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# PRENATAL DIAGNOSIS OF SICKLE CELL ANEMIA USING PCR AND RESTRICTION ENZYME Dde I*

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**SUMMARY: Gürgey A, Beksaç S, Mesci L, Çakar N, Karakaş Ü, Kutlar A, Altay Ç. (Departments of Pediatrics, Obstetrics and Gynecology, and Histology, Hacettepe University Faculty of Medicine, the Faculty of Engineering, Hacettepe University, Ankara and the Protein Chemistry Laboratory Medical College of Georgia, Atlanta, Georgia, USA). Prenatal diagnosis of sickle cell anemia using PCR and restriction enzyme Dde I. Turk J Pediatr 35: 159-162, 1993.**

**Prenatal diagnosis of sickle cell anemia was carried out in four fetuses using DNA technology. Fetal chorionic villus specimen were obtained at the 10th week of pregnancy from women at risk of giving birth to children with sickle cell anemia. Whole cellular DNA was obtained and the part of the DNA presumed to have a mutation increased after PCR was performed. After the application of Dde I restriction enzyme, mini gel electrophoresis was performed. The study of the electrophoretic patterns of the DNA indicated that one of the four fetuses was unaffected, one was a carrier and the remaining two were affected.** *Key words: prenatal diagnosis, sickle cell anemia, polymerase chain reaction.*

Sickle cell anemia is prevalent in Africa and in blacks of African origin. It is also seen in some Mediterranean countries[1]. In some regions of Turkey, the prevalence of sickle cell anemia is reported to be around 15 percent[2].

There is high mortality and morbidity associated with this disease. At present there is no known cure for sickle cell anemia. Eradication of this disease can only be accomplished by genetic counselling and prenatal diagnosis. Prenatal

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diagnosis of sickle anemia began in the early seventies by the use of fetoscopy. In vitro globin synthesis analysis was replaced by DNA analysis in the beginning of the eigthies[3-5].

Recent developments in DNA technology has made possible the use of this technique in small laboratories, which is a safe, sensitive, reliable and reproducible method [6]. Below is a report of the preliminary experiences we had in our center in diagnosing sickle cell anemia in which the polymerase chain reaction (PCR) and restriction enzyme Dde I methods were employed.

## Materials and Medhods

Chorionic villus biopsies were obtained from four women at the 10$^{th}$ week of pregnancy who were at risk of giving birth to children with sickle cell anemia.

The fetal and maternal parts of the chorionic villus specimens were separated under an inverted tissue microscope and the fetal chorionic villus tissue was placed in medium 199, obtaining cellular DNA[7]. PCR was performed by using synthetic oligoprimers (GGC, CAA, TCT, ACT, CCC, AG and ACA, TCA, AGG, GTC, CCA, TAG, AC) as templates close to the 5' and 3' ends of the mutant part of the B gene[8]. Amplified DNA products were digested with the specific restriction enzyme Dde I. The digested products were anlyzed in agarose gel containing 2% Nusieve agarose and 1% agarose (FMC Bio Products) in the presence of ethidium bromide. The electrophoretic pattern was examined under ultraviolet light. Restriction enzyme Dde I recognizes the CTAAG pattern sites and yields two bands of 150 and 201 bp in the part of the normal DNA obtained after carrying out the above procedure. In sickle cell disease, the presence of a CTGAG-CTGTG mutation removes the restriction recognition part, therefore, only one band of 351 pb is obtained from mutant DNA[8,9].

Our study revealed that one of the four fetuses was unaffected, one was a carrier and the remaining two had sickle cell anemia (Table I; Fig. 1).

Table I: Prenatal Diagnosis of Sickle Cell Anemia

| | Fragment (kb) (Dde I) | Genotype |
|---|---|---|
| 1. Fetus | 351 | SS |
| 2. Fetus | 351 | SS |
| 3. Fetus | 351, 201, 150 | SA |
| 4. Fetus | 201, 150 | AA |

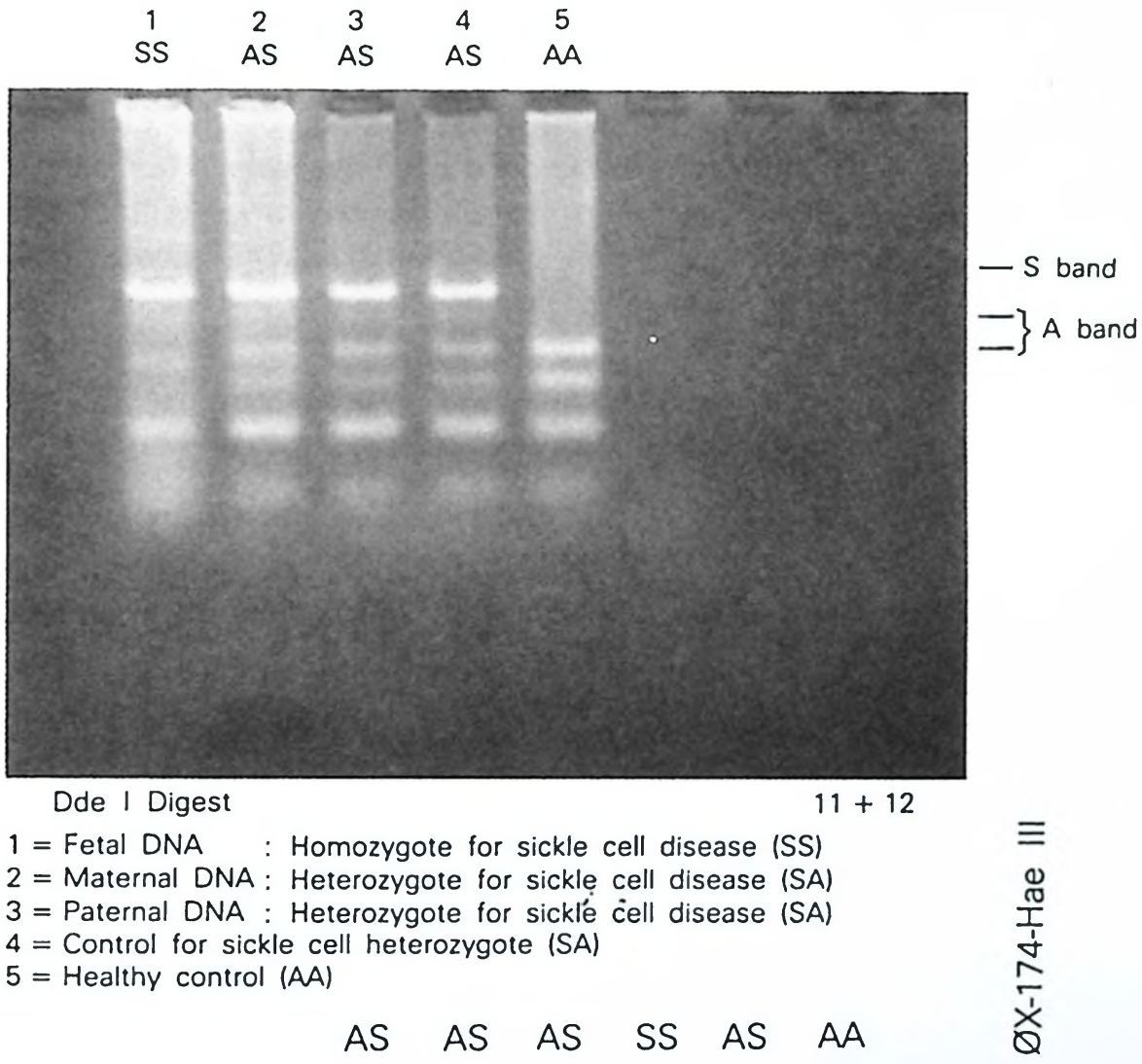


Fig. 1: Ethidium bromide stain of Dde I digested amplified DNA product from individuals with sickle cell trait (AS), sickle cell anemia (SS), normal $\beta$-globin genes (AA), and marker (ØX-174-Hae III).

## Discussion

Hemoglobinopathies commonly occur in Turkey and prenatal diagnoses using fetoscopy and in vitro globin synthesis analysis have been available in our center for the last eight years[4, 10-12]. However, the total number of women with at-risk pregnancies who seek prenatal diagnosis is unexpectedly low. Fetoscopy is usually performed between the 18-19 week of pregnancy, and would-be mothers probably do not want to wait that long for a prenatal diagnosis. Besides, termination of pregnancy at this stage also creates medical and psychological problems. We hope that prenatal diagnosis in which DNA technology is employed, will encourage more women with at-risk pregnancies and also other would-be mothers to seek prenatal diagnoses. Several DNA methods are used to detect a sickle cell mutation, but all require PCR[8]. Our study indicates that mutations which cause changes in recognition sites of restriction enzymes can easily be detected.

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# AMBULATORY ELECTROCARDIOGRAPHIC MONITORING IN HEALTHY NEWBORN INFANTS*

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**SUMMARY: Alpay F, Çeliker A, Lenk MK, Özcan O, Tanındı Ş, Işık E. (Department of Pediatrics and Cardiology, Gülhane Military Academy of Medicine, Ankara, Turkey). Ambulatory electrocardiographic monitoring in healthy newborn infants. Turk J Pediatr 35: 163-170, 1993.**

**Twenty-four hour ambulatory electrocardiographic monitoring is a useful method to document dysrhythmias and to assess treatment response. Various studies have been done in the pediatric age-group to determine normal heart rate values.**

**In this study we determined the heart rate and rhythm patterns of 25 healthy newborn infants whose ages ranged between 3-10 days (mean 6.5 days). There were 15 males and 10 females. The maximum heart rate in these infants was 175-231 beats/min (207 ± 14), minimum heart rate 60-121 beats/min (93 ± 16) and the average heart rate was 130-161 beats/min (143 ± 9). Five infants (20%) demonstrated marked sinus dysrhytmia, seven infants (28%) had ventricular premature contractions, two infants (8%) had supraventricular premature contractions, and five infants (20%) showed junctional rhythm disturbance. The sinus pause did not exceed 1.2 sec and there was no evidence of atrioventricular conduction disorders, or supraventricular-ventricular tachycardia attacks.**

**Our results were consistent with previous studies carried out in newborn infants. Dysyhythmias were detected during 24-hour ambulatory electrocardiographic monitoring in our study group. Since they were generally benign, they need no treatment.**

*Key words: 24-ambulatory electrocardiographic monitoring, newborn infants, dysrhythmia.*

Cardiac dysrhythmias are relatively rare in newborns because resting surface electrocardiograms (ECGs) are usually performed over a short time period[1-6]. Since 24-hour ambulatory ECG monitoring (AEM) is now available, various types of dysrhythmias, including those which are relatively benign such as sinus pause and second degree type 1 atrioventricular block have been detected in healthy newborns[1-6].

Several AEM studies have been conducted in special populations of healthy newborn infants[7-10], and results indicate that there seems to be a wide range of bradydysrhythmias and tachydysrhythmias in the normal neonate[1-6].

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This paper reports the results obtained from AEM studies performed in 25 healthy full-term neonates to determine heart rate and detect the presence of dysrhythmias. The birthweights of the neonates did not exceed 2500 g.

## Material and Medhods

Healthy newborn infants were defined as those presenting with no structural heart defects or symptoms of hypoglycemia, hypocalcemia, hyperbilirubinemia, polycytemia and septicemia. Between 1 Nowember 1990 and 30 June 1991, AEM was obtained for 25 babies (15 males, 10 females), aged 3 to 10 days (mean 6.5 days); born in the Obstetrics Unit of Gülhane Military Academy of Medicine. The results of the cardiovascular examination and regular electrocardiography were normal for all subjects.

AEM was performed using a two channel 24-h portable tape recorder (Hewlett-Packard, U.S.A.). The tapes were played and analyzed using an ECG high-speed analyzer. Recordings with interference were deleted full disclosure of all ECGs were not obtained. The analyzer was only able to determine RR and PP interval changes. Thus several supraventricular and ventricular premature beats, sinus pause, heart rate trend curves and heart rate histograms were easily interpreted (Fig. 1).

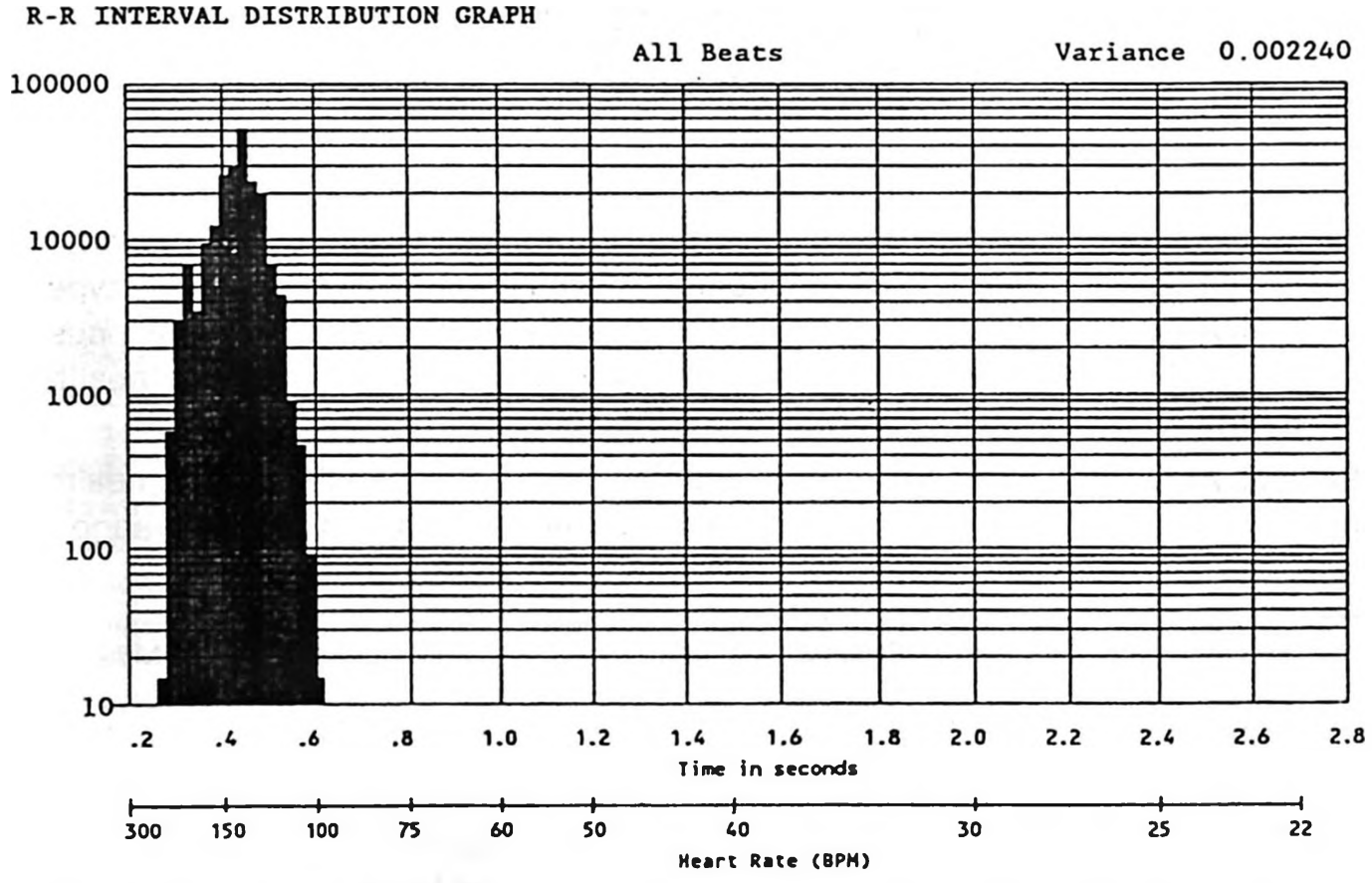


Fig. 1: R-R interval distribution graph. It shows a striking relatively narrow R-R interval distribution. BPM: beat/min.

Sinus arrhythmias were classified using the criteria of Brodsky et al[11]; irregular sinus arrhythmias with adjacent cycle lengths varying by 10% to less than 50% (mild), 50% to less than 100% (moderate), and 100% or more (marked). Supraventricular and ventricular premature contraction were defined according to the P wave, PR interval, QRS interval and T wave changes[6-12]. First degree atrioventricular block (AV) was defined as a PR interval of 1.8 s or more. The Wenckebach type AV block was defined as gradual prolongation of the PR interval terminating in a nonconducted P wave a dropped ventricular beat[6-12]. Mobitz II AV block and complete heart block are described elsewhere[6-12].

## Results

All infants were predominantly in sinus rhythm and showed a minimal continuous irregularity of rate (Fig. 2). The heart rate fluctuated widely in the newborn infants. The maximum heart rate was 207 ± 14 beats/min (range 175-231),

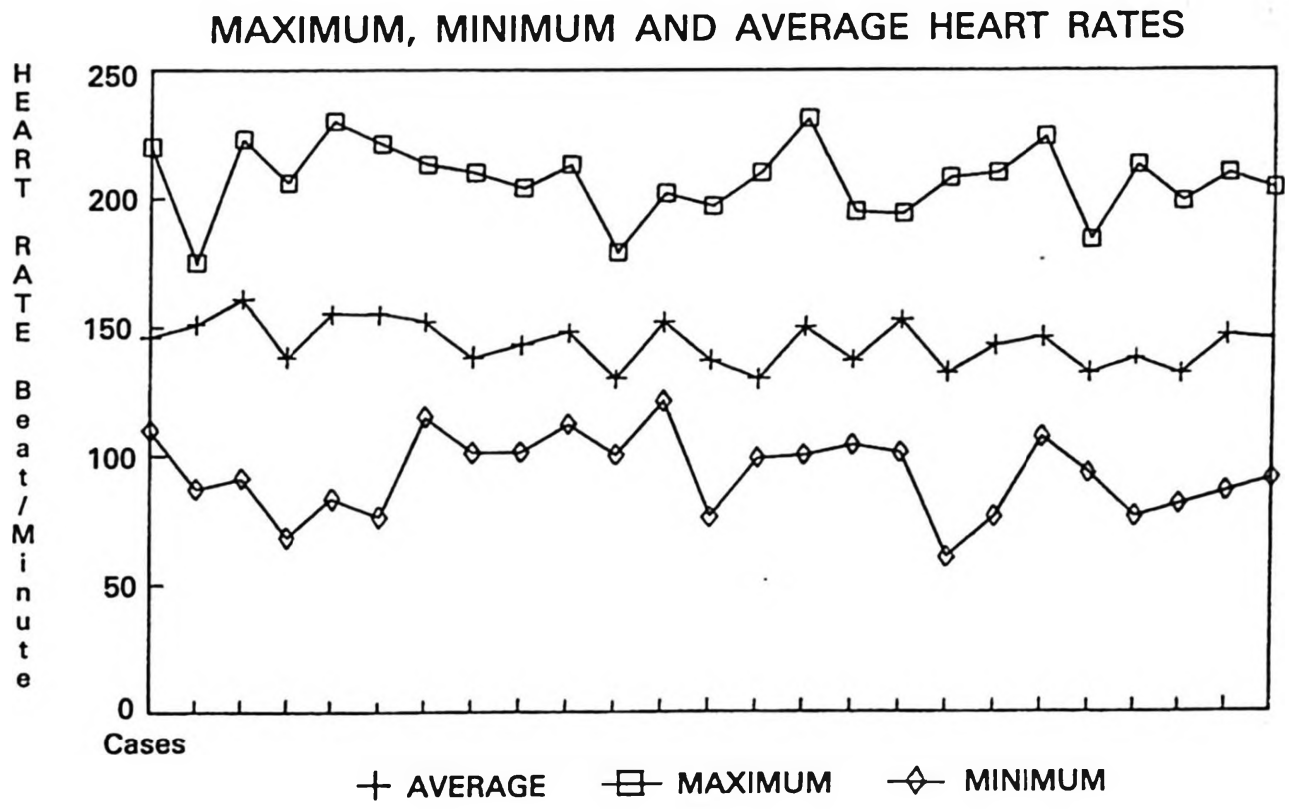


Fig. 2: Maximum, minimum and average heart rates. Heart rate values are shown for all cases. Each point represents one subject.

minimum heart rate 93 ± 16 beats/min (range 60-121) and average heart rate was 143 ± 9 beats/min (range 130-161) (Table I).

Mild or moderate sinus arrhythmias were detected in all subjects, however, marked sinus arrhythmias were determined in only five neonates (20%). Sinus

pause was not detected in our study population beyond marked sinus arrhythmias (Table II).

Ventricular premature contractions (VCPs) were the most common type of dysrhythmia observed in our study group (Fig. 3). Seven infants (28%) had at least one VPC/24 hours. There was a 12% incidence of subjects with five VPCs or more. There was no bigeminy, trigeminy or ventricular tachycardia.

Supraventricular premature contractions (SVPCs) were the second common type of dysrhytmia observed. Two full-term newborns (8%) had several SVPCs during

Table I: Clinical and 24-Hour Ambulatory Electrocardiographic Data in Full-Term Neonates

| | Mean ± SD | Range | |
|---|---|---|---|
| Age (day) | 6.5 ± 1.9 | 3 - 10 | |
| Weight (g) | 3232 ± 4.1 | 2500 - 3920 | |
| Hemoglobin (g/dl) | 18.7 ± 2.9 | 14.2 - 26 | |
| | | | Previous Reports[1-6] |
| Minimum Heart Rate (beat/min) | 93 ± 16 | 60 - 121 | 55 - 75 |
| Maximum Heart Rate (beat/min) | 207 ± 14 | 175 - 231 | 147 - 240 |
| Average Heart Rate (beat/min) | 143 ± 9 | 130 - 161 | |

Table II: Dysrhythmias Detected During Ambulatory-24 Hour Electrocardiographic Monitoring

| | No of Patients | % | Previous Reports % |
|---|---|---|---|
| Sinus Dysrhythmia | 25/25 | 100 | 100 |
| Marked | 5/25 | 20 | ? |
| Premature Ventricular Contractions | 7/25 | 28 | 6 - 17 |
| Premature Atrial Contractions | 2/25 | 8 | 2 - 33 |
| Junctional Rhythm | 5/25 | 20 | 18 - 70 |
| Sinus Pause | – | – | 5 |
| AV Block | – | – | 4 - 6 |

AV: atrioventricular

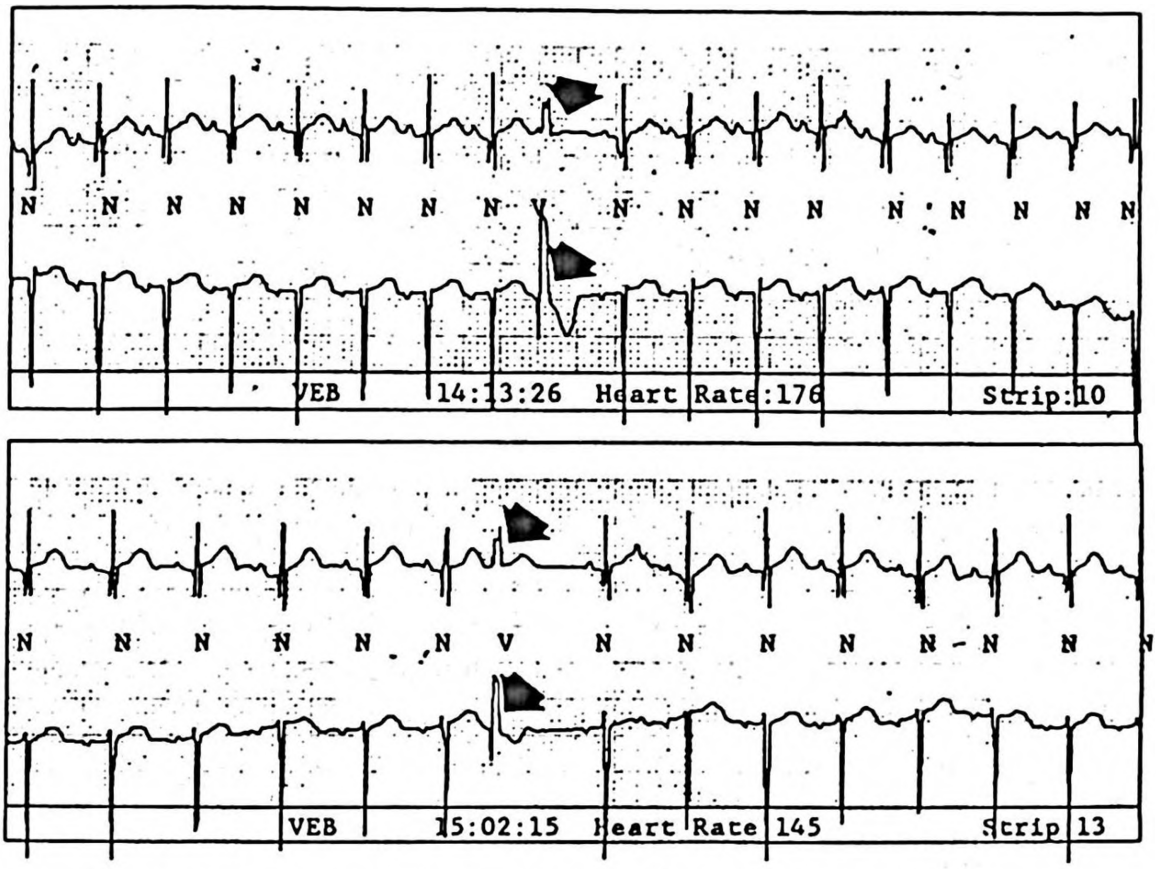


Fig. 3: Ventricular premature contractions (black arrow) in a newborn infant.

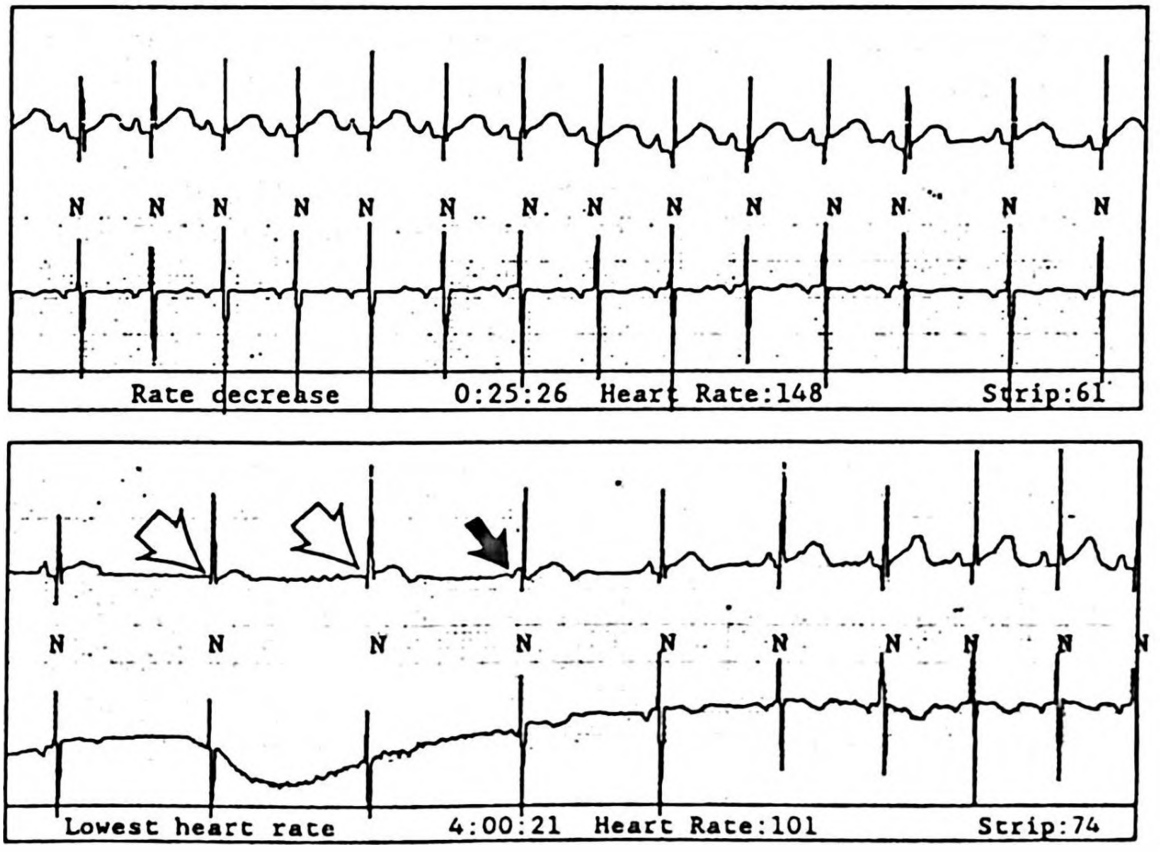


Fig. 4: Junctional escape (white arrow) and supraventricular escape (black arrow) in a newborn during the lowest heart rate episode.

AEM. Supraventricular tachycardia attacks were not noted in the study group. Junctional rhythm disturbance was observed in five infants (20%) during nighttime recordings. This rhythm was usually detected during the lowest heart rate episode (Fig. 4). There was no evidence of first and second degree type 1 block (Wenckebach) in our study group.

## Discussion

The heart rate range detected in these healthy newborn infants were similar to previously reported studies[1-6]. The mean lowest rate measured by AEM was 93 beats/min, with a standard deviation of 16. Heart rates as low as 60 beats/min were recorded over shortime periods and 20 percent of these infants experienced brief episodes of junctional escape rhythms during their lowest rates. Sudden episodes of bradycardia are a distinct feature of cardiac rhythm in this age group[1-6]. Studies have not been able to discern if there is a correlation between heart rate and respiratory pattern changes in sudden infant death syndrome[7-10].

Southall[2] found that 72 percent of healthy newborn infants had sinus pauses, namely complete sinoatrial exit block, sinus arrest, 2:1 sinoatrial block and sinoatrial Wenckebach block with the longest escape interval being 1.8 s. On the other hand Nakashima[1] did not find any sinus pause attacks in his newborn group. Montague[5] reported a sinus pause attack that lasted 840 milliseconds in one newborn subject although we did not detect any sinus pauses, in our infant group various sinus arrhythmias were present. We do not have full disclosure of 24 hour ECGs, but it not affect the results.

There is a greater incidence and frequency of VPCs over a 24-hour period in older children than in younger children[6-11]. Nakashima[1] reported an 18 percent incidence of VPCs in his newborn infant series. VPCs were also a common occurrence in our study group; seven newborns (28%) had at least one VPC during the 24-hour AEM. In various other studies the incidence of VPCs in infants series has also been reported to be low[1-5]. In conclusion, our results may be due to different recording and playback scanner units which are more sensitive.

Montague et al[5] conducted a study to investigate cardiac rhythm in 29 newborn infants with a mean age of 3.5 days. They found that only two infants experienced a SVPC which occurred at the beginning of the monitoring. However, Nakashima[1] reported a high incidence of SVPCs in healthy neonates. Of 80 full-term infants studied using 24-hour AEM, Southall et al[4] detected SVPCs in nine of these infants. They concluded that cardiac rhythm seems to be more stable in infants than in older children and adults and speculated that premature contractions disappear soon after the hemodynamic state is stabilized in the first few days of life[1-4]. In another study, Southall[2] reported that 85 percent of dysrhythmias can not be detected after 12 weeks of age. In our study group, the incidence of SVPC

was 8 percent. We were unable to explain the relatively low incidence of SVPCs detected in our newborn infant group.

It is very difficult for pediatricians to ascertain whether cardiac dysrhythmias or abnormal findings on the ECG of newborn infants are a prediction of Sudden Infant Death syndrome[7-10]. The results of our study offered no clues regarding Sudden Infant Death Syndrome. AV block was not demonstrated in our newborn infant group, although an incidence of 4-6 percent has been reported in various studies[2-4]. There was also no detection of AV block in the patients Nakashima[1] and Montague et al[5] studied. Since it is known that the incidence of AV block increases with age, these findings are not surprising[1,6].

A few studies, involving relatively large groups of infants documented infrequent supraventricular and ventricular attacks of tachycardia[3,4,9]. Nakashima[1] and Montague et al[5] did not detect these types of dysrhythmias in their newborn infant groups. Our results were consistent with these studies.

In conclusion the dysrhythmias detected in our newborn infants group during 24-hour AEM were generally benign and needed no treatment. We found no evidence of conduction disturbances or sinus node dysfunction in the infants. Our results indicate that the newborn infants we studied have a relatively stable hemodynamic status. It must be stressed, however, that dysrhythmias, other than the above-mentioned, must be managed appropriately.

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# ETIOLOGICAL AND FUNCTIONAL EVALUATION OF THE PEDIATRIC POPULATION WITH SPINAL CORD INJURIES*

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*Osman Başgöze MD**, Rıdvan Özker MD***

**SUMMARY: Dinçer F, Çeliker R, Sözay S, Başgöze O, Özker R. (Department of Physical Medicine and Rehabilitation, Hacettepe University Faculty of Medicine, Ankara, Turkey). Etiological and functional evaluation of the pediatric population with spinal cord injuries. Turk J Pediatr 35: 171-175, 1993.**

**Spinal cord lesions are the result of many etiological factors and are associated with motor, sensorial and autonomic dysfunctions. The subjects evaluated were a total of 217 paraplegics and quadriplegics who had been enrolled in a rehabilitation program during the last five years conducted by the Department of Physical Medicine and Rehabilitation of the Hacettepe University Faculty of Medicine. Forty-three of these patients were in the pediatric age group. In this clinical trial, the patients with spinal cord injuries were examined according to their age, sex, etiological factors and functional status. Frankel's scale was used for functional assessment. The mean age of patients was 9.19 ± 4.19 years and varied between 1-16 years.**

**The results of our classification according to etiological factors were as follows: Nineteen patients with tumors (44.1%), nine patients with infections (20.93%), five patients with congenital abnormalities (11.63%), five patients with vascular lesions (11.63%), four patients with trauma (9.30%), and one patient with a degenerative central nervous system disorder (2.32%). The effect of the rehabilitation program on the functional status of the patients is discussed.** *Key words: spinal cord injury, childhood, rehabilitation.*

Various etiological factors are involved in the development of spinal cord lesions which are associated with motor, sensorial and autonomic dysfunctions. Optimal management of spinal cord injured patients requires a multidisciplinary team. Physicians involved in the care of these patients deal not only with the physical disability, but also with socioeconomic and psychologic aspects of the problem[1]. Secondary complications such as diminished pulmonary function, immobility, osteoporosis and urinary and bowel dysfunction are common[2]. Trauma is now one of the most frequent causes of spinal cord injury, but tumour, infection, disc herniation, spinal stenosis, congenital abnormalities such as meningomyelocele, spina bifida, diastematomyelia and vascular lesions may also be an etiological factor. These factor not only effect adult patients but also patients in the pediatric age group[1-6].

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Rehabilitation together with medical and surgical treatment, has afforded the spinal cord injured patient the chance for a longer and better life. The most important therapeutic tool in rehabilitation is exercise. It plays a role in preventing complications, maintaining health and improving function. It is maximally effective when done at the right time, in the proper form and sufficiently[1,4]. The management of the disabled child is based on two fundamental factors: (1) the nature of the child's specific physical and functional impairment, (2) knowledge of normal development[3].

The aim of this study is to determine the distribution of paraplegic and quadriplegic patients in the pediatric age group according to age, sex and etiological factors and to evaluate the functional status before and after completion of the rehabilitation program by using Frankel's scale.

## Material and Methods

In this retrospective study, patients referred to the Department of Physical Medicine and Rehabilitation of the Hacettepe University Faculty of Medicine between 1985-1990 were investigated. A total of 217 patients were admitted to the rehabilitation program during this period and 43 of them were in the pediatric age group. Of these 43 patients, there were 41 (48.8%) females and 22 (51.2%) males. The ages ranged between one and 16 years, with a mean age of 9.19 ± 4.19 years. The patients were evaluated according to age, sex, etiology, lesion location and functional status. Frankel's scale was used to determine the functional status of the patients before and after completion of the rehabilitation program[1,7,8]. According to this scale Grade A consists of injuries in which no motor or sensory function is present below the level of the lesion. Grade B consists of injuries in which there is complete motor paralysis below the level of the lesion with some preservation of sensory function including sacral sparing. Grade C consists of injuries in which there is some motor power present below the level of the lesion, but of no practical use. Grade D consists of injuries in which there is useful motor power below the level of the lesion allowing the patient to move the lower limbs and walk with or without aid. Grade E consists of injuries in which there are no neurological symptoms such as weakness, sensory loss or sphincter disturbance. This classification system is based on the presence of sensation or motor function below the level of the lesion.

## Results

The distribution of patients according to age and sex is presented in Fig. 1. The breakdown of spinal cord injuries according to etiology shows that 19 patients (44.29%) had tumors, nine (20.93%) had infections, five (11.63%) had vascular lesions, five (11.63%) had congenital abnormalities (three cases meningomyelo-

cele and two cases with syringomyelia), four (9.30%) had traumas, and one (2.32%) had a degenerative central nervous system disorder.

Evaluation according to clinical status shows that 24 patients (55.82%) had incomplete paraplegia, nine (20.93%) had complete paraplegia, nine (20.93%) had incomplete quadriplegia and one (2.32%) had complete quadriplegia. Lesions were at the thoracal level in 55.93 percent of the patients.

The functional status of the patients was evaluated using Frankel's scale before and after entry into the rehabilitation program. Before treatment ten of the patient (23.25%) were classed in Grade A, five (11.3%) in Grade B, 23 (53.49%) in Grade C and five (11.63%) in Grade D. After completion of the rehabilitation program, neurological improvement of at least one degree according to Frankel's scale was noted in 40 percent of Grade A patients, in 80 percent of Grade B patients and in, 82.6 percent of Grade C patients (Table I).

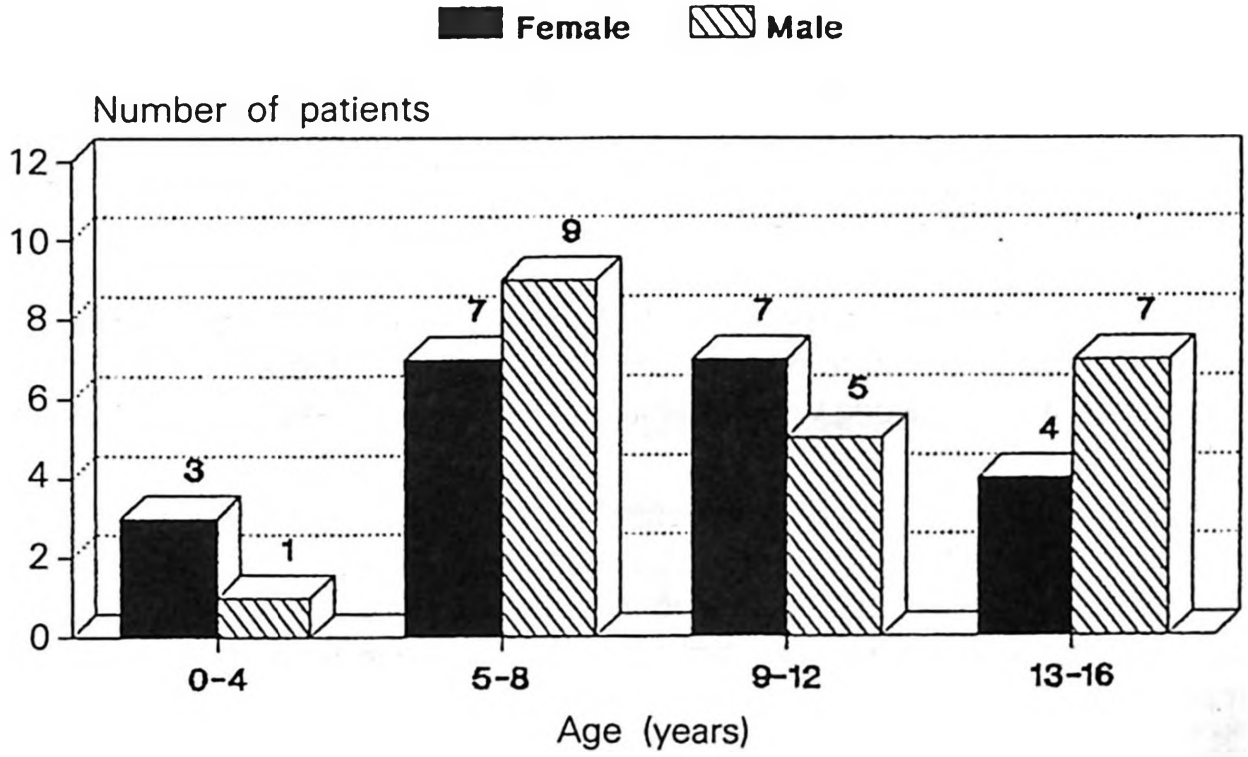


Fig. 1: Distribution of patients according to age and sex.

Table I: Functional Status of Patients Before and After Completion of the Rehabilitation Program According to Frankel's Scale

| After | A | B | Before | C | D | Total | Improvement | % |
|---|---|---|---|---|---|---|---|---|
| A | 6 | – | | 3 | 1 | 10 | 4 | 40 |
| B | – | 1 | | 1 | 3 | 5 | 4 | 80 |
| C | – | – | | 4 | 19 | 23 | 19 | 82.6 |
| D | – | – | | – | 5 | 5 | – | – |

## Discussion

Despite significant advances in medical technology, traumatic spinal cord injuries continue to be incurable, costly and catastrophic. The treatment of choice, therefore, is the proverbial "ounce of prevention". Teenagers, therefore, are an ideal target population for a spinal cord injury prevention program since they can be reached through the school system[9]. Spinal cord injury is one of the leading causes of disability in children and young adults[10].

In our study we observed that female and male children are equally exposed to spinal cord injury (Fig. 1). In the pediatric population, the 5-8-year-old age group is at high risk of spinal cord injury (16 patients), followed by the 9-12-year old age group (12 patients) and the 13-16-year-old age group (11 patients). Reports in the literature indicate that trauma is the most frequent cause of paraplegia and quadriplegia[1,8,10-12].

However, in our clinical trial we found tumors to be the most frequent cause. The Hacettepe University Hospital is a referral center for many complicated cases. The second most frequent cause of spinal cord injury in our patient series was infection. Spinal cord infections are generally secondary to trauma and pulmonary and urinary tract infections. These primary causes mostly occur in the first and second decades[5].

Since the thoracal region is the longest segment of the spinal cord, there are a great many locations where lesions can occur, and therefore, this region has attracted our attention along with other investigators. Our results parallel those in the literature[13,14].

Rehabilitation of a patient is a function of the following: age, quadriplegia, complete lesions, immobilization and such complications as pulmonary embolism, deep vein thrombosis, spasticity and decubitus ulcers[8,15,16]. Paraplegic and quadriplegic patients classed as Frankel's Grade A are in the high risk group for complications[16].

According to Frankel's scale, neurological improvement was more significant in Grade C patients following completion of the rehabilitation program. Eighty-two percent of Grade C patients were classed as Grade D after completion of rehabilitation. Forty percent neurological improvement was noted in Grade A patients and 80 percent in Grade B patients. The five patients in Grade D remained in the same category after treatment. Since Grade D includes a wide variety of neurological signs, it is not always possible for a patient to proceed to Grade E a category where there is no motor or sensory dysfunction. Most patients in Grade D are discharged when they gain adequate function even though they might not have proceeded to Grade E. The improvement rate was lower in Grade A patients when compared with those in Grades B and C, which

may be because complete lesions and complications are more frequent in Group A.

Spinal cord injuries account for more deaths and disabilities in the 1 to 44-year-old age group than all communicable diseases and other conditions. We must stress that rehabilitation for paraplegic and quadriplegic patients is one of the most important subjects for physicians of Physical Medicine and Rehabilitation.

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The Turkish Journal of Pediatrics 35: 177-180, 1993

# MEASUREMENT OF TESTICULAR VOLUME BY ULTRASONOGRAPHY*

*Ender Arıtürk MD***, Mustafa Özateş MD****

**SUMMARY: Arıtürk E, Özateş M. (Departments of Pediatric Surgery and Radiology, Dicle University Faculty of Medicine, Diyarbakır, Turkey). Measurement of testicular volume by ultrasonography. Turk J Pediatr 35: 177-180, 1993.**

**Twenty-five patients with scrotal hernia and hydrocele were studied. The testicular sizes of the patients were measured by using ultrasonography and the sliding caliper was used for calculations during surgery. The actual testicular volumes and those obtained by ultrasonography were not statistically different ($p > 0.05$). It is suggested that ultrasonography may be an alternative to orchidometers.** *Key words: scrotal ultrasonography, testicular volumes.*

In the past ten years, ultrasonography (USG) has been an essential method for diagnosing urological diseases. The use of high frequency real-time probes facilitates the visualization of superficial organs. Ultrasonography is most useful in evaluating the causes of chronic symptoms and scrotal enlargement, however, acute conditions can also be investigated by using this method with some success[1]. In addition, USG may also provide valuable information on testicular size[2,3]. Testicular volume may be calculated clinically measuring the length and width of the testis, and then using the formula devised by Lambert ($0.71 \times L \times W^2$)[4,5].

This study was undertaken with the aim of comparing the actual testicular volume with the volume obtained by using ultrasonography.

## Material and Methods

Twenty-five patients with the diagnosis of hydrocele and scrotal hernia comprised the study population. Ultrasonography was performed on all patients the day before surgical intervention. The testicular volume was calculated by measuring the length and width of the testis seen on the scan. All scans were done with a Toshiba diagnostic real-time scanner and a 7.5 MHz linear transducer (Fig. 1). The testicular volume was calculated by using the above-mentioned formula. The measurements were taken by only one observer and the testes were measured

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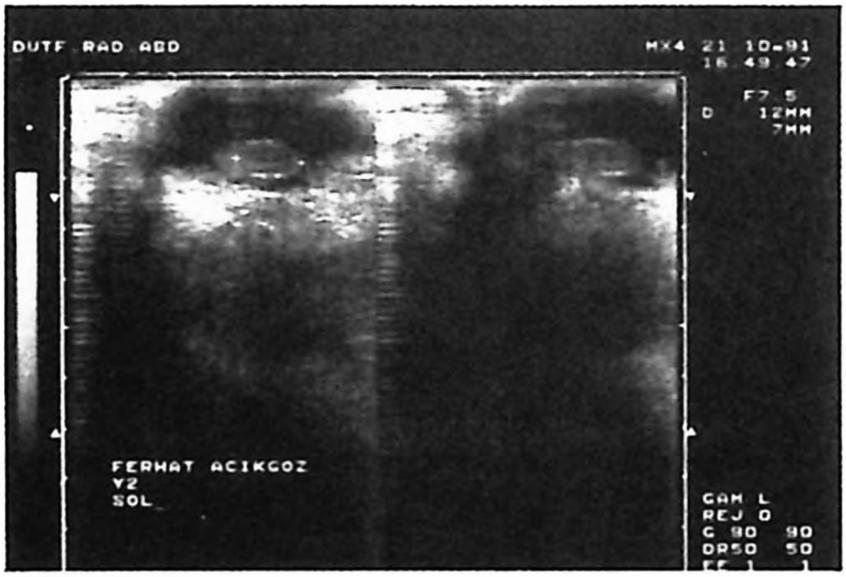


Fig. 1: Ultrasonographic appearance of the testis.

three different times. The actual testicular volumes were assessed in the operating room. The testicular size was determined by the use of a sliding caliper during routine herniorrhaphy or hydrocelectomy (Fig. 2). The actual testicular volume was calculated by using the formula, $0.71 \times L \times W^2$. The Student's t test was used to compare the mean sonographic testicular volumes with the actual volumes of the testes.

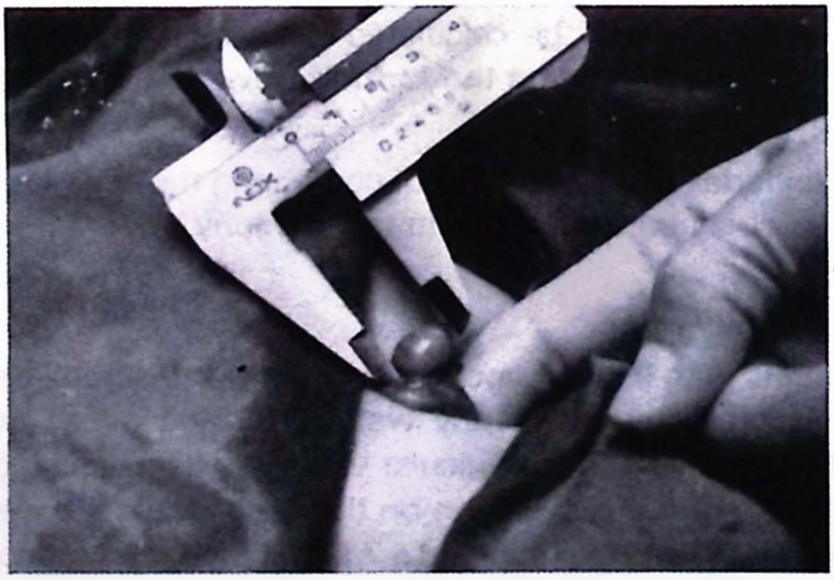

Fig. 2: Calculation of the actual testicular size by using the sliding caliper during surgery.

## Results

The actual testicular volumes and those obtained by using USG are presented in Table 1. The paired Student's t test revealed no differences between the groups ($p > 0.05$).

Table I: Ultrasonographic and Actual Measurements of the Testicular Size and Volume

| Case & Age | Length (mm) | | Width (mm) | | Volume (0.71 × L × W²) (mm³) | |
|---|---|---|---|---|---|---|
| | US | Act | US | Act | US | Act |
| NA 5 yrs | 19 | 19.4 | 11 | 12.1 | 1632.29 | 2016.65 |
| CM 2.5 yrs | 18 | 17.5 | 7 | 6.8 | 626.22 | 574.53 |
| EA 2.5 yrs | 14 | 13.8 | 11 | 10.5 | 1202.74 | 1080.23 |
| SL 10 yrs | 20 | 19.8 | 10 | 9.8 | 1420 | 1350.13 |
| GP 2.5 yrs | 14 | 14.6 | 7 | 7.2 | 487.06 | 537.37 |
| OSA 15 yrs | 22 | 21.8 | 11 | 11.5 | 1890.02 | 2046.97 |
| YT 2.5 yrs | 12 | 12.4 | 6 | 6.9 | 306.72 | 419.16 |
| CA 12 yrs | 16 | 15.6 | 12 | 11.8 | 1635.84 | 1542.22 |
| OA 9 mo | 13 | 12.8 | 6 | 5.4 | 332.28 | 265.01 |
| VD 2 mo | 12 | 12.6 | 7 | 7.3 | 417.48 | 476.73 |
| SY 11 yrs | 15 | 14.6 | 6 | 5.5 | 384.40 | 313.57 |
| MC 10 mo | 16 | 16.1 | 6 | 6.2 | 408.96 | 439.41 |
| AD 9 mo | 18 | 17.5 | 6 | 6.4 | 460.08 | 508.93 |
| VT 3.5 yrs | 17 | 16.9 | 7 | 6.8 | 591.43 | 554.83 |
| MC 6 yrs | 10 | 9.6 | 6 | 5.7 | 255.60 | 221.45 |
| PA 6 mo | 16 | 15.4 | 8 | 7.7 | 727.04 | 648.28 |
| KL 11 yrs | 18 | 17.6 | 10 | 9.4 | 1287.00 | 1104.15 |
| SP 12 yrs | 14 | 13.6 | 10 | 9.5 | 994.00 | 871.45 |
| MÖ 9 yrs | 19 | 18.2 | 12 | 11.6 | 1942.56 | 1738.78 |
| MS 2 yrs | 24 | 23.2 | 8 | 7.6 | 1090.56 | 951.42 |
| KM 2.5 yrs | 16 | 15.4 | 8 | 8.3 | 727.04 | 753.24 |
| AK 7 mo | 13 | 12.8 | 7 | 6.9 | 452.27 | 432.68 |
| AA 2 yrs | 13 | 13.6 | 7 | 7.3 | 452.27 | 514.57 |
| FA 2 yrs | 12 | 12.3 | 7 | 7.4 | 417.48 | 478.22 |
| AT 6 yrs | 13 | 13.4 | 6 | 6.8 | 332.28 | 439.93 |
| | $\bar{X}$ : 0.024 | | $\bar{X}$ : 0.152 | | $\bar{X}$ : 7.348 | |
| | SD : 0.574 | | SD : 0.445 | | SD : 123.964 | |
| | t : 0.2090 | | t : 1.707 | | t : 0.296 | |
| | $p > 0.05$ | | $p > 0.05$ | | $p > 0.05$ | |

## Discussion

Several orchidometers have been used to determine testicular volume. The Prader orchidometer is the one most used, however, Prader[6], noted than in its practical use there was considerable variation in the values obtained because of the

method and the investigator. The Schonfeld's orchidometer is also used widely[7,8] However, there are reports indicating that this instrument tends to overestimate the actual size of the small testis, while it underestimates large testicular volumes. Another such instrument is the Tachiara orchidometer[9], which consists of a graded series of punched out elliptical rings. The testes with its attachments and scrotal skin are evaluated together and are associated with considerable variability. Ultrasonography which is readily available, is a suitable, non-invasive method and gives accurate data concerning dimensions of organs. This method can also be used during the follow-up of patients when evaluating the effect of therapy on testicular integrity. In addition it gives valuable information regarding the changes in the testes and orchidopexies. Ultrasonography can be considered to be a suitable method for quantitative determination of testicular volume.

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# CHRONIC CONSTIPATION: A CAUSE OF RECURRENT URINARY TRACT INFECTIONS*

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**SUMMARY: Romańfczuk W, Korczewski R. (Pediatric Clinic, Institute of Clinical Medicine, Rzeszów, Poland). Chronic constipation: a cause of recurrent urinary tract infections in children. Turk J Pediatr 35: 181-188 1993.**

**In this study children hospitalized for chronic constipation were investigated for urinary tract problems. The study group consisted of 180 children, 62 girls and 118 boys, suffering from recurrent urinary tract infections. In a number of the patients, radiological and ultrasonographic examinations revealed various urinary tract anomalies. When patient presents with a problem of the urinary tract, it is necessary to consider constipation as a possible cause. In many cases the treatment of chronic constipation may be the most important remedy for pyuria, bacteriuria and enuresis.**
*Key words: chronic constipation, children, recurrent urinary, tract infection.*

Colon disturbances comprise a variety of pathological processes occurring more and more often in everyday pediatric practice. It seems that constipation, either chronic or acute, is the most important problem in terms of colon pathology in children and adolescents. Nowadays this is a social problem resulting from the influences of civilization and family predispositions[1-4].

Despite the vast literature on the subject, constipation still remains a problem that has not been thoroughly investigated and is not fully understood. Most researchers tend to use the term "constipation" to describe the occurrence and difficult passage of hardened, dry stool. Constipation usually denotes fewer bowel movements than normal, although the term seems more suitable for denoting the kind of stool rather than the frequency of its passage[1,3,4]. In Poland, chronic constipation in children includes habitual, functional constipation and that caused by different forms of hypoganglionosis and aganglionosis of the colon.

The classical complications associated with chronic constipation include: progressive loss of the urge to defecate; painful bowel movement; mechanical trauma of the anal mucosa caused by the passage of large amounts of hard feces through the anus; fecal soiling resulting from overflow; encopresis; and secondary changes in the anatomy of the colon (dolichocolon or considerable dilatation). However, more and more often, urinary tract problems accompany the condition. Not until the 60s and 70s did reports appear in the literature referring to recurrent

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urinary tract infections, incontinence or enuresis in children suffering from chronic constipation[2,4-6]. The frequent occurrence of morphological anomalies such as hydronephrosis, vesicoureteral reflux, bladder and urethra deformities, and functional disturbances such as uninhibited bladder contractions, have also attracted the attention of investigators[5-8].

In this paper we present observations concerning urinary tract anomalies in children hospitalized for chronic constipation resistant to ambulatory treatment.

## Material and Methods

The current investigators studied children between the ages of 3 and 17 who were admitted to the Rzeszow Pediatric Clinic, between 1983 and 1989 for chronic constipation. The group comprised 180 children 62 girls and 118 boys (female to male ratio 1:1.9). The ratio of town children to those from the country was 1.2:1. The average observation period was 13.5 months, however in every case it was more than six months from the date the child was discharged from the hospital.

The diagnosis of chronic constipation was established on the basis of interview, clinical symptoms, physical examination including rectal palpation, and endoscopy of the large bowel, rectoscopy and/or sigmoidoscopy. In some cases, radiological examinations of the colon along with barium enema studies using double contrast, abdominal ultrasonography or pathomorphological examinations were done, histochemical specimens of mucosae and submucosae were obtained, or Quinton's probe or deep rectal biopsies were done during endoscopy of the colon.

After Hirschsprung's disease was ruled out and the functional character of the constipation was diagnosed, medical treatment included daily cleansing enemas often preceded by manual disimpaction of feces from the rectum, laxatives and purgatives. A high-fibre diet and a special bowel training program were also prescribed. The treatment also included consultation with a psychologist as well as the child's parents, who were educated as to the nature of the problem as well as treatment and prevention methods.

Detailed information was obtained pertaining to urological problems of each child. If recurrent urinary infections, enuresis and other miction problems were present, repeated general urinalysis and two bacteriological urine cultures were done to determine urea and creatine levels in the blood serum. Radiological examinations of the urinary tract, intravenous urography and miction cysto-ureterography were sometimes necessary. Since 1988, ultrasonographic study of the urinary tract in such patients has been routine.

## Results

Within the investigated group of children with functional chronic constipation, an additional diagnosis of recurrent urinary tract infections was established in 41 girls (66%) and 30 boys (25%).

In 27 girls (44%) and 40 boys (34%) the interview revealed a history of enuresis. Tables I, II and III list the urinary tract abnormalities accompanying the intestinal disorders.

The patients were divided into three groups. The first group comprised children with the diagnosis of functional megacolon syndrome by endoscopic and radiological examination. The second group consisted of children with elongated colons or extra loops of the large intestine. The third group comprised children in whom no colorectal anomalies were found.

Table I: Urinary Problems in Children with Chronic Constipation (Total: 180, girls: 62, boys:118)

| | Children with chronic constipation | | | | | |
|---|---|---|---|---|---|---|
| | Grils | | Boys | | Total | |
| | n | % | n | % | n | % |
| Recurrent urinary tract infections | 41 | 66.13 | 30 | 25.42 | 71 | 39.44 |
| Enuresis | 27 | 43.55 | 40 | 33.89 | 67 | 37.22 |

Table II: Girls with Chronic Constipation and Urinary Problems

| Chronic constipation | N | EN | ER | UTI | UTI + ER | USG/RTG | PSYCHE |
|---|---|---|---|---|---|---|---|
| Functional megacolon | 22 | 10 | 14 (63.6%) | 12 (54.6%) | 8 (36.4%) | 4 (18.2%) | 13 |
| Dolichocolon | 14 | 5 | 5 (35.7%) | 12 (85.7%) | 5 (35.7%) | 4 (28.6%) | 5 |
| Normal colon | 26 | 6 | 8 (30.8%) | 17 (65.4%) | 7 (26.9%) | 5 (19.2%) | 6 |
| Total | 62 | 21 | 27 (43.6%) | 41 (66.1%) | 20 (32.3%) | 13 (20.9%) | 24 |

Abbreviations:
N : Number of patients
EN : Encopresis
ER : Enuresis
UTI : Urinary Tract Infections
USG/RTG : Abnormal results of USG/RTC
PSYCHE : Psychological deviations

Table III: Boys with Chronic Constipation and Urinary Problems

| Chronic constipation | N | EN | ER | UTI | UTI + ER | USG/RTG | PSYCHE |
|---|---|---|---|---|---|---|---|
| Functional megacolon | 47 | 16 | 16 (34%) | 11 (23.4%) | 7 (14.9%) | 5 (10.6%) | 13 |
| Dolichocolon | 31 | 9 | 9 (29%) | 8 (25.8%) | 3 (9.7%) | 5 (16.1%) | 7 |
| Normal colon | 40 | 14 | 15 (37.5%) | 11 (27.5%) | 8 (20%) | 8 (20%) | 11 |
| Total | 118 | 39 | 40 (33.9%) | 30 (25.4%) | 18 (15.3%) | 18 (15.3%) | 31 |

Abbreviations:
N : Number of patients
EN : Encopresis
ER : Enuresis
UTI : Urinary Tract Infections
USG/RTG : Abnormal results of USG/RTC
PSYCHE : Psychological deviations

The following urinary tract anomalies were found in three girls and four boys with functional megacolon syndrome: ambilateral vesicoureteral reflux, dilatation of the pyelocaliceal systems (unilateral or bilateral), hydronephrosis with megaureter, decidedly increased echogeneity and indistinct parenchyma structure in both kidneys (Figs. 1, 2). Eight patients (4 girls, 4 boys) with elongation of the colon or with extra loops in the large intestine also had urinary tract anomalies involving

Fig. 1: Intravenous urography demonstrating hydronephrosis with megaureter.

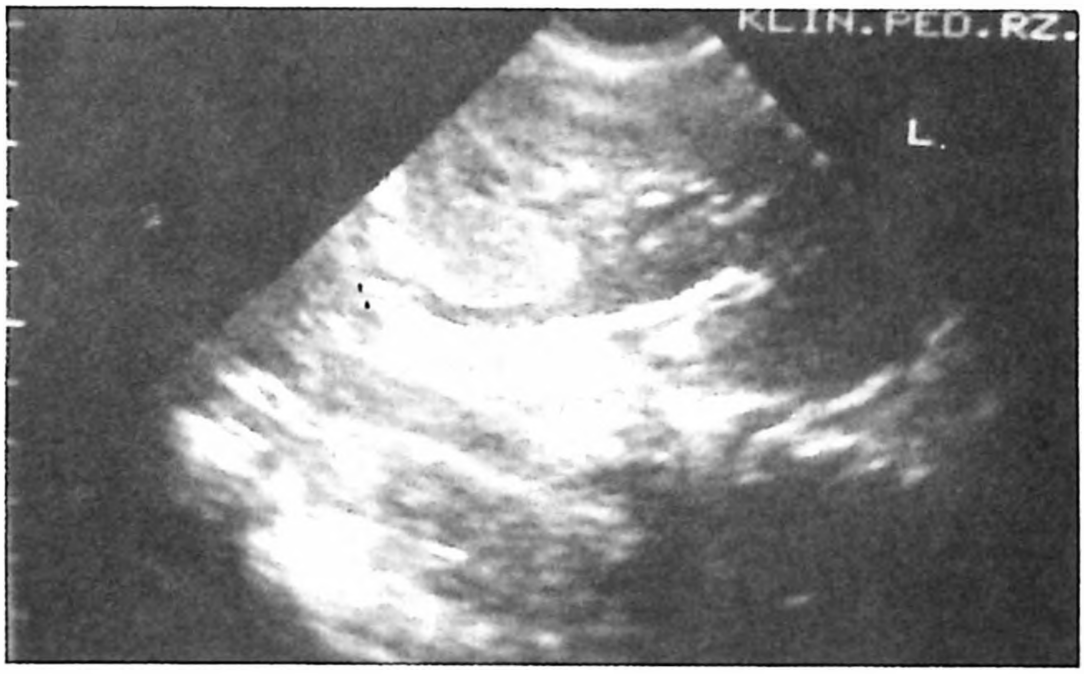


Fig. 2: USG demonstrating dilatation of the pyelocaliceal system in the left kidney.

ambilateral vesicoureteral reflux with simultaneous hypertrophy of the anterior lip of the bladder neck, and bilateral or unilateral dilatations of the kidneys' collecting systems. In one girl with dolichocolon, a lowered left kidney was also found. The greatest number of urinary tract anomalies was found in the group with no anatomic anomalies of the colon. All of the children suffered from chronic constipation which was believed to have a psychogenic origin since no other explanation could be found. Urinary anomalies were observed in as many as 20% of patients in this study. The most common anomalies were: explicit signs of urinary retention in one or both kidneys, and echogenic accumulation, shadows or deposits in the renal parenchyma.

Despite the long duration of colon and urinary problems, symptoms of functional disturbances of the kidneys were found in none of the cases under investigation. The kidneys of children functioned efficiently, and the blood urea and creatine concentrations were normal.

## Discussion

Chronic constipation as an etiologic factor in recurrent urinary tract infections and enuresis in children has recently been discussed in the literature[1,2,4-9]. In our study group of 180 children, persistent pyuria was found in more than 66% of girls and 25% of boys. In all cases, E. coli was the infecting organism responsible for the significant bacteriuria.

Researchers have reported a frequent association between recurrent pyuria and/or bacteriuria, and chronic constipation[1,3,4,5,7,10]. The studies showed that

this association occurred in 13%[1] to 45%[5] of patients, and, as in our group, girls were predominant (ratio of 2:1).

Enuresis was the most common complaint reported by parents on the children's admission to our clinic (27 girls and 40 boys). Enuresis most often occurred at night while the child was sleeping (64 children). Only one girl and two boys experienced diurnal enuresis. The prevailing type was secondary enuresis which recurred several months or even years after full control over miction was achieved. Various investigators have reported a similar ratio, although in none of the available sources did we find a clear distinction between the particular types of enuresis.

The considerable variety of developmental anomalies found in our study confirms earlier observations[1,5,6,8]. Elongation of the urethra, irregularities in bladder wall contours, hydronephrosis, vesicoureteral reflux and anomalies in the base plate were the most frequent abnormalities found in Shopfner's study of 39 children aged 5-13 years in 1968[6]. On the basis of radiological and ultrasonographic examinations, we found such anomalies in 11 girls and 12 boys. The most frequent anomalies were hydronephrosis, dilatation of the pyelocalieceal system and vesicoureteral reflux.

The factors favorable to the development of recurrent urinary tract infections include, among others, an increase in the absolute number of bacteria in the region of the external opening of the urethra (e.g. with intensive fecal soiling), disturbances in miction causing easier bacterial penetration into the baldder lumen, and disturbances in the bladder's defense mechanisms caused by urine retention or increased endovesical pressure". All of these conditions occur in children suffering from chronic constipation and secondary fecal incontinence, and therefore are fully relevant to our investigation.

In advanced chronic constipation in children and infants, chronic urine retention and urinary tract infection may occur. Bentley[2] went so far as to say that chronic constipation may in some cases lead to terminal renal failure. We observed no such case, normal levels of urea and creatine suggested undisturbed functional efficiency of the kidneys.

A number of authors have tried to account for the frequent presence of urinary tract anomalies in patients with chronic constipation. The predominant view is that such anomalies are caused by chronic urine retention. The rectum, considerably overloaded with hard fecal masses, is said to exert direct pressure on the bladder, resulting in obstructive uropathy. The blockage leads to recurrent urinary tract infections and hydronephrosis. The phenomenon of irregular contours of the bladder and its displacement on x-ray was described by Neuman[5] and Lloyd-Still[4]. However, another group of researchers see the disturbances in the process of maturation of the bladder base plate as the main cause of

hydronephrosis, vesicoureteral reflux and urethral elongation[6]. The plate is the most important part of the bladder because of its role in the mechanism of miction, producing the "triangle canal" which elongates the urethra towards the cephalad. When miction finishes, the walls of the triangle canal return to their primary position[11]. In children with habitual constipation, the dilated rectum rests on the sacral bone and exerts pressure on the bladder. It undergoes compression and moves down below the pubic symphysis, and the base plate becomes cone-shaped and undergoes front and upper displacement. This makes the formation of the normal triangle canal and the intracystic segments of the ureters impossible. The final effect is the formation of bladder-ureter refluxes, vesicoureteral refluxes and hydronephrosis[6]. Chronic distention of the bladder secondary to urine retention caused by problems with miction only contributes to the formation of hydronephrosis in these cases.

The treatment of recurrent urinary tract infections and enuresis is often unsuccessful. In nearly half of patients, the infections linger and very often recur, despite intensive medical treatment. In these cases total regression of the renal problems may be achieved only after normalizing the activity of the colon. The results of a number of authors[4,5,8], as well as our own experience, confirm the significance of regulating bowel movements in the treatment of recurrent pyuria, bacteriuria and enuresis. This therapy consists of the use of certain chemotherapeutic agents, preferably in accordance with an antibiogram together with dietary regulations, a fiber-rich diet, bowel movement training and medicine to restore a proper bowel movement pattern (e.g. lactulose).It is sometimes necessary to use a more invasive approach, suc as cleaning the bowel with enemas or manually disimpacting the colon clogged with feces.

Defective bladder activity and recurrent urinary tract infections cause gradually increasing damage to the upper urinary tract with progressive renal insufficiency. To make a detailed estimation of the urinary tract, it is necessary to do clinical, radiological and neurological examination. The neurological examination should determine the functional state of the muscles of the perineum, buttocks and lower extremities; the sensation within the perineum and the tone of the anal sphincters per rectum; the anal reflex; and the bulbo-cavernous reflex. A more detailed evaluation of the anal and uretheral sphincters and the detrusor muscle may be obtained by electromyographic and urodynamic cystometry and uroflometry examinations[5,8,11,12]. The considerable cost of the electromyographic and urodynamic methods encourages pediatricians to make more efficient use of their own assessment skills.

It is important to consider chronic constipation in every patient with a urinary tract infection. Unfortunately, this factor is often overlooked by doctors and parents, while radiological eaminations of children with recurrent urinary tract infections are frequently not preceded by an examination per rectum. Doctors often opt for

cleaning of the bowels and artificial disimpaction of the colon instead of attending first to fecal impaction, the most significant symptom of chronic constipation.

Urinary tract infections are observed not only in children with advanced and obvious constipation but also in children with latent problems of defecation. Therefore, in each case of functional disturbance of the colon, one must evaluate the urinary tract, and vice versa. Treating chronic constipation may often be the most important activity in the therapy of recurrent urinary tract infections and enuresis.

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# OXANDROLONE THERAPY IN SKELETAL DYSPLASIA*

*Atilla Büyükgebiz MD***, İlhami Kovanlıkaya MD****

**SUMMARY: Büyükgebiz A, Kovanlıkaya İ. (Departments of Pediatrics and Radiology, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Oxandrolone therapy in skeletal dysplasia. Turk J Pediatr 35: 189-196, 1993.**

**Three patients with short stature and different forms of skeletal dysplasia were treated with oxandrolone, 1.25 mg/day. All patients had a normal growth hormone response (10 ng/ml) to the insulin-induced hypoglycemia test (ITT). After one year of follow-up, it was noted that the pretreatment growth rate which was 2.5 cm/yr in the patient with spondyloepiphyseal dysplasia congenita (chronological age, 13 4/12 yr) had increased to 6.7 cm/yr after one year of treatment, while the pretreatment growth rate of the patient with hypochrondroplasia (chronological age, 12 7/12 yr) which was recorded at 2 cm/yr had risen to 5.3 cm/yr. The patient with multiple epiphyseal dysplasia (chronological age, 9 5/12 yr) had a pretreatment growth rate of 1.5 cm/yr which had risen to 8 cm/yr after the same period of treatment. An increase was noted in the height standard deviation score for chronological age and in the height standard deviation score for bone age in all patients. After one year of therapy, all patients were observed to still be in the prepubertal stage.**

**Oxandrolone therapy seems to be useful in the treatment of short stature seen in skeletal dysplasia.**

**However, a more lengthy study is needed in order to assess the efficacy of treatment with regard to adult height prognosis and to determine the optimal dosing required.**
*Key words: skeletal dysplasia, growth, oxandrolone therapy.*

Human skeletal dysplasias are a heterogenous group of disorders that result in disproportionate short stature. While individually rare these developmental defects of the skeleton give rise to a significant proportion of children with moderate to severe short stature. The understanding and appreciation of the heterogeneity within the skeletal dysplasias led to a systematic description of over 100 different forms which have been primarily classified on the basis of clinical and radiographic changes[1,2].

The aim of this study was to evaluate the use of oxandrolone a non-aromatizable androgen in the treatment of three different types of skeletal dysplasias which manifest short stature spondyloepiphyseal dysplasia congenita, hypochondroplasia and multiple epiphyseal dysplasia and to ascertain the effect of the drug on the growth of these children.

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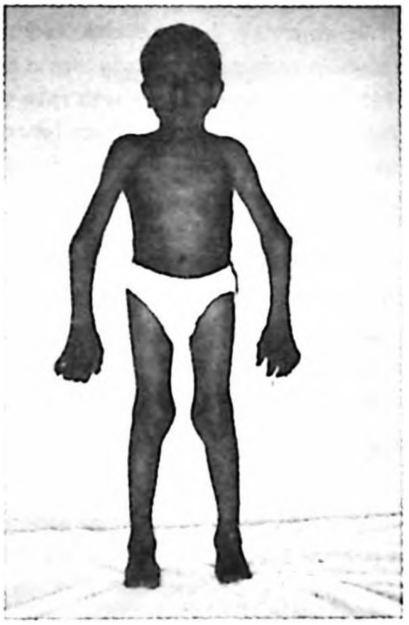
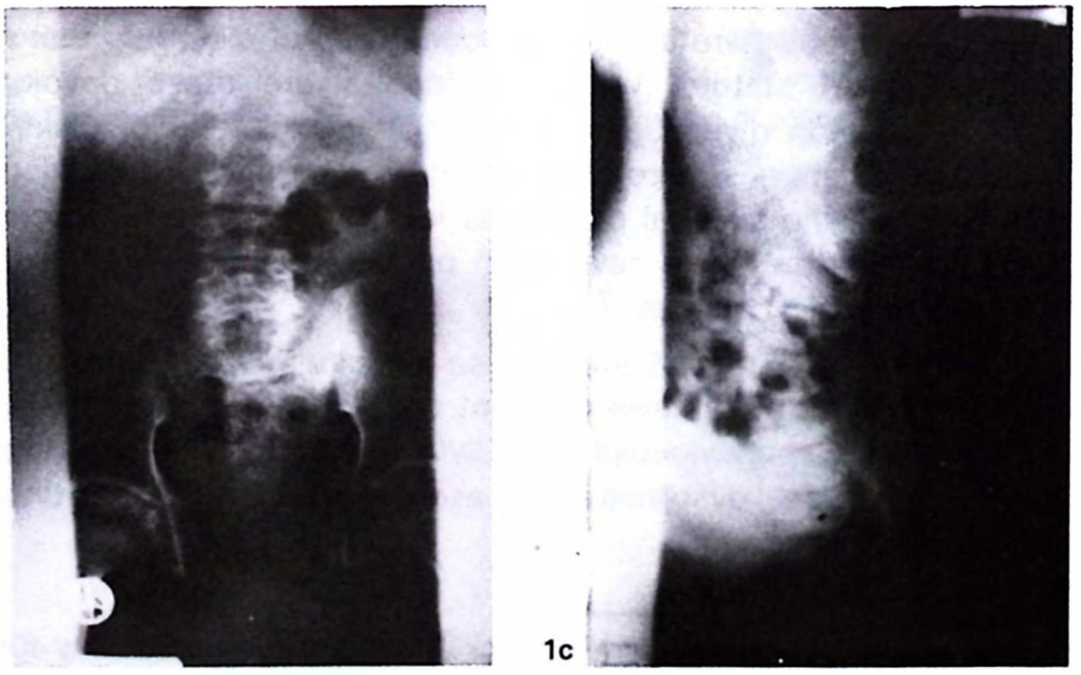
## Material and Methods

C.K., a 13 4/12-year-old boy was referred to our hospital for evaluation of short stature. His height was 128.5 cm (below the third percentile) and trunk/lower extremity ratio was 61/67.5 cm. His arm span was 133 cm, and the skeletal age was determined at 15 years. The testicular volume was 3 × 3 ml, and he was classified as being in Tanner's Pubic Hair Stage 1[3]. He was the second male child born to parents who were first degree relatives (Fig. 1a). Radiographic studies revealed spondyloepiphyseal dysplasia congenita (Figs. 1b, c).

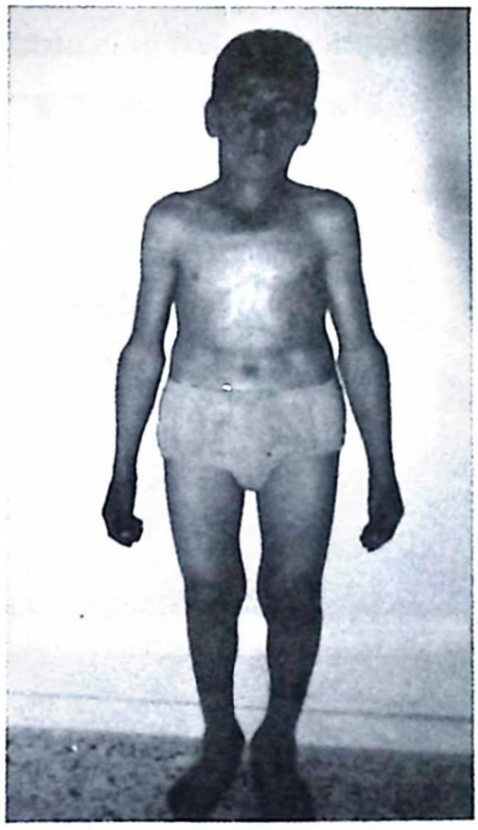




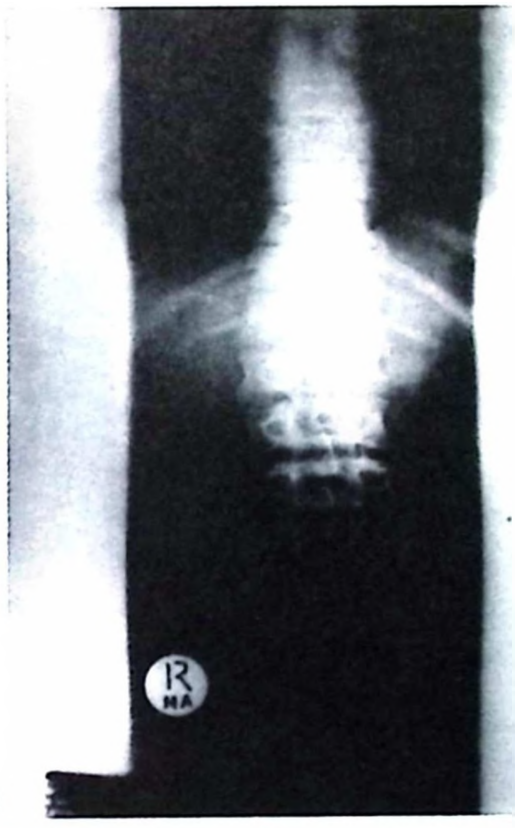
1b 1c

Fig. 1a, 1b, 1c: Spondyloepiphyseal dysplasia congenita patient. Roentgenographs show thoracic and lumbar vertebral bodies that illustrate a flat irregular wedged-shaped anterior portion. The proximal femoral epiphyses are deformed.

A.A., a 12 7/12-year-old boy, was referred to us because of short stature. His height was 117 cm (below the third percentile) and the trunk/lower extremity ratio was 57/60 cm. His arm span was 120 cm and the bone age was determined at ten years. His testicular volume was 2 × 2 ml and he was classed as being in Tanner's Pubic Hair Stage 1. He was the third born male child of parents who were first-degree relatives (Fig. 2a). Radiographic studies revealed hypochondroplasia (Figs. 2b, c).



2a

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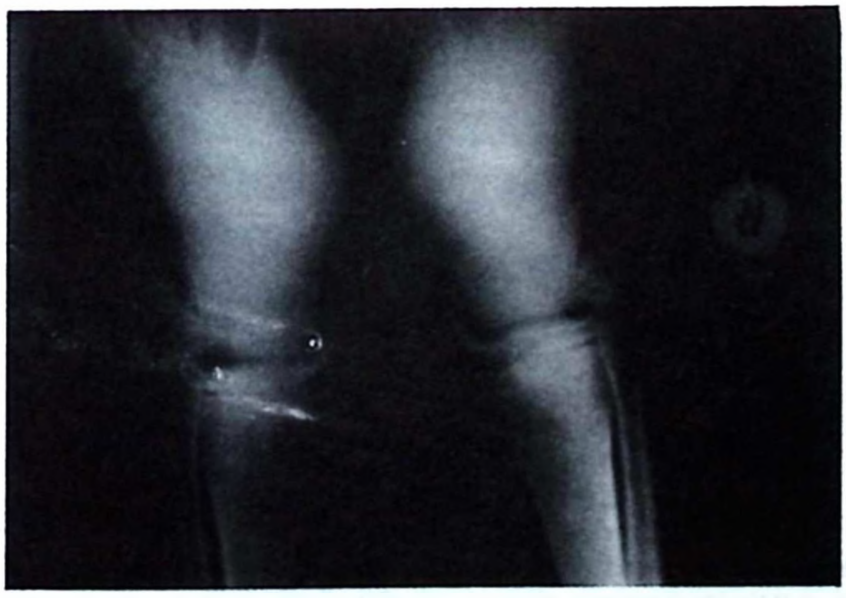
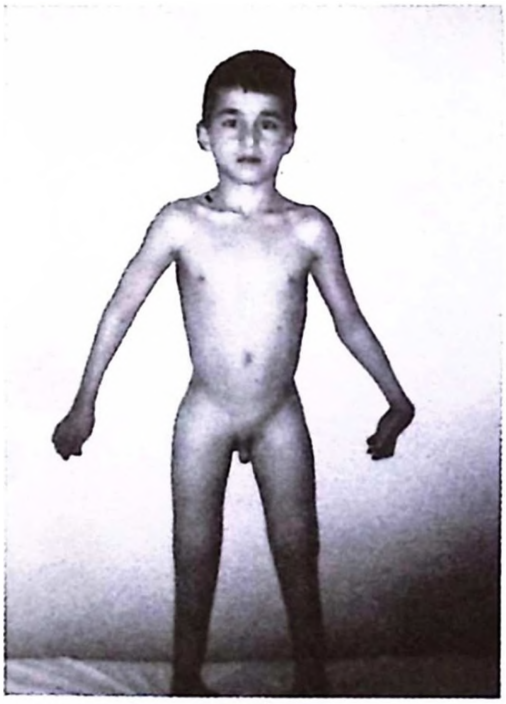


2c

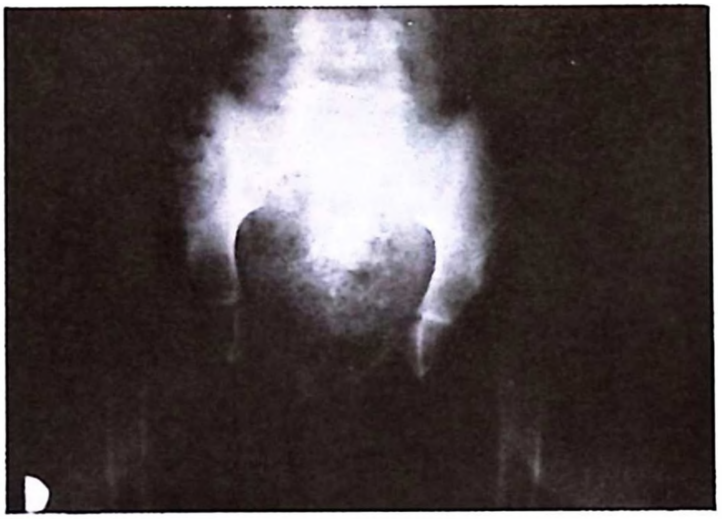
Fig. 2a, 2b, 2c: Hypochondroplasia. Roentgenographs show typical narrowing of the interpediculate distances of the L4, L5 vertebra and bilateral elongated distal ends of the fibula in respect to the tibia.

C.G., a 9 5/12-year-old-boy had a height of 110 cm (below the third percentile) the trunk/lower extremity ratio was 61/49 cm and he had an armspan of 115 cm. He had multiple skeletal deformities and a congenital hip dislocation. He had flexion contracture of both fingers and elbows and a talipes equinovarus deformity (Fig. 3a). His skeletal age was determined at eight years. The child had cryptorchidism and was classed as being in Tanner's Pubic Hair Stage 1.

There was no history of consanguinity in the family. Radiographic studies along with a physical examination led to the diagnosis of multiple epiphyseal dysplasia (Figs. 3b, c).



3a



3b

3c

Fig. 3a, 3b, 3c: Multiple epiphyseal dysplasia. An anteroposterior radiogram of the pelvis shows bilateral marked acetabular dysplasia, dislocation of the hips and hypoplasia of the proximal femoral epiphyses. On the tibia-fibula roentgenograms there are markedly irregular, deformed tarsal bones. The lateral part of the lower tibial epiphyses is thinned and deformed.

Insulin-induced hypoglycemia tests (ITT) using 0.15 units of insulin/kg body weight primed with testosterone were performed on all patients which yielded normal results (growth hormone levels above 10 ng/ml). All measurements were taken with a Harpenden stadiometer and bone ages were determined using the method devised by Greulich-Pyle[4]. The patients were followed for two years prior to initiation of therapy. The growth velocity of the patient with spondyloepiphyseal dysplasia congenita was 2.5 cm/yr, the patient with hypochondroplasia 2.0 cm/yr, and the patient with multiple epiphyseal dysplasia 1.5 cm/yr.

After obtaining patient and parental consent, oxandrolone therapy was administered to the patients with skeletal dysplasia in a dose of 1.25 mg/day. These patients were followed up for one year.

## Results

A noticeable increase was observed in the growth rates of all patients during the first year of oxandrolone therapy (Tables I-III). The increase was most marked in the patient with multiple epiphyseal dysplasia whose growth rate increased from 1.5 to 8.0 cm/year, while the growth rate of the patient with spondyloepiphyseal dysplasia congenita increased from 2.5 to 6.7 cm/year, and the patient with hypochondroplasia increased from 2 to 5.3 cm/year. These patients all remained in the prepubertal stage and the bone ages did not unduly accelerate during this period of treatment, but positive correlations were found, both pretreatment and posttreatment, with respect to height standard deviation scores, and bone age and chronological age values in all patients during the said period.

Table I: The Results of Oxandrolone Therapy in Spondyloepiphyseal Therapy

| | Pretreatment | One Year Postreatment |
|---|---|---|
| Chronological age* (yrs) | 13 4/12 | |
| Bone age** (yrs) | 13 | 13 6/12 |
| Height (cm) | 128.5 | 135.2 |
| Upper/Lower (cm) | 61/67.5 | 63.7/71.5 |
| Growth rate (cm/year) | 2.5 | 6.7 |
| Height SDS*** for BA | −3.18 | −2.66 |
| Height SDS for CA | −3.48 | −3.32 |
| Testicular vol (ml) | 3 × 3 | 3 × |
| Pubic Hair (Tanner) | 1 | 1 |

* Chronological age: CA
** Bone age: BA
*** Standard Deviation Score: SDS

Table II: The Results of Oxandrolone Therapy in Hypochondroplasia

| | Pretreatment | One Year Postreatment |
|---|---|---|
| Chronological age (yrs) | 12 7/12 | |
| Bone age (yrs) | 10 | 11 |
| Height (cm) | 117 | 122.3 |
| Upper/Lower (cm) | 67/60 | 59/63.3 |
| Growth rate (cm/year) | 2.0 | 6.3 |
| Height SDS for BA | −3.17 | −2.60 |
| Height SDS for CA | −4.45 | −4.25 |
| Testicular vol (ml) | 2 × 2 | 3 × |
| Pubic Hair (Tanner) | 1 | 1 |

Table III: Results of Oxandrolone Therapy in Multiple Dysplasia

| | Pretreatment | One Year Postreatment |
|---|---|---|
| Chronological age (yrs) | 9 5/12 | |
| Bone age (yrs) | 7 | 8 |
| Height (cm) | 110 | 118 |
| Upper/Lower (cm) | 91/49 | 62/56 |
| Growth rate (cm/year) | 1.5 | 8.0 |
| Height SDS for BA | −1.92 | −1.43 |
| Height SDS for CA | −3.98 | −3.30 |
| Testicular vol (ml) | undescended | undescended |
| Pubic Hair (Tanner) | 1 | 1 |

## Discussion

Skeletal dysplasias are quite a common cause of short stature and have a wide sprectrum of severity. Most individuals affected with these disorders are recognized by their disproportionate short stature[1,2].

Spondyloepiphyseal dysplasia congenita is characterized by shortness in stature. It has an autosomal dominant mode of inheritance. Afflicted individuals have a short trunk, lag in epiphyseal mineralization and flattening of the vertebral bodies (Figs. 1b, c)[1,2,5-8].

Multiple epiphyseal dysplasia is one form of skeletal dysplasia. It is transmitted in an autosomal dominant mode of inheritance and has a wide variety of expression.

Short femoral neck, acetabular dysplasia, irregular epiphyses are some roentgenographical characteristics of the disease (Figs. 3b, c)[1,2,5-8].

Hypochondroplasia is another form of skeletal dysplasia and one of the causes of disproportionate short stature. Roentgenographically, it is characterized by narrowing or constant interpedincular distances of the lumbar spine, long distal fibula and short distal ulna (Figs. 2b, c).

An autosomal dominant mutant gene etiology has been implicated in this disorder. In some cases there is a direct inheritance pattern transmitted by affected individuals, while in the majority of cases, the presumed mutation seems to have ocurred sporadically and the unaffected parents are of advanced age.

Final height attainment in adults ranges between 118.5 cm and 153 cm[1,2,5-8]. To date, there has been no known medical treatment to improve the height of these patients except for the use of biosynthetic human growth hormone which was found to be promising in acceleration of growth velocity in hypocondroplasias[9]. Oxandrolone, an anabolic steroid, administered in low doses promotes pharmacologically stimulated growth hormone release and it may be that the growth-promoting effect is by a direct effect on the tissues or via an increase in growth hormone secretion or a combination of both[10,11]. Loche et al[12], have recently demonstrated that there is an increased growth hormone response to growth hormone releasing hormone (GHRH) during oxandrolone therapy. Stanhope et al[10,13], have hypothesized that oxandrolone in 1.25 mg/day and 2.5 mg/day dose regimens induces a sustained rise in physiological growth hormone secretion and that the mode of action of this drug in producing a sustained growth acceleration is to increase endogenous physiological growth hormone secretion. Oxandrolone is especially used to treat patients with constitutional delay of growth and puberty and patients with Turner syndrome. Its growth-promoting effect has been reported by investigators[10,14-16]. Three forms of skeletal dysplasia were described in our study in which oxandrolone was the anabolic steroid used to induce growth in our patients. No deleterious side effects were observed, and at the follow-up one year later, all patients were found to still be in the prepubertal stage.

Our results suggest, but do not prove, that oxandrolone therapy may be useful in the treatment of short stature in early pubertal boys with skeletal dysplasia. However, further studies are needed to determine the optimum dosage of the drug and long-term side-effects.

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# FETAL THYMUS DEVELOPMENT IN VITAMIN D DEFICIENT RATS*

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**SUMMARY: Yurdakök M, Hazıroğlu R, Topaloğlu H. (Department of Pediatrics, Hacettepe University Faculty of Medicine, and Department of Veterinary Pathology, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey). Fetal thymus development in vitamin D-deficient rats. Turk J Pediatr 35: 197-199, 1993.**

**The morphological characteristics of the thymus in vitamin D-deficient rats were evaluated in utero. The thymus weight, thymus weight/body weight ratio and histological findings were found to be similar when the vitamin D-deficient rats were compared with the control animals. It is therefore concluded that vitamin D is not required for the morphological development of rat thymus.** *fetus, vitamin D deficiency, rats, thymus.*

It has been demonstrated that 1.25-Dihydroxy-vitamin $D_3$ [1.25 $(OH)_2D_3$ inhibits the production of medullar thymocytes which mediate the maintenance of peripheral blood T lymphocytes, and that vitamin D prevents glucocorticoid-induced thymocyte death[1,2]. These findings suggest that vitamin D is involved in the maturation of the thymus, which prompted us to study the effect of vitamin D deficiency in the development of the thymus in animals.

## Material and Methods

A vitamin D-deficient state was induced in pregnant rats and the thymus of the offspring were examined. There-week-old weaning female albino rats were maintained in the absence of ultraviolet light on a vitamin D-free diet containing 1% calcium and 0.8% phosphorus and deionized water for four weeks[3]. The female rats were then mated with normal males. After copulation, the pregnant rats were maintained on the same vitamin D-free diet under the same conditions. until delivery. The vitamin D-sufficient control rats were obtained by rearing rats on a similar protocol with a diet containing vitamin D. On the day of delivery, all the animals and their offspring were sacrificed. Neonates were weighed, and their thymuses were removed, weighed and submitted for histologic studies. In addition, the proximal tibiae of the mothers and their offsprings were examined histologically to confirm the presence of rickets.

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## Results

There were 26 siblings in the control group, and ten in the study (vitamin D-deficient) group, with no deaths occuring in either group.

The birth weights (BW), thymus weights (TW) and TW/BW ratios were similar in both groups (Table I, $p > 0.05$).

Histological examinations of the thymus sections revealed that the shape of the lobuli, interlobular septae, cortices, medullar differentiation, number of cells and Hassall corpuscules were similar in both groups.

Table I: Thymus Weight and Body Weight in Study and Control Groups

| (n: 26) | Control Group (n: 26) | Study Group (n: 10) | Statistical Significance |
|---|---|---|---|
| Body weight (g) | 3.49 ± 0.33 | 3.53 ± 026 | I |
| Thymus weight (mg) | 2.41 ± 0.49 | 2.50 ± 0.53 | I |
| BW / TW | 0.69 ± 0.26 | 0.72 ± 0.18 | I |

BW / TW: Body weight / thymus weight.

I: Insignificant ($p > 0.05$).

## Discussion

The biological action of 1.25-dihydroxy vitamin $D_3$ [1.25-$(OH)_2$ $D_3$] on bone and mineral metabolism is mediated via its binding to specific receptors in the intestinal bone and kidney cells. Specific intracellular receptors for 1.25 $(OH)_3$ $D_3$ are also found in cells from other normal and neoplastic tissues, indicating that this hormone may have biological effects[4,5]. The larger, dividing medullary thymocytes, but not the smaller, nondividing cortical thymocytes have intracellular receptors for 1.25 $(OH)_2$ $D_3$ and Provvedini et al[1] have shown that medullary thymocytes which mediate maintenance of peripheral blood T lymphocytes were inhibited by 1.25 $(OH)_2D_3$. In another study, Cohen and Duke[2] have shown that vitamin D prevented glucocorticoid-induced cell death of thymocytes.

The results of this study demonstrate that the thymus of vitamin D-deficient rats in utero had no defects. Thus vitamin D is probably not required for the morphological development of the thymus. However, further studies are needed to evaluate the thymic functions of vitamin D-deficient rats.

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# TYPE I GLYCOGENOSIS WITH RENAL TUBULAR DYSFUNCTION (Presentation of Two Cases)*

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**SUMMARY: Yüce A, Coşkun T, Koçak N, Özsoylu Ş, Özalp İ, Göğüş S. (Gastroenterology and Metabolism Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Type I glycogenosis with renal tubular dysfunction (presentation of two cases). Turk J Pediatr 35: 201-204, 1993.**

**Two patients with hepatic glycogenosis associated with Fanconi syndrome are presented. Both patients were treated with a neutral phosphorus solution, an oral alkaline solution, cholecalciferol and uncooked cornstarch. The proximal renal tubular functions were corrected in the patient who used cornstarch properly, which may indicate a causal relationship between Fanconi syndrome and glycogenosis.** *Key words: glycogenosis, Fanconi syndrome.*

The association of Type I glycogenosis with proximal renal tubular dysfunction (Fanconi syndrome) is known to be a rare entity [1,2]. Of the 171 cases of gycogenosis studied in our unit, only two cases of proximal renal tubular dysfunction were found. Since this type of association is rarely seen, we decided to present the clinical and laboratory findings of these two cases.

## Case Reports

### *Case 1*

A two-year-old boy born to consanguineous parents was admitted to the hospital for evaluation of growth retardation. Physical examination revealed a child with a doll-like face. His height was 72 cm (below the 3rd percentile) and his weight was 10 kg (below the 3rd percentile). He had a protuberant abdomen and the liver was palpable four cm below the right costal margin. Clinical findings of rickets were present.

Laboratory studies revealed normal hemoglobin and leukocyte counts but the results of urinalysis showed glucosuria and proteinuria. Generalized aminoaciduria, hypocalcemia (6.6 mg/dl), hypophosphatemia (1.4 mg/dl), elevated alkaline

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phosphatase level (18.8 BU, control < 14 BU), hypoglycemia (46 mg/dl), elevated blood lactate level (19.6 mg/dl) and metabolic acidosis ($HCO_3^-$ 13.98 mEq/L) were detected. Hyperlipidemia (1180) mg/dl) and hypercholesterolemia (260 mg/dl) were present. Plasma urea, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), electrolytes, protein, and albumin values were all within the normal range. X-ray of the wrist showed active rickets. A liver biopsy revealed swollen and vacuolated hepatocytes and minimal fibrosis in the portal areas, consistent with glycogenosis.

The patient was treated with a neutral phosphorus solution, an oral alkaline solution, cholecalciferol (5000 U/day) and uncooked cornstarch (2 g/kg/meal). Six years later, his liver was plapable 10 cm below the costal margin. Aminoaciduria, glucosuria, proteinuria and rickets were no longer present and the exhibited accelerated growth.

*Case 2*

A four-month-old girl, the product of a consanguineous marriage, was admitted to the hospital for evaluation of growth retardation. Physical examination, revealed that all anthropometric measurements were below the 3$^{rd}$ percentile (height 56 cm, weight 4100 g). She had a doll-like face and protuberant abdomen. The liver, had a soft consistency, and was palpable 3 cm below the right costal margin. She exhibited clinical manifestations of rickets.

Laboratory data revealed that the hemoglobin and leukocyte counts were normal. Glucosuria, aminoaciduria and a trace of proteinuria were present. Calcium was 10.2 mg/dl, phosphorus 2.4 mg/dl and alkaline phosphatase was found to be elevated (627 IU/L). Hypoglycemia (25 mg/dl) an elevated blood lactate level (71 mg/dl) and metabolic acidosis $HCO_3^-$ 16.34 mEq/L) were noted. Total lipid (840 mg/dl) and cholesterol (242 mg/dl) levels were elevated. Plasma urea, uric acid, ALT, AST, electrolytes, protein, and albumin, however, were all within the normal range. Wrist X-ray showed active rickets. A liver biopsy revealed palely, stained, swollen hepatocytes, compatible with glycogenosis.

She was given a neutral phosphorus solution, oral alkaline solution, cholecalciferol (10000 U/day) and an uncooked cornstarch (2 g/kg/meal) diet. But she did not comply with the therapeutic measures. Therefore, persistent aminoaciduria, hypophosphatemic rickets and metabolic acidosis did not change in four years. Her liver became progressively enlarged, and was palpable 9 cm below the right costal margin. Her growth was significantly retarded.

## Discussion

Both our patients had marked hepatomegaly, a doll-like face, fasting hypoglycemia, metabolic acidosis, hyperlactemia and hyperlipidemia. Although enzyme

studies could not be performed to determine the type of glycogenosis, clinical and laboratory findings were compatible with Type I glycogenosis[3]. In addition to glycogenosis, typical findings of Fanconi syndrome characterized by glucosuria, generalized aminoaciduria, metabolic acidosis and hypophosphatemic rickets were present in these two cases. Although this association has been known since Fanconi and Bickel's first reported such a combination[4], we diagnosed only two cases with this combination out of 171 cases of glycogenosis over the past fifteen years.

Nephropathy characterized by glomerulosclerosis and proteinuria without glucosuria and aminoaciduria has ben described in older patients with Type I glycogenosis[5]. However our patients were in the pediatric age group. Renal biopsies, were not available in our patients. Since they had glucosuria and aminoaciduria in addition to proteinuria, we believed the underlying pathology was most likely different from that resulting in glomerulosclerosis. Although rickets may cause generalized aminoaciduria, our patients also had proteinuria and glucosuria which are not expected in rickets.

Case I, who had used the therapy properly, showed signs of improvement in tubular dysfunction, and also in the amount of proteinuria. Chen et al[2] also reported improvement in tubular dysfunction of a patient with Type I glycogenosis who had received intensive cornstarch therpy. They suggested that the low incidence of Fanconi syndrome found in patients with glycogenosis may be due to prompt initiation of a cornstarch diet.

Cornstarch therapy was also found to be useful in controlling the clinical and metabolic manifestations of Type I glycogenosis and researchers have suggested that it be used as therapy at an early age[2,6].

Although Fanconi syndrome has been known to occur in association with various types of glycogenosis[1,2,7-11], the underlying defect of this metabolic association is unknown. Some of these patients also have a defective galactose metabolism[9-11]. The association of Fanconi syndrome and abnormal galactose metabolism in glycogenosis has been explained by a mitochondiral defect[9].

We conclude that tubular dysfunction occurs rarely in hepatic glycogenosis and therapy consisting of uncooked cornstarch may be effective not only in the controlling the clinical and biochemical abnormalities of the disease, but may also control proximal renal tubular dysfunction. Improvement in proximal renal tubular dysfunction was observed in one of our patients after initiation of uncooked cornstarch therapy with glycogenosis which may indicate that there is a causal relationship between hepatic glycogenosis and Franconi syndrome rather than it being a coincidental finding.

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The Turkish Journal of Pediatrics 35: 205-207, 1993

# A CASE OF TRANSIENT HYPERPHOSPHATASAEMIA FOLLOWING VITAMIN D – DEFICIENT RICKETS*

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**SUMMARY: Kutilek Š, Štěpan J, Bayer M. (Departments of Pediatrics and Internal Medicine Charles University 1st Faculty of Medicine, Prague, Czechoslovakia). A case of Transient hyperphosphatasemia following vitamin-D deficient rickets. Turk J Pediatr 35: 205-207, 1993.**

**A seven-month-old boy with a diagnosis of vitamin-D deficiency rickets is presented. After treatment with vitamin-D, biochemical and radiological improvement was noted. At 14-months of age the plasma akaline phosphatase activity valve was found to be elevated even though rickets was not present. A diagnosis of hyperphosphatasemia was established. Although rare in infancy, transient hyperphospha tasemia may occur following vitamin-D deficient rickets. This possibility should be considered when evaluating laboratory results in such cases.** *Key words: vitamin-D deficient rickets, transient hyperphosphatasemia, alkaline phosphatase.*

Transient hyperphosphatasemia of infancy and early childhood is a rare syndrome characterized by a temporary, isolated elevation of alkaline phosphatase activity (ALP), values in particular the bone isoenzymes. Once coupled with metabolic bone disease, it might concern the physicien, however, such an association is purely coincidental.

## Case Report

The patient was a boy of gypsy origin who at birth weighed 2350 g. At the ages of one, three and five months, he was repeatedly treated for bronchopneumonia. Whether the child had received the usual vitamin-D (calciferol) prophylaxis was uncertain, since the family was constantly on the move and also because they were uncooperative.

At the age of seven months, he was admitted to our department because of bronchopneumonia which was treated successfully with Ampicillin. A diagnosis of vitamin D-deficient rickets was established. There was an elevation of bone isoenzyme in serum ALP activity and the X-ray of the wrist showed typical signs of rickets. The boy was treated with four doses of Ergocalciferol (300.000 IU at the age of eight months, dose). He was discharged at the age of eight months, after

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six weeks of hospitalization. The ALP activity had decreased to normal values (Fig. 1) and the wrist X-ray had showed improvement of the rickets.

The boy was followed up for two months. His serum calcium (Ca) and phosphorus (P) concentrations and ALP activity level were within reference ranges. The wrist X-ray taken at the age of nine months showed no signs of rickets. The family did not turn for a periodic follow-up, but the boy was again examined at the age of 14 months.

We were concerned because the serum ALP activity value was 126.9 ukat/1. The serum Ca, P concentrations and ALT, AST, GMT activity values were all within reference ranges and there were no signs of rickets on the wrist X-ray.

The $^{99}Tc$ bone scan was normal. The boy had no signs of hepatopathy, malabsorption or renal disease. 85% of the increased ALP activity was caused by bone isoenzyme. This activity has rapidly decreased (Fig. 1).

We found no causal relationship between the rickets and hyperphosphatasemia.

Since then, the boy has suffered three episodes of respiratory infections but there were no signs of skeletal or liver involvement.

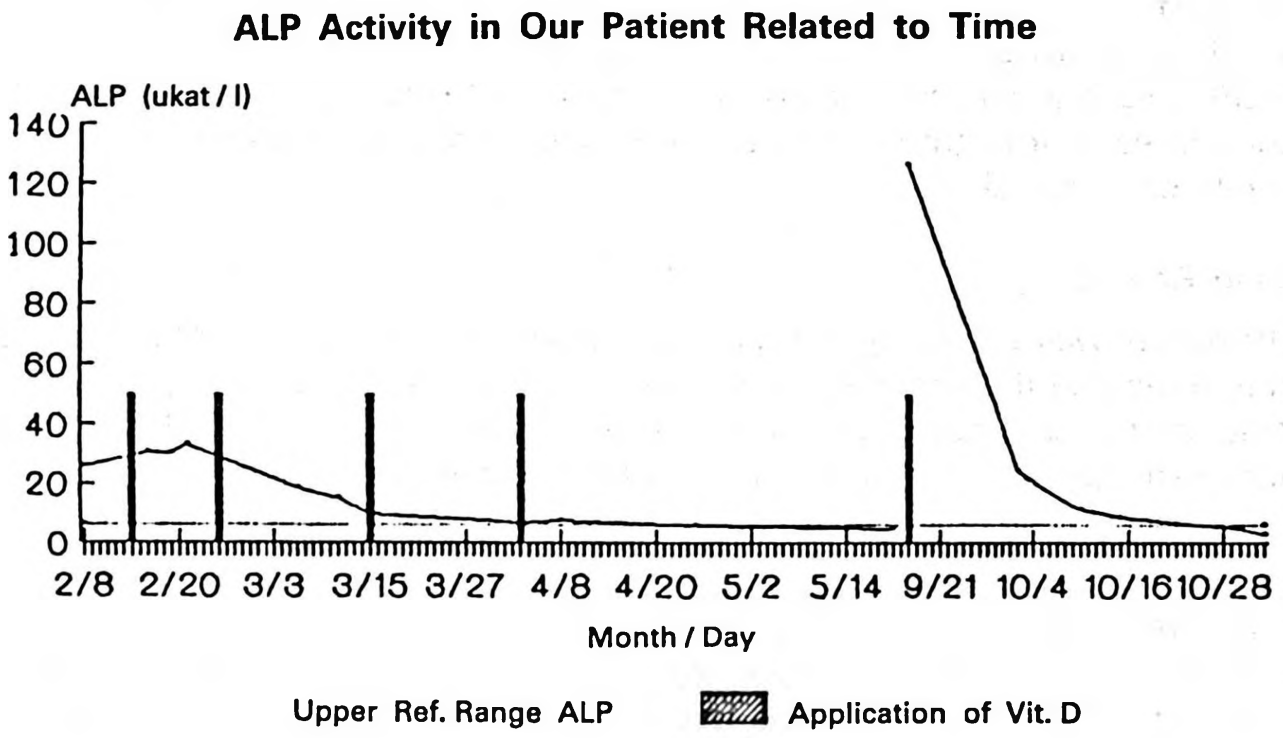


Fig. 1: Change in the serum alkaline phosphatase activity value following vitamin-D treatment and in the follow-up period.

## Discussion

The first report of transient hyperphosphatasaemia of infancy and early childhood was described in 1954[1], however more detailed studies and articles have been published in the last decade[2-6].

Kraut et al[4] has devised the following criteria for transient hyperphosphatasemia:

1. An age under five years.
2. Variable symptoms with no relation to ALP increased activity.
3. No evidence of bone or liver disease.
4. Elevation of serum ALP activity in bone and liver.
5. A return to normal of ALP activity valves within four months.

The etiology remains unclear. Three mechanisms may be responsible for the increased ALP activity in serum[6]:

1. Excessive synthesis or release of enzyme from its tissue of origin.
2. Activation of normal amounts of circulating enzyme.
3. Impaired clearance of the enzymes from the circulation.

Frank and Kruse[3] proposed that this syndrome had an infactious origin. A case of transient hyperphosphatasaemia in a girl with vitamin-D dependent rickets was described in 1986[2].

In conclusion, we reported the case of transient hyperphosphatasemia developing after vitamin-D deficient rickets, this with the aim of informing other physicians of this possibility, which is coincidental, but could arise in children with metabolic bone disease.

Abbreviations in The Text

ALP – Alkaline Phosphatase
ALT – Alanine-aminotransferase
AST – Aspartate-amino Transferase
GMT – Gamma-glutamyl transferase

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# TORTICOLLIS WITH HIATUS HERNIA IN CHILDREN*
## Sandifer Syndrome

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**SUMMARY: Şenocak M.E, Arda İ.S, Büyükpamukçu N. (Department of Pediatric Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey). Torticollis with hiatus hernia in children: Sandifer syndrome. Turk J Pediatr 35: 209-213, 1993.**

**Sandifer syndrome is an unusual combination of gastroesophageal reflux with or without hiatal hernia and torticollis. It can be considered to be part of an expanded syndrome of unusual presentation of gastroesophageal reflux. After successful surgical repair of the gastroesophageal reflux and the hiatus hernia, there is a gradual but permanent disappearance of the torticollis. An infant with this extremely rare syndrome is presented along with a review of the related literature.** *Key words: Sandifer syndrome, torticollis, gastroesophageal reflux, hiatus hernia.*

The Sandifer syndrome is an uncommon disease characterized by an unusual combination of gastroesophageal reflux (GER) with or without hiatal hernia and torticollis. Torticollis disappears completely after surgical correction of the hiatal hernia and gastroesophageal reflux.

In this paper a 5 ½-year-old girl with a history of torticollis, failure-to-thrive, vomiting, and anemia is decribed with the aim of reminding the clinician that this rare entity should be included in the differential diagnosis when investigating patients presenting with these symptoms.

## Case Report

A 5 ½-year-old girl was referred to our hospital because of a history of vomiting which began when she was four-months-old, and failure-to-thrive. The vomitus was mostly curdy, but had occasionally become blood-stained. She had been vomiting at least five times a day. Physical examination revealed a pale, irritable child with a weight of 8.1 kg (below the 3rd percentile). She had a left-sided torticollis, but the head could be brought easily to a normal position (Fig. 1). No abnormalities of the sternocleidomastoid muscle were found.

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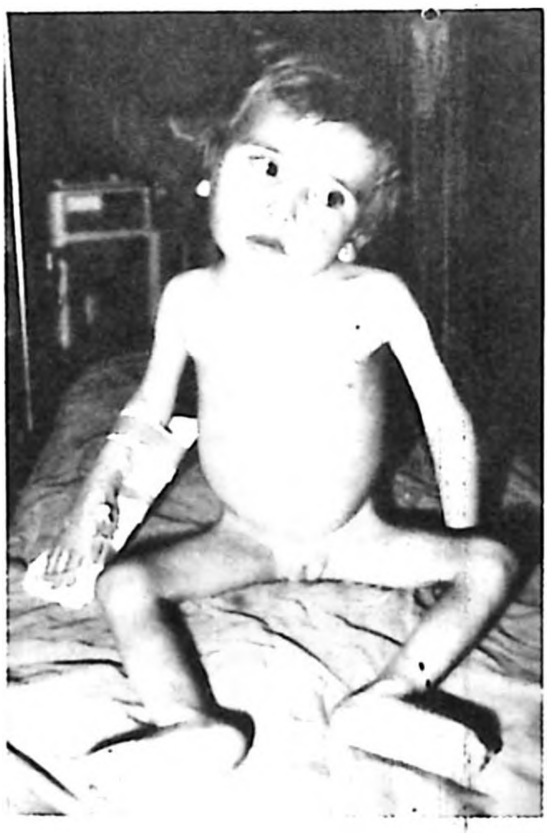

Fig. 1: Photograph showing left-sided torticollis.

The hemoglobin level was 7.3 g/dl, denoting an iron-deficiency anemia. Guaiac positive stools were noted. Roentgenograms of the cervical spine were normal and scoliosis was not present.

An upper gastrointestinal series revealed a small sliding hiatus hernia with evidence of reflux and esophagitis. The distal esophagus was moderately narrowed and irregular, suggestive of stricture (Fig. 2). Esophagoscopy at the time

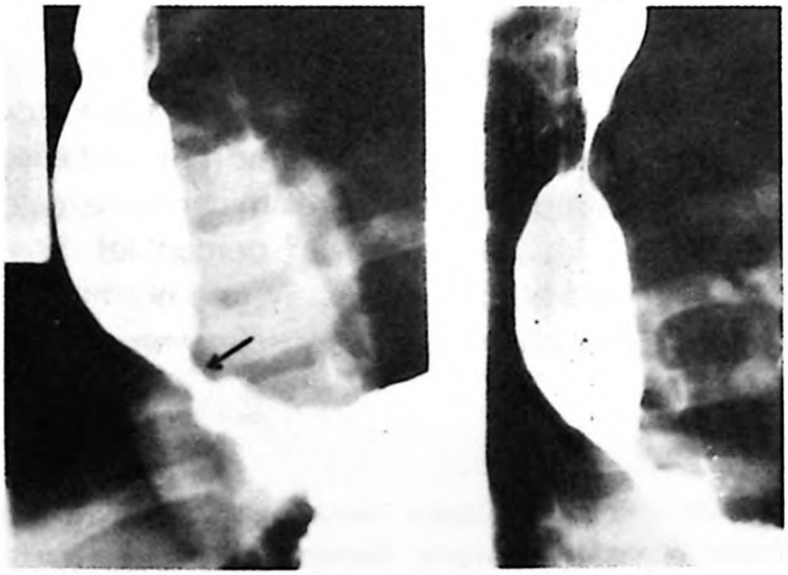

Fig. 2: Preoperative esophagogram demonstrating a small hiatus hernia with a moderately narrowed and irregular (arrow) distal esophagus.

of operation showed moderately severe esophagitis with bleeding in the lower one third of the esophagus.

At laparotomy, the entire thickness of the lower esophagus was inflamed and a small hiatal hernia was found. A repair of the hernia and a Nissen fundoplication were performed. The patient had a good recovery. There was a rapid gain in weight and the emesis ceased. Intestinal bleeding stopped post-operatively. Torticollis began within four months to gradually disappear almost completely without surgical intervention. Preoperatively the patient could only tolerate fluids, whereas at present, she heartily eats all kinds of food. A follow-up ten months later revealed that the patient was symptom-free. The barium swallow showed no evidence of hiatal herniation, GER, or esophagitis (Fig. 3). The head and neck postures were normal.

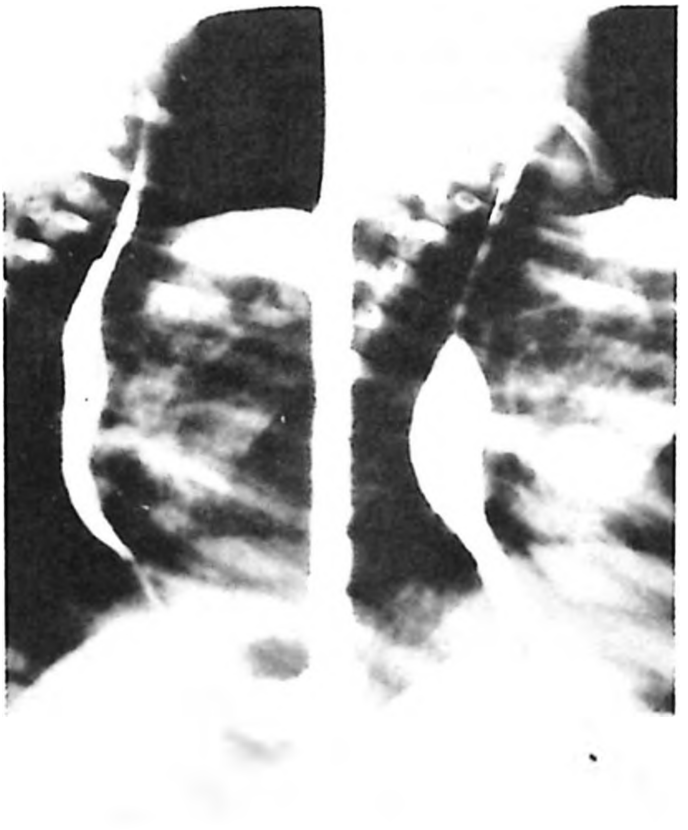

Fig. 3: Postoperative esophagogram showing normal passage of barium into the stomach without evidence of hiatus hernia or GER.

## Discussion

The exact incidence of GER in the general pediatric population is unknown. The initial symptom of GER in infancy is vomiting, and Carré reported it to be present in 90 percent of his patients by the sixth week of life[1].

The recognition of the unusual association of GER, with or without hiatus hernia, with torticollis and abnormal posturing of the upper part of the trunk has been

attributed to Dr. Paul Sandifer[2]. The original description of five patients with this syndrome was by Kinsbourne in 1964[3]. Since then additional cases have been reported[2,4-8]. This exceedingly rare syndrome was seen only in one of our patients during the last twenty years.

The presence of head tilt distinguishes this syndrome from other cases of hiatal hernia with reflux esophagitis and iron deficiency anemia. The cause of torticollis is still unknown. The disappearance of torticollis after successful repair of hiatus hernia and / or GER indicates that it occurs due to an attempt to relieve the pain of esophagitis resulting from GER[8]. We believe that torticollis causes the adoption of an abnormal posture because of repeated reflexive head tilting secondary to cronically occuring GER over a long period of time.

Since torticollis is a common problem in infancy, evaluation for GER should be made if none of the other causes listed in Table I can be documented. However, the normal sternocleidomastoid, muscle a and normal cervical roentgenographic examination should alert the physician to the possibility of GER and/or hiatus hernia as the underlying causes of torticollis. An esophagogram, radionuclide studies with Tc 99m sulfur colloid in feedings, 24-hour esophageal pH monitoring and / or esophageal manometric studies are sufficient to confirm the diagnosis.

**Table I: Causes of Torticollis**

| | |
|---|---|
| Abnormalities of the sternocleidomastoid muscle | (hematoma and / or fibrosis, congenital shortening) |
| Congenital anomalies of cervical vertebrae | (hemivertebrae, dysplasias, fusion anomalies) |
| Traumatic abnormalities of cervical vertebrae (rotatory subluxation) | |
| Inflammatory conditions of the neck (tuberculosis, rheumatoid arthritis) | |
| Tumors of the spinal canal or posterior fossa | |
| Abnormalities of extraocular muscles | |
| Abnormalities of the vestibular apparatus | |

Most patients reported in the literature with Sandifer syndrome have been treated surgically. After repair of the hiatus hernia and the GER, the gastrointestinal bleeding ceases immediately, and the iron deficiency anemia disappears within about six weeks. As mentioned above, the torticollis gradually disappears with complete and permanent return of normal motion and appearance of the head and neck by six months.

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The Turkish Journal of Pediatrics 35: 215-220, 1993

# OSTEOMYELITIS RELATED TO BCG VACCINATION: CASE REPORT*

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*Osman Küçükosmanoğlu MD*****, Namık Özbek MD****

**SUMMARY: Göçmen A, Özçelik U, Kiper N, Küçükosmanoğlu O, Özbek N. (Pediatric Thoracic Diseases Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Osteomyelitis related to BCG vaccination: case report. Turk J Pediatr 35: 215-220, 1993.**

**A nine-month-old girl with osteomyelitis related to BCG vaccination is presented. The patient did not have any clinical evidence of immunodeficiency. Her X-ray examination showed a lytic lesion of the right tibia and of the left ulna. Histopathological examination of biopsy material revealed a chronic granulomatous inflammatory reaction. She improved with intermittent short course antituberculosis therapy.** *Key words: BCG vaccine, osteomyelitis.*

Although BCG vaccination against tuberculosis is widespread in the world, very few complications have been reported following inoculation with this vaccine[1-3]. The most common adverse reaction to BCG vaccination is enlargement and / or suppuration of the lymph nodes, with drainage at the inoculation site[3-4]. There are also case reports describing subcutaneous abscess and localized osteitis caused by BCG vaccination[5-7]. It is estimated that the incidence of these complications is approximately 1 per 1 million vaccinees, but that the rates are higher in the Scandinavian countries, especially in Finland[5].

The above-mentioned should be considered in the differential diagnosis of bone infections in young children.

We wish to report a rare case of an infant with a lytic lesion in the ulna and in the tibia which improved after intermittent short course antituberculosis therapy.

## Case Report

A nine-month-old girl was admitted to the hospital for evaluation of swellings of the right knee and of the left elbow and limitation of movement. She was born in

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the hospital and her birth weight was 3200 g. She was vaccinated with BCG at one week of age. She was breastfed. When two months old, an enlarged left axillary lymph mode was noticed. At six months of age, it suppurated and healed within 15 days. She had no history of recurrent infections, oral moniliasis, diaper rash, or fever and neither was there a record of tuberculosis in her family. She had a 1.5 month history of right knee pain and had difficulty in taking steps. She subsequently developed swellings on her left arm and elbow. Prior to admission, she had received non-specific antibiotic therapy and a cast was applied, but no improvement was noted. Another physician, who suspected tuberculosis, recommended surgical intervention. The patient was noted to have had no weight loss, anorexia or night fever and sweats.

Physical examination revealed an infant in a good general condition. Her body temperature was 36.3 °C, pulse 156 / min, body weight 7300 g (10-25$^{th}$ percentile) and height 74 cm (75-90$^{th}$ percentile). There was slight scar formation in the left axillary area. Her right leg was painful and swollen. The left arm was tender and painful on palpation and there was swelling of the bony areas movement was restricted in both extremities.

Laboratory findings revealed a haemoglobin of 7.50 g / dl, and the white blood cell count was 12.400 / mm$^3$. The blood smear contained 26% polymorphonuclear leukocytes, 70% lymphocytes 3% monocytes, and 1% eosinophils. The thrombocyte count was normal. The erythrocytes showed hypochromia and microcytosis. Immunoglobulin concentrations were: Ig A:32.3 mg / dl (33.7 ± 12.6), Ig G:802 mg / dl (705.8 ± 168.2) and Ig M:168 mg / dl (104.2 ± 29). The nitroblue tetrazolium test was normal. Tuberculin and other skin tests were positive. Chest X-ray was normal, while X-ray examination of the extremities showed a lytic lesion of the right tibia and periosteal reaction and a lytic lesion of the left ulna.

On the second day of hospitalization, a biopsy was performed on the right proximal tibia. Periosteal thickening was detected. There was a small amount of fragile, lobulated, blood-tinged, white colored, material in the lesion. The Acid-fast bacilli stain did not yield any organisms and no growth was noted on the Lowenstein culture medium. Histopathological examination of the biopsy revealed a chronic granulamatous inflammatory reaction.

Isoniazid (INH) and rifampin (RMP) were each administered in a daily dose of 10 mg / kg for the first fifteen days. This dose was continued twice weekly for a period of nine months. Within the first three months, radiological studies revealed a definite improvement in the patient's condition. She was able to take a step. The local pain and swelling had completely disappeared. At the end of sixths month of treatment, the patient was able to walk without difficulty. Clinical examination revealed no pathological findings. Treatment was discontinued at the end of the ninth month.

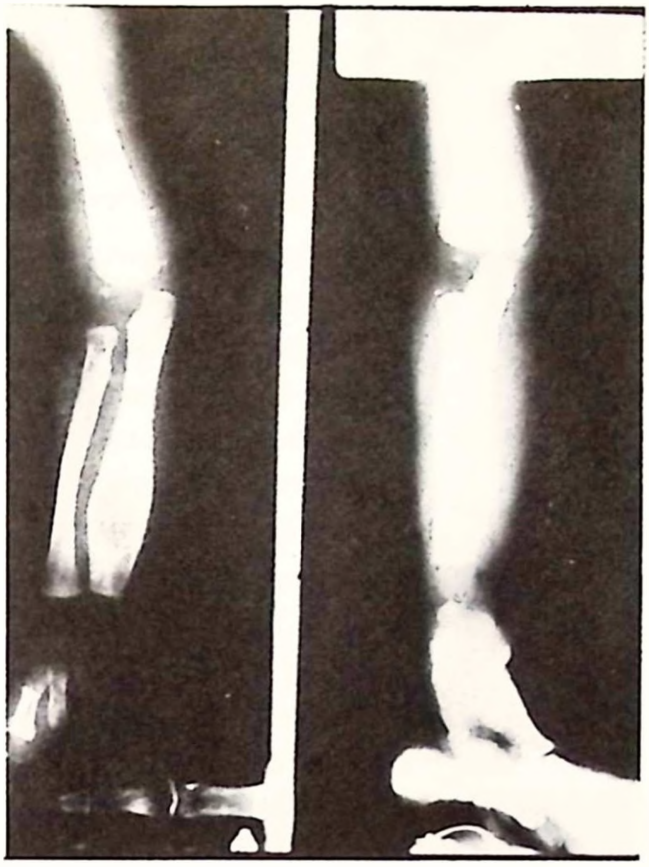

Fig. 1: Anteroposterior and lateral roentgenogram of left forearm. Single lytic lesion and periosteal reaction on ulna.

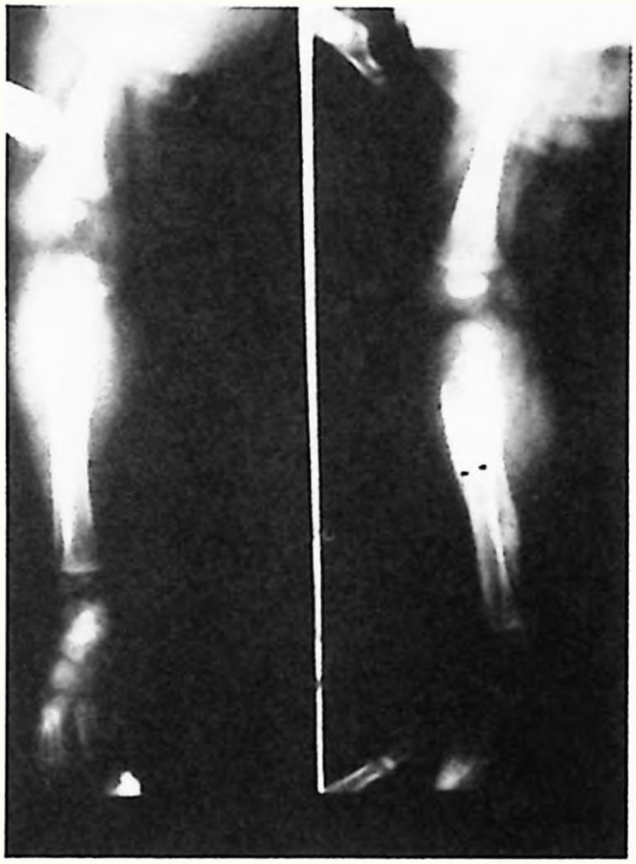

Fig. 2: Anteroposterior roentgenogram of lower extremities. Single lytic lesion on right tibia.

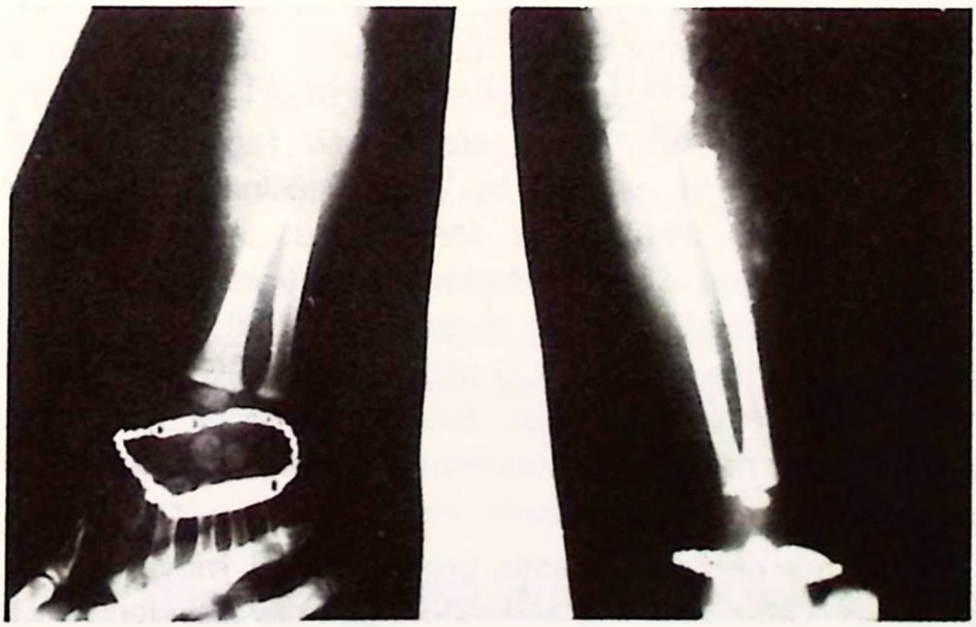

Fig. 3: Anteroposterior and lateral roentgenogram of left forearm (after treatment). There is no lytic lesion or periosteal reaction on ulna.

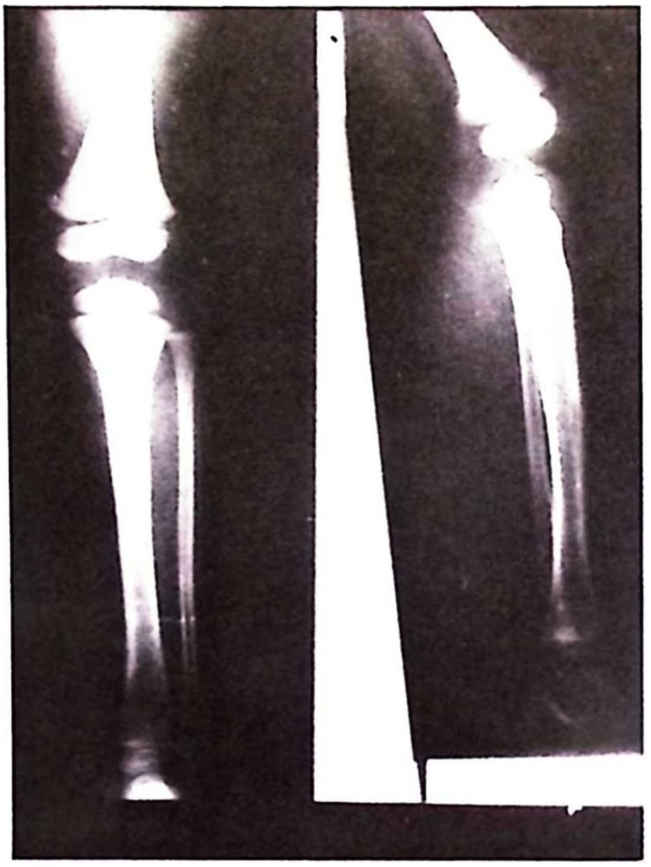

Fig. 4: Anteroposterior and lateral roentgenogram of lower extremities (after treatment). No lytic lesion is present.

## Discussion

There are few reports in the literature of severe and disseminated BCG complications, with the exception of thymolymphatic deficiencies with or without hypogammaglobulinemia or agammaglobulinemia, leukocyte and monocyte deficiencies with chronic granulamatous disease and immune deficiencies resulting from other factors[8-10]. Vaccination with BCG is a routine practice in our country as in many others[5]. There are a few reports regarding regional osteomyelitis complications caused by BCG vaccination which have been detected in the sternum, fibula, ulna, femur, talus and vertebrae. The most common site of localization is reported to be the extremities (70%)[1,5-7]. There is no male or female preponderance. It is presumed that in Finland and in other Scandinavian countries the rate of BCG osteitis and BCG arthritis is higher than 1 / 1.000.000 vaccinees[6], while in Spain, this rate is reported to be 1 / 80.000[7]. In young children, lesions may usually develop from several months to three years after vaccination.

The initial symptoms of osteomyelitis are restricted movement and pain on palpation of bones. Later, swelling and induration may develop[6,7,9]. Our patient, like most, did not show signs of thymolymphatic deficiency or hypogammaglobulinemia. However, the possibility of transient immunodeficiency caused either by

a viral, fungal or bacterial infection, should be considered in these types of cases[1]. The clinical findings in BCG-osteomyelitis progress more slowly than in staphylococcal or streptococcal osteomyelitis. In patients with BCG-osteomyelitis, the C-reactive protein value, leukocyte count and erythrocyte sedimentation rate are generally near normal[5-7].

There are no specific roentgenologic characteristics of BCG-osteomyelitis or BCG-arthritis. X-ray findings may resemble those seen in osteosarcoma and osteomyelitis. Radiologically, the typical lesion is a single lytic zone[6], which may have sclerosis or a periosteal reaction[6,7]. However, in our patient two lytic lesions were detected in different bones. The roentgenologic examination should include all extremities, the vertebral column and the lung. It is accepted that radiological improvement after three months of therapy is a common feature in BCG-osteomyelitis[1,5,6], which is consistent with our case. Even though reports in the literature suggest that therapy consisting of three drugs is nearly 100 percent successful[1,5-7], we used intemittent antituberculosis chemotherapy comprising two drugs, and we achieved a successful result. As to our knowledge, this is the first time such a regimen has been reported in the literature. This protocol consisted of a daily dose of INH + RMP for 15 days, which was followed by the same dose twice weekly for a period of nine months.

Despite the fact that the BCG species could not be identified in our case, as in many other cases reported in the literature[6,7], diagnosis was made on the basis of clinical history. There was no record of contact with tuberculosis bacillus, the chest X-ray was normal, and the X-rays of the extremities along with the histopathological examination were abnormal. In addition, the patient had a rapid response to treatment.

In conclusion, this condition should be considered in the differential diagnosis of osteolytic lesions in children vaccinated with BCG less than three years prior to the onset of symptoms.

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The Turkish Journal of Pediatrics 35: 221-226, 1993

# STUDIES OF IMMUNE RESPONSE IN A PATIENT WITH SELECTIVE COMPLETE C1q DEFICIENCY*

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**SUMMARY: Berkel Aİ. (Immunology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Studies of immune response in a patient with selective complete C1q deficiency. Turk J Pediatr 35: 221-226, 1993.**

**Immune response in a six-year-old girl with a rare complement defect, namely selective complete C1q deficiency, was studied. Her cell-mediated immune response (delayed hypersensitivity skin tests, E, EAC rosettes, in vitro lymphocyte transformation with phytohemagglutinin), isohemagglutinin titers, serum immunoglobulin levels, antibody titers to tetanus, Epstein-Barr virus, keyhole limpet hemocyanin, pneumococcus and bacteriophage ø x 174 were all normal. However, she had abnormal kinetics of the antibody response to bacteriophage following primary and secondary immunizations. Her antibody titers reached a peak four weeks after primary immunization and three weeks after secondary immunization, while the titers of controls peaked at two weeks and one week, respectively. In this study we could not prove the hypothesis that defective antibody response caused recurrent infections in this patient. An alternative hypothesis is the possible role of defective clearance of immune complexes in the development of severe infections. Patients with selective complete C1q deficiency form immune complexes with bacterial or viral antigens, thus activating the classical complement pathway. As a result, very low C1q levels decrease even further, leading to a severe immunodeficiency and recurrent infections. Future studies in this area may help to explain the mechanism(s) responsible for infections in this rare type of complement defect.** *Key words: immune response, complement deficiency, C1q deficiency.*

Complement defects are associated with the impairment of complement-related functions which result in decreased host resistance, leading to infections[1]. Selective complete C1q deficiency is a rare complement defect associated with vasculitis, lupus-like syndrome, infections and renal disease. Symptoms include malar rash, chronic skin infections, lesions of mucous membranes, fever and recurrent infections[2]. Most patients have circulating immune complexes and develop glomerulonephritis. Many die of either renal failure or serious infections, including sepsis and / or meningitis[3,4].

The aim of this study was to determine the immune response in a six-year-old girl with selective complete C1q deficiency who died of a sepsis-like illness, and to

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ascertain whether her infection was caused by an abnormal antibody response due to a complement defect. Our studies have shown that the patient's cellular and humoral immunity were normal except for abnormal kinetics of the antibody response to bacteriophage ø x 174. Her alternate pathway activity was intact[5].

Previously described methods were used to assess serum and surface membrane immunoglobulins (SmIg), isohemagglutinins, autoantibodies [smooth muscle (SMA), antimitochondrial (AMC), antinuclear (ANA)], antibody titers to Epstein-Barr virus (EBV), pneumococci and tetanus, E and EAC rosettes, delayed hypersensitivity responses [purified protein derivative (PPD), Candida albicans, streptokinase-streptodornase (SKSD), phytohemagglutinin (PHA)] and lymphocyte transformation (with PHA)[6]. Antibodies to bacteriophage ø x 174 and keyhole limpet hemocyanin (KLH) were determined by administering $2 \times 10^9$ plaque-forming units of bacteriophage / kg of body weight intravenously and a 1000 µg KLH (0.4 ml) intramuscularly[7,8]. The same dosage of bacteriophage was repeated six weeks later with the aim of detecting secondary antibody response. The patient was immunized against pneumococcus by a single 0.5 ml dose of pneumococcal vaccine (Pneumovax, Merck Sharp and Dohme, Westpoint, PA) which contained 14 pneumococcal serotypes, each one 50 µg. Serum samples were obtained at various intervals for antibody studies and were frozen and stored at −70 °C until assayed. Written consent from the parents was obtained. All antibody studies were performed four months prior to her death, while the patient was on maintenance prednisone therapy.

## Case Report

The patient's clinical findings were described previously[5]. She had been in good health until 4.5 years of age, when an erythematous malar rash and oral ulcerations developed. At the age of five, she suffered from episodes of fever, gingivitis, stomatitis, pyodermia and had a gluteal abscess. Following immunologic evaluation, a diagnosis of selective complete C1q deficiency was established on the basis of the absence of hemolytic complement ($CH_{50}$) and C1q activity. After a four-week-course of treatment with prednisone (2 mg / kg / day), the skin lesions and fever disappeared. However, they recurred each time corticosteroid therapy was discontinued. The patient was therefore put on maintenance prednisone therapy (alternate daily doses of 5 mg and 10 mg prednisone). The following laboratory tests were either normal or negative on several occasions: urinalysis, renal and liver functions, serum elctrolytes, LE cell, cryoglobulins, direct Coombs, hepatitis B surface antigen ($HB_s$ Ag), anti $HB_s$ antibody, antistreptolysin-O (ASO) titer, C reactive protein (CRP), erythrocyte sedimentation rate, nitroblue tetrazolium test (NBT), antidesoxyribonucleic acid (anti DNA), antiribonucleic acid (anti RNA), and anti-extractable nucleic acid (anti ENA) antibodies. Serum immune complexes were positive on several determinations. Two and one half years after

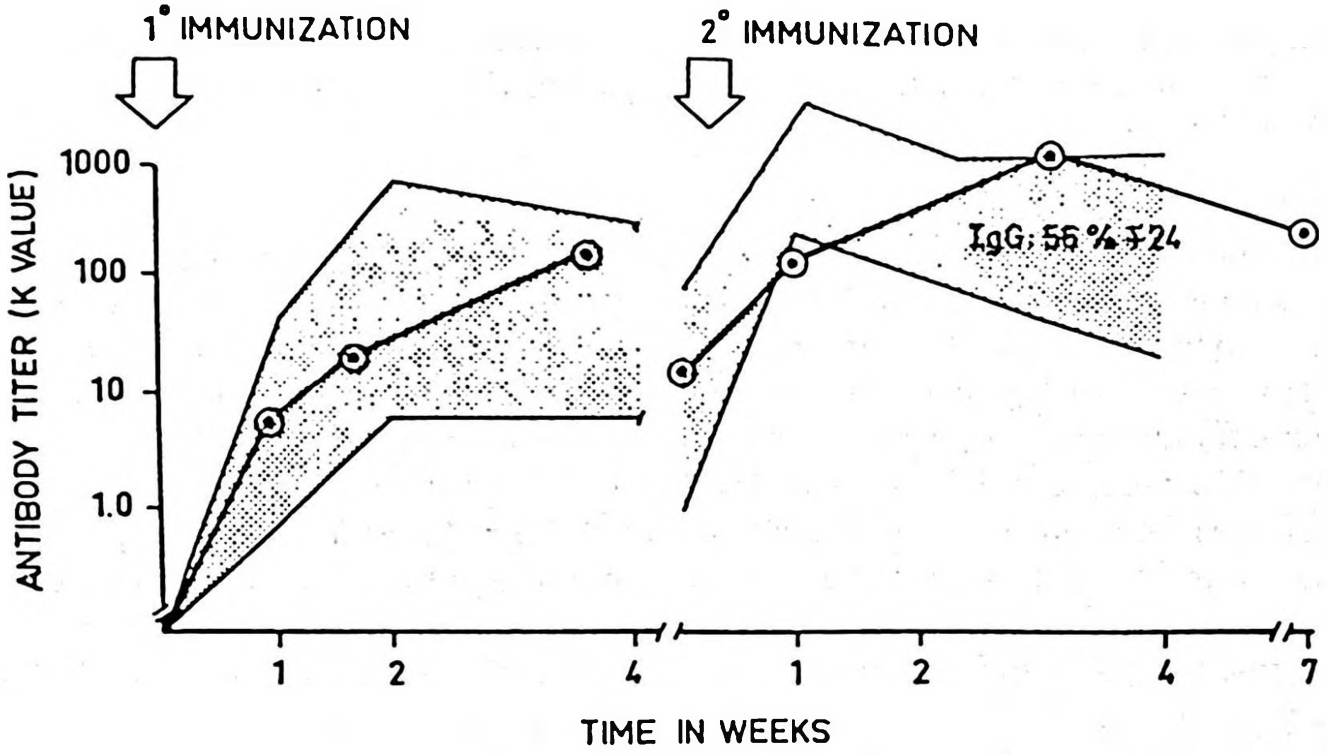
diagnosis, the patient died at home of a sepsis-like illness. Consent was not given for a postmortem examination. The family history for the disease was negative.

## Results

Cell-mediated immunity studies revealed positive delayed hypersensitivity skin reactions to two (PHA and SKSD) of four antigens tested. The percentage of E rosette formation was 52% (normal 65 ± 8%). In vitro transformation response to PHA was normal (patient 62.186 cpm; normal 28.989-116.682). The results of tests for humoral immunity revealed that 15.6 percent of circulating lymphocytes had C3b receptors (EAC rosettes) (normal 17.5 ± 6.1%) and 17.5 percent had surface membrane immunoglobulins (normal 14.6 ± 4.2%). The isohemagglutinin titer was normal (the patient was blood group A and the serum anti B titer was 1:32). Serum immunoglobulin levels were normal (IgG value was 1400 mg / dl; normal 680-1270 mg / dl, IgA 155 mg / dl; normal 33-148 mg / dl and IgM 155 mg / dl; normal 75-206 mg / dl). Antibody determinations revealed ANA to be positive while ASM and APC antibodies were negative.

Antibody response to tetanus toxoid showed a four-fold increase from the initial value of one unit rose to 16 units which comprised the IgG type (normal is four-fold increase or more). Antibody titers of the IgG type against various antigens of EBV were normal: anti-viral capsid antigen (VCA) 1:80 (normal $> 1:10$), anti-early antigen (EA) diffuse (D) $< 1:10$, restricted (R) $< 1:10$ (normal $> 1:10$ in acute infection), anti-nuclear antigen (EBNA) 1:80 (normal $> 1:10$). Antibody response to eight out of 12 tested pneumococcal serotypes (types 3,4,7,8,14,18,19,23) was normal (more than a 400 ng / ml increase from the pre-immunization level). The response was low for serotypes 1,6A,9 and 12, although the patient had protective levels (over 200 ng / ml) for serotypes 9 and 12. The patient had a good IgM and IgG antibody response to KLH when compared with the serum titer of the immunized healthy controls. IgM antibody titer was positive for seven successive serum dilutions starting with 1:50 (normally positive for fewer tubes in serial dilutions). IgG antibody titer was positive in diluted serum (normally positive in serial dilutions).

The patient's antibody response to bacteriophage ø x 174 is seen in Figure 1. She had a normal clearance of phage and showed a near normal primary response, the highest titer being observed at about four weeks while the peak titer in the normal controls was reached at two weeks. Her secondary response showed a high antibody titer of 1023, which was reached at day 22. The peak secondary response of the normal controls was at day 7. She was able to produce IgG. The sample obtained 22 days after the secondary immunization contained 22% IgG (Fig. 1).




Responses to primary (1°) and Secondary (2°) immunization with Bacteriophage øx 174 in normal controls (shaded areas) and in the patient with selective complete C1q Deficiency (0 circles). Primary and secondary responses were present in the patient, but were rather delayed. Usually the peaks of the primary and secondary antibody responses are at two weeks and one week respectively, while these peaks in the patient were at four weeks and three weeks. In the controls 56 ± 24 percent (mean ± 1 S.D.) of the antibody in the secondary response consisted of IgG whereas the patient had a value of 22%.

## Discussion

Complement plays an important role in host defense against viral and bacterial infections. In many instances, complement deficiencies have been associated with increased susceptibility to infection[1]. This was also observed in the patients with selective complement C1q deficiency whom we have followed. Three of these patients died of sepsis or meningitis and one of bronchopneumonia and renal failure[2,4]. The role played by antibody deficiency in susceptibility to infection has not been investigated due to the limited number of individuals with this defect.

Complement defects which occur naturally provide an opportunity to evaluate the role of complement on antibody response. Previous studies have suggested that normal function of the early components of the classical complement pathway has an important role in the induction of T dependent antibody production. A reduced antibody response to T dependent antigens has been observed in $C_4$ deficient men and animals, and also in mice following complement depletion with the cobra venom factor[9-11]. In vivo complement depletion suppresses the production of IgG and other T dependent antibody classes much more than the

relatively T dependent production of IgM[11]. Once normal priming has occurred, depletion during secondary immunization has a less marked suppression effect on the antibody response[11].

In our patient, we found no abnormality in cellular or humoral immunity except for abnormal kinetics of the antibody response to bacteriophage. The titers increased continuously during the primary response (in controls, antibody titers persistently peaked at two weeks post phage injection) and after secondary immunization, the peak was observed at three weeks rather than at one week (Fig. 1).

The C1q level in patients with selective complete C1q deficiency is very low but not zero (about one percent of normal)[3], and the half life of C1q is short (3.4 hours)[2]. The low C1q level may be sufficient to ensure formation of enough antigen-antibody-complement (C3b) complexes to stimulate antibody production and induce maturation of antigen specific memory cells. Therefore this study did not confirm our hypothesis of a defective antibody response as the mechanism for recurrent infections in this patient. An altenate hypothesis is the possible role of defective opsonization in the development of sepsis or other severe infections in this deficiency. Patients with complement defects may have defective clearance of antigen-antibody complexes by the reticuloendothelial system, due to failure of opsonization of immune complexes resulting from complement deficiency[12]. In our patient, circulating immune complexes were positive on several occasions. It has also been shown that early components of complement are sufficient for viral neutralization[13,14], and C1q may react directly with viral particles[15]. It is conceivable that our patient formed immune complexes with some viral antigens utilizing a small amount of C1q which results in the activation of the classical complement pathway and brings the already low C1q level to an even lower or maybe undetectable level leading to severe immunodeficiency and recurrent infection. In addition to normal cellular and humoral immunity, activation of the alternative complement pathway may also compensate for this. In order to prove the latter hypothesis, comprehensive immunologic studies in patients with selective complete C1q deficiency are necessary and may offer some clues for the mechanism(s) responsible for infections in this rare type of complement defect.

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# CHILD HEALTH THE GENOME PROJECT AND PHENYLKETONURIA*

*Charles R. Scriver MD***

**SUMMARY: Scriver CR. (deBelle Laboratory for Biochemical Genetics, McGill University Montreal Children's Hospital Research Institute, Montreal, Quebec Canada). Child health, the genome project and phenylketonuria. Turk J Pediatr 35: 227-237, 1993.**

**Child health and life expectancy improved together in many societies in the 20th century. As the incidence of diseases with major extrinsic causes declined, the heritability of disease as a whole increased. Accordingly, the genetic (intrinsic) causes of disease have become important. The genome project is an international venture out of which will come new knowledge about the biological basis of health and disease, and technologies to apply that knowledge in medicine and other disciplines. Phenylketonuria (PKU) at one time was seen only as a rare inborn error of metabolism causing severe mental retardation. Yet, the gene frequency is about 1% in Caucasian and Oriental populations, higher still in some populations with particular histories that have enhanced gene frequency. These genetic facts make PKU a paradigm in human genetics. This genetic disease, for which it was thought there was nothing to be done, has yielded to inquiries at clinical, metabolic, protein (enzyme) and DNA (PAH gene) levels. Results have shown that, early diagnosis (by population screening) and treatment (by low phenylalanine diet) largely prevents mental retardation. DNA analysis reveals particular associations between PKU mutations, RFLP haplotypes and populations; these associations are relevant for counselling. PKU illustrates well how medical science, molecular biology and gene mapping can improve knowledge and contribute to child health.** *Key words: heritability, genomics, phenylalanine hydroxylase, PAH gene, phenylketonuria.*

When social conditions and the quality of life improve in human society, a consequence is increased heritability of the diseases that continue to affect individuals and families. Increased heritability, in this sense, means increased relevance of genetic causes of disease among all causes. Another consequence

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The text is a summary of two lectures delivered at Hacettepe University Medical School on June 8 and 9, 1993. The lectures were entitled: From Følling to Nucleotide Sequences and Back; and Child Health in the Year 2000. The relevance of The Human Genome Project.

Many of the themes referred to here are documented in Development Brain Dysfunction Vol: 6 (Parts 1-3), 1993 an issue of that journal carrying an excellent article on PKU in Turkey and addressing all major themes.

of improved quality of life is decline in the perinatal mortality rate, improved neonatal survival rates and increased life expectancy. The result will be a change in population structure. The former relative excess of persons of pre-reproductive age will decline and there will be a relative increase in older-aged subjects. It could be said that improvements in child health care will increase the need for and relevance of knowledge relating to geriatric illnesses and the biological problems of senescence.

A necessary consequence of increased longevity, in the absence of any other change in population dynamics, will be population growth. This is one of the major problems facing the human species in some parts of the world now and on planet earth as a whole in the future. The most humane option to curtail population growth is the "fifth freedom"; it is the option to control reproduction in the context of personal choice.

These events, as I have described them, are some of the results of increased knowledge relating to health and disease and the applications of that knowledge. It has been said that science is an attack on ignorance and that what is not known is more important in science than what is known. Society tolerates scientists and their activities on the understanding that the facts will be converted into innovations, technologies and other applications to benefit society and individuals. To put it another way, the legacies of science are hypotheses (arrays of concepts), databases (arrays of facts), and innovations (arrays of technologies). In their particular way, the health sciences reflect this broad view. The lectures gave two illustrations: 1. The disappearance of mental retardation associated with phenylketonuria; and 2. The Human Genome Project.

*A Place for Molecular Genetics and Biology in Medicine:* The Medical Research Council of Canada (MRC) recently published its strategic plan. The document was written following a national consultation with scientists and the public. It contains some interesting phrases; here are two:

"Our potential for understanding the genetic bases for health and disease has never been greater. The prospective diagnostic and therapeutic benefits of molecular biology, including the evolution of stunningly creative frontiers of biotechnology, seem practically boundless. In it self, the multinational Human Genome Project, in which MRC is an active partner, promises to give us a virtual atlas of the human gene family, whose orderly expression is central to life and health".

"The boundaries of disciplines overlap, are redrawn as common, erode, disappear. The system is pluralistic in other ways. Research takes place in a wide variety of settings, from "dry labs" (office areas), to "centers of excellence" that may exist only electronically. The range of specialists, contributing expertise to

health issues, now includes lawyers and philosophers, engineers and physicists, clergy and social scientists, even historians".

The evolution of our knowledge about PKU and its applications in the past half decade in Quebec, for example (and in Turkey now), well illustrate these points of view. As for the Genome Project, which has been a cause for concern among many scientists and citizens, *The Economist* had a much more sanguine view (The Economist, Oct. 24, 1992, p. 96-98). The writer of the article entitled "The end of the beginning" expresses the opinion that the Genome Project is a search for data which in due course will be converted into tools for science, including the health sciences. Two interesting comments appear in the article.

"An entirely new field is being born, one that takes as its study not individual genes, but sets of them: genomics as opposed to genetics".

"When the genome project's nominal goal is reached, and a complete human genome is sequenced, nobody will notice. They will be too busy ..... finding out what it does and how it does it.

It is worth noting that a new journal with the title *Genomics* was established only 6 years ago and is now overflowing with articles every month. In the case of knowledge about PKU, it has advanced more rapidly since the mid-'80s, when tools were made available to study mutant genotypes and their expression in PKU, than in the previous 50 years following discovery of the disease in 1934.

*Phenylketonuria: Multiple Beginnings and Endings:* Phenylketonuria (PKU) is a classical Mendelian disorder; by this we mean that its principal cause is mutation in a single gene. On the other hand, PKU is a classical multifactorial disease; by this we mean that it has an equally important nutritional component of cause. The mental retardation associated with the Mendelian disorder is dependent on exposure to an essential nutrient: L-phenylalanine in the protein component of the diet. This fact permits treatment of the Mendelian disorder and the prevention of mental retardation by restriction of dietary phenylalanine. In brief, mutation and experience are each necessary and sufficient causes of the mental retardation in PKU[1].

PKU illustrates three important strategies in the study of genetic diseases. The first identifies, by segregation analysis, that a particular phenotype (disease) behaves as a Mendelian trait; in the second, one can work inward from the clinical disease to dissect phenotype at the levels of metabolite, biochemical reaction and polypeptide. With molecular biological techniques, one can get to the gene from the polypeptide through the corresponding cDNA. In the third approach, called reverse genetics by some people and positional clone (or expression cloning) by others, one works outward from the gene to explain the various levels of phenotype. The history of PKU illustrates all of these strategies.

*Historical Perspectives:* Asbjorn Følling discovered PKU in 1934. The story is well known. It is worth remembering, however, that this man, carefully trained in chemistry and nutrition and who had read articles by Garrod, used his knowledge and training to study the new disease. He recognized: a chemical signal for the disease; its origin in a disturbance of phenylalanine metabolism; the relationship between dietary phenylalanine and dishomeostasis; an association between the disorder and mental retardation; occurrence of the disorder in siblings; and a segregation pattern compatible with autosomal recessive inheritance. Dr. Følling's letter describing the new disorder brightened the final days of Garrod's life. Garrod was experiencing the loneliness of the long distance runner, and his concepts were not being used by colleagues in medicine. The fact that someone else now recognized their relevance was warmly recognized by Garrod.

Følling also identified the pathway affected by the abnormality in phenylketonuria. Others came to investigate that and other aspects of the disease. Judah Quastel and Lionel Penrose contributed to that knowledge and gave the name by which the disease is now known. George Jervis, among others, identified the enzyme defect and demonstrated a deficiency of phenylalanine hydroxylating activity in patients' livers. Seymour Kaufman, who has devoted more than a quarter century to delineating the precise defect in phenylalanine hydroxylase activity, opened up our knowledge of the hydroxylating reaction, thereby setting the scene for the discovery of other Mendelian disorders at other loci affecting components controlling the synthesis and recycling of the tetrahydrobiopterin cofactor.

Lionel Penrose was a contributor to many of these developments. As a geneticist whose particular interest was hereditary forms of mental retardation, he developed many important questions relevant to our analysis of PKU as a biological problem. It was Penrose who affirmed the necessary and sufficient conditions in the pathogenesis of the disease. He predicted that PKU would be treatable[2]. He also asked a fundamental question: If the disease has such an effect on the homozygote, and it is autosomal recessive, why is the gene so frequent in human populations? (He recognized that its frequency was about 1%). He also asked why this gene was not randomly distributed in human populations. (He noted a predilection for Celtic-derived populations). These fundamental questions[2] could not be answered until the era of molecular genetics had arrived. In the mean time, Penrose opened the door to proposals for the early diagnosis and treatment of PKU.

*Screening and Treatment:* Simple technological innovations made mass screening of the newborn infant to detect a chemical signal (hyperphenylalaninemia) a feasible social option. Robert Guthrie and colleagues were responsible for the most widely used test-a bacterial inhibition assay. It can be said that newborn screening for PKU and allied disorders has become the most widely applied genetic technology in human history. Society sees it as beneficial.

The rationale for newborn screening, in this case, is to achieve early diagnosis and an opportunity for intervention with a treatment that will prevent incipient disease from becoming established. The rationale was translated into practice when it became known that, by the use of a semi-synthetic diet low in phenylalanine, the metabolic phenotype could be controlled and near-normal homeostasis restored. The credit for this important development goes primarily to Louis Woolf, Horst Bickel, Marvin Armstrong and their colleagues. Thereafter it was shown that initiation of the diet within the first month of life was compatible with normal or near-normal intellectual development in the affected patient. Two important new developments in our understanding of PKU followed:

It soon became apparent that neonatal hyperphenylalaninemia is not always the equivalent of PKU. Some forms of hyperphenylalaninemia associated with deficient phenylalanine hydroxylase activity have only a mild metabolic phenotype-sometimes called non-PKU hyperphenylalaninemia. We have tended not to treat these persons. Other forms of hyperphenylalaninemia are associated with mutations at loci encoding the enzyme responsible for synthesis of tetrahydrobiopterin and the enzyme associated with recycling of dihydrobiopterin. Once the tetrahydrobiopterin-deficient forms of hyperphenylalaninemia were recognized, one could never counsel the patient and family with confidence until they were ruled out. Accordingly, national programs must now have access to the diagnostic tests to identify disorders of tetrahydrobiopterin synthesis and regeneration.

Another problem surfaced; it was maternal hyperphenylalaninemia. Half the patients with hyperphenylalaninemia are female. If the phenylalanine concentration is not in the normal range when a female conceives and carries a fetus, the product of that conception is at high risk of impaired brain development, microcephaly and other congenital malformations. The major legacy of successful PKU-prevention programs in the first generation is the need for effective programs to prevent the consequences of maternal hyperphenylalaninemia in the second. Most national programs now have registers of patients with hyperphenylalaninemia and prospective programs for the management of women at risk.

*Phenylalanine Hydroxylase Genotypes:* A new era of knowledge began when Savio Woo and colleagues cloned the PAH gene-first in rats, then in humans. It was shown that mutation at a single locus was sufficient to explain classical PKU; that one polypeptide was sufficient to form an effective homopolymer with phenylalanine hydroxylating activity in the presence of tetrahydrobiopterin; and that mutations causing PKU mapped to the PAH locus on chromosome 12q22-24.1.

The PAH gene encompasses more than 90kb of nucleotide sequences has a promotor region recently characterized, contains 13 exons, and has a suite of

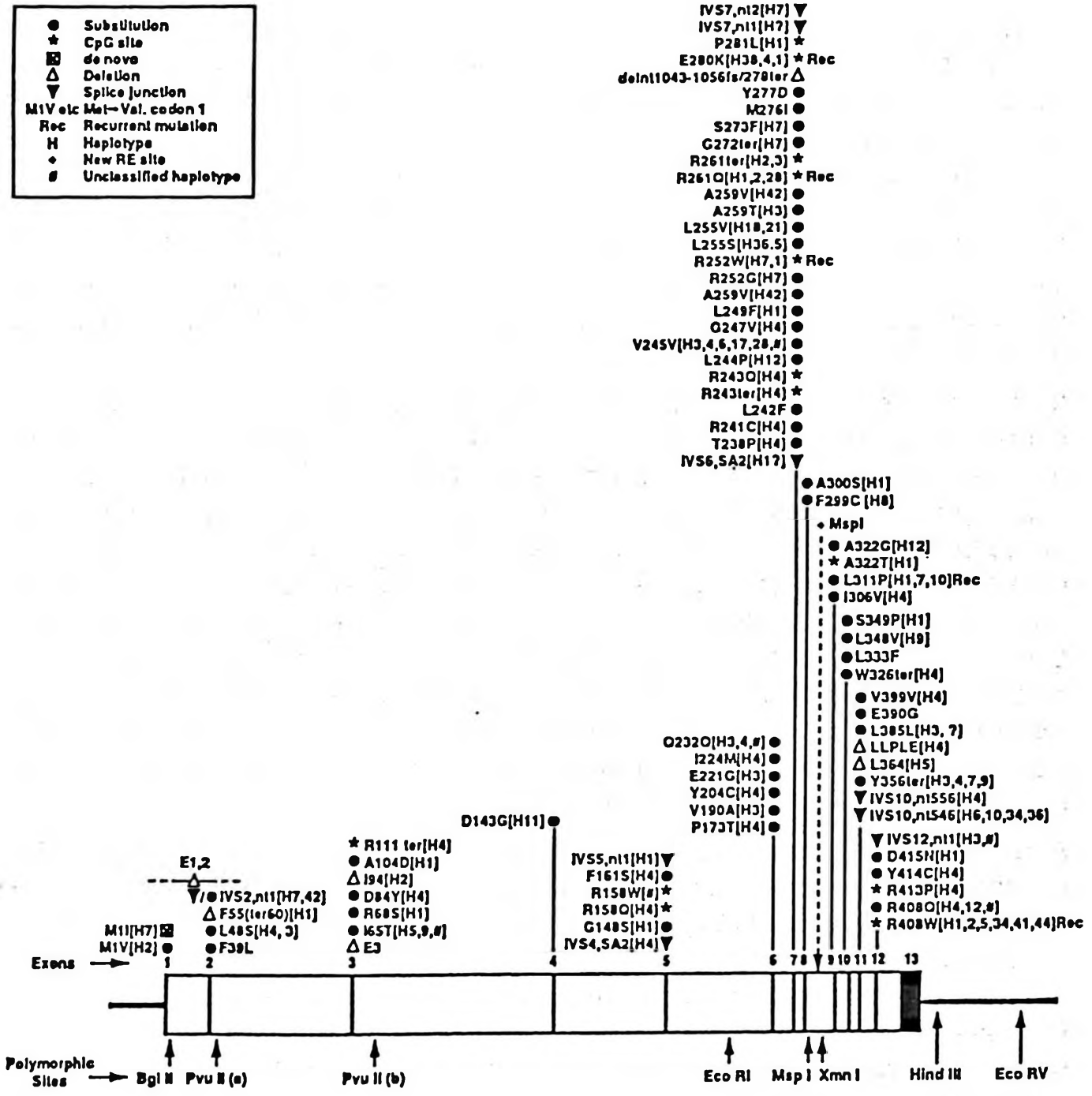


Fig. 1: Distribution of exons along 90 kb of genomic DNA encoding the human PAH gene in the chromosome region 12q.22-q24.1. Details about the 5' promoter region, not shown in the figure, are now available. Polymorphic restriction sites used in RFLP haplotypes are shown below the gene. Exons are numbered in Arabic numerals above the gene. Alleles are named by the following conventions. For missense mutations, the first letter and number following indicate the normal amino acid residue and codon number, respectively; the letter following is the replacement residue. For nonsense mutations, the suffix 'ter' (termination) is used after the codon affected. For deletion mutations, the prefix symbol, Δ, or the letters del (deletion), indicate the residue or exon affected. For splice-junction mutations, the intron affected (e.g. IVS2), the nucleotide involved (e.g. nt1,nt546) or region of the intron (e.g. SA2 for 3' end of junction) are named. Other symbols are explained on the figure.

polymorphic markers-7 of which are diallelic and one (in the Hind III region at the 3' end of the gene) is multiallelic. These markers provide informative haplotypes. Analysis of the gene with the corresponding probes and primers for PCR amplification of segments within the gene has identified a great many mutations associated with the phenylketonuria phenotype (Fig. 1); over 100 are now known. The mutations are non-randomly distributed in human populations and have both inclusive and exclusive associations with the RFLP haplotypes. Accordingly, it is possible to analyze the PAH locus and to study their histories and the corresponding histories of the populations with which they associate. The PAH locus has thus become very powerful for the study of human population genetics.

The molecular information has been put to work in other ways. For example, expression analysis of constructs has identified that some PKU mutations generate null phenotypes of unstable proteins or non-functional homopolymers. Others affect the specific activity of the enzyme, and these are of particular interest because they will identify residues essential for enzyme activity. There is correspondence between the severity of the mutational effect on enzyme activity, the degree of hyperphenylalaninemia in the absence of treatment, and the requirement for restriction of phenylalanine in the diet[3]. These broad correlations have predictive value for management of the patient.

Mutation analysis shows that some mutations are public in their distribution, meaning that they are prevalent in particular geographic regions and populations, e.g. R408W, IVS12,ntI, R261Q and IVS10,nt546 in Europe. Careful analysis of the association between mutation, haplotype and population is beginning to reveal information about the origns of these mutations in human history. Exon 7 is an important region in the gene, and mutation in this exon is a prevalent cause of PKU not because this exon is hypermutable, but probably because it is critical for the function of PAH. A number of mutations affect CpG dinucleotides, a hypermutable sequence in the human genome. Deletions or duplications as causes of mutation, are not unusually frequent in the PAH gene. Recombination within the gene may explain the occurrence of particular mutations on a different haplotype (e.g. R408W on haplotypes 1 and 2). But R408W also occurs on haplotype 44 in Orientals; here, recurrent mutation at a CpG dinucleotide is the likely explanation. Mean while, by means of the relevant PKU mutations, it may be possible to date the origin of recombinant European chromosomes. The findings are important and we are reminded that Penrose posed the corresponding questions long ago.

*Histories of Genes: Histories of Populations:* One of the hypotheses posed by Penrose was that PKU had a Celtic origin in Europeans. (Penrose did not know about the exceptionally high prevalence of PKU in Turkey nor about its occurrence in Orientals at frequencies resembling those in Europeans). In this regard, our

own analysis of PKU mutations in Quebec populations is relevant. Quebec represents a region founded by Europeans in the New World, first by French settlers in the 17th and early 18th centuries, then by British settlers between the mid-18th and-20th centuries, and finally by non-French Europeans and others in the late 20th century. These various founding families and their descendants have clustered in different geographic regions of the province. Accordingly, the molecular analysis of contemporary PKU chromosomes is likely to reveal stratification-and it does. At three regional centers caring for PKU patients identified by the provincial newborn screening program, we found three sets of PKU patients with three different sets of mutations. PKU, at these different centers, is three different sets of diseases according to its cause (mutation). These findings may have implications for the refinement of treatment.

We have found something else. We noticed that some mutations are actually shared by the three populations with their distinctive historical origins. At first this was puzzling, until we recognized that one of the shared mutations (IVS12nt1) had a broad European origin compatible with the origins of all varieties of founders. Another shared mutation, R408W on haplotype 1, could be traced to ancestors (Irish or Scottish) of Celtic origin in each family. Accordingly, we hypothesized that this particular mutation, very common in Quebec, was in fact the "Celtic chromosome" proposed by Penrose. Subsequent studies (Eisensmith, personal communication, 1993) show that R408W on haplotype 1 is the major PKU mutation in contemporary European populations with strong Celtic ancestry. Studies such as this indicate how founder effects and subsequent genetic drift explain the origin of PKU in populations whose histories are compatible with expansion in relative genetic isolation. I suspect that analysis of PKU mutations in Turkish populations will yield analogous findings and will have the corresponding relevance for the management of the disease.

*New Beginnings:* Evidence is accumulating that eraly diagnosis and treatment, with the best possible methods now available, does not accomplish all that we would desire in PKU. Abnormalities are identifiable by magnetic resonance imaging in the brain of treated PKU patients. These abnormalities are fewer, the better the treatment is. They imply that we must refine treatment, be more rigorous with it, and probably impose more burdens on families. It is not surpising, then that among affected families there is a gradually increasing demand for prenatal diagnosis. This demand is already apparent in Turkey. Until recently it was not possible to contemplate prenatal diagnosis for PKU. Genotype analysis now makes it possible. Identification of heterozygotes at risk in families is technically difficult at the phenotype level for a variety of reasons. It is feasible (some say easy) by analysis of haplotypes in informative families. Prenatal diagnosis, indirectly by haplotype association or directly by mutation analysis, is increasingly accessible and relevant in some societies.

*Envoie:* We return then to themes raised by Garrod, the person who highlighted inherited susceptibility to disease; by Følling who showed that PKU was a significant cause of mental retardation; and by Penrose, who asked basic questions about the origin and significant of PKU mutations in human populations. (We may yet discover that PKU mutations conferred a selective advantage on heterozygotes at some time in human history). This knowledge was not reason for despair; ways were discovered to achieve universal screening and early diagnosis of individuals harboring PKU mutations and to treat them before brain development was irreversibly impaired. It is fair to say that PKU, the disease, has disappeared from societies where the technologies are available; the associated genes continue to be transmitted at unchanging frequencies. Meanwhile, other problems attached to the disease continue, notably the issue of maternal hyperphenylalaninemia and the challenge to improve treatment of the phenotype. Physicist John Ziman said that "reliable knowledge" will serve as a basis for action, can lead to correct prediction and is relevant to choices of action that matter. All that we know about PKU fits the definition of "reliable knowledge".

I return to the theme of molecular biology in medicine and the relevance of the Genome Project, to close with some basic points of view. The basic question in medicine remains: why does this patient have this disease now? It is the question that distinguishes between the patient with the disease and the incidence of the disease; it also distinguishes the patient with the disease from the disease the patient has. Many of us work with a medical model which recognizes that manifestations of disease (the substrates for diagnosis and treatment) have their origin in a deviant cellular process (pathogenesis of the disease) which, in turn, originates in a cause which is both intrinsic (biological) and extrinsic (experience). To understand pathogenesis and cause is to have access to prevention. Medical education puts primary emphasis on the manifestation of disease. Interest in prevention is to some extent counter-intuitive, because if all diseases could be prevented there would be no need to practice medicine as we know it.

The history of PKU is one of the great illustrations of this theme. I said at the beginning that if we could improve social and environmental conditions, and thus the quality of life, we would increase the heritability of disease. Extrinsic causes can change almost overnight; intrinsic causes (mutations) change at a glacial pace. It follows that to know the human genome and its mutations will be increasingly relevant to an understanding of the biological basis of health and disease. This will have profound implications for medical education and the way we design and practice health care.

One can think about medicine in three frameworks of time and organism. When we meet with the patient, we recognize an individual in the present moment (now). But the individual belongs to a family which has a recent history and may

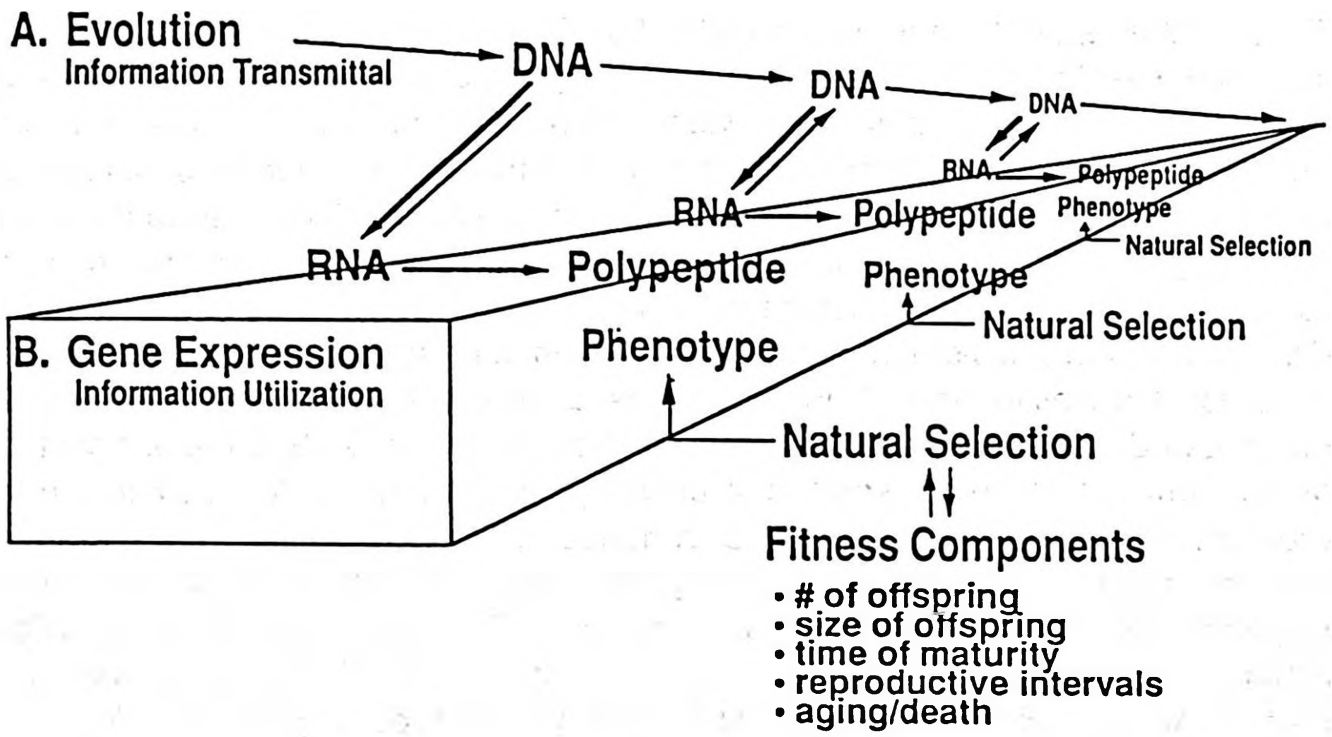


Fig. 2: Two biological paradigms: evolution, or information transmittal; and gene expression, or information utilization. The central dogma of biology links one to the other. Selection acts on phenotype to select an object (the corresponding gene). Fitness components which are the substance of 'life histories' are the products of natural selection.

have a future one if the person is or becomes a parent. Finally, the family belongs to a species (Homosapiens) whose demic expansion and evolution through its genetic footprints passed on to contemporary human populations. Accordingly, we are interested in two paradigms of genetics (Fig. 2). In one we are considering transmittal of information; this is the paradigm of evolution. The study of PKU contributes to the documentation of human evolution. It also describes an important gene that has been conserved and has evolved through mammalian and earlier species. The gene is a marker for history. In the other paradigm, we are concerned with utilization of information: how genotype becomes phenotype. The association between mutant genotypes and the corresponding phenotypes in PKU is but one of a hundred ways to illustrate how change in information can explain disease. The link between the two paradigms (transmittal and utilization) is of course the central dogma of biology; DNA makes RNA makes protein.

It all becomes manifest in what some call "life histories", which are the fundamental factors underlying the biology of individuals and populations. As practitioners of health care, we are contributing to the life histories of the human species. Accordingly, we are modifying the evolution of our own species. These are major responsibilities to consider in our roles as members of our species, as members of families, as individuals, whether we are patients or well persons, and as citizens no matter where we live on planet Earth.

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# To The Editor*

*Lütfiye Mesci BS**, Aytemiz Gürgey MD***, Çiğdem Altay MD****

Recent progress in DNA technology, such as the establishment of PCR using allele-specific oligoprimers, made it possible to apply this technique quickly and efficiently in prenatal diagnosis in small laboratories such as ours[1]. Recently, we noticed that oligoprimers prepared in the detection of frame shift codon 8, 9 (FSC89) mutation also give positive PCR reaction with the FSC 8 mutation. Dinucleotide (AA) deletion in codon 8 in FSC8 and insertion of G in FSC8,9 create similar shifts in the distal codons as follows:

| | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|
| Normal: 5' | AAG | TCT | GCC | GTT | ACT | GCC | CTG.....3' |
| FSCB : 5' | – | GTC | TGC | CGT | TAC | TGC | CCT.....3' |
| C 8,9 : 5' | AAG | GTC | TGC | CGT | TAC | TGC | CCT.....3' |

Therefore it was not surprising to detect these mutations by using the same primer matching with the newly created codons. (Fig. 1).

As we have previously reported, FSC 8 mutation is usually associated with thalassemia intermedia[2]. Therefore, differentiation of these two mutations from each other may be critical in genetic counseling and prenatal diagnosis in countries like ours where these two mutations are present. FSC 8 was shown to be associated with haplotype IV (according to Orkin's classification) and a positive recognition site for restriction enzyme Xmn at promotor region of $\gamma_G$. Therefore, in the differentiation of these two mutations, searching for an Xmn recognition site or using allele-specific oligoprimers with a 3 end matching with the normal sequence of codon 8 would be helpful.

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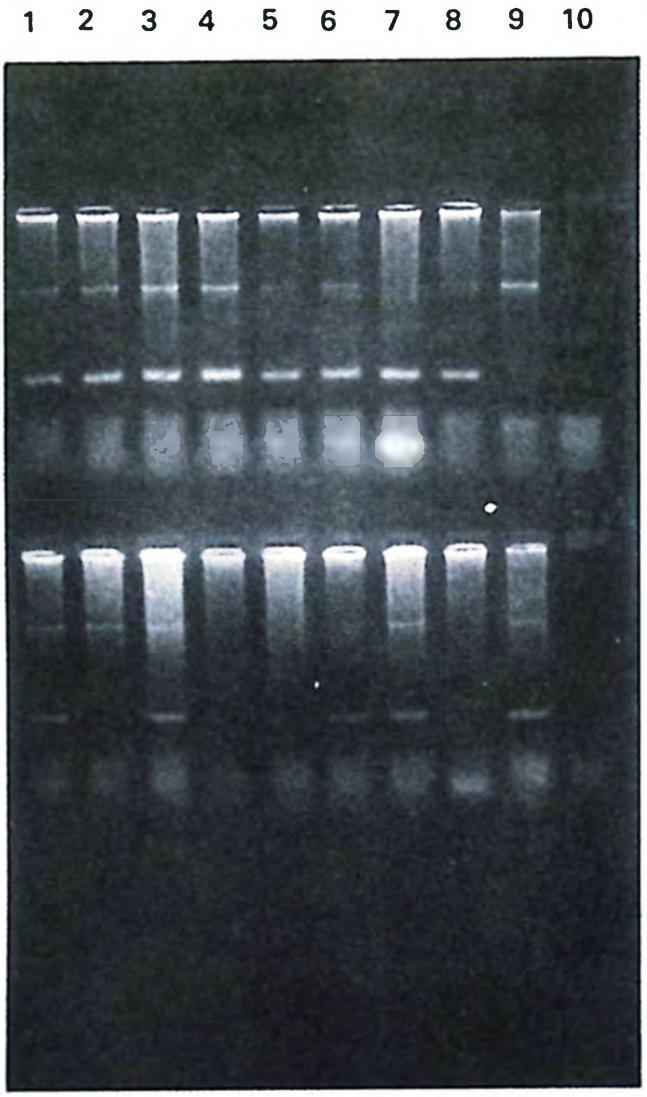


Fig. 1: 1) FSC8 heterozygote. 2) FSC8 homozygote. 3) FSC8 heterozygote. 4) FSC89 homozygote. 5) FSC8 homozygote. 6) FSC89 heterozygote. 7) FSC89 heterozygote. 8) FSC8 homozygote. 9) Control. 10) Blank.

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