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NEUROPHYSIOLOGICAL STUDIES OF PATIENTS WITH CLASSICAL PHENYLKETONURIA: EVALUATION OF RESULTS OF IQ SCORES, EEG AND EVOKED POTENTIALS*

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SUMMARY: Coşkun T, Topçu M, Üstündağ I, Özalp I, Renda Y, Ciğer A, Nurlu G. (Nutrition-Metabolism and Neurology Units, Department of Pediatrics, and Department of Neurology, Hacettepe University Faculty of Medicine, Ankara Turkey. Neurophysiological studies of patients with classical phenylketonuria: evaluation of results of I.Q. scores, EEG, and evoked potentials. Turk J Pediatr 35: 1-10, 1993.

Neurophysiological studies were conducted in 42 patients with classical phenylketonuria. The results of the intelligence quotient scores, electroencephalogram, visual evoked potentials and brain-stem auditory evoked potentials were evaluated. When compared with the controls, the subjects demonstrated a significant prolongation in VEP P_1 and BAEP I-V interpeak latencies and an increase in VEP N_1P_1 amplitudes. No relationship was found between these pathological responses and metabolic control. However, the observation of normal intelligence quotient scores in 14 out of 18 patients who displayed a pathological prolongation in P_1 latencies led us to the conclusion that evoked potentials may have a significant role in the determination of neurophysiological defects and that even cases with good metabolic control may have some obscure neurophysiological dysfunction which should be evaluated more carefully. *Key words: phenylketonuria, intelligence quotient, electroencephalogram, evoked potentials.*

Classical phenylketonuria (PKU) is a metabolic disease which emerges as a result of a decrease or lack of phenylalanine hydroxylase enzyme activity. The disease leads to mental retardation, if not treated early[1]. It is a known fact that patients may mature to an intelligence level within normal limits if they are diagnosed early and started on a carefully controlled diet[2-4].

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Previous studies reveal that certain neurophysiological deficits, in particular learning, perception and perspective disabilities observed in patients with normal intelligence, indicate that minimal damage, undetected by the electroencephalogram (EEG) and intelligence quotient (IQ) tests, remain in spite of early diagnosis and treatment[5-7].

The pathogenesis of mental retardation in PKU is not clearly understood. Although certain theories have been advanced, the well known pathology is that it results from a disorder in myelinization[8-10].

Evoked potentials, defined as electrical responses of the central nervous system (CNS) to an external stimulant, are practical and sensitive tests used to observe the development of illnesses interrelated with myelin disorders[11-13].

In this study, we used evoked potentials along with the IQ test and EEG with the aim of determining the role and significance of evoked potentials in the diagnosis of neurophysiological disorders in patients with classical PKU.

Material and Methods

Forty-two patients with classical PKU, ranging in age from one to ten years were selected for this study. The patients were divided into three groups on the basis of the time of restricted diet initiation and the degree of metabolic control.

Patients who received therapy within a period of two months were classed as the "early treatment group", those with phenylalanine levels lower than 7.5 mg/dl on the average prior to the tests were classed as the "satisfactory metabolic control group", while the remaining patients were classed as the "poor metabolic control" group.

The first group consisted of nine patients who were treated early and whose metabolic control was satisfactory. The average phenylalanine level in this group was 5.80 mg/dl. The female/male ratio was 5/4 and mean age 1.91 ± 0.40 years.

The second group consisted of 23 patients who were treated early and whose metabolic control was poor. The average phenylalanine level was 11.96 mg/dl. The female/male ratio was 11/12 and mean age 3.98 ± 0.57 years.

The third group consisted of ten male patients who were treated late in the course of the disorder. The average phenylalanine level was 12.30 mg/dl, and mean age 5.80 ± 0.76 years.

The control group consisted of 23 patients. Since there was a wide range of age in these three groups, the second group and the controls were divided into two subgroups, namely groups a and b. Subgroup a consisted of patients over 3.5 years and subgroup b consisted of those under 3.5 years.

The IQ was measured, EEG was taken and visual evoked potentials (VEP) and

brain-stem auditory evoked potentials (BAEP) were studied in all patients. In 19 of the controls, the VEP and BAEP were studied and an EEG was taken while in four controls only the VEP and BAEP were studied. The evoked potentials were tested using Medelect ST.10 ER94A equipment.

VEP: The electrodes were positioned on the occipital area and on the forehead at a distance of 80 cm from the monitor and the TV checkerboard using 50' checks on a 30° visual angle screen. A stimulus was applied once or as multiples of 128 with two alterations per second. The potential values between 10-100 Hz wave lengths were recorded and the average responses were calculated. The eyes were tested separately. The first positive wave P_{100} was considered primary data in evaluating VEP waves and taken as "latency". The amplitude values of N_1P_1 components were held by calculating the distances between two extreme points.

BAEP: The electrodes were positioned over the mastoid and vertex and both ears were stimulated separately by applying 10 stimuli/sec alternating clicks at one and multiples of 1024 (150-3000 Hz interval). A sound of 75 dB intensity was produced and placed by the stimulated ear. In evaluating the results, I-V interpeak latency, which was the central transmission time, was used.

EEG: Routine traces which were recorded by the electrodes positioned according to the 10-20 international system, taken by 8 channel mono and bipolar connections were evaluated using Nihon-Kohden equipment.

Psychometric evaluations: Either the Bayley Intelligence Development Test, Stanford Binet or Wechsler tests were given to measure intelligence quotient scores.

Analysis of variance (ANOVA), one-way ANOVA, Student's *t* test and a correlation test were used for statistical analysis.

Results

VEP: Table I displays the mean values of P_1 latency of the patient and control groups and their statistical analyses.

Out of a total 42 patients, delay in P_1 latency was detected in at least one eye of 22 patients and a statistically significant difference was noted only between the values of the patients in group II and the control values (Fig. 1; $p<0.05$). The other groups and sub-groups did not display a statistically significant difference either with each other or with the control group.

Table II shows VEP N_1P_1 amplitude values and the results of statistical studies. No statistically significant differences were found in N_1P_1 values between the groups. Amplitudes over 20 μV which could be qualified as giant amplitudes were obtained in 15 out of a total 42 patients.

Table I: VEP P_1 Latency Values in the Patient Groups and in the Control Group

	Mean P_1 Latency Values (msec)				
	Left Eye		Right Eye		
Groups	$\bar{X}$	SD	$\bar{X}$	SD	p
I	118.64	8.25	120.13	8.70	I vs II n.s.
II	128.85	6.82	131.67	7.08	I vs III n.s.
III	118.42	9.13	121.98	8.62	I vs C n.s.
C	105.53	1.25	104.59	1.16	II vs C < 0.05
					III vs C n.s.
	F: 3.476		F: 4.56		

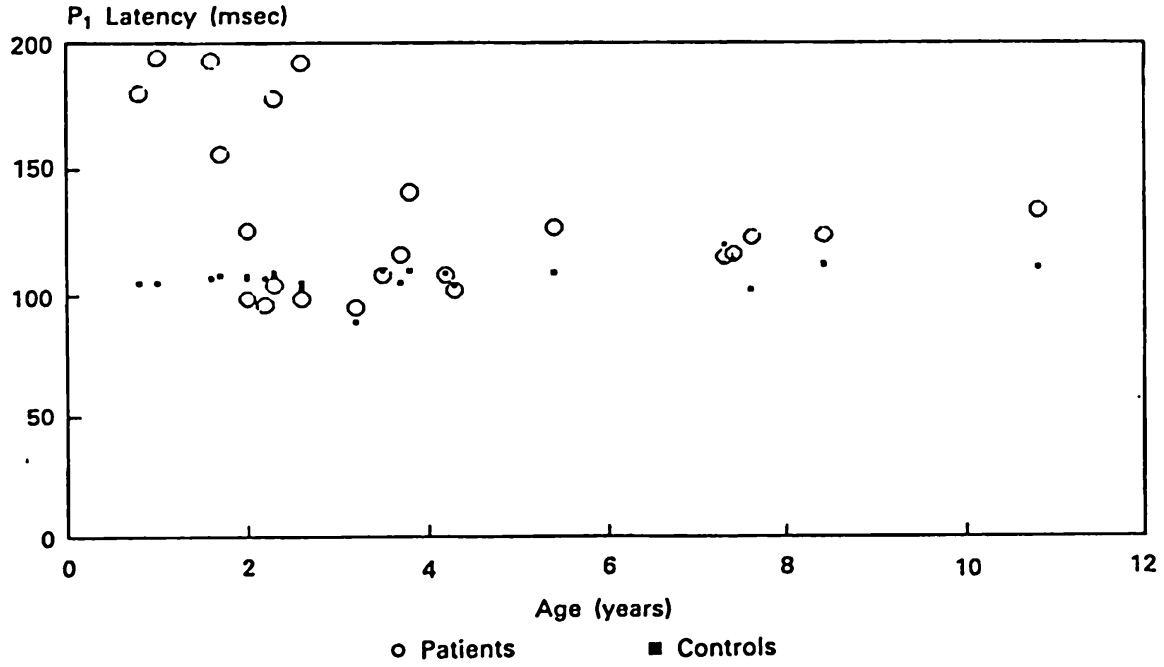

Fig. 1: VEP P_1 latencies according to age in group II and control group.

Table II: VEP N_1P_1 Values in the Patient Groups and in the Control Group

	Mean N_1P_1 Amplitude Values (μV)			
	Left Eye		Right Eye	
Groups	$\bar{X}$	SD	$\bar{X}$	SD
I	16.33	1.34	15.68	1.51
II	18.25	1.99	19.31	1.84
III	15.61	2.56	12.71	2.71
C	17.94	1.75	17.16	1.71
	F: 0.3107 n.s.		F: 1.6278 n.s.	

BAEP: Table III gives I-V interpeak latency values. Although a definite prolongation relative to the control group values was observed in all groups, a significant difference was noted only in the left ear between group I and the control group (Fig. 2; $p<0.05$). This result is due to the difference in the values of the control group.

Table III: BAEP I-V Interpeak Latency Values in the Patient Groups and in the Control Group

	Mean I-V Interpeak Latency Values (msec)					
	Right Ear			Left Ear		
Groups	$\bar{X}$	SD	p	$\bar{X}$	SD	p
I	4.27	0.13	n.s.	4.27	0.09	I vs II n.s.
II	4.07	0.07	n.s.	4.18	0.07	I vs III n.s.
III	4.14	0.06	n.s.	4.15	0.03	II vs III n.s.
C	3.99	0.04	n.s.	3.97	0.05	I vs C < 0.05
						II vs C n.s.
						III vs C n.s.
	F: 2.2278 $p > 0.05$			F: 3.0432 $p < 0.05$		

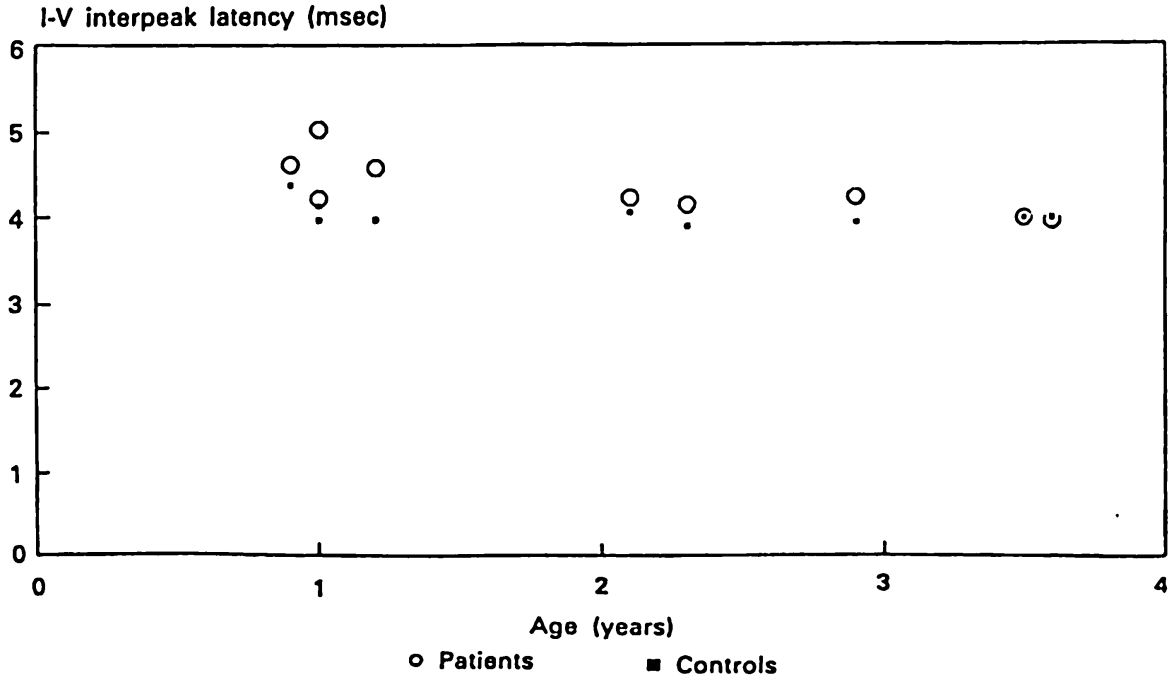

Fig. 2: BAEP I-V Interpeak latencies according to age in group I and control group.

EEG: The EEG traces were evaluated in terms of ground paroxysmal activity and asymmetry between the two hemispheres. The EEG was found to be pathologic in 17 out of 42 patients. Of the nine patients in group I, three had paroxysmal activity and one had paroxysmal activity with dysrhythmia (44%). Of the 23 patients in group II, seven had paroxysmal activity and one had paroxysism together with dysrhythmia (23%). Of the ten patients in group III, four had paroxysmal activity, one had paroxysism and dysrhythmia and one only had dysrhythmia (60%). Of the 19 controls in whom EEG tracings were taken, one had dysrhythmia and another had latent paroxysmal activity (10.5%). A statistically significant difference was found on the EEG between those who received late therapy and the control group. No relationship was found between convulsions and EEG disorders.

Psychometric evaluation: IQ and development scores were as follows: 102.11 ± 4.84 in group I (on early and rigid diet), 93.30 ± 4.08 in group II (poor metabolic control), and 28.90 ± 4.08 in the late therapy group. Four patients in group III could not be tested due to severe mental retardation. A statistically significant difference was found between groups I and III and groups II and III (Table IV).

Table IV: Mean IQ Values in Patient Groups

	Mean IQ Values and Development Scores		
Groups	$\bar{X}$	SD	p
I	102.11	4.84	I vs II n.s.
II	93.30	4.08	I vs III < 0.05
III	28.90	8.15	II vs III < 0.05

F: 41. 8887

When evaluated in terms of their IQs, all patients in group I, while only 60.9 percent of patients in group II were found to have an IQ within normal limits. These findings were statistically significant for all groups (Table V).

Statistical analyses were performed with regard to the IQ, VEP and BAEP results of the sub-groups delineated by age, but no significant difference was found. Negative correlation values were found for age and all other data, whereas a positive correlation coefficient of 0.001 was found between VEP P_1 latencies and BAEP I-V interpeak latencies.

Table V: Comparison of Psychometric Test Results in Terms of IQ

IQ Score	Group I		Group II		Group III	
	n	%	n	%	n	%
Mentally retarded (< 68)	–	0.00	2	8.70	10	100.00
Borderline (68-89)	–	0.00	7	30.40	–	0.00
Normal (> 90)	9	100.00	14	60.90	–	0.00
Total	9	100.00	23	100.00	10	100.00

for all groups $p < 0.05$

Discussion

Mental development in PKU is influenced by various factors, the two most important of which are early dietary treatment and optimal metabolic control. The results of previous studies indicate that patients who receive treatment within the first two months of life can attain an IQ within normal limits, while those with an average blood phenylalanine level below 7.5 mg/dl achieve maximum IQ capacity[14-18]. Nevertheless, in spite of a normal IQ, PKU patients are usually confronted with common problems such as learning disabilities, poor mathematical comprehension, changes in behaviour and a poor attention span[5,16,19,20]. IQ tests and EEG have been frequently used to diagnose patients with mental retardation and convulsions.

The use of evoked potentials which play an important role in the diagnosis and monitoring of patients, in particular those with a myelinization disorder dates back to the early 1980s. A limited number of such studies of PKU patients are reported in the literature. Hecox et al[21] was first to report that the BAEP I-V interval was longer in a two-year-old untreated patient than in treated patients. Creel and Buehler[22] reported a definite prolongation in P_1 latencies of VEP applied to untreated adult patients. Recent studies have investigated the relationship between evoked potential responses and metabolic control. Giovannini et al[23] could not find a significant difference between the patients and controls in a 14-case series, but which when evaluated in terms of metabolic control, they observed that P_1 values were significantly lower in the group with good metabolic control.

Meanwhile, in a study of 41 adolescents with hyperphenylalaninemia, Korinthenberg et al[24] showed that there was a definite prolongation in P_1 latencies and a variation in BAEP responses between patients and controls, but no correlation was reported between continuation of the diet therapy, phenylalanine concentrations, EEG or IQ test results. They claimed that the prolongation in the latencies is an indicator of demyelinization in PKU. The existence of a strong positive correlation between P_1 latency and BAEP I-V interpeak latency and the prolongation in latencies lead us to believe that there is extensive dismyelinization in PKU. Another observation which we have not come across in previous studies is the increase in VEP N_1P_1 amplitudes of the patients. Although not statistically significant, this observation is interpreted as being a possible indicator of increased hyperexcitability in PKU patients. Some studies have shown that the transmitter changes lead to giant amplitudes by disturbing synaptic transmission[25]. There are also studies reporting that toxic metabolites inhibit transmitter synthesis[9]. This hyperexcitability might explain the EEG pathologies and convulsions observed in patients with PKU.

As for the VEP and BAEP values in our study, no significant results were obtained for any of the groups when the sub-groups established on the basis of homogenous age distribution were compared with each other. This finding can be interpreted in two ways: 1) The increase in the number of subjects due to disaggregation into sub-groups, 2) Evoked potential responses display pathology independent of metabolic control. Pathological responses also in patients with good metabolic control can be accepted as proof of the fact that the brain can not totally be protected even in cases where normal IQ scores are attained through early therapy and rigid diet. In this respect, it is clear that evoked potentials may play an important role where IQ tests are inefficient in monitoring patients with PKU, in particular those having normal IQ scores.

Different results in various studies, though limited in number, support the second explanation[22-24]. Another fact which supports this explanation is that a pathological response was obtained in only four of our patients who were mentally retarded and diagnosed late.

The most significant and interesting finding in our study was that the IQ scores were within normal limits in all but four of the 18 patients in groups I and II having prolongation in P_1 latencies. The prolongation in P_1 latencies in a PKU patient with a normal EEG and IQ score should be interpreted as an indicator of pathologic myelinization.

This was a preliminary study, which for more concrete interpretations, should be supported by similar studies carried out in a larger series of age-matched patients and controls.

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REFERENCES

1. Tourian A, Sidbury JB. Phenylketonuria and hyperphenylalaninemia. In Stanbury JB, Wyngaarden JB, Fredrickson DS, et al. *The Metabolic Basis of Inherited Disease* (5th ed). New York: McGraw, 1983, pp 270-286.
2. Williamson ML, Koch R, Azen C, Chang C. Correlates of intelligence test results in treated phenylketonuric children. *Pediatrics* 68: 161, 1981.
3. Dobson JC, Kushida E, Williamson M, Friedman EG. Intellectual performance of 36 phenylketonuria patients and their nonaffected siblings. *Pediatrics* 58: 53, 1976.
4. Smith I, Beasley M. Intelligence and behavior in children with early treated phenylketonuria: a report from the MRC/DHSS phenylketonuria register. *Eur J Clin Nutr* 43 (Suppl 1): 1, 1988.
5. Pennington BF, van Doorninck WJ, McCabe LL, McCabe ER. Neurophysiological deficits in early treated phenylketonuric children. *Am J Ment Defic* 89: 467, 1985.
6. Brunner RL, Jordan MK, Berry HK. Early-treated phenylketonuria: neuropsychologic consequences. *J Pediatr* 102: 831, 1983.
7. Stevenson JE, Hawcroft J, Lobascher M, et al. Behavioural deviance in children with early treated phenylketonuria. *Arch Dis Child* 54: 14, 1979.
8. Dobbing J, Sands J. Quantitative growth and development of human brain. *Arch Dis Child* 48: 757, 1973.
9. Menkes JH. The pathogenesis of mental retardation in phenylketonuria and other inborn errors of amino acid metabolism. *Pediatrics* 39: 297, 1967.
10. Menkes JH. Cerebral proteolipids in phenylketonuria. *Neurology* 18: 1003, 1968.
11. Greenberg RP, Ducker TB. Evoked potentials in the clinical neurosciences. *J Neurosurg* 56: 1, 1982.
12. Chiappa KH, Ropper AH. Evoked potentials in clinical medicine (first of two parts). *N Engl J Med* 306: 1140, 1982.
13. Chiappa KH. Brainstem auditory evoked potentials in clinical neurology. In Courjon J, Mauguiere F, Revol M (eds). *Clinical Applications of Evoked Potentials in Neurology*. New York: Raven, 1982, pp 157-202.
14. Dobson JC, Williamson ML, Azen C, Koch R. Intellectual assessment of 111 four-year-old children with phenylketonuria. *Pediatrics* 60: 822, 1977.
15. Naughten ER. Continuation vs. discontinuation of diet in phenylketonuria. *Eur J Clin Nutr* 43 (Suppl 1): 7, 1989.
16. Holtzman NA, Kronmal RA, van Doorninck W, et al. Effect of age at loss of dietary control on intellectual performance and behavior of children with phenylketonuria. *N Engl J Med* 314: 593, 1986.
17. Michals K, Azen C, Acosta P, et al. Blood phenylalanine levels and intelligence of 10-year-old children with PKU in the national collaborative study. *J Am Diet Assoc* 88: 1226, 1988.
18. Pietz J, Benninger C, Schmidt H, et al. Long-term development of intelligence (IQ) and EEG in 34 children with phenylketonuria treated early. *Eur J Pediatr* 147: 361, 1988.
19. Hackney IM, Hanley WB, Davidson W, Lindsao L. Phenylketonuria: mental development, behavior, and termination of low phenylalanine diet. *J Pediatr* 72: 646, 1968.

20. Koff E, Boyle P, Pueschel SM. Perceptual-motor functioning in children with phenylketonuria. *Am J Dis Child* **131**: 1084, 1977.
21. Hecox KE, Cone B, Blaw ME. Brainstem auditory evoked response in the diagnosis of pediatric neurological diseases. *Neurology* **31**: 832, 1981.
22. Creel D, Buehler BA. Pattern evoked potentials in phenylketonuria. *Electroencephalogr Clin Neurophysiol* **53**: 220, 1982.
23. Giovannini M, Valsasina R, Villani R, et al. Pattern reversal visual evoked potentials in phenylketonuria. *J Inherited Metab Dis* **11**: 416, 1988.
24. Korinthenberg R, Ullrich K, Füllen Kemper F. Evoked potentials and electroencephalography in adolescents with phenylketonuria. *Neuropediatrics* **19**: 175, 1988.
25. Schafer EW, McKean CM. Evidence that monoamines influence human evoked potentials. *Brain Res* **99**: 49, 1975.

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FREQUENCY OF THE IVS-10nt546 MUTATION IN 44 TURKISH PHENYLKETONURIA PATIENTS*

*Meral Özgüç PhD**, İmran Özalp MD***, Turgay Coşkun MD****, Engin Yılmaz MS*****, Hayat Erdem PhD******, Şükrüye Ayter PhD*******

SUMMARY: Özgüç M, Özalp İ, Coşkun T, Yılmaz E, Erdem H, Ayter Ş. (Departments of Medical Biology and Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Frequency of the IVS-10nt546 mutation in 44 Turkish phenylketonuria patients. Turk J Pediatr 35: 11-14, 1993.

The newly identified point mutation in intron 10 of the phenylalanine hydroxylase gene activates a cryptic splice site and results in an in-frame insertion of nine nucleotides between exons 10 and 11 of the processed transcript. This mutation is observed in association with haplotype 6 of phenylketonuria chromosomes. Since in Turkey, haplotype 6 is observed in 40 percent of mutant phenylketonuria alleles, the aim of this study was to establish the incidence of this particular mutation. Forty-four classical phenylketonuria patients were studied and the frequency of the intron 10 splicing mutation was determined to be 31 percent. *Key words: phenylketonuria, IVS-10nt546 mutation.*

Classical phenylketonuria (PKU) is an autosomal recessive disease which results from a deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH). Since the isolation of the full-length cDNA for PAH[1], a multiple of different mutations have been reported in the PAH gene[2]. However, the mutant genotypes thus far reported account for only a fraction of all the mutant alleles and most of the mutations causing the disease, especially in the Mediterranean countries, remain unknown[3].

A particular pattern emerged from studies of PKU families in southern Europe, where haplotype 6 was found to be frequent[4]. In a previous study, it was found that 40 percent of the mutant Turkish PKU alleles are of haplotype 6 origin[4].

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Haplotype 6 is associated with the severe form of phenylalanine hydroxylase deficiency.

Recently a G to A transition in 3' of intron 10 of the phenylalanine hydroxylase gene was identified[5]. This mutation is in linkage with mutant RFLP (Restriction Fragment Length Polymorphism) haplotype 6 and is also seen on the rare haplotypes 10 and 36 in Bulgarians and Bulgarian Turks[6].

The mutation activates a cryptic splice site that leads to an in-frame insertion of nine nucleotides between exons 10 and 11 of the processed transcript. Moreover, this mutation creates a Dde I restriction endonuclease site that is easily detectable in a simple, nonradioactive assay based on PCR (Polymerase Chain Reaction) amplification and restriction analysis.

Since haplotype 6 is a common haplotype of mutant PKU chromosomes in Turkey, we chose to screen for the Dde I site mutation in Turkish PKU patients.

Material and Methods

Forty-four classical PKU patients followed at the Metabolism and Nutrition Unit of the Department of Pediatrics at Hacettepe Children's Hospital were studied for this mutation. Genomic DNA was extracted from peripheral blood samples by standard procedures[7]. One microgram of genomic DNA was amplified by using oligonucleotide primers (AP 255: 5' TGC AGC AGG GAA TAG TGA TC: 62-43 bp upstream of exon 11 and AP 264: 5' TAG ACA TTG GAG TCC ACT CTC: 79-99 bp downstream of exon 11 and 30 cycles were completed. One cycle of amplification consisted of denaturation (94 °C, 30 sec.) annealing (57 °C, 1 min.) and extension (72 °C, 1 min.).

PCR products were checked by 1.5% agarose gel electrophoresis and the amplified fragments were visualized with ethidium bromide under ultraviolet light. Ten microliters of the amplified product were digested by Dde I restriction enzyme (20 U of the enzyme incubated at 37 °C for a period of 16 hours). Restriction fragments were observed using ethidium bromide stained with 4% Nusieve agarose gel[8].

Results

When the PCR products are digested with Dde I enzyme, electrophoretically separable fragments of different lengths are produced which represent different alleles (Fig. 1).

The amplification results in a product of 295 bp. In the presence of the mutation, the 295 bp PCR product is cleaved into two fragments of 246 bp and 49 bp. Of the 44 PKU subjects, 11 (25%) were homozygous for this mutation and 6 (6.8%) were heterozygous. The total frequency was (28/88 alleles) 31 percent.

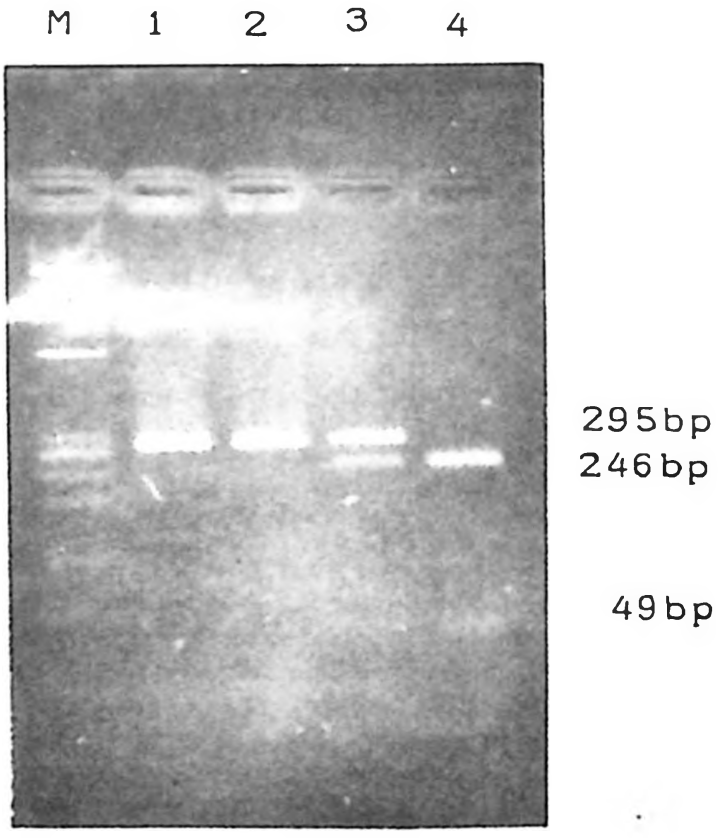

Fig. 1: Dde I restriction digestion results of amplified DNA from PKU patients. M: X174 Hae III digest size marker DNA, 1: PCR product, 2: homozygous normal, 3: heterozygous individual, 4: homozygous PKU patient (carries Dde I site on both chromosomes).

Discussion

The newly identified mutation in intron 10 of the phenylalanine hydroxylase gene activates a cryptic splice site and results in an in-frame insertion of 9 nucleotides between exons 10 and 11 of the processed transcript.

Molecular analysis in different European countries has revealed that the majority of PKU alleles are associated with a limited number of haplotypes. However, significant differences in the geographical distribution of particular haplotypes have been observed[9].

In this northern part of Europe, haplotype 3 accounts for a large proportion of PKU alleles, while it is nearly absent in Mediterranean populations[10]. However in the southern part of Europe, haplotype 6 is more dominant[4,11].

The splicing defect in intron 10 (IVS-10nt546) of the phenylalanine hydroxylase gene is strongly linked to haplotype 6 in different ethnic groups[6].

In addition, this mutation was present in PKU alleles of some rare haplotypes confined to specific populations (haplotype 10 in Bulgarians and haplotype 36 in Turks). These findings, together with the previously reported data on PKU haplotype distribution, suggest that in southern Europe, the splicing defect in

intron 10 of the phenylalanine hydroxylase gene is a common cause of PKU. The simple nonradioactive diagnostic test described here can be used for prenatal analysis and for carrier detection of PKU. Until more mutations specific to the Turkish population can be determined, haplotype 6 mutation can be used as an initial direct screening test.

REFERENCES

1. Kwok SC, Ledley FD, DiLella AG, et al. Nucleotide sequence of a full-length complementary cDNA clone and amino acid sequence of human phenylalanine hydroxylase. *Biochemistry* **24**: 556, 1985.
2. John SW, Rozen R, Scriver CR, et al. Recurrent mutation, gene conversion, or recombination at the human phenylalanine hydroxylase locus: evidence in French-Canadians and a cataloge of mutations. *Am J Hum Genet* **46**: 970, 1990.
3. Okano Y, Wang T, Eisensmith RC, et al. Phenylketonuria missense mutations in the Mediterranean. *Genomics* **9**: 96, 1991.
4. Konecki LU, Schlotter M, Yaylak C, et al. DNA haplotype analysis at the phenylalanine hydroxylase locus in the Turkish population. *Hum Genet* **81**: 373, 1989.
5. Dworniczak B, Aulehla-Scholz C, Kalaydjieva L, et al. Aberrant splicing of phenylalanine hydroxylase mRNA, the major cause for phenylketonuria in parts of southern Europe. *Genomics* **11**: 242, 1991.
6. Kalaydjieva L, Dworniczak B, Aulehla-Scholz C, et al. Phenylketonuria mutation in southern Europeans. *Lancet* **337**: 865, 1991.
7. Ostrer H, Hejtmancik JF. Prenatal diagnosis and carrier detection of genetic diseases by analysis of deoxyribonucleic acid. *J Pediatr* **112**: 679, 1988.
8. Lynch JR, Brown JM. The polymerase chain reaction: current and future clinical applications. *J Med Genet* **27**: 2, 1990.
9. Kalaydjieva L, Dworniczak B, Kucinskas V, et al. Geographical distribution gradients of the major PKU mutations and the linked haplotypes. *Hum Genet* **86**: 411, 1991.
10. Lyonnet S, Caillaud C, Rey F, et al. Molecular genetics of phenylketonuria in Mediterranean countries: a mutation associated with partial phenylalanine hydroxylase deficiency. *Am J Hum Genet* **44**: 511, 1989.
11. Konecki DS, Lichter-Konecki U. The phenylketonuria locus: current knowledge about alleles and mutations of the phenylalanine hydroxylase gene in various populations. *Hum Genet* **87**: 377, 1991.

The Turkish Journal of Pediatrics 35: 15-21, 1993

DELETION ANALYSIS OF DUCHENNE MUSCULAR DYSTROPHY*

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SUMMARY: Erdem H, Ayter Ş, Özgüç M, Topçu M, Topaloğlu H, Renda Y. (Department of Medical Biology, and the Neurology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Deletion analysis of Duchenne muscular dystrophy. Turk J Pediatr 35: 15-21, 1993.

DNA of 15 patients with Duchenne muscular dystrophy (DMD) were analyzed for deletions within the DMD gene by using recombinant DNA technology. Deletion frequency was 47 percent and six of the deletions occurred in the region of probe 7 + 8. Only one of the deletions was observed in the region of probe 9-7, and no deletions were found in the regions of probe 30-1, 30-2 and 47-4 (5b + 6). The frequency of deletions found in the Turkish DMD patients corresponds to frequencies reported for other populations. *Key words: Duchenne muscular dystrophy, DNA, cDNA probes, deletion analysis.*

Duchenne muscular dystrophy (DMD) is an X-linked recessive, neuromuscular disease characterized by progressive muscular weakness. The onset of weakness usually occurs between two and three years of age. Affected males are usually confined to a wheelchair by the age of 12, and do not survive beyond their late teens[1,2].

Until recently, as is the case with many other human genetic diseases, the biochemical basis for DMD was totally unknown. To isolate the DMD gene a "reverse genetic" approach was employed. Subsequent to the characterization of the gene on the basis of its chromosomal position, the entire cDNA for the DMD gene was isolated and found to contain 14,000 nucleotides and more than 65 exons. Dystrophin protein, the product of the gene, consists of 3685 amino acids[3-7]. Dystrophin is located in the sarcolemmic membrane of normal skeletal muscle but is absent in the muscle of DMD patients[8-12].

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The frequency of intragenic deletions of the dystrophin gene is about 60 percent in DMD patients. Partial gene duplications have also been reported in a small number of patients (5%). In the remaining patients (30 to 35%), without detectable deletions or duplications, the nature of molecular lesions leading to the DMD phenotype remains unknown[13-15]. The high incidence of deletion mutations in patients suffering from DMD that can be identified by means of dystrophin cDNA probes has provided a direct method for genetic diagnosis that permits a high degree of diagnostic accuracy in more than 60 percent of families studied[16-22].

Material and Methods

Seventeen patients with DMD were evaluated for intragenic deletions by using 30-1, 30-2, 47-4, 44-1, 9-7 cDNA probes.

DNA extraction and digestion: 10 ml of peripheral blood was drawn in 13 nmol/L EDTA and leukocytes were separated by centrifugation and lysed in 155 mM NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM EDTA in the presence of 10 mg/ml proteinase K overnight. After phenol-chloroform extractions, DNA was precipitated using ethanol 4U Hind III restriction enzyme per microgram of DNA was used for four hour digestion[23].

Southern Blotting: The resulting fragments were separated by means of electrophoresis through 0.8% agarose gels in Tris-borate, EDTA buffer overnight and at 1V/cm, denatured in 1.5 M NaCl and 0.5 M NaOH, neutralized in 0.5 M Tris-HCl (pH: 7) and 3 M NaCl and transferred overnight to nylon (Hybond) filters by Southern Blotting[23,24].

Labelling of cDNA probes and hybridization: The cDNA probes were radioactively labelled using $\propto$ ^{32}P dCTP (800 or 3000 Ci/mol) (Amersham) and commercial oligolabelling kits (Amersham). The filters were hybridized overnight with 30-1, 30-2, 47-4, 44-1, 9-7 cDNA probes that had been labelled with $\propto$ ^{32}P. Following washing at 65 °C in a 4 percent phosphate buffer, 10 ml EDTA, and 1% SDS, blots were exposed to X-ray film (X-OMAT Kodak) for 3-5 days at −70 °C[24,25].

Results

Normal Hind III restriction fragment patterns are shown in Fig. 1. The deletion of one or more of the fragments shows the deletion region within the DMD gene. There is no need to use probe 12-14, since it corresponds to the 3' untranslated sequences of the transcript of the last exon[21].

Due to low DNA concentration, lane 12 was cancelled. Lane 16 and 17 were affected siblings so they had the same results, therefore only one of them was validated in the results. Although 17 patients were studied, the data of these two were cancelled and 15 of them are being discussed.

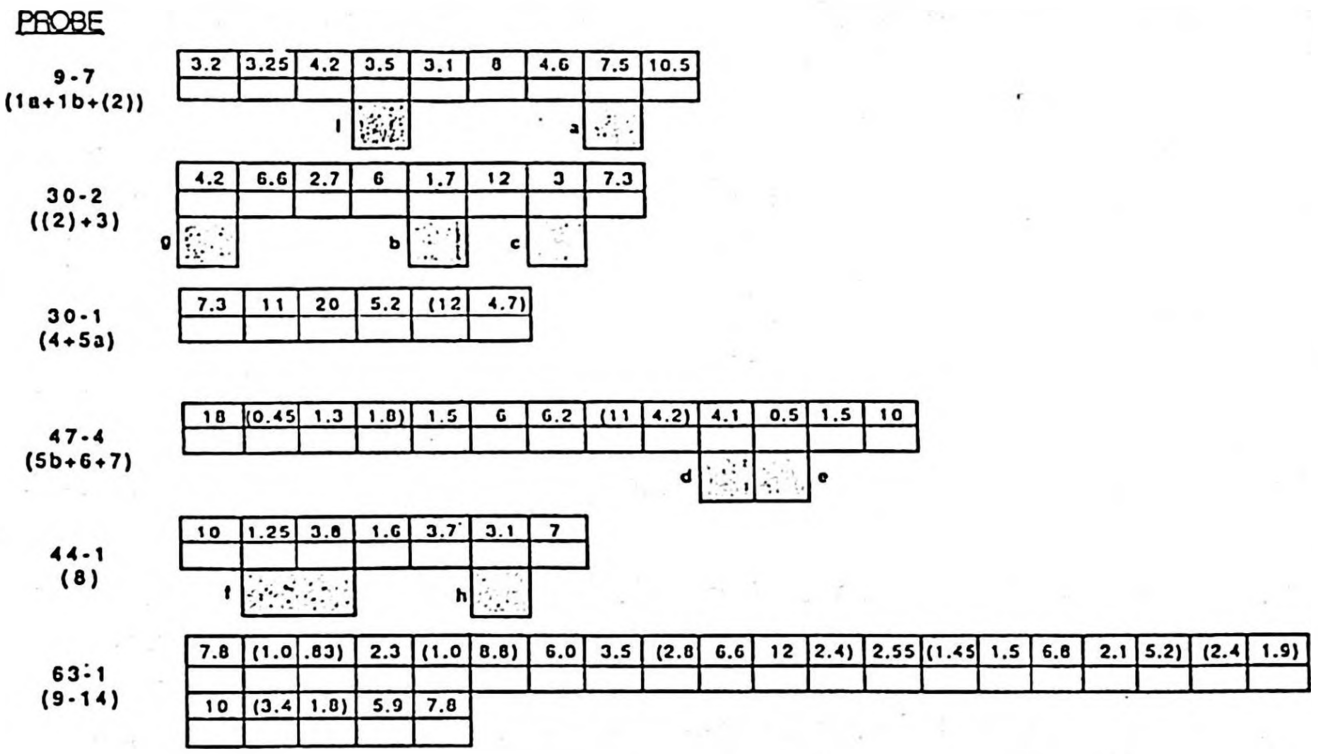

Fig. 1: Normal Hind III restriction patterns. Hind III fragments arranged in order and black bars indicate amplification sites.

20, 12+11, 7.3, 5.2, 4.7 kb fragments were analyzed with probes 30-1 and 12, 7.3, 6.0, 6.6, 4.2, 3, 2.7 1.7 kb fragments were analyzed with probe 30-2 No deletion was observed by using probes 30-1 and 30-2.

Probe 47-4 contains 5b+6+7 kb fragments digested with Hinc II restriction enzyme. The seventh region was isolated and mixed with probe 44-1 (region 8) and used together for hybridization for a better resolution.

By using probe 47-4 (5b+6) 18, 11, 6.2, 6, 4.2, 1.8, 1.5, 1.3 and 0.5 kb fragments were analyzed. As a result, none of the patients had any deletions within the probe 47-4 (5b+6) region.

Normally 8 Hind III fragments (10.5, 8.5, 8, 7.5, 4.6, 4.2, 3.25+3.20, 3.1 kb) are revealed by probe 9-7. In lane 12, results were not clear due to a low DNA concentration, therefore this patient was not included in the results. In the first 7 lanes, foreign DNA contamination was seen between 7.5-4.6 kb fragments. Except for the 3.25+3.20 fragment in lane 5, all fragments were deleted. No deletion was observed in the remaining patients (Fig. 2).

10, 7, 4.1, 3.8+3.7, 3.1, 1.6, 1.5, 1.2, 0.5 kb fragments were analysed with probe 44-1+47-4 (7+8). The 10 kb fragment in lane 8, 13, 14, 10 kb, 3.8+3.7 kb fragments in lane 4 and 6, 1.6 kb, 3.8+3.7 kb fragments in lane 7 were deleted (Fig. 3).

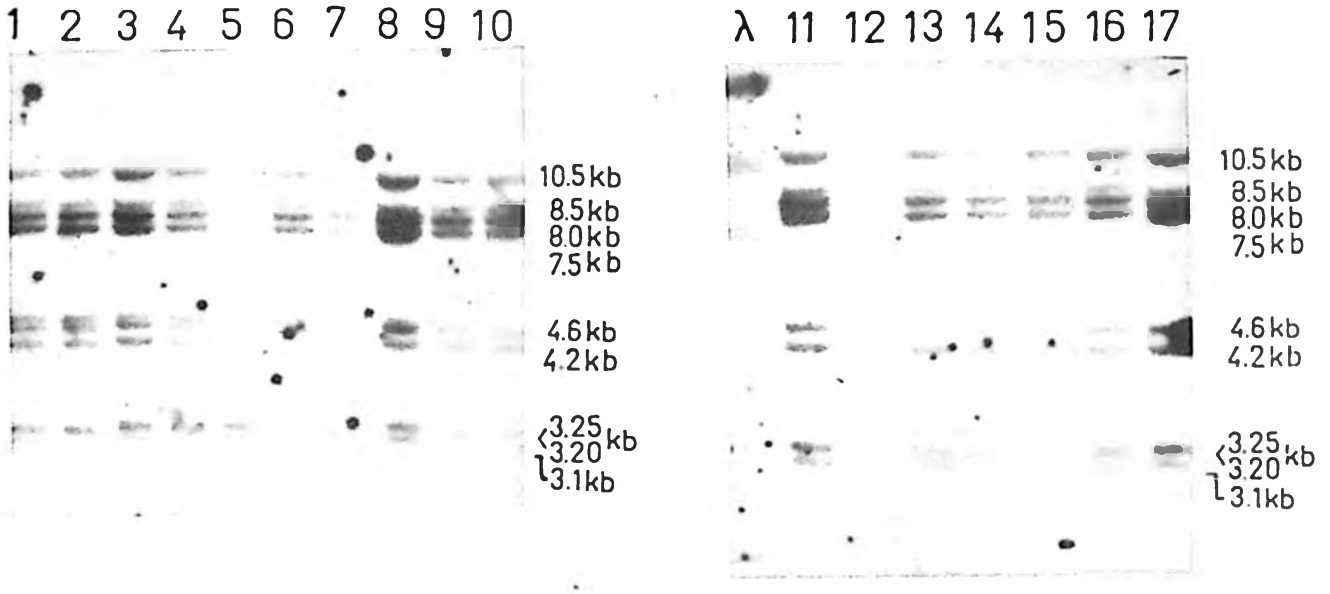

Fig. 2: Autoradiographs of two Southern Blots hybridized with probe 9-7.
λ: Lambda Hind III digested size marker.

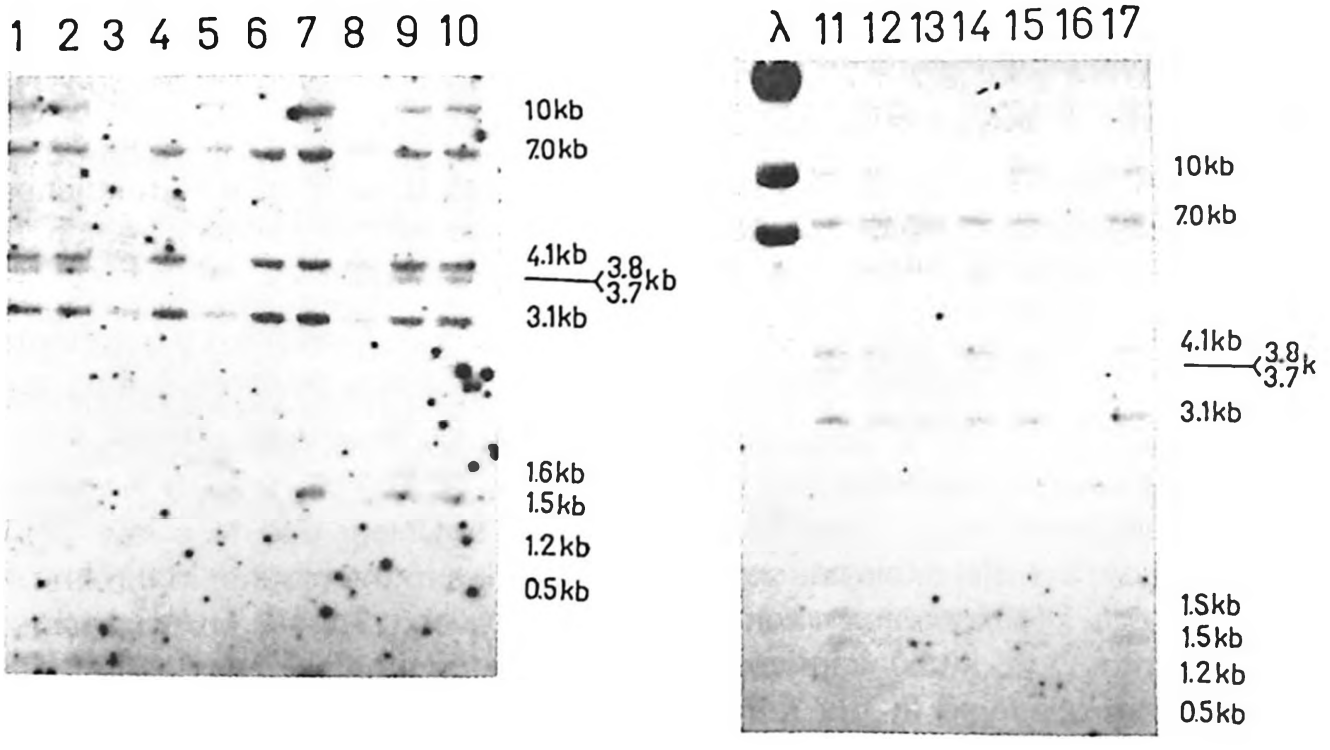

Fig. 3: Autoradiographs of two Southern Blots hybridized with probe 44 – 1 + 47 – 4 (7 + 8) λ: Lambda Hind III digested size marker.

Discussion

Since the DMD gene locus is very large and recombinations between the DMD mutations and the intragenic flanking markers are frequent, the use of linked RFLPs (restriction fragment length polymorphism) in diagnosing DMD is less accurate. The detection of deletions in the genes of DMD patients by using cDNA probes offers the highest reliability for diagnosis. With cDNA clones as probes, DMD gene deletions have been shown to be the cause of muscle dystrophy in 60 percent of DMD patients[12-14,17,22]. One alternative in detecting these deletions is the polymerase chain reaction (PCR)[26-29]. However, since 40 percent of patients have point mutations or deletions that are too small to be detected either by using the Southern Blot technique or PCR, these families should be studied with more traditional linkage analyses.

We found a 47 percent frequency of deletions in our Turkish DMD patients which is similar to other groups reported[30-32]. Although deletions arise heterogeneously they are generally large and are concentrated around the probe 7+8 and 9-7 regions of the gene. Deletions were detected in 7 out of 15 patients. The majority of deletions were shown using the probe 7+8 (lanes 4, 6, 7, 8, 13 and 14). A smaller concentration of deletions were found in the proximal region of the gene corresponding to probe 9-7 (lane 5).

The frequency of deletion mutations observed in the Turkish DMD alleles was similar to that reported in studies made of other populations. Thus the use of the above-mentioned cDNA probes can be used accurately for diagnosis and genetic counselling in our population. With the characterization of the mutations in the remaining DMD lesions, more accurate diagnoses will be available for a wider range of DMD families.

REFERENCES

1. Monaco A, Kunkel M. Cloning of the Duchenne/Becker muscular dystrophy locus. In Harris H, Hirschorn K (eds). *Advances in Human Genetics. New York;* Plenum Pub. 1988, pp 61.
2. Davies KE, Read AP. *Molecular Basis of Inherited Disease.* Washington: IRL Press, 1988.
3. Boyd Y, Buckle VJ. Cytogenetic heterogeneity of translocations associated with Duchenne muscular dystrophy. *Clin Genet* 29: 108, 1986.
4. Gutmann DH, Fischbeck KH. Molecular biology of Duchenne and Becker's muscular dystrophy: clinical applications. *Ann Neurol* 26: 189, 1989.
5. Worton R, Duff C. Duchenne muscular dystrophy involving translocation of the dmd gene next to the ribosomal RNA genes. *Science* 224: 1447, 1984.
6. Ray PN, Belfall B, Duff C, et al. Cloning of the breakpoint of an X;21 translocation associated with Duchenne muscular dystrophy. *Nature* 318: 672, 1985.
7. Koenig M, Hoffman EP, Bertelson CF, et al. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell* 50: 509, 1987.

8. Koenig M, Monaco AP, Kunkel LM. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell* 53: 219, 1988.
9. Monaco AP. Dystrophin, the protein product of Duchenne/Becker muscular dystrophy gene. *TIBS* 14: 412, 1989.
10. Campbell KP, Kahl SD. Association of dystrophin and an integral membrane glycoprotein. *Nature* 338: 259, 1989.
11. Hoffman EP, Knudson CM, Campbell KP, Kunkel LM. Subcellular fractionation of dystrophin to the triads of skeletal muscle. *Nature* 330: 754, 1987.
12. Arata K, Sugita H. Dystrophin and the membrane hypothesis of muscular dystrophy. *TIBS* 10: 437, 1989.
13. Forrest SM. Cross GS, Speer A, et al. Preferential deletion of exons in Duchenne and Becker muscular dystrophies. *Nature* 329: 638, 1987.
14. Darras BT, Blattner P, Harper JF, et al. Intragenic deletions in 21 Duchenne muscular dystrophy (DMD)/ Becker muscular dystrophy (BMD) families studied with the dystrophin cDNA: location of break points on Hind III and Bg/II exon-containing fragment maps, meiotic and mitotic origin of the mutations. *Am J Hum Genet* 43: 620, 1988.
15. Monaco AP, Bertelson CJ, Liechti-Gallati S, et al. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. *Genomics* 2: 90, 1988.
16. Koenig M, Beggs AH, Moyer M, et al. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am J Hum Genet* 45: 498, 1989.
17. Passos-Bueno MR, Rapaport D, Love D, et al. Screening of deletions in the dystrophin gene with the cDNA probes Cf23a, Cf56a, and Cf115. *J Med Genet* 27: 145, 1990.
18. Lindlöf M, Kiuru A, Kääriänen H, et al. Gene deletions in X-linked muscular dystrophy. *Am J Hum Genet* 44: 496, 1989.
19. Kimber RD, Hyland VL, Haan EA, Mulley JC. Deletion analysis for Duchenne (and Becker) muscular dystrophy. *Aust Paediatr J* 25: 292, 1989.
20. Gillard EF, Chamberlain JS, Murphy EG, et al. Molecular and phenotypic analysis of patients with deletions within the deletion-rich region of the Duchenne muscular dystrophy (DMD) gene. *Am J Hum Genet* 45: 507, 1989.
21. Liechti-Gallati S, Koenig M, Kunkel LM, et al. Molecular deletion patterns in Duchenne and Becker type muscular dystrophy. *Hum Genet* 81: 343, 1989.
22. Speer A, Spiegler AW, Hanke R, et al. Possibilities and limitation of prenatal diagnosis and carrier determination for Duchenne and Becker muscular dystrophy using cDNA probes. *J Med Genet* 26: 1, 1989.
23. Maniatis T, Fritsch EF, Sambrook J. *Molecular Cloning: A Laboratory Manual.* New York: Cold Spring Harbor Laboratory, 1982.
24. Williams JG, Patient RK. *Genetic Engineering,* Washington: IRL Press, 1988.
25. Hames BD, Higgins SJ, (eds). *Nucleic Acid Hybridization: A Practical Approach.* Washington: IRL Press, 1985.
26. Chamberlain JS, Gibbs RA, Ranier JE, et al. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic Acids Res* 16: 111, 1988.
27. Speer A, Rosenthal A, Bilwitz H, et al. DNA amplification of a further exon of Duchenne muscular dystrophy locus increases possibilities for deletion screening. *Nucleic Acids Res* 17: 4892, 1989.
28. Hentemann M, Reiss J, Wagner M, Cooper DN. Rapid detection of deletions in the Duchenne muscular dystrophy gene by PCR amplification of deletion-prone exon sequences. *Hum Genet* 84: 228, 1990.

29. Chamberlain JS, Gibbs RA, Rainer JE, Caskey CT. Multiplex PCR for diagnosis of Duchenne muscular dystrophy. In Innis M, Gelfand D, Sninski J, White T (eds). *PCR Protocols and Applications-A Laboratory Manual.* New York: Academic Press, 1989.

30. Lindlöf M, Kiuru A, Käärriänen H, et al. Gene deletions in X-linked muscular dystrophy. *Am J Med Genet* 44: 496, 1989.

31. Cooke A, Lanyon WG, Wilcox DE, et al. Analysis of Scottish Duchenne and Becker muscular dystrophy families with dystrophin cDNA probes. *J Med Genet* 27: 292, 1990.

32. Sugino S, Fujishita S, Kamimura N, et al. Molecular-genetic study of Duchenne and Becker muscular dystrophies: deletion analyses of 45 Japanese patients and segregation analyses in their families with RFLPs based on the data from normal Japanese families. *Am J Med Genet* 34: 555, 1989.

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HEMOLYTIC-UREMIC SYNDROME (HUS): A CLINICOPATHOLOGICAL STUDY OF 15 CASES*

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SUMMARY: Tınaztepe K, Akkök N, Tınaztepe B. (Nephropathology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Hemolytic-uremic syndrome (HUS): A clinicopathological study of 15 cases. Turk J Pediatr 35: 23-36, 1993.

Although hemolytic-uremic syndrome (HUS) is a clinico-pathological entity, renal biopsies are usually not indicated for diagnosis, and therefore, studies concerning the histological aspects of the syndrome are few. This study mainly describes the morphological characteristics of 15 tissue-diagnosed sporadic cases of HUS. The ages of the patients ranged between 10 mos. to 15 yrs., with five being under two. The male/female ratio was 2:3. The prodromal phase was present in 10 patients (67%) with gastrointestinal symptoms in four patients (27%) with neurological symptoms, and in three patients (20%) with upper respiratory infections. Five patients had HUS associated with diarrhea (D+) (three infants and two children), while the remaining ten patients (two infants and eight children) had no diarrhea (D−). *E.coli* was identified in the stool of four of the D+ cases, one of which was also associated with Shigella. The shortest clinical course was 14 days and the longest 55 days in 13 patients. The disease recurred after three months in one patient, and on three occasions in 15 months after onset of HUS in the other.

Fourteen patients died and one biopsy-diagnosed case recovered after the acute phase. All patients had anemia (Hb 3.4-10 g/dl) and acute renal failure. Seven cases demonstrated Burr cells, eight cases had thrombocytopenia and six cases oliguria/anuria. Microscopic hematuria was detected in four cases and gross hematuria in two cases. All patients revealed proteinuria and azotemia (40-200 mg/dl).

Five/five (100%) cases had decreased creatinine clearance, 12/14 (86%) cases had increased uric acid levels, 9/14 (64%) cases had an electrolyte imbalance.

Light microscopy revealed microangiopathic type involvement of the glomeruli in all cases. According to additional findings, the cases were classed into three histological groups: type 1 showing cortical necrosis (3 cases), type 2 predominant glomerular and arteriolar involvement (11 cases) and type 3 predominant arterial involvement (1 case). All cases were considered primary HUS except for one which was associated with membranous glomerulonephritis. (D+) HUS cases were predominantly of the microangiopathic type, similar to the (D−) group; the latter being contrary to the literature. Hypertension was present in 67% of cases and there was no correlation found between the clinical duration of HUS and the histological type. All five patients studied immunohistologically revealed a nonspecific type fibrinogen deposition. Extra-renal microangiopathy was demonstrated in the adrenals, stomach, pancreas, liver and skin in two necropsies studied. *Key words: hemolytic-uremic syndrome, thrombotic microangiopathy, acute renal failure.*

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The hemolytic-uremic syndrome (HUS) represents one of the major causes of acute renal failure in childhood, especially in infancy. The syndrome characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure has a characteristic renal vascular pathology[1-3]. The etiology varies, and in the majority of cases, the cause remains unknown. Whatever the cause, the end result is extensive injury of the renal microvasculature[4]. For practical purposes, cases can be divided into two main clinical types: those associated with prodromal diarrhea (D+) and the others without diarrhea (D−). D+ HUS has an acute presentation with bloody diarrhea which carries a better prognosis[5,6]. An association between D+ HUS and Verocytotoxin producing *Escherichia coli* (VTEC) was first demonstrated by Karmali et al[7,8]. Serotype 0157:H7 is now recognized as the most important cause of HUS in this group[9], but other serotypes can also be found[6,7]. A wide spectrum of other organisms including *Shigella, Salmonella,* enteroviruses and other infectious agents have been hitherto reported to be associated with D+ HUS[10]. The rare D− (atypical) form may be familial, sporadic or relapsing and carries a poor prognosis[6].

A great deal of information concerning the histopathological findings of HUS in children is reported in the studies conducted by Levy et al[11] and by Habib et al[12] in France. These studies have revealed three major morphological patterns of renal injury in such patients:

1. Thrombotic microangiopathy (TMA) with predominant glomerular involvement,
2. Thrombotic microangiopathy (TMA) with predominant arterial involvement,
3. Cortical necrosis (CN).

Similar morphological patterns have also been described by some other authors using slightly different nomenclature[13,14].

Since a diagnostic renal biopsy is rarely indicated, there are few studies in the literature describing the histopathological aspects of HUS. The present study reports the spectrum of clinical and renal pathological findings of 15 children with tissue-diagnosed HUS. The extent of extrarenal involvement is also reported in two of these cases.

Material and Methods

Fifteen cases with a tissue diagnosis of HUS and whose ages varied from 10 mos to 15 yrs were studied retrospectively for a clinicopathological evaluation over a twelve-year period from 1978 to 1990. Of these, five cases (33%) were in the 0-2 year-old age group, three cases (20%) were in the 3-5 year-old age groups, four cases were in the 6-12 year-old age group and three cases were in the 13-15 year-old age group. Fourteen of these patients died and one survived. A light microscopical study of the renal tissue was carried out in all cases, while

additional fluorescence microscopical studies were available in five of them. The samples of renal specimens were needle biopsy in one case, postmortem material in six cases (one incisional, five needle samples), a unilateral nephrectomy in six cases and an autopsy in two cases.

The renal tissues were fixed both in formalin and in Duboscq-Brasil solution. The sections were stained with hemotoxylin-eosin, periodic acid-Schiff (PAS), trichrome and Jones silver stains.

Immunofluorescence microscopical studies were carried out on fresh frozen tissues. The monospecific fluoresceinated antisera used were: anti-IgA, anti-IgG, anti-IgM, anti-C3 and anti-fibrinogen.

The patterns of renal injury described by Habib et al[12] were determined for each specimen, which were defined as follows (Figs. 1-7):

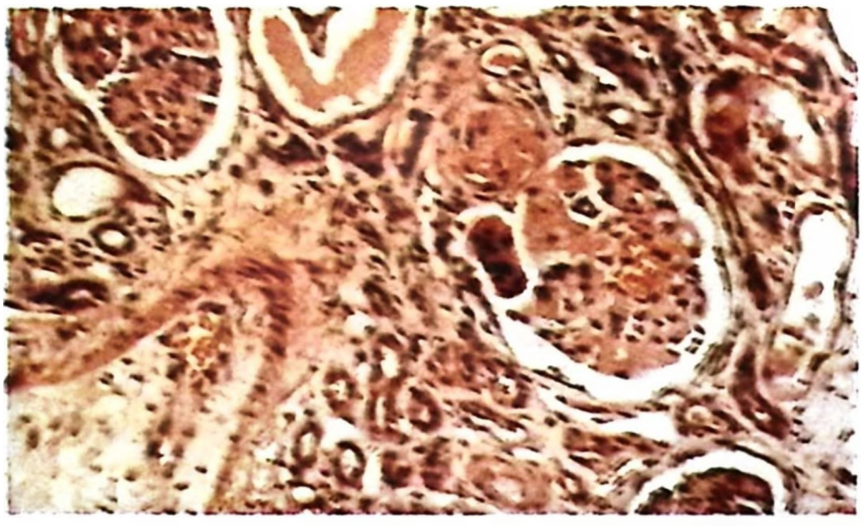

Fig. 1: Thrombosis of an arcuate artery with subintimal edema and a glomerulus with thrombotic microangiopathy (HE × 200).

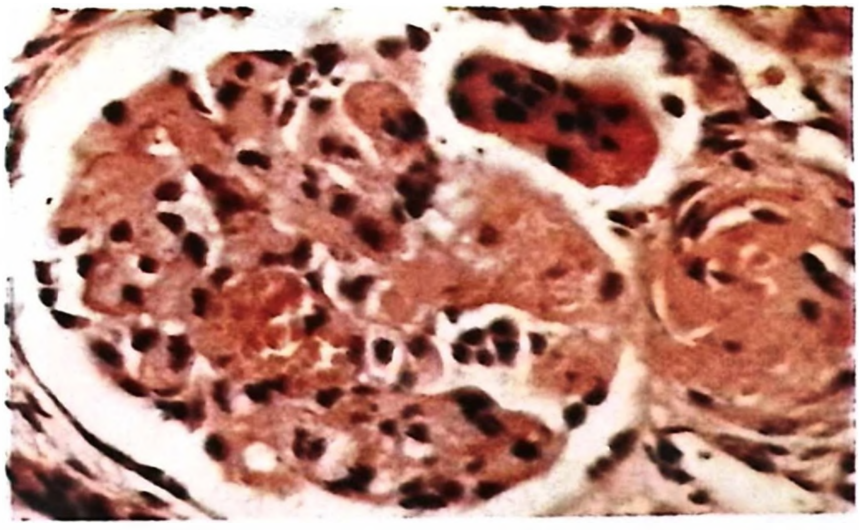

Fig. 2: Glomerulus showing widespread thickening of the capillary walls; there is no increase in cellularity. The afferent arteriole is occluded by fibrin-like material (HE × 350).

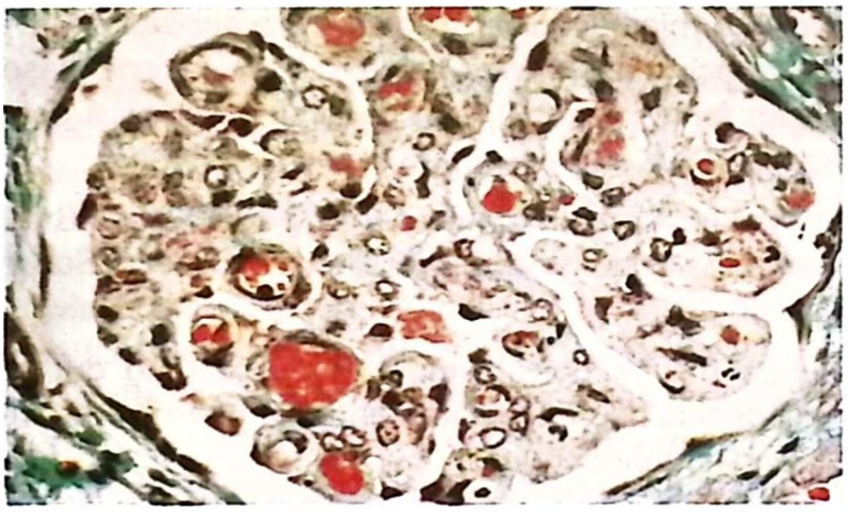

Fig. 3: Glomerulus showing narrowing of the lumina of the glomerular capillaries with thrombi, subendothelial and intraluminal fibrin deposition and widened subendothelial space (Trichrome light-green × 350).

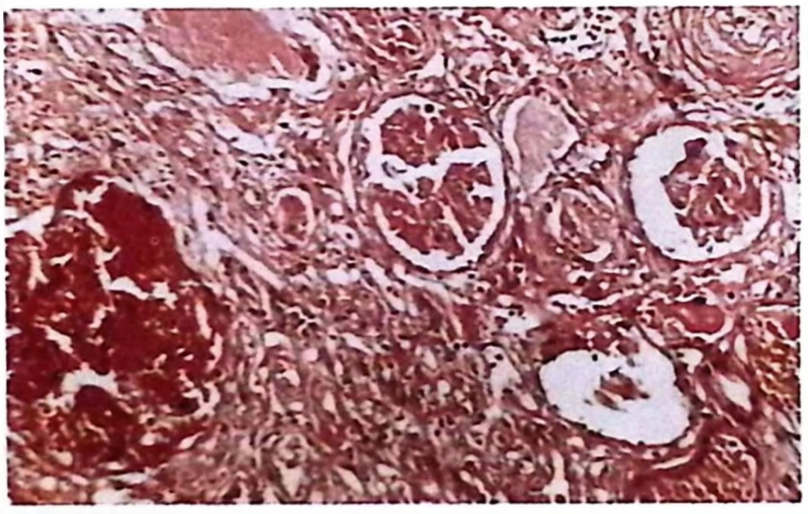

Fig. 4: Four heterogenously involved glomeruli. The one at the bottom shows fibrinoid necrosis, while the others are segmentally involved. The lumen of the artery in the corner is narrowed, showing an "onion-skin" pattern (HE × 150).

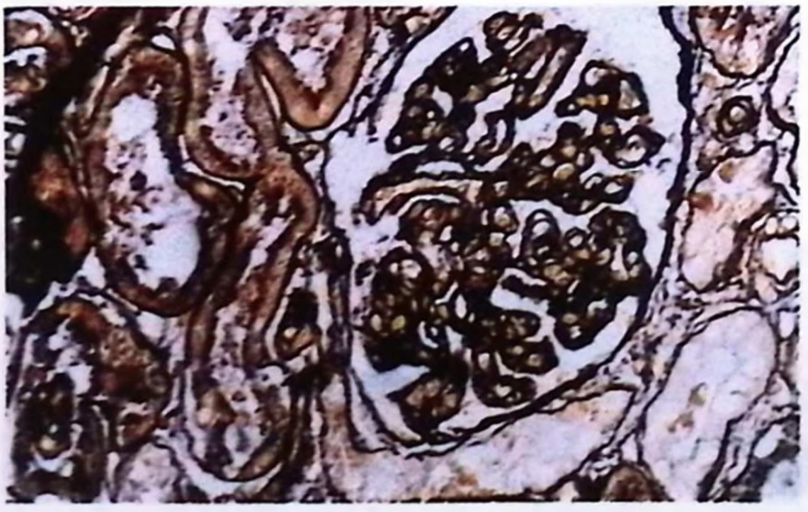

Fig. 5: Thickening and "double-contour" appearance of the glomerular tuft. Similar changes are also seen in the tubular basal membranes (John's Silver × 250).

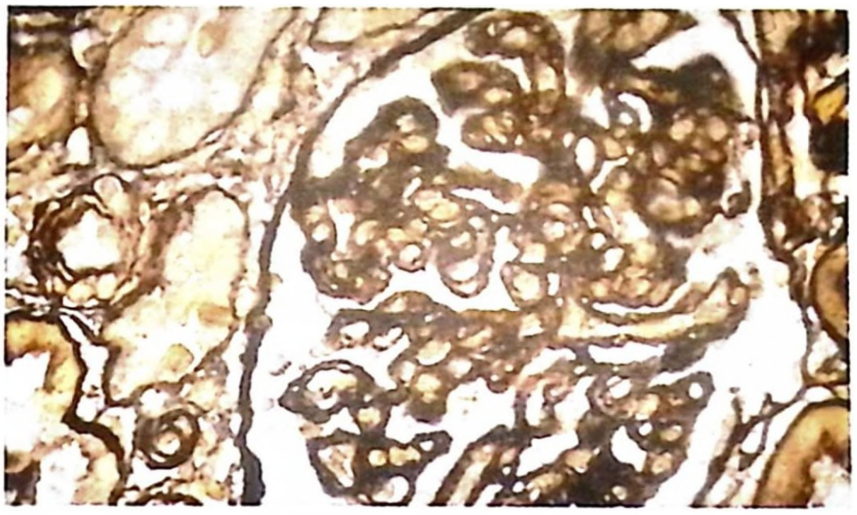

Fig. 6: Double contours are clearly visible in the glomerular tuft (John's Silver × 350).

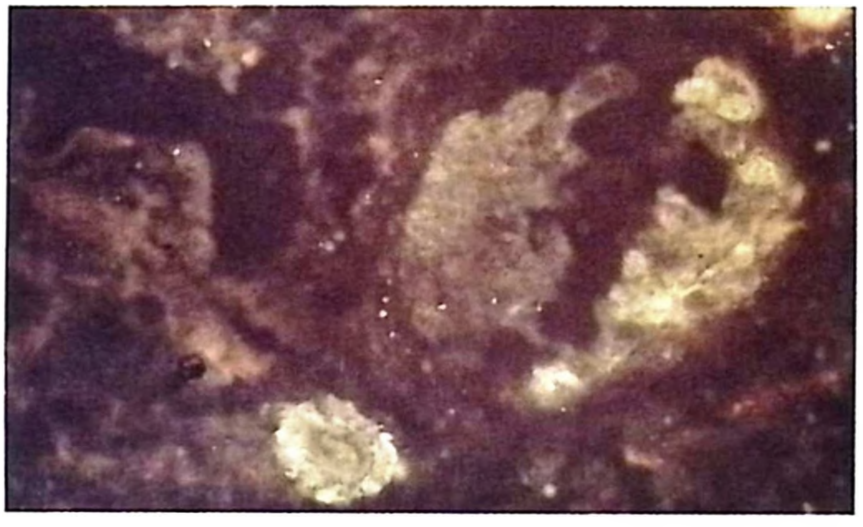

Fig. 7: Immunofluorescent appearance of HUS. Deposits of IgM in the pre-glomerular arteriole wall in the basal membrane zone of the glomerular capillary walls showing segmental distribution and a partial granular pattern.

TMA with predominant glomerular involvement: These specimens showed swelling and detachment of the endothelial cells which resulted in thickening of the glomerular capillary walls and narrowing of the capillary lumina. The number of affected glomeruli varied considerably and additional glomerular lesions were present, including so-called "paralytic glomeruli" with extremely dilated and congested capillary lumina and "double-contours" due to widening of the subendothelial space. The renal arteries, with the exception of an occasional thrombus in the glomerular arterioles, were not involved in the specimens classified as glomerular TMA.

TMA with predominant arterial involvement: These specimens showed renal tissue in which the arterioles and interlobular and arcuate arteries were narrowed or obstructed by swelling of the endothelium and/or thrombi. Fibrinoid necrosis,

intimal swelling and proliferation, "onion-skinning" due to fibrous replacement of the thickened intima and laminar intimal fibroplasia were found.

Cortical necrosis: This lesion was characterized by a recent or old infarction of all cortical structures in a portion of the renal tissue, sometimes accompanied by calcification of the infarcted region.

Extrarc al studies: Two cases were examined at autopsy (one complete and one partial). Liver tissue was examined in ten cases, muscle in two cases and the gastrointestinal tract in one case (Fig. 8).

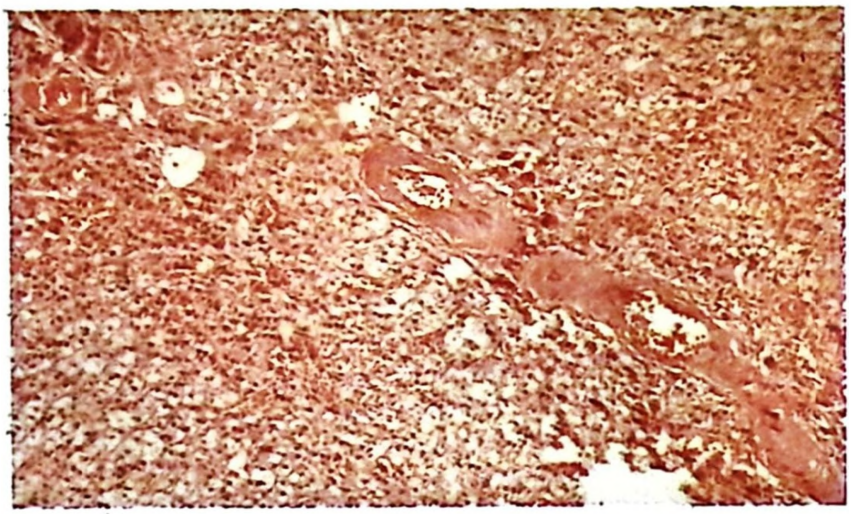

Fig. 8: Adrenal microangiopathy with deposition of fibrinoid material in the capillary wall and occlusion of the lumen is seen (HE × 200).

Results

Clinical Findings

Of 919 pediatric renal biopsies evaluated at the Hacettepe Children's Hospital between the years 1978 and 1990, one case was diagnosed by biopsy as having HUS. Among the intra vitam tissue-diagnosed pediatric nephrologic diseases in our center, the rate of HUS was found to be 0.1 percent, while the rate of HUS among postmortem pediatric material was 10 percent (14/147).

The ages of the patients at the onset of disease ranged from 10 months to 15 years in our study. Five were infants and seven were young children. The male/female ratio was 2/3. Five children had prodromal diarrhea (D+ HUS) and the remaining ten were (D−) cases. Five of the (D−) cases had gastrointestinal symptoms other than diarrhea (abdominal pain, vomiting), three cases presented with upper respiratory tract infection (20%) and two cases showed no distinct prodromal symptoms (14%). The (D+) group consisted of three infants and two children, while there were two infants and eight children in the (D−) group. E.coli infection was identified in the stool specimens of four of the (D+) cases, and the

fifth (D+) case, had both *E.coli* and *Shigella* infections. No specific infection was identified in any of the (D−) cases. Two children had recurrent HUS, one of whom had a previous diagnosis of membranous glomerulonephritis. This case was classified as having the secondary type of HUS.

Anemia was present in all cases. Thrombocytopenia was present in eight cases (53%) and Burr cells were demonstrable in seven cases (47%). All 15 children had acute renal failure and proteinuria. Five of them had hematuria (33%) and six showed oligoanuria (40%). All cases had microangiopathic hemolytic anemia and azotemia (100%), but thrombocytopenia was present in only eight of them (53%). Complications of acute renal failure, a high level of uric acid, electrolyte imbalance and congestive heart failure were present in 12/14, 9/14 and 8/15 cases, respectively.

Hypertension developed in ten patients (67%) and there was no response to therapy in seven. Eighty percent of the (D−) and forty percent of the (D+) cases had hypertension. It developed in two TMA cases with predominant arterial involvement (one associated with cortical necrosis), in seven of the 11 cases with predominant glomerular involvement and in one of two cases with cortical necrosis. Symptoms of central nervous system (CNS) involvement were observed in ten cases, such as alterations in the level of consciousness (four cases), focal or generalized convulsions (four cases) and decerebrate rigidity (two cases). Gastrointestinal hemorrhages were observed in three cases.

Fourteen of the patients died, twelve during the stage of acute renal failure and two after progressing to end-stage renal failure. Only one patient survived and was followed for four years (Table I).

Histopathological Findings:

Thrombotic microangiopathy (TMA) which is essential in the diagnosis of HUS was noted in all cases. Cortical necrosis was associated with TMA in three cases and arterial involvement was associated with TMA in two cases.

Histopathological Classification:

1. TMA with predominant glomerular involvement was noted in 11 patients (73%) and arteriolar involvement was also present in four of them. The percentage of glomeruli presenting the typical pattern of TMA varied in our study (< 25% in one case, between 25 and 50% in four cases, between 50 and 75% in five cases and > 75% in one case). Other glomeruli appeared normal. Paralytic glomeruli were also observed.
2. TMA with predominant arterial involvement was noted in two patients. In one of these cases, it was seen in combination with cortical necrosis. Interlobular and arcuate arteries showed almost complete occlusion of the lumina due to

Table I: Summary of Selected Clinical and Laboratory Findings of 15 Children With Hemolytic-Uremic Syndrome

Number of patients	15
Male / Female ratio	6/9
Age at presentation:	
Mean	60 months
Range	10 months-15 years
Type of prodrome:	
Diarrhea	5 (33%)
Other gastrointestinal symptoms	5 (33%)
Convulsions and unconsciousness	4 (27%)
Upper respiratory tract infection	3 (20%)
Anemia (Hb less than 11 g/dl)	15 (100%)
Thrombocytopenia (less than 100,000/mm^3)	8 (53%)
Burr cells	7 (47%)
Proteinuria	15 (100%)
Anuria-oliguria	6 (40%)
Hypertension (more than 95 percentile)	10 (67%)
Outcome:	
Chronic renal insufficiency	1 (7%)
Mortality	14 (93%)

thrombosis, intimal edema, intimal proliferation and thickening of the muscular walls.

3. Cortical necrosis was noted in three nephrectomy cases. It was widespread in all cases

In four cases, renal tissue was examined during the first three weeks of the disease and early patterns of renal injury were found. In contrast, in the nine cases in whom renal tissue was examined after the first three weeks of the disease, late patterns of renal injury were noted, with arterial involvement being present in two of these cases.

In the recurrent HUS cases, renal tissue was examined three and fifteen months after the onset of disease. Both early and late patterns of renal injury were noted in these cases and one of them also had cortical necrosis.

Table II: Histopathologic Groups and Age Distribution of 15 HUS Cases

Histopathologic Groups	Age Groups	
	Infancy (0-2 Years)	Childhood (3-16 Years)
TMA with predominant glomerular involvement	4	7
TMA with predominant arterial involvement	–	2 (one case with cortical necrosis)
Cortical necrosis	1	1
Total	5	10

Relationship between age, prodrome and pattern of renal injury (Fig. 9):

Of five children under 24 months of age, four had TMA with predominant glomerular involvement and one had cortical necrosis. Of ten children over 24 months of age, seven had TMA with arterial involvement, one had cortical necrosis and two had TMA with predominant glomerular involvement (one case revealed a combination of cortical necrosis and TMA with predominant arterial involvement).

Of five children with prodromal diarrhea (D+), four had TMA with predominant glomerular involvement and one had cortical necrosis. Of ten children without

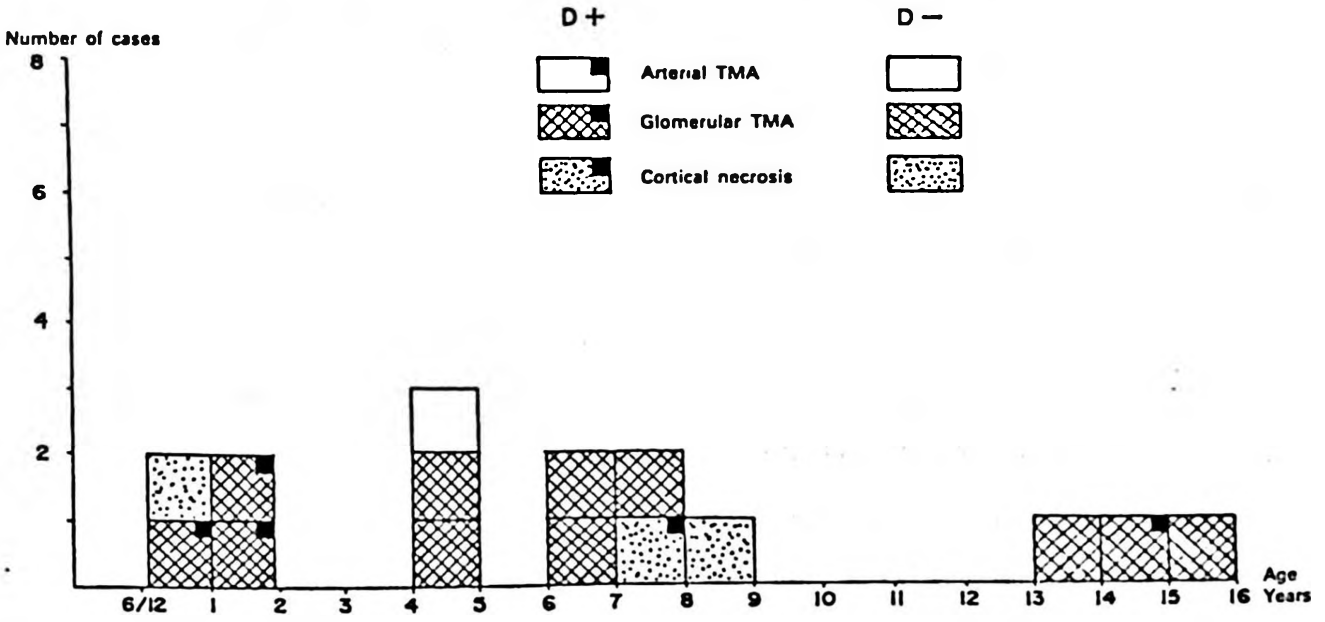

Fig. 9: Distribution of three histopathologic patterns of HUS according to age and prodromal presence (D +) or absence (D –) of diarrhea.

prodromal diarrhea (D−), seven had TMA with predominant glomerular involvement, two had TMA with predominant arterial involvement and two had cortical necrosis (in one patient it was associated with arterial involvement).

Only one patient with glomerular TMA survived, and in this case, 25 percent of the glomeruli were involved in which thrombotic microangiopathic changes were minimal.

Renal Immunofluorescence Findings:

Immunofluorescence studies were performed on one biopsy with TMA and predominant glomerular involvement and on four necropsies (two with cortical necrosis, one with TMA and predominant arterial involvement and one with TMA and glomerular involvement). Staining for fibrinogen-related antigen was detected in the glomeruli of all five cases. No specific pattern of staining was observed. Diffuse, broken, linear and/or granular glomerular basement membrane deposits were noted. CD23 deposition was found in three cases and IgM in two. Of the cases with HUS secondary to membraneous glomerulonephritis, immunofluorescence findings revealed the diagnostic pattern of membraneous glomerulonephritis (Table III).

Table III: Immunohistological Findings in Five HUS Patients

Case No.	Histopathological Groups	Immunofluorescence Findings Immunoreactant	Localization
1	TMA with predominant arterial involvement	Fibrinogen IgM	GBM* zone
2	Cortical necrosis	Fibrinogen IgM C_3	Glomeruli and arterioles
7	Cortical necrosis (Secondary to membraneous glomerulonephritis)	Fibrinogen IgM IgG granular IgA	GBM zone
8	TMA with predominant glomerular involvement	Fibrinogen C_3	GBM zone
13	TMA with predominant glomerular involvement	Fibrinogen C_3	Glomeruli and capillaries of interstitium

* GBM: Glomerular basement membrane.

Extrarenal Findings:

Extrarenal microthrombi were identified in two necropsy cases. They were present in the adrenals, gastrointestinal tract and pancreas of one case and in the liver and skin of the other. Diffuse brain edema was observed in one patient whose CNS was examined.

Discussion

Since the hemolytic-uremic syndrome is a syndromic condition, its clinical and pathological findings are heterogeneous. It occurs in infancy and early childhood[1,2,13,15,16]. In our study, we also found it to occur in these age groups. Most of the patients in our series were in the 0-2 year-old age group (33%), the mean age at onset being 60 months. HUS is usually associated with diarrhea, and the majority of patients in large series have (D+) type HUS[11,15-17]. In our series, 33 percent constituted (D+) cases and 67 percent (D−) cases. The (D+) HUS type occurs principally in infants, while the (D−) type is seen mostly at a later age[18]. In our series, 60 percent of the (D+) group were infants, while only 20 percent of the (D−) group were infants.

On clinical examination, microangiopathic hemolytic anemia and azotemia were found in all our patients, whereas, thrombocytopenia was present in only 53 percent, which may be due to the fact that thrombocytopenia varies according to the stage of the syndromic condition. Since the duration of thrombocytopenia may be short, it can be easily overlooked and thrombocytosis may therefore be seen as a rebound phenomenon following the thrombocytopenic period[3]. In our series, disseminated intravascular coagulation (DIC) was detected in four patients with thrombocytopenia because of the presence of elevated fibrin degradation products in the blood and prolonged PT/PTT values. Fibrin thrombi were noted in the glomerular capillaries of the glomeruli of two cases with a clinical diagnosis of DIC.

Hypertension develops in HUS and can be severe. Uncontrollable hypertension carries a poor prognosis[19]. Severe hypertension occurs in older patients with (D−) type HUS[12,20]. Our findings were consistent with these reports. Hypertension was present in 80 percent of (D−) cases and 40 percent of (D+) cases studied. Although hypertension is known to develop more frequently in older patients than in infants, we found it to occur almost equally in infants (60%) and older children (70%).

Thoenes and John[21] reported a strong correlation between the arterial form of TMA and hypertension. Severe hypertension developed in two of our cases with arterial TMA. This result is in accordance with the study done by the above-mentioned authors. In our study, hypertension was prominent in both the cortical necrosis group and the TMA with predominant glomerular involvement

group. Therefore, hypertension is one of the major clinical findings in all histopathological groups.

Central nervous system (CNS) involvement, which is a serious complication of HUS, carries a poor prognosis. CNS involvement is reported in 25-90 percent of cases in various series studied[1,2,19]. In our series, it was noted in 10 cases (67%) having a severe clinical course and fatal outcome. A CNS examination was performed only in one patient in which microthrombi were not detected, but diffuse brain edema was present.

Upadhyaya et al[22] reported microthrombi in a wide distribution of organs including the brain and myocardium. Extrarenal thrombi were present in two cases examined in our series involving the adrenal glands, gastrointestinal tract and pancreas in one case and the liver and skin in the other. These sites are similar to those reported in large series[14,22]. Evidence of ischemia and focal areas of infarction with surrounding edema are frequently noted in the extrarenal organs[22]. We demonstrated similar findings in liver tissue of five of the 12 cases we examined.

Immunofluorescence microscopical studies revealed varying amounts of fibrinogen in all five cases studied (Table III). Stainings with antisera against fibrinogen, C_3 and IgM were observed mainly along the glomerular basement membranes and were also occasionally present in the arteriolar walls, as reported in the literature[2,14].

Since the cases we studied had mostly fatal outcomes, a correlation between histopathological groups and prognosis could not be made. Habib et al[12] has suggested that prognosis is poor in cases in which more than 50 percent of the glomeruli are involved and that sequelae have been observed in these patients if they survive. Less than 25 percent of the glomeruli were involved in only one patient that survived in our study, but late sequelae developed such as hypertension and a decrease in the creatinine clearance level.

Summary

This retrospective clinicopathological study consists of 15 cases of tissue diagnosed hemolytic-uremic syndrome (HUS) observed over a twelve-year period of experience. HUS constituted 0.1 percent (1/919) of renal biopsies and 10 percent (14/147) of renal necropsies in a Hacettepe University series. The age distribution varied from 10 months to 15 years, with a mean age of 60 months.

Of 15 HUS cases (33%), five had (D+) type with prodromal diarrhea and two had a recurrent clinical course, in one of whom it was secondary to membranous glomerulonephritis. All patients had anemia and acute renal failure. Burr cells were detected in seven cases (47%), thrombocytopenia was seen in eight cases (53%),

oliguria/anuria was present in six cases (40%) and hematuria was observed in five cases (33%).

Light microscopy revealed microangiopathic type involvement of the glomeruli in all cases. Classification based on light microscopic studies showed:

1. Thrombotic microangiopathy with predominant glomerular involvement (11 cases),
2. Thrombotic microangiopathy with predominant arterial involvement (two cases),
3. Cortical necrosis in three cases (one case in combination with arterial TMA).

All five patients studied immunohistologically revealed a non-specific type of fibrinogen deposit and few other immunoreactants. Extrarenal microangiopathy was demonstrated in the adrenals, stomach, pancreas, liver and skin in two necropsies studied.

REFERENCES

1. Gianantonio C, Vitacco M, Mendilaharzu F, et al. The hemolytic-uremic syndrome. *J Pediatr* 64: 478, 1964.
2. Kaplan BS, Levin M, de Chadarevian JP. The hemolytic-uremic syndrome. In Edelmann CM Jr (ed.). *Pediatric Kidney Disease* (2nd ed) Vol. II. Boston: Little, Brown and Co, 1992, pp 1383-1405.
3. Heptinstall RH. Hemolytic uremic syndrome, thrombotic thrombocytopenic purpura and systemic sclerosis (systemic scleroderma). In Heptinstall RH (ed). *Pathology of the Kidney* (4th ed) Vol. II. Boston: Little, Brown and Co, 1992, pp 1163-1233.
4. Neild G. The haemolytic-uraemic syndrome: a review. *Q J Med* 241: 367, 1987.
5. Dolislager D, Tune B. The hemolytic-uremic syndrome: spectrum of severity and significance of prodrome. *Am J Dis Child* 132: 55, 1978.
6. Milford DV, Taylor CM. New insights into the haemolytic uraemic syndromes. *Arch Dis Child* 65: 713, 1990.
7. Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* 19: 619, 1983.
8. Fitzpatrick MM, Dillon MJ. Current views on aetiology and management of haemolytic uraemic syndrome. *Postgrad Med J* 67: 707, 1991.
9. Bitzan M, Moebius E, Ludwig K, et al. High incidence of serum antibodies to Eschericia coli 0157 lipopolysaccaride in children with hemolytic-uremic syndrome. *J Pediatr* 119: 380, 1991.
10. Bogdanović R, Čobeljić M, Marković M, et al. Haemolytic-uraemic syndrome associated with Aeromonas hydrophila enterocolitis. *Pediatr Nephrol* 5: 293, 1991.
11. Levy M, Gagnadoux MF, Habib R. Pathology of hemolytic-uremic syndrome in children. In Remuzzi G, Mecca G, de Gaetano G (eds). *Hemostasis, Prostaglandins and Renal Disease.* New York: Raven, 1980, pp 383-397.
12. Habib R, Levy M, Gagnadoux MF, Broyer M. Prognosis of the hemolytic uremic syndrome in children. In Hamburger J, Crosnier J, Grünfeld JP, Maxwell MH (eds). *Advances in Nephrology.* Vol. XI. Chicago: Year Book Medical Publishers, 1982, pp 99-130.
13. Srivastava RN, Moudgil A, Bagga A, Vasudev AS. Hemolytic uremic syndrome in children in northern India. *Pediatr Nephrol* 5: 284, 1991.

14. Argyle JC, Hogg RJ, Pysher TJ, et al. A clinicopathological study of 24 children with hemolytic uremic syndrome: a report of the Southwest Pediatric Nephrology Study Group. *Pediatr Nephrol* 4: 52, 1990.

15. Milford DV, Taylor CM, Guttridge B, et al. Haemolytic uraemic syndromes in the British Isles 1985-8: association with Verocytotoxin-producing Escherichia Coli. Part 1: clinical and epidemiological aspects. *Arch Dis Child* 65: 716, 1990.

16. Martin DL, Mac Donald KL, White KE, et al. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. *N Engl J Med* 323: 1161, 1990.

17. Sieniawaska M, Korniszewska J, Gura C, et al. Prognostic significance of certain factors in the haemolytic-uraemic syndrome. *Pediatr Nephrol* 4: 213, 1990.

18. Drummond KN. Hemolytic uremic syndrome-then and now. *N Engl J Med* 312: 116, 1985.

19. Robson WL, Fick GH, Wilson PC. Prognostic factors in typical postdiarrhea hemolytic-uremic syndrome. *Child Nephrol Urol* 9: 203, 1988.

20. Bohle A. Investigations into the morphology of the hemolytic uremic syndrome. In Bulla M (ed). *Renal Insufficiency in Children.* New York: Springer-Verlag, 1982, pp 1-6.

21. Thoenes W, John HD. Endotheliotropic (hemolytic) nephroangiopathy and its various manifestation forms (thrombotic microangiopathy, primary malignant nephrosclerosis, hemolytic-uremic syndrome). *Kidney Wochenschr* 58: 173, 1980.

22. Upadhyaya K, Barwick K, Fishaut M, et al. The importance of nonrenal involvement in hemolytic-uremic syndrome. *Pediatrics* 65: 115, 1980.

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PLASMA CHROMIUM LEVELS IN SMALL-FOR-GESTATIONAL-AGE NEWBORN INFANTS*

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*Gülşen Erdem MD*****, *Aytekin Temizer PhD******

SUMMARY: Yurdakök M, Özer D, Erdem G, Temizer A. (Department of Pediatrics, Hacettepe University Faculty of Medicine, and the Department of Analytical Chemistry, Hacettepe University Faculty of Pharmacy, Ankara, Turkey). Plasma chromium levels in small-for-gestational-age newborn infants. Turk J Pediatr 35: 37-40, 1993.

Plasma chromium (Cr) levels were determined in 24 preterms and 18 full-term newborn infants. There was no statistically significant differences in plasma Cr levels between the preterm and full-term infants. Plasma Cr levels were similar in small-for-gestational-age infants and in infants with hypoglycemia compared with healthy infants. *Key words: chromium, hypoglycemia, newborn, plasma, small-for-gestational-age.*

Hypoglycemia in small-for-gestational-age (SGA) infants is a multifactorial phenomenon. The factors which are implicated in this condition include depletion of liver glycogen, decreased fat and protein reserves, functional hyperinsulinism, decreased fat oxidation, a low gluconeogenic rate and increased glucose demand as a result of asphyxia at birth, and a relatively large brain mass. In most SGA infants there is little evidence of a primary endocrine abnormality responsible for the hypoglycemia. However, further research, particularly related to hormone receptor activities, is needed to clarify this point[1].

Chromium (Cr), a trace element essential for humans and animals, is involved in normal carbohydrate metabolism which potentiates the action of insulin[2-4]. Improvement of glucose intolerance has been demonstrated in malnourished children, especially in those with Cr deficiency, by the supplementation of inorganic Cr[5-7]. Since information pertaining to Cr deficiency is very limited, we decided to measure the plasma levels in SGA newborn infants with the aim of

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contributing to an explanation regarding the pathogenesis of hypoglycemia in these infants.

Material and Methods

Twenty-four preterm and 18 full-term infants who were selected at random were enrolled in this study. Eight of the preterm infants and eight of the full-term infants were SGA, while the rest were appropriate-for-gestational age (AGA) infants (Table I). None of the infants had severe congenital malformations. Infants with infectious diseases[3] and inborn errors of metabolism were excluded from the study on the basis of their clinical and laboratory findings including blood cultures and plasma and urine amino acid analyses.

Table I: Some Features of the Newborn Infants

		SGA Infants	AGA Infants
Preterm	M/F	5/3	13/3
	GA (w)	35.4 ± 1.7	35.3 ± 0.9
	BW (g)	1641 ± 383	2199 ± 183
Full-term	M/F	5/3	6/4
	GA (w)	39.2 ± 1.1	39.7 ± 1.1
	BW (g)	2443 ± 220	34.15 ± 440

M/F: male/female, GA: gestational age, w: week, BW: birth weight.

Hypoglycemia was defined as a blood sugar level under 20 mg/dl in the preterm infants and 30 mg/dl in the full-term infants on the first day of life. To determine the serum Cr levels, blood was drawn from the infants on the first day of life prior to the administration of intravenous fluids. These samples were collected in plastic syringes having siliconized needles attached to heparinized polyvinylchloride tubing. After the blood was centrifuged for 15 min at X 2000 g, the plasma was carefully removed with a sterile polystyrene pipette, and 1 ml aliquots were stored in sterile polypropylene tubes at −20 °C. The plasma Cr level was assayed by atomic absorption spectrophotometry (Perkin Elmer 3030) as previously described[8]. All results were expressed as the mean ± SD and statistical analysis was performed by using the Student's *t* test.

Results

We found no difference in plasma Cr levels between the preterm and full-term infants and in SGA and AGA infants (Table II). Plasma Cr levels were also similar in the newborn infants with hypoglycemia and in those with normal blood glucose levels (Table III).

Table II: Plasma Cr Concentrations (μg/l) in the Newborn Infants

	SGA Infants	AGA Infants	Total
Preterm	34.61 ± 27.02 (n : 8)	36.62 ± 23.43 (n : 16)	35.61 ± 25.68 (n : 24)
Full-term	43.14 ± 20.28 (n : 8)	26.99 ± 24.63 (n : 10)	35.33 ± 22.46 (n : 18)
Total	31.80 ± 24.02 (n : 16)	38.87 ± 24.09 (n : 26)	

$p > 0.05$ in all comparisons.

Table III: Plasma Cr Concentrations (μg/l) in Hypoglycemic and Normoglycemic Newborn Infants

	Infants With Hypoglycemia	Infants With Normoglycemia	Total
Preterm	41.22 ± 24.26 (n : 8)	30.25 ± 23.40 (n : 9)	35.76 ± 23.53 (n : 17)
Full-term	56.92 ± 12.92 (n : 5)	29.61 ± 22.84 (n : 15)	43.26 ± 18.09 (n : 20)
Total	45.50 ± 22.34 (n : 13)	29.84 ± 22.54 (n : 24)	

$p > 0.05$ in all comparisons.

Discussion

Although it is known that Cr potentiates the action of insulin, the mechanism of this effect has not been clearly understood. Cr is essential for the peripheral action of insulin at the cellular level. The increase in insulin efficiency with supplementary Cr may be related to improvements in receptor number or affinity or both. This may cause the rapid disappearance of Cr from the circulation following an intravenous glucose load in healthy individuals. It has been reported that Cr supplementation improves impaired glucose tolerance in man[3].

Cr deficiency does not appear to be related to low fasting blood glucose levels. Impaired glucose utilization in patients with protein and calorie malnutrition is not solely due to a deficiency of a single trace element, i.e. Cr. But a positive effect

may be seen on glucose tolerance after the administration of Cr when the plasma Cr level is lower than normal[5,6]. In this study we could not find a difference in plasma Cr levels between the SGA newborn infants or the hypoglycemic infants when compared with the controls.

Pregnancy is characterized not only by significantly lowered serum Cr levels, but also by a decrease in the Cr content of hair on the scalp[9]. The role of Cr in fetal metabolism has not been clearly defined. Our results show that plasma Cr concentrations are not affected by the duration of gestation or intrauterine nutrition.

REFERENCES

1. Senior B, Sadeghi-Nejad A. Hypoglycemia: A pathophysiologic approach. *Acta Paediatr Scand* Suppl 352: 1, 1989.
2. Uusitupa MI, Kumpulainen JT, Voutilainen E, et al. Effect of inorganic chromium supplementation on glucose tolerance, insulin response, and serum lipids in noninsulin-dependent diabetics. *Am J Clin Nutr* 38: 404, 1983.
3. Anderson RA, Bryden NA, Polansky MM. Serum chromium of human subjects: effects of chromium supplementation and glucose. *Am J Clin Nutr* 41: 571, 1985.
4. Anderson RA, Polansky MM, Bryden NA, et al. Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. *Metabolism* 36: 351, 1987.
5. Hopkins LL, Ransome-Kuti O, Majaj AS. Improvement of impaired carbohydrate metabolism by chromium (III) in malnourished infants. *Am J Clin Nutr* 21: 203, 1968.
6. Carter JP, Kattab A, Abd-el-Hadi K, et al. Chromium (III) in hypoglycemia and impaired glucose utilization in kwashiorkor. *Am J Clin Nutr* 21: 195, 1968.
7. Gürson CT, Saner G. Effects of chromium supplementation on growth in marasmic protein-calorie malnutrition. *Am J Clin Nutr* 26: 988, 1973.
8. Varma A. *Handbook of Atomic Absorption Analysis.* Vol. I. Florida: CRC Press, 1984.
9. Hambidge KM, Rodgerson DO. Comparison of hair chromium levels of nulliparous and parous women. *Am J Obstet Gynecol* 103: 320, 1969.

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THE DISTRIBUTION OF HAPTOGLOBIN TYPES IN VARIOUS REGIONS OF TURKEY*

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SUMMARY: Aksoy M, Erdem Ş. (Hematology Unit, Department of Internal Medicine, Istanbul University Faculty of Medicine at Çapa, Istanbul, and the Biology Division of the Turkish Scientific and Technical Research Council, Gebze, Kocaeli, Turkey). The distribution of haptoglobin types in various regions of Turkey. Turk J Pediatr 35: 41-43, 1993.

The distribution of haptoglobin (Hp) types and the gene frequencies of Hp1 in various regions' of Turkey are discussed. The Hp1 gene frequency ranged between 20.4 percent for Turks living in the eastern part of Turkey and 32.6 percent in those who either are at present living in or have migrated from Western Thrace. Generally, the Hp1 gene frequency in the Turkish population is 26.5 percent, a figure similar to that recorded for Asians. Minor variations in the Hp1 gene frequencies in various parts of Turkey may be assumed to have been caused in certain regions by intermingling with the local populations. *Key words: haptoglobin, regional distribution, Turkey.*

Smithies'[1] discovery that haptoglobins (Hp) undergoing starch gel electrophoresis yielded five distinct patterns led to heightened universal interest in these globins. Furthermore, this characteristic immediately found an application in studies relating to the distribution of Hp types in various populations and ethnic groups. It is well known that data regarding the frequency of occurrence of blood types in a population was formerly used in such studies. However, several investigators have emphasized that Hp typing has proved to be as effective as the former method in these research projects. The distribution of the major types of Hp in various populations living on different continents is quite uniform. We may cite as an example the Hp 1 gene frequency which is between 35 and 45 percent in Europe[1], but generally higher in Africa, ranging between 40 to 87 percent. In contrast to these figures, the Hp 1 gene frequency in the Asian population ranges from 8 to 39 percent. Even though there are some exceptions, the Hp 1 gene frequencies are remarkably similar throughout Europe and Asia.

The distribution of Hp variants in the Turkish population on the whole, and in some regions of Turkey, have been researched since 1966, and the results obtained

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have been published[2-5]. The purpose of this paper is to summarize and discuss the results of these studies.

Material and Methods

Haptoglobin typing was determined in the following manner: A total of 919 blood samples were collected from unrelated Turkish people living in various regions of Turkey. Starch gel electrophoresis was carried out on 500 blood samples according to the method devised by Smithies[6], while the remaining 619 blood samples were subjected to acrylamide gel electrophoresis. Equipment used were the microzone electrophoresis cell and the Kodel 113 acrylamide cell accessory produced by the Spinco Division of Beckman Instruments, Inc, California, U.S.A.

Results and Discussion

As has been documented, the Hp 1 gene frequency in European populations ranges between 34 (in Greece) and 45 percent (in the Basque region of Spain)[1]. On the other hand, the Hp gene frequency in Asian populations is lower than the figure recorded in those in Europe, ranging between 7 (in the Irulas tribe in India) and 33 percent (in the Ghasgai in Iran)[1]. Naturally there are some exceptions associated with the higher values recorded for the Hp 1 gene frequency in Asians[1]. The Hp 1 gene frequency for Turks living in Asia Minor is 26.5 %, which is well within the range recorded for Asians[2]. However, it may be noted that the Hp 1 gene frequency exhibits interesting regional variations among Turks. The Hp 1 gene frequency in 135 Turks living in Antalya, a city on the eastern coast of the Mediterranean, was found to be 26 percent[3]. A study conducted in 102 apparently healthy Turks selected at random who were either still living in or had migrated from Western Thrace revealed that the frequency of the Hp 1 gene was 32.6 percent, which incidentally was the highest percentage recorded for any Turkish population[4]. This figure can perhaps be explained by assuming that Turks intermingled with other Europeans formerly living in these regions[4]. The authors of this paper, in association with their colleagues, carried out a study on abnormal hemoglobins, beta-thalassemia and Hp 1 gene frequencies among Turks living in Van, a city close to the eastern border of Turkey[5] and found that the Hp 1 gene frequency was 20.4 percent. This study encompassing 159 Turks living in this region revealed a figure that was the lowest of any region in Turkey.

REFERENCES

1. Giblett ER. *Genetic Markers in Human Blood.* St. Louis: Mosby, 1969, pp 95-100.
2. Erdem Ş, Aksoy M. A note on the distribution of haptoglobin types in Turkey. *Hum Hered* 25:18, 1975.

3. Aksoy M, Dinçol G, Erdem Ş. Haemoglobin variants, beta-thalassemia glucose 6-phosphate dehydrogenase deficiency and haptoglobin types in Turkish people living in Manavgat, Serik and Boztepe (Antalya). *Hum Hered* 30:3, 1980.
4. Aksoy M, Kutlar A, Kutlar F, et al. Survey on haemoglobin variants, beta-thalassaemia, glucose-6-phosphate dehydrogenase deficiency, and haptoglobin types in Turks from Western Thrace. *J Med Genet* 22:288, 1985.
5. Aksoy H, Bayraktar Y, Yüzbaşıoğlu T, et al. Frequencies of abnormal hemoglobins, high A_2 beta-thalassemia and distribution of haptoglobin types in Van and its vicinities. *Doğa* 10:10, 1986.
6. Smithies O. Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. *Biochem J* 61:629, 1955.

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PROTEIN C AND ANTITHROMBIN III IN CHILDREN WITH ACUTE LEUKEMIA*

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SUMMARY: Atlıhan F, Karakaş Z, Batun S. (Department of Pediatrics, Dicle University Faculty of Medicine, Diyarbakır, Turkey). Protein C and antithrombin III in children with acute leukemia. Turk J Pediatr 35: 45-51, 1993.

In this study Protein C (PC) and antithrombin III (AT III) levels in childhood acute leukemia were investigated. The mean PC activity levels in 19 newly diagnosed cases of acute leukemia were significantly lower as compared with the normal controls ($p < 0.05$). A significant increase was found ($p < 0.01$) in the patients in remission. Prior to treatment 78.8 percent of patients had decreased PC activity levels, but all patients had normal PC activity during remission.

Decreased PC activity levels were found to be independent of the leukocyte count and liver function.

No statistically significant difference was found in the AT III antigen levels between the untreated patients, the patients in remission and the control group. Our results indicate that apart from thrombocytopenia, low PC activity levels and alterations in fibrinolysis and coagulation may be responsible for the hemorrhagic manifestations observed in cases of acute leukemia. *Key words: protein C, antithrombin III, acute leukemia.*

Hemostasis is a complex mechanism involving local reactions in blood vessels, platelet activities and interactions of specific coagulation factors which circulate in the blood.

Four basic systems are involved in the regulation of coagulation: the fibrinolytic system, endothelial cells, protein C (PC) and antithrombin III (AT III)[1-3].

Protein C is a vitamin K dependent plasma glycoprotein that, when activated by thrombin and thrombomodulin, acts as a major inhibitor in blood coagulation. Activated PC has an anticoagulant effect by inactivating factors Va and VIIIa. It also facilitates fibrinolysis by neutralizing an inhibitor of the plasminogen activator (Fig. 1)[2-7].

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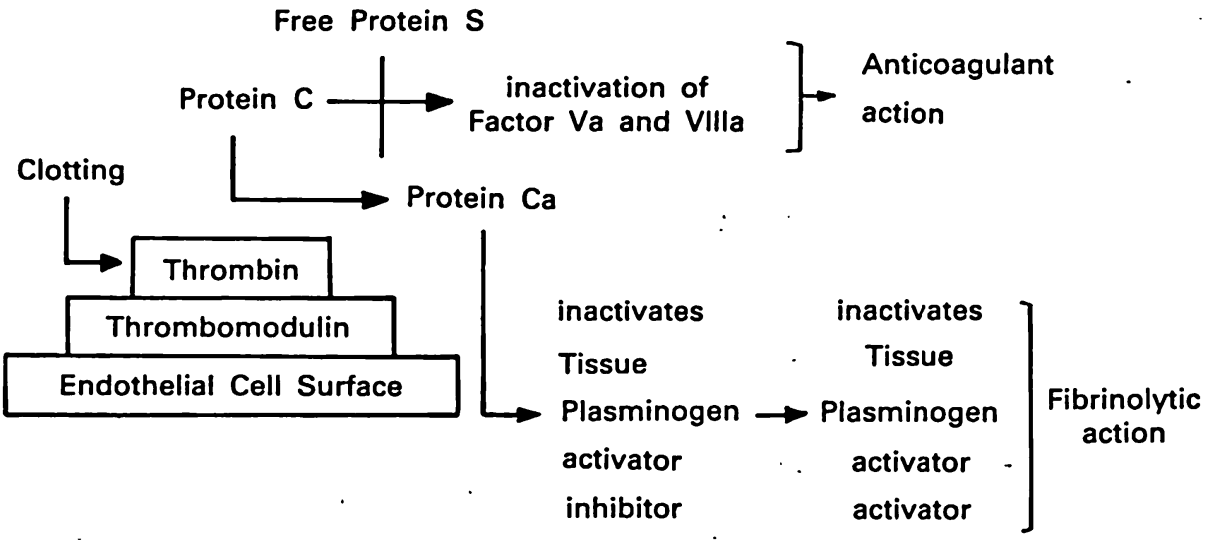

Fig. 1: Anticoagulant and fibrinolytic action of protein C.

Thrombin binds to thrombomodulin, a receptor protein on the surface of endothelial cells. The thrombin-thrombomodulin complex rapidly activates protein C.Protein Ca has an anticoagulant effect by inactivating factors Va and VIIIa. It also facilitates fibrinolysis by neutralizing an inhibitor of plasminogen activator.

AT III is a globulin which mainly inactivates thrombin, while factor Xa is a major inhibitor of blood coagulation[2, 8]. Hemostatic disorders occur very frequently in patients with acute leukemia.

Forty to sixty percent of patients demonstrate minor or major signs of bleeding at diagnosis. Thrombocytopenia cannot be considered the only cause of these hemorrhagic manifestations, but also changes in extravasal structures, the coagulation system, and fibrinolysis have been implied. In various studies, different data describing alterations in coagulation and fibrinolysis in acute leukemia have been reported. Therefore, it is difficult to evaluate the role of the deficiency in coagulation factors and alterations in fibrinolysis during hemorrhagic episodes in the acute leukemias[9-11].

The present study aims at investigating PC and AT III levels in childhood acute leukemia and to determine whether these two major coagulation inhibitors play a role in the hemorrhagic manifestations of this disease.

Material and Methods

The PC and AT III levels were studied in 19 children diagnosed as having either acute lymphoblastic leukemia (ALL) or acute non-lymphoblastic leukemia (ANLL). These levels were measured upon admission to the Department of Pediatrics of Dicle University Faculty of Medicine prior to initiation of treatment. The ages of

the patients ranged between 2.5 and 13 years. The control group consisted of 12 age-and-sex matched healthy children.

A diagnosis of acute leukemia was established after examining peripheral blood and bone marrow samples using conventional cytochemical stains. Acute leukemia was classified morphologically according to the criteria proposed by the FAB Group[12].

Plasma samples were analyzed in 19 children before treatment was initiated, and in 14 of these children, determinations were repeated after remission was achieved.

Blood samples were collected in sodium citrate (9 parts blood:1 part 0.11 mol/l anticoagulant) using a 5 ml syringe. Plasma was obtained by centrifugation at 1500 g for 10 minutes. Blood samples were frozen and stored at − 20 °C. PC activity was determined by the coagulometric method using a commercial kit (Behring Diagnostics). With the use of standard human plasma (Behringwerke Diagnostics), a reference curve was drawn on double logarithmic graph paper and the results were expressed as percentages. The normal range was 70-140 percent. An AT III assay was determined in plasma by the radial immunodiffusion technique using the Nor-Partigen AT III antigen kit (Behring Diagnostics). The results were expressed as a percentage of a normal pool. The normal range was 73-100 percent.

The diagnosis of disseminated intravascular coagulation (DIC) was confirmed by a decreased fibrinogen (< 300 mg/dl) and platelet count (< 100000/mm^3), prolonged partial thromboplastin time and an elevation of fibrin degradation products. Enzymatic synthesis function of the liver was evaluated by the following tests: SGPT, SGOT, prothrombin time and protein electrophoresis. The Student's *t* test was used for statistical analyses.

Results

Seventy-nine percent of the patients studied had ALL and 21.2 percent had ANLL. The mean age of the patients was 7.60 ± 3.59 years, and the mean age of the control group was 7.75 ± 3.49 years.

Mean PC concentrations were 52.15 ± 30.93 percent in the newly diagnosed cases of acute leukemia and 101.32 ± 16.46 percent in the control group. PC activity was significantly lower in the cases of acute leukemia as compared with the controls ($p < 0.01$). Mean PC activity levels in the 14 patients in remission were 94.5 ± 20.99 percent (Table I). A significant increase was found in the PC levels of the patients in remission as compared with the untreated patients ($p > 0.01$), but no statistical significant difference was found when comparing these values with those of the control group ($p > 0.05$). Of 19 patients, 14 (78.8 %) had decreased PC activity levels prior to treatment, but all patients in

Table I: Plasma Protein C Activity in Patients with Untreated Leukemia, in Patients in Remission and in the Control Group

	n	Protein C (%) (Range)	t	P
Control	12	101.32 ± 16.46 (85 - 140)	5.75	< 0.01
Leukemia	19	52.15 ± 30.93 (3 - 120)	4.68	< 0.01
Remission	14	94.5 ± 20.99 (74 - 140)		

remission had normal PC activity levels. The mean PC activity levels were 60.86 ± 26.07 percent in ALL patients and 19.5 ± 27.73 percent in ANLL patients. Mean PC activity was found to be significantly lower in the ANLL group. ($p < 0.05$).

The enzymatic synthesis function of the liver was evaluated. Mean PC activity levels were 42.62 ± 21.90 percent in eight patients with liver dysfunction and 56.3 ± 38.71 percent in ten patients with normal liver function. There was no statistical significant difference found between these groups ($p > 0.05$). PC activity was 50.4 ± 12.05 percent in patients with leukocyte counts over 50000/mm^3 and 52.42 ± 35.62 percent in patients with leukocyte counts below 50000/mm^3. There was no statistical significant difference found between these groups ($p > 0.05$).

Mean AT III antigen levels were 106.36 ± 23.03 percent in the cases of acute leukemia prior to treatment, 107.92 ± 19.83 percent in the cases in remission and

Table II: Plasma AT III Antigen Levels in Patients with Untreated Leukemia, in Patients in Remission and in the Control Group

	n	AT III (%) (Range)	t	P
Control	12	121.16 ± 17.42 (100 - 152)		
Leukemia	19	106.36 ± 23.03 (73 - 150)	2.02	> 0.05
Remission	14	107.92 ± 17.93 (77 - 141)	0.20	> 0.05

121.61 ± 17.42 percent in the control group. There was no significant difference statistically between these groups ($p > 0.05$). (Table II).

There was no significant statistical difference found between the AT III antigen levels of the patients with ALL and those with ANLL.

Discussion

Various coagulation and fibrinolysis disorders due to disease or to therapy are known to occur in children with acute leukemias. The two major inhibitors in coagulation, PC and AT III, have not been extensively investigated in pediatric cases of acute leukemia, and therefore, with the aim of determining whether they have a pathophysiological role in hemorrhagic manifestations, the PC and AT III levels were measured in 19 children newly diagnosed as having acute leukemia.

In our study, mean PC activity levels were significantly lower than in the normal controls, with 78.8 percent of the patients having PC levels below the normal range. Rodeghiero et al[13] in their study conducted in 58 patients with acute leukemia reported that the mean PC antigen concentrations were slightly lower than in the normal controls, with about one third of patients having PC values below the normal range. In our study, we evaluated the functional activity of PC. Marlar and co-workers[14] determined both the PC antigen and activity levels in DIC and showed that functional activity decreases more rapidly than antigen levels. Many studies have shown that PC activity decreases progressively during the initial stages of DIC, but that it gradually returns to normal in nonfatal cases[5, 14, 15]. In our study, only one patient had DIC, therefore, low PC activity levels cannot be explained by DIC.

Rodeghiero and co-workers[13] observed no decrease in PC antigen levels in patients with DIC resulting from leukemia and where there was no liver involvement. But they observed a decrease in PC antigen levels in patients with DIC who had liver disease as a consequence of the reduced synthesis by the liver. In the present study, we tried to determine whether a decrease in PC levels correlated with liver dysfunction by measuring the SGOT, SGPT, and PT levels and protein electrophoresis. No significant difference was found between PC activity levels of patients with liver dysfunction and those with normal liver function. PC concentrations were also low in 80 percent of the patients with normal synthesis function of the liver. Therefore, we cannot conclude that liver dysfunction alone accounts for low PC activity levels as Griffin et al[15] and Rodeghiero et al[13] have stated.

Some authors have suggested that the release of specific protease inhibitors derived from leukemic cells that possess fibrinolytic and procoagulant activity are responsible for the alterations in fibrinolysis in acute leukemias. In patients with untreated acute leukemia, widely varying changes in parameters of coagulation

and fibrinolysis have been reported. Enhanced fibrinolytic activity has been reported by Brakman et al[9] in contrast to the reports of Guarini et al[10], who explained the reduction of tPA (tissue plasminogen activator) activity by degradation of proteases from the leukemic cells or inactivation by some inhibitors. A new protease inhibitor of the hemostatic system that has been recently described is able to slowly inactivate PCa generated within the blood. The second new protease inhibitor reported can rapidly neutralize the action of urokinase or tissue type plasminogen activator[12]. In conclusion, the decreased levels of PC activity in untreated cases of leukemia can be explained by proteases and inhibitors released from the leukemic cells. Obtaining normal PC levels during remission also supports our hypothesis. We attempted to find a correlation between leukocyte counts and PC activity, but we found decreased PC activity levels independent of leukocyte counts. We also found PC activity to be significantly lower in ANLL cases in comparison with ALL cases, which is probably due to the proteases released from the primary granules of myeloid leukemic cells[9, 10, 16].

There was no statistical significant difference found between the AT III antigen levels of the untreated leukemic patients, the remission group and the control group. AT III antigen levels were within the normal range in all patients. There was also no difference found between the AT III antigen levels in the ANLL and ALL groups. There was no correlation found between AT III, the number of circulating blast cells and liver function. Rodeghiero et al[13] found normal AT III antigen and activity levels in cases of acute leukemia and reported that AT III activity was significantly lower only in the acute myeloblastic leukemia (AML) group when compared with the controls. Other studies on AT III indicate that this inhibitor is usually normal[13, 17]. In conclusion, we can say that there are varying changes in parameters in the coagulation of fibrinolysis in pediatric patients with acute leukemia. Our results indicate that besides thrombocytopenia, low levels of PC activity and alterations in fibrinolysis and coagulation may be responsible for the hemorrhagic manifestations observed in cases of acute leukemia. However further studies are needed to prove this point.

REFERENCES

1. Behrman RE, Vaughan VC III. Schema of coagulation. *In Nelson Textbook of Pediatrics* (13th ed). Philadelphia: Saunders, 1987, pp 1065-1067.
2. Yurdakök K, Oran O, Tekinalp G. Antithrombin III ve Protein C. *Katkı* 8:3, 263, 1987.
3. Oktay K. Protein C yapısı, fizyolojisi ve trombotik hastalıklardaki rolü. *Yeni Tıp Dergisi* 5:66, 1986.
4. Clouse LH, Comp PC. The regulation of hemostasis: the protein C system. *N Engl J Med* **314**:1298, 1986.
5. Mannucci PM, Vigano S. Deficiencies of protein C, an inhibitor of blood coagulation. *Lancet* 2:463, 1982.

6. Esmon CT. Protein-C: biochemistry, physiology, and clinical implications. *Blood* 62:1155, 1983.
7. Nardi M, Karpatkin M. Prothrombin and protein C in early childhood: normal adult levels are not achieved until the fourth year of life. *J Pediatr* 109: 843, 1986.
8. Rosenberg DR. Physiology of coagulation: the fluid phase: In Nathan DG, Oski FA. (eds). *Hematology of Infancy and Childhood* (3rd ed) Vol: 2. Philadelphia: Saunders, 1987, pp 1255-1266.
9. Brakman P, Synder J, Henderson ES, Astrup T. Blood coagulation and fibrinolysis in acute leukaemia. *Br J Haematol* 18:135, 1970.
10. Guarini A, Mussoni L, Gugliotta L, et al. Depressed fibrinolysis in patients with acute leukaemia. *Br J Haematol* 66:327, 1987.
11. Reitz M, Witzke G, Egbring R, Gutjahr P. Coagulation factors and proteinase inhibitors in the plasma of children with acute lymphoblastic leukoses. Behaviour before and during treatment according to Protocol I of the Cooperative Leukaemia Study COALL-80. *Klin Wochenschr* 62:1165, 1984.
12. Sallan ES, Weinstein JH. Childhood Acute leukemia. In Nathan DG, Oski FA (eds). *Hematology of Infancy and Childhood.* (3rd ed) Vol. II Philadelphia: Saunders, 1987, pp 1028-1063.
13. Rodeghiero F, Mannucci PM, Viganò S, et al. Liver dysfunction rather than intravascular coagulation as the main cause of low protein C and antithrombin III in acute leukemia. *Blood* 63:965, 1984.
14. Marlar RA, Endres-Brooks J, Miller C. Serial studies of protein C and its plasma inhibitor in patients with disseminated intravascular coagulation. *Blood* 66:59, 1985.
15. Griffin JH, Evatt B, Zimmerman TS, et al. Deficiency of protein C in congenital thrombotic disease. *J Clin Invest* 68:1370, 1981.
16. Goldschmidt B, Koós R. Metabolism of fibrinogen in children with acute lymphoblastic leukaemia. *Eur J Pediatr* 143:140, 1984.
17. Liebman HA, Wada JK, Patch MJ, McGehee W. Depression of functional and antigenic plasma antithrombin III (AT-III) due to therapy with L-asparaginase. *Cancer* 50:451, 1982.

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THE EFFECT OF EXOGENOUS SURFACTANT REPLACEMENT THERAPY ON LUNG FUNCTION*

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SUMMARY: Yüksel B, Greenough A. (Department of Child Health, King's College Hospital, London, England). The effect of exogenous surfactant replacement therapy on lung function. Turk J Pediatr 35: 53-57, 1993.

Exogenous surfactant replacement therapy, regardless of type, reduces mortality and morbidity. We have reviewed the existing data to determine the effect this form of treatment has on lung function. Several studies have demonstrated that surfactant replacement therapy improves compliance, but the timing and magnitude of the effect is variable. The effect is dependent firstly on the type of surfactant used, natural surfactant being associated with a more rapid effect than artificial surfactant; secondly whether the surfactant is given as rescue or therapy as prophylaxis and thirdly the gestational age of the infant treated. Preliminary evidence also suggests that surfactant replacement therapy may influence lung function in the long term, since infants treated with surfactant rather than a placebo have lower airways resistance and higher specific conductance at follow-up. These preliminary reports are encouraging because they suggest that exogenous surfactant replacement therapy may have the additional benefit of reducing chronic respiratory morbidity associated with premature birth. *Key words: lung function, premature birth, respiratory distress syndrome, surfactant.*

Surfactant deficiency was recognized as the cause of respiratory distress syndrome many years ago[1]. Unfortunately, early studies of surfactant replacement therapy yielded disappointing results[2, 3]. In the early 1980s, however, both a natural[4] and a artificial surfactant[5] were used apparently with good clinical effect. Over the last decade there has been an explosion in the number of surfactants available[4-10]. Their efficacy has been assessed in a large number of trials, which have demonstrated that exogenous surfactant replacement therapy, regardless of type, reduces mortality[11-14] and morbidity[9, 14-16]. We have reviewed the existing data on the effect of surfactant replacement therapy on lung function. Our aims were firstly to determine factors influencing the acute effects of exogenous surfactant on lung function and secondly to assess if this therapy was likely to affect the long term respiratory morbidity frequently associated with premature birth[17].

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Lung function abnormalities in surfactant deficient infants

Surfactant is a surface tension lowering agent which promotes alveolar stability[18] and protects epithelial surfaces[19]. As a consequence, the presence of an adequate surfactant monolayer reduces the work of breathing[19]. In surfactant deficiency, the lungs become atelectatic, with a decrease in lung compliance[19]. Lung function measurements reveal affected infants to have both a low compliance and low functional residual capacity (FRC)[20]. Thus surfactant replacement therapy would be predicted to improve both these lung function abnormalities.

Acute changes in lung function following surfactant replacement therapy

A variety of studies have demonstrated that surfactant replacement therapy is associated with an improvement in compliance, but that the timing and magnitude of the effect is variable[21-24]. Natural surfactant has an immediate effect on lung function, with the improvement in compliance occurring at the same time as the reduction in requirement for respiratory support[22]. These rapid effects may occur as a consequence of natural surfactant being recruited into the existing alveolar monolayer and thus immediately "functional". In contrast, artificial surfactants have a slower onset of action[5, 9, 21, 25]. No significant difference was noted between infants given an artificial lung expanding compound (ALEC) or placebo in the resuscitation period[26] or one hour after treatment[27]. The administration of ALEC was associated with an improvement in lung compliance only six hours after the initial therapy had been given. The improvement in lung function became more significant at 24 hours[21]. Artificial surfactants, rather than being recruited into the monolayer, may instead be taken up into the Type II pneumocytes where they are utilized to produce the infant's own surfactant which subsequently affects lung function. This sequence is likely to explain the apparent delay in effect of the artifical surfactant; as endogenous surfactant is recycled with a "cycle-time"[28] which is similar to the duration of the delay of action of ALEC on lung compliance.

Whether surfactant is given as prophylaxis or as rescue therapy, it also influences its effect on lung function. The administration of ALEC is only associated with an improvement in compliance when given as prophylaxis[21], but not as rescue therapy[29]. The latter trial, however, was small and the possibility of a type II error cannot be ignored. The effect of exogenous surfactant on lung function is also influenced by gestational age. A significant improvement in compliance following ALEC was only noted in infants of less than 30 weeks of gestation, but not in those greater than that level of maturity[9].

More recently, the effect of surfactant on FRC has been assessed. One study demonstrated, in response to human surfactant, an immediate increase in FRC

but, surprisingly, a reduction in respiratory compliance[30]. This combination of changes in lung function was explained by the possible alteration in the shape of the static recoil pressure characteristics of the diseased lungs which could occur as a result of treatment[31]. The FRC of the surfactant-deficient ventilated infant, however, would be predicted to be low. Thus, it is surprising that any single treatment could increase the FRC to such a level that the position on the pressure/volume curve would be so shifted as to be associated with a reduction in compliance.

Longer term effects of surfactant replacement therapy on lung function

Measurements of compliance at 7 and 28 days after treatment have suggested that the effect of surfactant on lung function may be short-lived[24, 32]. No significant differences in compliance were noted at 7 days following administration of ALEC or placebo[21]. Similarly, pulmonary resistance was significantly decreased at 2, 3 and 14 days following surfactant, but there was no difference at 28 days, compared to controls[24]. In contrast, a recent study has found that lung function measured by plethysmography at 7 months of age was better in infants given Exosurf than those administered placebo. The former group had significantly lower airways resistance and higher specific conductance[33]. These apparently conflicting results may be explained by the known effects of surfactant. The lower survival rate of the placebo groups may suggest that the "control" infants with severe lung function abnormalities die during the perinatal period. Thus, this would bias the lung function data obtained immediately outside the perinatal period in favour of the controls. Surfactant replacement therapy also, however, reduces acute respiratory complications, such as air leak, an important determinant of the degree of abnormality of lung function in the first year of life[34]. Thus it is not surprising that Exosurf-treated infants were found to have significantly better lung function at 7 months of age[33]. Abnormal lung function at 6 months of age is a highly specific predictor of chronic respiratory morbidity persisting into the second year of life[34]. Thus, these preliminary reports[33] are extremely encouraging because they suggest that exogenous surfactant replacement therapy may reduce the chronic respiratory morbidity associated with premature birth[17].

REFERENCES

1. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. *Am J Dis Child* 97:517, 1959.
2. Robillard E, Alaire Y, Dagenais-Prerusse P, et al. Microaerosol administration of synthetic beta-gamma-dipalmitoyl-L-alpha-lecithin in the respiratory distress syndrome: a preliminary report. *Can Med Assoc J* 90:55, 1964.
3. Chu J, Clements JA, Cotton EK, et al. Neonatal pulmonary ischemia. Part I: Clinical and physiological studies. *Pediatrics* 40:709, 1967.

4. Fujiwara T, Maeta H, Chida S, et al. Artificial surfactant therapy in hyaline membrane disease. *Lancet* 1:55, 1980.
5. Morley CJ, Bangham AD, Miller N, Davis JA. Dry artificial lung surfactant and its effect on very premature babies. *Lancet* 1:64, 1981.
6. Shapiro DL, Notter RH, Morin FC 3d, et al. Double-blind, randomized trial of a calf lung surfactant extract administered at birth to very premature infants for prevention of respiratory distress syndrome. *Pediatrics* 76:593, 1985.
7. Speer CP, Robertson B, Curstedt T, et al. Randomized European multicentre trial of surfactant replacement therapy for severe neonatal respiratory distress syndrome: single versus multiple doses of Curosurf. *Pediatrics* 89:13, 1992.
8. Hallman M, Merritt TA, Schneider H, et al. Isolation of human surfactant from amniotic fluid and a pilot study of its efficacy in respiratory distress syndrome. *Pediatrics* 71:473, 1983.
9. Morley CJ, Greenough A, Miller NG, et al. Randomized trial of artificial surfactant (ALEC) given at birth to babies from 23 to 34 weeks gestation. *Early Hum Dev* 17:41, 1988.
10. Stevenson D, Walther F, Long W, et al. The American Exosurf Neonatal Study Group I. *J Pediatr* 120:53, 1992.
11. Ten Centre Study Group Ten centre trial of artificial surfactant (artificial lung expanding compound) in very premature babies. *Br Med J* 294:961, 1987.
12. Soll RF, Hoekstra RE, Fangman JJ, et al. Multicenter trial of single-dose modified bovine surfactant extract (Survanta) for prevention of respiratory distress syndrome. Ross Collaborative Surfactant Prevention Study Group. *Pediatrics* 85:1092, 1990.
13. Dunn MS, Shennan AT, Hoskins EM, et al. Two-year follow-up of infants enrolled in a randomized trial of surfactant replacement therapy for prevention of neonatal respiratory distress syndrome. *Pediatrics* 82:543, 1988.
14. Collaborative European Multicentre Study Group Surfactant replacement therapy for severe neonatal respiratory distress syndrome: An international randomized clinical trial. *Pediatrics* 82:683, 1988.
15. Merritt TA, Hallman M, Bloom BT, et al. Prophylactic treatment of very premature infants with human surfactant. *N Engl J Med* 315:785, 1986.
16. Konishi M, Fujiwara T, Naito T, et al. Surfactant replacement therapy in neonatal respiratory distress syndrome. A multi-centre, randomized clinical trial: comparison of high-versus low-dose of surfactant TA. *Eur J Pediatr* 147:20, 1988.
17. Greenough A, Maconochie I, Yüksel B. Recurrent respiratory symptoms in the first year of life following pretern delivery. *J Perinat Med* 18:489, 1990.
18. Jobe A, Ikegami M. Surfactant for the treatment of respiratory distress syndrome. *Am Rev Respir Dis* 136:1256, 1987.
19. Van Golde LM, Batenburg JJ, Robertson B. The pulmonary surfactant system: biochemical aspects and functional significance. *Physiol Rev* 68:374, 1988.
20. Hjalmarson O. Mechanics of breathing in respiratory distress syndrome. In Raivio KO, Hallman M, Kouvalainen K, Volimoki I (eds). *Respiratory Distress Syndrome.* London: Academic Press, 1984, pp 171-185.
21. Morley CJ, Greenough A. Respiratory compliance in premature babies treated with artificial surfactant (ALEC) *Arch Dis Child* 66:467, 1991.
22. Davis JM, Veness-Meehan K, Notter RH, et al. Changes in pulmonary mechanics after the administration of surfactant to infants with respiratory distress syndrome. *N Engl J Med* 319:476, 1988.
23. Couser RJ, Ferrara TB, Ebert J, et al. Effects of exogenous surfactant therapy on dynamic compliance during mechanical breathing in preterm infants with hyaline membrane disease. *J Pediatr* 116:119, 1990.

24. Bhutani V, Abbasi S, Long WA, Gerdes JS. Pulmonary mechanics and energetics in preterm infants who had respiratory distress syndrome treated with synthetic surfactant. *J Pediatr* 120:S18, 1992.

25. Morley CJ. On behalf of the trial collaborators. Ten centre trial of artificial surfactant (ALEC) in very premature babies. *Br Med J* 294:991, 1987.

26. Milner AD, Vyas H, Hopkin IE. Effect of exogenous surfactant on total respiratory system compliance. *Arch Dis Child* 59:369, 1984.

27. Milner AD, Vyas H, Hopkin IE. Effects of artificial surfactant on lung function and blood gases in idiopathic respiratory distress syndrome. *Arch Dis Child* 58:458. 1983.

28. Jacobs H, Jobe A, Ikegami M, Jones S. Surfactant phosphatidylcholine source, fluxes, and turnover times in 3-day-old, 10-day-old, and adult rabbits. *J Biol Chem* 257:1805, 1982.

29. Greenough A, Morley CJ, Wood S, et al. Effect of surfactant on lung function in preterm neonates. *Second International Surfactant Symposium.* Basel: Karger, 1984.

30. Edberg KE, Ekström-Jodal B, Hallman M, et al. Immediate effects on lung function of instilled human surfactant in mechanically ventilated newborn infants with IRDS. *Acta Paediatr Scand* 79:750, 1990.

31. Goldsmith LS, Greenspan JS, Rubenstein SD, et al. Immediate improvement in lung volume after exogenous surfactant: alveolar recruitment versus increase distention. *J Pediatr* 119:424, 1991.

32. Goldman SL, Bosque E, McCann E, Lewis K. Pulmonary mechanics in premature infants one month after treatment with synthetic surfactant. *J Pediatr* 120:S25, 1992.

33. Yüksel B, Greenough A, Gamsu HR. Respiratory function at follow-up after neonatal surfactant replacement therapy. *Early Hum Dev* 1992, (in press).

34. Yüksel B, Greenough A. Persistence of respiratory symptoms into the second year of life: predictive factors in infants born preterm. *Acta Paediatr Scand* 1992, (in press).

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FAMILIAL SICK SINUS SYNDROME IN TWO SIBLINGS*

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SUMMARY: Çeliker A, Oto A, Özme Ş. (Cardiology Units, Departments of Pediatrics and Internal Medicine, Hacettepe University Faculty of Medicine, Ankara, Turkey). Familial sick sinus syndrome in two siblings. Turk J Pediatr 35: 59-64, 1993.

Two siblings with sinus node disease are presented. The patients were severely affected and required permanent pacing. A cardiac electrophysiologic study was conducted in Case 2, which revealed an atrioventricular conduction disturbance in addition to sinus node dysfunction. The parents and other siblings showed no evidence of sinus node disorder. The occurrence of this disease in these siblings suggests an autosomal dominant mode of inheritance with variable penetrance. *Key words: sick sinus syndrome, familial.*

A disturbance in sinus node function is a common cause of symptomatic arrhythmias and may result from failure of sinus automaticity, or the inability to propagate impulses from the sinus node to the atrium, or a combination of both[1-4]. This type of arrhythmia rarely occurs in childhood, but is frequently associated with extensive atrial operations[5]. There are many other etiological or associated conditions. The cause of sinus node dysfunction may be classified as intrinsic or extrinsic[6-11].

In view of the multifactorial etiology of sinus node dysfunction, it is not surprising that no single underlying histological abnormality has been identified, but rather a variety of findings has been reported[1-4, 12, 13]. Among these factors, familial occurrence is rarely involved[6-11]. In this report two siblings with sinus node dysfunction are presented.

Case Reports

Case 1

A three-year-old girl was apparently well until the age of two, when she developed syncopal attacks. Physical examination revealed bradycardia and a systolic murmur which was believed to be associated with ventricular septal defect. The

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following year, she had frequent syncopal episodes and a routine examination was unremarkable except for a heart rate of 80/min, with frequent irregularity. The ECG showed basically a junctional rhythm (Fig. 1). The telecardiogram revealed slight cardiomegaly. The M-mode echocardiogram was normal. She was hospitalized at the age of three because of syncopal attacks. Exercise and the administration of atropine and orciprenaline did not significantly increase the heart rate, which confirmed the diagnosis of sinus node dysfunction. It was then decided that a permanent pacemaker be inserted in the patient. An epicardial demand pacemaker was used and continuous pacing was achieved at a rate of 100/min. Following insertion of the demand pacemaker she had no syncopal episodes. The last telecardiogram taken was normal and her last ECG showed paced rhythm with sinus node dysfunction.

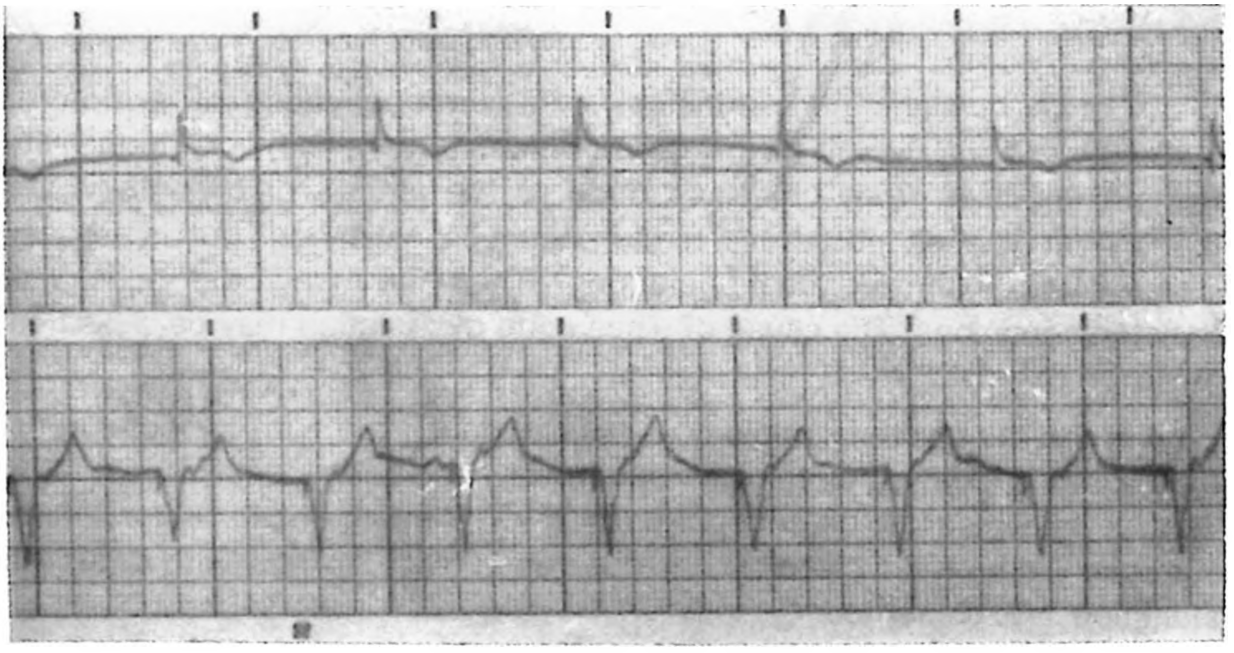

Fig. 1: Top panel shows junctional rhythm and absent P waves. The bottom panel demonstrates pacemaker rhythm.

Case 2

An 11-year-old boy was admitted to the hospital with dizziness. He had been apparently well until five months prior to admission. A routine examination revealed bradycardia, and a systolic murmur was heard in the fourth left intercostal space. The ECG revealed sinus bradycardia with frequent sinus arrest and an infranodal rhythm at a rate of 42/min (Fig. 2). On some strips a normal sinus rhythm was observed. Exercise did not noticeably increase the heart rate. The two-dimensional echocardiogram revealed left ventricular dysfunction with a normal cardiac anatomy. The telecardiogram showed an enlarged heart. Before the permanent pacemaker was implanted, a cardiac electrophysiologic study was

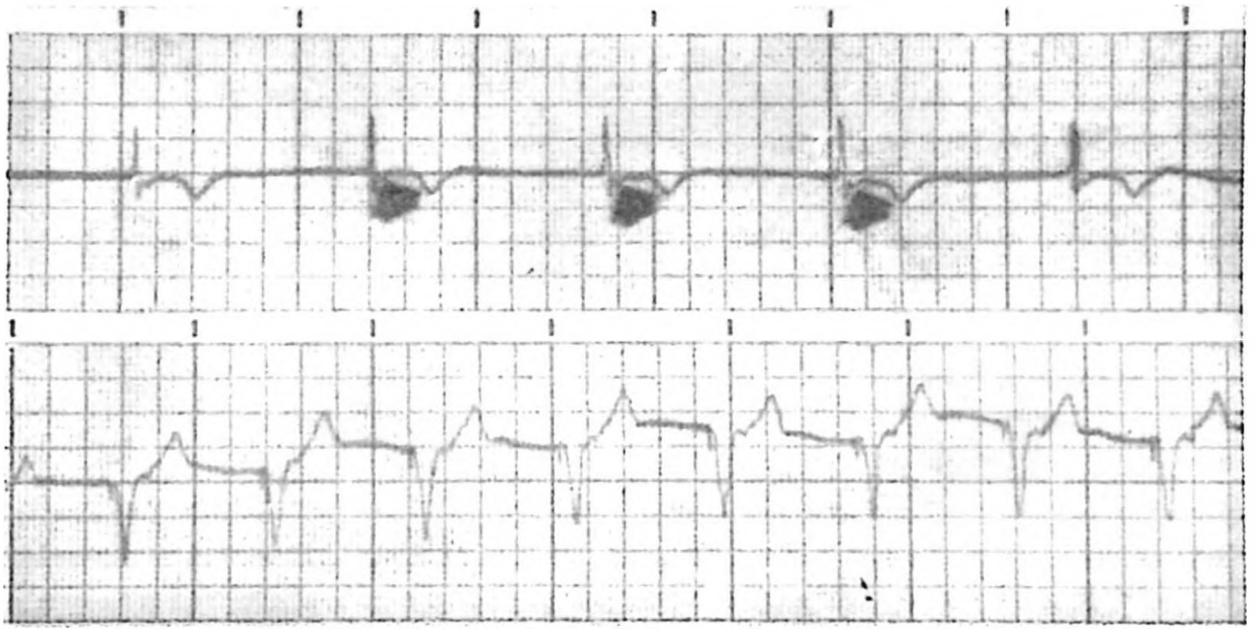

Fig. 2: A low junctional rhythm is noted on the first ECG. Retrograde P waves follow the QRS complex (black arrow). Second panel shows a pacemaker rhythm which was achieved with a VVIR pacemaker.

performed to evaluate sinus node and conduction functions. The study revealed the presence of an abnormal functioning sinus node associated with abnormal A-V conduction time (Fig. 3). A transvenous ventricular rate responsive pacemaker (Telectronics Meta MV) was implanted and adjusted after exercise testing to optimize the child's life style. He showed no signs of dizziness and was discharged on the 20th day of hospitalization.

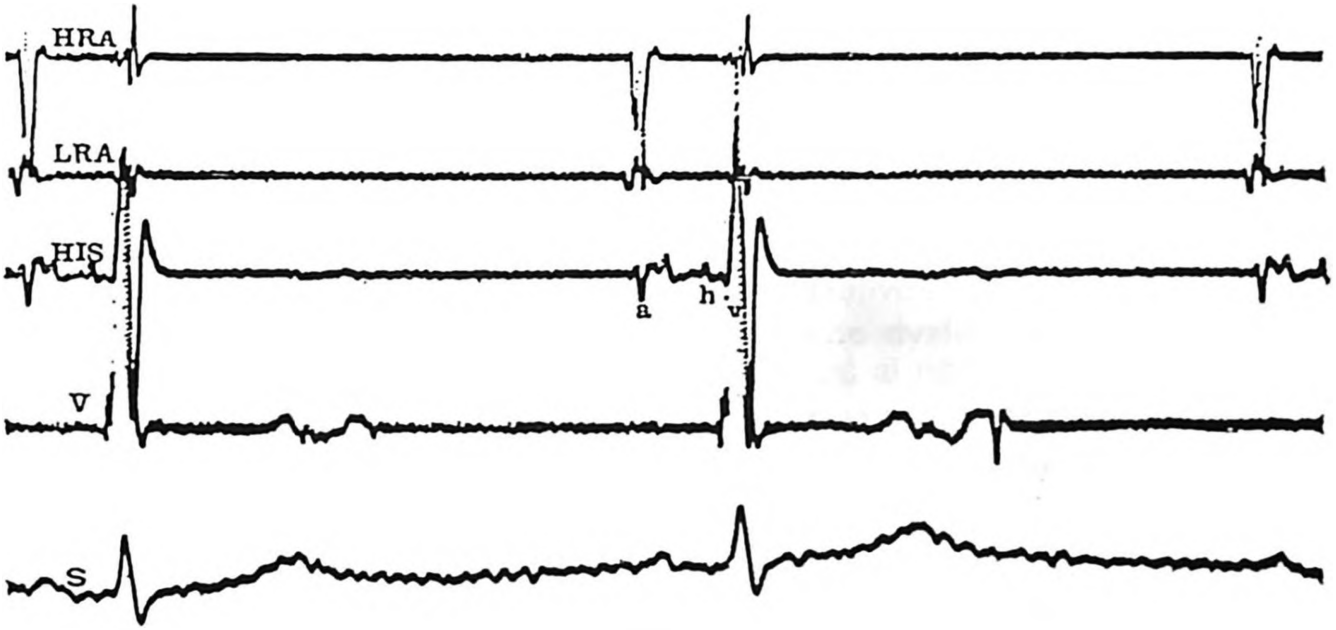

Fig. 3 a:

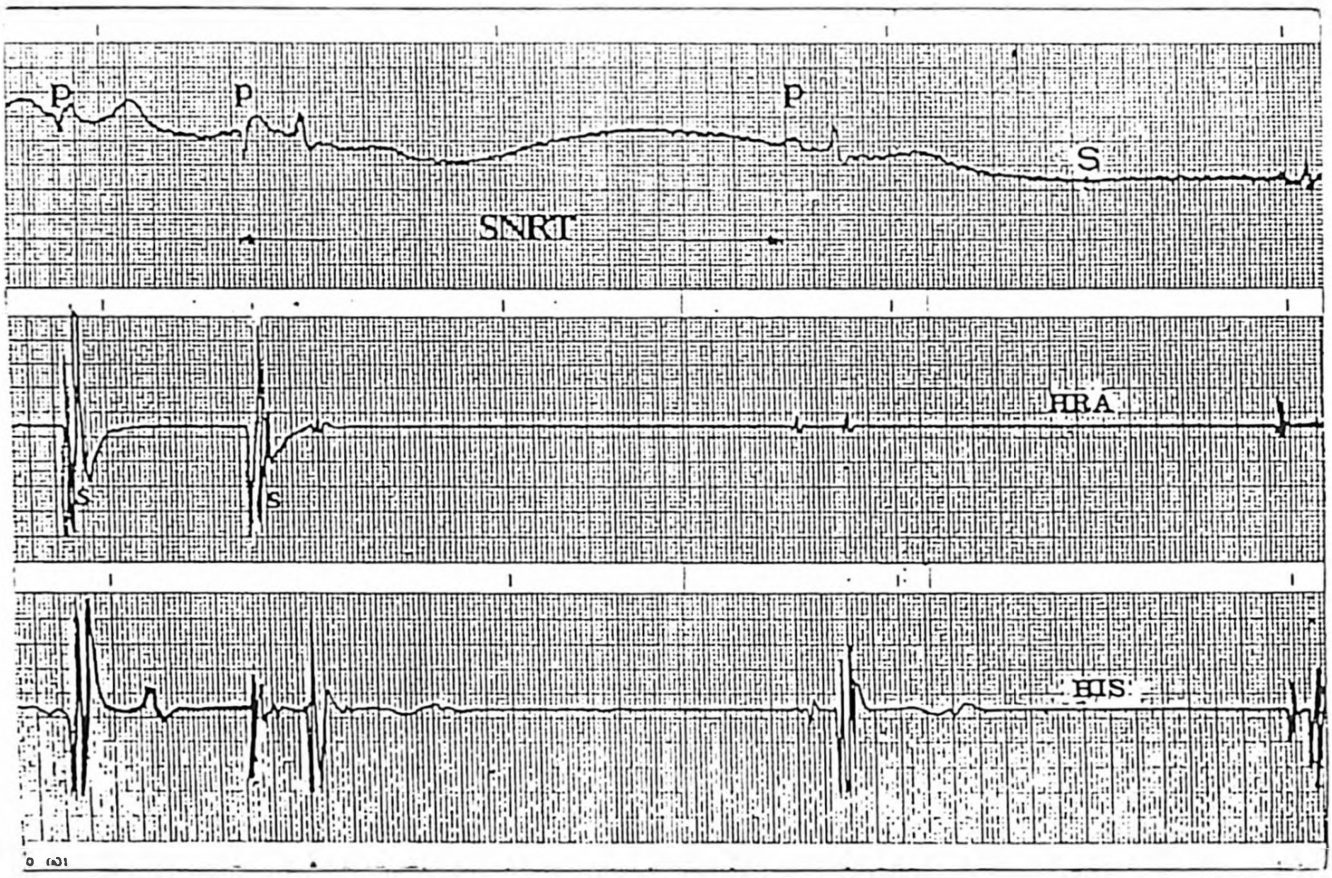

Fig. 3 b:

Fig. 3: Cardiac electrophysiologic study.
a) His bundle recordings revealed abnormally prolonged AH interval (AH = 130 msec).
b) SNRT, which was measured from the surface ECG, was prolonged (SNRT = 2200 msec, CSNRT = 1040 msec).
HRA : high right atrium, LRA : low right atrium, V : right ventricular apex, S : surface.

The other four siblings in the family are healthy. ECGs and exercise tests were normal in these sibs. The parents also evidenced no cardiovascular symptoms.

Discussion

Sinus node dysfunction is an uncommon rhythm disorder in childhood[2, 3]. The disorder occurs sporadically, although it may be frequently noted after cardiac surgery, especially due to the changing atrial anatomy[5].

Since atrial redirection operations have been performed more frequently in recent years, sinus node dysfunction is a relatively rare occurrence in our postoperative care unit. We were able to demonstrate sick sinus syndrome in two siblings, which might indicate a familial occurrence of this disorder.

Dysfunction of the sinoatrial node may manifest itself in several patterns[1, 2]. Etiologies commonly described in this syndrome include cardiomyopathies and surgical injury to the sinoatrial node[5-11]. Some authors have described familial forms of this syndrome[6-11], which may be associated with the fibrosis of nodal or conduction tissue or myocardial disease[6-11]. Allensworth et al[6] found persistent atrial standstill in three siblings and a right atrial biopsy showed interstitial and perivascular deposits of amyloid in one. Although the fibrous degeneration of the sinus node is the main pathologic process in the nonfamilial form of this syndrome, in some patients an associated anomaly of the AV node is also detected[4, 12-13]. These diverse findings may be explained by the varying spectrum of the disease. However there are only a few detailed pathologic studies that discribe the familial form of this syndrome and which suggest that a degenerative process is involved in its etiology[6, 8]. Since we did not perform any pathologic examinations in our patients, we cannot offer any explanation about the etiology of this syndrome. However, we can speculate that fibrous degeneration may be the etiologic mechanism implicated, since pacemaker therapy was successful[9].

It is suggested that the familial sinus node disorder is inherited in a pattern corresponding to an autosomal dominant trait with variable penetrance[6-11]. We are also in accord with this hypothesis because the patients we studied were two siblings.

Since node dysfunction is frequently associated with AV conduction disturbances[1-4]. This concomitat abnormality remains undetected and the surface ECG is within normal limits[1-4]. On the other hand, cardiac electrophysiologic parameters of sinus node dysfunction are not pathologic in certain patients with symptomatic bradycardia which is suggestive of sick sinus syndrome[1-3]. Thus it is recommended that sinus node and AV conduction functions be investigated in these patients so that a precise diagnosis can be established, one allowing for the proper choice of a pacemaker[1-3, 5]. Therefore, we conducted a complete electrophysiologic study in Case No. 2, and we detected abnormal AV conduction in addition to sinus node dysfunction.

In conclusion, given the hereditary character of this condition and the high incidence of conduction disturbances in healthy relatives, it seems advisable to evaluate the family of any patient with sinus node dysfunction, especially of childhood onset.

REFERENCES

1. Rankin AC, Rae AP. Sinus and Atrial Arrhythmias. In Macfarlane PW, Lawrie TD, (eds). *Comprehensive Electrocardiology. Theory and Practice in Health and Disease.* (1st ed) Vol. II. New York: Pergamon Press, 1989, pp 881-914.
2. Beder SD, Gillette PC, Garson A, et al. Symptomatic sick sinus syndrome in children and adolescents as the only manifestation of cardiac abnormality or associated with unoperated congenital heart disease. *Am J Cardiol* 51:1133, 1983.
3. Yabek SM. Evaluation of sinus node automaticity and sinoatrial conduction in children with normal and abnormal sinus node function *Clin Cardiol* 1:136, 1978.
4. Bolognesi R, Benedini G, Affatato A, et al. Electrophysiological evaluation of the sinus node in patients with atrioventricular and/or intraventricular conduction defects. *J Electrocardiol* 22:297, 1989.
5. Wolf GS, Tamer D, Arrhythmias following cardiac surgery. In Roberts NK, Gelband H (eds). *Cardiac Arrhythmias in the Neonate, Infant and Child* (2nd ed). Norwalk: Appleton-Century-Crofts, 1983, pp 253-296.
6. Allensworth DC, Rice GJ, Lowe GW. Persistent atrial standstill in a family with myocardial disease. *Am J Med* 47:775, 1969.
7. Spellberg RD. Familial sinus node disease. *Chest* 60:246, 1971.
8. Nordenberg A, Varghese PJ, Nugent EW. Spectrum of sinus node dysfunction in two siblings. *Am Heart J* 91:507, 1976.
9. Ward DE, HO SY, Shinebourne EA. Familial atrial standstill and inexcitability in childhood. *Am J Cardiology* 53:965, 1984.
10. Lorber A, Maisuls E, Palant A. Autosomal dominant inheritance of sinus node disease. *Int J Cardiol* 15:252, 1987.
11. Barak M, Herschkowitz S, Shapiro I, Roguin N. Familial combined sinus node and atrioventricular conduction dysfunctions. *Int J Cardiol* 15:231, 1987.
12. Thery C, Gosselin B, Lekieffre J, Warembourg H. Pathology of sinoatrial node. Correlations with electrocardiographic findings in 111 patients. *Am Heart J* 93:735, 1977.
13. Evans R, Shaw DB. Pathological studies in sinoatrial disorder (sick sinus syndrome). *Br Heart J* 39:778, 1977.

The Turkish Journal of Pediatrics 35: 65-67, 1993

CARDIAC ARREST: AN UNUSUAL SIDE-EFFECT OF INTRAVENOUS ORNIDAZOLE*

*Hasan Özen MD***, Gülten Seçmeer MD***, Güler Kanra MD***
*Mehmet Ceyhan MD****, Nilgün Kurucu MD*****

SUMMARY: Özen H, Seçmeer G, Kanra G, Ceyhan M, Kurucu N. (Department of Pediatrics, Hacettepe University Institute of Child Health, Ankara, Turkey). Cardiac arrest: an unusual side-effect of intravenous ornidazole. Turk J Pediatr 35: 65-67, 1993.

Nitroimidazoles have been used alone or in combination with other antibacterial agents in the treatment of brain abscess. They are associated with certain adverse reactions related primarily to the gastrointestinal tract and central nervous system. But, as far as we know, cardiac arrest due to ornidazole has not been reported in the literature.

In this study, we presented a case in which cardiac arrest occurred as an unusual side-effect of intravenous ornidazole administered to a ten-year-old girl suffering from brain abscess. *Key words: ornidazole, cardiac arrest.*

Anaerobic microorganisms such as Bacteroides, Fusobacteriums and Gram (+) cocci are the agents responsible for brain abscess in 85-88 percent of cases. In patients with congenital cyanotic heart disease, the incidence of brain abscess is 2-6 percent, and infections caused by multiple microorganisms are frequent[1]. In addition, microaerophilic and anaerobic streptococci can cause up to 45 percent of brain abscesses.

In the treatment of brain abscess, 5-nitroimidazoles have been used alone or in combination with other antibacterial agents because of their bactericidal activity and perfect penetration into cerebrospinal fluid. The nitroimidazoles have some adverse reactions related primarily to the gastrointestinal tract and central nervous system. In this study, we report an unusual adverse reaction to ornidazole, that to our knowledge, has not been documented in the literature.

Case Report

A ten year-old girl was brought to the hospital for evaluation of vomiting and headache of ten days' duration. Her past history revealed that she had been under

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medical supervision for a condition that had been diagnosed as congenital heart disease (dextrocardia and double outlet right ventricle of the transposition type) when she was two and a half years old.

Physical examination revealed bilateral grade 2 papilledema, left-sided facial paresis of the central type and left hemiparesis. The cranial computerized axial tomography revealed a 5 × 5 cm abscess located in the right frontal lobe. The patient underwent surgery immediately, and the abscess was aspirated through a burr hole.

Treatment consisted of benzyl penicillin G combined with chloramphenicol and ornidazole (Biteral®, Roche) 20 mg/kg/day in three divided doses (i.v. infusion in 50 cc fluid in 30 minutes or longer). No other medication was given to the patient. Alpha-hemolytic streptococci, beta-hemolytic streptococci, *N. meningitidis* and anaerobic Gram (+) rods (unidentified) were cultured from the surgical specimen. The determination of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, calcium, phosphate, blood urea nitrogen, uric acid and creatinine concentrations were found to be within normal limits.

On the 13th day of therapy, the patient's body temperature rose to 39 °C, and dyspnea and increased cyanosis were noted two hours after the administration of benzyl penicillin G and ornidazole. Since we thought this was an adverse reaction to benzyl penicillin G, it was discontinued. Although all the symptoms promptly disappeared six hours after the first reaction, identical signs and symptoms were observed during the infusion of ornidazole, and in addition, cardiac arrest occurred. Chloramphenicol was not administered. Cardiac rhythm returned to normal within 30 seconds as a result of cardiac massage and the establishment of adequate ventilation with ambu. The blood pressure was 110 mm Hg systolic and 80 mm Hg diastolic. Flushing, urticaria or other signs of allergic reactions were not observed. Ornidazole therapy was discontinued.

Discussion

The nitroimidazoles, particularly metronidazole, tinidazole and ornidazole are used to treat many serious infections caused by Gram (+) and Gram (−) anaerobic bacteria and protozoa *(T.vaginalis, E.histolytica, G.lamblia).* It is also given prophylactically. However, some well-known dose-dependent adverse reactions, which disappear shortly after treatment is discontinued, have been associated with the use of these drugs[2-6].

Many reports document the adverse effects of metronidazole and tinidazole, while there are very few concerning the side-effects of ornidazole. In a report from Australia, thirty-six suspected reactions to tinidazole were presented and ten of them were considered to be due to hypersensitivity reactions. Reported manifestations include generalized urticaria, facial, periorbital or laryngeal edema,

hypotension, bronchospasm and dyspnea[4]. In our patient, only fever, irregular respiration, increased cyanosis and dyspnea were noted. There were no dermatologic signs.

In a study from Norway, high-dose tinidazole administered intravenously to one patient caused unconsiousness lasting 10 seconds followed by a spasm in one arm. In addition a drop in systolic blood pressure was observed in this patient, however, the total IgE level was normal. This reaction was not considered a type I allergy, but a direct toxic reaction to the drug[5]. Furthermore, tachycardia and bradycardia were observed in four of five cases receiving Tiberal® (ornidazole) tablets. In one of these cases, there was persistent atrial flutter and extrasystoles, but because these patients were taking multiple medication, the exact cause was not specified and, therefore, not reported (Roche, personal communication, 24 April 1989).

After a search of the literature, we discovered that fever was a rare reaction to this drug, and that no case had been reported in wihch cardiac arrest had occurred. The cardiac arrest that occurred in our patient is considered by us to be a direct toxic effect of the drug.

REFERENCES

1. Patrick CC, Kaplan SL. Current concepts in the pathogenesis and management of brain abscesses in children. *Pediatr Clin North Am* 35: 625, 1988.
2. Webster LT Jr. Drugs used in the chemotherapy of protozoal infections. In Gilman AG, Goodman LS, Rall TW, Murad F (eds.). *The Pharmacological Basis of Therapeutics* (8th ed). New York: Pergamon, 1990, pp 1008-1021.
3. Scully BE. Metronidazole. *Med Clin North America* 72: 613, 1988.
4. McEwen J. Hypersensitivity reactions to tinidazole (Fasigyn). *Med J Australia* 1: 498, 1983.
5. Aase S, Olsen AK, Roland M, et al. Severe toxic reaction to tinidazole. *Eur J Clin Pharmacol* 24: 425, 1983.
6. Roe FJ. Safety of nitroimidazoles. *Scand J Infect Dis* (Suppl) 46: 72, 1985.

The Turkish Journal of Pediatrics 35: 69-73, 1993

THE ROLE OF HIGH-DOSE INTRAVENOUS METHYLPREDNISOLONE IN THE TREATMENT OF VARICELLA ASSOCIATED THROMBOCYTOPENIC PURPURA*

*Dolly Yafet Aji MD***, Nüvit Altınkaya MD***, Ayşe Güler MD****
*Yusuf İzzet Ayhan MD****, Ayten Erginel MD*****, Asım Cenani MD*****

SUMMARY: Aji DY, Altınkaya N, Güler A, Ayhan YI, Erginel A, Cenani A. (Department of Pediatrics, İstanbul University Faculty of Medicine at Cerrahpaşa, İstanbul, Turkey). The role of high-dose intravenous methylprednisolone in the treatment of varicella associated thrombocytopenic purpura. Turk J Pediatr 35: 69-73, 1993.

Two children with varicella associated thrombocytopenia are presented. Case 1, evidencing no mucosal or parenchymal bleeding was administered prednisone 2 mg/kg/day therapy after having been put under observation for a period of one week without medical treatment. There was an increase in the platelet count during the period of therapy culminating in a return to normal on the eighth day.

Case 2, who was in poor clinical condition at presentation, was treated with high-dose intravenous methylprednisolone (HIVMP) 30 mg/kg/day, resulting in the platelet count returning to a normal level (180,000/mm^3) on the second day of therapy.

HIVMP therapy is recommended in the treatment of life-threatening cases of thrombocytopenia because of its rapid action, limited side-effects, and its low cost.
Key words: thrombocytopenia, purpura, varicella, platelets, methylprednisolone.

Varicella is generally characterized by a mild viral infection with infrequent complications. Even though varicella associated thrombocytopenia is a rare entity, almost half of the cases of thrombocytopenia seen in combination with infections occur because of varicella[1]. Thrombocytopenia develops either when the rash appears or a few weeks later. However, it has also been reported during the incubation period. It has a sudden onset, with the platelet count usually falling below 20,000/mm^3. Spontaneous recovery usually occurs within a few weeks to a few months[2]. The clinical picture varies between mild transitory purpura and a fatal fulminant hemorrhage. The most dangerous complications are intracranial and intraparenchymal bleeding.

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There are different approaches regarding the treatment of this disease. This report describes two patients with varicella associated thrombocytopenia whose treatment protocols differed.

Case Reports

Case 1

A 4-year-7-month old girl was admitted with a three-day history of bruising on her body. She contracted varicella 12 days prior to admission. Physical examination revealed petechiae and ecchymoses scattered over the trunk and extremities. Crusts due to varicella were present. There was no evidence of mucosal bleeding, hepatosplenomegaly or lymphadenopathy. A complete blood count was normal except for a platelet count of 10,000/mm^3. Results of urinalysis were within normal limits. Bone marrow examination revealed hypercellularity with an increased number of megakaryocytes and no thrombocyte production. Platelet antibodies could not be documented because of technical reasons.

The patient was put under observation for one week without being given any therapy, but no increase in the platelet count (10,000/mm^3) was noted. Then prednisone therapy in a dose of 2 mg/kg/day was initiated. The platelet count rose to 40,000/mm^3 by the fourth day, and it reached 160,000/mm^3 by the eighth day. After two weeks of therapy, the dosage of prednisone was tapered off and then discontinued. Recovery was uneventful and the platelet count was found to be normal at subsequent follow-ups.

Case 2

An 8-year-old girl was admitted for evaluation of lethargy, fever, cough, easy bruising and bloody urine of three days' duration. Physical examination revealed a stuporous girl with ecchymoses and petechiae scattered all over her body.

Varicella crusts were present. There was a hematoma on each eyelid and nasal and oral bleeding were present. The urine was dark red in color. The heart rate was 100/min, blood pressure 100/60 mmHg, respiration rate 32/min and body temperature 38 °C. Crepitations were heard over the right lung. Hepatosplenomegaly and lymphadenopathy were absent.

Initial laboratory studies revealed a hemoglobin level of 10.9 g/dl, hematocrit 35%, While Blood Cell count 17,400/mm^3 with a predominance of neutrophils. The platelet count was 20,000/mm^3. Urinalysis revealed trace protein, numerous red cells, a few white cells and no casts. Chest x-ray revealed lobar pneumonia of the right lung. A bone marrow specimen showed abundant megakaryocytes with no thrombocyte production. Platelet antibodies could not be demonstrated because of technical reasons.

A few hours after admission the patient's condition worsened, and there was a deterioration in consciousness. Stiff neck with increased deep tendon reflexes and hyperpnea developed, clinically suggesting subarachnoidal hemorrhage. Lumbal puncture could not be accomplished because of bleeding at the puncture sites. Computed tomography could not be performed because of technical and economic reasons.

The patient was administered high-dose intravenous methylprednisolone (HIVMP) therapy in the following doses: 30 mg/kg/day for three days, followed by 20 mg/kg/day for four days. During the following four-week period decreasing doses of methylprednisolone of 10 mg/kg/day, 5 mg/kg/day, 2 mg/kg/day and 1 mg/kg/day were administered each for one week, respectively. The patient also received penicillin and gentamicin to treat her pneumonia. Although the occurrence of viral pneumonia is always a possible complication in varicella, the patient's fever and leukocytosis with a predominance of neutrophils, suggested a bacterial origin of the disease. The platelet count rose to 180,000/mm^3, on the second day of therapy and her state of consciousness improved. The ecchymoses gradually faded and the hematuria disappeared. During the course of therapy, the platelet count remained above 150,000/mm^3 and only once, when the dose was reduced to 5 mg/kg/day, did it fall to 50,000/mm^3, but rapidly returned to normal levels. The pneumonia was no longer present. Side-effects encountered during therapy were weight gain of two kilograms and slight blood pressure elevations which responded well to therapy. There was no fall in the platelet count after the cessation of therapy.

Discussion

Different methods of therapy are suggested when treating infection associated thrombocytopenia such as clinical observation along with supportive therapy, oral prednisone therapy (2 mg/kg/day), high-dose intravenous methylprednisolone therapy, intravenous gamma globulin therapy and splenectomy. The platelet count generally returns to normal levels within two weeks even without therapy[3]. Intravenous gamma globulin causes a rapid increase in the platelet count[4]. This treatment which is costly in Turkey, is associated with such side-effects as hemolysis and ABO alloimmunization. Platelet transfusion has no beneficial effect because transfused platelets are rapidly destroyed by antibodies[1]. The use of steroids in treating varicella infection is controversial because it can lead to a spread of infection, increase in mortality, hemorrhagic rash, and a deterioration of the microscopic pathology of uncomplicated varicella[5]. On the other hand, corticosteroids increase the platelet count by causing immunosuppression, decreasing platelet antibody production, causing a hemostatic effect on the vessel wall and inhibiting phagocytosis in the accessory cell system[6,7]. Steroids have been used in varicella associated thrombocytopenia. They can only have an

effect on immune-mediated thrombocytopenia, but do not influence direct thrombocyte-virus interaction[2,7].

Recent reports in the literature indicate that the platelet count returns to normal within three days in patients with idiopathic thrombocytopenic purpura who are treated with high-dose intravenous methylprednisolone (HIVMP)[8,9]. Since this treatment is relatively less costly than gamma globulin therapy, and yields comparably good results, it is emphasized that it be the treatment of choice in the management of patients with idiopathic thrombocytopenic purpura. It is also stressed that clinical observation without medical therapy have particularly no difference in their effect on increasing the platelet count[8,9]. We had to resort to the use of corticosteroids because the first patient had varicella for a period of 12 days, while the second patient was suffering from the illness for 15 days. Case 1 was in good clinical condition at presentation. She had no mucosal bleeding, and therefore, was put under observation without any medical therapy for one week. However, no increase in platelet count was noted. It was then decided that prednisone 2 mg/kg/day be administered and her platelet count returned to normal within a period of eight days of therapy.

We previously administered HIVMP to a patient with thrombocytopenia caused by an innoculation with the Measles-Mumps-Rubella vaccine who made a rapid recovery after treatment[10].

On the other hand, Case 2 was in poor clinical condition at presentation. She had impaired consciousness and active mucosal hemorrhaging. We therefore, decided to treat her with HIVMP and we observed with satisfaction that the platelet count rapidly increased.

After our experience with these two cases, we are of the opinion that when a life-threatening hemorrhage is present in patients afflicted with thrombocytopenia, HIVMP therapy is the treatment of choice. Moreover, this therapy has the advantage of being relatively inexpensive, gives prompt results, and is associated with few side-effects.

REFERENCES

1. Feusner JH, Slichter SJ, Harker LA. Mechanisms of thrombocytopenia in varicella. *Am J Hematol* 7: 255, 1979.
2. Yeager AM, Zinkham WH. Varicella-associated thrombocytopenia: clues to the etiology of childhood idiopathic thrombocytopenic purpura. *Johns Hopkins Med J* 146: 270, 1980.
3. Ware R, Kurtzberg J, Friedman HS, et al. Chronic immune-mediated thrombocytopenia after varicella infection. *J Pediatr* 112: 742, 1988.
4. Lauro WJ, Goldsmith PM, Frenkel LD, et al. Intravenous gammaglobulin treatment of profound herpes varicella zoster associated thrombocytopenia. *Ann Allergy* 56: 240, 1986.
5. Finkel KC. Mortality from varicella in children receiving adrenocorticosteroids and adrenocorticotropin. *Pediatrics* 28: 436, 1961.

6. Beham ES, Taft LI. Idiopathic thrombocytopenic purpura in children: results of steroid therapy and splenectomy. *Aust Paediatr J* 8: 311, 1972.
7. Dixon RH, Rosse WF. Platelet antibody in autoimmune thrombocytopenia. *Br J Haematol* 31: 129, 1975.
8. Özsoylu Ş. High-dose intravenous methylprednisolone for the treatment of childhood chronic idiopathic thrombocytopenic purpura. *Pediatr Rev Commun* 2: 29, 1987.
9. Özsoylu Ş. Elevation of PAIgG on intact and lysed platelets in the case unresponsive to intravenous gammaglobulin. *Scand J Haematol* 37: 456, 1986.
10. Altınkaya N, Aji DY, Güler A, et al. Kızamık, kızamıkçık, kabakulak aşısından sonra trombositopenik purpura. *Cerrahpaşa Tıp Fakültesi Dergisi* 21: 441, 1990.

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