studied prospectively. With this purpose, we included only those episodes of severe hypoglycaemia that caused loss of consciousness or convulsions and required outside assistance.

PATIENTS AND METHODS

Patients

Thirty-four children with insulin-dependent diabetes mellitus who had been treated with ICIT for more than 1 year were considered for inclusion in this study. This represents more than 25% of the diabetic children seen in our clinic during this time. All patients have received two daily injections of highly purified porcine insulin (conventional insulin therapy with Actrapid MC and Monotard MC, Novo-Nordisk, Copenhagen, Denmark) since the onset of diabetes. Intensified conventional insulin therapy was started in this study population after an average duration of diabetes of 3.5 SD 2.5 years. After the institution of ICIT each child received semisynthetic human insulin (Novo-Nordisk, Copenhagen, Denmark) injections four times a day: three times Actrapid HM Penfill 30 minutes before meals by means of NovoPen, and Ultratard HM hypodermic injection at 10 P.M. Patients and their families received instruction in the management of their diabetes by a physician, a nurse, and a dietitian. Patients followed their usual diabetic diet, which consisted of 25% fat, 15% protein, and 60% carbohydrate given as three meals and three snacks. Patients were encouraged to test their blood glucose levels with reagent strips and reflectance meters two or three times daily (before breakfast and supper and at bedtime). Each child was taught to adjust the short-acting insulin dose according to the blood glucose concentration before the insulin injection. Close follow-up was performed at our clinic at 2-mo intervals. In cases of intercurrent illnesses or any problem, children were seen as often as once a week. In addition, they were requested to come to the clinic or contact the physician immediately in the event of severe hypoglycaemia. There were no clinical symptoms or anamnestic data indicating vascular complications or manifest neuropathy in any of the children.

Study design

The study was conducted prospectively over a 1-yr period. At each clinic visit, a detailed information was obtained for the children and their parents on any episode of severe hypoglycaemia that occurred in the interval since the last visit, in case this was not reported immediately after the episode. Children with one or more episodes of severe hypoglycaemia during the study period were compared to nonhypoglycaemic population. Comparisons between the two groups were made by comparing sex, age, duration of diabetes, fructosamine levels determined at each clinic visit, insulin doses, insulin antibody bindings and autonomic function tests. Informed consent was obtained from all subjects and their parents before the study.

Autonomic function tests

Five cardiovascular tests were performed before the initiation of ICIT and 1 yr thereafter. Each diabetic child was examined - in the lying position - for the mean resting heart rate for a period of 1 min, for hyperventilatory arrhythmia (beat-to-beat variation) during five maximal inspirations and expirations, for increase in heart rate and fall in systolic blood pressure during 1 min standing after the lying position, and for rise in diastolic blood pressure after a 1-min sustained maximal handgrip /15, 16, 17/. Heart rates were determined with a routine ECG device (Medicor ER 31-A, Budapest, Hungary) by R-R intervals in standard leads, for blood pressure measurements a digital blood pressure device (Omron HEM-400 C, Tokyo, Japan) was used.

To obtain a reference range of cardiovascular tests, 30 healthy age-matched children were used as control group. Confidence limits of 95% (2SD) from means of control subject were considered as limits of normality. If the deviation from the mean in the control group exceeded 2SD, the values of the diabetic children were considered as abnormal /18/.

Analytic methods

Serum fructosamine was mesured by the Roche Fructosamine kit (Roche, Basle, Switzerland). The mean and SD for nondiabetic children was 2.0 SD 0.3 mmol/l. Fasting serum C-peptide concentration was determined by the Mallincrodt kit (RIA-mat C-peptide, Dietzenbach, FRG). The normal fasting value is 0.17-0.99 mmol/l. Insulin antibody binding was estimated by a radiological method (Isotest, Budapest, Hungary,). The upper limit for healthy subjects was below 20%.

Statistical analysis

Data and results are expressed as mean and SD. Data were tested by Student's paired and unpaired t-tests or by Wilcoxon signed rank test and Mann-Whitney rank sum test when data were not normally distributed. The frequency of cases with the nonhypoglycaemic group was assessed by chi-square test with Yates correction.

RESULTS

Twelve hypoglycaemic episodes were recorded in 7 patients (hypoglycaemic group) and 27 children had no hypoglycaemia (nonhypoglycaemic group) during a 1-yr period of ICIT (Table I). Out of the 7 hypoglycaemic patients, one child had 3 hypoglycaemic episodes, three children had 2 hypoglycaemic episodes, and three patients had 1 hypoglycaemic event. There

TABLE I

Clinical data of diabetic children studied (mean ± SD)

	Diabetic children nonhypoglycaemic group	hypoglycaemic group	Total patients
n	27	7	34
Hypoglycaemia (events yr^{-1})	0	≥ 1	0.35 ± 0.3
Gender (M/F)	12/15	3/4	15/19
Age (years)	13.2 ± 3.2	13.5 ± 4.0	13.4 ± 3.5
Diabetes duration at the institution of ICIT (years)	2.8 ± 2.2	5.4 ± 2.5*	3.5 ± 2.5
Basal C-peptide (nmol l^{-1})	< 0.1	< 0.1	< 0.1
Insulin antibody binding (%)	7.5 ± 5.0	8.7 ± 5.8	8.3 ± 5.2

ICIT: intensified conventional insulin therapy
* : $p < 0.02$

were no significant differences in respect of sex distribution, age and insulin antibody binding between the two diabetic groups. At the time of institution of ICIT the hypoglycaemic group had a significantly longer duration of diabetes than the nonhypoglycaemic group (5.4 SD 2.5 years vs. 2.8 SD 2.2 years, $p<0.02$). All patients in the two groups had no detectable basal C-peptide secretion. After the initiation of ICIT a significant improvement of metabolic control was registered in both of two diabetic groups. Fructosamine concentrations and the mean daily insulin doses were similar in the two groups throughout the study period (Fig. 1). There was no difference in the average

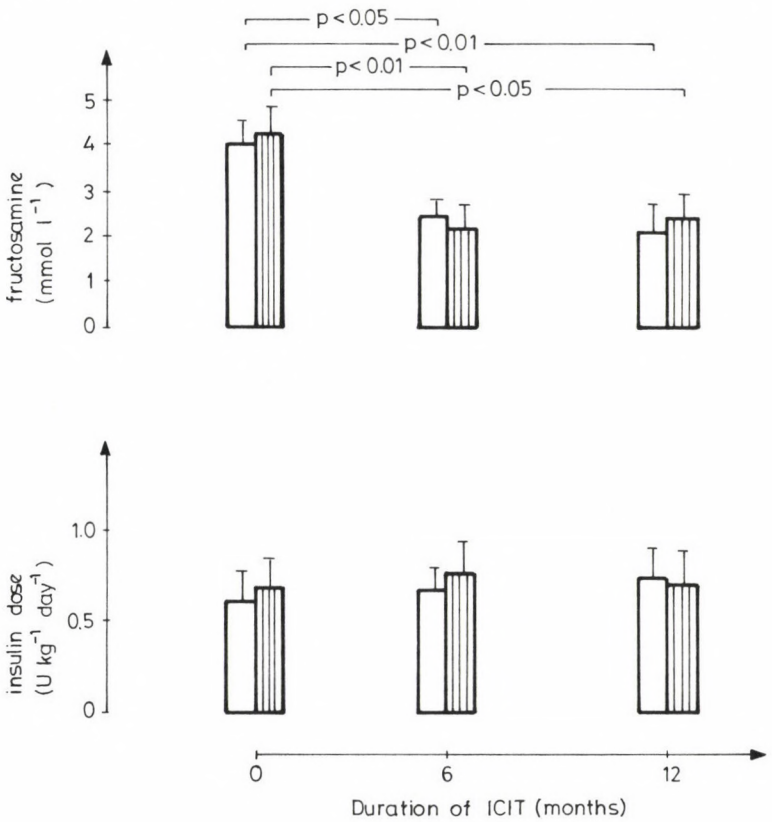

Fig. 1. Fructosamine levels and insulin doses in nonhypoglycaemic (□) and hypoglycaemic (▨) diabetic children during intensified conventional insulin therpy (ICIT)

frequency of blood glucose measurement between the two groups (2.5 SD 0.3 estimations/day vs. 2.3 SD 0.4 estimations/day).

Results of five cardiovascular tests obtained at 1-yr interval did not differ significantly. The individual values for hyperventilatory arrhythmia and orthostatic decrease in blood pressure in the two groups of diabetic children are shown in Figure 2. The hypoglycaemic group showed a significantly decreased hyperventilatory arrhythmia (before ICIT: 16.1 SD 3/min vs. 24.4 SD 5/min, $p<0.01$; 1 yr thereafter: 17.3 SD 3/min vs. 26.0 SD 5/min, $p<0.01$) and a pronounced orthostatic decrease in blood pressure (before ICIT: 13.2 SD 4 mmHg vs. 6.0 SD 4 mmHg, $p<0.01$; 1 yr thereafter: 12.3 SD 4 mmHg vs. 5.6 SD 4 mmHg $p<0.01$) compared to the nonhypoglycaemic group. There were no significant differences in the average values of

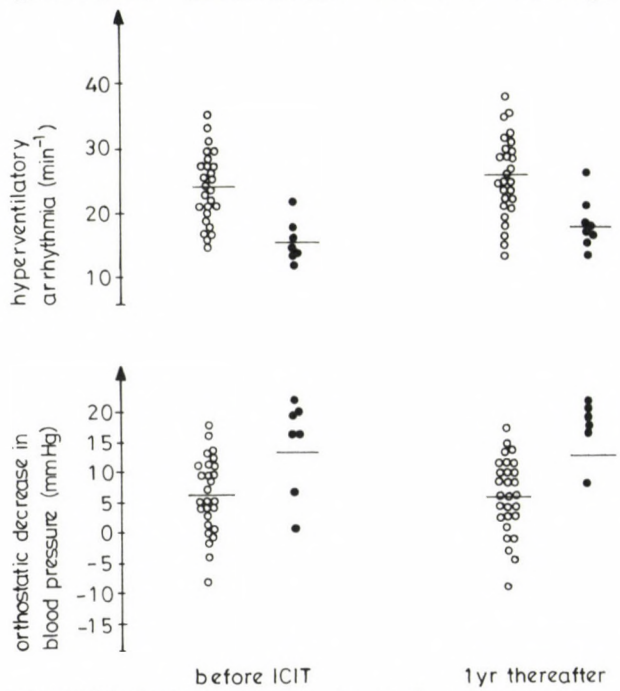

Fig. 2. Hyperventilatory arrhythmia and orthostatic decrease in blood pressure in nonhypoglycaemic (○) and hypoglycaemic (●) diabetic children during intensified conventional insulin therapy (ICIT). (Horizontal bars represent mean values; hypoglycaemic vs nonhypoglycaemic: $p < 0.01$)

resting heart rate, standing/lying heart rate ratio, and increase in blood pressure during sustained handgrip when comparing hypoglycaemic group with nonhypoglycaemic group (data are not shown).

Abnormal cardiovascular tests were found in 6 of seven hypoglycaemic children and 13 of twenty-seven nonhypoglycaemic patients. Three or more abnormal test results were found in 5 patients of the hypoglycaemic group and 1 of the nonhypoglycaemic group. All the five hypoglycaemic patients having 3 or more abnormal tests showed abnormal hyperventilatory arrhythmia and abnormal orthostatic decrease in blood pressure simultaneously. Coexistent abnormalities in these two parameters were not shown in the nonhypoglycaemic group (5/7 vs. 0/27, $p<0.001$).

DISCUSSION

In our 1-yr prospective study, ICIT provides an optimized glycaemic control in insulindependent diabetic children. Although fructosamine levels approached normal in all patients, it tended to remain in the upper normal range. Almost 20% (7/34) of this study population showed 1 or more severe hypoglycaemic episodes in a single year. This hypoglycaemic group had a longer duration of diabetes at the time of initiation of ICIT, and a more expressed cardiovascular autonomic dysfunction than the nonhypoglycaemic group.

The incidence of severe hypoglycaemia in insulin dependent diabetes mellitus is controversal. Goldstein et al. /19/ documented that the severity of hypoglycaemic episodes was related to the degree of blood glucose control. In the first 12 months of the Diabetes Control and Complications Trial, patients receiving intensive insulin therapy reported a threefold increase in severe hypoglycaemic events compared with

those patients receiving conventional therapy /7/. Furthermore, results from this diabetes trial suggest a relationship between the occurrence of severe hypoglycaemia and disease duration. Muhlhauser et al /20/ and Schiffrin et al /6, 21/ did not find a higher incidence of severe hypoglycaemia in intensively treated than in conventionally treated diabetic subjects. According to Canadian authors /22/ severe hypoglycaemia in diabetic children does not appear to be related to long-term blood glucose control. In our present study overzealous attempts to achieve normoglycaemia as cause of severe hypoglycaemia can be excluded because long-term control and insulin dosages did not differ in those with and those without severe hypoglycaemia. However, the occurrence of severe hypoglycaemia in our patient population treated with ICIT seems to be not frequent if we take into account that in a recent study by Daneman et al /23/, 31% of 311 children treated conventionally were found to have had at least one severe hypoglycaemic episode. Furthermore, some other studies on adult patients with less optimal blood glucose control reported a similarly high frequency of severe hypoglycaemia per year /24, 25, 26/. Until recently, hypoglycaemia was considered as an acute event of inadequate diabetes management with no long-term consequences. However, recent studies document that irreversible brain damage or intellectual impairment may be the consequence of severe hypoglycaemia /27, 28, 29, 30/.

Neurological complications of diabetes mellitus have been well-known for a long time, including dysfunction of the peripheral (somatic) and autonomic nervous system. Neurological complications in the subclinical stage appear initially as reversible functional disturbances, but later, the clinical symptoms are irreversible having poor prognostic consequences /15/. Clinically, diabetic autonomic neuropathy can manifest as gastrointestinal, genitourinary, and cardiovascular symptoms, but it may cause troubles also in pupillary reflex response to light, secretory function of sweat glands, and in the physiologic defense mechanisms against hypoglycaemia /10, 14/. There are two components of the hypoglycaemia response affected by the autonomic system in both the normal and diabetic

individual /31/. The first is the complex of clinical signs of declining blood sugar level, resulting from catecholamine release and the typical adrenergic symptoms. This phenomenon is known as hypoglycaemic awareness. The second component to the physiologic response to hypoglycaemia is biochemical in nature. With the decrease of blood glucose concentration to abnormally low levels, there is normally an increase in counterregulatory hormone (glucagon and epinephrine) secretion. This process is referred to as hypoglycaemic responsiveness. It is known that patients with insulin-dependent diabetes may lose either their hypoglycaemic awareness, hypoglycaemic responsiveness, or both /8, 9, 32/. The mechanism for the loss of hypoglycaemic awareness and responsiveness is unclear. There is some evidence to suggest that central (hypothalamic) activation of counterregulation may be diminished in insulin-dependent diabetes /33/. Other studies suggest that a reduction in sensitivity of peripheral beta-adrenergic receptors is a factor causing loss of symptoms of hypoglycaemia /34/. It is possible that the induction of tight blood glucose control using intensified insulin therapy lowers the blood glucose threshold for the release of counterregulatory hormones in response to hypoglycaemia /35/, nevertheless, an association with increased insulin antibody binding has also been proposed /9/. Many data suggest that hypoglycaemia unawareness and unresponsiveness may be due to disturbed function of autonomic nervous system since parasympathetic and sympathetic innervation play role in glucagon and epinephrine secretion during hypoglycaemia /10, 11, 12, 13, 14, 36/. Recently, concern has arisen that human (as opposed to beef or pork) insulin may cause more frequent and/or severe hypoglycaemia in association with reduced warning symptoms /40/. In our prospective study it was not aimed to analyse the difference in frequency of hypoglycaemia between conventionally and intensively treated patients (e.g. animal vs. human insulin periods), because of only retrospective data have been available on hypoglycaemia occurrence during the conventional treatment period. In spite of this, our results do not support the above mentioned concept, since there was no difference between hypoglycaemic and nonhypoglycaemic groups

concerning type of insulin used before ICIT period. A very recent study also concluded that human insulin use is not associated with either increased frequency of hypoglycaemia or reduction in awareness of hypoglycaemia /41/.

According to previous studies, some extent of autonomic nervous system dysfunction may appear relatively early during the course of childhood diabetes /18/, and subclinical signs of autonomic neuropathy are demonstrable in a relatively considerable proportion of diabetic children and adolescents /37, 38, 39/. Our present study suggests that autonomic dysfunction may play a role in the development of some severe hypoglycaemias in diabetic children treated with ICIT, especially, if the institution of this regimen happens after several years of diabetes duration. Nevertheless, further studies are necessary to prove the possible causal relationship between autonomic dysfunction and impaired defense mechanisms against hypoglycaemia in diabetic children.

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Acta Paediatrica Hungarica 32 (2), pp. 149-157 (1992)

SEX HORMONE BINDING GLOBULIN (SHBG) IN CHILDREN WITH OBESITY

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Received 27 September 1991

Serum sex hormone binding globulin (SHBG) concentration of children with obesity was measured and relationships between SHBG level and body mass index (BMI), waist hip ration (WHR), serum insulin, C-peptide, thyroid hormones (thyroxine - T_4, triiodothyronine - T_3/ sexual hormones (testosterone - T, oestradiol - E_2) were investigated.

Significant negative correlations were found between SHBG concentration and BMI, serum insulin, C-peptide concentration; significant positive concentrations were found between BMI and serum insulin, C-peptide concentration. Thyroid hormone and sexual hormones did not associate with SHBG levels.

These results suggest that insulin hypersecretion has an important role in determining the reduction of SHBG production in obesity.

INTRODUCTION

Sex-hormone binding globulin (SHBG) is a circulating glycoprotein with a molecular weight of around 100.000 daltons. SHBG is thought to be synthetized in the liver; in the circulation its biological function is the transport of sex-steroid hormones. SHBG bind testosterone (T) and oestradiol (E_2) with a high affinity and this binding is considered to be an important factor in determining the free, active concentrations of these hormones /3/. SHBG levels themselves are thought to be regulated by the concentration of androgens and oestrogens; the former inhibit, while the latter stimulate SHBG production /1, 3/. Thyroid hormones are also known

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to stimulate SHBG production: increased concentration of SHBG is seen in hyperthyroidism, while hypothyroidism is associated with decreased SHBG levels /18/. A recent investigation found that insulin inhibits SHBG production in a human hepatoma cell line, suggesting an alternative regulating mechanism in SHBG production which is dependent on insulin levels /17/.

Obesity is commonly associated with low serum SHBG concentration in both adult- and childhood /4, 6, 7, 14/. Although a significant negative correlation exists between serum SHBG and body weight /4/, the mechanisms involved are unknown at present. In this study, therefore, our aim was to define the factors determining serum SHBG concentration in childhood obesity.

PATIENTS AND METHODS

Thirty-nine obese children (15 girls and 24 boys) were considered in this study. Their age was 10.0 - 14.5 years (girls 13.1 $\pm$ 2.1, boys 12.8 $\pm$ 2.7). None had diabetes, other endocrinopathies or systemic diseases. None of them was taking any medicament. All girls had a normal menstrual history since their menarche, and all boys had secondary signs of puberty (P2 - P5). Body mass index (BMI: body weight, kg divided for height, m^2) was calculated, the waist and hip girth were taken and their ratio (WHR) was also calculated to define the body fat distribution.

A single blood sample for hormonal determinations was obtained in the morning after an overnight fast. Serum SHBG was measured by the immunoradiometric assay (IRMA) of Farmos G. Ltd. Serum insulin and C-peptide determined by radioimmunoassay (RIA) kits supplied by Izinta and Serono. Serum thyroxin and triiodothyronine (T_4 and T_3) were measured by RIA methods developed in our university. Serum T and E_2 were determined by dissociation enhanced lanthanide fluoroimmunoassay (DELPHIA) purchased from Pharmacia. All assays were performed in duplicate.

Statistical analyses were done with CBM computer programme.

RESULTS

BMI and WHR values, fasting SHBG concentrations and the results of hormone measurements are demonstrated on Table I. BMI was slightly higher in the subgroup of the girls, whereas WHR was higher in the subgroup of the boys; the differences were significant ($p < 0.05$). Serum SHBG concentration did not differ significantly in the two subgroups, and no significant differences in hormone results were found between the two sexes. Therefore, the relationships between serum SHBG concentrations and other parameters were investigated in the united group.

Relationships between serum SHBG concentration and BMI, WHR values and hormone results are shown in Table II; the correlation coefficients are demonstrated. A significant negative correlation was found between BMI values and serum SHBG concentrations ($r = 0.46$, $p < 0.01$), WHR values and serum SHBG concentrations did not correlate. Negative correlations were found between serum insulin and SHBG concentrations, furthermore between serum C-peptide and SHBG concentrations ($r = -0.33$ and $r = -0.34$; $p < 0.05$).

No relationships were found between serum SHBG and T_3, T_4 concentrations and, similarly, serum SHBG concentrations were not associated with T and E_2 levels. It is also demonstrated in Table II that strong correlations were obtained between BMI values and serum insulin levels, moreover, between BMI values and serum C-peptide concentrations ($r = 0.55$ and $r = 0.54$; $p < 0.01$).

DISCUSSION

Obesity both in childhood and in adulthood, is often associated with a decreased circulating SHBG concentration /4, 6, 7, 14/. In this study there was not any opportunity to compare the SHBG concentrations of obese children to the results obtained in the age matched healthy controls. In a

TABLE I

BMI and WHR values, results of SHBG and hormone measurements
(X ± SD)

Parameters	Girls (n = 15)	Boys (n = 24)	Girls and boys (n = 39)
BMI (kg/m^2)	33.87 ± 4.82$^+$	26.67 ± 4.33$^+$	31.28 ± 4.92
WHR	0.90 ± 0.08$^+$	0.96 ± 0.07$^+$	0.94 ± 0.08
SHBG (nmol/l)	16.97 ± 7.60	21.48 ± 17.36	19.75 ± 14.45
Insulin (u/U /ml)	38.70 ± 33.41	28.42 ± 25.86	32.19 ± 28.87
C-peptide (ng/dl)	4.34 ± 4.09	3.46 ± 2.73	3.80 ± 3.29
T_4 (nmol/l)	111.5 ± 18.8	126.0 ± 24.3	121.3 ± 23.7
T_3 (nmol/l)	2.5 ± 0.46	2.70 ± 0.47	2.63 ± 0.47
E_2 (pg/ml)	79.08 ± 65.63 (n = 12)	-	-
T (pg/ml)	-	692.4 ± 515.2 (n = 13)	-

$^+$ $p < 0.05$ (Student T-test)

TABLE II

Correlations between BMI, WHR values, hormone results and SHBG concentrations

Correlations	r	n	P
BMI - SHBG	-0.46	39	< 0.01
WHR - SHBG	-0.08	39	NS
Insulin - SHBG	-0.33	39	< 0.05
C-peptide - SHBG	-0.34	39	< 0.05
T_4 - SHBG	0.24	39	NS
T_3 - SHBG	0.23	39	NS
T - SHBG	0.14	13	NS
E_2 -SHBG	0.12	12	NS
BMI - Insulin	0.55	39	< 0.01
BMI - C-peptide	0.54	39	< 0.01
WHR - Insulin	0.09	39	NS
WHR - C-peptide	0.07	39	NS

r = correlation coefficient
n = number of cases
p = strength of significance
NS = non significant

previous study SHBG concentration of healthy 11-14 year-old children was found to be 60 ± 6 nmol/ml, and 40.4 ± 11.8 nmol/ml values were measured in a group of normal adults /11, 14/. Therefore, our results are in accordance with data showing decreased SHBG concentrations in childhood obesity.

In the present study it was found that serum SHBG concentrations were negatively correlated with BMI values indicating the degree of obesity. Similar association between the degree of obesity and circulating SHBG levels was demonstrated by others both in childhood and in adulthood obesity /4, 6/. Significant negative correlations were found between serum insulin and SHBG, and between serum C-peptide and SHBG concentrations. The role of insulin in regulating SHBG production is supported by several data. Insulin hypersecretion is a common feature in obesity, and obese women with low SHBG level have significantly higher glucose stimulated insulin and C-peptide levels than those observed in obesity with normal SHBG level /14/. Furthermore, serum SHBG concentrations fall throughout the pubertal period and a close negative correlation exists between serum insulin and SHBG levels /8/ Recently it has also been demonstrated that insulin is able to inhibit SHBG production from cultured hepatoma cell lines /17/.

Beside serum insulin concentrations, C-peptide levels were also negatively correlated with serum SHBG levels. It is well known that insulin and C-peptide metabolism can differently be altered in obesity because of the decreased hepatic clearance rate of insulin and insulin resistance /10/. In our work, although the basal serum insulin and C-peptide levels were measured, equally strong correlations were found between serum insulin SHBG, and between serum C-peptide and SHBG levels. It can be suggested that insulin hypersecretion has the main role in determining the reduction of SHBG production. Therefore, the C-peptide/insulin ratio was not demonstrated.

The effect of thyroid hormones on SHBG production was demonstrated by several works /1, 12, 18/. Serum SHBG concentration is low in hyperthyroidism and it is elevated in hyperthyroidism /1/. Measuring the serum SHBG level can be used

for assessing the peripheral effect of thyroid hormones /12, 17/. In obesity serum T_4 and T_3 levels are generally found in the normal range, although nutritional factors can influence thyroid hormone (mainly on T_3) levels /5/. In our work normal thyroid hormone levels were measured in obese children and there was not any association found between serum thyroid hormones and SHBG concentrations.

It is well known that oestrogens increase SHBG levels and androgens decrease them /3/. It was also demonstrated that serum SHBG concentration was not elevated in androgen insensitivity, and cyclicity cannot be demonstrated in SHBG concentrations during menstrual cycle /9, 13/. In our work E_2 and T measurements were performed only in a few cases; sexual hormone levels and serum SHBG concentrations did not associate.

Previous data have indicated that high insulin level is associated with increased overall and upper body obesity /15/. For this reason WHR was calculated. However, WHR did not associate with serum insulin, C-peptide levels and SHBG concentration. This finding can be explained by the fact that the WHR may be inappropriate in puberty, since pelvic widths may change rapidly /2/ and this index reflect the variation rather than adipose tissue distribution.

Our results suggest that decreased serum SHBG concentration cannot be explained by the changes in thyroid and sexual hormones in obesity; insulin level has a fundamental role in determining the reduction of SHBG production.

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Acta Paediatrica Hungarica 32 (2), pp. 159-165 (1992)

DETERMINATION OF HAIR TRACE ELEMENTS IN CHILDHOOD CELIAC DISEASE AND IN CYSTIC FIBROSIS

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Received 29 November 1991

Applying proton-induced X-ray emission authors investigated the hair trace element contents in 10 children with acute celiac disease after 3 to 6 and 12 months long gluten-free diet; in 9 children with cystic fibrosis and in a control group (6 children) of the same age.

There was no difference in Cu, Fe, Ca, Cl values between the examined groups.

The Zn contents of the hair are significantly low in the group with acute celiac disease after a short-term diet and also in the group with cystic fibrosis, the data approach the normal range only after a year's diet.

The significant rise of hair potassium contents is well indicated in patients with acute celiac disease and this rise may be due to the destruction of cell cuticles. In case of cystic fibrosis there is no significant rise of hair potassium value.

INTRODUCTION

In the course of our former examinations it was observed that children suffering from celiac disease, having clinical symptoms but not yet having been placed on a diet, typically have hair without any luster.

The diameter of these children's strands of hair and the cuticle erosion score were significantly different from those of the control group even after 3 months' dieting.

In order to understand the biochemical reasons behind these micromorphological differences, the Zn, Cu, Fe, K, Ca, Cl

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contents of the hair of children suffering from celiac disease were examined first in the acute period of the disease, then at various times during the glutenfree diet. The results were compared with the trace element contents of the hair of healthy children and children with cystic fibrosis.

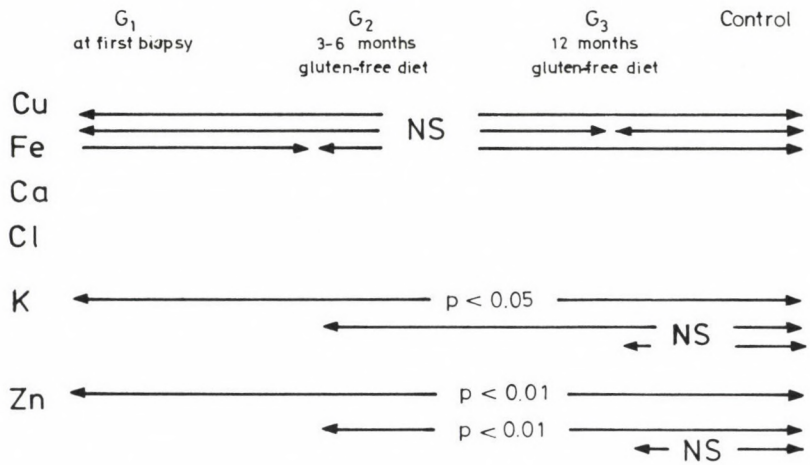

Fig. 1. Hair trace element content of children with celiac disease compared to healthy controls

MATERIAL AND METHOD

The hairs were removed from the frontal third of the crown and they were analyzed at 4 cms above the root. The groups of the examined children were the following: Group 1 was made up of 10 children aged from 1.5 to 10 years. Their hairs were examined at the time of the first intestinal biopsy. The same patients made up Group 2 as well after being on a glutenfree diet for 3 to 6 months. In Group 3: the same children were examined after being on a glutenfree diet for 12 months. 9 children suffering from cystic fibrosis (3 to 12 years of age) made up Group 4. Group 5 was the control group of 6 healthy children within a similar age bracket.

The examinations were carried out by using proton induced X-ray emissions (PIXE) /5, 13/. The hairs were exposed to a concentration of protons of 2.5 MeV energy. As a result of this, the trace elements in the hair emitted radiation characteristic of them. Using a semiconductor Si/Li, the energy of the radiation was detected. This energy determined the

elements while its intensity determined the elements' concentrations. The spectrum gained this way was assessed with the help of a computer. The differences in the thicknesses of the strands of hair were corrected by a correlative sulphur atom, as the inner standard, which correlates with the mass of hair. A x^2 test was used for the statistical analysis of the data.

RESULTS

There was no difference between the groups concerning their Cu, Fe, Ca, Cl values.

The Zn contents were lower (16.6±10.78; $\bar{x}$±SD, $P<0.01$) compared to the control groups (56.2±16.6; $\bar{x}$±SD) and the group of patients who had been on diet for 3 months (23.9±12.59; $\bar{x}$±SD, $P<0.01$). After a year on diet this difference was not observable any longer (40.4±27.2; $\bar{x}$±SD). The Zn value in the hair of patients with cystic fibrosis was also significantly lower (34.9±19.35; $\bar{x}$±SD) than that of the control group (56.22±16.6; $\bar{x}$±SD, $P<0.05$).

In the groups of patients suffering from celiac disease and cystic fibrosis, the serum Zn values were at the low end of the normal range and they were not in correlation with the Zn contents of the hairs.

The potassium contents of the hair in patients with celiac disease were significantly higher compared to the control group ($p<0.05$) and during the diet it indicated a downward tendency.

The increased potassium values measured in the hairs of the group with cystic fibrosis (14.5±12.78; $\bar{x}$±SD) did not differ significantly from those of the healthy children, but this increased value was significantly lower than that of the group with celiac disease.

DISCUSSION

The analysis of hair trace elements is a wellestablished method of assessing the nutritional conditions of the organism

TABLE I

Determination of hair elements of children with celiac disease and cystic fibrosis (* $p < 0.05$, ** < 0.01)

		Cl	K	Ca	Fe	Cu	Zn
G_1	$\bar{x}$	219.747	45.88*	25.067	14.545	31.04	19.59**
	±SD	265.042	39.30	8.82	4.74	1.455	10.78
G_2	$\bar{x}$	109.77	32.12	24.295	13.634	3.207	23.974**
	±SD	83.127	31.66	14.22	6.994	1.13	12.599
G_3	$\bar{x}$	123.71	16.16	23.86	20.517	14.643	40.484
	±SD	56.89	8.007	15.94	14.477	18.54	27.826
G_4	$\bar{x}$	260.401	14.49	30.005	9.233	5.465	34.91*
	±SD	191.34	12.73	48.548	4.610	3.145	19.35
G_5	$\bar{x}$	263.035	7.978	19.087	12.498	5.424	56.222
	±SD	216.38	4.218	5.211	4.874	2.020	16.65

/4, 12, 16/. Different hair preparations, hair-dyes and also the environmental conditions have an effect on the measured data /8/ and the trace element contents of the hair change at various distances from the hair-root. The trace element contents of the hair vary significantly in infancy /9/, so the data can only be compared with those of children within a similar age bracket. The determination of hair trace elements measured at the same distance from the hair-root in children of the same age (whose hair was treated similarly), however, gives information on long-term metabolic processes of the organism /10/.

It has become familiar from publications that very often there is no correlation between the Zn contents of the hair and that of the plasma /1, 11/, since the plasma always indicates the acute values of the day, therefore more suitable for detecting permanent effects. Our studies as well as those of others have long revealed that Zn deficiency detected from serum as yet in patients with acute celiac disease can be considered as a result of a chronic process, which is also reinforced by low Zn contents of hair.

Apart from many other factors, Zn deficiency also contributes to slow development and any other symptoms of patients with celiac disease.

However, the significant increase of hair potassium contents in acute celiac disease is a new result.

Alkali and halogen elements do not link with tissue proteins /13/, the significantly high potassium value may be due to increased cell destruction and only after a long diet can this value approach the normal range. There is no such significant rise in hair potassium contents in patients with cystic fibrosis. The signs referring to inner metabolic disturbances should be further investigated.

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Acta Paediatrica Hungarica 32 (2), pp. 167-187 (1992)

IN VITRO MOTILITY INVESTIGATIONS AND ELECTRON MICROSCOPIC OBSERVATIONS ON CHILDREN'S UPPER URINARY TRACT MUSCLE WALL

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Received 29 November 1991

This study was performed as a part of a longer research programme on urinary tract smooth muscle layer in children. All the children whose samples were investigated underwent surgery for urinary tract malformations. Specimens were taken from different segments of upper urinary tract during surgical intervention. Specimens were investigated by either in vitro motility tests or electron microscopy or both of them. Basic patterns of tissue strips were recorded after incubation of varying duration and then tested by administering neurotransmitter agents like noradrenaline and acetylcholine-bromide. Microstructure of samples were examined electron microscopically. Investigations were performed in order to find correlation between microarchitecture and motility patterns of urinary muscle wall. Factors influencing urinary muscle motility, characteristic features of impaired musculature and its possible regeneration are discussed too. Microhistological deteriorations inhibit spontaneous smooth muscle motility but muscle contractility proved by administering noradrenaline and acetylcholine-bromide remained in some extent. Taking into consideration that smooth muscle is able to regenerate and rebuild close contacts pediatric surgeon and urologist should spare kidney parenchyma as far as it is possible.

INTRODUCTION

Two-three decades ago microhistology of urinary tract smooth musculature and its nerve supply were made clear by electron microscopy at that time and later, factors exerting influences on smooth muscle contractibility were investigated. Moreover a

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lot of examinations were performed by electron microscopy to discover impaired structure of urinary tract smooth muscle layer of patients suffering from urinary tract malformations. For this reason the present study's aim is to find correlations between motility patterns gained by organ bath tests and microstructural features observed by electron microscopy on upper urinary tract samples obtained from children at surgery.

MATERIALS AND METHODS

Two types of investigations were performed in this study:
a) "in vitro" motility tests in tissue bath
b) electron microscopic examinations.

Samples were taken from different segments of 28 children's upper urinary tracts during surgical intervention (Fig. 1). Children's ages range from 2 weeks to 13 years with a mean of 19 months. All of them suffered from urinary tract malformations and underwent either reconstructive surgery (ureteropelvic plastics, ureteroneocystoplastics with or without shortening and tailoring, urinary diversions and undiversions) of nephroureterectomies, or heminephrectomies with ureterectomies. Main data are listed in Table I.

Removed specimens underwent motility or electron-microscopic or both investigations depending on the availability of skilled technical assistance (Table I).

For in vitro motility investigations specimens were cut into strips of 2 cm in length and 0.5-0.7 cm in width to be thin enough to allow diffusion of neurotransmitter agents to all the muscle cells and free of adherent fat and adventitial tissues in order to avoid self excition of the muscle cells through their own vegetative nerves. Strips were continuously moistened with Tyrode solution during preparation and incubation. For stable patterns incubation occurred at $4^{o}C$ for one, 24, 48 and 72 hours. Samples incubated longer than one hour were obtained from the same sites of patients' upper urinary tracts as duplicates, triplicates, etc. Strips were mounted in water jacketed organ bath: 46 of 89 strips after one hour, 29 after 24 hours, 11 after 48 hours and 3 after 72 hours of incubation. Strips were suspended under 1 gram tension and connected to a transducer for recording contractions. Tissue bath was filled with 10 ml Tyrode solution, thermostated at $37^{o}C$, aereted continuously with 95% oxygen and 5% carbon dioxide. Contractions were provoked directly by administering neurotransmitter agents to organ bath fluid. As neurotransmitter agents noradrenaline (Noradr) and acetylcholine-bromide (Ach-Br) were administered in increasing concentration (Noradr: $0.99x10^{-6}$, $1.97x10^{-6}$, $3.94x10^{-6}$, $7.88x10^{-6}$, $15.76x10^{-6}$ M and Ach-Br: $3.68x10^{-6}$, $7.37x10^{-6}$, $14.74x10^{-6}$, $29.48x10^{-6}$, $58.97x10^{-6}$ M). After each administration organ bath was washed out and filled with fresh

TABLE I

Distribution of patients and their samples investigated by motility tests and electron microscopy

Diagnosis	No. of patients investigated by motility tests	No. of patients investigated by electron microscopy	No. of patients investigated by motility tests and electron microscopy	Sites of samples	No. of samples investigated by motility tests	No. of samples investigated by electron microscopy
Stenotic pyelo-ureteric junction	3	2	5	calyx	10	3
				pyelum	18	9
				pyeloureteric junction	7	4
				ureter-upper	6	3
				middle-segment	7	2
				lower	6	2
Stenotic uretero-vesical junction	2	3	3	pyelum	1	2
				pyeloureteric junction	1	1
				ureter-upper	1	1
				middle-segment	4	2
				lower -stenotic	5	2
				-non stenotic	3	2
Vesico-uretero-renal reflux	1	1	1	pyelum	2	-
				ureter-upper	1	2
				middle-segment	1	2
				lower	4	2
Duplex kidney and ureter	1	1	2	pyelum	2	2
				ureter-upper	2	2
				middle-segment	3	4
				lower	3	3
Other malformations	-	2	1	ureter-upper	-	1
				middle-segment	-	3
				lower	2	3
Total	7	9	12		89	57

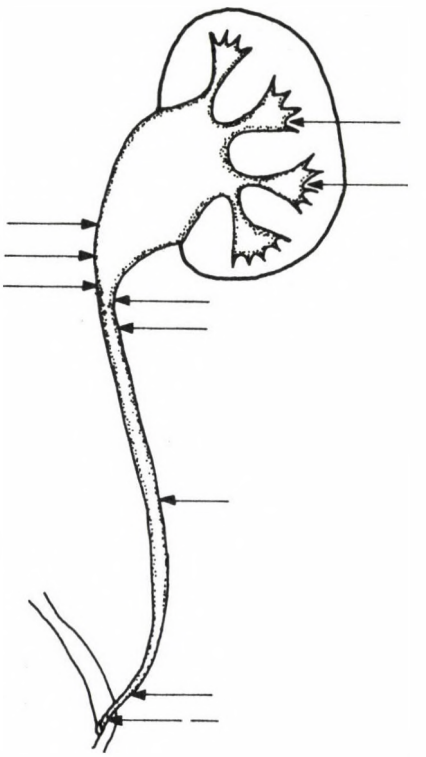

Fig. 1. Sites from where samples were taken for in vitro motility tests and electron microscopic examinations

Tyrode solution. Stable patterns of strips and effects by administering neurotransmitter agents were recorded by RADELKIS pen recorder.

Samples in sizes of about 2x1x1 mm blocks were taken from whole thickness of walls of ureters, pyelums and calyces for electron-microscopy. To be fresh enough specimens were cut off immediately after cessation of blood supply and immersed in 2% phosphate buffered glutaraldehyde (p_H 7.2), postfixation occurred in 1% phosphate buffered osmium tetroxide (p_H 7.2) then samples were dehydrated in graded ethanol and embedded in DURCOPAN (Fluka AG). Ultrathin sections were cut, mounted on copper grids, stained by uranil acetate and leadcitrate, and at last examined under either JEOL 100-B or JEOL 100-C electron-microscope.

RESULTS

At first stable patterns of strips were observed in tissue bath then contractions induced by Noradr and Ach-Br were tested. Statistical analyses of our materials could not be done because data failed to show any condition to analyse at all, and conclusions could be drawn from our observations only for surgical practice and follow-up.

Spontaneous long lasting rhythmic and slow contractions were observed during testing strips obtained from either unimpaired or mildly deteriorated pyelums and ureters. (Fig. 2 A, B, 4 C, 6 A, B, C).

Specimens removed from dysplastic ureters and ureters with seriously deteriorated muscle microstructure exhibited no spontaneous rhythmic contractions when testing them in organ bath (Fig. 3).

All strips taken from calyces, pyelums, pelviureteric junctions, and upper middle ureter segments but those from dysplastic ureters contracted by administering Noradr to organ bath fluid after 1 and 24 hours incubation (Fig. 4 A, B, C, D, E).

No strips contracted after 72 hours of incubation. Only two samples contracted by administering Noradr to organ bath fluid after 48 hours of incubation, both of them were from middle ureter segments. None of all above mentioned specimens contracted when administering Ach-Br.

Strips obtained from non-stenotic ureterovesical junctions contracted by administering both neurotransmitter agents used in this study (Fig. 5 A, B).

Stenotic ureterovesical segments did not contract at all.

It can be ascertained:

Strips taken from pyelums and pelviureteric junctions had higher contractions than those from calyces and ureters of the same patients.

The more concentrated Noradr was administered the more extensive contractions were achieved in all strips. That means contractions were dose-related as well as in some extent those depended on microstructure of smooth muscle layer (Fig. 6 A, B, C).

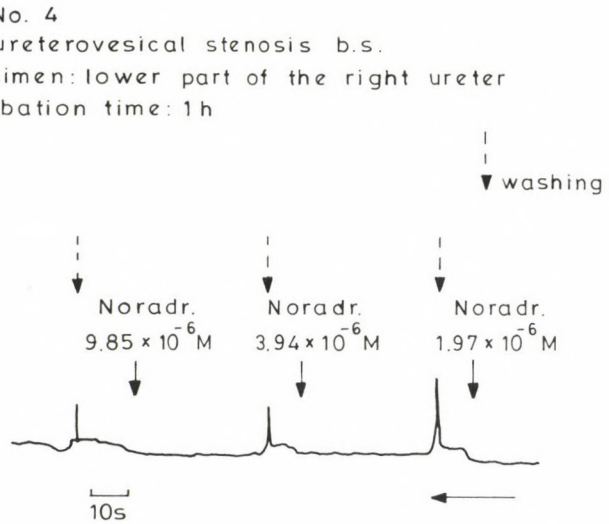

Fig. 2.A. Patient (Pt.) No. 4. Between two contractions provoked by administering Noradr into tissue bath fluid slow rhythmic contractions of a strip taken from lower segment of mildly deteriorated ureter are seen (b. s. = both sides)

DISCUSSION

Transport of urine along the upper urinary tract depends on:

a) quantity of excreted urine stimulating peristalsis /9/.

b) microhistological architecture of urinary tract muscle layer considering intact close contacts which are responsible for intercellular impulse transmission (3, 4, 6, 7, 8, 11, 23, 25, 32, 33).

c) influences of vegetative nervous system on urinary tract smooth muscle cells (4, 7, 12, 24, 27, 28, 30, 31, 32, 35, 36, 37).

d) locally released prostaglandins co-ordinating intercellular impulse transmission /1, 2, 5, 7, 29, 44, 45, 46/.

e) damages of inner coat of protective transitional epithelium due to local microtraumas resulting in entry of urine and exposure of the contractile elements to nonphysiological environment /47, 49/,

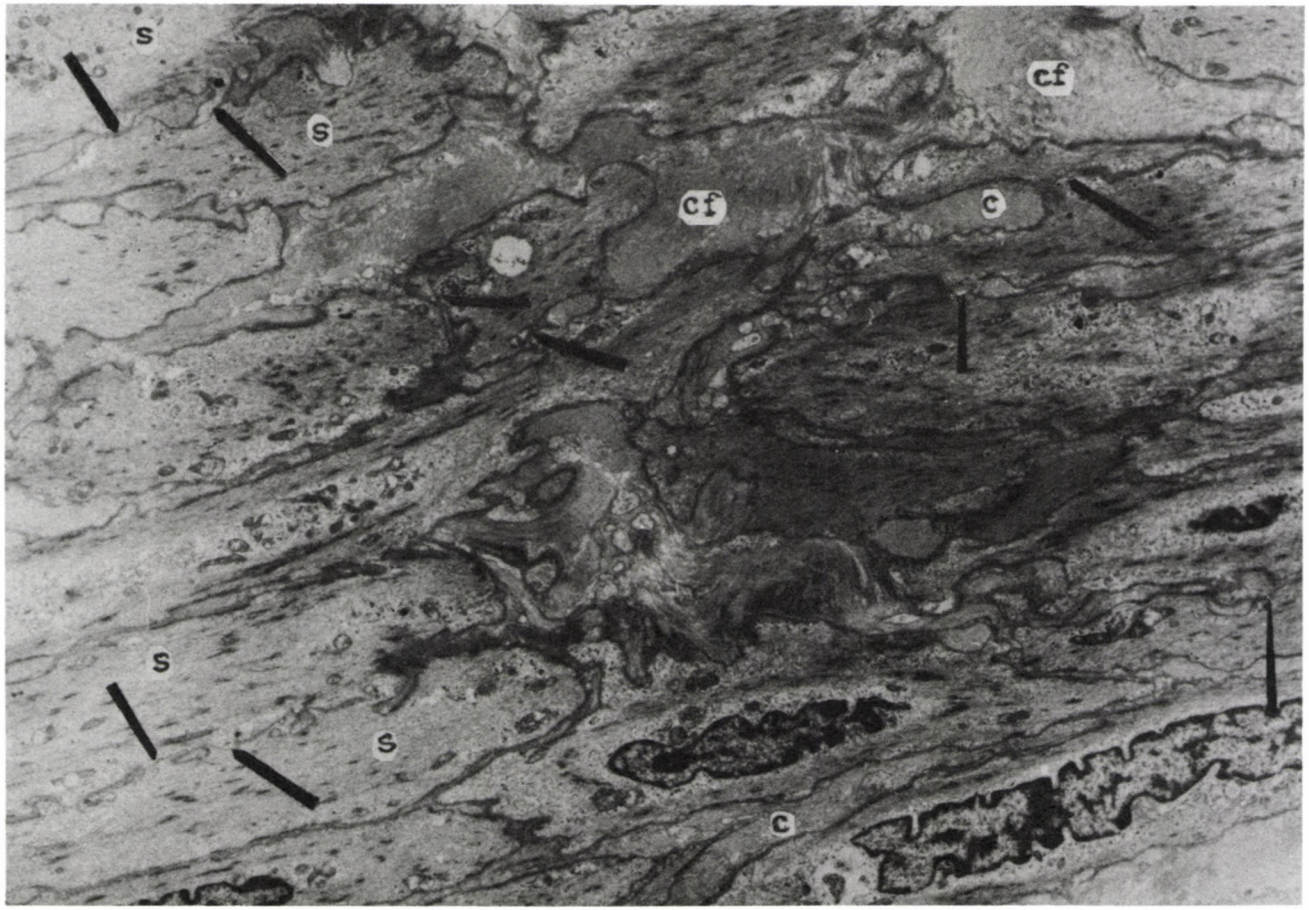

Fig. 2.B. Electron micrograph shows slightly increased collagen (c) and collagen fibres (cf) amid muscle cells (s). Smooth muscle cells are linked by close contacts (large arrows). Mildly impaired muscle layer (X4000)

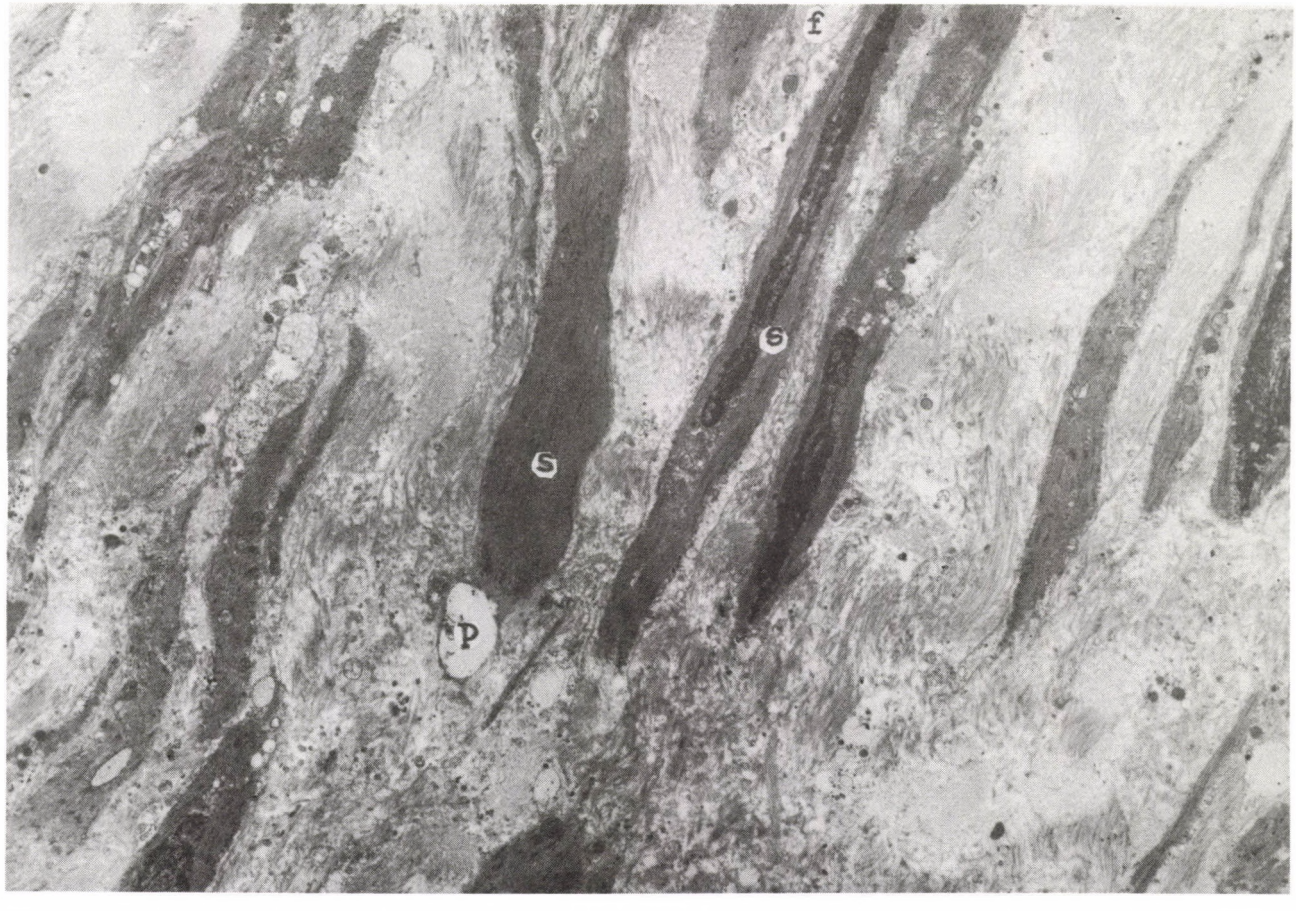

Fig. 3. Pt. No. 24. Very seriously damaged musculature of the pyelic wall. Smooth muscle cells are disconnected by large amount of collagen fibres. No close contact is seen at all. Perishing muscle cell (p) and fibroblasts (f) point out: the muscle layer has been damaging for a long while (X4000)

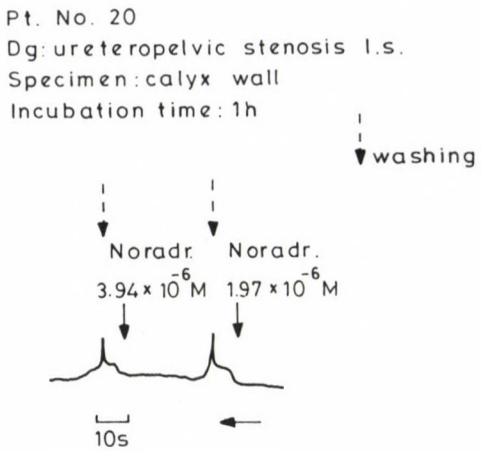

Fig. 4.A. Pt. No. 20. Contractions of moderately impaired calyx wall

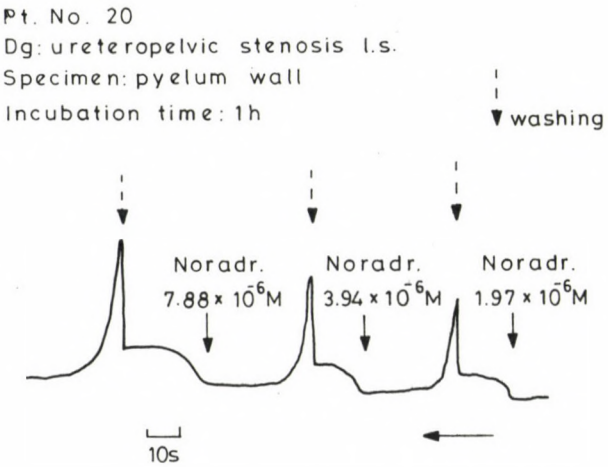

Fig. 4.B. Contractions of moderately damaged pyelum wall

Fig. 4.E. Unimpaired smooth musculature of the middle ureter segment (X4000)

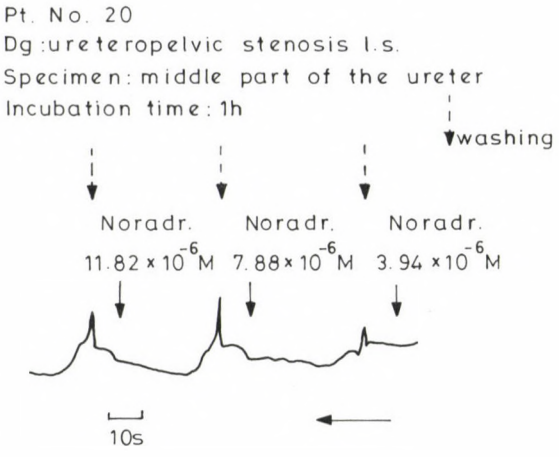

Fig. 4.C. Spontaneous and induced contractions of unimpaired middle ureter segment (l.s. = on the left side)

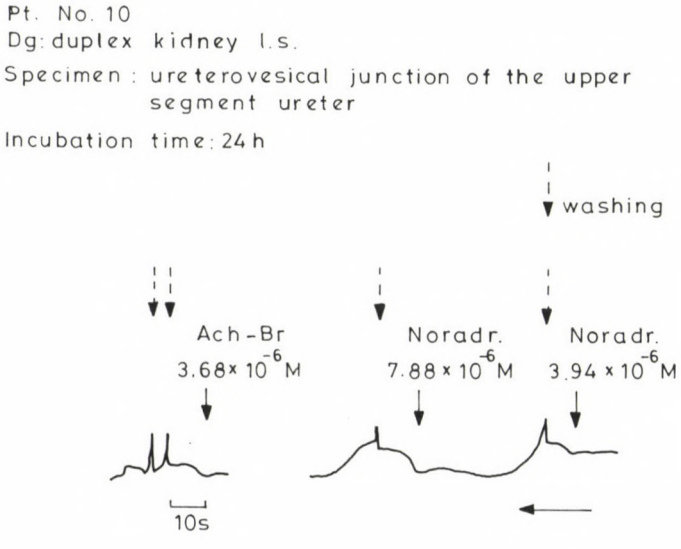

Fig. 5.A. Pt. no. 10. Distended ureterovesical junction of duplex kidney's upper segment ureter shows no spontaneous contraction but contracted by administering both of neurotransmitter agents used in this study

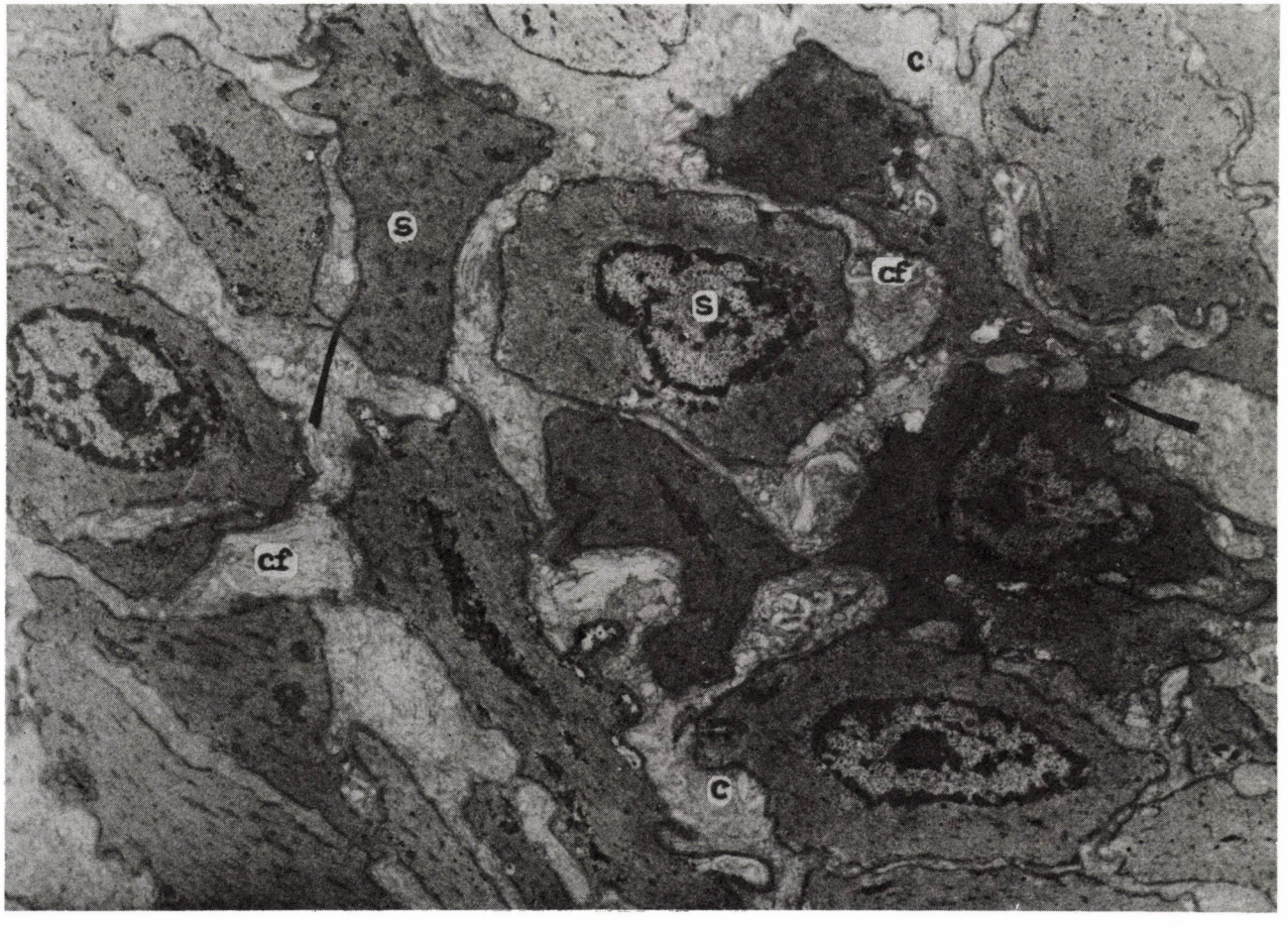

Fig. 4.D. Moderately impaired smooth muscle microstructure is characterized by increased intercellular collagen and collagen fibres disjoining smooth muscle cells. Close contacts are seen sparsely. (X4000)

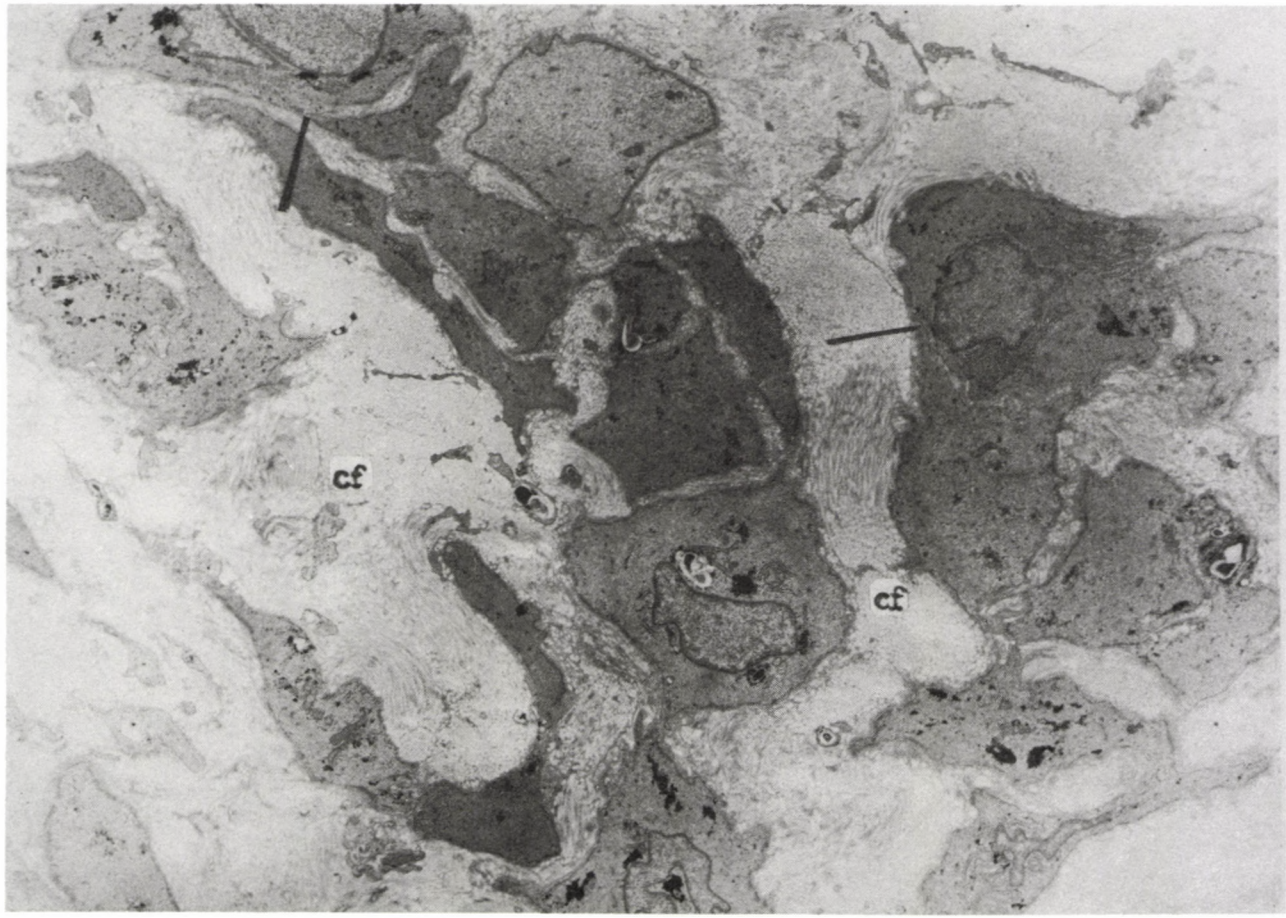

Fig. 5.8 Seriously damaged muscle microstructure. Large amount of collegen fibres are seen and close contacts are found very sparsely. Muscle bundles are not totally destroyed (X4000)

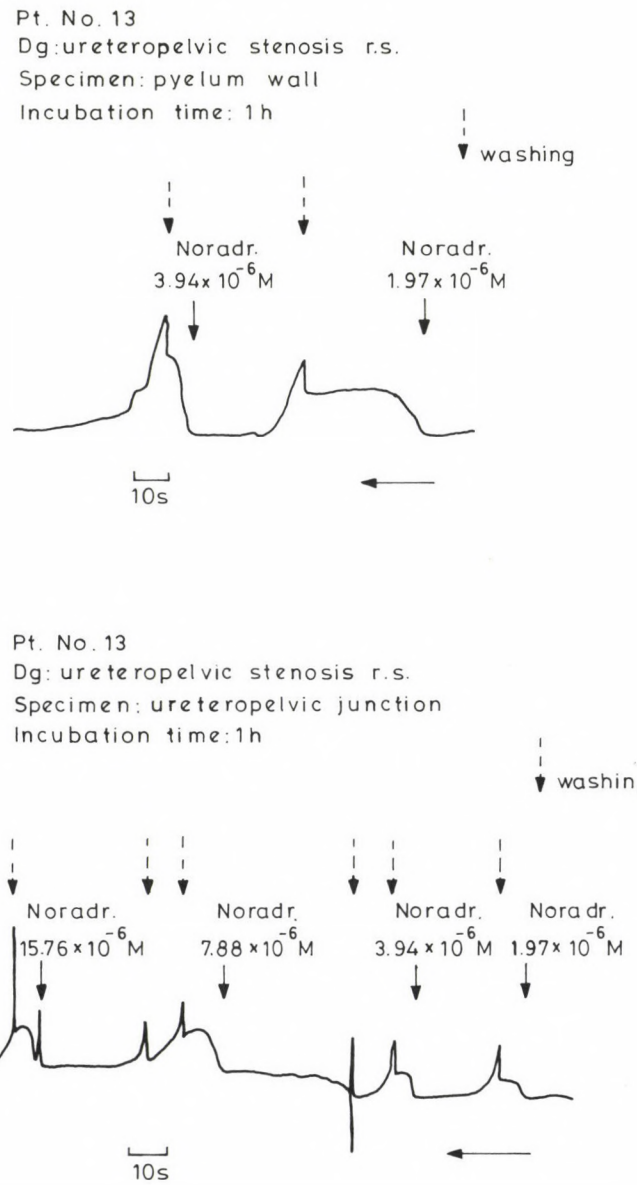

Fig. 6.A,B. Pt. No. 13. Dose-dependent contractions evoked by administering Noradr (r.s. = on the right side)

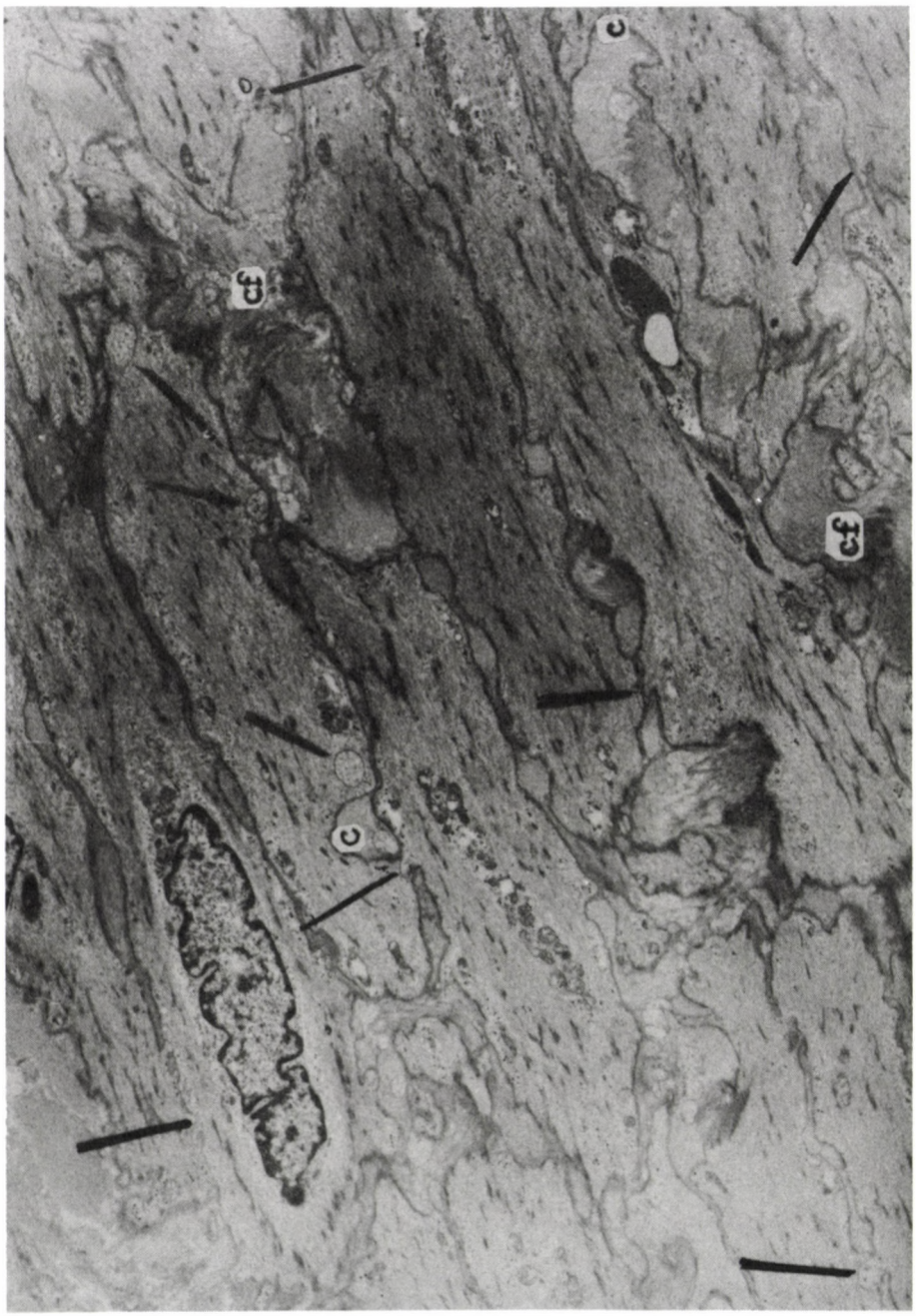

Fig. 6.C. Mildly deteriorated smooth muscle layer of the pelvic wall (X4000)

f) endo- and exotoxins of uropathogenic bacteria influencing peristalsis directly or indirectly /48/.

Human urinary tract musculature consists of smooth muscle cells forming muscle bundles. Muscle cells are separated by thin sheets of connective tissue elements and muscle bundles are demarcated by collagen fibres. Smooth muscle cells are joined by close contacts (gap junctions, nexus close contacts, nexuses, tight contacts, close approaches). Close contacts are 200 nm in width and in this region cell walls lie in close apposition with no intervening basement membrane (Fig. 2 B, 4 D, E and 6 C). Close contacts are electric and metabolic coupling sites of low resistance /3, 19, 33/.

Pacemaker cells in minor calyces and pyelum wall are reactive to tension in calyx and pyelum and co-ordinate peristalsis along upper urinary tract. There are "P" "pale" smooth muscle cells in the ureter wall, they are thought to be pacemaker cells too. /8, 13, 14, 15, 16, 17, 18, 19, 26, 27/.

Smooth muscle cells receive nerve supply from vegetative nervous system. One nerve-ending stimulates indirectly about one hundred muscle cells and stimuli spread through close contacts from one cell to another without decreasing /3, 37/.

Smooth muscle cells are transformed into myofibroblasts and fibroblasts by increasing tension inside urinary tract. Fibroblasts produce collagen fibres /18, 20, 21, 22, 23, 34, 39/. Furthermore derivates of urinary tract infections worsen the situation by inducing muscle cells to produce elastin and collagen /23, 49/. Hereby the microstructure of the muscle layer deteriorates and close contacts are disrupted resulting in the smooth musculature's loss of its co-ordinated function. The seriousness of impairments vary and can be classified as mild, moderate, serious and very serious damages /20, 39/.

Recent research made clear that deteriorated urinary smooth muscle layer can regenerate. Regeneration means that increased fibres and intercellular collagen and elastin disintegrate, muscle cells rebuild close contacts by extending cytoplasma processes /10, 11, 38, 40/.

Lastly muscle dysplasia should be mentioned: this is a congenital malformation inside the smooth muscle cells. Myosin fibrils are not found in the cytoplasma. Ureter of this type of anomaly is not able to transport urine at all /41, 42, 43/.

CONCLUSIONS

This study has shown that structural changes extending throughout the upper urinary tract muscle layer inhibit spontaneous motility, but muscle strips can be induced to contract directly by administering neurotransmitter agents in tissue bath fluid. It means that smooth muscle cells preserve contractibility except those having no contractible fibrils i.e. myosin (thick filaments) in cytoplasma.

Taking into consideration that smooth musculature is able to regenerate and rebuild close contacts to some extent, it can therefore be said that pediatric surgeons and urologists should try to spare kidney parenchyma as much as it is possible.

Application of the above mentioned in vitro findings to pediatric surgical and urological activity: they serve as additional but very important factors in deciding whether to perform and what type of surgical intervention is necessary in children's maldeveloped urinary tracts.

ACKNOWLEDGEMENTS

I wish to express thanks to Mrs Judit Nagy, Mrs Erzsébet Balásházy (Institute of Biochemistry, Szeged), Mrs Sirpa From (Electron Microscopic Laboratory, Turku) for their excellent technical assistance and Professor Emeritus Ferenc Guba (Institute of Biochemistry, Szeged), Assistant Professor Lauri Pelliniemi (Head of Electron Microscopic Laboratory, Turku) for use of the facilities of their Electron Microscopic Laboratory.

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Acta Paediatrica Hungarica 32 (2), pp. 189-197 (1992)

ABOUT DR_{beta}-RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSIS IN KIDNEY TRANSPLANTATION IN CONNECTION WITH A PAEDIATRIC PATIENT

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Received 28 December 1991

A retrospective analysis of the HLA-DR antigens was performed at the DR_{beta} DNA locus by the means of restriction fragment length polymorphism (RFLP) in the case of a two-times kidney transplanted paediatric patient and in 16 adult kidney donor recipient pairs in order to prove the importance of DNA molecular analysis in those cases where the serological identification is poor. The child and her grafts (first from her mother, the second from a cadaver donor) carried the DRw6 antigen which serologically can very poorly be defined. According to DR serotyping before transplantation both the child and the cadaver kidney proved to be DR5,6, while the DNA analysis revealed mismatches; the child possessed the two subtypes: $13b^1$ and 14a of the DRw6 antigen only and none of the DR5, the cadaver kidney proved to belong to the DR4 antigen group instead of DR5, and furthermore to a different subtype of the DRw6 ($13a^3$) than the recipient. The DNA analysis of other 16 adult donor-recipient pairs also underlined the importance of the DR_{beta} RFLP analysis in cases where the transplantation antigens could be poorly defined.

INTRODUCTION

The antigens of the HLA-D region have been implicated in the regulation of the immune response and allograft rejection in clinical transplantations. Fortunately, partial matching between donor and recipient may be sufficient to achieve high

graft survival rates in the presence of immunosuppressive therapy.

The influence of HLA-DR matching on cadaver graft survival is more pronounced than that seen with A, B matching. Recent advances in molecular biology make possible to characterize different antigens according to the individually characteristic cleavage sites for certain restriction endonucleases of their genes. Restriction Fragment Length Polymorphism (RFLP) analysis of the HLA-DR gene has been developed into a powerful tool for genomic tissue typing /1, 2, 5, 18/. It has been suggested that this methodology can detect donor-recipient incompatibilities, which may be important in influencing the graft function.

The serological methods fail to easily discriminate between the subtypes of DR 2 (DRw 15, DRw 16), DR 3 (DRw 17^1, 17^2, 18), DR 4 (4^1, 4^2), DR 5 (DRw 11^1, 11^2, 12^1, 12^2), DRw 6 ($13a^1$, a^3, a^4, $13b^1$, $13b^2$, 14a, 14b) and DR 7 (7^1, 7^2) antigens.

Several studies demonstrated /7, 8, 14/ that the kidney graft survival in DRw6-positive patients was significantly lower than in DRw6-negative ones. In contrast these reports, Hoitsma et al. /9/ found no significant differences in overall renal allograft survival between DRw6-positive and negative recipients.

The richness of the subtypes of DRw6 antigen can not be discriminated by serotyping. In a girl with two kidney transplantations and in adult patients also, we reviewed the $HLA\text{-}DR_{beta}$ matching, the number of rejection episodes and the allograft outcome in order to highlight the usefulness of the RFLP analysis in kidney transplantation. We decided to compare the pretransplant HLA-DR serology with the DR_{beta}-DNA-analysis and the number of rejection episodes in 8 adult donor-recipient pairs selected by compatibility for DRw6 antigens, and in 8 pairs selected for other antigens.

PATIENTS AND METHODS

N.A. 12-year-old girl was introduced into the chronic haemodialysis and renal transplantation program because of a chronic renal failure originated from a Henoch-Schönlein nephropathy. She had got her first kidney graft from her mother. The mother's HLA antigens were: A: 2,11; B: 22,35; DR: 3.6; the patient's were: A: 2,11;, B: 22,x; DR: 5.6. This graft had to be removed 1.5 year later because of a chronic rejection and acute vascular rejection. The child's second kidney transplantation was performed on 19. August 1988 using a cadaver kidney (HLA-A: 2,11; -B: 5,14; -DR: 5,6). This graft had to be removed 1 month later because of an accelerated vascular rejection and chronic rejection. The HLA serotyping (HLA-A, -B, -DR) was carried out by a standard microcytotoxicity assay /16/. ABO compatibility, sharing at least two HL-A,B antigens and one DR antigen, as well as a negative T-cell cross-match test, were the prerequisites for allograft transplantation. The patient and her grafts were analysed by DNA-DR_{beta} and DQ_{beta}-RFLP technique after transplantation using Southern blot analysis /15/. DNA was extracted either from frozen donor spleen or from recipient peripheral leukocytes /10/. Genomic DNA was digested with Taq I, Bam HI, Bgl II, Pst I, Eco RI, Hind III restriction endonucleases, electrophoresed in 0.7% agarose gel and blotted onto a nitrocellulose membrane /11/. Nitrocellulose membrane was hybridized with ^{32}P-labelled cDNA probes (6): p-II-beta-1 (DQ_{beta}) and p-II-beta-3 (DR_{beta}) (kindly provided by Dr. P. Peterson, Uppsala).

Eight adult donor-recipient pairs selected by compatibility for DRw6 antigen (Group A) and 8 donor recipient pairs selected by compatibility for other DR antigens (Group B) were also examined by DNA-DR_{beta}-RFLP analysis. The number of rejection episodes and graft survival was also determined.

All patients had been on maintenance dialysis before the kidney transplantation. All grafts for the adult transplantations were taken from cadavers.

The initial immunosuppressive regimen was triple therapy: cyclosporine 12 mg/kg (later decreased, monitored with TDX), methylprednisolone 1 mg/kg (decreasing after the 3rd day) and Azathioprine (below 150 mg/day). The rejection episodes were treated with high-dose steroid therapy (500 mg methylprednisolone daily, for 3-5 days) followed by the steroid recycle. The rejection episodes were confirmed by the clinical course and histopathology.

Statistical evaluation was performed with Student's t test.

RESULTS

The child had got a maternal graft matching for one antigen each of all HLA-loci. At the DR locus the 14a subtype of the DRw6 antigen was of maternal origin. The second DR antigen by RFLP analysis revealed a discrepancy with the DR5 serotyping ($13b^1$, another subtype of DRw6) (Table I). This graft survived 1.5 year and had to be removed because of a chronic rejection. She had altogether four rejection episodes. The second kidney graft was found serologically compatible at DR antigens (DR 5,6), but this proved to be incorrect by RFLP analysis (cadaver graft had 4^1, $13a^3$ antigens). There was also a discrepancy in the serologically not determinable DRw6 subtypes (donor: $13a^3$, recipient: $13b^1$, 14a) (Table I). This graft survived 1 month, the number of rejection episodes was one, the graft had to be removed because of an accelerated vascular rejection and a chronic rejection. In the case of adult kidney transplantations, the results of the serological HLA-DR typing as compared to the RFLP analysis the number of rejection episodes and the allograft outcome in Group A (8, serologically DRw6 compatible donor-recipient pairs) versus Group B (8 donor-recipient pairs, selected by compatibility for other than DRw6 antigens) were as follows:

1. The number of mismatches by the DR_{beta}-RFLP was significantly higher in Group A than in Group B ($p = 0.001$).
2. The number of rejection episodes was slightly higher in Group A (2.9 ± 1.6) than in Group B (1.5 ± 0.53), , ($p = 0.04$).
3. The two-year graft survival was 25% in Group A, while 88% in Group B (Table I).

DISCUSSION

Nowadays the DNA-RFLP analysis has demonstrated that the polymorphism of the HLA-D region genes is greater than suggested by the results of the conventional serological and cellular techniques.

This method can reveal donor-recipient mismatches which may be important in influencing the graft function. The HLA-DR_{beta}-typing of renal allograft patients and donors and its correlation with pretransplant serotyping have been examined in a number of laboratories (3-4, 12-13, 17, 19).

The polymorphism detected by RFLP analysis of Taq I enzyme digested DNA, hybridizes with DR_{beta} cDNA probe correlates quite well with the phenotypic polymorphism recognized by serology and mixed lymphocyte culture.

All the recognized serologic DR specificities, DR1 to DRw18 can be distinguished by RFLP analysis except for $DR7^2$/9 /2/. However, using Hind III/DR_{beta} RFLP the distinction of 7^2 (DQw9, Dw11) can be achieved.

However, DR serology can not discriminate the subtype specificities of DR2, 3, 4, 5, w6, 7 antigens, and, this is of special interest in the case of DRw6 because of its numerous (seven at present) subtypes. Identification of DRw6 splits as $13a^1$, $13a^3$ etc. required Taq I/DR_{beta}-RFLP and also examination with more restriction enzymes and DQ_{beta} probe, so as to be informative due to the linkage disequilibrium between DR and DQ loci (1-2, 4-5).

Our paediatric patient proved to have this poorly determinable DRw6 antigen. At her pretransplant serotyping one out of two DR antigens was categorized incorrectly (DR5).

Two subtypes of the DRw6 antigen have been found by RFLP: $13b^1$ and 14a, the latter being of maternal, the former ($13b^1$) of paternal origin, respectively.

We emphasize this situation because of the "high responder" status of the recipients having DRw6 antigen, well-known from some laboratories /8, 14/.

The possible association of the allograft survival with the HLA-DNA-RFLP-matching of the kidney donor-recipient pairs has been demonstrated in cases of transplantations to adults.

Many factors may influence the graft outcome, including the HLA-matching, immunosuppressive therapy, sex and blood group of the recipients, presensitisation, etc. No single factor plays a predominant role and the relative effect of each factor may be difficult to demonstrate.

Opelz G. et al /12/ found that eliminating the typing mistakes from the determination of HLA-DR antigens, the success rate of HLA-matched cadaver transplants in nearly as good as that of HLA-matched sibling transplants. They showed that the high typing error rate was responsible for many unexpected transplant failures, but they have not examined separately the DRw6 selected donor-recipient pairs.

TABLE I

Serological and DR_{beta}-RFLP analysis and allograft outcome in adult renal transplanted donor-recipient pairs (Group A and Group B) and those of a child with two successive grafts

No.	Patients	HLA-DR serol.	HLA-DR serol. match.	DNA RFLP*	DNA match.	Allograft outcome No. of rej. episodes[xx]	Allograft outcome graft survival[+]
				Group A			
1	D	5, 6	1	$13a^1$, 14a	1	4	1 yr
	R	6		$13a^3$, 14a			
2	D	6	1	8, $13a^4$	0	2	>2 yr
	R	6		10, 14a			
3	D	5, 6	1	11^1, $13a^4$	0	1	2 yr
	R	6, 7		7^1, $13a^1$			
4	D	6, 7	1	15, 7^2	0	2	1.5 yr
	R	6		$13a^3$, $13a^4$			
5	D	5, 6	1	$13a^1$, 14a	0	5	1 yr
	R	3, 6		10, 17^1			
6	D	6	1	8, $13a^4$	0	5	1 yr
	R	5, 6		11^1, 7/9			
7	D	1, 6	1	1, 14a	0	3	2 mo
	R	2, 6		11^1			
8	D	6, 7	1	7^2, 15	0	1	2 wk
	R	6		$13b^1$, 9			
				Kidney graft to a child[++]			
child		5, 6		$13b^1$, 14a			
			1		1	4	1.5 yr
1st graft		3, 6		14a, 18			
2nd graft		5, 6	2	4^1, $13a^3$	0	1	1 mo

TABLE I. Cont.

No.	Patients	Group B HLA-DR serol.	HLA-DR serol. match.	DNA RFLP*	DNA match.	Allograft outcome No. of rej. episodes**	graft survival+
1	D	1	1	1, 9	1	1	3 yr
	R	1		1, 4^1			
2	D	2, 6	1	15, 16	1	1	>2 yr
	R	2, 7		7^1, 16			
3	D	2	1	4^1, 16	0	1	>2 yr
	R	2, 6		$13a^1$, 15			
4	D	2, 5	1	11^1, 15	1	2	>2 yr
	R	4, 6		4^1, 11^1			
5	D	1, 7	1	1, 7/9	1	1	>4 yr
	R	6, 7		1, $13a^1$			
6	D	1, 7	1	1, 7/9	1	2	2 yr
	R	1		1, 4^1			
7	D	2, 6	1	14b, 15	1	2	>1.5 yr
	R	2, 4		4^1, 15			
8	D	2, 5	1	12, 15	1	2	>2 yr
	R	2		15			

Legends to Table I

Group A: 8 donor-recipient pairs selected by compatibility for DRw6 antigen
Group B: 8 donor-recipient pairs selected by compatibility for other DR antigens
D = donor, R = recipient
RFLP = Restriction Fragment Length Polymorphism
*: DNA-RFLP matching less in Group A versus Group B (p= 0.001)
**: slightly more rejection in Group A versus Group B (p = 0.04)
+: Two-year graft survival less in Group A versus Group B (25% vs 88%)
++: diverse serological and DNA-RFLP data together with poor graft survival

The polymerase chain reaction (PCR) amplifies DNA sequences several millin-fold so within a few hours we can characterize the genes of the transplantation antigens. This makes PCR a valuable method in clinical practice for improving the success rate of kidney transplantations, especially in the presence of DRw6 antigen. Even if performed retrospectively, detection of mismatches by RFLP can be of predictive value concerning allograft rejection.

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Acta Paediatrica Hungarica 32 (2), pp. 199-200 (1992)

BOOK REVIEW

Physician's Guide to Sunscreens. Ed. by N.J. Lowe Marcel Dekker, Inc. 1992, New York, 232 pages, 24 illustrations, tables

The reader can take a very well proportioned and edited book into his hand, the 15 subchapters within the 4 main chapters are written by the best American professionals within those specific fields, with the exception of the 7th subchapter which A.R. Young (London) has written on 5-Methoxypsoralen containing sunscreens. At the back of the book a concise table of selected American skin and lipstick sunscreens along with their trade names, manufacturer and sun protector factor (SPF) can be found. This is a selection of products with a SPF of 15 or above, as these meet the requirements of present day, according to the latest scientific findings. In the introduction the editor expresses the importance of wide spread enlightening on the connection between skin cancer and severe sun exposure. Besides this, accelerated skin aging also suggests the necessity of sunscreens everyday use, on our environmental catastrophe close earth, in the state of growing ozone holes. The second chapter deals with photobiological aspects, the third with sunscreen ingredients and formulations. The fourth main chapter discusses the biological evaluation of sunscreen products. According to past examinations just the cancer causing effects of UVB wavelengths, which make up a minimal part of sunrays, had been proved. In 1988 it was proved for the first time that the UVA wavelengths (315-400 nm) are also carcinogen. As UVA occurs in a significantly large amount in sunrays, furthermore it reaches human skin through cloudy skies, it is important to know that this type of wavelength is the most important from the point of view of sun caused skin damage. It causes damage to the collagen and blood vessels within the dermis, as shown in L. H. Kligman and A. M. Kligman's chapter. A series of histological and electron microscopic examinations also proved

that skin aging caused by light can be well differentiated from the atrophic phenomenon of elderly people sunscreened skin. Pathak, a professor from Harvard University analysed the skin's natural sunscreening mechanisms. He convincingly proved how much the skin's pigment content, according to which human-skin can be divided into VI types, can determine the occurrence of not only basalomas but also the still almost incurable melanoma. R.S. Stern (Harvard) established that the protective effect of sunscreening agents related to every geographical place and risk group result in a similar percentage of tumor reduction. When using sunscreens their side effects should be taken into account. Their occurrence is more predominant with those products with a higher sunscreen factor, as they are prepared from a combination of more materials and furthermore they are of a high concentration. Mainly contact sensitisation effect and more seldom photocontact allergy have been recorded. This problem, however, occurs in about 1-2% altogether. The monograph's special value is that for the reader who is not acquainted with photobiology it is also clear that every Caucasian person needs sunscreen. The proof that a considerable part of the radiation which is harmful to our health and later also responsible for cancer are endured during our childhood, is considerably striking. For this reason I strongly recommend the book for pediatricians and pediatric dermatologists.

J. Mátyás Baló

EDITORIAL.

Acta Paediatrica Hungarica published a paper entitled "Effects of prostaglandin E_2 on the newborn respiratory system" written by E. Princzkel, L. Vojcek, L. Karmazsin, LG. Lampé (Debrecen Medical University), AC. Turnbull (Oxford) in Vol. 31. number 3, pp 337-355, 1991. In March 1992 Professor Karl J. Hittelman, Ph.D., Associate Vice Chancellor, University of California, San Francisco informed the editor of Acta Paediatrica Hungarica that with all probability plagiarism has been committed by the above-mentioned article, being entirely identical with the original earlier paper of Guerra FA, RD Savich, LD Wallen, CH Lee, RI Clyman, FE Mauray and JA Kitterman "Prostaglandin E_2 causes hypoventilation and apnoe in newborn lambs" issued in Journal of Applied Physiology, 64 (5): 2160-2166, 1988. The reviewers of manuscript did not know about the Dr Kitterman's paper, thus the editor could not prevent the plagiarism. However, upon the letter of Professor Hittelman, immediate measures were taken and the alleged charge was investigated by the Ethical Committee of Debrecen Medical University. Unfortunately, the investigations of Professor L. Kovács, Vice Rector of University confirmed the act of plagiarism. Appropriate steps against Drs. E. Princzkel and L. Vojcek "authors" of the paper have been taken: they have been dismissed from the University. Professors Lampé and Karmazsin did not know about the previous paper of the Kitterman group and thought that the experiments had been carried out in Oxford. Drs Princzkel and Vojcek declared that Professor Turnbull who was their professional host in Oxford did not know anything about the manuscript and its submission to the Acta Paediatrica.

Together with the decision of the Ethical Committee of the Debrecen University and the issuing of this editorial we regard this regrettable case as closed.

M. Miltényi
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Prof. Dr. M. Miltenyi, Editor
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June 10, 1992

Dear Prof.Miltenyi:

My co-authors and I thank and commend the officials of Debrecen Medical University and Acta Paediatrica Hungarica for their rapid and thorough investigation of this sad and unfortunate incident and for their definitive action to rectify the situation. The responses of the University and the journal are tributes to the ethics and integrity of our colleagues in the Hungarian scientific community.

Joseph A. Kitterman, MD
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ESPE CLINICAL FELLOWSHIP IN PEDIATRIC ENDOCRINOLOGY*

The European Society for Pediatric Endocrinology has established a Clinical Fellowship. The aim is to promote the development of patient care and clinical research in pediatric endocrinology in Europe. The programme is primarily designed for European Centers where the possibilities for training in pediatric endocrinology are not available in the home situation. The fellowship consists of a 1-to 2-year clinical training in a European Center with longstanding tradition of excellence in pediatric endocrinology. Applications for a short-term fellowship aiming to improve quality in the management of a specific pediatric endocrine problem will also be taken into consideration. Financial support will be sufficient for a family. The deadline for application (i.e. submission of the completed application form) is **August 15, 1992** (postmarked). The fellowship will begin effectively in Spring, 1993.

Further information and application forms can be obtained from the chairman of the fellowship committee at the following address :

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* This programme is sponsored by Ares-Serono.

TEL AVIV UNIVERSITY אוניברסיטת תל-אביב
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DEPARTMENT OF PATHOLOGY החוג לפתולוגיה

TO PEDIATRICIANS TREATING INFANTS AFFECTED BY

WOLMAN DISEASE

SUGGESTIONS FOR EFFECTIVE TREATMENT

For over a year a female infant affected by Wolman disease and treated by the dietary treatment described below survived far beyond the 3-4 months predictable life span. Although otherwise normal, with time she developed EFA (essential fatty acid deficiency) which can be, and is now, treated by an established procedure which is not antagonistic to the dietary treatment.

The treatment consists of strict cessation of breast feeding and strict avoidance of foods containing any fats and oils (triglycerides and cholesterol esters). Formula feeding should be as free as possible of neutral lipids, but should contain all vitamins.

In order to avoid dermatological complications and stunted growth due to EFA deficiency, about 10 microliter (1/100 of ml) of sunflower oil should be lightly rubbed, once daily on the skin of one arm in infants below 5 kgs in body weight. Infants 5-10 kgs in weight should be treated by daily doses of 20-25 ml. In bigger children the dose should be about 2 mg/day/kg body weight. The site of oil smearing should be alternated on consecutive days between arms, forearms, thighs and legs.

At present it is not known whether this percutaneous administration of EFA should continue uninterrupted, or if 3-4 weeks of treatment should be followed by intermission of 1 or more weeks. It is proposed that this point should be tested by experience in different cases.

In order to be able learn about best treatment modalities from a sizable group of patients, it is suggested that treatment of patients and its effects be reported to me every 3-6 months. A report in the name of all participants (after consultation, of course) will be published in due time. Alternately, pediatricians of a country or an area can combine their efforts and results and publish them independently. Also in this case, I would be grateful for information about the cases, which information will be considered confidential.

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April 1, 1992

רמת אביב, תל-אביב 69978, ת.ד. 39040, טל. 5459811, 03-412275, פקסימיליה: 03-410173.

INSTRUCTIONS TO AUTHORS

Acta Paediatrica Hungarica publishes articles on clinical observations and original research of paediatric subjects and related fields. Articles are subject to editorial revision. Two complete copies of the manuscript including all tables and illustrations should be submitted. Manuscripts should be typed double-spaced with margins at least 4 cm wide, 25 lines to a page, 50 character spaces to a line. The first page should include the title, authors' names and name of the institution where the work was done. On a separate sheet an abstract of not more than 200 words should be supplied; it should not contain abbreviations or references.

All manuscripts must be accompanied by the following written statement, signed by each of the authors: "The undersigned authors transfer all copyright ownership of the manuscript entitled (title of article) to the Akadémiai Kiadó, Budapest in the event the work is published. The undersigned authors warrant that the article is original, is not under consideration by another journal, and has not been previously published. For the examinations included in the paper the permission of the local ethical committee has been obtained."

Abbreviations should be spelled out when first used in the text. *Drugs* should be referred to by their WHO code designation (Recommended International Nonproprietary Name); the use of proprietary names is unacceptable. The *International System of Units* (SI) should be used for all measurements except blood pressure.

References should be numbered in alphabetical order and only the numbers should appear in the text (in parentheses). The list of references should contain the name and initials of all authors (the use of et al instead of authors' names is not accepted). For journal articles the title of the paper, title of the journal abbreviated according to the style used in Index Medicus, volume number, first page number and year of publication should be given; for books, the title followed by the publishers and place and year of publication.

Examples:

Royer P: Metabolism and action of vitamin D in the fetus. Acta Paediatr Hung 25:161, 1984

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Index: 26.021

Acta Paediatrica Hungarica

VOLUME 32, NUMBER 3, 1992

Akadémiai Kiadó, Budapest

ACTA PAEDIATR HUNG HU ISSN 0231-441X

ACTA PAEDIATRICA

HUNGARICA

A Quarterly of the Hungarian Academy of Sciences

Editor

M. MILTÉNYI

Acta Paediatrica publishes original reports of studies in paediatrics and borderline fields.

Manuscripts and editorial correspondence should be addressed to

ACTA PAEDIATRICA HUNGARICA

Bókay J. u. 53.

H-1083 Budapest, Hungary

Subscription information

Orders should be addressed to

AKADÉMIAI KIADÓ

P.O. Box 245

H-1519 Budapest

Acta Paediatrica Hungarica is published in yearly volumes of four issuse by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences

Prielle Kornélia u. 19—35.

H-1117 Budapest

Acta Paediatrica Hungarica is abstracted/indexed in Biological Abstracts, Excerpta Medica, Index Medicus, La Presse Médicale

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PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

Acta Paediatrica Hungarica 32 (3), pp. 203-217 (1992)

UNIDENTIFIED MULTIPLE CONGENITAL ABNORMALITIES IN TWINS. A POPULATION-BASED HUNGARIAN STUDY

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Received 10 January 1992

Of 1038 index patients with multiple congenital abnormalities, 34 were twins. This 3.3 per cent is higher than the Hungarian birth rate of about 2.1 per cent. However, after the exclusion of cases with congenital abnormality association of low birth weight newborn infants and with genital anomalies of the male, the twin birth rate was 1.8 per cent. Thus, the unidentified multiple congenital abnormalities have no common cause with twinning.

INTRODUCTION

There is now a growing amount of evidence indicating that the frequencies of congenital abnormalities (CAs) may be higher in twins /e.g., 9, 10, 12, 14/. The general observations are the following: /i/ for CAs due to general origin, the concordance values decrease in the order: monozygotic twins (MZ), dizygotic twins (DZ) and singletons; for those due to polygenic origin, the concordance values are in the order: $CAs_{MZ} > CAs_{DZ} = CAs_{singletons}$ /9, 15/ /ii/ deformations occur more frequently as a consequence of uterine constraints in both MZ and DZ twins /4, 7, 11/ and /iii/ in MZ twins, there is an excess of two groups of CAs; the first of these are part of the MZ twinning such as conjoined twins, some amorphous twins and some early embryonic field defects (e.g., caudal regression-

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sirenomelia, holoprosencephaly /14/. The second group is related to and caused by differences in vascular interchanges between MZ twins (e.g., differences in vascular supply, reverse flow, vascular disruptions leading to limb defects, hemifacial microsomia etc. /15/.

Data suggestive of higher frequencies of multiple congenital abnormalities (MCAs) in twins have also been reported in the literature, but the differences appear to be small and non-significant /15, 16/. However, to our knowledge, the question of whether MCAs have some causal factors in common with twinning has not been systematically explored. Since twinning represents a special situation in which a number of factors (including spatial constraints in the uterus, shorter gestation period, low birth weight, etc.) play a role, it was thought of interest to undertake a population-based study to investigate whether there exists a causal relationship between twinning and unidentified MCAs.

MATERIAL AND METHODS

A multiple congenital abnormality (i.e., concurrence of two or more CAs in the same individual) is the end result of errors in two or more different morphogenetic processes. A population-based MCA evaluation program (within the framework of the Center for Congenital Anomaly Control) has been in existence in Hungary since 1973 the overall objectives of which are /i/ to accumulate data on CA-syndromes and CA-associations which are of value in prognosis and genetic counseling and /ii/ to provide sensitive indicators of teratogenic and mutagenic factors in the environment. To achieve these objectives, efforts are continuously made to improve the validity and completeness of registration of MCAs and to increase the rate of identified MCA-entities. Details on how the program operates are given in reference 5.

The MCA Registry comprised 2733 cases with unidentified MCAs between 1973 and 1980. Our plan was to study on 1384 cases, i.e., 50.6 per cent of the study material. A specially designed post-paid questionnaire with an explanatory letter was mailed to parents in 1982 and 1983. Mothers were asked to inform us about numbers, dates and outcomes of all their pregnancies and the health condition of their children. To be certain, we requested all medical documents concerning their pregnancies and their children's CAs. We accepted only those questionnaires in which every question concerning the CAs in first degree

relatives, especially those in sibs was correctly answered. When there were problems with the response, district nurses were asked to visit the families and seek clarification. /1/ 109 letters (7.9 per cent) proved to be undeliverable. /2/ In the remainder of the cases where there was no response, another questionnaire and letter were sent and we asked for the help of the district nurses in order to obtain the data of these families. However, 166 parents (12.0 per cent) either failed or refused to respond. Some of their children lived in foster homes or with foster parents. /3/ 71 cases were excluded from further analysis. /i/ Eight cases were registered twice. /ii/ Some misclassifications and a few misdiagnosis of MCAs were detected later, i.e., after the notification by clinicians in 31 cases. /iii/ Finally a nosological diagnosis was achieved by detailed clinical examination of different experts later 32 cases. (At the time of notification these recognizable MCA-entities were not identified.) Thus, the study sample involved 1038 children (including 34 twins) with unidentified MCA. In this approach, index cases, i.e., affected twins were designated as twins "A".

The birth data collected by the Hungarian Central Statistical Office for total births and healthy twins (1973-1980) and by Budapest Twin Registry (1970-1979) /13/ were used by some comparative analysis. For statistical evaluation of the data chi-square statistics ($X^2_{[y]}$) (with Yates' correlation), the Fisher's exact test (F_e) and t-test (with Welch modification for non-normal distribution of birth weights (t_w) were employed.

RESULTS

There were 34 twins in the study sample of 1038 cases with unidentified MCAs and this gives an occurrence rate of 3.28% which is significantly higher than the national figure of 2.06% for the study period (28 593 twins in 1 388 525 births; X^2 of 7.6; $0.05 > p < 0.01$). Their MCA profiles and birth weights are given in Table I. It can be seen that oout of the 34 twins, one was a concordant twin-pair (these were conjoined twins), 2 were semi-concordant (i.e., one component CA was found in twin "B") and the remainder were discordant (i.e., predominantly only the index twin "A" was affected; note there were 3 cases 3 "B" twins affected in this group). Thus, out of 33 (the conjoined twin pair is counted as one), there were 6 affected "B" children, giving a rate of a 18.2% /6/33/.

The sex-ratio of index twins is 0.545 (18 males out a total

of 33) and this corresponds to what has been known from studies of MCAs /5/. The sex-ratios for twins of unlike-sex and of like-sex are, respectively, 0.778 and 0.458. However, among twins "A" of the unlike-sex group, the number of affected females is less than expected; among affected twins "B", there is a male preponderance (sex ratio of 0.667).

A careful examination of the birth weights given in the last two columns of Table I will show that, within given pairs, these figures are generally similar, irrespective of whether the "B" twins have CA or not. In Fig. 1, the estimated mean birth weights of the index twins ("A") with MCAs with those of "B" twins with and without CAs are compared. Additionally, comparable data for other newborn groups are also presented. As can be seen, the mean birth weights increase from 1457 g for "B" twins with CA to 1782 g for the "A" twins with MCA to 1876 g for the "B" twins without CA. All these mean birth weights are lower than that (2280 g) for healthy twins (Budapest Twin Registry) and lower still relative to those of singletons with and without CA.

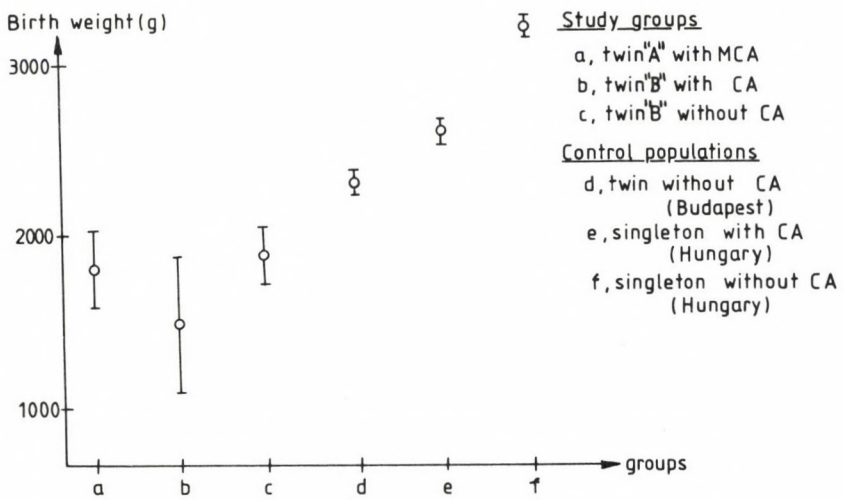

Fig. 1

Tests of significance in mean birth weights gave the following values:

/i/ "A" twins with MCAs versus healthy twins from the Budapest Twin Registry: (group a versus group d in Fig. 1) $t = 8.76$; $P \ll 0.001$.

/ii/ "B" twins without CA versus healthy twins (group c and d): $t = 4.68$; $P < 0.001$.

/iii/ Affected versus non-affected singletons (groups e and f): $t = 24.3$; $P \ll 0.001$.

For zygosity determination the most desirable method would be the examination of the actual genotype. However, it was not possible to do this in the majority of our cases either at birth or later because of the large number of stillbirths and perinatal deaths. It is well known that Weinberg method is applicable only to large populations. Nevertheless we attempted to use it for the estimation of zygosity in 33 twin pairs studied (Table III). The numbers of observed DZ and MZ was 18 and 15, respectively in the study sample. The observed number of MZ was higher than expected /11/, however, the difference was not significant ($P = 0.45 > p > 0.05$) because of small number of cases.

Our data permit an analysis of the common genetic component. All twin pairs are classified as like-sexed or unlike-sexed pairs. All unlike-sexed twin pairs are dizygotic. However, dizygotic pairs are equally likely to be like-sexed or unlike-sexed. The remaining like-sexed twin pairs are considered to be monozygotic. The numbers of concordant, semi-concordant and discordant pairs with MCA in unlike-sexed and like-sexed groups are shown in Table IV. As will be clear, there are no significant differences in the concordance between monozygotic and dizygotic twins. It would therefore seem that common genetic factors do not play a significant role in the origin of these unidentified multiple CAs. However, it should be borne in mind that identified CA-syndromes and CA-associations were excluded from this study material.

The so-called registry diagnosis was attempted in these unidentified MCAs on the basis of international literature

TABLE I

The data of twin index patients

	SEX "A"	SEX "B"	Component CAs "A"	Registry-diagnosis	"B"	Birth weight "A"	Birth weight "B"
	M	F	CCM /persistent ductus arteriosus/ + Hypospadias /glans/	LBW-association	-	2.350	2.100
	M	F	Hypospadias /penis/ + Cystic kidneys /bilateral/	-	-	1.400	2.200
xx	F	M	CCM /transposition of great vessels/ + Hypoplasia of lungs	-	Hypoplasia of lungs	1.200	1.100
	F	F	CCM /ventricular septal defect/ + congenital inguinal hernia /right/	LBW-association	/Mental retardation/	1.970	2.400
	M	F	CCM /persistent ductus arteriosus + atrial septal defect/ + Hydrocephalus	-	-	2.250	2.050
	M	M	CCM /persistent ductus arteriosus/ + Congenital inguinal hernias /bilateral/	LBW-association	-	1.300	1.500
	M	M	Spina bifida cystica + Exomphalos + Anal atresia	Extrophy of cloaca	-	1.670	2.050
	M	M	CCM /persistent ductus arteriosus/ + Hydrocephalus + Undescended testes /bilateral/	LBW-association	-	1.500	1.750
	M	M	Spina bifida cystica + Undescended testes /bilateral/	-	-	2.250	2.000

	M	F	Limb reduction /aplasia of tibia, right + clubfoot/ + poly-syndactyly of hand /right/	Hypoplasia of tibia with polydactyly syndrome	-	3.000	2.700
	F	F	CCM /atrial septal defect/ + Ear anomaly /+deafness/ + congenital inguinal hernia /left/	-	-	1.300	1.500
	M	M	CCM /atrial septal defect/ + Congenital inguinal hernias /bilateral/	-	-	1.450	1.750
	M	M	Congenital dislocation of hips /bilateral/ + Undescended testes /bilateral/ /Mental retardation/	-	-	1.500	1.500
	M	M	CCM /verticular septal defect/ + Teratoma /lipoma in temporal region, left/	-	-	1.700	2.000
y	F	F	Limb reduction /absence of metatarsus III., bilateral/ + CCM /ventricular septal defect/	-	Hydrocephalus	1.710	1.600
	F	F	Limb reduction /hands, absence of finger I-III., left, absence of II-IV., right/ + Other limb anomaly /elephantiasis, foot, left/ + Congenital dislocation of hip /right/ + Clubfeet /bilateral/	-	/Mental and somatic retardation/1	1.800	1.500
X	M	M	Exomphalos /total/ + CCM /ventricular septal defect/ + Cystic kidneys /multicystic, left/	Conjoined twin /CCM-CK/ association/	Exomphalös /persistent ductus arteriosus/ + Hydrocephalus + Cystic kidneys /bilateral/ /hydrops/	1.900	
	M	M	Exomphalos + CCM /persistent ductus arteriosus/ + Hydrocephalus + Cystic kidneys /bilateral/ /+hydrops/		Exomphalos /total/ CCM /ventricular septal defect / + Cystic kidneys /Multicystic, left/	950	

	SEX "A"	SEX "B"	Component CAs "A"	Registry-diagnosis "A"	Registry-diagnosis "B"	Birth weight "A"	Birth weight "B"
	F	F	CCM /ventricular septal defect/ + Congenital inguinal hernia /right/	LBW-association	-	1.250	1.750
	F	F	CCM /persistent ductus arteriosus/ + Congenital inguinal hernias /bilateral/	LBW-association	-	1.750	1.730
	M	F	CCM /ventricular septal defect/ + Hydrocephalus	-	-	1.900	1.600
y	F	M	CCM /persistent ductus arteriosus/ + Congenital dislocation of hips /bilateral/ + Teratoma /Cyst. of lig. testis/ + Congenital inguinal hernias /bilateral/	LBW-association	Cleft palate	1.050	1.350
	F	F	CCM /pulmonary stenosis/ + Dentatio praecox + /Umbilical hernia/	-	-	1.980	1.620
y	M	M	Hypospadias /glans/ + Congenital inguinal hernias /bilateral/ + umbilical hernia	GAM	CCM /Ventricular septal defect/	2.100	2.220
	M	F	Inguinal hernia /left/ + Undescended testes /left + aplasia testes/	GAM	-	2.900	2.250
	F	F	CCM /Ventricular septal defect/ + Congenital inquinal hernia /bilateral and umbilical hernia/	-	-	2.400	2.600
	M	F	Congenital inquinal hernia /left/ + Undescended testes /left + hydrocele/	GAM	-	2.750	2.400

	F	F	CCM /persistent ductus arteriosus/ + Megaloureter, left + Congenital inguinal hernia /left/	LBW-association	-	2.100	2.250
	M	M	CCM /persistent ductus arteriosus/ + Congenital inguinal hernias /bilateral/ /+Mental retardation/	LBW-association	-	1.000	1.100
	M	M	Cleft lips /bilateral +cleft palate/ + CCM /Tetralogy of Fallot/	-	-	1.150	1.400
	F	F	CCM /transposition of great vessels + aortic stenosis, valvular/ + Agenesis of kidneys /bilateral/	-	-	2.400	2.100
	F	F	CCM /persistent ductus arteriosus + ventricular septal defect/ + Congenital dislocation of hips /bilateral/	LBW-association	-	1.650	1.650
	F	F	CCM /persistent ductus arteriosis/ + Hypoplasia of abdominal muscles	Prune-belly defect	-	1.220	1.200
xx	F	F	Exomphalos + CCM /arterial septal defect, type II./	-	Exomphalos	1.800	1.100

CCM = congenital cardiovascular malformation
LBW = Ca-association of newborn with low birth weight
GAM = genital anomalies of the male
CCM-CK = congenital cardiovascular malformation - cystic kidney association
x = concordant
xx = semi-concordant
y = discordant

TABLE II

Sex ratio of twins A and twins B in the study sample

Twin	Like-sexed				Unlike-sexed				Total			
	M	F	Σ	Sex ratio	M	F	Σ	Sex ratio	M	F	Σ	Sex ratio
"A"	11	13	24	0.458	7	2	9	0.778	18	15	33	0.545
Affected "B"	2	2	4	0.500	2	0	2	1.000	4	2	6	0.667
Non-affected "B"	9	11	20	0.450	0	7	7	0.000	9	18	27	0.500
Subtotal	11	13	24	0.458	2	7	9	0.222	13	20	33	0.394

TABLE III

Concordance in estimated MZ and DZ twin pairs based on sex

Twin pairs	Unlike-sexed	Like-sexed			Total			$p/F_e/$
	DZ	DZ+	MZ+	Σ	DZ	MZ	Σ	
Concordant	0	0	1	1	0	1	1	0.45 NS
Semi-concordant	1	1	0	1	2	0	2	
Discordant	8	8	14	22	16	14	30	0.42 NS
Total	9	9	15	24	18	15	33	

+Calculated on the basis of Weinberg method

TABLE IV

Concordance in estimated MZ and DZ twin pairs based on sex

Twin pairs	Unlike-sexed	Like-sexed			Total			$p/F_e/$
	DZ	DZ+	MZ+	Σ	DZ	MZ	Σ	
Concordant	0	0	1	1	0	1	1	0.45 NS
Semi-concordant	1	1	0	1	2	0	2	
Discordant	8	8	14	22	16	14	30	
Total	9	9	15	24	18	15	33	

+Calculated on the basis of Weinberg method

(e.g., hypoplasia of tibia with polydactyly syndrome, [McKusick catalog: No 18877] and extrophy of cloaca) and our previous studies /1, 2, 5/. Of the 34 twins with MCAs, 7 had CA-association and low birth weight /5/, 3 genital anomalies in the male /2/, 2 congenital cardiovascular malformation-cystic kidney association /1/, one extrophy of cloaca and one hypoplasia of tibia with polydactyly syndrome. Thus different CA-entities were identified in 41.2% of twins with MCAs using registry diagnosis.

DISCUSSION

The occurrence of index twins "A" was significantly higher in our study sample of MCAs cases. Similar increases in MCAs in twins, (although non-significant) have also been reported in the literature /15, 16/. Differences in ascertainment and sample size may be responsible for differences between our studies and those of others.

The considerably higher rate of affected twins "A" among multimalformed babies (X^2 = 7.1; 0.05 > p < 0.01) is suggestive of some causal connection between twinning and MCAs. There is an increased likelihood for CAs in twin gestation because they are actually two persons from the same pregnancy /8/. In this way, the relative population frequency of DZ versus MZ twins of 2/3 to 1/3, may cause the incidence of any CA in twins which corresponds to 5/3 times incidence of singletons. To eliminate this problem, 34 cases of the study sample have to divide by 5/3, so the number of index twins "A" became only 20.4. It is not significantly higher than that of the population at large ($X^2_{[y]}$ = 0.05; P > 0.01).

Our analysis did not provide evidence for a role of mendelian factors or chromosomal aberration in the origin of unidentified MCA. It seems however that the excess of twins among unidentified MCA cases can be explained by the high occurrence of CA-association with low birth weight (LBW)

newborns and genital anomalies of the male (GAM). The lower birth weight and preterm birth which are characteristic for twins may have a triggering factor in the pathogenesis of these CA-entities. All cases with CA-associations with LBW and GAM were discordant in our study sample which is another argument against genetic liability. After the exclusion of this CA-association (10 cases), GAM (3 cases) and conjoined twins (2 cases), the occurrence of unidentified MCAs in twins is only 1.83 per cent it is even slightly lower than the Hungarian twin birth rate of 2.1%. The point is that after the exclusion of the effect of lower birth weight and preterm birth there is no causal connection between the genesis of twinning and unidentified MCAs.

In conclusion, the unidentified MCAs in our study sample do not provide evidence for common cause with twinning but suggest that twinning can trigger the combination of some CAs due to lower birth weight and preterm birth.

ACKNOWLEDGEMENT

We gratefully thank to Professor K. Sankaranarayanan, Silvius Laboratory, Leiden for the thorough review of the manuscript.

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EFFECT OF THYROXINE ADMINISTRATION ON RENAL FUNCTIONS IN NEWBORN INFANTS WITH PERINATAL ASPHYXIA

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Received 11 December 1991

The study was undertaken to assess the influence of thyroxine given to improve respiratory adaptation in asphyxiated neonates on the recovery of compromised renal functions.

Two groups of infants with perinatal asphyxia were selected for the study. Group I consisted of 8 infants treated conventionally, while Group II included 7 infants who in addition to standard therapy were administered 50 μg thyroxine at admission and repeated 24 hours later. Their respective mean gestational ages were 38.7 weeks (range: 34-42 weeks) and 37.4 weeks (range: 34-41 weeks). The studies were performed on days 1, 7 and 14 and the results compared to those obtained in 13 healthy neonates with the gestational age of 39.2 weeks (range: 38-41 weeks) (Group III).

Asphyxiated neonates had significantly higher plasma uric acid, xanthine, hypoxanthine and creatinine levels ($p < 0.05$), while their GFR proved to be markedly reduced ($p < 0.01$) when compared to the values of healthy controls. Moreover, there was a significant elevation of urinary excretion of NAGA ($p < 0.001$), urine osmolality ($p < 0.05$), PENa, FECa, RFI ($p < 0.05$) in infants presenting with perinatal asphyxia. Renal tubular responsiveness to aldosterone measured as TTKG was also found to be depressed ($p < 0.025$). In response to thyroxine therapy renal functional recovery appeared to be accelerated as indicated by the lower plasma creatinine level, lower rate of fractional electrolyte and urinary NAGA excretion and improved reactivity to aldosterone on days 7 and/or 14 as compared to those obtained in neonates presenting with asphyxia but without thyroxine therapy. The results seem to suggest that thyroid hormones may have an important role in the recovery of renal functions in newborn infants suffering from perinatal asphyxia.

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INTRODUCTION

Newborn infants suffering from perinatal asphyxia may have compromised renal functions resulting from renal vasoconstriction/hypoperfusion, direct hypoxic cellular injury, or both. Ischemic, toxic and obstructive acute renal failure has been shown to be associated with depleted cellular ATP levels and thyroxine treatment has been demonstrated to accelerate recovery of renal functions and restoration of cellular ATP pool /5, 17, 22/. In addition to these experimental data, the beneficial effect of thyroxine on impaired renal functions has also been substantiated in pediatric patients with shock-related acute renal failure /21/.

Several reports indicate that newborn infants with hyaline membrane disease have biochemical hypothyroidism /7, 10, 13, 15/, thyroid hormones enhance fetal lung maturation /3, 19, 20/ although thyroxine administration to neonates suffering from hyaline membrane disease and perinatal asphyxia does not appear to consistently improve respiratory functions /1, 2, 16/.

In view of these clinical and experimental evidences the present study was undertaken to assess 1./ the influence of thyroxine treatment on the recovery of glomerular and tubular functions in newborn infants with perinatal asphyxia and 2./ to determine whether the accelerated recovery of renal functions if any, is the result of improved respiratory functions or rather it may be accounted for by the direct effect of thyroxine on the kidney.

PATIENTS AND METHODS

Subjects

Two groups of newborn infants presenting with perinatal asphyxia and a group of healthy neonates were selected for the study. The diagnosis of perinatal asphyxia was made in infants who had Apgar score of 7 or less at five minutes of age. Asphyxiated infants received intermittent positive pressure ventilation through endotracheal tube or face mask and $NaHCO_3$ bolus in a dose of 1-2 mEq/kg of solution containing 4.2%

$NaHCO_3$ and 20% glucose in water. After this treatment in the delivery room they were transported to our perinatal intensive care unit where they were alternately assigned to either conventional therapy (supplemental oxygen and intravenous fluid administration, Group I) or to conventional therapy completed with thyroxine (Group II). 50 µg thyroxine was given on admission and repeated the same dose 24 hours later (L-Thyroxine, Henning, Berlin) as applied by Amato et al /1/.

Respiratory support, arterial blood pressure (Kontron Ultrasonic Blood Pressure Monitor), transcutaneous pO_2 and pCO_2 value (Hellige Oxacapnomonitor) and acid-base balance (Radiometer ABL 330) have been routinely monitored. There was no statistically significant difference between the two asphyxiated groups with respect to pregnancy complications, instrumental delivery, birth weight, gestational age, one- and five-minute Apgar scores, age on admission, intravenous fluids received prior to and following entry into the study and need for respiratory support to maintain PaO_2 greater than 50 Hgmm. The healthy neonates (Group III) were cared for in the maternity unit. They were exclusively breast-fed and only 5% glucose in water was added to provide the required fluid intake. There was no significant difference between gestational age of the three groups of newborns. The clinical data of the patients studied are presented in Table I.

Study design

The study was carried out on days 1,7 and 14 to determine the perinatal asphyxia-related changes in renal functions and the influence of thyroxine administration on their postnatal course. For assessing the severity of hypoxic insult the patients survived plasma levels of xanthine, hypoxanthine and uric acid /4/ were measured. To compare the postnatal development of glomerular and tubular functions in the different groups 24-hour urine collection was performed and blood was taken at midpoint to determine glomerular filtration rate, fractional excretion of sodium, potassium and calcium, urine flow rate, as well as urine and plasma osmolality.

Laboratory assays and calculations

Plasma and urine sodium and potassium were determined by flame photometry, creatinine by the modified Jaffe method /11/ and osmolality by freezingpoint depression using Knauer osmometer. Calcium concentration in the plasma and urine was measured complexometrically. Endogenous creatinine clearance, fractional electrolyte excretion and renal failure index (RFI) were calculated using standard formulae. To estimate renal tubular responsiveness to aldosterone the recently introduced transtubular potassium gradient (TTKG) was calculated according to the formula of West et al /25/ as follows:

$$TTKG = \frac{\text{K urine: (U/P) osmolality}}{\text{plasma K}}$$

Urinary aldosterone excretion measured by radioimmunassay /23/ was used as an estimate of daily aldosterone excretion.

Hypoxic tubular injury was further defined by determination

TABLE I

Clinical characteristics of newborn infants with perinatal asphyxia receiving conventional (Group I) or conventional plus thyroxine therapy (Group II) and those of healthy control neonates (Group III) Data are given as means and ranges of individual values

Group	n	GA weeks	BW g	Apgar score 1 min	5 min	positive pregnancy history	complicated delivery	age at admission hr	Oxygen head box	delivery CPAP	PEEP	duration of O_2 therapy days	neurological symptoms at follow-up
I	8	37.1 (34-42)	2774 (1900-3800)	2.1 (1-4)	5.3 (4-7)	3	2	4.3 (1-9)	3	3	2	2.3 (1-5)	3
II	7	37.4 (34-41)	3106 (2350-4300)	2.4 (0-4)	5.0 (3-7)	4	3	2.9 (1-6)	2	3	2	2.8 (1-7)	3
III	13	39.2 (38-41)	3652 (2710-3750)	9.0 (8-10)	9.2 (8-10)	1	0	-	-	-	-	-	-

BV = birth weight
CPAP = continuous positive airway pressure
GA = gestational age
PEEP = positive end-expiratory pressure ventilation

of urinary excretion of NAGA, a lysosomal enzyme, which is released from the damaged proximal tubular cells /8/. For statistical evaluation the paired and unpaired Student's t-tests were used and the values were presented as means ± SEM. Approval of the institutional ethical committee and informed parental consent were obtained for the study.

RESULTS

The time of supporting respiration was the same in both groups with asphyxia (3-7 days). In case of CPAP ventilation the PEEP was 4 H_2Ocm, the FiO_2 was 1.0 - 0.4 in both groups, respectively. The parameters of IPPV ventilation were the following: in the Group I: FiO_2: 1.0-0.4, PEEp: 4-1 H_2Ocm, PIP_{max}: 35 H_2Ocm, frequency: 60-20/min, in the Group II: FiO_2: 1.0-0.35, PEEP: 4-2 H_2Ocm, PIP_{max}: 32 H_2Ocm, frequency: 60-20/min.

Plasma xanthine, hypoxanthine and uric acid levels as markers of hypoxic stress are shown in Table II. It can be seen that in response to perinatal asphyxia there was a marked increase in plasma xanthine level which was followed by a steady decline, the rate of decline, however, was more rapid in infants on thyroxine treatment. Similarly, plasma hypoxanthine level tended to be elevated in infants presenting with asphyxia on day 1, it fell with advancing age, the fall being more pronounced in infants receiving thyroxine. Plasma uric acid level proved also to be higher in infants recovering from perinatal asphyxia, thyroxine therapy had no apparent influence on its subsequent fall.

Table III demonstrates that as a result of higher fluid intake urine flow rate was higher in infants of asphyxiated than in the control group and increased with age irrespectively of thyroxine therapy. Urine osmolality decreased accordingly without discernible difference between the groups with or without thyroxine administration. Plasma creatinine concentration was significantly elevated in asphyxiated neonates and it remained at about the same level all-over the study. In contrast, when thyroxine therapy was applied plasma creatinine concentration declined at a constant rate until the end of the 2nd week to approach the values of healthy neonates.

TABLE II

Plasma xanthine, hypoxantine, and uric acid levels of newborn infants with perinatal asphyxia receiving conventional (Group I) or conventional plus thyroxine therapy (Group II) and those of healthy control neonates (mean ± SE)

Group	Postnatal age (days)	Xanthine (μmol/l)	Hypoxanthine (μmol/l)	Uric acid (μmol/l)
I	1	26.0 ± 3.4[a]	50.0 ± 11.9	545.2 ± 35.9[a,b]
	7	17.9 ± 2.9	45.6 ± 11.7	215.1 ± 13.5[b]
	14	10.0 ± 1.4[a]	33.2 ± 8.0	148.3 ± 14.5[a]
II	1	23.5 ± 1.7[a,b,A]	56.0 ± 9.4[c]	579.0 ± 81.8[a,b]
	7	7.0 ± 0.9[b]	22.7 ± 4.0[c]	215.8 ± 40.2[b]
	14	10.4 ± 2.0[a]	22.9 ± 3.7	136.6 ± 31.8[a]
III	1	18.0 ± 1.5[A]	42.9 ± 2.3	504.5 ± 34.1[c]
	7	16.7 ± 1.4	41.6 ± 2.2	284.9 ± 32.9[c]
	14			

Significant differences within groups (a,b,c)
Significant differences between groups (A)
a,b: $p < 0.001$
c,A: $p < 0.05$

TABLE III

Parameters of renal functions in newborn infants with perinatal asphyxia receiving conventional (Group I) or conventional plus thyroxine therapy (Group II) and those in healthy control neonates (means ± SE)

Group	Postnatal age (days)	Urine flow rate ml/kg	Urine osmolality mOsm/kgH_2O	Plasma creatinine µmol/l	GFR ml/min.x1.73m^2
I	1	2.0 ± 0.3^{a,b,B}	179.2 ± 18.6^{a,c}	80.8 ± 3.7^{d,B}	21.6 ± 2.8^{B}
	7	3.3 ± 0.4^{b,c}	108.3 ± 18.0^{c,C}	107.6 ± 11.3^{d,C,E}	18.2 ± 1.4^{c,C,E}
	14	3.7 ± 0.5^{a,c}	73.2 ± 8.0^{a}	81.7 ± 11.3	32.5 ± 5.0^{c}
II	1	1.4 ± 0.4^{a,b,A}	210.8 ± 21.9^{a,c}	74.9 ± 8.9^{A}	24.8 ± 2.2^{F}
	7	3.3 ± 0.5^{b,C,D}	111.9 ± 21.4^{c,C}	66.7 ± 9.1^{C}	30.2 ± 3.7^{C}
	14	3.4 ± 0.5^{a}	74.5 ± 20.4^{a}	58.8 ± 5.4	34.2 ± 6.3
III	1	0.2 ± 0.04^{b,A,B}	233.8 ± 30.9^{b}	52.4 ± 1.9^{A,B}	34.3 ± 2.4^{F,B}
	7	1.6 ± 0.2^{b,D}	84.1 ± 9.2^{b}	50.3 ± 4.2^{E}	41.8 ± 2.7^{E}
	14	-	-	-	-

Significant differences within groups (a,b,c,d)
Significant differences between groups (A,B,C,D,E,F)
a,b,B,C,E,F: $p < 0.001$
d,A: $p < 0.025$
C: $p < 0.01$

Approximately reciprocal changes were seen in endogenous creatinine clearance: i.e. it was depressed in asphyxiated neonates and appeared to recover earlier when thyroxine was given.

Table IV summarizes the results of determination of several markers of renal tubular functions. Urinary NAGA excretion was about three times higher and remained practically unchanged in infants presenting with asphyxia when compared with that of one-week-old healthy neonates (because of technical reasons we could not get values on the first day). Thyroxine administration had no influence on its postnatal course. The fractional sodium excretion in the asphyxiated newborns was in the normal range as well, but the decrease was faster in those treated with thyroxine. Fractional potassium excretion was about the same in all groups irrespective of age, perinatal asphyxia and thyroxine therapy. Fractional calcium excretion in asphyxiated neonates exceeded that of healthy controls, however its age-related changes were not significantly influenced by thyroxine. Essentially the same pattern was seen for renal failure index: it was elevated in infants with asphyxia and the rate of its decline remained unaffected by thyroxine.

Renal tubular responsiveness to aldosterone was estimated by calculating TTKG as reliable measure of aldosterone-induced distal tubular potassium secretion and by the simultaneous measurement of daily aldosterone excretion (Figure 1). When compared to healthy neonates TTKG in asphyxiated newborn infants indicated impaired distal tubular potassium secretion. In response to thyroxine treatment only a slight, statistically insignificant improvement occurred. The failure of the distal tubule to secrete potassium after perinatal asphyxia becomes even more evident when the simultaneous daily aldosterone excretion is considered. Infants suffering from perinatal asphyxia had significantly increased urinary aldosterone excretion, therefore higher TTKG could have been expected. In face of the increased urinary aldosterone excretion the markedly depressed TTKG in infants with asphyxia can be regarded as a clear evidence for the asphyxia-related severe impairment of this distal tubular function.

TABLE IV

Parameters of renal tubular functions in newborn infants with perinatal asphyxia receiving conventional (Group I) or conventional plus thyroxine therapy (Group II) and those in healthy control neonates (means ± SE)

Group	Postnatal age (days)	Urinary NAGA excretion (μmol/day)	FENa (%)	FEK (%)	FECa (%)	RFI
I	1	48.0 ± 6.3	0.48 ± 0.17	6.1 ± 0.6	0.7 ± 0.1^{B}	1.1 ± 0.5^{a}
	7	49.3 ± 7.8^{E}	0.46 ± 0.08^{F}	4.7 ± 0.7	1.0 ± 0.1^{E}	0.6 ± 0.2^{c}
	14	52.9 ± 10.8	0.24 ± 0.09	4.3 ± 0.7	0.6 ± 0.1	0.2 ± 0.02^{a,c}
II	1	42.0 ± 13.5	0.45 ± 0.10^{b}	5.5 ± 1.0	0.5 ± 0.1	1.0 ± 0.3^{a,A}
	7	56.9 ± 11.8^{D}	0.37 ± 0.08	4.9 ± 0.9	0.8 ± 0.1^{C}	0.8 ± 0.2^{d}
	14	47.7 ± 17.1	0.14 ± 0.05^{b}	4.5 ± 0.9	0.8 ± 0.3	0.2 ± 0.07^{a,d}
III	1	-	0.34 ± 0.08	6.4 ± 0.3	0.3 ± 0.1^{B}	0.3 ± 0.07^{A}
	7	15.5 ± 5.1^{D,E}	0.23 ± 0.03^{F}	7.1 ± 0.7	0.4 ± 0.1^{C,E}	0.3 ± 0.07
	14	-	-	-	-	-

Significant differences within groups (a,b,c,d)
Significant differences between groups (A,B,C,D,E,F)
a,A,D,E: $p < 0.001$
b,B,C: $p < 0.02$
d,F: $p < 0.025$
c: $p < 0.01$

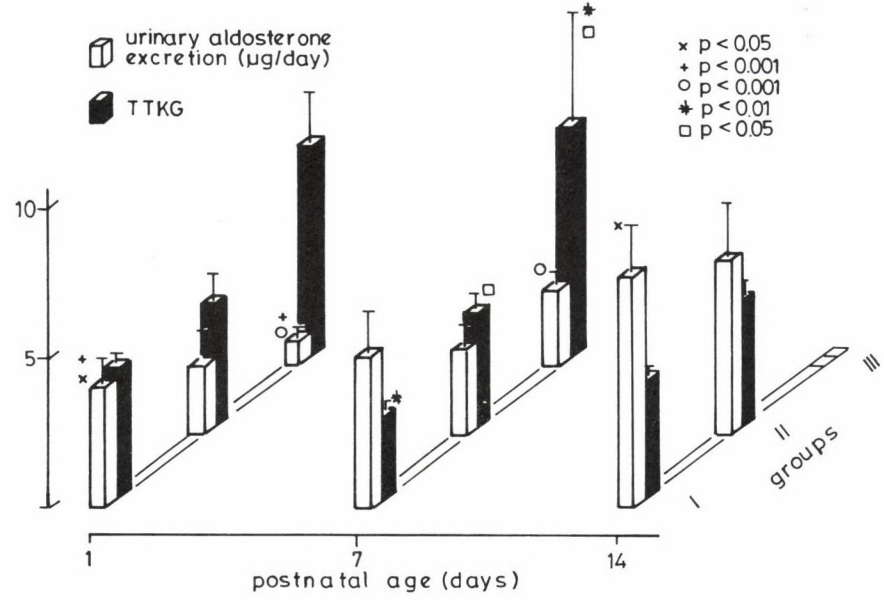

Fig. 1. Urinary aldosterone excretion and TTKG in newborn infants with perinatal asphyxia receiving conventional (Group I) or conventional plus thyroxine therapy (Group II) and those of healthy control neonates (Group III), tertial bars represent standard error of the mean

DISCUSSION

The results presented in this study provided evidence that thyroxine administration in the immediate postnatal period to neonates surviving perinatal asphyxia had no apparent influence on respiratory adaptation as judged by the unaltered oxygen requirement and respiratory support. On the other hand, thyroxine therapy was demonstrated to accelerate the recovery of GFR and some tubular functions. It seems reasonable to assume, therefore, that the beneficial effects of thyroxine on renal functions are unlikely the result of improved oxygenization but rather they may be accounted for by its direct stimulation of cellular metabolism within the kidney.

In spite of the fact that considerable body of evidence supports the view that thyroid hormones accelerate lung maturation, recent clinical study by Amato et al failed to demonstrate any beneficial influence of postnatal thyroxine administration on the course of idiopathic respiratory distress syndrome in premature infants /1/. The results of our present study are consistent with this observation and the experimental protocol we applied provided us the unique opportunity to get information about the possible role of thyroxine in the recovery of renal functions from hypoxic depression in asphyxiated human neonates. The hypoxic renal impairment caused by perinatal asphyxia was indicated by the significantly elevated plasma creatinine level and decreased GFR and by the increased urinary excretion of NAGA, increased fractional excretion of sodium and calcium and by the increase of RFI. In an attempt to define the effect of asphyxia on distal tubular function we calculated TTKG a reliable marker of renal tubular responsiveness to aldosterone. In agreement with several recent reports we could demonstrate that GFR and some indices of proximal tubular function recovered or approached to the control value earlier when thyroxine was given. Concerning the influence of thyroxine on the renal response to aldosterone apparent conclusion can not be drawn. The decrease of TTKG was similar in both groups of asphyxiated neonates, however, the daily aldosterone excretion in the thyroxine-treated infants

was lower than in those without thyroxine. Nearly identical TTKG-values in face of reduced rate of aldosterone production may imply some improvement of renal tubular aldosterone reactivity.

Great effort has been made to define the cellular mechanisms leading to ischemic renal damage and to accelerated recovery after thyroxine administration. Recent data from studies on pathophysiologic events during acute ischemic cell injury have revealed that reduction of cell ATP, elevation of cytosolic Ca^{++}, phospholipid degradation and abnormal production of reactive oxygen metabolites are the major factors responsible of cellular level for the complex process of hypoxic acute renal failure /24/. It has been demonstrated that cellular high energy phosphate, in particular ATP pool is markedly depleted and the ability of the kidney to resynthesize ATP is diminished following acute renal injury /12/. ATP provides energy for vital cell functions including membrane-bound Na-K-ATP-ase activity which is responsible for regulating sodium and water transport across cell membranes and maintaining cellular integrity. Since treatment of acute renal failure with either ATP-$MgCl_2$ or thyroxine resulted in almost identical accelerated restoration of cellular ATP levels and recovery of renal function, it is reasonable to assume that this salutary effect of thyroxine is mediated through repletion of cellular ATP pool /18/. As an alternative possibility it has also been suggested that thyroxine stimulates incorporation of phospholipide into cell membrane /3/, therefore it may contribute to the maintenance of membrane stability and cellular integrity /6/.

In conclusion, our results show that thyroxine administration to neonates presenting with perinatal asphyxia may accelerate the recovery of compromised renal functions without significant influence on pulmonary adaptation. In view of this favourable effects of early thyroxine therapy further studies are needed to define more clearly the criteria for the selection of patients to and the optimal timing and dosage of thyroxine administration.

ACKNOWLEDGEMENT

The authors thank the HENNING GmbH, Berlin for providing the thyroxine.

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Acta Paediatrica Hungarica 32 (3), pp.235-239 (1992)

BICARNESINE-TREATED CARNITINE DEFICIENT MYOPATHY: CLINICO-CHEMICAL INVESTIGATIONS

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Received 29 November 1991

Authors report on a Bicarnesine replacement therapy in an infant girl patient suffering from carnitine deficient myopathy diagnosed at 1 year of age. The hypotonic patient's motoric functions improved and she became able to walk as a result of therapy applied, but the pathological process generalized to encephalomyopathy. Free and esterified carnitine were determined from the serum and muscle biopsymaterial. After the Bicarnesine-supplementation the serum carnitine fractions elevated.

INTRODUCTION

Carnitine deficiency as a lipid storage myopathy was first published by Engel and Angelini /7/. Isolated muscle type /1/a, 1/b, 13, 14, 6/ systhemic carnitine deficiency /12, 8/ and secondary carnitine deficient syndromes were differentiated.

The molecular weight of the quaterner amine carnitine is 162 Dalton. That is synthetized in liver tissue and transported by carnitine acyltransferase to the cytosol taking part in the transport of the long chain fatty acids as a carrier through the mitochondrial membranes.

The case of a carnitine deficient girl patient will be published discussing the clinical chemical diagnostic methods and the therapeutic possibilities.

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CASE REPORT

O.G. (b.29.Aug.1982) female patient's familial and perinatal history was negative (birth weight 3050 g). In the late newborn stage (from 12th day) she had apnoeic attacks, transitoric systolic heart murmur, hepatomegaly, generalized hypotonia, paradox ventillation movements, CO_2 retention. She was suspected to have metabolic myopathy.

Laboratory findings: serum CK 37 U/l, KN 3 mmol/l, uric acid 317 µmol/l, Na 133 mmol/l, K 5.6 mmol/l, Cl 102 mmol/l, gamma-GT: 25 U/l, alk.phosphatase 76 U/l, serum and urine aminoacid-chromatography normal.

Ophthalmological findings:strabismus convergens, epicanthus, fundus oculi -10.0 D myopia. CSF tapping results were as follows: Pándy negative, total protein, glucose in CSF normal, lactic acid 0.6 mmol/l, pyruvate 34 µmol/l (normal), - se lactic acidosis and subacute necrotising encephalopathy (Leigh's) can be excluded. Serum lactate 1.1 mmol/l, pyruvate 107 µmol/l (normal), NH_3 50 gamma%, arylsulphatase 33.6 U (normal).

EGG: monotonous fast theta activity without any specific abnormality. The only sign of the maturation of her cortical activity during the last 6 years - between 3 and 9 years of age - is an increased response (desynchronisation) for photic stimulation. - Electromyography: neurogen atrophy. ENG: n. peroneus mcv 20 m sec on the left side, DL not measurable, without any denervation signs. - Muscle biopsy was made at 1 y. of age, after detecting the carnitine deficiency Bicarnesine (Labaz Laboratoire, solution 100 mg/kg/day) substitution was given. After 8 months of carnitine substitution the level of serum free and esterified carnitine was found elevated. Muscle biopsy: No 3365/1983: the diameter of the muscle fibers decreased, 6-12 µ, these had normal polygonal shape, except some ovaloid or circle ones showing so-called acidophil necrosis. Hypertrophized muscle fibers were not found. Mild perivascular fibrosis was seen, polymyositis, malignancy and lipid storage myopathy can be excluded.

Dg: Muscle atrophy in early stage.

Echocardiography normal, no cardiomyopathy.

The girl is mildly retarded but has developed virtually no speech. Audiometry verified severe sensorineural hearing loss. A hearing aid was provided at three years without any success in speech. From four years on she attended special nursery class where she is being trained in the use of the Bliss symbolic language system to assist communication.

Biochemical investigations and results

The free and esterified carnitines (O-acylcarnitine) were measured from serum and muscle biopsy specimen according to Bieber's and Lewin's method /2/. The serum carnitine fractions were extremly decreased, the free carnitine was not detectable in the muscle tissue, while the esterified carnitine level diminished as comparad to the controls (Table I).

TABLE I

Biochemical results

	Carnitine free µmol/l	Carnitine esterified µmol/g
Controls (n=10)	32.1	44.6
serum	S.D. ± 12.4	± 15.1
muscle (n=5)	1.25	1.3
	S.D. ± 0.44	± 0.56
O.G. (patient) serum	2.11	11.2
muscle	0.0	0.32
O.G. (patient) 8 months after starting of Bicarnesine treatment serum	18.6	26.22

DISCUSSION

Our girl patient proved to be a case of primary type carnitine deficiency excluding the secondary types (hepatic fibrosis, haemodialysis). The initial muscle type carnitine deficiency in spite of the delayed introduction of carnitine supplementation progrediated, so generalized carnitine deficiency the so-called carnitine deficient encephalomyopathy has developed.

Ware et al.'s /17/ case (14 y.o. male patient with systemic carnitine deficiency) showed hypoglycemia, hepatic laesion and progressive myopathy. In the primary carnitine deficient myopathic cases the muscle carnitine was under 20% of the controls /4/. The carnitine supplementation may be particularly effective /3/.

Lipid storage myopathies might occur without any enzyme deficiency /11/. Laesion of the beta-oxidation of the fatty acids does not lead to lipid storage myopathy /16/.

In our case lipid storage myopathy was not detected and carnitine acyl-transferase activity was not determined.

DiMauro and DiMauro /5/ have published muscle carnitine palmitoyltransferase deficiency with myoglobinuria, others have described /10/ partial deficiency of the above mentioned enzyme.

Early diagnosis of the genetically determined carnitine deficiency would be important as prognostic factor.

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Acta Paediatrica Hungarica 32 (3), pp. 241-255 (1992)

DECREASE IN THE ANTIOXIDANT CAPACITY OF RED BLOOD CELLS IN CHILDREN WITH CELIAC DISEASE

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Received 3 January 1992

The erythrocyte glutathione metabolism of 11 children with acute celiac disease (CD), 11 children under gluten free diet with CD and 5 children with cow's milk allergy was compared to that of 11 children with nutritive iron deficiency and to 22 healthy children as controls. Erythrocyte glutathione (GSH) content of celiac children was elevated and the glutathione disulfide (GSSG) level was significantly decreased as compared to normal controls. Erythrocyte GSSG/GSH ratio in acute CD differed also from the one in iron deficiency. In vitro oxidative load of acetylphenylhydrazine proved the impaired glutathione stability of the erythrocytes in acute CD and cow's milk allergy. A parallel rise of methemoglobin and hemichrome level of blood cells was seen. Further on, the selenium content of the red blood cells of CD patients decreased. All alterations of the erythrocyte tended to normalize during the dietetic period.

These data suggest a reduced protective capacity of erythrocytes in CD and in cow's milk allergy in childhood against oxidizing stresses.

INTRODUCTION

Several lines of evidence indicate an immunologic mechanism for the gluten sensitivity. The pronounced mucosal changes may develop a few hours after gluten challenge /5/ and during this process local inflammatory changes /9, 17/ take place, as well as a significant increase of neutrophil chemotactic activity of sera and a degranulation of mast cells /14/. Inflammatory cells are known to induce free radical production which in

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themselves can cause direct damage to biological compounds if protective mechanisms are impaired.

We wanted to study the relation between the tissue damage induced by gluten and the signs of oxidant injury of patients with celiac disease (CD). Understanding the role of toxic O_2 metabolites in complex biological systems has been limited by the lack of direct methods for detecting this highly reactive compounds in vivo. Therefore we investigated the glutathione redox system, oxidative derivates of hemoglobin (methemoglobin and hemichrome) and selenium content of erythrocytes of celiac patients in acute period, after gluten free diet and, gluten challenge parallel with histological examinations.

MATERIALS AND METHODS

22 children with CD (corresponding to the ESPGAN criteria) /18/, further on 5 patients with cow's milk protein intolerance, 11 children with nutritive iron deficiency and 26 healthy children before minor surgical intervention were studied. Of 22 CD patients, 11 had active disease and 11 were in remission. In all CD patients and in 2 cases with cow's milk protein intolerance, small bowel biopsy was performed. At the same time a 2 ml blood sample, by adding EDTA, was taken for biochemical investigations.

To assess the selenium status, in another series of patients, altogether 24 children with CD on gluten containing diet and 25 CD patients on gluten free diet for at least 1 year and 15 matched healthy children were investigated.

The concentration of reduced glutathione (GSH) and oxidized glutathione (GSSH) was measured by specific glutathione reductase enzyme reaction immediately after blood sampling by the method of Tietze /27/. The value of GSSG was defined separately according to Akerboom and Sies /3/, after alkylation of GSH with N-ethyl-male-imide (NEM). The removal of NEM was performed by Sephadex gel-filtration. The stability of GSH was measured by Beutler's original method /8/. The incubation of whole blood in 5 mg/ml acetyl-phenylhydrazine lasted for 1 hour at 37°C. The oxidation state of hemoglobin was defined according to Szebeni et al /26/ by reading the light absorption spectra of the previously refrigerated haemolysate at 560, 600, 630 and 700 nm. Haemolysate of washed erythrocytes was used to determine the selenium concentration by a fluorometric method using 2,3-diaminonaphtalene as the complexing agent /15/.

The statistical significance between the determined parameters was analysed by Student's "t" test. Differences at $p < 0.05$ were considered significant.

These studies were approved by the Scientific Committee and the Ethical Council of the Szeged University, and by the Division of Clinical Pharmacology of the Hungarian National Pharmaceutical Institute.

RESULTS

The reduced glutathione levels of erythrocytes in control and celiac patients are presented in Fig. 1. The results are expressed in μmol/g hemoglobin. GSH content was higher in red blood cells of CD patients and of nutritive iron deficiency cases, respectively, as compared to the healthy control group. Red cell oxidized glutathione (GSSG) also proved to be elevated in the nutritive iron deficiency group compared to normal controls, while patients with CD showed markedly lower values (Fig. 1/B). The erythrocyte GSSG/GSH redox ratio of children with CD also decreased significantly compared to the control groups during the course of the diet (Fig. 1/C).

In vitro treatment with acetyl-phenylhydrazine (APH) caused a significant decrease in the glutathione content of erythrocytes of CD children on gluten challenge and of patients with cow's milk allergy, while during gluten free diet the values of CD children did not differ from those of controls (Fig. 2). During diet, the rise of erythrocyte glutathione stability could be proved in 6 patients with CD and in one case of cow's milk allergy (Fig. 3).

Gluten and milk load respectively induced an increase of the oxidation derivates of hemoglobin. There was a marked elevation in the values of instable methemoglobin and hemichrome in blood, while these alterations disappeared during the diet (Fig. 4).

Fig. 5 presents the selenium (Se) content of the washed erythrocytes of CD patients and of healthy controls. The Se level of red blood cells of CD patients on gluten challenge was 51% lower than in the control group (228 $\pm$ 158 ng/gHb and 465 $\pm$ 136 ng/gHb, respectively; $p < 0.001$). During one year or more of gluten free diet, blood selenium increased by 15% (298 $\pm$ 149 ng/gHb); however it did not reach the full normal

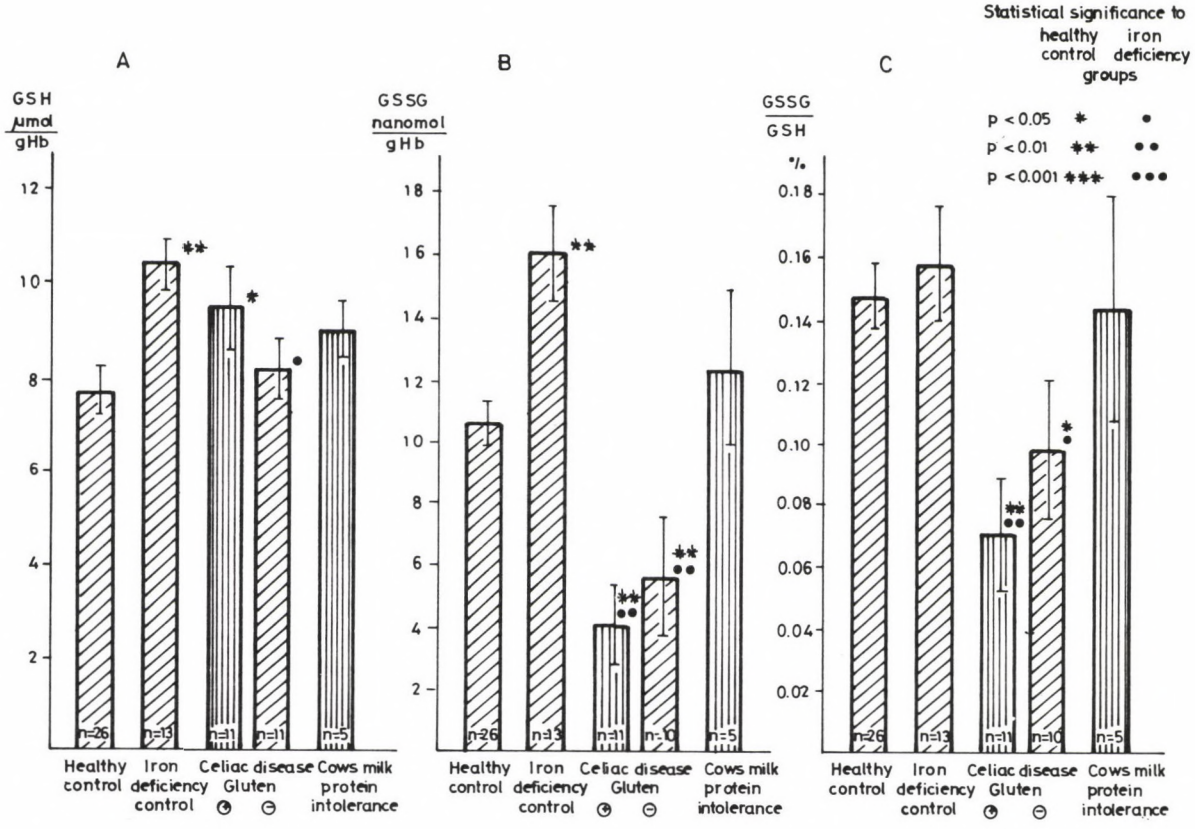

Fig. 1. Reduced and oxidized glutathiones of erythrocytes and their molar ratio ($\bar{X} \pm SE$)

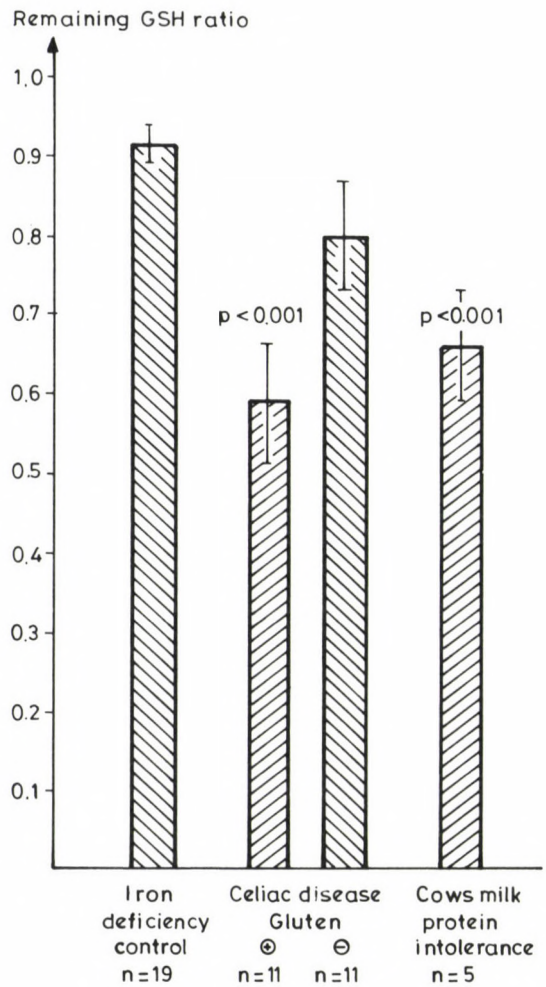

Fig. 2. Change in glutathione stability in red blood cells induced by acetyl-phenylhydrazine ($\bar{X} \pm SE$)

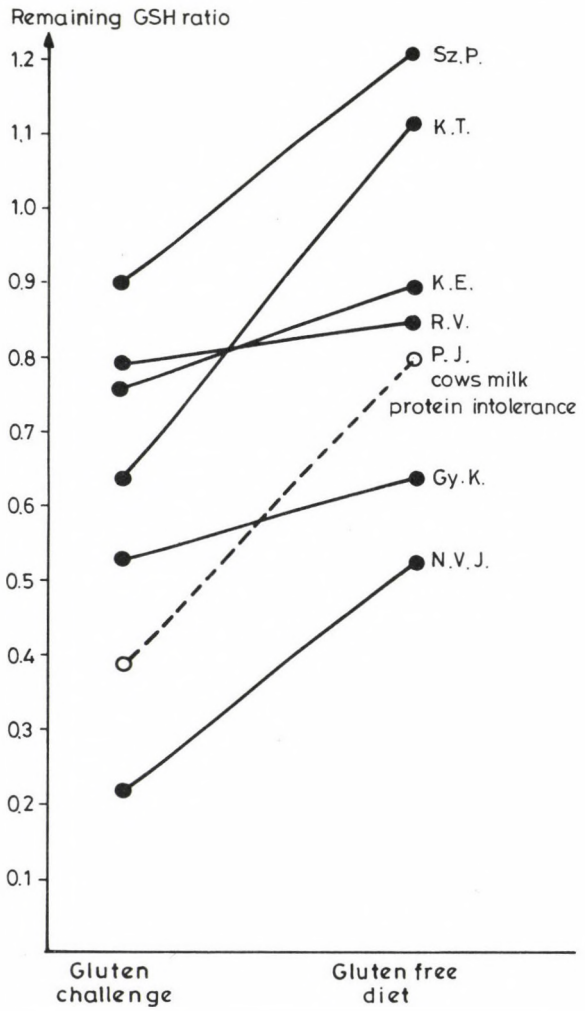

Fig. 3. Change in glutathione stability of erythrocytes in celiac patients on gluten free diet

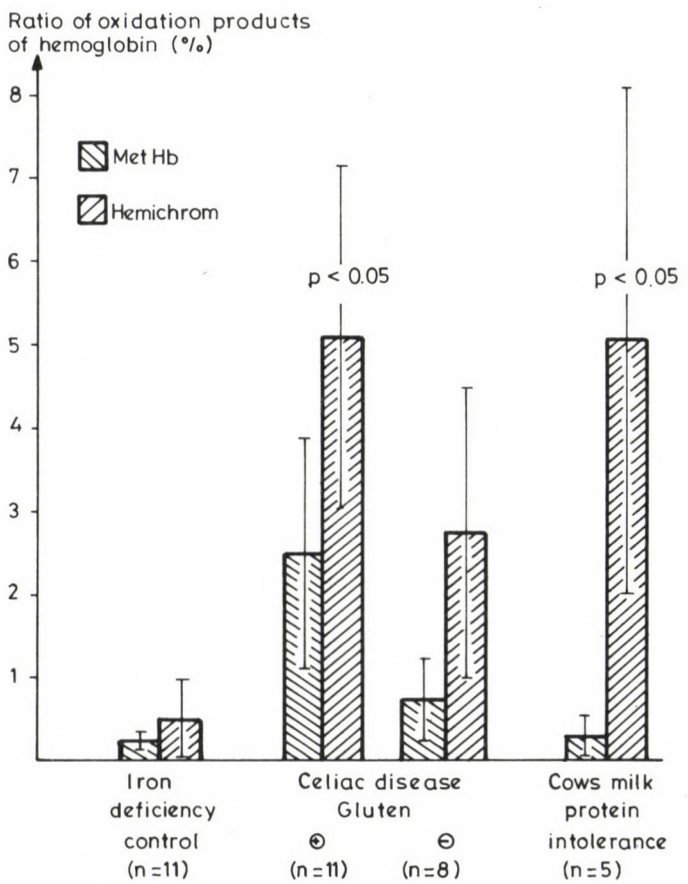

Fig. 4. The ratio of the oxidation products of hemoglobin ($\bar{X} \pm SE$)

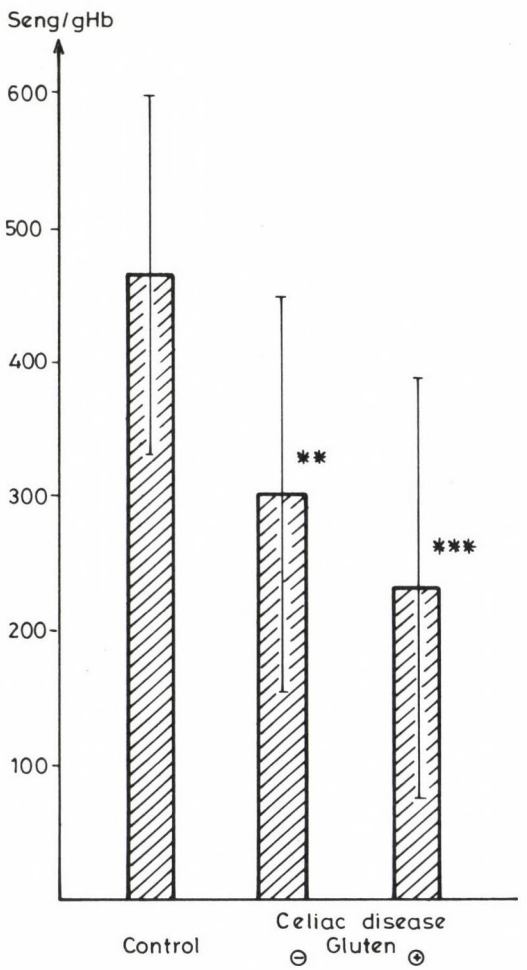

Fig. 5. Red blood cell selenium content of CD patients on gluten challenge and on gluten free diet

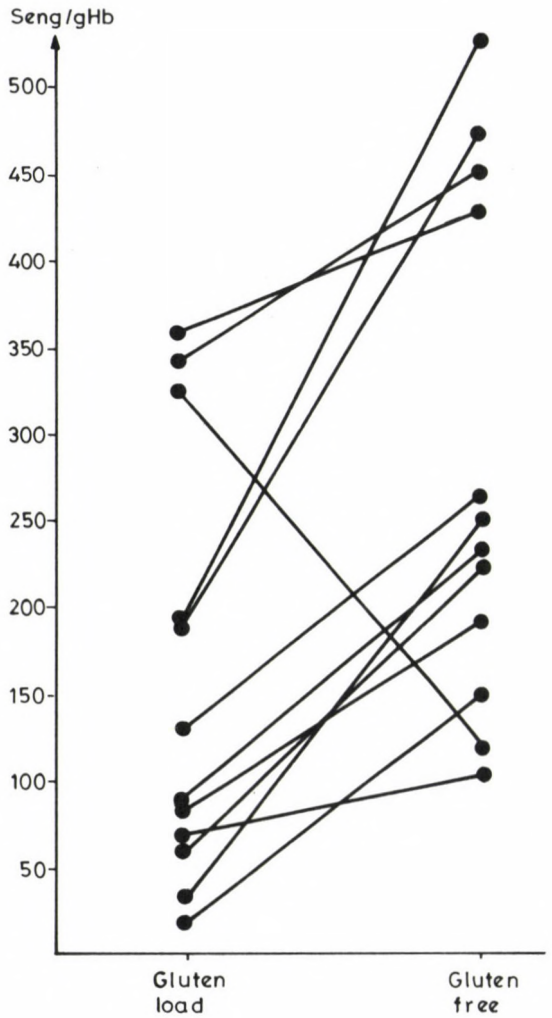

Fig. 6. Changes of the erythrocyte selenium content of celiac patients on gluten free diet

blood Se level (Fig. 6).

Histological findings of CD patients in acute period and after gluten challenge showed subtotal atrophy.

DISCUSSION

Glutathione is the most abundant low molecular weight thiol-containing compound in living cells. Its reduced form contributes to the viability of erythrocytes by stabilizing the thiol groups of membrane enzymes and hemoglobin /4/ and by acting as a reducing agent for hydroperoxides and free radicals, thus protecting the red cells against oxidative damage /19/.

Most of hydrogen peroxide is removed by reaction with reduced glutathione to form oxidized glutathione and water catalyzed by the selenium containing enzyme glutathione peroxidase (GSH-Px) /25/:

$$2\ GSH + H_2O_2 \xrightarrow{GSH\ Px} GSSG + 2\ H_2O$$

Therefore the investigation of the glutathione system is a sensitive index of the <u>in vitro</u> oxidative processes /1/.

Iron deficiency in itself may influence erythrocyte glutathione metabolism /16/. In nutritive iron deficiency, the increased function of glutathione synthetase enzyme is responsible for an elevation of the content for the red blood cells /22/. This process compensates for the deficient function of hemoglobin, as a redox system, present in a lower concentration than the physiological one. As iron deficiency is a usual accompanying symptom of acute CD and cow's milk allergy, we wanted to ascertain whether the changes of the erythrocyte glutathione metabolism in CD are a consequence of iron deficiency exclusively, or whether other factors may also play a role. Thus a second control group of patients with nutritive iron deficiency was formed. In our observation, erythrocyte GSH content elevated significantly in the CD group on gluten challenge and in nutritive iron deficiency, but the difference of GSSG values between the two groups emphasizes the

role of factors other than merely iron deficiency.

Acute oxidant stress induces the decrease of GSH in the red blood cell and increases the GSSG level /7/. If, however, the oxidant stress is long lasting, the compensation process results in the increase of GSH. This phenomenon was observed - under laboratory conditions - by administering methylene blue to rabbits /20/. The same changes can be seen during human submaximal exercise /12/. Elevated blood sulfhydril levels provide an accessfully marker of increased oxidant exposure /6/. The elevated erythrocyte GSH of CD patients on gluten load can be regarded as a consequence of a permanent oxidant stress, as well. This explanation was supported also by the fact that the glutathione status of the red blood cells normalized during the diet.

The impaired defense of erythrocytes against oxidative stresses in CD and cow's milk allergy was proved by the in vitro acetylphenylhydrazine loading test of blood which showed a definitive decrease of erythrocyte GSH level compared to the controls. This type of glutathione in vitro instability of red blood cells improved during the diet.

During gluten and milk challenge the presence of instable methemoglobins and hemichrome is another evidence of decreased erythrocyte defensive capacity against oxidative stress. This instability of red blood cells against oxidative stress could also explain the frequent manifestation of methemoglobinaemia found in dermatitis herpetiformis patients treated by Dapson and by other medicaments with oxidative effect /2/.

Similar but milder metabolic changes of erythrocytes of cow's milk allergy patients suggest that the presence of chronic oxidative stress effects is not a unique phenomenon of CD: it probably occurs during mucosal destruction.

The definite decresae of erythrocyte GSSG/GSH ratio of CD patients on gluten challenge refers to the decreased activity of GSH-Px. This enzyme, besides removing hydrogen peroxide in vivo, acts together with superoxide dysmutase (SOD) and catalase; but GSH-Px was found to be much more effective against oxygen-derived free radicals than catalase and SOD /21/. GSH in itself has a low grade reductive capacity. If

GSH-Px decreases GSH protection is not significant, which leads to the destruction of the membranes /11/.

Since GSH-Px destroys H_2O_2, low levels of this enzyme may permit increased histamine release by mast cells, as well.

There is a synergic effect in the defence against peroxidation injury of the cell between vitamin E and GSH-Px /24/. In animal experiments tocopherol can only prevent haemolysis induced by peroxide, while GSH-Px prevents also the oxidation of hemoglobins by peroxides /23/. An accepted screening method of vitamin E deficiency, the peroxide haemolysis test /10/ proved to be negative in acute CD /13/. In spite of this, we have to admit that in the results obtained in the present study a partial vitamin E deficiency might have some role.

The selenium deficiency in the presented patients can be regarded as a definite factor in the increased sensitivity of the erythrocytes against oxidative stresses in acute CD. The same biochemical alteration of erythrocytes were seen in children with selenium deficiency in China /29/. Selenium deficiency may lead to a decreased activity of GSH-Px and, on the other hand, Se also has a direct antioxidant property. Ward found reduced Se in serum in treated and untreated patients /29/. In our studies the selenium was determined in blood cells which is more reliable parameter of the true Se depletion of the body, and we obtained a gradual restoration of the Se deficiency during gluten free diet, which may indicate that Se deficiency can be - at least in part - a consequence of mucosal damage in CD.

Future studies would be needed to clear the exact role of free radicals in tissue damage. To lessen these pathological processes, the therapeutic use of antioxidants and scavangers i.e. selenium and vitamin E should be a question of consideration.

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Acta Paediatrica Hungarica 32 (3), pp.257-267 (1992)

Ki-1 POSITIVE (ANAPLASTIC, LARGE CELL) LYMPHOMA (CASE REPORTS AND REVIEW)

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Received 27 January 1992

Ki-1 positive (anaplastic, large cell) lymphoma is a subgroup of non-Hodgkin lymphomas identified recently by Ki-1 (or BER-H2) (CD 30) monoclonal antibody. The clinicopathological features of two such pediatric cases of lymph node origin described here, and also the available literature emphasize the heterogenous nature of Ki-1 positive lymphomas, in almost every respect. Nevertheless, the Ki-1 antibody serves as an important diagnostic tool to differentiate lymphomas from other anaplastic, large malignancies.

INTRODUCTION

Probably, non-Hodgkin lymphomas (NHL) are the best examples how latest technologies (e.g. monoclonal antibodies for phenotyping, gene-rearrangement studies) can lead to the reclassification of malignancies, sometimes, with the identification of new subclasses. The value of any such attempt depends mainly on its relevance to prognosis. Recently, Ki-1 monoclonal antibody (CD 30) was reported to be specific to neoplastic cells of Hodgkin's disease /41/, but the expression of Ki-1 antigen was also observed on a group of NHLs, showing reactivity to antibodies Ki-1 or BER-H2 (the latter is a paraffin-reactive Ki-1 analogue). Although, these Ki-1 positive (anaplastic, large cell) lymphomas are morphologically high grade malignancies /22, 28, 29, 43/, their appearance, in every respect, seems to be rather heterogenous. Here, we describe two pediatric cases and discuss some clinicopathological features of this entity.

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CASE REPORTS

Case 1. - V.A. 10 years old boy. Admission was necessary due to severe abdominal pain. Physical examination revealed a tumor, mainly in the left side of the abdomen. Accordingly, ultrasound showed inhomogenous solid area in connection with the intestinal tract. Besides, two metastases of 2 cm of diameter appeared in the liver. Laboratory results, chest X-ray, bone marrow and liquor were negative. The abdominal mass (about 8 cm in diameter) has been removed with a segment of jejunum. - Histologically (2505/84), large neoplastic cells infiltrated the mesenterial lymph nodes, with only a small percentage of normal host cells. The large, oval or irregularly shaped nuclei contained one or more prominent nucleoli and were surrounded by pale cytoplasm. Mitotic figures, necrotic areas were not infrequent, while fibrosis was not obvious. Besides HLA-DR (Dako) and LCA (Bio-Genex) positivity, IgM and kappa light chain monoclonality suggested diffuse NHL of B-cell (possibly centroblastic) origin. Parts of the tumor has been xenotransplanted and fully characterized as established line /24/. The gene-rearrangement study showed bigenotypic character (haplotypes for IgH: R/R, IgL-kappa: R/D, IgL-lambda: G/R) /25/, but the tumor cells expressed only IgL-k mRNA (unpublished). Retrospectively, the tumor cells were highly positive with BER-H2 (Dako) monoclonal antibody (Fig. 2). - Patient has been treated as a B-cell lymphoma of stage III /30/ according to the protocol of POG (high dose Cyclophosphamide plus high-dose Methotrexate with coordinated intrathecal prevention - 35). The disease-free period now surpassed 6 years.

Case 2. - H.A. 8 years old boy. Four years earlier his nephrosis syndrome was treated effectively by steroid. Recently, he was vaccinated against rubella and two weeks later lymphadenomegaly developed in the ipsilateral axillar region. Although, the lesion histologically proved to be negative, it progressed towards the subclavicular region and within a month reached about 8 cm of diameter. Besides, a nut-sized tumor appeared in the submandibular area. Laboratory and clinical data showed normochrome anemia, increased sedimentation (40 mm/hr), a left-shifted granulopoiesis with preserved erythro- and thrombopoiesis in the bone marrow. Chest X-ray, abdominal ultrasound and bone-scintigraphy were negative. Total protein in the serum (83 g/l), as well as IgG fraction (15.4 g/l) increased, while IgA and IgM decreased. - Biopsy (2284/91) was taken from the axillary region. Normal lymph node structure was recognized only in small areas, since the lesion was diffusely infiltrated by tumorous tissue. Most of the tumor cells had large, rather irregular nucleus, loose chromatin, prominent nucleolus, and slightly basophilic cytoplasm. Multinuclear giant cells were inconspicuous, while mitotic figures were numerous. Host elements appeared as delicate bands of connective tissue, with limited extent of fibrosis, scattered small group of plasma cells and relatively few,

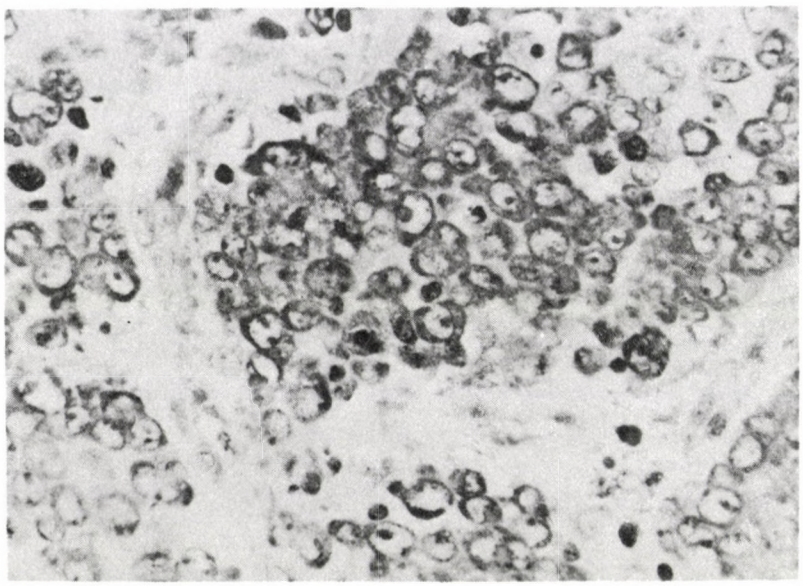

Fig. 1. Ki-1 positivity in large cell lymphoma (case 2) (x240)

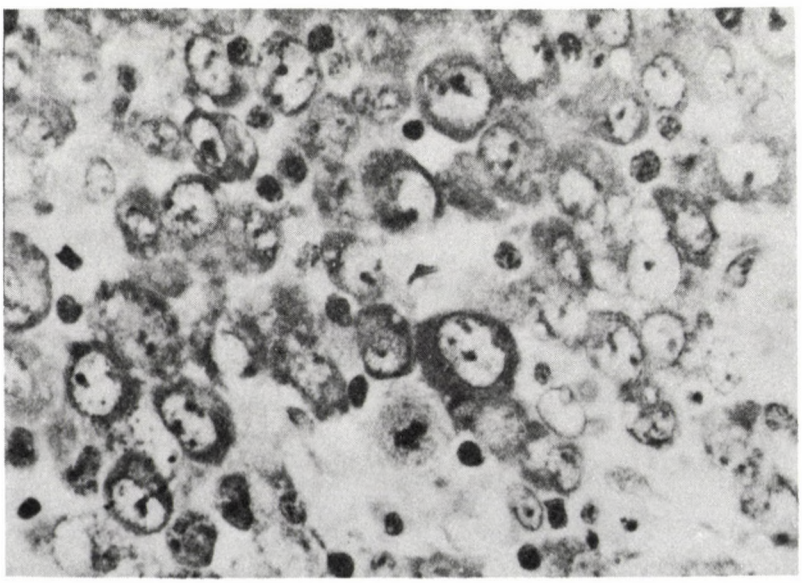

Fig. 2. Ki-1 positivity in large cell lymphoma (xenotransplanted tumor - HT 117 - from case 1) (x450)

randomly distributed macrophages and lymphocytes. Electronmicroscopy could not find cell-connecting structures. Immunohistochemistry showed intensive granular cytoplasmic positivity with BER-H2 (Fig. 1), and positive staining with EMA (epithelial membrane antigen - Amersham and pan-T (Dako). Pan-B, LCA, vimentin (Amersham), cytokeratin (wide spectrum, Dako) and lysozime (Dako) remained negative. IgH gene preserved germline configuration, while TCR gene indicated unexpected polyclonality. (DNA for gene-rearrangement study was isolated from AMeX-fixed, paraffin-embedded biopsy material - 39). Therefore, the exact geno- and phenotype is still obscure. - The treatment was designed for Ki-1 positive large cell lymphoma in stage II according to BFM protocol. (Cyclophosphamide and Prednisolone for 5 days followed by three cycles of combination chemotherapy - high-dose Ifosfamide and Cyclophosphamide, Cytozin arabinoside, Vumon, Adriablastin, Methotrexate, Prednisolone or Oradexon, including intrathecal prophylaxis with Cytozin arabinoside, Methotrexate and Methylprednisolone). Complications (pancytopenia and infections) were successfully resolved. The boy is in disease-free remission since 4 months.

DISCUSSION

Ki-1 positive lymphoma represents 1-3% of NHLs /6, 11/, and can occur at any age group with peaks in second-third and fifth-seventh decades /7, 34/. In different reports the frequency of childhood cases ranged between 15 to 20% /7, 44/. In one-half of the cases the presenting complaint was lymphadenopathy, in others the involvement of lymph nodes and skin appeared together, or extranodal origin (most frequently skin only) was observed /1, 7/. Infiltration of bone marrow can also be present /7, 8/.

The first impression of a large (anaplastic) cell tumor comes from histology. Typically, at the early stage, the affected lymph nodes are only partially involved by the neoplastic process invading preferentially sinuses and paracortical areas, sparing more-or-less the follicles, but in other cases, presumably in more advanced stage, diffuse infiltrate may afface the nodal architecture. Accompanying fibrosis (both interstitial and in bands) could be different. In general, the nuclei of tumor cells are indented or lobulated, with one or several prominent nucleoli. Eccentric lobated nuclei sometimes express a wreathlike configuration.

The cytoplasm is usually abundant, finely granular, but occasionally may resemble "signet-ring cell" lymphoma, containing endocytotic vacuoles /1, 27/. Reed-Stenberg-like giant cells and mitoses are rather frequent. According to the morphological appearance two subtypes can be distinguished (relative polymorphy or monomorphy), without obvious relationship to immunophenotype /7, 8/.

Before the introduction of Ki-1 antibodies many of these cases were recognized as malignant histiocytosis, Hodgkin's disease (mainly the nodular sclerotic form), metastatic carcinoma, malignant melanoma /1, 44/, regressing atypic histiocytosis (nodulo-ulcerative proliferation of atypic histiocytes in the skin) /13/ or sinusoidal large cell lymphoma /32/. The list clearly indicates the differential diagnostic problems of morphologic evaluation, including in samples obtained by fine needle biopsy /46/.

If immunohistochemistry reveals Ki-1 positivity, the evidence for lymphoma is almost unequivocal. Among non-lymphoid tumors Ki-1 positivity was observed only in embryonal carcinomas /14/. (Sometimes, due to cross-reaction, diffuse cytoplasmic false positive staining may appear in pancreas or salivary gland carcinomas using BER-H2). It should be emphasized that only cases in which all or nearly all large cells express Ki-1 antigen could be regarded as Ki-1 positive lymphoma.

Concerning the most important clonal markers, clonal rearrangement of TCR- or Ig-genes were present in 70% of cases, the remaining showed null-cell genotype. Most of these lymphomas have T-cell, less have B-cell phenotype, but either both or none of the specific markers could also be expressed /10, 16, 19, 44/. Evaluation of other markers can be difficult. It is not infrequent, that LCA is negative and EMA is positive. Vimentin and S-100 may also show positivity, but cytokeratin, lysozime, melanocyte-specific antigen and desmin are always negative /10, 14, 16, 45/. Ki-1 positive lymphoma cells may express many antigens which are characteristic on histiocytes (vimentin, CD 68, alpha-antitrypsin, alpha-1-antichymotrypsin), therefore in such cases gene-rearrangement study is essential

to rule out true histiocytic origin /2, 3, 5, 6, 10, 17/. The distinction between Ki-1 lymphoma and Hodgkin's disease is made upon the later's LeuM1 (CD 15) positivity /1, 21/. Fixation or embedding may denaturate Ki-1 antigen resulting in the failure to recognize Ki-1 lymphoma, when only paraffin-embedded material is available. Cytogenetic studies found association of Ki-1 lymphoma with a chromosomal translocation involving 5q35 /t(2;5)(p23;q35)/ in most cases /12, 23, 28, 36/ but in others either this translocation was not present or different one /t(9;14)/ was described /22, 26/.

The clinical aspects of Ki-1 lymphoma are at least as heterogenous as the above discussed features. Some studies suggest that the prognosis in childhood and in elderly patients, respectively, and in adults with skin involvement is relatively good /1, 21/. The evaluations in reported cases are hampered by the short follow-ups. In one study the median survival time was 13 months for 41 patients /7/. Most of them received polychemotherapy (usually CHOP), some in combination with radiation, or radiation was given only. Young patients (age below 40), or those in stage I and II had better prognosis. Another group using combination chemotherapy achieved complete remission in two, and no remission in one child /40/. Similarly, chemotherapy (different in each cases, presumably due to the different initial diagnosis) led to complete remission in two out of six children (the longest survival was 30 months till the end of the observation period) /21/. Long survivals (14-25 yrs) were reported in a retrospective analysis for three patients (aged 2,28,49) /37/. Spontaneous remissions are extremely rare in large cell lymphomas /8, 15/. In another retrospective study of 31 children, a disease-free survival of 74% was found using different protocols /18/. At evaluation it is important to prove the "de novo" nature of Ki-1 lymphoma, because both Hodgkin's disease and T-cell lymhoma with low malignancy can transform into Ki-1 lymphoma /45/. In one study the antecendent history of different lymphoproliferative diseases was apparent in 7 out of 24 patients /34/.

The review of accumulated experiences suggests that Ki-1 positive large cell lymphomas are characterized by clinical, morphologic, immunopheno- and genotypic heterogeneity. While more retro- and prospective studies are necessary to establish the clinical relevance and significance of the presence of Ki-1 antigen on lymphoma cells, at the level of morphology, however, the Ki-1 monoclonal antibodies proved their diagnostic value, established their place in diagnostic protocols, especially in cases of large cell malignancies.

ACKNOWLEDGEMENT

Authors express their sincere thanks to Janos Földi, MD for his excellent gene-rearrangement study.

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Acta Paediatrica Hungarica 32 (3), pp. 269-285 (1992)

ADOLESCENT MOTHERS: DO THEY BREAST-FEED LESS?

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Two groups of mothers with infants between sixty and eighty-nine days of age, from the same Health Area of the city of Havana were studied. The first group's age was nineteen years or less and members of the second one were twenty to twenty-five years old. The sociocultural level of the older group was significantly more favourable. The proportion of mothers exclusively breast-feeding was lower among the teenagers, and the frequency of complete weaning by the end of the third month was higher in their group. Duration of breast-feeding, either exclusive or combined with bottle-feeding was also shorter in the group of adolescent mothers, and frequency of diarrheal episodes was higher among their infants; body weight for age and body weight for length showed a shift towards lower percentile channels in these infants. Such differences were evident despite of that both groups of mothers had the same opportunity to receive information about the advantages of breast-feeding and there were no differences in the frequency and quality of prenatal care.

INTRODUCTION

Pregnancy among teenagers is frequently seen nowadays, though rates vary from country to country /5, 6, 16/. This fact has public health implications because early motherhood has been related to physical and psychosocial risks run by the children /29, 34/, a higher risk of death /7, 14, 32/, cognitive and behaviour problems /1/ and influence upon other developmental aspects /8, 11/ included. In addition, pregnancy may affect the nutritional status of the still growing

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adolescent mother who has increased needs for energy and protein /4, 18/ to face own and fetal needs for growth.

In several countries, the prevalence and duration of breast-feeding remains low despite of all efforts made to promote this practice /33/, while in many others an increasing trend has been observed /13, 28/.

There is some evidence that adolescent mothers are less likely to breast-feed /3/, although with active counseling they are capable of successful lactation /19/.

The question whether this decline of breast-feeding is related with an increased incidence of pregnancies at earlier ages arises; the aim of the present study is to investigate this possibility by comparing infant feeding practices and several social, cultural and educational factors in mothers of two age groups, one of them being under twenty.

PATIENTS AND METHODS

Two groups of fifty mothers each, with infants between sixty and eighty-nine days of age were selected from the same Health Area of the city of Havana. The criteria for selection of the subjects appear in Table I.

Each mother was interviewed according to a previously designed protocol, and each infant was measured for body weight and length, according to the International Biological Programme /31/. Three anthropometric criteria were used for assessing growth and nutritional status: body weight and body length for age, and body weight for length. Cuban standards were used as reference values /12, 17/. The chi-square test was used for group comparison.

RESULTS

Figure 1 shows the distribution of the father's age significant differences exist between the two groups, the proportion of teenagers being higher in the first one. The paternal schooling level also showed highly significant differences (Fig. 2): partners of teenagers have lower educational levels. The maternal level of schooling, however, showed no differences. There were significant differences in

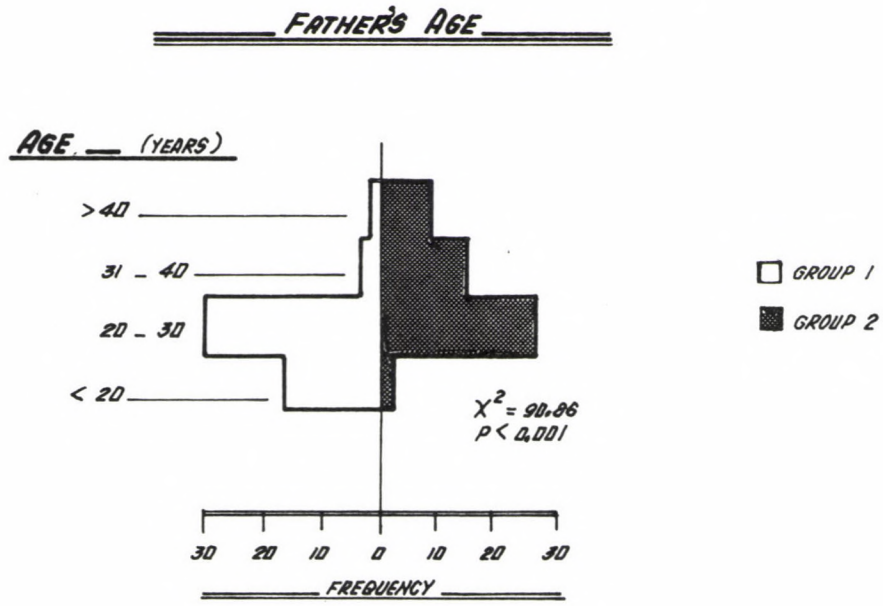

Fig. 1. Distribution of paternal age in the two study groups. Note the higher proportion of teenagers in Group 1

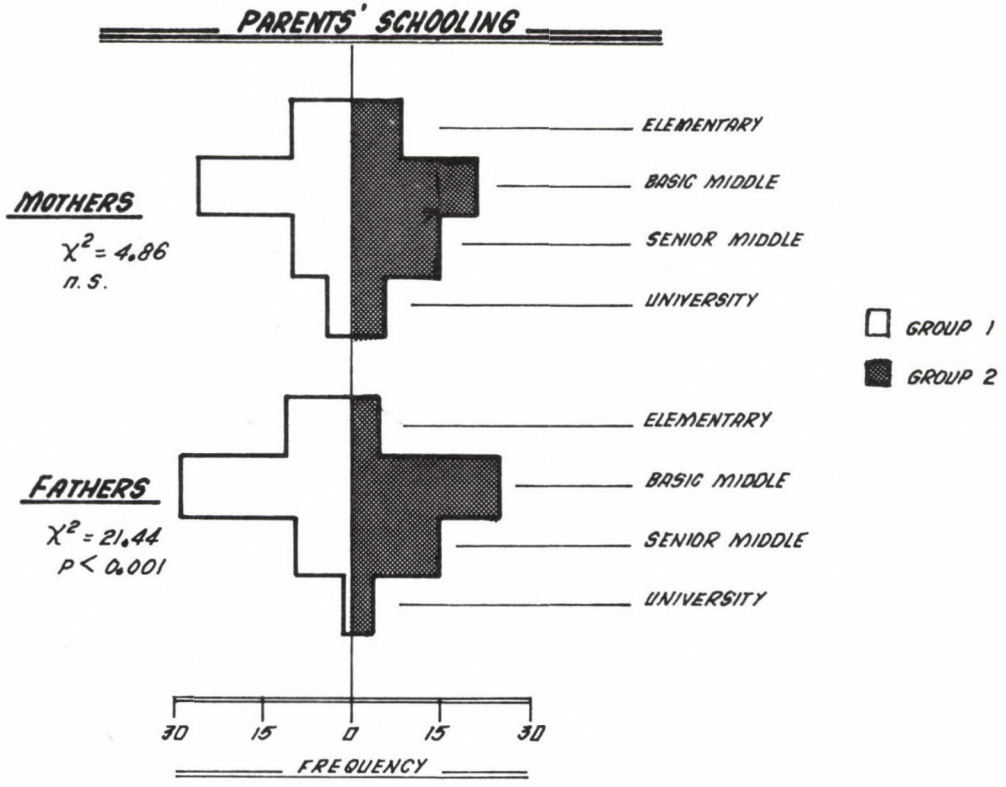

Fig. 2. Parental schooling. Fathers associated with teenager mothers have lower educational levels

parental professions between the two groups, paternal and maternal alike (Table II).

When considering the couple's status, again significant differences were found: the proportion of unmarried and divorced mothers was higher among adolescents (Table III).

Modes of feeding before the actual age of each infant were recorded in the same protocol by recall. Immediately after birth, there were no differences between the two groups, but they became significant at thirty, sixty and eighty-nine days: teenagers breast-feed less frequently and wean earlier (Fig. 3).

Causes of weaning differed between the two groups as shown in Table IV. It is of interest to observe that the major differences found correspond to: self decision; infant's disease and the statement that the infant refused the breast.

No differences regarding prenatal care were observed: the mean number of prenatal consultations was 6.54 ± 1.08 for teenagers and 6.62 ± 0.95 for the older group; the mean gestational age at the first prenatal consultation was 15.02 ± 4.11 and 14.36 ± 3.67 weeks, respectively. No differences regarding first information about advantages of breast-feeding or regular information about breastfeeding practice or preparation for breast-feeding were found either, as can be seen in Table V and VI.

Diarrheal episodes were more frequent among teenagers' infants, but there were no differences regarding their number. More children from group 1 needed hospitalization because of diarrhoea (Fig. 4).

Hygienic and environmental conditions differred significantly: deficient and poor conditions were more frequent in the teenagers group (Fig. 5).

Differences in the percentile distribution of body weight and length for age, and for body weight for length were found. A shift towards lower channels is evident in the adolescent group (Fig. 6).

TABLE I

Criteria of selection of the two groups of mothers

Criterion	Group 1	Group 2
Mothers' age	19 years	20 to 25 years
Infants' age	60 to 89 days	60 to 89 days
Pregnancy and delivery	Normal full term	Normal full term
Birth weight	at least 2500 g	at least 2500 g
Parity	First delivery (only child)	First delivery (only child)

TABLE II

Parents

Profession occupation	Fathers		Mothers	
	Group 1	Group 2	Group 1	Group 2
A) Worker	24	11	6	10
B) Student	8	11	9	14
C) Both	17	28	12	18
D) None	1	0	13	8

$X^2 = 9.60$ $X^2 = 10.35$

$p < 0.05$ $p < 0.05$

TABLE III

Couple's status

Status	Group 1	Group 2
Stable	28 (56%)	37 (74%)
Single	16 (32%)	5 (10%)
Divorced	6 (12%)	8 (16%)
Total	50	50

$X^2 = 11.12$
$p < 0.005$

TABLE IV

Reason for weaning (as referred by the mother)

Cause	Group 1 n = 26	Group 2 n = 15
Not enough milk	26.9%	26.7%
Infant was unsatisfied	11.5%	26.7%
The infant refused breast	26.9%	13.4%
Doctor's prescription	3.9%	20.0%
Self decision	11.5%	0.0
Infant's disease	15.4%	6.6%
Mother's disease	3.9%	6.6%

$X^2 = 19.97$
$p < 0.05$

TABLE V

First information about the advantages of breast-feeding

		Group 1	Group 2	
WHEN ?	Prenatal	45	47	$X^2 = 2.03$
	At delivery	5	3	n.s.
	Never	0	0	
WHERE ?	Health Center	34	30	$X^2 = 3.54$
	At home	13	14	n.s.
	In hospital	3	6	
WHO ?	Obstetrician	5	8	$X^2 = 2.94$
	Pediatrician	2	2	n.s.
	Nurse	32	27	
	Relative	11	13	

TABLE VI

Regular information about breast-feeding and its advantages

WHEN ? WHERE? WHO ?	Group 1	Group 2
Prenatal consultations	5	8
Home visits	32	34
Radio/ TV Programs	11	23
Written Press	4	7
Mass Organizations talks	7	11
None	0	0

$X^2 = 6.82$

n.s.

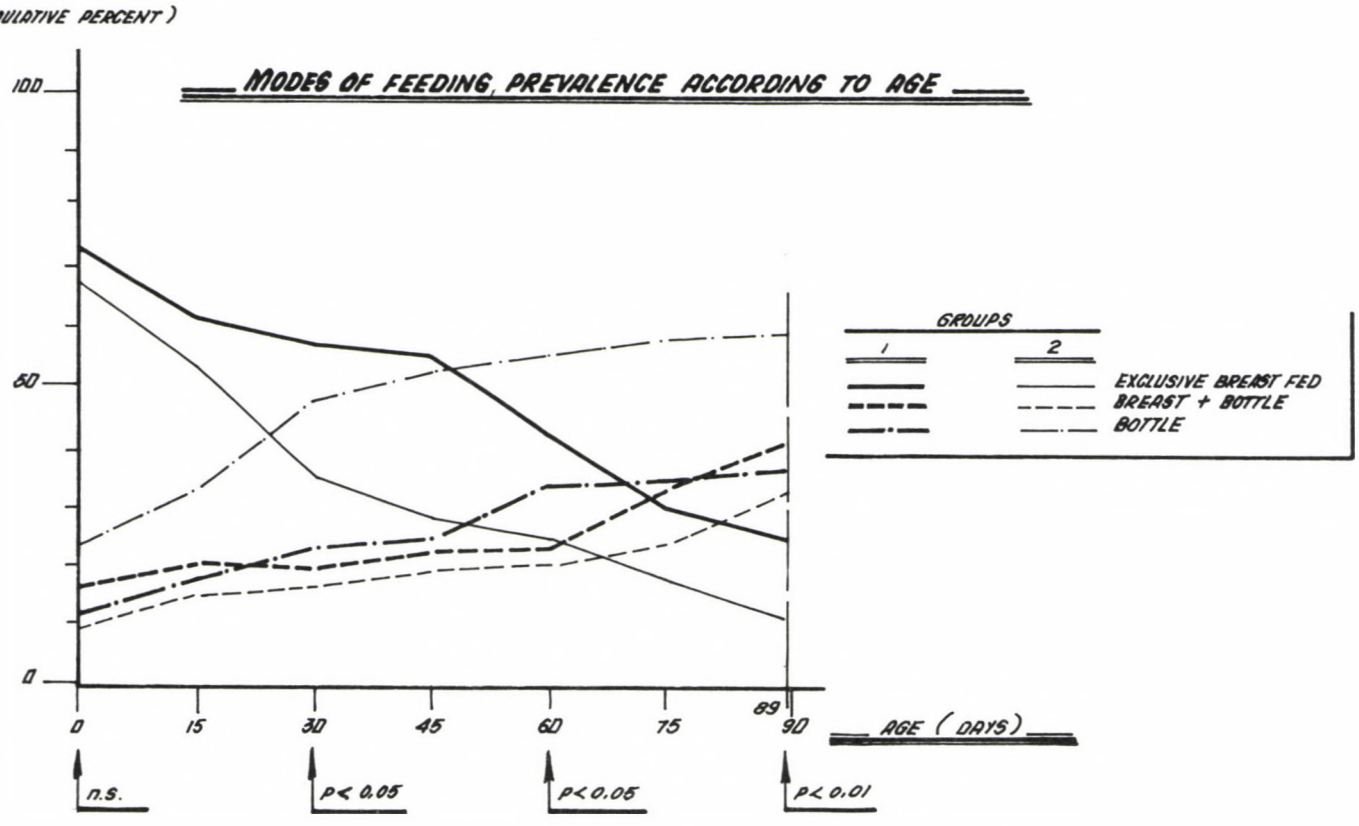

Fig. 3. Modes of feeding up to the time of study in the two study groups. The difference becomes significant after 30 days of age. Teenagers breast-feed less frequently and wean earlier

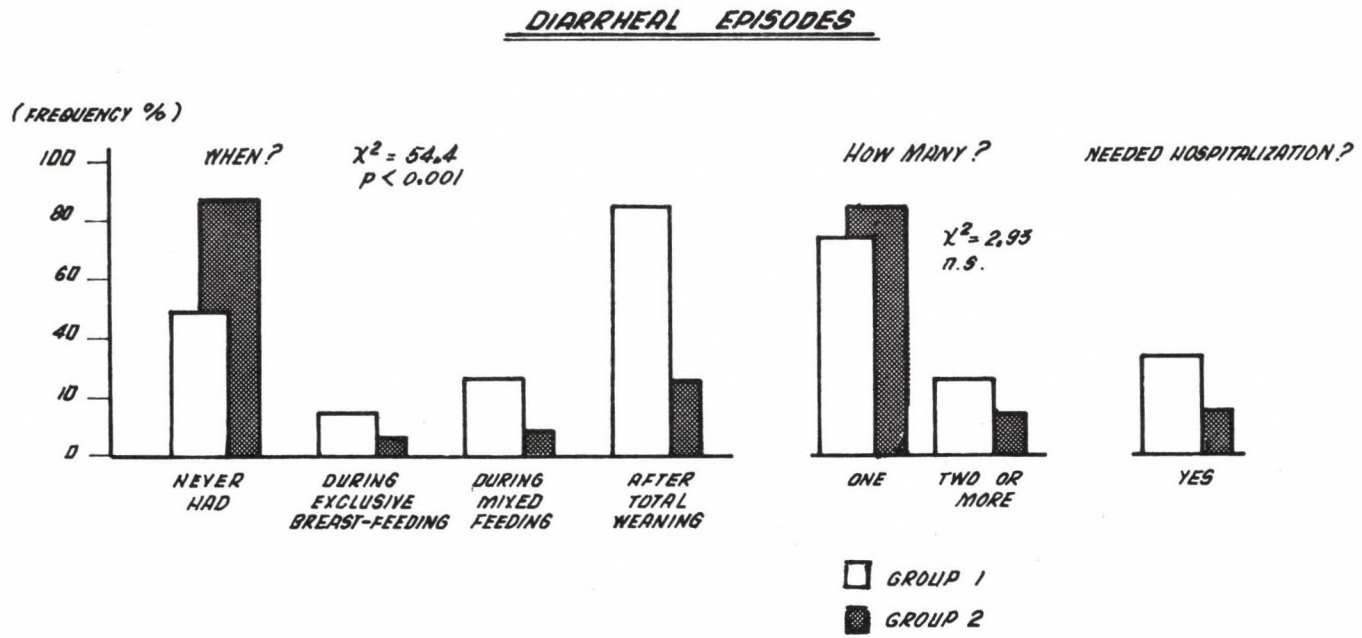

Fig. 4. Incidence of diarrhoeal episodes: higher in Group 1

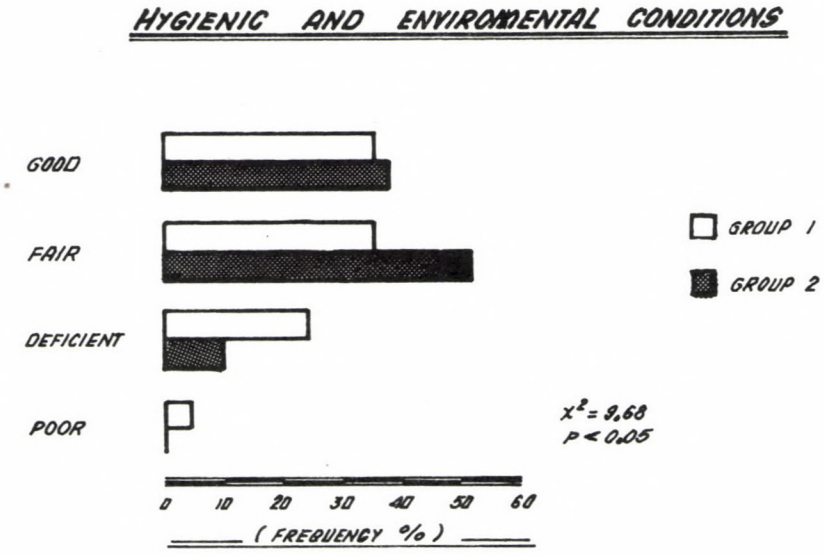

Fig. 5. Hygienic and environmental conditions of the two study groups. The classification in four categories was based upon house conditions, degree of overcrowding, quality of water supply, sanitary services, wastage disposal and presence of insects or rodents.

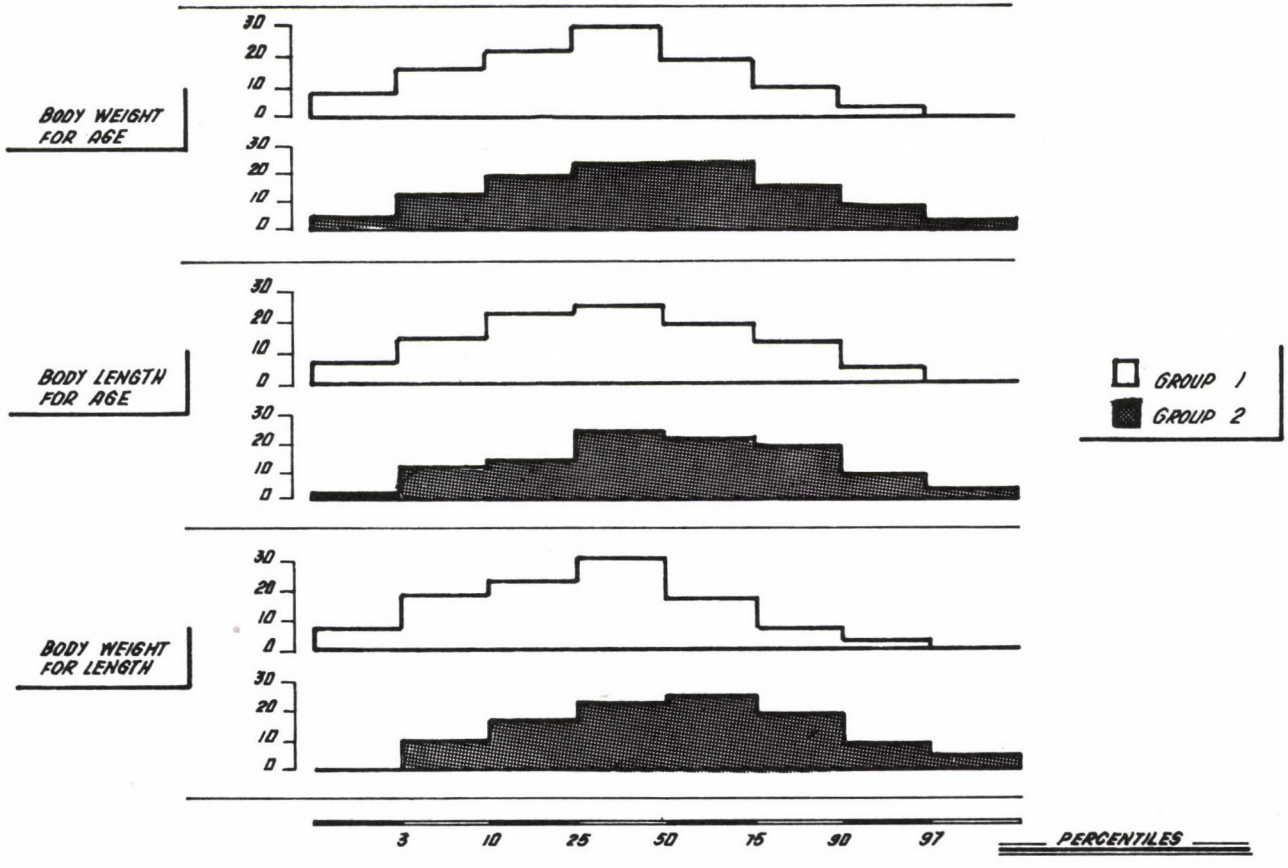

Fig. 6. Percentile distribution of anthropometric measurements: a shift towards lower channels is observed in infants born to teenagers

DISCUSSION

The results of this preliminary study show that infants of teenager mothers are breast-fed in a lower proportion than those of mothers aged 20 to 25 years. In Uruguay, Bauzá, Díaz-Rosselló and Bielawski /3/ studied 239 mothers aged 12-19 years who attended Health Centers in Montevideo, and found that by 30 days of age, 45% of the children of teenager mothers and 34.3% of those of older mothers were completely weaned, while the proportions increased to 79.4 and 67.8 by the end of the third month. Mean duration of exclusive breast-feeding in children of adolescent mothers was 63.6 days and for those of older mothers 72.7 days. By analysing in an epidemiological approach the association between different variables to breast-feeding practices, Díaz-Rosselló and Bauzá found a significant association between mother's age and failure to start breast-feeding /10/. In Mauritius, the age of the mother was found to be positively correlated with the length of lactation: the proportion of women still breast-feeding at any age of the child increases with increasing maternal age /25/.

The two study groups significantly differred in regard to several social, cultural, economical and environmental variables possibly influencing feeding practices. Lorenzo et al /20/ and Rivara /26/ found that the majority of teenagers's husbands were of similar age but there was a sizeable group who were older, older fathers cohabiting with teenager mothers are, however, more similar to adolescent fathers than to men of their age living with women beyond twenty /24/.

A strong association between attained educational level and the frequency and duration of breast-feeding has been found /22, 23, 28/. The absence of a difference regarding the level of schooling between the two groups could be attributed to the fact that girls are more stable in the educational system after they have completed the Junior High School (about 15 years of age). Similar results were obtained by Bauzá et al /3/. In our study, the educational level of mothers seems to have no influence on breastfeeding practice.

The proportion of fathers exclusively doing physical work was higher in Group 1 while the proportion of teenager mothers who neither studied nor worked was higher. The educational level of men living with teenager mothers was significantly lower.

A significant proportion of early pregnancies come from instable couples. In Britain, four fifths of pregnancies in teenagers are conceived out of a wedlock /6/. In Cuba, a study carried out in Havana showed that 56.4 % of pregnant teenagers were not married, 17.6 % lived alone and 6 % were divorced /14/. In this study the proportion of stable couples, whether legally married or not, was significantly lower in the adolescent group while the number of single mothers living apart from the infant's father, was significantly higher. There is evidence that the success of lactation is influenced by the couple's stability: when the father has a positive attitude to the pregnancy, maternal weight gain and the infant's birth weight are significantly higher /21/; on the contrary, illegitimate babies are less likely to breast-feed /15/.

Most of the adolescent mothers express positive attitudes toward the fetus and the infant /8/, although many of them are psychologically immature and their identification with the maternal role is not firmly developed; this may influence attitudes toward breast-feeding. The difference between the two study groups in respect of reasons for weaning were mainly found in aspects attributed to the child.

General and specific prenatal care is usually worse among teenagers /3, 27/: many of them seek for medical care at advanced gestational age or do not regularly attend to prenatal clinics. However, in our study no differences were found in gestational age at the time of the first visit or in the number of visits or in the quantity or quality of the information about breast-feeding received before delivery, which reflects the good quality and availability of our Mother and Child Health Programs.

Hygienic and environmental conditions were inferior for adolescents and their offspring. The interaction of these factors with the social, cultural and educational background

characteristic of teenager mothers contributed to a higher incidence of diarrhoeal episodes. Poor families are less likely to breastfeed from birth on /2/; low socio-economic status rather than the adolescent mother's young age itself has been considered as the major factor responsible for the intellectual deficit seen in children of teenagers /11/. This interaction results in the shift of body weight for age and body weight for length towards lower percentile channels, in this group higher rates of low birthweight, infectious and nutritional diseases; and infant mortality can be expected /14/.

Teenagers becoming parents seem to constitute a special group, not only from the biopsychological point of view, but also because of a peculiar social and cultural background resulting in a lower frequency and duration of breastfeeding.

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Acta Paediatrica Hungarica 32 (3), pp. 287-288 (1992)

BOOK REVIEW

Atlas of the Pediatric Ultrasound. Edited by Reinhard D. Shultz, Ulrich V. Willi. Thieme Verlag, 1992. 204 pages, 1532 illustrations.

This book is a translation of the German edition, published in 1990. The authors are well-known pediatric radiologists from all over the world (Fawer CL, Forster I, Gassner I, Jequier S, Schulz RD, Seitz K, Vergesslich K, Willi UV).

The atlas is organized on the basis of frequently encountered symptoms and typical clinical investigations. The case arrangement is not "encyclopedic" and is somewhat arbitrary. It is result of situations in which the indication for sonography is based not on a defined set of symptoms but on a more complex inquiry, of situations that require a differential diagnosis.

Cases that have the same diagnosis but a different morphologic presentation are shown side-by-side. The size of the pictures are small, but informative.

The atlas has 23 chapters, with a very short introduction before every chapter, and sometimes an examination-table of the indications of the investigation. The cerebral disease and the spinal tract chapters are illustrated not only by ultrasound, but by CT and MR pictures as well. In the chapter "Abnormal Thoracic Findings" there are only very few pictures about cardiac investigations. A separate chapter deals with ultrasound diagnosis of abdominal pain. There is a very rich illustration of the abdominal masses, it is a great help in the differentation between the different morphological pictures of the same histological tumors. A separate chapter deals with the ultrasound difference in blount abdominal traumas. In the chapter on the gastrointestinal disease the ultrasound pictures are completed by x-ray and pictures and drawings. The urinary tract diseases comprise a major part of the atlas. It is very useful to see pictures about the normal kidney in a separate chapter. The chapter about the neonatal and infant hip is a great help in

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everyday work.

The structure of the atlas is unusual, but the distribution according to symptoms is very useful in practical work. The comments on the pictures are detailed.

The well presented book is published by Thieme Medical Publishers. It is a great help for colleagues who have a special interest in pediatric ultrasound, but for pediatricians, and pediatric surgeons as well.

Éva Kis, MD

INSTRUCTIONS TO AUTHORS

Acta Paediatrica Hungarica publishes articles on clinical observations and original research of paediatric subjects and related fields. Articles are subject to editorial revision. Two complete copies of the manuscript including all tables and illustrations should be submitted. Manuscripts should be typed double-spaced with margins at least 4 cm wide, 25 lines to a page, 50 character spaces to a line. The first page should include the title, authors' names and name of the institution where the work was done. On a separate sheet an abstract of not more than 200 words should be supplied; it should not contain abbreviations or references.

All manuscripts must be accompanied by the following written statement, signed by each of the authors: "The undersigned authors transfer all copyright ownership of the manuscript entitled (title of article) to the Akadémiai Kiadó, Budapest in the event the work is published. The undersigned authors warrant that the article is original, is not under consideration by another journal, and has not been previously published. For the examinations included in the paper the permission of the local ethical committee has been obtained."

Abbreviations should be spelled out when first used in the text. *Drugs* should be referred to by their WHO code designation (Recommended International Nonproprietary Name); the use of proprietary names is unacceptable. The *International System of Units* (SI) should be used for all measurements except blood pressure.

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Erlandsen SL, Meyer EA (eds): Giardia and Giardiasis. Plenum Press, New York 1984

Detter JC: Biochemical variation. In: Textbook of Human Genetics, ed Fraser O, Mayo O, Blackwell Scientific Publications, Oxford 1975, p. 115

Tables should be comprehensible without reference to the text. The headings should be typed above the table.

Figures should be identified by number and author's name. The top should be indicated on the back, and the approximate place of the figure in the text. Captions should be typed on a separate page.

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Index: 26.021

Acta Paediatrica Hungarica

VOLUME 32, NUMBER 4, 1992

Akadémiai Kiadó, Budapest

ACTA PAEDIATR HUNG HU ISSN 0231-441X

ACTA PAEDIATRICA

HUNGARICA

A Quarterly of the Hungarian Academy of Sciences

Editor

M. MILTÉNYI

Acta Paediatrica publishes original reports of studies in paediatrics and borderline fields.

Manuscripts and editorial correspondence should be addressed to

ACTA PAEDIATRICA HUNGARICA
Bókay J. u. 53.
H-1083 Budapest, Hungary

Subscription information

Orders should be addressed to

AKADÉMIAI KIADÓ
P.O. Box 245
H-1519 Budapest

Acta Paediatrica Hungarica is published in yearly volumes of four issuse by

AKADÉMIAI KIADÓ

Publishing House of the Hungarian Academy of Sciences
Prielle Kornélia u. 19—35.
H-1117 Budapest

Acta Paediatrica Hungarica is abstracted/indexed in Biological Abstracts, Excerpta Medica, Index Medicus, La Presse Médicale

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ELECTROLYTE AND GLUCOSE CONCENTRATION IN PLASMA AND CEREBROSPINAL FLUID MEASURED PARALLEL IN PATHOLOGIC NEWBORN INFANTS

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Received 8 September 1992

Cerebrospinal fluid and plasma sodium, potassium, chloride, calcium and glucose concentration were measured parallel in 14 pathological newborn babies of gestational age and birthweight of 36.3±4.3 wks and 2410±890 g, respectively, at the age of 37.8±4.4 wks post-conceptionally. Whilst potassium, calcium and glucose level is much lower in the cerebrospinal fluid than in the plasma, similar sodium and higher chloride concentration was found in the cerebrospinal fluid. The significant positive correlation between plasma and cerebrospinal fluid glucose and sodium levels proves the lack of a functioning barrier for these compounds. On the other hand, cerebrospinal potassium level varied within a narrow range, independently of the plasma-concentration and the maturity of the studied babies. Pathophysiological implications of the results are further discussed in short.

INTRODUCTION

Cerebrospinal fluid (CSF), apart from its role in mechanical protection, serves as a metabolic pool and sink for the central nervous system. In spite of the fact that CSF is the third most commonly used body fluid for diagnostic evaluation, our present knowledge on the development and regulation of its homeostasis is still fragmentary. In principle, the composition of CSF depends on the plasmaconcentration of the various constituents and also the biochemical milieau of the brain. Transport between the different compartments is, of course, influenced by the function and/or maturity of the blood-CSF, blood-brain and the brain-CSF barriers.

It is known that potassium and calcium concentration in the brain interstitial fluid and CSF may well affect metabolism and

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electrophysiological activity of the nervous tissue cells and synapses /4, 6/. Increased CSF potassium concentration has been thought to be a possible causative factor in the pathogenesis of cerebral infarcts /4, 9/. Decreased relative CSF glucose level characterizes not only purulent meningitis, but can also be observed in case of other pathology, like in patients with subarachnoideal haemorrhage or posthaemorrhagic hydrocephalus, certainly due to disorders of transport mechanisms.

The objective of the present study was to collect more data on the regulation of CSF composition in newborn babies of various gestational ages, suffering from various perinatal pathology. Understandably, normal control values had to be missed.

PATIENTS AND METHODS

Concentration of sodium, potassium, chloride, calcium and glucose have been measured simultaneously in plasma and CSF of 14 newborn infants of 36.3 ± 4.3 (29-41) weeks gestational age (±SD) and 2410 ± 890 (1270-3950) grams birthweight. All babies suffered from perinatal pathology, which was related to hypoxia-asphyxia in 9, major non-neurologic malformation in 3 and septicemia in 2 cases. The lumbar puncture and blood sampling were parts of the diagnostic studies, performed at the postconceptional age of 37.8 ± 4.4 (30-43) weeks. All CSF samples evaluated were macroscopically clear. Bacterial meningitis could be excluded by culturing and cytology. For biochemistry routine laboratory methods were used. All babies in study were fed banked human milk or received glucose-saline drip infusion, according to their age or need at the time of the investigation.

For statistical analysis standard mathematical methods were used.

RESULTS

Results of the study are shown in the Table. It is seen that like in adults, in the CSF of the newborn babies a similar sodium, a higher chloride and much lower potassium, calcium and glucose level could be observed as an average, than that in the plasma. Furthermore, it is to be noted that the mean CSF glucose

TABLE I

Electrolyte and glucose concentration in plasma and cerebrospinal fluid measured parallel in 14 pathological newborn infants

	mean	SD	SE	range
Sodium (mmol/l)				
plasma	137	5.9	1.6	(130-147)
CSF	138	6.3	1.7	(124-146)
R	1.01	0.04	0.01	(0.39-1.09)
Potassium (mmol/l)				
plasma	4.4	0.6	0.1	(3.7 - 5.7)
CSF	3.0	0.3	0.1	(2.7 - 3.7)
R	0.68	0.09	0.03	(0.50- 0.81)
Chloride (mmol/l)				
plasma	99	3.7	1.0	(91-105)
CSF	116	6.1	1.6	(103-124)
R	1.16	0.06	0.01	(1.04-1.23)
Calcium (mmol/l)				
plasma	2.20	0.45	0.12	(1.5 - 3.0)
CSF	1.52	0.37	0.10	(0.6 - 2.0)
R	0.71	0.20	0.09	(0.28-1.00)
Glucose (mmol/l)				
plasma	5.1	1.1	0.3	(3.4 - 7.6)
CSF	2.5	1.2	0.3	(0.9 -4.8)
R	0.46	0.16	0.04	(0.22-0.65)

R = CSF/plasma concentration ratio

level was less than the 50 percent of the plasmaconcentration, what is more, in a few patients even much less concentrations were detected as shown by the lower range values.

On correlation analysis a significant positive correlationship ($r=0.7806$, $p<0.001$) was found between plasma and CSF glucose level, furthermore plasma and CSF sodium concentration ($r=0.6627$, $p<0.01$). No such relationship could be observed between the levels of any other parameters measured in the two bodyfluids. It has also been tested whether the maturity of the babies, as expressed by their postconceptional age, is related to the CSF/plasma concentration ratio of the electrolytes and glucose, but no such relationship was found.

DISCUSSION

The composition of CSF is similar to an ultrafiltratum, which may, however, be modified by the active transport of various compounds. In healthy adults, sodium, chloride and magnesium concentration of CSF is higher, whilst potassium, calcium, phosphate and hydrocarbonate concentration is lower than that in the plasma, similarly to the lower organic crystalloid (glucose, amino acids) levels in the cerebrospinal fluid. Any changes in the composition of CSF may be due to alterations in plasmaconcentrations of the various constituents, or may reflect metabolic changes in the cerebral extracellular fluid. Both possible mechanisms largely depend on the functional maturity or damage of the various barriers.

In the present study a close relationship has been found between plasma and CSF glucose concentration in the 30-43 postconceptional weeks old pathological babies, which shows that glucose transport is not influenced by the blood-CSF barrier. Another point to note is the lack of significant correlation between CSF/plasma glucose ratio and the maturity of the babies. This means that the lacking barrier function for glucose is probably not related to immaturity, at least in this age group. The mean glucose concentration in CSF was less than 50 percent of that in the plasma, what is more, in a few cases it was only a third or fifth of the blood glucose level, measured parallel. This observation proves that in pathological newborn infants relatively low CSF glucose level (<50 percent of blood glucose) not necessarily indicates purulent meningitis. This phenomenon may well be caused by transport disorders, as it was suggested by others /3, 9/ in case of posthaemorrhagic hydrocephalus or other kinds of intracranial pathology.

No difference between plasma and CSF sodium concentration was found. The lack of a higher CSF sodium level in comparison to plasma level, and also the significant correlationship found by us and previously by Stutchfield and Cook /9/ between plasma and CSF sodium concentration suggests in this age group at least that active transport does not influence CSF sodium concentration, it

is primarily plasma level dependent. This observation may have practical importance in case of iatrogenic and any other kinds of hypernatraemias, considering suggested links between rised CSF sodium concentration and different types of cerebral haemorrhages.

Both CSF chloride concentration and CSF/plasma chloride concentration ratio were closely similar in the study babies to normal adult values. Increased CSF chloride level indicates a functioning active chloride transport mechanism, which must be fully matured at the range of postconceptional age of the babies studied by us.

It is well known that in the central nervous system extracellular potassium and calcium are important factors in neuron excitability and synaptic transmission. Disorders in their homeostasis result in pathological electric activity, cellular damage and abnormal clinical symptoms /5, 7/. Edvinson et al./4/ hypothesise that increased CSF potassium concentration causes persistent vasoconstriction of the pial arteliolae and small arteries, which is a pathogenic factor in the development of cerebral infarcts. In the adult, potassium concentration of brain interstitial fluid and CSF is well regulated and completely independent of the plasma concentration, due to an efficient blood-CSF and blood-brain potassium barrier /1, 8/. The results of the present study prove that the CSF potassium regulation in the human newborn is already mature. Potassium level of the CSF was found to be not related to plasma potassium concentration and varied between narrower ranges than plasma potassium level. It is to be emphasized, however, that all study babies were normopotassemic, on the one hand, and we did not investigated the effect of acidosis on CSF potassium concentration, on the other hand.

Results of animal experiments showed a gradually decreasing calcium concentration in CSF and brain extracellular fluid, from the foetal to adult age /7/. In the foetus CSF passively reflects plasma calcium concentration, the function of blood-CSF barrier for calcium develops only later, with advancing maturation. In the adult CSF calcium level is normally half of the plasmaconcentration /8/. In the newborn infants we studied a

relatively higher CSF concentration could be observed, as shown by the mean CSF/plasma concentration ratio of 0.71. At the same time, however, no statistically significant correlation could be found between CSF and plasma calcium levels, which means that the transport is limited by a functioning barrier.

The evaluation of the results of our study needs special care since the studied group of patients has not been homogeneous in respect of gestational age or pathology. The reason of this is the principle that examination of CSF is rarely necessary on clinical ground even in seriously ill newborn infants. Another difficulty in the interpretation is the lack of normal control values. Nevertheless, the variable postconceptional age of the studied babies has provided a possibility to evaluate the findings in relation to functional maturity. In general, of course, the possible effect of pathology on the various parameters studied could not be excluded. But again, this would mean that our observations reflecting active regulatory functions are valid in the case of pathology, as well.

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Acta Paediatrica Hungarica 32 (4), pp. 299-307 (1992)

PROLACTIN AND DOPAMINE CONCENTRATIONS IN THE CEREBROSPINAL FLUID DURING THE NEONATAL PERIOD

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Received 10 April 1992

Prolactin (PRL) has been detected in the cerebrospinal fluid (CSF) in humans and the absolute level appears to reflect the serum PRL concentration. Because PRL is thought to be involved in the regulation of brain water and electrolyte content attempt has been made to determine CSF and plasma PRL and dopamine (DA) concentrations, osmolality, and sodium level in 21 newborn infants undergoing lumbar punction because of apneic spells, fever, or perinatal asphyxia. The mean of gestational age was 36.5 weeks (range: 31-41) and birthweight was 2572 g (range: 1140-3550). The lumbar puncture was performed at the 8.3 postnatal day (range: 1-38). The plasma concentration of PRL was 106.52 ± 14.43 ng/ml, significantly higher than the CSF PRL level (43.24 ± 7.39 ng/ml, $p < 0.01$). This elevated level was observed in all individual cases. DA concentration in the plasma was much higher than the value detected in the CSF (64.75 ± 13.83 vs 8.64 ± 0.72 ng/ml, $p < 0.01$). No difference was observed between the sodium content of the CSF and plasma (138.94 ± 1.28 vs 138.04 ± 1.03 mmol/l), however, the osmolality of the plasma tended to be higher than the CSF osmolality (286.7 ± 3.81 vs 276.76 ± 2.19 mosm/kg, $p < 0.05$). In the CSF osmolality, PRL, DA, and sodium concentrations did not show any correlation.
In conclusion: in the CSF PRL probably does not play a primary role in controlling the osmolality and sodium content. PRL in CSF seems to be independent from CSF DA concentration.

INTRODUCTION

Prolactin (PRL) together with other pituitary hormones has been repeatedly detected in the cerebrospinal fluid (CSF) in

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humans /3, 19/ and several other mammalian species /6, 10, 16, 18, 23 25/. The absolute CSF PRL level appears to reflect the serum concentration regardless of the cause of elevation in the serum PRL /5, 19/. Conditions of natural hyperprolactinemia, such as pregnancy and neonatal life, are accompanied by an increase in CSF PRL /3/. PRL does not cross the blood-brain barrier /29/ but enters the CSF by a specific PRL receptor-mediated transport mechanism /30/. The likely site of the transport mechanism is the choroid plexus /21, 30/. PRL receptors exist on the epithelial cells of this tissue /20, 30/, which interfaces between blood and CSF. Since binding to the choroid plexus decreases with age the effect of PRL on the choroid plexus function may be more important during the perinatal than later stages of development /20/. PRL may exert a physiological effect directly on the choroid plexus, which is the principal site of CSF production. The possibility that PRL has a significant role in the water and electrolyte balance of the brain of premature animals has been suggested by Coulter /7/. To investigate the role of PRL in the regulation of liquor composition, in the present study we decided to examine the PRL concentrations in the CSF and plasma during the neonatal period. The sodium concentrations together with the osmolality and dopamine (DA) levels of the plasma and CSF were also measured.

MATERIAL AND METHODS

Twenty-one newborn infants were enrolled in the study. The mean of the gestational age was 36.5 weeks (range: 31-40) and the birthweight was 2572 g (range: 1140-3550). As a part of clinical examination spinal tap and CSF evaluation was indicated because of apneic spells, fever, perinatal asphyxia or intracranial haemorrhage. In the case of positive bacterial cultures, or blood in the CSF, the patients were excluded from the study. The clinical data are shown in Table I.

Plasma and liquor Na concentrations were measured by flamephotometry, the osmolality was detected by Knauer osmometer. The concentrations of PRL were determined by RIA method /4/. The plasma and CSF DA levels were measured by the method of Hahn /15/. Statistical analysis was performed using Student's t-test. The results are expressed as a mean $\pm$ SEM.

TABLE I

Clinical data of the enrolled patients

Gestational age (weeks)	36.5 (31-41)
Birth weight (g)	2572 (1140-3550)
Apgar score 1 minute	8.1 (1- 9)
Time of lumbar puncture (days)	8.3 (1-38)
N	21

RESULTS

The plasma concentration of PRL was 106.52 ± 14.43 ng/ml, significantly higher than the CSF PRL level (43.14 ± 7.39 ng/ml, $p < 0.01$). This elevated level was observed in all individual cases (Fig. 1). DA concentration in the plasma was much higher than the value detected in the CSF (64.75 ± 13.83 vs 8.64 ± 0.72 ng/ml, $p < 0.01$). No difference was observed between the sodium levels of the CSF and plasma (138.94 ± 1.26 vs 138.04 ± 1.03 mmol/l), however, the osmolality of the plasma tended to be higher than the CSF osmolality (286.7 ± 3.81 vs 276.76 ± 2.19 mosm/kg, $p < 0.05$). Results are listed in Table II.

DISCUSSION

The present study - in agreement with previous data (3,19) - provided further evidence that PRL is present in the CSF, however, in the plasma the concentration is significantly higher during

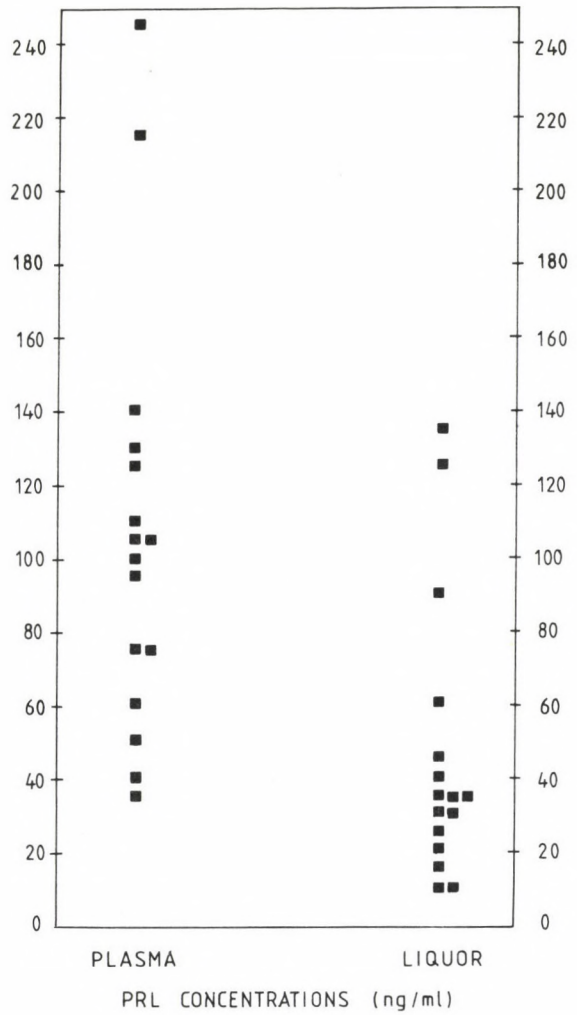

Fig. 1. The individual plasma and CSF PRL values

TABLE II

Plasma and liquor PRL, DA, Na concentrations and osmolality (mean ± SEM). * $p<0.01$, ° $p<0.05$

	Plasma	Liquor
PRL concentration (ng/ml)	106.52±14.43 n=16	46.65±7.39 * n=21
DA concentration (ng/ml)	64.75±13.83 n=8	8.64±0.72 * n=21
Na concentration (mmol/l)	138.04±1.03 n=13	138.94±1.26 n=18
Osmolality (mosm/kg)	286.70±3.81 n=8	276.76±2.19 ° n=21

the neonatal period. We found no correlation between PRL and DA concentrations of CSF. Exogenous DA exerts an inhibitory effect on the secretion of PRL even in sick preterm neonates /26/ and results in a significant elevation of CSF DA concentration /27/. The latter observation suggests that DA crosses the blood-brain barrier in the neonate.

Although PRL does not cross the blood-brain barrier /29/, it could be detected in the CSF. The accessibility of PRL to CSF is unique among polypeptide hormones. In comparison GH, LH, ACTH, and PTH, do not significantly accumulate in the CSF reflecting an increased hormone level in the blood /1, 6, 10/. Walsh et al. demonstrated that PRL is transported from the circulation to CSF by a specific PRL receptor mediated process /30/. In the rat, hyperprolactinaemia results in an elevation in the concentration of CSF PRL /5, 19/, suggesting that CSF PRL is of pituitary origin. However, a recent report demonstrated that hypophysectomy, which suppressed concentration of PRL in the plasma, did not alter its baseline or cycling pattern of CSF /8/. CSF PRL may be derived from two sources:

1. from the transport of PRL from the vasculature to CSF,

2. from prolactin-containing cells localized in the central nervous system.

Estradiol replacement to the hypophysectomized female rat normalized the CSF PRL suggesting that CSF PRL is regulated by an estrogen dependent mechanism at an extrapituitary site and that hypothalamic immunoreactive-PRL may be one source of CSF PRL /8/. The role of PRL in the CSF is far from clear. However, PRL has been suggested to regulate its own secretion by the anterior pituitary via short negative-feedback loop acting on the dopaminergic nerve terminals in the median eminence /2, 14, 24/.

During the neonatal period the PRL serves as a regulator of water and electrolyte homeostasis /11, 12/. Moreover, Coulter and Lorenzo et al. suggested that PRL regulates the water and electrolyte balance of the brain /7, 20/. One site of its action could be the choroid plexus which contains PRL receptors and is responsible for the CSF secretion /20, 30/. Besides PRL, other hormones like vasopressin and atrial natriuretic peptide (ANP) are involved in this process /9, 13/. Acute alteration of serum osmolality has an effect on both CSF formation rate and brain water content /17/. Recently Tulassay et al. demonstrated that ANP content of CSF correlated with CSF pressure but not with sodium concentration or osmolality /28/. In patients suffering from empty sella syndrome the impairment of CSF dynamics was found. A correlation between elevated intracranial pressure and hypothalamo-hypophyseal control of PRL secretion may exist in these patients /22/.

In conclusion: liquor PRL was found to be lower than plasma PRL. We could not demonstrate a correlation between the osmolality and PRL or DA concentrations in the CSF, however, the intracranial pressure was not measured. On the basis of these observations DA probably primarily does not control the PRL content of CSF. Further studies are needed to elucidate the possible role of CSF PRL in the regulation of CSF formation and in the control of water and electrolyte balance of brain during early human development.

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Acta Paediatrica Hungarica 32 (4), pp. 309-317 (1992)

THE DISTRIBUTION OF LYMPHOCYTE SUBPOPULATIONS IN AN INFANT WITH PRRIMARY INTESTINAL LYMPHANGIECTASIA

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Received 13 October 1992

Authors analysed in an infant with primary intestinal lymphangiectasia the number of intraepithelial lymphocytes and the distribution of T lymphocyte subpopulations in the jejunal mucosa with immunohistochemical methods. It was established that the number of intraepithelial lymphocytes and of the cells belonging to the various T lymphocytes markedly decreased in the patient compared to controls, however the decrease of the CD4 cells was less than that of CD8 cells, thus the CD4/CD8 ratio increased. Authors conclude that the increase of CD/4/CD8 ratio in the jejunal mucosa may explain the absence of frequent gut infections in intestinal lymphangiectasia.

INTRODUCTION

Intestinal lymphangiectasia (IL) is a rare disorder characterized by dilated lymphatics within the small intestinal mucosa, which can easily rupture and in consequence a protein-losing enteropathy occurs /1, 2/. This abnormality may be primary or secondary to conditions such as tuberculosis, tumour, or retroperitoneal fibrosis /3, 4/.

In Hungary some case reports on this disease have also been published /5, 6, 7/. Kósnai et al. through the presentation of their cases gave a very thoroughful summary of the diagnosis and therapy of IL /5/.

IL is sometimes associated with an immune deficiency state with abnormalities in both humoral and cellular immune system

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/8, 9/ due to loss of immunoglobulins /10, 11/ and T cells /12/ into the intestinal lumen. Despite the primary abnormality of the gut as the cause of the immunodeficiency in IL, there is no increased susceptibility of gut infections /8/. This observation suggests that the local gut immunity is not impaired in this condition.

Earlier only surprisingly little attention has been paid to the local gut immunity in IL. Immunohistological analysis of the small intestinal mucosa in IL was reported only by Myszor et al. /10/ who observed a decreased number of intraepithelial lymphocytes (IEL), but found the number of immunoglobulin containing cells in the lamina propria to be normal.

Until now the distribution of lymphocyte subpopulations was not analysed in this disease. In the present work we counted the number of IEL and the distribution of lymphocyte subsets in the small intestinal mucosa of our patient with IEL and the results were compared to those found in healthy controls.

PATIENT AND METHODS

Case report

The six months old male infant was admitted to our department in 1991. From his family history it is remarkable that his brother was born with diaphragmatic hernia and despite the surgical correction he died at age of 13 days. The pregnancy was undisturbed with our patient, he was born with Caesarian section on term because of imminent foetal asphyxia with a birthweight of 3870 gram. He was exclusively breastfed until four months. Since that time he has got also cow's milk. Until his admission, gliadin-containing food was not administered. His development was quite normal, and he did not have any other disease. Two weeks before his admission he developed watery diarrhoea and then swelling of his eyelids and lower limbs was noticed.

Physical examination revealed oedema of his feet and eyelids. Organomegaly was not noted. Laboratory investigation revealed a very low serum total protein (31 g/l). Serum electrophoresis: albumin: 0.46, $alpha_1$-globulin: 0.1, $alpha_2$-globulin: 0.19, beta-globulin: 0.17, gamma-globulin: 0.08. Immunoelectrophoresis: IgG: 1.2 g/l, IgA: 0.26 g/l, IgM: 0.46 g/l. From his other laboratory findings: serum Ca: 1.98 mmol/l, serum P: 1.9 mmol/l, alkaline phosphatase: 45 U/l haematologi-

cal status was normal, absolute lymphocyte count: 1840. Erythrocyte sedimentation rate was normal, proteinuria was not observed.

Abdominal ultrasonography revealed a small quantity of ascites, but otherwise showed a quite normal situation. The clinical and laboratory observations suggested the probability of a protein-losing enteropathy. A small intestinal biopsy was done at the ligamentum Treitz with a two ports Crosby capsule. The mucosal biopsy specimen revealed fenestration of the normal architecture of jejunal villi by large dilated lacteals (Fig. 1), thus the pathological diagnosis of IL was established. As the secondary origin of lymphangiectasia was excluded, the diagnosis was primary IL.

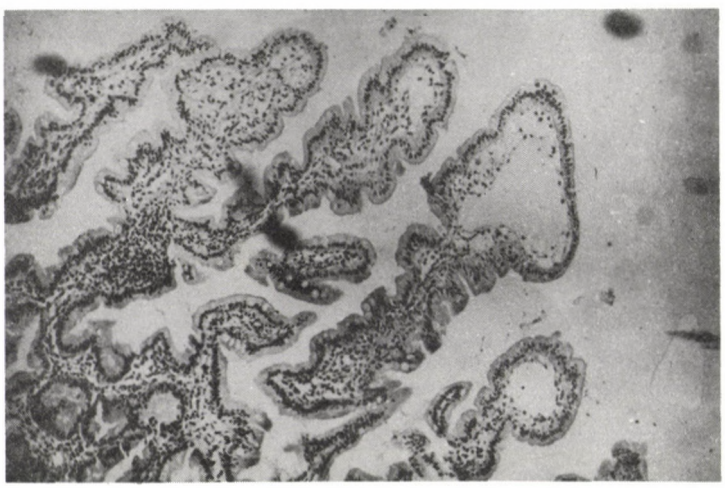

Fig. 1. Jejunal mucosal biopsy specimen (haematoxylin-eosin, magnification x67). The normal architecture of jejunal villi is partially distorted by dilated lacteals

Rapid clinical improvement was observed beside feeding with formula containing exclusively medium-chain triglicerides (Humana MCT), oedema and diarrhoea were rapidly ceased. One year after onset of disease his total serum protein became 60 g/l.

Methods

One of the two biopsy specimens was used for routine histology and the other one for immunohistochemical study. Serial frozen sections were cut at 5 micrometers from each tissue block, as it was published earlier /13/. Monoclonal antibodies and a sensitive immunoperoxidase method was used to determine the numbers of T lymphocyte subsets in the jejunal biopsy specimens /14/. A panel of commercially available mouse monoclonal antibodies was used. CD3 positive T cells were

determined by Leu 4 (Becton Dickinson, Mountain View, Ca, USA) monoclonal antibody. CD4 and CD8 surface antigens were identified by T4 (Coulter Immunology, Hialeah, Fl, USA) and OKT 8 (Ortho Diagnostic Systems, Raritan, N. J., USA) respectively. TCR delta 1 (T cells Sciences Inc., Cambridge, MA, USA) recognizing constant region of delta chain of T cell receptor (TCR) was used to identify all gamma/delta TCR positive T cells. Alpha/beta TCR positive T cells were detected by monoclonal antibody F 1 (T cell Sciences Inc.).

The slides were evaluated using a Leitz-Ortholux light microscope. The numbers of cells in the jejunal mucosa were counted at X900 magnification using a 0.045x0.045 mm graticule placed in the eyepiece of microscope. The cell densities were expressed as cell per mm in the surface and crypt epithelium and as cell per mm^2 in the lamina propria /15/. The number of IEL was counted in the haematoxylin-eosin specimens which were done for routine histology. We compared the cell densities of our patients to those of our earlier reported controls /13, 14/. The control group consisted of 13 children with mean age of 8.2 years (range: 0.65 to 15.2) who were studied for growth retardation. They had neither symptoms nor findings of gastrointestinal disease. The jejunal mucosa was normal in each of them.

RESULTS

The number of IEL was very low in the patient, it was only about one tenth of the lowest value found in the controls (Table I).

The peroxidase-labelled cells were easily identified in the immunohistochemically stained slides. The distribution of lymphocyte subpopulations and the CD4/CD8 ratios in the surface epithelium and the Lieberkühn crypts are summarized in Table I. It is statable that in the surface epithelium the numbers of all different lymphocyte subsets with the exception of gamma/delta TCR positive T cells were lower than the lowest values found in the controls. In the patient the number of CD4 positive cells was less decreased than that of CD8 cells, thus the CD4/CD8 ratio was higher than that in any controls. In the crypts of patient the numbers of cells belonging to the different lymphocyte subpopulations were lower than the mean of those in the controls with the exception of CD4 positive cells. Consequently the CD4/CD8 ratio became elevated compared to the mean of controls.

In the lamina propria of the patient the density of each lymphocyte subset was lower than the lowest value of that in the controls, and so the CD4/CD8 ratio was higher than the highest value of that observed in the controls (Table II).

TABLE I

The numbers of IEL and of cells belonging to different T lymphocyte subpopulations, as well as the CD4/CD8 ratios in the intraepithelial spaces of jejunal mucosa of our patient and of controls

	Patient	Controls (n=13)	
		mean ± SEM	range
Surface epithelium (cells/mm)			
IEL	1.5	30.0 ± 3.3	16.5 - 56.2
CD3	1.4	29.9 ± 3.6	15.4 - 54.0
CD4	0.4	1.5 ± 0.4	0.6 - 3.6
CD8	1.0	26.3 ± 0.02	11.6 - 54.6
alpha/beta TCR	0.9	19.9 ± 4.1	15.8 - 58.2
gamma/delta TCR	0.3	3.5 ± 1.2	0.0 - 6.2
CD4/CD8 ratio	0.45	0.066 ± 0.019	0.01- 0.21
Crypt epithelium			
CD3	2.2	7.1 ± 1.1	2.4 - 12.5
CD4	0.6	0.4 ± 0.1	0.0 - 1.5
CD8	2.1	6.9 ± 1.0	1.4 - 11.6
alpha/beta TCR	2.1	6.8 ± 0.6	2.1 - 13.1
gamma/delta TCR	0.3	2.1 ± 1.1	0.0 - 3.6
CD4/CD8	0.26	0.052 ± 0.024	0.01- 0.28

The sum of the alpha/beta and gamma/delta positive T cells was nearly similar to the number of CD3 positive cells in the intraepithelial spaces and in the lamina propria of both the patient and controls. The sum of the CD4 and CD8 cells was similar to the number of CD3 cells in the intraepithelial spaces of the patient and of controls.

TABLE II

The numbers of cells belonging to different T lymphocyte subpopulations and the CD4/CD8 ratio in the lamina propria of jejunal mucosa of our patient and controls (cells/mm^2)

	Patient	Controls (n=13)	
		mean ± SEM	range
CD3	440	1970 ± 189	889 - 2709
CD4	405	1502 ± 160	837 - 2394
CD8	131	919 ± 104	219 - 1646
alpha/beta TCR	414	1770 ± 176	920 - 2802
gamma/delta TCR	39	96 ± 22	56 - 122
CD4/CD8 ratio	3.1	1.69 ± 0.22	1.0 - 1.9

DISCUSSION

The systemic immunodeficiency in IL is due to low circulating T cell numbers and hypogammaglobulinaemia consequent on loss of lymphocytes and immunoglobulins into the intestinal lumen. The consequences include decreased cell mediated and humoral immunity /8, 9/. In patients with IL, however, there is no propensity to gut infection such as the chronical bacterial infections and giardiasis seen in patients with immunodeficiency states /10/. Our case also confirms this observation, namely in our patient despite marked hypogammaglobulinaemia no gastrointestinal infection was observed. These findings suggest that the local immune system of gut works satisfactorily in this disorder.

We confirmed the earlier report /10/ that the number of IEL is strongly decreased in IL. The absence of frequent infection despite reduction of IEL can be explained by the fact that the lymphocytes in the intraepithelial spaces are mainly suppressor

type /16/ and are not playing a significant role in combating infection. The increase of CD4/CD8 ratio als helps the defence against gut infection.

Although in the lamina propria we observed a very marked decrease in all types of lymphocyte subsets, the elevation of CD4/CD8 ratio in concequence of the less decrease of CD4 than CD8 cells, could explain that less CD4 positive cells can insure a suitable immune defence. With the elevation of CD4/CD8 ratio in IL might be explained by the nearly normal density of immunoglobulin containing cells in the lamina propria of patients with IL, which was reported earlier /10/. A critical step controlling extravasation of lymphoid cells in the small intestinal mucosa is the binding of lymphocytes to vascular endothelium at sites of lymphocyte exit from the blood. Several adhesion molecules, both on the lymphocyte and endothelial cell surfaces, are required for this interaction /17/. In pathological state, expression of these molecules may change. It can be supposed that in IL the number of those endothelial determinants increase for which the CD4 T cells have receptors, and therefore the extravasation of these cells to the mucosa may increase, partly compensating the loss of them to the intestinal lumen.

The decrease of alfa/beta and gamma/delta TCR positive cells are nearly similar in IL which does not suggest a special role for gamma/delta positive cells in this disorder contrary to the observations in coeliac disease /14/.

On the basis of the immunohistological analysis of the jejunal mucosa of our patient, it can be concluded that the increase of CD4/CD8 ratio in both the lamina propria and intraepithelial spaces may explain the absence of frequent gut infections in IL despite the significant decrease of lymphocytes.

ACKNOWLEDGEMENTS

This work was supported by grants from the Hungarian National Science Fund (No. 1122) and the Sigrid Juselius Foundation, Finland.

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Acta Paediatrica Hungarica 32 (4), pp. 319-324 (1992)

SEROTONIN CONCENTRATION IN OFFSPRING OF PARENTS SUFFERING FROM PREMATURE CORONARY ARTERIAL DISEASE

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Received 12 June 1992

Thirty-one 3 to 14 years old offspring of parents who had an acute myocardial infarction before age of 45 and 42 healthy children without any family history of cardiovascular disease were investigated. A significant increase in plasma free serotonin concentration was seen in endangered children. From these results it may be supposed that vascular endothelium is dysfunctional in offspring of parents suffering from premature coronary arterial disease.

INTRODUCTION

Several recent studies show that the interactions of platelets with the vessel wall are obviously relevant both in the pathogenesis and in the clinical manifestation of atherosclerosis. Endothelial cells due to their critical anatomical position between the circulating blood and the media of the vascular wall, play an important regulatory role. Platelets remain inactivated in healthy blood vessels and do not adhere or aggregate. Aggregation of platelets leads to the release of their contents. Important among these substances is serotonin.

The present study was undertaken to determine whether the plasma free serotonin concentration altered in children whose parents had an unquestionable acute myocardial infarction before the age of 45.

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MATERIALS AND METHODS

Thirty-one 3 to 14 years old offspring, 16 girls and 15 boys, of parents suffering from premature coronary arterial disease were involved in the study. In addition 42 healthy children of the same age, 26 girls and 16 boys, hospitalized because of various complaints for observation to the pediatric department, without any family history of cardiovascular diseases, as controls, were investigated. Blood samples were collected by venepuncture after fasting overnight at 7-8 a.m. after 15 minutes quiet bed-rest. None of the children were taking any medication before start of the study. Children affected by metabolic diseases, epilepsy, mental or motor retardation of any origin, were excluded. Plasma free serotonin determination was performed according to Shellenberger and Gordon's method /12/. Platelet count was studied by Medicor PS-5 analyser. All values are expressed as mean $\pm$ SEM. Statistical significance between group means was assessed by Student's t-test.

RESULTS

The groups were not divided into subgroups because there was no statistically significant difference between the serotonin level in children whose both parents or one parent and grandparents had premature coronary arterial disease and in children whose one parents had an early acute myocardial infarction. As shown in Table I, a significant increase in levels of plasma free serotonin was seen in offspring of parents suffering from early coronary arterial disease compared to controls. There was no difference with respect to platelet count (Table II). No gender difference was seen in the examined parameters.

DISCUSSION

It is generally recognized that serotonin has complex actions on the cardiovascular system /2, 13, 14, 15/. It is accumulated in platelets by both active transport and passive diffusion from circulating plasma and stored in dense granules

TABLE I

Plasma free serotonin levels in children (µg/dl)

	Control		Endangered	
	Girls	Boys	Girls	Boys
n	26	16	16	15
$\bar{x}$	6.6	6.9	10.1^{xx}	9.5^{x}
SEM	0.5	0.5	0.9	1.1

x = $p < 0.05$
xx = $p < 0.01$ compared to control

TABLE II

Platelet count in cildren (G/l)

	Control		Endangered	
	Girls	Boys	Girls	Boys
n	26	16	16	15
$\bar{x}$	258.7	265.0	219.6	222.9
SEM	14.1	21.1	16.5	11.0

within the platelets. Serotonin is taken up by platelets, where it is stored, and by vascular endothelial cells, where it is inactivated enzymatically. An elevated serotonin concentration will be present if uptake mechanism are inhibited, and the turnover of platelets is accelerated or when the vascular endothelium becomes dysfunctional or damaged. We have shown a statistically significant increase in plasma free serotonin concentration in children with risk of cardiovascular disease. From this result it must be supposed that there is an endothelial dysfunction in children of parents suffering from premature coronary arterial disease. Endothelium appears to have a critical role in modulation of vascular responses to vasoactive substances that are released from aggregating

platelets /1,8/. Platelets do not adhere to healthy, intact endothelium, however, the injured endothelium activities circulating platelets to adhere sub-endothelial structures of damaged vessel /1, 3, 6, 7/. It has already been suggested that endothelium is dysfunctional in early atherosclerosis /5/. The platelet-aggregation leads to the release and local accumulation of serotonin that promotes further platelet aggregation, coronary artery vasoconstriction and reduction in coronary blood flow /2/. Atherosclerosis appears to increase responsiveness to serotoninergic vasoconstrictor responses, probably in part by producing a functional defect in endothelium /3, 5, 6/. Many factors can damage the endothelium and contribute to the pathogenesis of atherosclerosis. Among them, oxygen-free radicals considered to be the most active /4/. It seems that both exogenous and endogenous oxygen-free radicals react with the endothelial membrane and damage the endothelium, as shown by an increase in malondialdehyde. Raised concentration of lipid peroxides, induced by free radical formation, have been found also in children of parents suffering from early acute myocardial infarction /11/.

From this study and by reason of our previous examinations /9,11/, we concluded that in children with high-risk of cardiovacular diseases, there is an endothelial dysfunction, which is equivalent to the early and critical stage of the atherosclerotic process.

Repeated injury of vessel-wall, caused by oxidation products of dietary lipids, smoking, diabetes, hypertension, stress, and materials originating from platelets, lead to the development of lipid-rich atherosclerotic lesions /10/. Therefore, it would be necessary to emphasize the importance of eliminating environmental influences and change life-style, as early as possible, in exposed families. If child has a parent, who had an acute myocardial infarction before the age of 45, all other risk factors should be altered.

ACKNOWLEDGEMENT

We thank Mária Almási for expert technical assistance.

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Acta Paediatrica Hungarica 32 (4), pp. 325-332 (1992)

INSULIN RESISTANCE IN OBESE BOYS WITH ACANTHOSIS NIGRICANS

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Received 4 January 1992

Insulin resistance was investigated in three obese boys with acanthosis nigricans and their results were compared to those obtained in non-acanthotic obese patients. Blood glucose immune reactive serum insulin and C-peptide during oral glucose tolerance test and ^{125}I-insulin binding investigated. Obese patients with acanthosis nigricans were more insulin resistant than simple obese controls. Insulin binding studies performed in two acanthotic patients suggested that one of them had insulin resistance type A, and the second patient had insulin resistance type B. According to the results acanthosis nigricans can serve as a marker for severe insulin resistance in obesity.

INTRODUCTION

Acanthosis nigricans (AN) is a dermatosis with unknown etiology characterized by a velvety, smooth thickening of the skin, accentuated skin markings and varying degrees of hypertrophy of the epidermis. Histologic findings are hyperkeratosis, papillomatosis and hyperpigmentation /10, 15/.

AN can occur at any age both in males and females but its exact incidence is unknown. The appearance of this dermatosis is a various omen in adults, in many of whom internal malignancy develops. However, malignancy associated with AN has not been reported in children /10, 11/. Some patients with AN in childhood and adolescence have been described as lean, whereas others were obese /16/. Children with AN often have different congenital syndromes and endocrinological abnormalities /10, 11/. AN may be associated with insulin

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resistance caused by a decreased number of insulin receptors, antireceptor antibodies and postreceptor defects /1, 5, 9/.

Recently three boys with obesity and AN were admitted to our department. Since the insulin resistance is also a common feature in childhood obesity /8, 13/, present work was performed to evaluate the insulin resistance of these obese acanthotic boys and to compare their results to those obtained from weight and age matched non-acanthotic obese boys.

PATIENTS AND METHODS

The clinical data from acanthotic obese patients (AO1, AO2, AO3) are presented in Table I. They are 6, 11 and 13 years old. The bone age was equal to chronological age in one of our patients, and it was accelerated in the two other cases. The degree of overweight was considerable in all of them. They had been obese for 2-4 years. The signs of puberty were presented in two of our cases. At the time of the study acanthotic skin lesions were observed in areas of neck and axillae and, in one of our patients, they could be seen in antecubital and inner thigh areas, too. The patients and their parents could not determine the exact time of the acanthotic lesions. Family occurrence of AN was not mentioned. The simple obese control group consisted of 7 children with obesity ranging in age from 7-13 years with weight ranging between 160-190% of ideal body weight.

An oral glucose tolerance test (OGTT) with a 50 g/m^2 glucose load was performed after an overnight fast. Blood was sampled at 0, 30, 60, 90, 120 and 180 m after the glucose ingestion and plasma glucose was determined by the glucose oxidase technique. Serum immune reactive insulin (IRI) and C-peptide were measured by commercial radioimmunoassay (RIA) methods (IRI: Izinta, Hungary, C-peptide: Serono). The study of ^{125}I-insulin binding to red blood cells was performed by a modification of the method of Gambhir et al. /4/; non-specific binding was determined at 10^5 ng/ml of insulin and subtracted from total binding. The presence of insulin receptor antibodies in sera of acanthotic patients was evaluated by determining if patient-serum could decrease the insulin binding to red blood cells obtained from normal persons.

RESULTS

The results are summarized in Table II and in Fig. 1. In upper part of the table the basal blood glucose. IRI and C-

TABLE I

Clinical data of patients with obesity and acanthosis nigricans

	AO1	AO2	AO3
Age (year)	6	11	13
Bone age (year)	6	14	14
Height (cm)	125	163	161
Body weight (kg)	40	82	80
Body mass index - BM (kg/m^2)	26.5	30.8	31.6
Degree of obesity (percent of ideal b.w.)	160	165	167
Puberty	P0	P3	P4

peptide concentrations and Σ glucose, Σ IRI and Σ C-peptide values during OGTT are demonstrated. Although the results obtained from acanthotic patients are individual values, a more marked reactive hyperinsulinaemia in all AO cases and a decreased glucose tolerance in two AO patients can be seen, compared to results of the simple obese group. The competition-inhibition curves of the ^{125}I-insulin binding studies are demonstrated in Fig.1. Specific radioactive insulin binding is decreased in AO1 and AO2 patients compared to nonacanthotic obese patients. Similarly, Σ C-peptide /Σ IRI ratio is decreased in acanthotic obese boys compared to the controls (Table I). It is also shown that inhibition dose of unlabelled insulin required to decrease maximal binding by 50% (ID_{50}) is very high in AO2 patient indicating a decreased hormonereceptor affinity. Serum of this patient could decrease reasonably (by 54.2%) the specific labelled insulin binding to red blood cells obtained from a normal person shown the presence of insulin receptor antibodies.

TABLE II

Results of blood glucose, serum IRI and c-peptide measurements during OGTT and insulin binding studies

Parameters		Simple obesity (n = 7; X ± SD)	Obesity with AN AO1	AO2	AO3
Blood glucose (mmol/l)	basal	4.88 ± 0.16	5.2	5.9	6.3
	Σ	33.1 ± 3.1	30.7	36.9	41.7
IRI (pmol/l)	basal	135.6 ± 25	197	157	275
	Σ	1892 ± 275	5247	3864	4968
C-peptide (nmol/l)	basal	0.31 ± 0.05	0.30	0.38	0.50
	Σ	4.51 ± 0.42	5.90	5.18	6.60
Σ C-peptide/Σ IRI (molar ratio)		2.65 ± 0.7	1.12	1.34	1.33
Specific binding of ^{125}-I- insulin %		5.10 ± 1.38	1.98	1.14	-
Bound insulin (pg/10^9 blood red cells)		2.27 ± 0.61	0.88	0.51	-
Inhibition dose-ID_{50} (10^{-6} mol insulin)		3.5 ± 0.5	4.2	14	-
Percental inhibition of AN sera (%)			11.2	54.2	

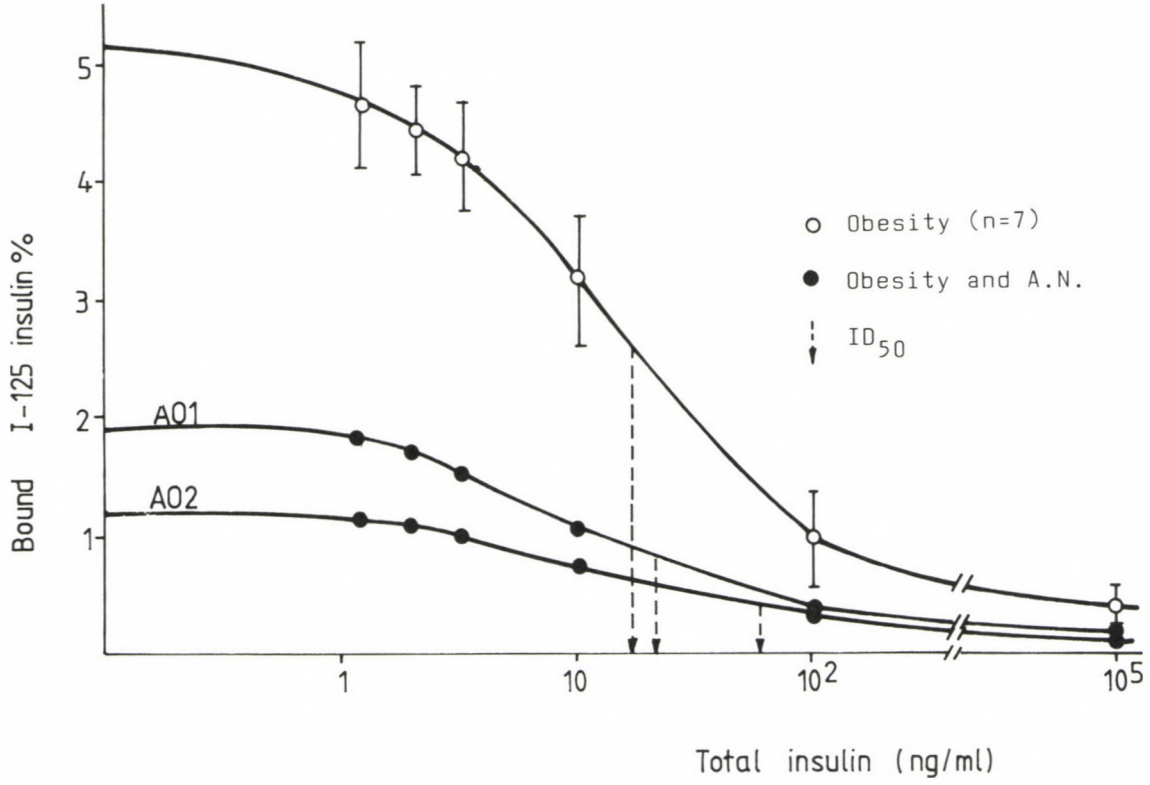

Fig. 1. Competition - inhibition curves of insulin binding studies in red blood cells obtained from patients with obesity and acanthosis nigricans and controls with simple obesity

DISCUSSION

In the past decade there have been reported by several authors a severe insulin resistance in AN /9, 10, 14, 15/. It was also demonstrated that insulin resistance is a common feature in obese patients /8, 9/. Since insulin resistance can be seen in lean acanthotic patients /16/, the question arises whether the AN could account for the insulin resistance seen in obese subjects with AN.

In our acanthotic obese patients basal and reactive serum IRI concentrations were more increased than those of non-acanthotic obese controls. The decreased ration of C-peptide and IRI during OGTT (Σ C-peptide/Σ IRI) is a further characteristic feature of insulin resistance in obesity /2/. A decreased Σ C-peptide/Σ IRI ration was found in our acanthotic obese patients compared to results obtained from nonacanthotic obese controls. These results suggest that insulin resistance is more severe in obesity with AN than in simple obesity. Recently several works have also reported a greater degree of insulin resistance in obese acanthotic subjects when compared with weight controls /14, 15, 16/. It was also demonstrated that insulin binding to skin fibroblast was significantly lower in the obese acanthotic subjects than in the simple obese patients /14/. It seems that AN can be considered as a marker for severe insulin resistance in obesity. In AN insulin resistance can be due to a receptor and a postreceptor defects /1, 5/. The receptor defect can be either type A characterized by a decreased number of insulin receptor per cell or type B where the presence of antireceptor antibodies and decreased insulin binding can be demonstrated. The receptor affinity is normal in AN with insulin resistance type A and it is decreased in AN with insulin resistance type B /3/.

To assess potential defects in insulin at the cellular level insulin binding to red blood cells and a possible decreasing effect of sera from acanthotic patients on insulin binding were tested in our AO1 and AO2 patients /4/. A marked decrease in

insulin binding to its receptor sites was found suggesting that the insulin resistance is due to alteration in insulin and its receptor interaction. The difference found in ID_{50} values of AO1 and AO2 patients indicates a decreased receptor affinity in the latter case, since there is an inverse relationship between ID_{50} and receptor affinity /3, 7/. Furthermore, in AO2 patient the defect in the insulin-receptor interaction appears to be secondary to circulating antibodies which are directed at the insulin receptors and impair their functions, since his serum could decrease the specific insulin binding to red blood cells obtained from a normal person. Our results suggest that AO1 patient has insulin resistance type A, and AO2 patient has insulin resistance type B. Unfortunately this characterization could not have been performed in AO3 patient.

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Acta Paediatrica Hungarica 32 (4), pp. 333-346 (1992)

THE USE OF HUMAN RECOMBINANT ERYTHROPOIETIN IN CHILDREN ON CHRONIC DIALYSIS

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Received 16 October 1992

Human recombinant erythropoietin (r-HuEPO, Eprex^{R}) was administered to 8 children with chronic renal failure and high transfusion requirement. The hormone was given i.v. 2-3 times per week at the end of the dialysis. The selected initial dose (160 U/kg/week) was gradually raised up to a maximum of 400 U/kg/week. Anaemia normalised by the tenth week. The aimed target haematocrit was 0.33, the average maintenance dose was 250-300 U/kg/week. The absolute reticulocyte count seemed to be a more sensitive indicator of the actual erythropoietin effect than the haematocrit level. No major adverse effects (convulsion, progressive hypertension, thrombosis) were observed during treatment with r-HuEPO.

The need for transfusions decreased dramatically, no transfusion was needed following the second week of treatment. The general condition improved substantially. In one hyperimmunized child the cytotoxic antibody titer decreased by 75 per cent.

INTRODUCTION

Renal transplantation is the treatment of choice of children with end stage renal failure. Even if this positive outcome occurs, it is usually preceded by a long period, sometimes years of chronic dialysis. Renal anaemia is one of the major problems encountered in children with chronic uraemia on renal replacement therapy.

The causes of renal anaemia in children are multiple:

1. The bone marrow of uraemic children is characterised by a significantly lower cellular density when compared to that of

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adults suffering from the same level of uraemia /12, 14/. It is hypothesized that the uraemic toxins inhibit the physiological development of the bone marrow. As a consequence renal anaemia of childhood is much more expressed as in later periods of life /12, 18, 19/.

2. Intestinal and extraintestinal blood losses can further accentuate the anaemia. These losses can reach the level of 2.6-6.7 l/1.73 m^2/year /12-14/.

3. The half-life of erythrocytes is inversely correlated to the raise of the serum carbamid nitrogen level. The mean half-life of the erythrocytes is 60-70% of the normal /2, 12, 14/.

4. The major site of erythropoietin production is the kidney. In end stage renal disease renal erythropoietin production is decreased or even disappears (nephrectomy). Thus the physiological stimulus for the bone marrow is insufficient or absent /2, 3, 8, 12/.

Until the introduction of erythropoietin treatment the management of the resulting severe anaemia consisted in repeated transfusions. Repeated blood transfusions not only account for transmission of blood-born infections (hepatitis, HIV, CMV), but can also lead to chronic iron overload and corresponding organ damage. Furthermore, multiple transfusions can lead to sensitization and development of cytotoxic antibodies reducing the probability of transplantation.

The introduction of erythropoietin offers a rational alternative for the treatment of renal anaemia. The hormone was first described by Paul Carnot in 1906. Since the late fifties it has been known that the major site of this hormone production is the kidney. In the early eighties the aminoacid sequence of the hormone was clarified, the gene was isolated and transferred in ovarian cells of the Chinese hamster. The cell cultures provided sufficient quantities of human recombinant erythropoietin (r-HuEPO) for clinical use /4, 5, 8, 9/. The first clinical studies were performed in 1986 /4, 5, 22/. The first Hungarian clinical trials began in 1989. We report about the results of the first paediatric administration of r-HuEPO in Hungary.

PATIENTS AND METHODS

We began to administer r-HuEPO (Eprex^{R}-Cilag) in October 1989. The results of the treatment of eight children are summarized in this study. Mean age at the beginning of the study was 12.2 (range 5.5-18) years. The male:female ratio was 2:6. Bilateral nephrectomy was performed in two patients because of uncontrollable hypertension. The underlying disease leading to renal failure and the main clinical data of the patients are listed in Table I. All patients were treated with chronic haemodialysis except patient 6. Haemodialysis was performed three times weekly (4-5 hours per session). r-HuEPO was administered intravenously at the end of the dialysis. One patient (No. 6) was treated with chronic ambulatory peritoneal dialysis (CAPD). She received r-HuEPO subcutaneously.

The dose of Eprex^{R} was raised at intervals of one-two weeks until the desired haematocrit (HTC) level was reached (30-32%). The beginning dose was 160 U/kg/week. Later on the dose was adjusted according to the actual HTC level ("open-dose" study).

The dose of Eprex^{R} was calculated as U/kg/week. The laboratory parameters (HTC, reticulocyte count, creatinine level) were measured with routine laboratory methods once weekly.

The mean and SEM of the laboratory data of the same treatment phase (same treatment week) of the patients were calculated.

RESULTS

The initial dose of r-HuEPO was based on the review of the literature /1, 4, 6, 10, 11, 13, 15, 16, 17, 20/. The initial dose of 1160 U/kg/week was gradually raised until the 6-7. week, to a maximal dose of 400 U/kg/week. The mean dose stabilised at 250-300 U/kg/week at about the fifth month of treatment. The data on the Eprex^{R} doses are represented on Fig. 1.

The mean basal HTC of our patients was 0.18 despite repeated transfusions. A substantial raise of the HTC level was observed after the sixth week of treatment, the highest values were observed at weeks 14-15. Later on the values stabilized at 0.33 (Fig. 2).

Before beginning the treatment with r-HuEPO the absolute reticulocyte count of our patients was $0\text{-}1x10^{9}$ signalling a

TABLE I

Data of the patients at the time of initiation of recombinant human erythropoietin (Eprex®) therapy

Number	Age (years)	Sex	Basic disease	Duration of previous dialysis (month)	Previous transfusion requirement (packed red cell)
1.[x]	18	♂	chronic GN	57	1.5 U/mo
2.[x]	11.5	♂	FSGS	32	2 U/mo
3.	13	♀	FSGS	20	1.5 U/mo
4.	10	♀	interstitial nephritis	18	2 U/mo
5.	12	♀	juvenile nephronophtisis	2	1 U/mo
6.[xx]	13	♀	RPGN	2	1 U/mo
7.	14.5	♀	FSGS	1.5	3 U/mo
8.	5.5	♀	FSGS	1	2 U/mo

Remarks:
x = after bilateral nephrectomy; "anephric patient"
xx = CAPD patient
FSGS = focal segmental glomerulosclerosis
RPGN = rapidly progressive glomerulonephritis

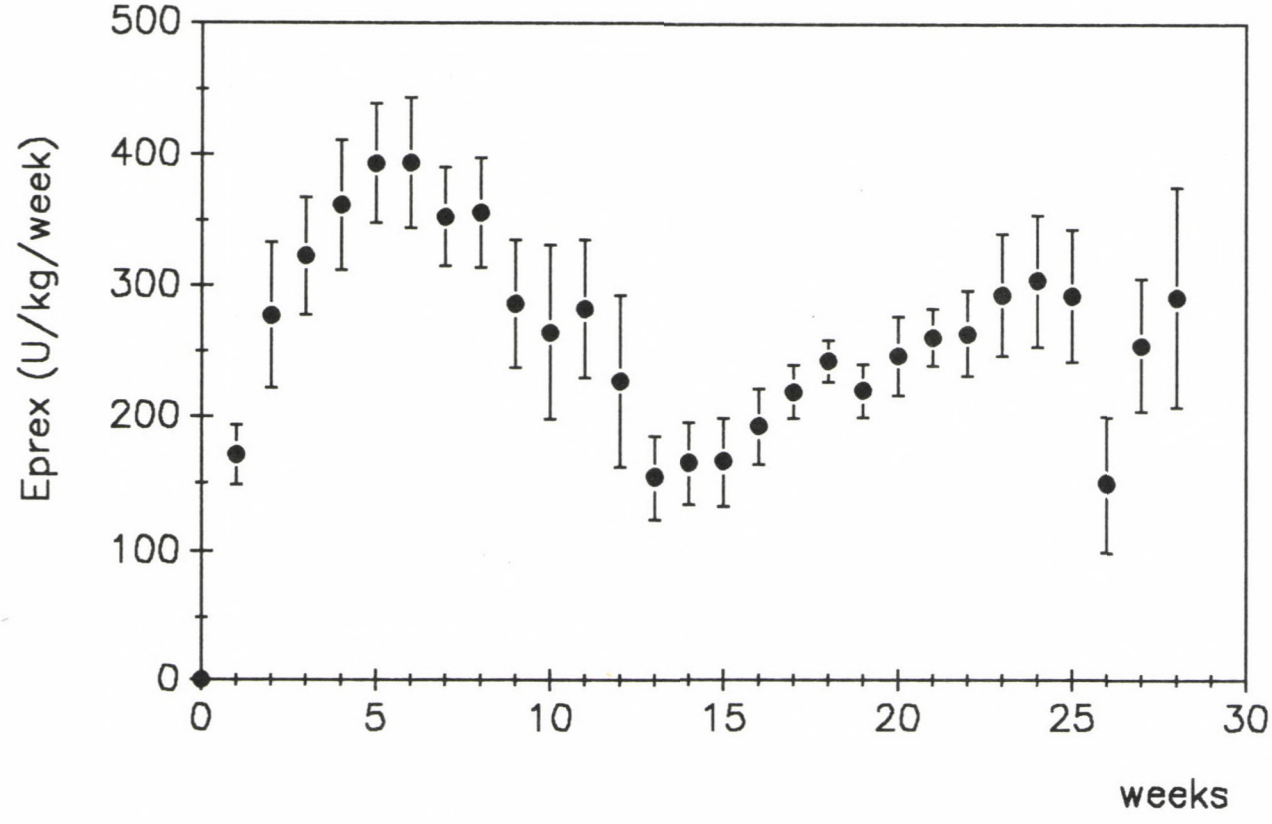

Fig. 1. Doses of Eprex^{R} during treatment (n = 8, mean ± SEM)

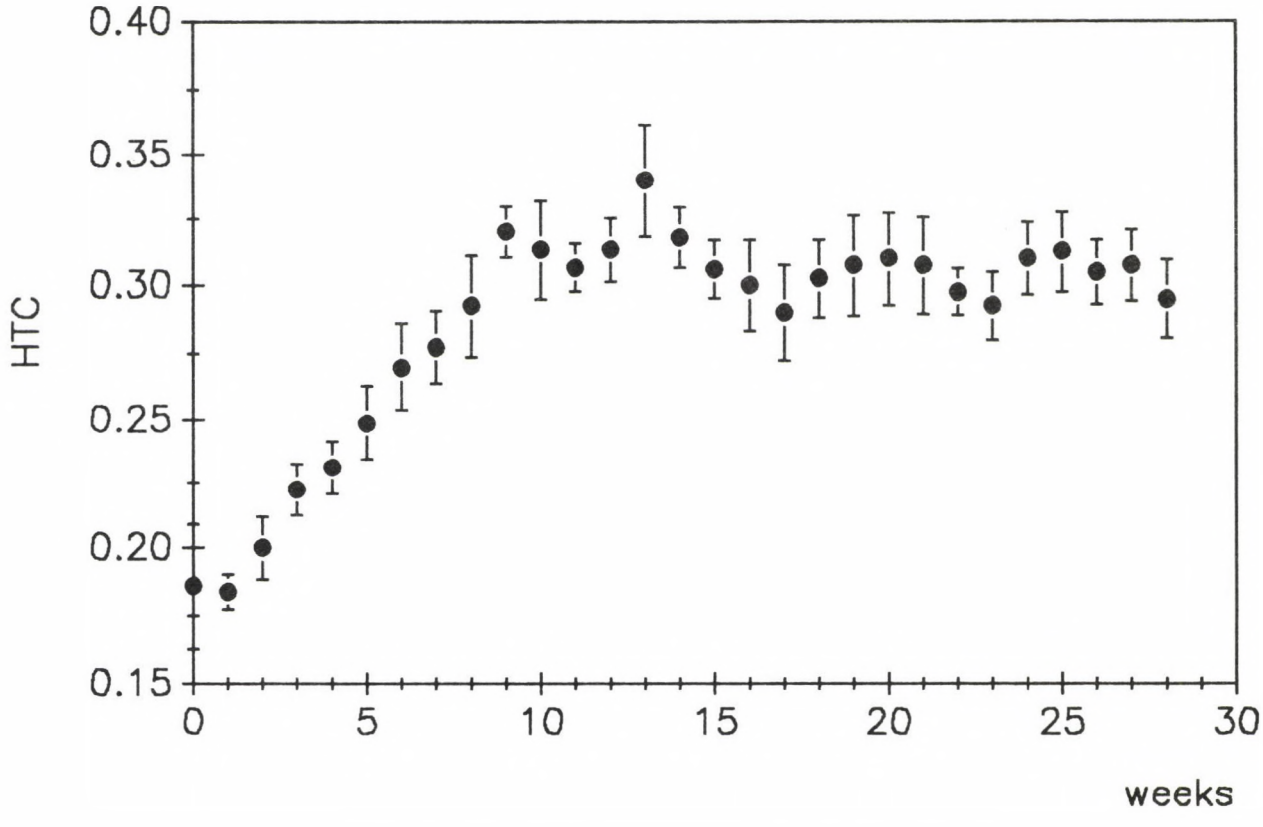

Fig. 2. Evolution of the haematocrit value during Eprex^{R} treatment (n = 8, mean $\pm$ SEM)

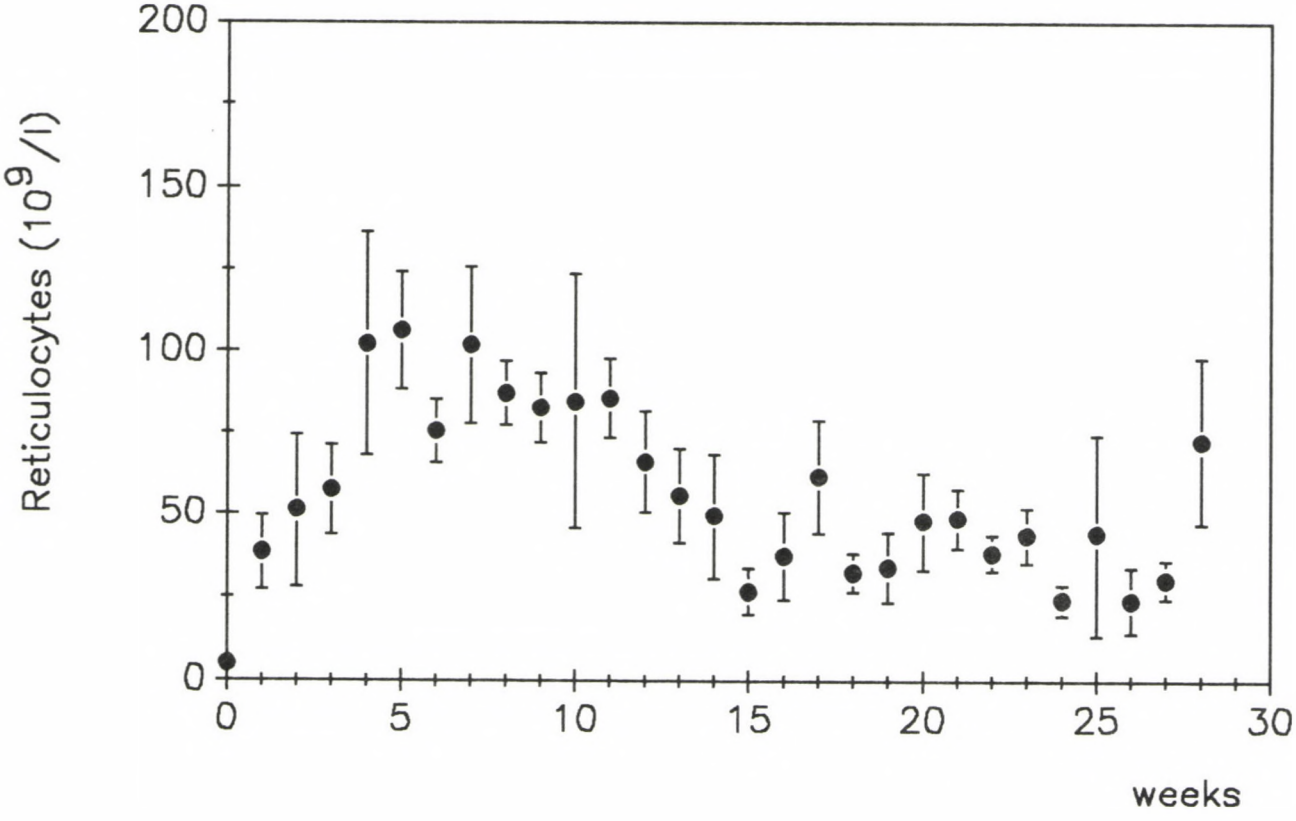

Fig. 3. Evolution of the reticulocyte count during Eprex^R treatment (n = 8, mean ± SEM)

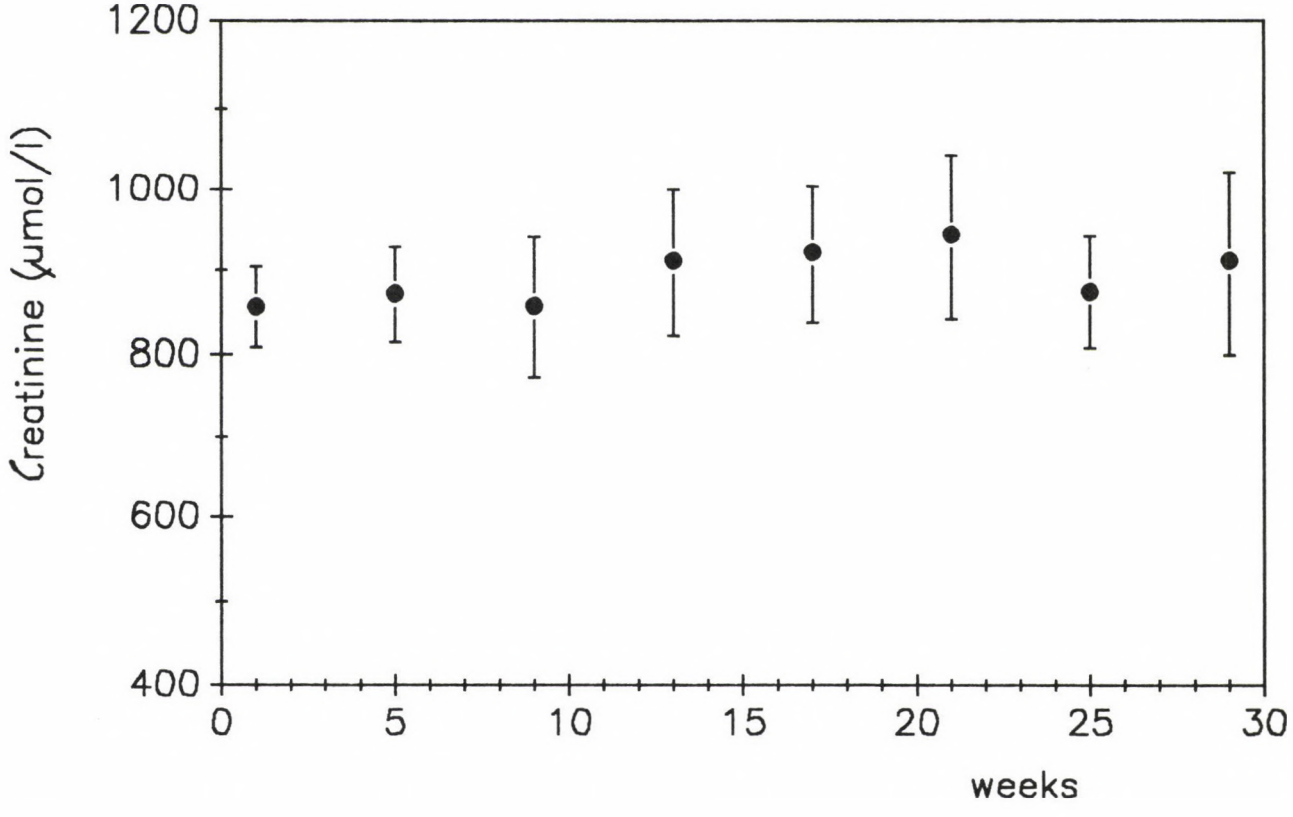

Fig. 4. Serum creatinine levels of Eprex^{R}-treated uraemic children (n = 8, mean ± SEM)

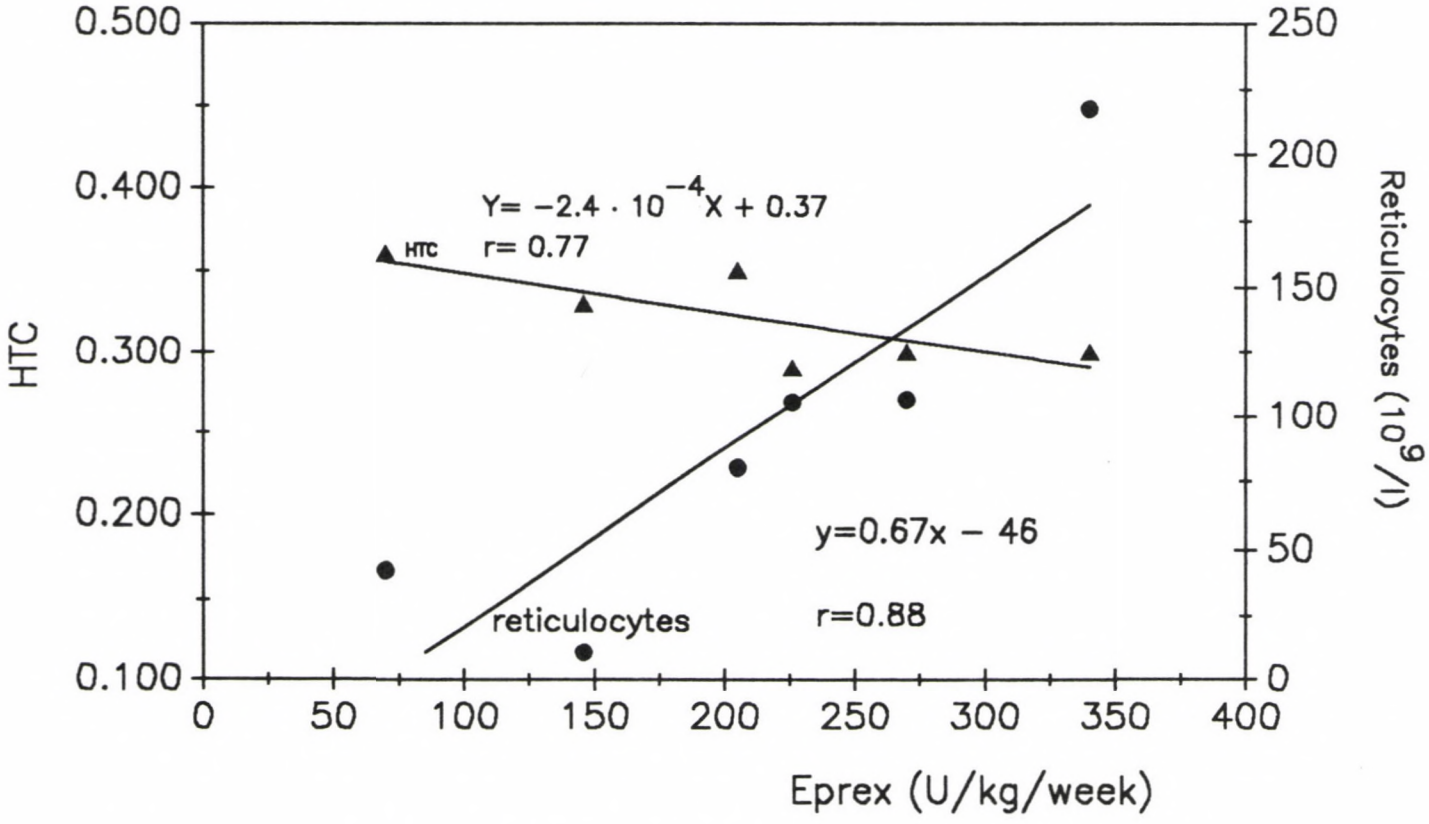

Fig. 5. Correlations between erythropoietin doses, haematocrit (HTC) and reticulocyte count during the tenth week of treatment ($n = 8$, mean $\pm$ SD)

serious depression of the erythrocyte production. At the fifth week of treatment a one-hundredfold raise of the reticulocyte count was observed, reaching a two-three-fold normal level. After the 15th week of treatment the values stabilised at about the normal level (Fig. 3).

Fig. 4 shows the correlations between the Eprex^{R} dose, HTC level and reticulocyte count at the tenth week of treatment. A strong positive linear correlation was observed between the dose of hormone and reticulocyte count ($y=0.67\ x-0.46$, $r=0.88$, $p<0.01$). A strong negative linear correlation was observed between HTC and the dose of r-HuEPO ($y=2.4\ 10^{4}x+0.37$, $r=0.77$, $p<0.01$).

The evolution of serum creatinine levels during treatment are shown on Fig. 5.

DISCUSSION

Erythropoietin is the treatment of choice of patients with end stage renal failure and the resulting severe anaemia /16, 18/. The site of the hormone synthesis is the cortical tubular cell /9/. Erythropoietin is the most effective stimulus of the bone marrow erythrocyte production.

All of our eight patients needed regular transfusions prior to the introduction of the hormone. As a major advantage of r-HuEPO treatment the need for transfusions fell to zero after several weeks of administration. Only patients N^{O} 4 and 7 needed one further transfusion during the first two weeks of treatment. The initial administration of 160 U/kg/week and its gradual increase up to 400 U/kg/week proved to be a safe dosage. No excessive raise of haematocrit level occurred. The target haematocrit value was set to 0.33 in order to prevent possible side effects (see later). The average maintenance dose was 250-300 U/kg/week.

The absolute reticulocyte count (Fig. 3) was found to be the most sensitive indicator of the efficacy of Eprex^{R}. The data summarized on Fig. 5 clearly indicate that the actual effect of the hormone is reflected by the evolution of the reticulocyte count rather than by haematocrit level.

Further complementary factors: iron, folic acid and vitamin B_{12} are needed to allow the full build-up of r-HuOPE effect /10/. Our previously polytransfused patients (N^{O} 1-4) needed iron replacement following 10-12 weeks of treatment, whereas patients without multiple transfusions (N^{O} 5-8) received iron supplementation after 5-8 weeks of treatment depending on their serum iron levels. Folic acid was routinely substituted because of the losses due to dialysis. Vitamin B_{12} deficiency was not observed.

The mean pre-dialysis serum creatinine levels of our patients were between 800-900 µmol/l. The raise of the haematocrit due to r-HuEPO treatment could have a negative effect on the efficacy of the dialysis /16/. No such side effect was detected in our series, the average creatinin level remained stable.

Several further side effects are reported following treatment with r-HuEPO: deterioration of the underlying hypertension, hypertensive encephalopathy, seizures, thrombosis (occlusion of the Cimino arteriovenosus access) and allergic reactions ("flu-like" syndrome) /5, 6, 17, 20/. None of the described complications were observed in our patients.

One further advantage of the treatment was observed in patient No. 1, who has been on haemodialysis for 6 years. His extreme levels of cytotoxic antibodies (nearly 100%) fell to 20% following eight months of Eprex^{R} administration.

In conclusion, erythropoietin administration in the current dosage proved to be a safe and effective alternative for the treatment of renal anaemia. The side effects of multiple transfusions could be spared, and the general condition and physical activity of the patients improved significantly.

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Acta Paediatrica Hungarica 32 (4), pp. 347-369 (1992)

A BEHAVIOURAL TERATOLOGIC STUDY ON OFFSPRING OF SELF-POISONED PREGNANT WOMEN

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Received 13 October 1992

Ninety-three cases born from women who attempted suicide by taking large doses of chemicals and eight cases who had mothers affected by a chemical poisoning as accident during pregnancy were studied. Data of birth weight and gestational time, congenital anomalies and postnatal disorders were obtained in cases and matched controls (sociological twins). Test examinations were performed in both cases - controls and their mothers in the home of families. The mothers of index children had a lower socioeconomic status and more were unmarried than the Hungarian pregnant population at large. The behaviour of index and control mothers showed some obvious differences. The bivariate analyses indicated some possible teratogenic effect of maternal poisoning, but it was not confirmed by multivariate analysis except lower birth weight. Thus differences between cases and control were explained mainly by the maternal behavioural factors.

INTRODUCTION

People who attempt suicide by taking large doses of chemicals or meet with an accident may provide an appropriate "experimental" epidemiological model for the study of the effects of chemicals in the human being /1, 2, 3/. Previously the self-poisoning model was used for the study of the mutagenicity of chemicals in humans /4, 5, 6/. The present paper reports on the teratogenic effect of chemicals. Until now only case reports of self-poisoned pregnant women have been published /7, 8/.

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We have examined the problem in three ways. First, a descriptive epidemiologic study was made in 142 self-poisoned pregnant women whose pregnancy outcomes were compared with their previous and subsequent pregnancy outcomes /9, 10/. Mental retardation occurred in 6.5% of 93 index children and 2.7% of 144 sibs. Furthermore three of 78 index children past the age of 10 (i.e., 3.8%) had obvious behavioural deviations. (Two of them were in prison.) Similar conditions were not found among sibs. These findings prompted us to undertake a second approach, involving the behavioural study of offspring of self-poisoned pregnant women, the results are summarised in this paper. However, there is a third approach: a longitudinal prospective teratogenic study on the offspring of self-poisoned pregnant women /11/.

Our purpose is to examine two questions raised by our previous study. First, it is possible to confirm the previously detected behavioural effect on offspring after severe chemical intoxication in the pregnancy of their mothers? Secondly, if the previous results were confirmed, is it possible to separate the teratologic and maternal-social effects of self-poisoning in the origin of behavioural deviation?

MATERIALS AND METHODS

In 1985, 276 pregnant women (the so-called index mothers) were ascertained by scrutinizing case histories of all poisoned women between the ages of 14 and 50 hospitalized in the Inpatient Clinic of Toxicological Internal Medicine, Sándor and Frigyes Korányi Hospital, Budapest from 1980 to 1984. The diagnosis of pregnancy was based on information given by the women or on gynaecologic examination which were performed in all cases with a missed menstrual period or on any other signs of pregnancy. We planned to visit all index mothers at home. However, 40 had untraceable new addresses. Of 236 index mothers who were visited 52 were dropped from the research due to the misdiagnosis or uncertainty of 51 pregnancies (women denied being pregnant at the time of the home visit; probably these pregnancies were terminated) and one pregnant women was a foreigner. The distribution of 184 pregnancy outcomes evaluated was as follows: induced abortion 31, fetal death 17, and livebirth 136. However, in the latter group three mothers and two children died, 11 mothers were in prison (4.0%), 12 children were adopted or in foster home and seven families

refused to cooperate. Of 101 remaining index mothers, 8 had an accident or overdosage of drugs during pregnancy but they were not separated from women who had self-poisoning during pregnancy because of the low number of this subgroup.

Our purpose was to have both sibling and matched controls. However, 101 index children had 117 sibs and their distribution was uneven, because 44 index children had no sibs and the sibs of 57 remaining index children were of varying ages. Thus, this approach was abandoned and one non-relative healthy control was selected for each index child from the kindergartens of Budapest districts where index children lived. These "sociological twin" controls were matched to the index children by age (month of birth), sex and birth order, and families were matched to index mothers by age, marital status, education (number of school classes), occupation, income and housing conditions.

Test examinations were performed in the home of the families in 1985-1990; the number of home visits was between 2 and 5 with a mean of 3.3. The Budapest Developmental Test /12/ was used for the estimation of the mental development of children under 5. The Behavioural Style Questionnaire /13/ was used for the examination of behavioural-temperament-scale in children aged 4-7 years. The drawing test was used for the study of performence in all children. The Home test /14/ was planned to estimate the family atmosphere and maternal behaviour in children with age under 3.5 years old. The personality of index mothers was examined by the colour-test of Lüscher /15/, the tree test of Koch /16/ and the signature test of Bánáti /17/.

The statistical analysis was carried out by using ...·2 and u test multivariate logit models modified to matched data.

RESULTS

a/ Personal data

The educational and occupational distributions of 101 index mothers are shown in Table I, 56 (55.4%) index mothers were married at the time of poisoning, however, 72 (71.3%) were married at the time of the study.

Of 101 index children, 57 were boys and 44 girls. Their age distribution is shown in Table II. The distribution of birth orders was as follows: 1=51(46); 2=27(39); 3=14(11); 4=8(3); 5=1(2). (Expected numbers based on Hungarian pregnant women having livebirths in the study period shown in brackets.)

TABLE I

Educational and occupational distribution of index mothers (Expected figures of education based on Hungarian pregnant women having livebirths are shown in brackets)

Education (Number of school classes)		Occupation	
4 or less	8 (3)	Unskilled	5
5-7	24 (4)	Semiskilled	15
8	12 (51)	Skilled	38
9-12	55 (31)	Clerk	40
≤13	2 (12)	Professional	3
Total	101 (101)		101

b/ Toxicological data

Of 101 index mothers, 93 (92.1%) attempted suicide by drugs and 8 (7.9%) had accidents (noxious mushroom poisoning 2, intoxication of carbon monoxide 2, pesticide 2, sulphuric acid 1) or an overdose of thrichlorethylene.

The maternal age and the gestational month at the time of poisoning are also shown in Table II. Self-poisoning occurred between the 7th week and the 32rd week of gestation.

The severity of poisoning is shown in relation to gestational trimesters in Table III. Familial and economic reasons were mentioned as a cause of self-poisoning by 39 and 47 index mothers, respectively, while 7 did not give any explanation.

The list of drugs used for self-poisoning is shown in Table IV. Of 93 self-poisoned mothers, 66 (70.9%) used one drug, 15 (16.1%) two drugs, 9 (9.7%) three and 3 (3.3%) four drugs. The most frequently used drugs are benzodiazepines (59 cases; 58.4%).

TABLE II

Distribution of age of index children, maternal age-groups (expected figures based on Hungarian pregnant women having livebirths in the study period are shown in brackets) and gestational month at the time of poisoning

Age of index children	No.	%	Age-group of mothers	No.	%	(%)	Gestational month	No.	%
1-2	15	14.9	15-19	24	23.7	(13.9)	2	7	6.9
3	26	25.7	20-24	34	33.7	(38.2)	3	22	21.8
4	13	12.9	25-29	26	25.7	(32.1)	4	22	21.8
5	16	15.8	30-34	11	10.9	(11.8)	5	20	19.8
6	18	17.8	35-39	4	4.0	(3.3)	6	22	21.8
7	13	12.9	40-44	2	2.0	(0.7)	7-8	8	7.9
Total	101	100.0	Total	101	100.0	(100.0)	Total	101	100.0

Table III

Distribution of severity of poisoning (how many times it exceeds the usual daily dose) at the function of gestational trimester

Severity of poisoning	Trimester			Total
	I	II	III	
mild (2-10x)	19	41	3	63[x]
moderate (11-20x)	3	6	1	10
severe (20-49[x])	5	15	2	22[xx]
very severe (≥50x)	2	2	2	6
Total	29	64	8	101

[x] including 2 accidents
[xx] including 6 accidents

TABLE IV

List of most frequently used drugs for self-poisoning

Drug	No	%
Diazepam	42	31.1
Phenobarbital	22	16.3
Promethazine	10	7.4
Nitrazepam	9	6.7
Chlordiazepoxide	8	5.9
Meprobomate	7	5.2
Glutemide	6	4.4
Others	31[x]	23.0
Total	135	100.0

x Human teratogenic drugs were not used for self-poisoning, however, drugs were combined with alcohol abuse in three cases

c/ Clinical data

Pregnancy complications are shown in Table V; comparable data were not obtained in control mothers. The mean birth weight of index children was significantly lower than that of control children ($u=1.84$; $p < 0.05$) (Table VI). Duration of gestation of index and control cases was not significantly different (Table VII).

The rate of congenital anomalies documented by medical discharge summaries or personal examination did not show significant differences between the study group and two control groups (Table VIII). The expected total birth prevalence of congenital anomalies is about 6.5 in Hungary.

The occurrence of major postnatal diseases was somewhat, but not significantly higher in the group of matched controls than in index children (Table IX).

d/ Results of behavioural tests of offspring

The Budapest Developmental Test (Table X) indicated a significant decrease ($p < 0.005$) in the Development Quotient (DQ) of 70 index children with age under 5 years compared with that of matched controls. The difference is explained mainly by the predominance of lower values (≤ 100) in index children (52.9% vs 15.7%). However, the only three children with scores over 131 were index children.

The Behavioural Style Questionnaire (BSQ) was used in children aged 4-7 years (Table XI). This test is based on 100 questions in order to evaluate nine features of development of children on a six-point scale. Table XI shows the summary of significant deviations between index children and their matched controls in features studied. Boys had significantly lower adaptability after the self-poisoning in all trimesters, while in girls the effect was seen only after self-poisoning in the first and third trimesters. A lower level of emotional behaviour (humour and depression) was seen in both sexes after poisoning in the second trimester (and in the first trimester of index

TABLE V

History of 101 pregnancies studied

Events	No.	%
Physical trauma	8	7.9
Smoking	36	35.6
Regular drinking	9	8.9
Disorders (pneumonia, neurosis, lues)	6	5.9
Threatened abortion	6	5.9
Other pregnancy complications (EHP, cervical incompetency)	4	4.0

TABLE VI

Mean birth weight (g) of index and control children as well as of sibs born from previous and subsequent pregnancies of their mothers

Group	Pregnancies					
	previous		study		subsequent	
	No.	$\bar{x}$	No.	$\bar{x}$	No.	$\bar{x}$
Study	80	3077	101	3046 $\pm$ 553	37	2808
Control	66	3101	101	3170 $\pm$ 478	42	3233

TABLE VII

Distribution of gestational time groups in index and control children

Children	-31 No.	%	32-36 No.	%	37-41 No.	%	42- No.	%	Total No.	%	χ^2_3	p
Index	2	2.0	10	9.9	86	85.1	3	3.0	101	100.0		
											4.75	>0.05
Control	1	1.0	3	3.0	95	94.1	2	2.0	101	100.0		

TABLE VIII

Distribution of congenital anomalies in index children, their sibs and matched controls

Congenital anomaly	Index children (N = 101)	Sibs of index children (N = 117)	Matched controls (N = 101)
Cong. hydrocephalus	0	1	0
Cong. cardiovascular malformation	2	3	2
Diaphragm defect	1	0	0
Cong. dislocation of hip	2	1	1
Clubfoot	1	2	0
Undescended testis	2	0	1
Cong. inguinal hernia	1	1	2
Total	9	8	6
%	8.9	6.8	5.9
χ^2_1	-	0.32	0.65
p	-	>0.05	>0.05

TABLE IX

Distribution of disorders in index children, their sibs and matched controls

Disorder	Index children (N = 101)	Sibs of index children (N = 117)	Matched controls (N = 101)
Meningitis	1	2[x]	0
Brain tumour	0	1	0
High myopia	0	1	0
Recurrent "cold"	4	1	4
Pneumonia	3	2	8
Bronchial asthma	0	2	0
Recurrent gastroenteritis	2	0	5
Other disorders of digestive system	0	1	0
Nephritis	0	1	0
Galactosemia	0	1	0
Jaundice	0	0	1
Drug allergy	1	0	0
Anaemia (severe)	2	1	2
IgA deficiency	0	0	1
Somatic retardation	1	2[x]	0
Total	14	14	21
%	13.9	12.0	20.8
χ^2	-	0.17	1.69
p	-	> 0.05	> 0.05

[x]one of these disorders occurred in one sib

boys' mothers). Girls showed some difference in the feature of advice and alienation after poisoning in the second trimester. Hyperactivity-hypermobility, which is a well-known symptom of minimal brain damage, was observed after self-poisoning in the second trimester in both sexes.

The drawing test is frequently used because it seems to be a playful exercise to children. Differences were not significant (Table XII) though the trend is obvious: control children had a higher proportion of results which were over average.

TABLE X

Results of Budapest Development Test in 70(5) index cases and matched controls under the age of 5. (Numbers of accidents are shown in brackets)

Group of Dg	Cases No.	Cases %	Controls No.	Controls %
-80	2	2.9	0	0.0
81-90	12	17.1	2	2.9
91-100	23(1)	32.8	9	12.9
101-110	10(2)	14.3	28	40.0
111-120	10	14.3	19	27.1
121-130	10(1)	14.3	12	17.1
131-	3(1)	4.3	0	0.0
Subtotal	70(5)	100.0	70	100.0
$\bar{X}$	104.1 ± 16.3		110.03 ± 10.2	
u	2.62			
p	< 0.005			

e/ Test results of mothers

The Home test data are summarized in Table XIII. There were significant differences between case and control boys on items I, IV, V and VI, and between case and control girls on items III, IV and VI. Overall differences were significant on all items except II. Thus, the familial environments of the study and control groups were significantly different.

Data from the test of Koch (Table XIV) showed a complicated pattern. While the proportion of infantilism was significantly more frequent in index mothers, traumasthenia was significantly higher in control mothers.

The results of the colour test of Lüscher (Table XV) demonstrated significantly higher rate of immature emotions, anxiety, obstinate and vegetative tension in index mothers.

TABLE XI

Summary of significant deviation of Behavioural Style Questionnaire's features (I: activity, II: rythm of the biological function, III: advance and alienation, IV: adaptability, V: intensity, VI: humour and depression, VII: persistence, VIII: deviability and absent mindedness, IX: low threshold of response) in index children aged 4-7 years from matched controls as a function of sex and gestational trimesters

	Case boys			Control boys	Case girls			Control girls
	no.	$\bar{X}$	S.D.	(N = 22)	No.	$\bar{X}$	S.D.	(N = 23)
First	IV. 6	82.5	12.2		IV. 6	72.0	12.8	
	VI. 6	66.7	13.8					
				I. 50.8 $\pm$ 4.4				I. 49.8 $\pm$ 4.5
Second	I. 14	40.3	6.2	IV. 41.2 $\pm$ 5.8	I. 14	40.9	6.7	III. 33.4 $\pm$ 3.9
	IV. 14	88.1	12.7	VI. 40.5 $\pm$ 4.3	III. 14	38.8	5.4	IV. 37.7 $\pm$ 7.7
	VI. 14	91.6	34.7		VI. 14	103.4	32.2	VI. 40.0 $\pm$ 4.9
Third	VI. 2	108.0	26.0		IV. 3	104.0	8.5	

TABLE XII

Results of the drawing test in 101 index children and 101 control children

Results of test	Index children						Control children					
	Boy		Girl		Total		Boy		Girl		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Under average	14	24.6	9	20.5	23	22.8	12	21.1	8	18.2	20	19.8
Average	30	52.6	25	56.8	55	54.4	27	47.4	23	52.3	50	49.5
Over average	13	22.8	10	22.7	23	22.8	18	34.6	13	29.5	31	30.7
Subtotal	57	100.0	44	100.0	101	100.0	57	100.0	44	100.0	101	100.0
	$\chi^2_2 = 1.12$		$\chi^2_2 = 0.53$		$\chi^2_2 = 1.63$							
	$p = 0.58$		$p = 0.77$		$p = 0.44$							

TABLE XIII

Results of home test in children under the age of 3.5 years

Items of test	Index cases						Matched controls						Difference		
	Boy (N=33)		Girl (N=18)		Total (N=51)		Boy (N=33)		Girl (N=18)		Total (N=51)		Boy	Girl	Total
	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	u	u	u
I. Emotional and verbal responsiveness of mother	8.09	2.04	8.17	2.71	8.12	2.28	10.00	0.83	9.12	2.32	9.67	1.38	-4.93xxxx	-1.12	-4.09xxxx
II. Acceptance of child's behaviour	5.70	1.64	6.06	1.47	5.83	1.58	5.59	1.70	6.00	1.33	5.74	1.56	0.26	0.13	0.28
III. Organization of the physical and temporal environment	4.91	1.08	4.78	1.23	4.86	1.13	5.31	1.29	5.65	0.84	5.444	1.12	-1.31	2.46x	-2.54xxx
IV. Provision of appropriate play materials	5.85	2.44	5.83	2.24	5.84	2.37	7.31	2.05	7.65	1.53	7.44	1.86	-2.56xx	-2.82xxxx	-3.72xxxx
V. Maternal involvement with the child	4.12	1.70	4.22	1.69	4.16	1.70	5.00	1.44	5.53	1.09	5.20	1.31	-2.21xx	2.74xxx	-3.39xxxx
VI. Opportunities for variety	2.94	1.52	3.17	1.12	3.02	1.38	3.93	1.05	3.88	0.83	3.91	0.97	-3.01xxxx	-2.34xx	-3.07xxx

xx $p < 0.05$
xxx $p < 0.01$
xxxx $p < 0.005$

TABLE XIV

Results of the tree (Baum) personality test of Koch in 101(8) index mothers and 101 control mothers. (Numbers of accidents are shown in brackets)

Items of test	Index mothers NO.	%	Control mothers No.	%	Difference
Inhibition	52(4)	51.5	40	39.6	$\chi^2_1 = 2.87$; $p = 0.09$
Traumasthenia	12(0)	11.9	30	29.7	$\chi^2_1 = 9.74$; $p = 0.001$
Impression mobility	40(4)	39.6	38	37.6	$\chi^2_1 = 0.08$; $p = 0.77$
Infantilism	49(3)	48.5	27	26.7	$\chi^2_1 = 10.21$; $p = 0.0001$

TABLE XV

Results of the colour (Farb) personality test of Lüscher in 101 (8) index mothers and 101 control mothers. (Numbers of accidents are shown in brackets)

Items of test	Index mothers No.	%	Control mothers No.	%	Differences
Immature emotions	40(2)	39.6	14	13.9	$\chi^2_1 = 17.09$; $p = 0.001$
Anxiety	53(7)	52.5	36	35.6	$\chi^2_1 = 5.01$; $p = 0.02$
Obstinate	58(5)	57.4	26	25.7	$\chi^2_1 = 20.87$; $p = 0.001$
Vegetative tension	52(5)	51.5	25	24.8	$\chi^2_1 = 15.30$; $p = 0.001$
Decreased vitality	52(3)	51.5	42	41.6	$\chi^2_1 = 1.99$; $p = 0.16$
Neurotic	38(0)	37.6	41	40.6	$\chi^2_1 = 0.19$; $p = 0.66$

Data from the signature test of Bánáti (Table XVI) indicated a higher rate of neurotic index mothers (which was not the case in the colour test). Intellectual performance did not show a significant difference; however, the trend indicates a lower level in index mothers.

Multivariate analysis

In the bivariate analysis of case-control studies a significant difficulty arises from the bias created by confounding factors. In order to rule out the confounding factors as much as possible, a multivariate analysis /18/ was performed. Adjusted relative odds and their 95% confidence limits were calculated from the estimates of model parameters and their standard errors /19/. The procedure for selecting variables to the final model was the following. First, the variables in the bivariate analysis were taken into a model. Second, those factors of which coefficient estimates were less than twice standard errors were excluded. Third, each one of these selected factors was added one at a time to the reduced model and included if the difference of deviances (between the two models) was greater than the 95% point of chi square distribution. Predictor variables were gestational age at the time of poisoning (I-II-III trimesters), severity of poisoning (mild and moderate - severe and very severe), type of drugs (benzodiazepines - barbitals - others), socioeconomic status of mothers ($\leq$8->8 class of schools) and maternal tests (under average - average - over average). The dependent variables were birth weight, gestation time and results of tests in offspring.

First, the maternal tests /15, 16, 17/ were evaluated and seven factors were developed on the basis of data from the study and control groups. These seven factors can explain 68% of standard deviation of the original variables. After the rotation of factors studied, the differentiation of study and control groups was correct in 74% of analyses. In particular, the control group showed a more homogeneous pattern (84% of analyses were correct). The differentiation was correct in 54% of analyses in the study group.

TABLE XVI

Results of the signature personality test of Bánáti in 101(8) index mothers and 101 control mothers (Numbers of accidents are shown in brackets)

Items of test	Index mothers No.	%	Control mothers No.	%	Differences
Irregular	19(1)	18.8	28	27.7	$\chi^2_1 = 2.25$; $p = 0.13$
Traumasthenia	59(4)	58.4	64	63.4	$\chi^2_1 = 0.52$; $p = 0.47$
Neurotic	36(3)	35.6	22	21.8	$\chi^2_1 = 4.74$; $p = 0.03$
Intellectual performance					
Under average	20	19.8	22	21.8	
Average	72(7)	71.3	61	60.4	$\chi^2_2 = 4.00$; $p = 0.13$
Over average	9(1)	8.9	18	17.8	

TABLE XVII

Summary of multivariate analysis. Adjusted means show the figure after the reduction of "final maternal factor"

Parameters	Index children		Matched controls		p
	Observed $\bar{x}$	Adjusted $\bar{x}$	Observed $\bar{x}$	Adjusted $\bar{x}$	
Birth weight	3061	3061	3170	3169	< 0.01
Gestation time	39.2	39.1	39.6	39.6	> 0.05
DQ	72.3	72.6	74.1	73.7	> 0.05
BSQ	1.79	1.79	1.82	1.82	> 0.05
Drawing	1.93	1.94	2.12	2.11	> 0.05
Home test	1.49	1.49	1.55	1.55	> 0.05

Second, the results of Koch, Lüchser and Bánáti tests were evaluated and later these were combined as a "final maternal factor". The latter showed a significant difference between index (3.43) and control (1.89) mothers ($p < 0.01$).

The multivariate analysis (Table XVII) indicated that after the exclusion of differences in mothers of the study and control groups, index children and controls showed a significant difference in birth weight ($\chi^2_1 = 7.67$; $p < 0.01$) but not in the results of behavioural tests. The decreased birth weight in the study groups showed a correlation with the severity of poisonings during pregnancy.

DISCUSSION

The mothers of index children had a lower socioeconomic status (based on educational level and occupational background) and more were unmarried than the Hungarian pregnant population at large (about 7% in the study period).

The severity of self-poisoning was lower than in self-poisoned females in general but the distribution of drugs for suicide attempts was similar to the general pattern /9/.

Obvious teratogenic consequences of self-poisoning during pregnancy were not apparent but the birth weight of index children was lower than that of matched controls. This indicates mild intrauterine growth retardation because gestational time did not show any significant difference between index cases and matched controls. These data confirm our previous results because the birth weight of babies born from subsequent pregnancies of index mothers was significantly lower than that of both control children and of babies born from previous pregnancies of index mothers /10/.

The possible teratogenic effect of maternal self-poisoning during pregnancy was indicated by /1/ the somewhat lower intellectual performance based on developmental quotient, /2/ hypermotility and /3/ lower adaptability in the bivariate analysis. All may be the symptoms of minimal brain damage or minimal cerebral dysfunction. However, no significant difference was found in multivariate analysis.

The multifaceted personality assessment of mothers showed a higher rate of emotional immaturity, anxiety, obstinate, vegetative tension and infantilism which may have some connection with their suicide attempts and probably with their parental attitudes. A higher rate of neurotic personality was indicated by one test (Bánáti) but not by another (Lüscher) in index mothers. The traumasthenia occurred at a significantly higher level in the control mothers. The Home test indicated some differences in mother-child connection e.g. the higher frequency of depressive character in index cases. Other significant differences in the items of the Home test between cases and controls may also be related to aspects of the mother's personalities. The point is that the behaviour of index and control mothers shows some obvious differences.

The multivariate analysis of children's data indicated that the differences between index children and matched controls are caused mainly by the maternal behavioural factors. The interaction between these biological and familial-sociological effects needs further analyses.

It would be convenient if there was no need for this kind of human terato-epidemiologic research because the plethora of experimental systems that have been developed over the past several years were so effective and widely used that people were not exposed to teratogenic chemicals. Unfortunately, this is not the case. Consequently, the harsh reality is that human beings provide indispensable information within teratology, too.

ACKNOWLEDGEMENT

Authors thank Prof. J.M. Joffe and Dr. Béla Buda for their useful comments and suggestions.

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Acta Paediatrica Hungarica 32 (4), pp. 371-378 (1992)

EWING'S SARCOMA IN THE OCCIPITAL BONE. CASE REPORT

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Received 13 October 1992

The head is a very rare primary site for Ewing's sarcoma which occurs most often in the long bones of the extremities and in the pelvis. This report describes an unusual case of Ewing's sarcoma arising from the occipital bone in a seven year old girl. The tumour compressed the venous sinuses, thus lowering the intracranial pressure resulted in temporary recovery which made the diagnosis difficult.

INTRODUCTION

Ewing's sarcoma is a rare tumour of the bone. It accounts for approximately 1% of all childhood neoplasms /15/. This tumour affects children and young adults. It occurs most frequently in the early portion to midportion of the second decade of life. Primary tumours may arise in every part of the skeleton, but most often in the long bones of the extremities (the humerus and femur) and in the pelvic bones /21/. The head and neck are very rare primary sites for Ewing's sarcoma /1, 4, 7, 11, 12, 18, 19/.

This case report describes a Ewing's sarcoma arising in the occipital bone of the skull, infiltrating the dura mater and causing the symptoms of raised intracranial pressure.

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CASE REPORT

The seven year old girl was admitted to hospital at the end of April 1990 with a two-days history of headache, nausea, vomiting and abdominal pain. She had no complaints until 2 days before admission. The parents said that 3 weeks prior to admission she had fallen off her bicycle, but she did not lose consciousness.

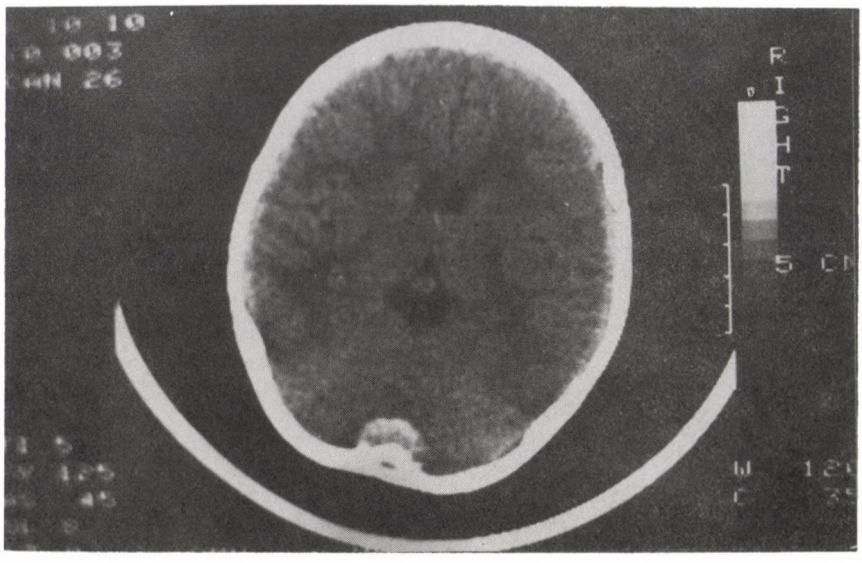
Physical examination findigs were normal, except for a slight abdominal pain in the right lower quadrant on deep palpation. There were no focal neurological findings. The abdominal pain soon disappeared, but on the 4th day of hospitalization intravenous fluids had to be administered for symptoms of mild dehydration. On the 6th day she began to complain about double vision. It was present looking in all directions. The fundoscopic examination revealed papillaoedema bilaterally. The patient was then transported to our department.

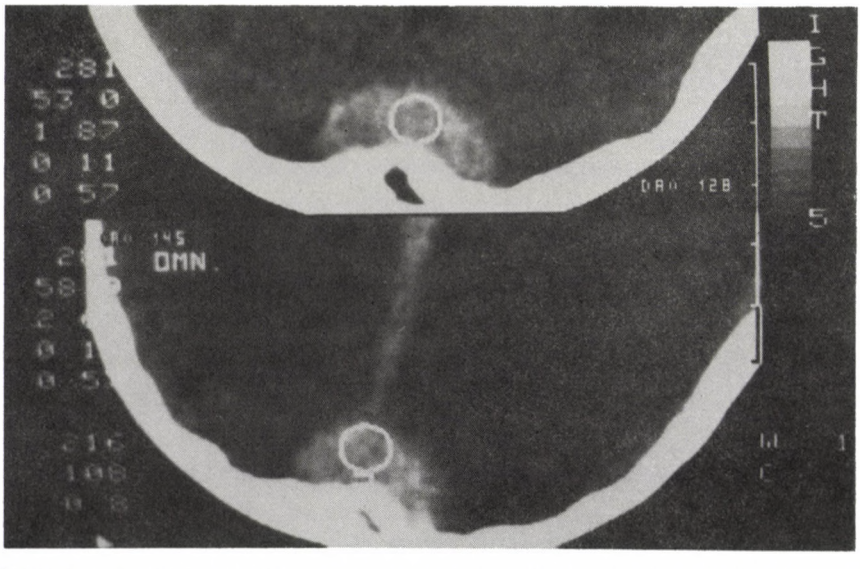
On addmission she was somewhat tired. The findings of the physical examination were normal. The detailed neurological examination found a slight truncal ataxia, diplopia and bilateral papillaoedema /10/. The clinical features were those of space-occupying tumour. The plain skull X-ray, EEG, CT brain scan, routine haematological and biochemical tests were normal. Lumbar puncture was done carefully under the protection of Mannitol, because of the raised intracranial pressure. The cerebrospinal fluid was normal.

As the child improved, during the intracranial pressure reducing therapy, she became symptom-free. She did not have complaints and with our diagnostic procedures we could not prove neurological disease, so we discharged her. She was in good health for the next six weeks and there were no signs of papillaoedema.

In July she was readmitted. She complained about a severe headache for 2 days with vomiting once or twice daily. The physical findings were normal with no focal neurological signs. The fundoscopic examination found bilateral papillaoedema again. Cerebral tumour was strongly suspected. The repeated CT scans of the head - with and without contrast enhancement - demonstrated cerebral oedema along with the obstruction of the ventricular system and a remarkable mass lesion round the sinuum confluens in the occipital area. The tumour was a slightly round mass, almost uniformly hyperdense, with a further enhancement after an injection of contrast medium (Figs 1-2). The magnetic resonance imaging of the head clearly showed the high-density space-occupying lesion in the occipital area (Fig. 3). Evaluation for extracranial manifestations revealed metastases in the lung. Bone marrow was normal. No evidence of another primary lesion was found using isotop whole-body bone scan.

A suboccipital craniectomy was performed. In the area of the protuberantia occipitalis externa the bone was soft, livid and infiltrated with necrotic tumour mass. The patient underwent a suboccipital resection of the tumour, which had attached to the





Figs 1, 2. Axial CT scans demonstrating the hyperdense tumour of the occipital bone in two different magnifications

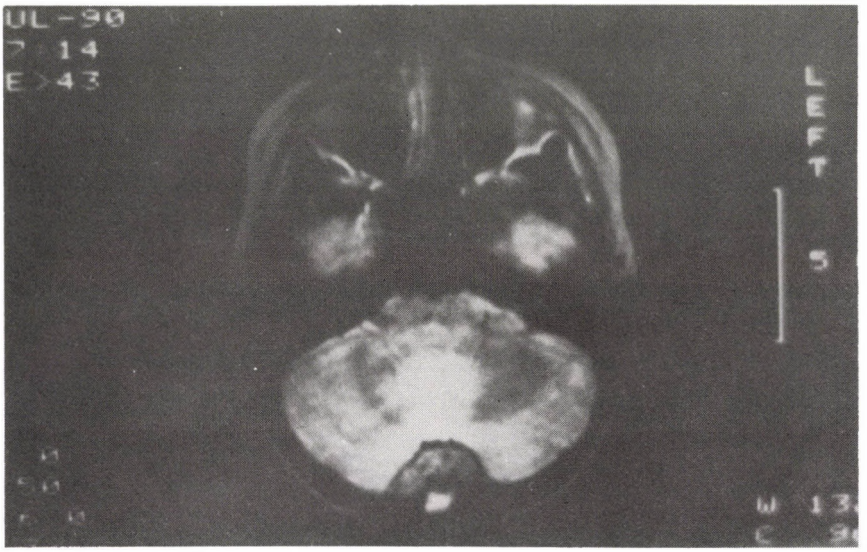


Fig. 3. Magnetic Resonance Imaging shows a high-density tumour in the occipital area

dura mater and infiltrated that. The location and the infiltrating extension of tumour did not allow radical extirpation.

The histological examination showed small round cell proliferation. The tumour cells had fairly wide, light stained cytoplasm, the nuclei were usually round and the structure of the nuclei was loose. The PAS (periodic acid - Schiff) reaction was positive. Immunohistochemical stain for LCA (leucocyte common antigen) was negative. Electronmicroscopical findings were not performed.

The patient received postoperative radiation therapy with 4860 cGy in sections to the occipital bone of the skull and a three-drug regimen chemotherapy (Vincristine, Adriamycine, Cyclophosphamide). The CT scan performed shortly after the irradiation therapy did not show any evidence of tumour shrinkage.

The child is now 2 years postoperative. There is not evidence of local recurrence, but she has pulmonary metastases.

DISCUSSION

Ewing's sarcoma is a rare, undifferentiated, small round to oval cell sarcoma of the bone. It represents about 10% of all the malignant primary bone tumours /13, 20/. This neoplasm

affects children and young adults most frequently. Its histogenesis is uncertain, although many authors think on an undifferentiated, mesenchymal cell of origin. It occurs most often in the long bones of the extremities and in the pelvic bones. The head and neck are unusual sites for primary skeletal Ewing's sarcoma and most cases described in these locations have arisen from the maxilla and the mandible /6, 16/. In the literature only a few references specific to the flat bones of the skull are available. An extensive review of primary Ewing's sarcoma involving the bones of the head and neck was published by Siegal et al. in 1987 /19/. They reported on 29 patients with Ewing's sarcoma in the head and neck region. In this group 11 patients had skull involvement, as the primary site of the tumour. Dahlin in a series of 299 Ewing's sarcoma found 2.3% to involve the bones of the head /5/. The occipital bone seems to be a very unusual site of origin of Ewing's sarcoma. If the cell of origin is an uncommitted mesenchymal cell, it is reasonable to assume that these mesenchymal stem cells exist throughout the body and reside in soft tissue as well as bone. This may help to elucidate such unusual primary sites as the occipital bone for Ewing's sarcoma.

This case report describes a Ewing's sarcoma arising in the occipital bone and infiltrating the dura mater in a 7 year old girl. In addition to location and age, also the clinical signs were uncommon and made this case unique. It took time to make the diagnosis. The most common presenting signs and symptoms of the Ewing's sarcoma were not present. We could not palpate the mass and/or swelling at the site of the tumour. As the appearance of the head Ewing's sarcoma differs in several respects from the so-called typical appearance, we could not find the characteristic X-ray findings of the Ewing's sarcoma (the distinct onion-ring appearance found in the long bones). The absence of the typical apperance was a pitfall for us. Only the second CT scan and the MRI helped us to make the diagnosis of an extradural space-occupying lesion.

A further unusual difficulty was that the tumour was located near the confluence of the sinuses and pressed by the mass,

causing venous flow disturbance and signs of increased intracranial pressure (vomiting, diplopia, papillaoedema). In this way it is understandable, why the child became symptom-free after the intracranial pressure reducing therapy.

Ewing's sarcoma is a diagnosis of exclusion /15/. The tumour has no unique morphological markers that would allow reliable distinction from the other small round cell tumours. This may cause considerable difficulties in the diagnosis. In young patients all the common solid tumours of childhood must be considered, when they present in their primitive or undifferentiated form (Ewing's sarcoma, primary bone sarcomas, rhabdomyosarcoma, lymphoma, metastatic neuroblastoma and primitive neuroectodermal tumours) /2/. In most cases, however, a clear distinction on purely histological grounds is possible.

Prognostic considerations: the size and site of the primary tumour, the site and the extent of metastatic disease, the extent of the soft tissue extension of the primary tumour and the LDH level are at present believed to be the most important prognostic factors for patients with Ewing's sarcoma /3, 8, 9, 14, 17/. There is a significant lower mortality for head and neck primaries compared with all other sites /19/. Patients with metastatic disease limited to the lung appear to far better, than those with metastatic disease in other sites.

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Acta Paediatrica Hungarica 32 (4), pp. 379-380 (1992)

BOOK REVIEW

Atlas of rare chest diseases in children Ed. by Rudnik J, Kurzawa R, National Research Institute of Mother and Child, 1990, 192 pages, 69 figures, 160 X-ray pictures

The idea prompting the publication of this book was the fourth meeting of the European Paediatric Respiratory Society held in Krakow 1983. One section of this meeting discussed the rare pulmonologic diseases. On this occasion the participants of the meeting have been requested by Professor Rudnik, president of the Organizing Committee to put at the disposal of the publisher all documentation of rare diseases.

The publishing of the book was much delayed by the unexpected death of Professor Rudnik - it appeared 7 years later than planned. Finally the complication of the book was undertaken by Professor Kurzawa who let the book come out in 1990. The presentation of the book satisfies every demand, it contains a great number of X-ray pictures and coloured histological figures of high quality. About two thirds of the material are coming from the Rabka Pulmonological Centre and the remaining one third from various countries of Europe.

The first chapter deals with the congenital malformations including 28 case reports. One of them is a patient suffering from immotile cilium syndrome described by Márialigeti and Appel. Several articles deal with the pulmonary cysts of various origin as well as with the possibilities of their surgical solution. An interesting case report was written by Zuk et. al in which a relaxed diaphrgam made the impression of a right-side pulmonary tumour.

The second chapter reports on the congenital malformations combined with inflammatory or tumorous processes. In the case report of Latos and coworkers the patient suffered from Ehlers-Danlos disease and the abnormal pulmonary connective tissue resulted in a polycystic degeneration in the lung presenting clinically with severe spasmodic coughing. The pathogenesis of the disease is based on the lack of transformation of

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procollagen into collagen. This chapter includes also the history of a patient with multiple bone lymphangioma and chylothorax treated by Cserháti et al.

In the third chapter authors write about the primary pulmonary tumours in childhood dividing them into 3 groups considering their localization: pulmonary, bronchial and mediastinal tumours. Among the described cases especially rare diseases are the Castelman tumour and the malignant chemodectoma.

The fourth chapter deals with pulmonary mycoses (actinomycosis and aspergilloma) while the fifth with chronic interstitial pneumonies of patients coming from the Helsinki Children's Hospital and Immunological Centre. The diseases are groupped according to clinical pictures:
a) no specific character, b) expressed bronchial obstruction,
c) desquamative lymphoid type.

The short sixth chapter is devoted to BCG dissemination in immunodeficient states. In one of the interesting cases described by Schuster et al. exclusively the humoral immundeficiency (IgA and IgG) without cellular components resulted in BCG dissemination following live measles vaccination. Hódsági et al. report on BCG dissemination in chronic granulomatosis.

In the seventh chapter diseases of various etiologies are discussed. Póder et al. presented a case in which a pesticide called paraquat caused toxicosis at first right side infiltration, then emphysema occurred. Fifteen months after toxicity fibromatous alterations dominated the picture.

The book is rich in illustrations, therefore it is convenient for searching and also finding analogies for rare thorax deformities. The descriptions of clinical pictures are short which made possible the great number of case reports, however, the reader may be unsatistied with the profoundness of description.

Endre Cserháti, MD

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PRINTED IN HUNGARY

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Abbreviations should be spelled out when first used in the text. *Drugs* should be referred to by their WHO code designation (Recommended International Nonproprietary Name); the use of proprietary names is unacceptable. The *International System of Units* (SI) should be used for all measurements except blood pressure.

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