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CLINICAL AND LABORATORY APPROACH TO A NEONATE SUSPECTED OF AN INBORN ERROR OF METABOLISM*

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Foreword

Metabolic and molecular advances have led to the identification of hundreds of inborn errors of metabolism, too many for most to recall from memory alone without referral to authoritative compilations of published works, often assembled in formidable (in size and scope) books weighing many pounds. Those encountering such disorders in neonates face unique and special challenges because of the fragility of their patients, the rapidity with which these conditions progress, and the fact that many of the expressions of inherited metabolic disturbances mimic other serious conditions, thereby delaying diagnosis and placing the newborn at serious risk for survival.

A simple algorithm to quickly establish the diagnosis of the numerous inborn errors of metabolism that have been reported would be helpful. However, because of the diversity of the conditions and defects that can occur, numerous subsets would have to be developed to cover the full spectrum of conditions that may be encountered.

In this report, conditions to consider when confronted with a newborn suspected of an inborn error of metabolism are grouped, based primarily on the presenting clinical findings in the neonate together with laboratory tests that can be performed by most clinical laboratories. Further workup to narrow the possibility should follow, confirming the diagnosis by special tests with consultation from specialists who are more familiar with these conditions. Establishing the diagnosis by specific enzymatic assay would require the involvement of specialists with the laboratory resources to perform the tests. The approach suggested is by no means exhaustive. It does not include genetic conditions that are unlikely to be expressed in the newborn period, nor those that are extremely rare in occurrence.

Only an abbreviated historical account is presented, omitting many important citations to individual papers from a large family of dedicated investigators. Many citations are to chapters in the three volumes of The Metabolic and Molecular Bases of Inherited Disease by Scriver, Beaudet, Sly and Valle, with the

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expectation that the reader would pursue other details cited in these works and those published since then, as may be clinically indicated, in their workup of any neonate suspected of an inborn error of metabolism.

Background

Garrod's[1] conceptualization of our chemical individuality proposed in 1908 stemmed from his study of alkaptonuria. Plumbing was not in use and the affected was readily identified as the urine turned dark upon standing. The constancy of this feature and its expression in only some members of families with higher rates of consanguineous marriages led Garrod to ascribe the trait to a recessively inherited inborn error of the metabolism of homogentisic acid, which he had identified as the compound in urine that darkened after oxidation[2,3].

It took 50 more years before the absence of homogentisic acid oxidase activity was demonstrated as the only defect in the degradation of phenylalanine and tyrosine in alkaptonuric liver biopsies[4]. In the intervening years, important advances leading to our understanding of the genetic basis of clinical diseases were made, including the proposal that the synthesis of enzymes was determined by genes, one gene for one enzyme[5] and that the primary amino acid sequence of a mutant protein would be altered as was found with the hemoglobin in sickle cell anemia accounting for the abnormal function of the protein[6]. Until recently, more than 300 inborn errors of metabolism, or IEMs, have already been identified.

Most arise from the inheritance of mutant alleles that are autosomally recessive. A few are due to a defective dominant allele or to maternally inherited mutant mitochondrial DNA or X chromosome. The majority are single base-pair substitutions that can affect transcription, translation, mRNA splice junctions, processing and translation, and regulatory sites. Less frequent mutations include gene deletions with or without abnormal insertions, duplications, inversions, and expansion or contraction of unstable repeat sequences[7].

Mutations lead to the synthesis of mutant proteins that either fail to function at all, function partially, with altered interactions with cofactors, or with increase in activity. Total absence of enzyme protein synthesis; abnormal protein processing, assembly, and secretion; or defective importation of normally synthesized enzymes into various intracellular compartments can also occur[8].

Collective data are available on the frequency of 8 IEMs in the US[9] (Table I). The most frequent is cystic fibrosis, which occurs once in every 2000 live births. Phenylketonuria (PKU) occurs once in 10-25.000 live births in the US while the incidence is almost three times higher in Turkey[10]. Galactosemia and maple syrup urine disease also occur with higher frequency in Turkey than in the US, perhaps as a result of a higher rate of consanguineous marriages in Turkey[11,12].

Table I: Frequency of IEMs by Newborn Screening

IEM	Frequency	
	USA[1]	Turkey[2]
Cystic fibrosis	1:2.000	
Congenital hypothyroidism	1:3.600-5.000	1:2,736
Congenital adrenal hyperplasia	1:12.000	
Phenylketonuria	1:10.000-25.000	1:4,500
Galactosemia	1:60.000-80.000	higher
Biotinidase deficiency	1:72.000-126.000	
Homocystinuria	1:50.000-150.000	
Maple syrup urine disease	1:250.000-40.000	higher

Live births.
1 from reference (9) and 2 from references (10) and (11).

Various strategies are available for treatmenat of many IEMs, the commonest being the restriction of intake of the substrate that cannot be metabolized. As this may not be sufficient in some cases, compounds have been identified that can link with specific metabolites through alternative pathways to produce nontoxic conjugates that can be eliminated by the kidneys[13-15]. Synthetic hormones and blood proteins can also replace those that may not be synthesized. Sometimes, dysfunctional enzymes can be activated by increasing the concentrations of the cofactors involved. Experience with organ transplantation is limited but promising for some storage disorders and cystic fibrosis. Somatic gene therapy may become available for other conditions in the future, as in adenosine deaminase deficiency[8].

Many of the signs and symptoms exhibited by newborns with IEMs are also seen after hypoxic injury to the brain, intracranial hemorrhage, or infection (Table II). Differentiating an IEM from such conditions could, therefore, be very challenging, as even a rash, an enlarged liver, an odor, or discoloration of the urine can occur in newborns suffering from certain infections as well as an IEM.

Table II: Some Presenting Symptoms of IEMs in Neonates

Neurological	Gastrointestinal	Other
poor sucking reflex	poor feeding/vomiting	dysmorphia
lethargy -> coma	vomiting/diarrhea	organomegaly
hypotonia/hypertonia		rash
opisthotonus	**Cardiopulmonary**	odor
tremors/myoclonus/seizures	hyper/hypoventilation	cataracts
hypothermia	apnea	jaundice
	bradycardia	

According to Dr. Özalp and her colleagues[11], the odds that an infant with such symptoms would have an IEM is almost four percent in Turkey. Thus, the possibility of an IEM should be entertained whenever an infant with symptoms suggestive of hypoxic injury to the brain, intracranial hemorrhage, or systemic infection is encountered.

Recommended Approach

Once alerted that an IEM might be possible, quickly assess the urgency of the clinical status of the newborn regarding the possibility of imminent decompensation from a cardiopulmonary or neurological standpoint. Stabilization with fluids, intubation and assisted ventilation may be necessary.

Whether symptoms developed after a normal period following birth is important to establish and would be known at the time of examination of the newborn. Similarly, maternal, familial and perinatal factors of importance would be known. If the neonate exhibits dysmorphic features, check the appearance of the parents.

Then, samples of blood, urine and, if possible, cerebrospinal fluid (CSF) should be obtained, setting aside aliquots, including the cells, for specific diagnostic tests. Table III lists the tests that would ordinarily be obtained from a clinical laboratory. Table IV lists the preferred manner of processing the various samples for specific diagnostic tests that could be done to confirm and establish the diagnosis of the specific IEM involved. Processing of blood samples for lactate and pyruvate and the ketone bodies should be done at the bedside to prevent interconversions that can occur if the blood is allowed to stand long before deproteinization.

Table III: Recommended Initial Laboratory Tests

Sample	Clinical Laboratory Tests	Special Laboratory Tests
Blood	Complete blood count, glucose, electrolytes, pH, arterial O_2/CO_2, BUN, lactate/pyruvate, β-hydroxy-butyrate/acetoacetate, uric acid, bilirubin, ammonia, liver enzymes	Amino acids, organic acids, fatty acids, carnitine and carnitine esters, enzyme assays on red blood cells (rbcs) or white blood cells,
Urine	Urinalysis including test for both reducing substances (Clinitab) and glucose (Gluco-strix), ketones-acetone (Acetab), ketoacids (dinitrophenylhydra-zine test), urobilinogen	Orotic acid, amino acids, organic acids, pterin forms, porphyrins, sulfite, as indicated
CSF	Protein, glucose, amino acids, lactate/pyruvate	
Other		CT of the head for possible brain edema and radiological exam of organs/tissues, as indicated to r/o infection, calcifications, abnormal size, or malformations

Table IV: Processing of Samples for Specific Diagnostic Studies

Sample	Procedure
Whole blood	Blot on filter paper, dry, store at room temperature
Plasma	EDTA, heparin, or Ca^{++} but not citrate, transport cold, or freeze and transport frozen
Serum	Transport cold, or freeze and transport frozen
Urine and CSF	Freeze, no additions
Red blood cells/ white blood cells	Remove plasma, refrigerate and transport cold, freeze only if washed with saline first and transport frozen
Skin biopsy	In culture media, or keep cold and transport cold, or freeze in liquid nitrogen and transport frozen
Postmortem tissues	Treat skin as above; other tissues freeze and transport frozen

Personally examine a fresh specimen of urine, or a recently wet diaper, smelling it for odors. The urinalysis should be complete and include a test for reducing substances (Clinitab), for glucose, and for ketoacids. The latter is usually done using an acidic 2,4-dinitrophenylhydrazine solution. A computed tomography (CT) of the head may be necessary to assess for brain edema depending on the clinical findings, and other radiological tests should be obtained as indicated following the initial clinical assessment.

After samples have been collected, a vitamin cocktail would be infused consisting of selected vitamins that serve as cofactors for enzymes that may be involved in some IEMs. These include vitamins B1, 2, 6 and 12, biotin and folate, mixed with 10% glucose and infused at 60-70 calories/kg over several hours (Table V). Improvement implies one of the ingredients in the cocktail was correcting an enzymatic step, in which case the treatment may have to be continued. Needless to say, monitoring the fluid and electrolyte balance and the status of the body fluids must continue throughout therapy.

Table V: Vitamin Cocktail for Initial Infusion*

Vitamin	Dose	Associated IEM
Thiamine (B_1)	10 mg	MSUD, Lactic acidemias
Riboflavin (B_2)	100-300 mg	Electron transport defects
Pyridoxine (B_6)	250-500 mg	B_6-dependent seizures, homocystinuria
Cobalamin (B_{12})	1-2 mg	Methylmalonic acidemia, Homo-cysteine methyltransferase defects
Biotin	20 mg	Organic acidurias
Folate	70-120 mg	Homocystinuria, Hyperphenyl-
or Folinic acid	12.5 mg	alaninemia

Definitive diagnostic tests are available for most of the known IEMs, but no single test covers all possible conditions. Often, tests are run by different laboratories, requiring undue volumes of blood and numbers of samples to be drawn. Furthermore, most tests take days to weeks before results become available. Therefore when dealing with a newborn suspected of an IEM one must rely on one's clinical judgement to narrow the differential diagnosis to a few conditions by taking into account the presenting clinical symptoms and several key laboratory findings.

In view of the large number and diversity of IEMs, are there clinical clues that can be used to narrow the possible conditions to a manageable few? Or, how would a neonate with an IEM present? Unlike older children with storage defects demonstrating the effect of time on the accumulation of unmetabolizable substrates that distort the face, body and intellect, most neonates with IEMs are not readily identifiable, with a few exceptions.

The majority will appear normal and referral may be because of a positive screening test or a family history of a previous sibling with such a disorder. Since the IEMs with the highest frequency are cystic fibrosis, cystinuria, and classical PKU, where no physical stigmata are present at birth, most newborns with IEMs will appear normal. However, 10-20 percent of newborns with cystic fibrosis present with meconium ileus[16], and vomiting suggestive of pyloric stenosis occurs in many PKUs[17].

IEMs can also appear as an acute metabolic disturbance following poor milk intake or vomiting, with the development of hyperventilation and the onset of progressive neurological abnormalities.

Some babies show predominantly neurological symptoms of a progressive nature, as though intoxicated either following or before the initiation of feeding.

Signs of other organ or tissue dysfunction could also be the manner of presentation, including severe, explosive diarrhea related to feeding, nonphysiological jaundice, or discoloration of the urine-which may turn dark as in alkaptonuria, or reddish as in the porphyrias.

Rarely, dysmorphia or other overt physical abnormalities can actually be present at birth as a result of in utero effects that impact organogenesis or growth which cannot be compensated for by maternal factors. Such prenatal effects can result in prematurity, smallness in size for the gestational age of the newborn, macrocephaly, a characteristic dysmorphia, gross body distortions, cataracts, and hepatomegaly or other organ size derangements.

A) Examples of A Few IEMs Presenting with Dysmorphia or Other Overt Physical Abnormalities Include

1. Neonates with hyperphenylalaninemia, not classical PKU, but due to cofactor recycling or cofactor synthesis defects, who may be small for their gestational age[18]. In addition, these newborns would exhibit various neurological abnormalities.

2. Newborns with glutaryl-CoA dehydrogenase deficiency or glutaric acidemia Type I may present with macrocephaly and only later develop a progressive dystonia and dyskinesia. The macrocephaly is associated with CT findings showing dilatation of the lateral ventricles and widening of the cortical sulci. Bouts of sudden onset of neurological deterioration with episodic ketotic hypoglycemia, hyperammonemia and liver abnormalities with a characteristic organic aciduria may also develop. This defect involves a mitochondrial matrix enzyme which transfers electrons to ubiquinone in the respiratory chain[19].

3. Abnormal facies occur in a number of IEMs, including:

a) Multiple acyl-CoA dehydrogenase deficiency or glutaric acidemia Type II, where the neonate presents with a high forehead, low set ears, hypertelorism, hypoplastic midface, rocker bottom feet, abdominal wall muscle defects, hypospadias and hepatomegaly. Metabolically, they exhibit severe hypoketotic or nonketotic hypoglycemia and metabolic acidosis, with fatty degeneration of the liver parenchymal, renal tubular epithelial, and myocardial cells. In this disorder, the metabolism of several amino acids and fatty acids is blocked, leading to the accumulation of specific organic acids in the urine including isovaleric acid in some patients, giving off the odor of sweaty feet[20].

b) Pyruvate dehydrogenase or PDH deficiency, where the neonate presents with the fetal alcohol facies. Presumably this resemblance stems from the effects of acetaldehyde found in alcohol toxicity which inhibits PDH, leaving these infants to develop severe, intractable lactic acidemia[21].

c) The cerebro-hepato-renal syndrome of Zellweger, where a Down syndrome-like facies with deformities is found. This disorder is due to defective targeting of enzymes bound for the peroxisomes, single membrane enclosed intracellular organelles, which lay empty due to failure of the enzymes, normally housed in the peroxisomes, to function. Another striking finding in these infants is the profound hypotonia they exhibit[20].

d) In another peroxisomal defect, rhizomelic chondrodysplasia punctata, gross abnormalities of the limbs, are exhibited right from birth. There is considerable shortening of the proximal portions of the limbs with vertebral and control nervous system (CNS) abnormalities. Unlike Zellweger, in this syndrome, some peroxisomal bodies can be found with some enzymes present but defects in plasmalogen biosynthesis and phytanic acid oxidation are selectively deficient[20].

e) In I-cell disease or mucolipidosis II, the neonate may be small with the characteristic clinical features already apparent at birth. These include coarse facial features with epicanthal folds, a flat nasal bridge, anteverted nostrils, macroglossia, restricted joint movement, hypotonia, hernias, talipes equinovarus, and congenital hip dislocations. There is abnormal lysosomal enzyme transport

in mesenchymal cells with the lysosomal enzymes secreted into the extracellular space. Targeting of lysosomal enzymes to the lysosomes is receptor-mediated through mannose 6-P04 markers on the enzymes. Of two enzymes that synthesize the mannose 6-P04 on the lysosomal enzymes in the Golgi complex, phosphotransferases appear to be defective in I-cell disease. Diagnosis is by assay of the serum for the activities of lysosomal enzymes with verification by assay of the enzymes in cultured fibroblasts and the ratio of extracellular as opposed to the intracellular activities of the enzymes[22].

4. In the X-linked oculocerebrorenal syndrome of Lowe, congenital cataracts are always found. It takes several weeks before the proximal tubular acidosis with bicarbonate wasting leading to the Fanconi syndrome is expressed by increase in phosphate, amino acid and glucose excretion. Failure to thrive, recurrent infections and polyuria with low urine osmolarity would be expressed later. Any infant with congenital cataracts should be checked for this syndrome by examination of the urine and blood for these abnormalities.

5. In Wolman's disease, hepatosplenomegaly of massive proportions and calcification with enlargement of both adrenal glands are observed in the first week of life. Persistent and forceful vomiting associated with marked abdominal distension and steatorrhea occurs in the first weeks. Diarrhea, jaundice or a persistent low-grade fever can also occur. Neurologically, no specific signs are detectable at birth, but a progressive loss of alertness develops within a few weeks after the onset of the other symptoms.

Deficient lysosomal acid lipase activity is the cause of this disease resulting in massive accumulation of cholesteryl esters and triglycerides in the tissues of the body. Liver function tests may be abnormal but other laboratory tests are not diagnostic. Calcifications of the adrenal glands and depressed adrenal responses are suggestive of the diagnosis. Biopsy of tissue is needed for confirmation. Diagnosis would then be established by demonstration of the deficiency of acid lipase activity in cultured skin fibroblasts, lymphocytes, or other tissues. Suppression of cholesterol and apolipoprotein B synthesis by 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors and cholestyramine treatment has not yet been tried in this fatal disorder[23].

6. Ceramidase deficiency of Farber's disease, a deficiency of a lysosomal acid ceramidase, can result in several phenotypic expressions that differ in severity and sites of the major tissue involvement but all of which result in a unique triad of findings: subcutaneous nodules, arthritis, and laryngeal abnormality frequently first noted as a hoarseness which may progress to aphonia and feeding difficulties.

Ceramides are intermediates in sphingolipid metabolism and are produced in the synthesis and degradation of gangliosides. In addition, ceramides are components of myelin and cellular membranes. Ceramidases degrade ceramide,

and acid, neutral, and alkaline ceramidases have been detected. In all phenotypes, granulomatous infiltrations are found in the subcutaneous, joint, and lung tissues, with parenchymal cells also accumulating storage material and being frequently involved in granulomatous reaction. The storage material is ceramides, a significant proportion of which are 2-hydroxy fatty acids that can be found in biopsied tissues. Also, ceramides accumulate in body fluids and deficiency of lysosomal acid ceramidase activity is detectable in white blood cells and cultured skin fibroblasts[24]. No specific therapy is available.

7. In galactosidosis, Type I, affected infants present with hydrops, edema, coarse facies, inguinal hernias, and telangiectases. Ocular abnormalities including a cherry red spot may also be present. Cardiac and renal dysfunction with proteinuria and hepatosplenomegaly occur.

This disorder is due to a combined deficiency of lysosomal β-galactosidase and neuraminidase because of a deficiency of a protective lysosomal protein that functions uniquely. With the β-galactosidase, the protective protein prevents the rapid proteolytic degradation of the enzyme. With the neuraminidase, association with the protective protein is a requirement for activation, while association with β-galactosidase confers stability. Affected individuals excrete an excessive amount of sialyloligosaccharides in their urine which can be detected by thin-layer chromatography. Confirmation would be by enzyme assay in white blood cells or cultured skin fibroblasts[25]. No specific therapy is available.

8. Three inherited defects of the connective tissues can be readily detected in the newborn period: osteogenesis imperfecta, Ehlers-Danlos syndrome and the dystrophic form of epidermolysis bullosa[26].

a) Osteogenesis imperfecta, dominantly inherited, can result in perinatal fractures, particularly of the long bones of the arms and legs, the ribs, or the small bones of the hands and feet. Blue sclerae are also present. There is defective production of Type I procollagen in this disease which can be demonstrated in cultured dermal fibroblasts. No treatment is available.

b) Ehlers-Danlos syndrome is characterized by marked skin fragility and hyperextensibility of both the skin and joints: More than 10 mutations are known but the exact defect that results is not known. In Type I, an autosomal dominant disorder, infants are often born prematurely because of premature rupture of the membranes. In Type VII, multiple joint dislocations and bilateral congenital hip dislocations occur. No treatment is available.

c) Epidermolysis bullosa is characterized by blistering of the skin, often present at birth, occurring within the epidermis at the dermal-epidermal junction or within the dermis below the basemet membrane, and is inherited by either dominant or recessive mechanisms. Progressive syndactyly from scarring of the skin and

mucosal involvement leading to esophageal strictures can lead to profound disability. Survival is limited both by infection and derangements of the gastrointestinal tract. No treatment is available.

Thus, some affected newborns can present with distinguishing features at birth. Careful physical examination could lead to the early recognition of these as well as other inherited disorders of metabolism.

B) Examples of IEMs Presenting with Predominantly Neurological Symptoms of A Progressive Nature Suggestive of Intoxication Include

1. Maple syrup urine disease or MSUD where the newborn develops increasing lethargy, hypertonia, and intermittent opisthotonus. Breast-feeding may delay onset of symptoms. The disorder results from failure to process the keto acids from the branched chain amino acids (BCAA), leucine, isoleucine and valine, to their respective CoA derivatives by a single enzyme complex which is a combined decarboxylase-dehydrogenase. The respective keto acids and amino acids accumulate and spill over into the urine[27] causing the urinary DNPH test to be strongly positive. (detected by dinitrophenylhydrazine (DNPH) test by adding an equal volume of 0.1% of DNPH in 2 N HCl to urine).

If this is found along with the characteristic odor, reduce the BCAA intake and provide high levels of thiamine until the aminogram is completed. Detection of increases in the BCAA and the presence of alloisoleucine in the aminogram of the blood should confirm the diagnosis of MSUD. (the nonprotein amino acid, L-alloisoleucine, is formed endogenously in MSUD from isoleucine through 3-methyl-2-oxo-pentanoic acid undergoing nonenzymatic racemization and transamination to L-alloisoleucine).

Computed tomography (CT) scans are normal at three days of age in affected asymptomatic MSUD infants identified by sibship advantage. By nine days, however, CT abnormalities can develop despite treatment, showing generalized brain edema. A unique localized intense edema involving the cerebellar deep white matter, the dorsal brain stem and cerebral peduncles is seen by imaging studies, suggestive of dysmyelination. The EEG is abnormal in newborns with classic MSUD with comb-like rhythms of 5 to 9 Hz spindle-like sharp waves over the cortical regions[28].

During acute decompensation, dialysis may be necessary. Leucine is responsible for the abnormal neurological signs and isoleucine is responsible for intensifying the odor. Therefore, leucine is key, and its levels should be monitored above all else. Treatment for MSUD is to avoid the substrates that cannot be metabolized using a BCAA free formula offering 2-3 grams of amino acids or protein equivalent/kg/day. Remember that the BCAA are essential, required for protein synthesis, and must be provided in the diet through natural foods to complement the amonut of protein ingested in the special formula. In cow's milk, isoleucine and valine contents are much lower than leucine. During correction of an acute

state, it is important to correct the isoleucine and valine levels to normal despite elevated leucine levels, or else catabolism will not reverse. Should a catabolic state result, insulin can be judiciously used to reverse catabolism. A trial of thiamine therapy giving 50-300 mg/d for three weeks is advisable[27].

2. Nonketotic hyperglycinemia. With a rapid progression of neurological abnormalities characterized by hypotonia, in contrast to MSUD, together with hypoventilation and abnormal eye movements and seizures, consider nonketotic hyperglycinemia. The neurological abnormalities are profound and intractable, developing after a brief normal period after birth with the hypoventilation leading to progressive respiratory failure. Increasing lethargy, poor feeding, intermittent ophthalmoplegia, segmental myoclonic jerks, hiccups, and coma also occur. The only laboratory finding of significance is a raised glycine level in the blood, urine and CSF. A CSF to blood ratio for glycine of > 0.08 is diagnostic. Many other IEMs are associated with elevated glycine levels due to accumulated metabolites that interfere with the hepatic glycine cleavage enzyme, but their CSF glycine content is usually normal.

Nonketotic hyperglycinemia is due to a defect in the glycine cleavage system, a four-peptide complex located in the inner mitochondrial membrane of the major organs which incorporates the carbon skeleton of glycine into purines, glutathione, creatine, and the precursor of heme and porphyrins[29]. Glycine is a neurotransmitter, inhibitory in the spinal cord and brain stem causing apnea and hiccupping early in the disease. It is an excitatory agonist of the N-methyl-D-aspartate (NMDA)-type glutamate receptor channel complex in the cortex explaining intractable seizures. Glycine binding enhances glutamate binding to the NMDA site, increasing the frequency of channel openings once glutamate is bound to the receptor. This promotes sustained stimulation of excitatory impulses and also blocks normal impulse traffic. Thus, neurological defects found in this disorder are due to both the inhibitory and stimulatory roles of glycine in the CNS.

Without the amino acid profile, a trial of protein intake reduction and benzoate to remove glycine as hippurate by an alternate pathway could be tried. Seizure management is difficult, and valproate must be avoided as it interferes with the synthesis of what little amounts of the glycine cleavage enzyme proteins that occur. Dextromethorphan, an antitussive whose major metabolites are moderately potent antagonists of the NMDA sites, has been used together with benzoate with some results on seizure control and reduction of glycine levels[29].

3. Sulfite oxidase deficiency or molybdenum cofactor deficiency. Where neurological abnormalities develop almost immediately after birth with intractable, tonic-clonic seizures, axial hypotonia, and peripheral hypertonia (a pyramidal syndrome with spastic tetraplegia, feeding difficulties, ± dislocated lenses), consider sulfite oxidase deficiency or molybdenum cofactor deficiency, which are almost universally fatal.

For diagnosis of sulfite oxidase deficiency, an increase in sulfite and low sulfate with increased sulfocysteine accumulation must be documented. Urinary sulfite

should be elevated as detected with a dipstick test (manufactured in the US, called Merckoquant Sulfite Test). Fresh urine is used as sulfite will be oxidized to sulfate at room temperature. Quantitative tests for sulfite (elevated), sulfate (diminished) and thiosulfate can be done by anion column chromatography, and S-sulfocysteine (increased) can be identified by amino acid analysis. Restriction of sulfur-containing amino acid intake, binding of sulfite with compounds like cysteamine, and the administration of thiamine may be tried[29].

Three enzymes require molybdenum as a component of a cofactor necessary for activity: sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. Molybdenum cofactor deficiency is also fatal. Symptoms appear within the first or second weeks of life, resembling sulfite oxidase deficiency, as described above. Diagnosis should show high levels of sulfite, thiosulfate and S-sulfocysteine, but near normal levels of sulfate, an absence of urothion (degradation product of molybdopterin) and elevation of xanthine and hypoxanthine. The severe neurological abnormalities seen with molybdenum cofactor deficiency are presumed to result from tha absence of sulfite oxidase activity. Measures to diminish sulfite production by restriction of the intake of the sulfur-containing amino acids plus the use of thiol compounds with the potential to bind sulfite, such as penicillamine or mercaptoethanesulfonate, have been tried without effect. Addition of cysteamine to absorb excess sulfite, thiamine, and inorganic sulfate to avoid a potential sulfate deficiency should also be tried in this disorder[30].

4. Infantile gangliosidosis. Affected patients exhibit neurological abnormalities shortly after birth with poor sucking and subnormal weight gain. Neurological deterioration develops with an exaggerated startle response and increased deep tendon reflexes and progresses to severe brain damage. Dysmorphia develops with time with hirsutism and joint deformities. Macuiar cherry-red spots are pathognomonic and hepatosplenomegaly also occurs. Storage of ganglioside G_{M1} occurs in the brain and visceral organs, and keratan sulfate, keratan sulfate-derived oligosaccharides and mucopolysaccharides accumulate in the liver and spleen. Mucopolysaccharides and various forms of galactose-containing oligosaccharides are markedly increased in the urine. The genetic defect is due to deficiency of β-galactosidase activity which can be detected in leukocytes and cultured fibroblasts[31]. No treatment is available, as of yet.

5. Tetrahydrobiopterin (BH_4) deficiencies. If the newborn is small for his gestational age and the neurological findings are mild but progressive, consider a cofactor defect in the enzymatic conversion of phenylalanine (PA) to tyrosine (TY), which is not PKU. The cilinical laboratory findings will be normal but the PKU test would return positive.

Widespread screening for PKU has identified a minority of patients with high PA levels who have normal liver PA hydroxylase or PAH activity. Their defect is at the level of the cofactor that is required by PAH.

The conversion of PA to TY requires not only the hydroxylase itself, but a cofactor which is produced in our bodies from folic acid (Table VI). Folate itself is not the cofactor but must be enzymatically converted to the active factor, tetrahydrobiopterin or BH_4, to serve as the cofactor required by PAH. The cofactor function of tetrahydrobiopterin in the hydroxylating reaction with aromatic amino acids relates to its ability to reduce molecular oxygen, provide electrons and oxidize to the quinonoid form of dihydrobiopterin. Consumption of tetrahydrobiopterin is stoichiometric during hydroxylation of phenylalanine. It is regenerated from the quinonoid form of dihydrobiopterin by dihydropteridine reductase.

Table VI: Phenylalanine Conversion to Tyrosine

Phenylalanine	Phenylalanine hydroxylase or PAH tetrahydrobiopterin or BH_4	Tyrosine
Dihydropteridine reductase recycles BH_4 after PAH reaction		
GTP cyclohydrolase + 6-pyruvoyltetrahydropterin synthase		BH_4 available for PAH and other enzymes involved in neurotransmitter synthesis

Tetrahydrobiopterin or BH_4 is made available for PAH by the actions of three enzymes: one that recycles it after it is altered during the conversion of PA to TY, and two separate enzymes that actually synthesize the cofactor from precursors such as guanosine-triphosphate (GTP). GTP is the major precursor of the pterin nucleus. The initial step in its conversion towards the pterin nucleus is catalyzed by GTP-cyclohydrolase I. Deficiency of GTP-cyclohydrolase I impairs BH_4 synthesis, leading to hyperphenylalaninemia. 6-pyruvoyltetrahydropterin synthase deficiency is the most prevalent form of hyperphenylalaninemia not due to PAH deficiency. Defects in each have been reported and differences amongst these result in differences in their neurological symptomatology (Table VII).

Table VII: Disorders of BH_4 Synthesis

A) GTP cyclohydrolase deficiency (shunts GTP to pterin pathway)
   1. *Clinical features*: progressive truncal hypotonia and limb hypertonia with seizures and intermittent hyperthermia.
   2. *Laboratory findings*: elevated serum phenylalanine levels, low blood and urinary neopterin and biopterin, low neurotransmitter levels, and normal liver PAH activity.

B) 6-pyruvoyltetrahydropterin synthase deficiency
   1. *Clinical features*: difficulty swallowing, oculogyric spasms, truncal hypotonia, limb hypertonia, hyperthermia, seizures, low birth weight, and abnormal white brain matter by MRI.
   2. *Laboratory findings*: elevated serum phenylalanine levels, high neopterin levels in plasma and urine, low biopterin levels, and normal liver PAH activity.

The same form of the cofactor is also required by other enzymes that synthesize important neurotransmitters resulting in diminished levels of these agents, accounting for the neuropathy. The neurological abnormalities found in defects of the enzyme that regenerates the cofactor and of the two enzymes that are needed to synthesize the specific cofactor are not seen in classical PKU, where the cofactor is available and recycled appropriately[32].

Distinction of these conditions from PKU can be made by checking the blood PA levels before and after an IV or oral dose of BH_4 (7.5 mg/kg). If the PA level drops, a cofactor enzyme problem may be the cause of the high PA levels and the neurological symptoms. Distinction between the enzymatic defects of BH_4 synthesis rests in the urinary pterin forms that are found (Table VII).

Treatment of these disorders by reducing phenylalanine intake, while ensuring adequate tyrosine intake, as in PKU, is not sufficient. Three other measures are necessary: 1. provision of folate, the precursor of the cofactor or folinic acid which crosses the blood-brain barrier more readily, 2. provision of certain amines that can be converted to the neurotransmitters by other routes, and 3. prescribing an inhibitor of enzymes that catabolize the neurotransmitters, such as carbidopa.

Each of these enzymes, unlike phenylalanine hydroxylase, is expressed in peripheral blood cells as well as by the liver (Table VIII), making it easier to collect a sample for enzymatic assay to confirm the diagnosis without a liver biopsy.

Table VIII: Tissues for Enzymatic Differentiation of the Hyperphenylalaninemias

Enzyme	Tissues
Phenylalanine hydroxylase	Liver
Dihydropteridine reductase	Liver, skin fibroblasts, red blood cells white blood cells platelets, amniocytes
GTP-cyclohydrolase	Liver, monocytes after phytohemagglutinin
6-pyruvoyl tetrahydropterin synthase	Liver, red blood cells

C) Examples of Disorders That Develop After A Brief Normal Period with Either Vomiting or Poor Feeding with Increasing Hyperventilation and Lethargy and Progressing to Coma, Which Necessitates Distinction of the Basis for the Hyperventilation. One of Several Patterns May be Encountered

1. Hyperventilation due to central drive resulting in respiratory alkolosis. Urea cycle defects. If there is no metabolic acidosis, but rather a respiratory alkalosis with the blood pH > 7.4, glucose is normal or slightly low, the ketones are not elevated and the BUN is low, check the blood ammonia and the urine for orotic

acid. With a low BUN, consider a urea cycle defect (above arginase, either carbamoyl phosphate synthase or CPS, ornithine transcarbamoylase or OTC, argininosuccinic acid or ASA synthetase or ASA lyase defects, or the hyperornithinemia, homocitrullinemia and hyperammonemia syndrome, or triple H syndrome) where the hyperventilation is centrally driven secondary to brain edema, leading to the respiratory alkalosis. (remember that with time, a metabolic acidosis and lactic acidemia could develop).

The clinical presentation of CPS, OTC, AS and AL deficiencies are very similar. The similarity of the clinical presentation relates to the hyperammonemia. All are inherited as autosomal recessive traits except for OTC deficiency which is sex-linked. Neonatal onset of symptoms is usually upon a perfectly normal, full-term pregnancy and delivery with no physical abnormalities upon birth, for at least the first 24 hours. Then, lethargy and poor feeding occur with the progressive addition of other symptoms, including vomiting, progression of lethargy, hypothermia, and hyperventilation, which can rapidly progress to a fatal outcome.

The urea cycle defects are treatable, but identification of the specific defect is necessary for proper therapy of the several defects that are included. Immediately, reduce protein intake. In the acute crisis, hemodialysis and the administration of phenylacetate with benzoate may be necessary to reduce the total ammonia load. For CPS and OTC, hemodialysis plus intravenous benzoate (0.25 g/kg) and phenylacetate (0.25 g/kg) diluted in 10 percent glucose would be infused over 24 hours. Hemodialysis should be repeated until ammonia is only three to four times normal, at which point treatment with oral sodium phenylbutyrate could follow. In ASA lyase defects, parenteral arginine could be used, with or without hemodialysis. For ASA synthetase deficiency, hemodialysis plus benzoate and phenylacetate with 10 percent arginine HCl at a dose of 0.66 g/kg/d would be used, as recommended[33]. For argininosuccinase deficiency, hemodialysis and 10 percent arginine HCl at a dose of 0.66 g/kg/d may be sufficient.

Chronically, dietary protein intake should be reduced to 1.5-2 g/kg/d. For CPS, OTC and AS deficiencies, 0.5 g/kg/d of phenylbutyrate could be given for conversion to phenylacetate, conjugation with CoA, and linkage with glutamine to form phenylacetylglutamine for excretion, which removes two nitrogens per molecule excreted. For ASA lyase defects, 0.5 g/kg/d of arginine enables removal of waste nitrogen as ASA for which renal clearance is the same as the GFR.

The triple H syndrome can present in neonates who appear normal with an uneventful course, if they are breast-fed. When fed a high protein formula, refusal to eat, vomiting, lethargy and even episodes of coma develop. Protein restriction to less than 1.2 g/kg/d with the possible addition of ornithine for enhancement of ornithine transport into the mitochondria is recommended.

Ammonia is toxic and hyperammonemia can produce severe brain edema where ammonia appears to be the only cause for the acute encephalopathy. Studies in normal, awake primates where concentrations of ammonia were progressively increased to five times normal resulted in progressive behavioral, physiological, biochemical and neurological findings similar to patients with hyperammonemia. As the ammonia levels rose, intracranial pressures rose with hyperventilation and the development of respiratory alkalosis. Brain edema with flattening of the cortical gyri and herniation of the cerebellar tonsils occurred[34].

Once the neonate is over the acute crisis, the long-term management would consist of a low protein diet together with other compounds that would help to reduce the nitrogen load, depending on the site of the enzyme block. For both ASA synthase and lyase, arginine would be limiting, and thus addition of arginine to the diet (0.4-0.7 g/kg/d) is recommended. Brusilow[33] recommends that arginine be provided to all patients with defects of the urea cycle except for those with an arginase defect, as arginine is necessary for various proteins in growth and turnover, as well as being a substrate for nitric oxide production. Normal plasma ammonia levels are important but the plasma glutamine can be used as a useful guide since it rises before the plasma ammonia does.

A reasonably accurate diagnosis can be made with the amino acid profile and the urinary orotic acid level. Clinically, differentiation between the first two steps in the urea cycle would depend on the urinary orotic acid levels. These would be higher in OTC deficiency, because the substrate which cannot be metabolized, carbamoyl phosphate, accumulates and is channelled to the formation of the pyrimidine nucleotides, of which orotic acid is an intermediate. The presence or absence of ASA should differentiate the synthase from the lyase defects for ASA. Urea cycle defects would also result in increased glutamine levels and decreased ornithine and arginine concentrations.

Long term, the components to be monitored in the blood would also depend on the site of the block. Should seizures be a problem, valproic acid should be avoided as it can aggravate the hyperammonemia by reducing the formation of N-acetylglutamate, which is required for activation of CPS.

2. Hyperventilation due to metabolic acidosis with hypoglycemia and lactic acidosis.

Following a similar catastrophic, life-threatening progression of symptoms after a brief normal period with increasing hyperventilation and increasingly progressive neurological abnormalities, the basis for the hyperventilation may be a metabolic acidosis where pH is < 7.3. If so, carefully consider the blood glucose, lactic acid, pyruvate and ketone body levels and the anion gap while a waiting the amino acid and organic acid results, as a large number of different disorders can do this and their tentative diagnosis may be possible upon recognition of the implications of these findings. Whether or not there is ketoaciduria would also be helpful to know.

Under a fed state, glucose should be in abundance with excess stored in the liver as glycogen for later release and utilization, between feedings and during a short fast. The two main organs that store glucose as glycogen are the liver and muscle.

Under fasting conditions, whether due to disinterest in feeding, lethargy and somnolence, or vomiting, because of an IEM, intracranial hemorrhage, or infection, the fuel status is strained. Sensors tigger the uncoupling of glucose from glycogen by phosphorolysis, which is the cleavage of the glycogen bond by orthophosphate or Pi, to form glucose-1-PO4. This form of glucose cannot diffuse out of the cell but must undergo several steps before release by the action of glucose-6 phosphatase located on the luminal side of the smooth endoplasmic reticulum. The enzymes that process glycogen to free glucose are present in the liver, but the critical final one, glucose 6-phosphatase, is absent in muscle (Fig. 1). Thus, despite its larger store of glycogen (due to the sheer number of muscles that we have), glucose cannot be released from glycogen by muscle, while it can from the liver. Instead, the phosphorylated glucose from glycogen in muscle is converted to lactate and pyruvate, which are released and transported to the liver for conversion to glucose by gluconeogenesis.

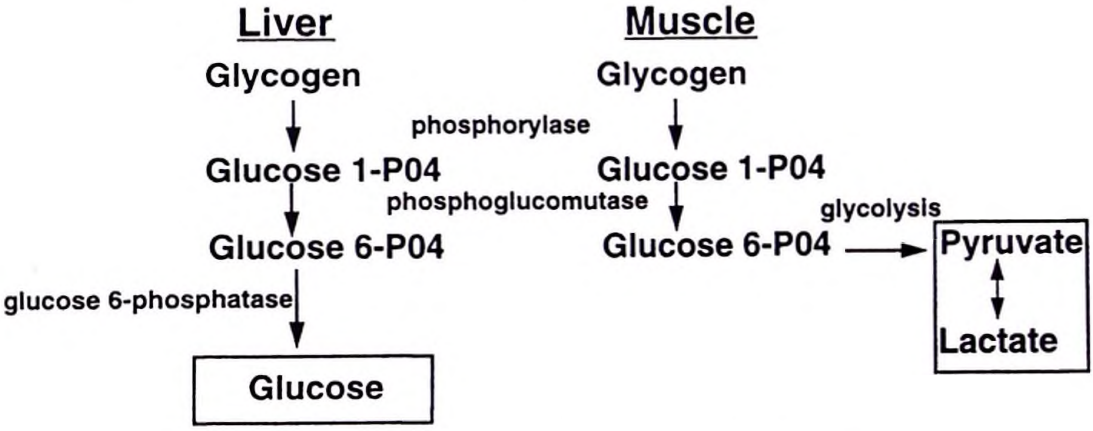

Fig. 1: The key enzymatic steps regulating glycogenolysis in the liver and muscle are in small letters. End-products of the muscle and liver are enclosed.

As fasting continues, lactate, pyruvate, and alanine released from peripheral tissues, particularly from muscle, are processed at an accelerated rate in the liver by gluconeogenesis (Fig. 2). Any block in gluconeogenesis could lead to back up of these intermediates, depending on the site of the block. Ketosis would occur under prolonged fasting or, if caloric needs are not met, as the fatty acids released by adipose tissue are also brought to the liver for oxidation and conversion to the ketone bodies for export and use by the brain, as well as by the heart and muscles.

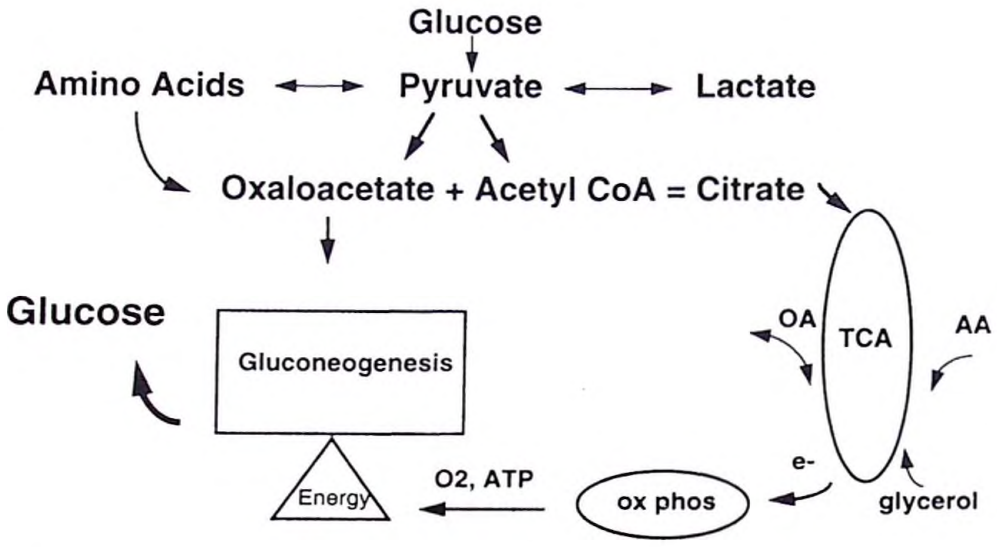

Fig. 2: Pyruvate entering the mitochondria can be converted to acetyl-CoA or oxaloacetate. Citrate, formed from oxaloacetate and acetyl-CoA, is metabolized in the tricarboxylic acid or TCA cycle, generating electrons that are transported by carriers, $FADH_2$ and NADH, to the electron transport complex for oxidative phosphorylation, generating ATP that is used as fuel in various cellular processes, including gluconeogenesis.

Gluconeogenesis is not the reversal of glycolysis or glycogenolysis, but requires its own specific enzymes. Pyruvate, or lactate and alanine after conversion to pyruvate, is sequentially acted upon enzymatically to form glucose 6-P04. The same terminal enzyme, glucose 6-phosphatase, is required to release free glucose, just as in glycogenolysis. Since this enzyme is present in the liver but not in muscle, the process of gluconeogenesis is a major function of the liver. Thus, under fasting conditions, glucose would be released by the liver by two processes, glycogenolysis and gluconeogenesis, whereas only lactate and pyruvate would be released by muscle.

Defect of glucose 6-phosphatase. Therefore, between feeding or during a short fast, defects of the enzymes of glycogenolysis result in mild to moderate hypoglycemia without a rise in lactic acid or pyruvic acid levels, as long as gluconeogenesis continues to occur in the liver. However, a defect of glucose 6-phosphatase results in profound hypoglycemia because glucose 6-P04, from either glycogenolysis or gluconeogenesis, would not be cleaved to free glucose. Lactic acid and pyruvic acidemia would occur as these alternative fuels continue to be released by muscle but cannot complete the cycle of gluconeogenesis. A defect at this site is widely referred to as von Gierke's disease or Type I glycogen storage disease.

Enzymes along key steps in gluconeogenesis include: pyruvate carboxylase or PC, phosphoenolpyruvate carboxykinase or PEPCK, fructose 1.6 biphosphatase, glucose-6 phosphatase, enzymes of the tricarboxylic acid or TCA cycle, or the electron transport chain. Defects with any of the rate-limiting enzymes of gluconeogenesis lead to severe hypoglycemia, metabolic acidosis, and severe lactic acidosis and pyruvic acidemia as these alternative fuels continue to be released by muscle but cannot be processed to glucose, causing them to accumulate. Ketosis would, of course, occur as fatty acids continue to be mobilized and oxidized by the liver at an accelerated pace.

Fructose 1.6 biphosphatase deficiency deserves special mention. This is a severe disorder of gluconeogenesis causing life-threatening episodes. Half of the patients exhibit symptoms by day one to four with hyperventilation, profound acidosis, irritability, somnolence or coma, apneic spells, dyspnea, tachycardia, muscular hypotonia, moderate hepatomegaly, and hypoglycemia. Lactate, ketones, alanine and uric acid levels are elevated in blood and urine. Liver function is usually within normal limits, as are renal tubular function and coagulation. This enzymatic lesion prevents the endogenous formation of glucose from the precursors lactate, glycerol and gluconeogenic amino acids such as alanine. For survival, the patient depends on exogenous glucose; therefore, treatment is with glucose and sodium bicarbonate to correct the acidosis. Oral glycerol provokes abnormalities like oral fructose, but the response to galactose is normal. Avoidance of fasting is critical for this autosomal recessively inherited disorder[35].

The normal lactate to pyruvate ratio is 25. That is, there should be 25 moles of lactate for every mole of pyruvate in the blood, under normal conditions. If both are increased but their ratio is about 25, there must be excessive production of both by the muscles with normal processing of lactate and pyruvate by the liver, as may occur after excessive muscular contractions during a seizure with no block in gluconeogenesis. If both are increased but the ratio is < 25, there must be a block in the processing of pyruvate.

In order for gluconeogenesis to occur, sufficient ATP must be produced. This requires a functioning tricarboxylic acid or TCA cycle linked to an electron transport mechanism. Pyruvate from various sources must be converted first to acetyl-CoA by PDH in the mitochondria, and then to citrate, an intermediate of the TCA cycle. This cycle provides intermediates for the biosynthesis of a number of compounds, including various amino acids and the porphyrins, while generating electrons that are carried by special carriers, NADH and $FADH_2$, the nicotinamide and flavin adenine dinucleotides that carry the electrons from the TCA cycle to the electron transport complex (Fig. 2). There, the electrons are transferred to oxygen by a process coupled to the phosphorylation of ADP producing ATP[21, 36, 37].

Glucose oxidation results in the synthesis of the 3-carbon intermediate, pyruvate, in the cytosol, which is further metabolized in the mitochondrial compartment via the TCA cycle (Fig. 2). Pyruvate entering the mitochondria is acted upon by PDH or pyruvate carboxylase, leading to the formation of acetyl-CoA or oxaloacetate, respectively.

In PDH deficiency, there is severe lactic acidemia as pyruvate can only be processed through pyruvate carboxylase to form oxaloacetate. With a block in PDH, pyruvate backs up, forming lactate, which is released in abundance in this disorder. The severity of the disease is a function of the severity of the lactic acidemia. The most severely affected infants who die before six months have intractable lactic acidemia, low residual activity of PDH complex with cystic lesions of the cerebral hemispheres, or cerebral atrophy.

Treatment for PDH is a high fat, low carbohydrate diet that is ketogenic to provide an alternative source of acetyl-CoA from the breakdown of fatty acids, which can still occur in the liver. The acetyl-CoA can also be converted to β-OH butyrate and acetoacetate, the ketone bodies, for ready transport to the brain, where it could be used by the brain mitochondria as acetyl-CoA and react with oxaloacetate to form citrate to enter the TCA cycle. Thiamine may also be beneficial. Dichloroacetate to activate residual PDH activity may be of some benefit in an acidotic crisis[21].

Pyruvate carboxylase deficiency. Pyruvate can also be acted upon by pyruvate carboxylase, a biotin-requiring enzyme, which converts pyruvate to oxaloacetate, important in several ways, including the formation of citrate by linking to acetyl-CoA and as substrate for gluconeogenesis (Fig. 2). Thus, defective pyruvate carboxylase or PC activity is associated with limits on 1 (gluconeogenesis and 2) the availability of oxaloacetate for conversion to citrate with consequences on the TCA cycle.

Gluconeogenesis can go on in PDH defects as PC is unaffected and there is another source of acetyl-CoA for interaction with oxaloacetate to form citrate, from the oxidation of fatty acid metabolism, which also occurs in the mitochondria. Thus, if fatty acid oxidation is adequate, sufficient citrate would be produced to run the TCA cycle despite the presence of a PDH defect[21].

TCA cycle or oxidative phosphorylation defects. Abnormalities due to mutations that affect the TCA cycle or the generation of ATP by oxidative phosphorylation cause various degrees of hypoglycemia and lactic acidemia, depending on the residual enzymatic activity present. Obviously, total blocks are incompatible with life. Distinction among disorders of the TCA cycle depend on the organic acid profiles in the urine. The genes for the five enzymes of oxidative phosphorylation are both nuclear and mitochondrial. Therefore, some defects can be acquired by maternally transmitted mutant mitochondrial DNA[37].

All of the defects with hypoglycemia would benefit from the administration of glucose and appropriate salts to correct the acidosis. Depending on the defect suspected, other measures could also be tried, including the administration of vitamins that serve as cofactors for the enzymes involved until the organic acid profiles are completed and the specific diagnosis is confirmed.

3. Hyperventilation with metabolic acidosis and severe anionic gap.

In another group of conditions where the degree of hypoglycemia is milder, there is no ketoaciduria, and the degree of the ketosis and lactic acidosis cannot account for the anionic gap, consider such organic acidemias as isovaleric acidemia with the odor of sweaty feet and elevated leucine levels, propionic acidemia, methylmalonic acidemia, or multiple carboxylase defects. Each would be distinguished by the specific abnormal organic acid accumulations found in the plasma and urine. Hyperammonemia can also occur in these disorders. Clinically, thrombocytopenia, neutropenia, and pancytopenia are also found during acute episodes of acidosis.

Immediately reduce the protein intake, and reverse the hypoglycemia and the acidosis. After the organic acid findings are in, take whatever specific steps are needed to restrict the intake of substrates that cannot be metabolized, removing offending metabolites through alternative pathways and augmenting residual enzyme activity by administration of appropriate cofactors that are involved, depending on the defect.

For isovaleric acidemia due to deficiency of isovaleryl-CoA dehydrogenase activity[38], L-leucine intake must be reduced. As leucine is an essential amino acid, too severe reduction can lead to undesirable consequences. Adjunctive therapy with glycine and L-carnitine have been used to reduce the plasma levels of isovaleric acid through the formation of isovalerylglycine[39] and isovalerylcarnitine[40] (250 mg/kg by gavage of glycine and 25 mg/kg/6h of carnitine). Follow up on 1.5-2 g/kg of protein plus glycine and carnitine. Isovalerylglycine is nontoxic and readily excreted; isovalerylcarnitine is less well excreted compared to isovalerylglycine. Diagnosis requires analysis of the organic acids. Isovaleric acid should be elevated in the absence of elevation of other short-chain acids[41, 42]. A pancytopenia can develop due to the isovaleric acidemia.

For propionic acidemia, patients present with dehydration, severe ketoacidosis, lethargy and coma developing within the first day of life. The profound metabolic acidosis is most striking. If the neonate survives the initial episode, intermittent bouts would occur in association with infection, constipation, and increased protein intake. Neurologic sequelae including dystonia, severe chorea, and pyramidal signs are frequently seen.

This disorder is due to a defect in the catabolism of several essential amino acids (isoleucine, valine, methionine and threonine), the odd carbon-chain fatty acids, cholesterol, uracil, and thymine by propionyl-CoA carboxylase activity.

Intake of the amino acids involved must be reduced without limiting growth. Catabolism of isoleucine, methionine and threonine accounts for 50 percent of the propionyl-CoA accumulating in affected patients. Other metabolites also contribute to the accumulation of propionyl-CoA, but gut bacteria may account for 20 percent or more. The serum propionic acid level can rise 100-fold. Pancytopenia can occur, which appears to be due to inhibition of bone marrow proliferation and maturation and, possibly, to a shortened red blood cell survival due to the propionic acidemia[43]. This enzyme is one of the biotinylated carboxylases, and while biotin administration can reduce the degree of propionic acidemia in some cases, improvement in an isolated propionyl-CoA carboxylase deficency has yet to be reported[44].

A low protein diet (0.5-1.5 g/kg/d) or selective reduction of the content of propionate precursors is used. Avoidance of fasting, biotin administration, and L-carnitine (100 mg/kg/d) may help to reduce the excretion of propionic acid[45]. To reduce gut bacteria production of propionic acid, metronidazole (10 mg/kg) has been used[46].

For methylmalonic acidemia, a primary block in the mutase fails to explain the acidosis, hypoglycemia, hyperglycinemia and the hyperammonemia that occur. It has been suggested that these features result from the effects of accumulated organic acids and esters in the mitochondria that inhibit other specific intramitochondrial steps. A pancytopenia can occur due to the methylmalonic acidemia[47]. For treatment, address the life-threatening problems of ketoacidosis, hypoglycemia, hyperammonemia and dehydration. Institute a restricted protein diet, restricting the amino acid precursors of methylmalonic acid and give 1-2 mg CN-cobalamin. L-carnitine supplements could be used to replete stores of free carnitine and enhance the reduction of methylmalonic acid[45], and the use of metronidazole[46] could have beneficial effects on reducing the gut as a source of methylmalonic acid.

Multiple carboxylase or biotin defects. Symptoms that are seen include tachypnea and hyperventilation, irritability, hypotonia, skin rash, etc. These disorders are associated with metabolic acidosis, mild to moderate hyperammonemia, and organic aciduria involving the substrates for the individual enzymes involved.

While the majority of IEMs involve one metabolic pathway, the biotin defects involve enzymes across all three classes of metabolites carbohydrate, fats, and amino acids resulting in defects in gluconeogenesis, fatty acid synthesis, the catabolism of the odd carbon length fatty acids, and the catabolism of leucine. Four carboxylases known to require biotin, a water soluble member of the vitamin B family as a prosthetic group, catalyze these pathways, including pyruvate carboxylase, propionyl-CoA carboxylase, β-methylcrotonyl-CoA carboxylase and acetyl-CoA carboxylase. Each carboxylase is synthesized as an inactive apoenzyme requiring biotinylation catalyzed by the enzyme, holocarboxylase

synthase. Three of the carboxylases are mitochondrial and one is cytosolic. When these enzymes are ready for normal catabolism, the biotin that is attached is conserved by recycling by a number of enzymes, the biotinidases, that are detectable in the blood. Mammals cannot synthesize biotin, which must be obtained from the diet. There is some evidence, however, that the gut bacteria also contributes as a source. The defects that can arise may involve the holocarboxylase synthase, one of each of the individual carboxylases, or the recycling enzymes, the biotinidases. If the holocarboxylase is involved, each of the individual carboxylases would also be affected. Treatment with biotin can reverse symptoms, if the apoenzymes are not affected[48].

4. Metabolic acidosis without ketonemia, the nonketotic hypoglycemias.

As fasting is prolonged, more and more fatty acids are released by adipose tissue and are taken up by the liver, enzymatically linked to CoA and then to carnitine for transport into the mitochondria, where the machinery for β-oxidation of the fatty acids are located, before reconversion back to the fatty CoA for β-oxidation in the mitochondria. Two-carbon acetyl-CoAs are enzymatically removed for conversion to the ketone bodies for export and use as the fuels, acetoacetate and β-OH butyrate, by the brain and muscle. In the blood, acetoacetate will spontaneously convert to acetone, noticed as the familiar sweet odor on the breath of patients in ketoacidosis. The acetyl-CoA can also be used in the TCA cycle after formation of citrate with oxaloacetate.

In the presence of metabolic acidosis and hypoglycemia, the presence of ketone bodies indicates that the enzymes of fatty acid β-oxidation and ketone body formation are present and working hard. In the presence of metabolic acidosis and hypoglycemia, the absence of ketone bodies signifies a block in either 1. the supply of acetyl-CoA for ketone body formation, as in PDH deficiency, or a fatty acid oxidation defect, or in 2. the processing of the acetyl-CoA for the exportable fuels, acetoacetate and β-OH butyrate.

Defects at selected enzymatic steps in the formation of the ketone bodies lead to nonketotic types of metabolic acidosis with hypoglycemia during fasting.

Fatty acid oxidation defects. With a defect in the steps that move the fatty acids into the mitochondria, the availability of glucose and of short chain fatty acids which do not require the addition and switching of CoA for carnitine and back to CoA, should allow sufficient acetyl-CoA for the TCA cycle and activation of PC for gluconeogenesis. A defect in the β-oxidation of a longer chain fatty acid would leave the short and medium-length fatty acids available for both the TCA cycle and gluconeogenesis to occur, as long as fasting is avoided. Also, the peroxisomal system uses an oxidase, not a dehydrogenase, on long-chain fatty acids, which could reduce long-chain ones to shortened fatty acids that may then by-pass the defect in the mitochondria. Depending on the chain length of

the fatty acid involved in fatty acid oxidation defects, ketosis may be absent or present. A medium chain length fatty acid oxidation defect is the most frequent derangement you are likely to see and is the one fatty acid oxidation with the highest chance of death on prolonged fasting. Long-chain fatty acids would still be broken down to provide some acetyl-CoA moieties, and PDH and PC could still function to provide some back up with gluconeogenesis and function of the TCA cycle, accounting for the mild hypoglycemia and mild ketosis that can be seen. Frequently, these children are misdiagnosed as a case of sudden infant death or Reye's syndrome. With each type of defect, the organic acids in the blood and urine and the carnitine status would have to be analyzed to pinpoint the lesion[49].

3-hydroxy-3-methylglutaryl-CoA lyase deficiency. If there is a metabolic acidosis with nonketotic hypoglycemia with hyperammonemia, increased liver size and liver enzymes, consider a defect involving 3-hydroxy-3-methylglutaryl-CoA lyase, which occurs with increased frequency in Arabic populations. Patients present with vomiting, hypotonia, and increasing lethargy, and coma develops in 10 percent. A bimodal age distribution is reported with 30 percent of cases presenting between two to five days of age, and 60 percent between three and 11 months. All have metabolic acidosis and 90 percent have hypoglycemia, both of which are very severe. Half of the patients have severe hyperammonemia and hepatomegaly with increase in the liver enzymes. The death rate is rather high at 20 percent. Patients are often misdiagnosed as Reye's syndrome until the characteristic organic acids are found in the urine. This defect leads to an inability to form acetoacetic acid and 3-hydroxybutyric acid, so patients show no ketosis, despite their acidosis[50].

As this is a block in the formation of the ketone bodies, fasting must be avoided. Treatment of acute episodes consists of glucose to control the hypoglycemia and appropriate electrolytes. A protein-restricted diet should be followed offering 1.5-2 g/kg/day, with restriction of fat to about 25 percent caloric intake.

Other nonketotic defects. A similar clinical presentation can be found in glutaric acidemia. Type I, in association with extrapyramidal symptoms and macrocephaly at birth due to glutaryl-CoA dehydrogenase deficiency, as well as in glutaric acidemia. Type II, which is associated with dysmorphia, prematurity and the odor of sweaty feet.

For glutaric acidemia Type I, treatment consists of reduction of protein or a lysine and tryptophan-low formula plus L-carnitine and riboflavin. Valproic acid has been tried to selectively increase GABA in synaptic areas by inhibiting GABA transaminase or succinic semialdehyde dehydrogenase, or by inhibiting GABA uptake by glial cells and nerve endings. Most importantly, one must rapidly treat the acidosis and hypoglycemia during intercurrent infections and catabolic states.

For glutaric acidemia Type II, or multiple acyl-CoA dehydrogenase deficiency with a neonatal onset, the patient is often premature, presenting by 24^{th}-28^{th} hour of life with hypotonia, hepatomegaly, severe hypoglycemia, metabolic acidosis, and the odor of isovaleric acidemia. Some neonates present with palpable kidneys due to cysts, facial dysmorphism, rocker bottom feet, muscular defects of the anterior abdominal wall, and anomalies of the external genitalia, including hypospadias. Most die in the first few weeks. Those who survive die within a few months after hypoketotic or nonketotic hypoglycemia and metabolic acidosis, with fatty degeneration of the liver parenchymal, renal tubular epithelial, and myocardial cells, and with accumulation of metabolites of compounds that are oxidized by enzymes that transfer electrons to electron transfer flavoproteins. Generally, treatment has not been very successful and has consisted of the use of diets low in fat and protein with supplementation of carnitine and riboflavin. The parenteral administration of methylene blue (2 g/kg/d) has also been tried as an artificial electron acceptor to remove flavin-bound electrons from residual acyl-CoA dehydrogenase with equivocal results[37].

D) Examples of Other Defects in the Newborn Based on Their Clinical Presentation

1. Jaundice without Severe Anemia

a) Galactosemia can arise from defects of one of three enzymes along the metabolic pathway that converts galactose to glucose: galactose 1-P04 uridyltransferase, galactokinase, and uridine diphosphate galactose 4-epimerase. Defects with these enzymes result in the accumulation of galactose and the development of toxicity symptoms such as failure to thrive, vomiting, and liver disease, beginning a few days after the ingestion of milk. Jaundice due to elevated unconjugated bilirubin often appears within a few weeks of life. Mild hemolysis suggestive of erythroblastosis fetalis can occur. Abnormal liver function tests and hepatomegaly with the full blown picture of liver disease with ascites, in the absence of portal hypertension or severe hypoalbuminemia, can also occur. Cataracts can be present within days of birth. Delay in development, neurological symptoms, and ovarian failure are late consequences of galactosemia.

As the primary dietary source of carbohydrate for the newborn is lactose, the principal disaccharide in mammalian milk which consists of glucose and galactose, the action of lactase in the intestinal tract would release sufficient galactose to produce symptoms in vulnerable newborns. The major metabolic pathway for galactose is its conversion to glucose without disruption of the carbon skeleton. Several enzymatic steps are involved. First, galactose is phosphorylated to form galactose1-P04 by galactokinase. Then, galactose 1-P04 reacts with UDP-glucose, producing UDP-galactose and glucose 1-P04, catalyzed by galactose 1-P04 uridyltransferase. A defect at this step is responsible for classic galactosemia. The UDP-galactose is finally acted upon by the epimerase, which inverts the hydroxyl group of the galactose moiety at the fourth carbon of the hexose chain to form UDP-glucose.

Laboratory findings include abnormal liver function tests, elevated blood and urine galactose, reducing substance in the urine, hyperaminoaciduria, albuminuria, and hyperchloremic metabolic acidosis, which could be due to the gastrointestinal disturbance that occurs in galactosemia, poor food intake, and/or as renal tubular defect in urine acidification that occurs in this disease. There is also an increase in the galactose 1-P04 content of the red blood cells. The diagnosis can be made in the circulating red blood cells (rbcs) by increase in galactose 1-P04 and by enzymatic assay for the specific enzymes involved.

Treatment is to restrict galactose intake by avoiding proprietary formulas as well as both human and cow's milk. Soybean based milks or a casein hydrolysate, Nutramigen, can be used instead[51].

b) Bile and bilirubin metabolic defects. Newborns have increased bilirubin levels due to the decreased erythrocyte half-life, diminshed hepatic capability to dispose of bilirubin, and diminished intestinal bacteria that degrade bilirubin to urobilinogen, with a greater surface-to-volume ratio of the bowel enhancing the intestinal absorption of unconjugated bilirubin compared to adults[52]. As a result, about half of all newborn become clinically jaundiced during the first five days of life. The serum bilirubin, largely unconjugated, can reach 10 mg/dl or more before decreasing to normal levels in seven to 10 days[53]. Plasma bilirubin concentrations are higher in breast-fed compared to formula-fed infants and will promptly fall upon discontinuation of breast-feeding. Levels as high as 15-24 mg/dl have been reported within 10-19 days of age without the development of kernicterus[54].

1. Crigler-Najjar syndrome Type I, due to the absence of hepatic bilirubin UDP-glucuronosyltransferase activity, manifests itself within the first few days of life with jaundice due to increased plasma concentrations of indirect-reacting bilirubin, which can lead to kernicterus and death. Apart from the jaundice and neurological impairment, other findings are not abnormal. Stool color, bilirubin production, the hematocrit, red blood cell (rbc) morphology and kinetics, as well as the liver function tests are normal. Aggressive phototherapy and plasmapheresis can avert kernicterus, if instituted in a timely manner, but long-term outlook is still very bleak. Liver transplantation appears to result in long-term survival.

2. Bile acid biosynthesis defects and cholestasis. Manifestations of cholestatic liver disease at birth with pale stools, dark urine, and progressive jaundice can occur because of defects in either 3β-hydroxysteroid-Δ^5-oxidoreductase/isomerase or 3-oxo-Δ^4-steroid 5β-reductase activity.

With the former derangement, 3β-hydroxystreid-Δ^5-oxidoreductase/isomerase, there is increased circulating conjugated bilirubin, liver transminases, alkaline phosphatase and low levels of vitamin E. Cholate and chenodeoxycholate are not detectable in the plasma. Diagnosis can be made by negative ion fast atomic

bombardment-mass spectrometry of a urinary bile acid fraction where a pattern corresponding to characteristic sulfated esters is found. A urinary test for the accumulating bile acids in urine can be made by the Lifschutz reaction, and enzymatic assay can be done on cultured skin fibroblasts. Treatment with chenodeoxycholic acid (250 mg/d) inhibits the rate-limiting enzyme in bile acid biosynthesis, cholesterol 7α-hydroxylase, and diminishes the production of toxic metabolites from cholesterol[55].

With 3-oxo-Δ^4-steroid 5β-reductase deficiency, jaundice, pale stools and dark urine are also seen within the first day or so of life. A marked increase in conjugated bilirubin and coagulopathy also occur. Fasting bile acids are elevated in the serum in contrast to the other defect leading to cholestasis in newborns. In this disorder, there is failure to convert two bile acid intermediates, 7α-hydroxy and 7α, 12α-dihydroxy 4-cholesten-3-one, to the corresponding 3-oxo-5β satured derivatives toward chenodeoxycholic and cholic acid formation. However, the side chain of the accumulated 3-oxo-Δ^4 bile acid precursors may be oxidized despite incomplete transformation of the ring nucleus, accounting for the increase in the serum bile acids that occurs. In affected newborns, the liver transaminases and the serum alkaline phosphatase are normal, in contrast to the former disorder. Definitive evidence for this diagnosis has yet to be obtained from enzymatic study. Treatment with exogenous bile acids inhibits cholesterol 7α-hydroxylase activity, preventing the accumulation of potentially toxic bile acid intermediates, while exerting a choleretic effect and improving intestinal fat absorption[55].

3. Copper transport defect or Menkes disease. Newborns with the X-linked classic Menkes disease with widespread disturbance in the intracellular transport of copper are often born prematurely and exhibit hypothermia and hyperbilirubinemia as neonates. Most appear normal but some can exhibit trichorrhexis nodosa, monilethrix, and unusual facies as neonates. The cerebral degeneration, vascular complications (subdural hematoma, arterial rupture, and thrombosis), and bone changes develop later. Serum copper and ceruloplasmin levels are low with marked differences from normal by two weeks of age. Liver copper content is low whereas gut tissue content is high. Treatment to reverse the copper deficiency can restore hepatic and serum levels to normal without reversal of the neurological damage that occurs. Copper histidinate treatment has been the usual form of copper administered[56].

2. Jaundice with Anemia

a) Glucose 6-P04 dehydrogenase deficiency. A number of defects can result in hemolytic anemia and jaundice in the neonate. Glucose 6-P04 dehydrogenase (G6PD) deficiency resulting from many allelic mutations of the gene is the commonest known enzymopathy affecting people worldwide. G6PD produces NADPH, the coenzyme that serves as a hydrogen donor in numerous enzymatic reactions, some of which are catalyzed by glutathione reductase, an enzyme

important in protection against oxidative damage. A defect in G6PD would reduce this ability and allow peroxide to accumulate, exceeding the ability of catalase to detoxify the peroxide, leading to hemolysis. Red cell morphology is affected and Heinz boides appear due to denatured protein adhering to the celluar membrane. Reticulocytosis and impaired liver function can also appear. There is striking variability in its expression, but kernicterus occurs with increased frequency amongst affected newborns in both Africa and Southeast Asia. Avoidance of oxidant drugs, treatment of hypoxia, sepsis and acidosis, prophylactic administration of phenobarbital to improve hepatic conjugation of bilirubin, and phototherapy or exchange transfusion could be used[57].

b) Pyruvate kinase deficiency. Severe neonatal anemia and jaundice occur. Red blood cell morphology is usually not affected in the neonate except in severe cases when macrocytosis and echinocytes can be detected. Erythrocyte osmotic fragility is normal, the Coombs test is negative, and cold agglutinins are not found. Red blood cell (rbc) life span is shortened and cells are selectively sequestered and destroyed in the spleen as well as the liver. Indirect bilirubinemia and decreased haptoglobin concentrations reflect the severity of the hemolytic process. Fecal urobilinogen excretion is increased. Splenectomy permits the newly formed cells to survive longer, and transfusions of red blood cells (rbcs) may be necessary from time to time. Enzyme assay on red blood cells (rbcs) must be done without contamination with leukocytes[58].

c) Intrinsic red blood cell (RBC) structural membranopathies. Hereditary spherocytosis is caused by the progressive loss of red blood cell (rbc) membrane surface. Defects of spectrin, ankyrin, pallidin and band 3 of the red blood cell (rbc) membrane have been found in this disorder. Affected infants develop jaundice and anemia within the first few days of life, requiring exchange transfusion to prevent kernicetrus. Once the hemoglobin is corrected by the transfusion, the progression of the anemia is much slower and the course of the disease can be reduced. Later, the spleen may enlarge. The presence of spherocytes and increased osmotic fragility are key diagnostic findings. Besides the anemia, the reticulocytes are increased and there is increase in unconjugated bilirubin in the blood. Fecal excretion of urobilinogen is increased. Splenectomy is the treatment for this disease[59].

3. Congenital Cyanosis or Methemoglobinemia

Over 400 human hemoglobin variants have been demonstrated, the majority of them due to single amino acid substitutions in one of the globin chains. Variants resulting in sickle cell and the thalassemias may express abnormalities in the first year of life, but rarely in the neonate. Among the clinical diseases resulting in the neonatal period are variants where 1) the hemoglobin levels are normal but cyanosis is noted at birth, such as in hemoglobin Bart, observed in Southeast

Asians or people of Mediterranean origin, which results in a syndrome that is fatal in utero, or soon after birth, or 2) there is an accumulation of methemoglobin due to hemoglobin M[60].

a) Hemoglobin Bart presents in a newborn who is pale and edematous with signs of cardiac failure and intrauterine hypoxia. Hepatosplenomegaly and other organ abnormalities may also be found. For diagnosis, the hemoglobin level, hematocrit, red blood cell (rbc) indices, hemoglobin electrophoresis, and estimation of the fetal hemoglobin, hemoglobin A_2 and hemoglobin Bart levels should be made, not only on the patient but also on family members. Further analysis by a hematological laboratory would be necessary to pinpoint the variant.

b) Congenital cyanosis can result from an inherited structural defect of the hemoglobin or due to a defect in one of the enzymes involved in maintaining the level of reduced hemoglobin within the normal range. The structural defect of hemoglobin resulting in congenital methemoglobinemia, a benign condition, is due to a dominant pattern of inheritance of hemoglobin M. Individuals with the enzymatic derangements are cyanotic from birth. Ten to 15 percent of total hemoglobin as methemoglobin produces the cyanosis, whereas 2.5-3 times that amount of deoxygenated hemoglobin results in a comparable degree of cyanosis. The degree of methemoglobinemia found in patients is well tolerated and patients are asymptomatic. Treatment is unnecessary, and the methemoglobinemia clears with parenteral methylene blue. Oral ascorbic acid and riboflavin are also reported to keep the methemoglobin level at five percent.

4. Hemophilia

Hemophilia presents as bruising and hematoma formation unaccountable by trauma, or as continued bleeding after circumcision or needle puncture in a male neonate. Various mutations of X-linked factor VII deficiency interfere with the production of normal levels of factor VIII. A history of other affected males in the extended family may be obtained. A prolonged partial thromboplastin time, normal prothrombin time, normal bleeding time, normal platelet count with a specific defect in factor VIII clotting activity, and no antibodies to factor VIII are diagnostic. Treatmenat is by replacement therapy using either blood derived factor VIII or recombinant products that are available, recognizing that the half-life of factor VIII is only about 12 hours[61].

5. Diarrhea

Disaccharidase deficiencies or intolerance cause severe diarrhea starting within hours or days of life, with dehydration occurring after ingestion of the disaccharide that is not cleaved by specific intestinal enzymes which are either diminished or absent (lactase or sucrase-isomaltase). Flatulence, borborygmi, and abdominal distension also occur. Reducing substances can be found in the feces. Breath

hydrogen is increased after ingestion of the suspected disaccharide, and chromatographic analysis of the stool would identify the disaccharide that is not cleaved. Avoidance of the offending disaccharide-containing formula is key[62].

6. Hormone Deficiencies

a) Congenital hypothyroidism. Inadequate production of thyroid hormone is due to a number of causes, only a few of which are genetic, but all of which can result in mental retardation, growth failure, deafness, and neurological abnormalities. The majority of affected newborns will be normal in appearance because of placental transfer of maternal thyroid hormone. Unless there is a prior history of an affected sibling, or persistence of neonatal jaundice, the clinician may not be aware of this diagnosis. Only with diligence and care can a low metabolic rate, poor peripheral circulation, bradycardia, constipation, or a goiter be noted. A free T_4, TSH, or both, tests can be done using the dried filter paper spot used in many screening programs. The free T_4 would be low and the TSH would be elevated. Treatment is with synthetic thyroid.

b) Congenital adrenal hyperplasia[63]. A number of enzymatic defects can affect the biosynthesis of the adrenal corticosteroids. The commonest is a 21-hydroxylase deficiency which causes decreased synthesis of cortisol. Corticotropin-releasing factor and ACTH levels increase, and the high ACTH levels increase the levels of the precursors of cortisol, of which 17 α-OH progesterone is a major component. The 17 α-OH progesterone has a sodium loss and potassium retention effect with water loss. The increased ACTH increases the production of androgens. This increased androgen production leads to the somatic and sexual precocity seen in affected newborns. In females, an incorrect sex assignment could result. In three-fourths of affected males, a salt-losing syndrome can develop. Danger of life-threatening adrenal crises arises which can result in nine percent mortality in the newborn period. This defect can be detected by neonatal screening tests run on blood spots collected on filter paper for increased 17 α-OH progesterone[9]. Treatment is by administration of cortisone with careful monitoring. Females may require surgery to correct ambiguity of the genitalia in the future.

Other inherited conditions that are readily detectable by physical examination (Down syndrome, albinism, etc.), some that are difficult to detect in the newborn (Marfan), and others that express themselves after the first few months of life have not been included in this report on a suggested clinical and laboratory approach to the diagnosis of an IEM in the neonate.

Would new screening tests be available to identify all affected neonates in the future? Newborn screening tests rely on time-dependent changes in the concentrations of substances in the blood for identification [phenylalanine for PKU,

leucine for MSUD, methionine for homocystinuria, galactose for galactosemia, 17-hydroxyprogesterone for congenital adrenal hyperplasia and thyroxine (T4) and thyrotropin (TSH) for congenital hypothyroidism]. In the US, it is recommended that screening be done after 24 hours of age but before day five or, if infant is discharged before at 24 hours of age, that the initial specimen be collected in the hospital and follow-up specimen be sent in by day seven to 21[9]. Recent policies on early discharge of postpartum mothers and their infants as a cost-saving strategy raise concerns of missing cases. Thus, the feasibility of genotyping from dried blood spots was explored for phenylalanine hydroxylase.

Dried blood spots as a source of genomic DNA for polymerase chain reaction (PCR) gave a low yield of PCR products, necessitating a two-step amplification procedure to be used with three percent different oligonucleotide primers. Ninety-six percent of mutant alleles were identified, not 100 percent[64]. For he work entailed and the four percent positive error rate, genotyping may not be suitable for widespread use as a screening tool for PKU. Whether genotyping for other disorders would result in tolerable positive error rates, or none at all, remains to be seen. (Interestingly, PKU appears to be caused by more than 200 mutations at the PAH locus, most of which are strongly associated with a specific restriction fragment length polymorphism or a variable number of tandem repeat haplotypes; five novel substitutions in the PAH genes were also found).

But, for the tools currently in use, a separate test is done for each disorder screened and only a few IEMs are included. Advances in the design of mass analyzers, new ionization techniques and efficient computing systems have led to tandem mass spectrometers (MS) which can analyze specific compound groups such as the amino acid and acylcarnitine mixtures without prior separation. Automated electro-spray ionizatilon tandem mass spectrometers can now be used for the diagnosis of amino acid and fatty acid IEMs from dried blood spots[65]. While this approach is bound to become more widespread in use, the range of disorders that can be identified is still limited.

A combination gas chromatography/MS (GC/MS) approach extending the disorders to a total of 22 IEMs is in use in Japan[66]. This approach requires pretreatment of the urine sample to remove urea with urease plus derivatization. The time required for analysis is still a bit long, allowing only 80 samples to be analyzed per instrument in a working day, but development is proceeding to automate and thus decrease the time required for GC/MS.

With families at risk, prenatal DNA analysis can be helpful. Amniocytes and chorionic villus samples can be used, but contamination of the chorionic villus by maternal decidua is possible when PCR amplification for restriction fragment length polymorphism (RFLP) or mutational analysis is used.

Finally, even if the diagnosis is readily apparent, or a screening test returns as positive, consult your local experts early, encourage parental adherence to the recommendations of a multidisciplinary team of professionals, and be prepared for a life-long relationship with your patient, complete with genetic counseling with the family and the patient as well.

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EPIDEMIOLOGY AND ANTIBIOTIC RESISTANCE OF GRAM-NEGATIVE URINARY PATHOGENS IN PEDIATRIC PATIENTS*

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SUMMARY: Gür D, Kanra G, Ceyhan M, Seçmeer G, Kanra B, Kaymakoğlu İ. (Infectious Diseases Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Epidemiology and antibiotic resistance of Gram-negative urinary pathogens in pediatric patients. Turk J Pediatr 1999; 41: 37-42.

In order to determine the etiological agents and the rate of resistance to various antibiotics, 209 consecutive Gram-negative bacteria isolated from children admitted to Hacettepe University Children's Hospital with urinary tract infections were investigated over a three-month period. Of these, 46 (22%) were nosocomial isolates. The most frequently isolated organism was E.coli (n: 141) followed by Klebsiella spp. (39), Proteus spp. (19), Pseudomonas spp. (8) and Enterobacter spp. (2). In vitro susceptibilities were evaluated by microbroth dilution method, following NCCLS guidelines. Overall, 75 percent of the isolates were resistant to ampicillin, 52 percent were resistant to TMP/SMX and 25 percent to cefuroxime. Amikacin was the most active aminoglycoside; 93 percent of the isolates were susceptible to this agent, while resistance to gentamicin was 21 percent. Resistance to ceftazidime and ceftriaxone was 12 percent and 19 percent, respectively. Overall, resistance to imipenem was one percent and to ciprofloxacin three percent. These in vitro results should be taken into account before initiating empirical therapy; broad spectrum antibiotics should not be used if the isolate is susceptible to the older drugs in order to prevent the increase in resistance. *Key words: antibiotic resistance, urinary tract pathogens.*

Empirical therapy of urinary tract infections (UTI) depends on knowledge of the causative agents and their susceptibility patterns to antimicrobial agents in each center. These factors may change over time and according to the location. Hence, periodic surveillance studies are required in each center to follow the frequency of the etiological agents and their susceptibility profile as well as to provide a guide to the clinician for empirical treatment. In this study, the prevalence of Gram-negative bacteria isolated from urine cultures in Hacettepe University Children's Hospital was investigated and their susceptibilities to commonly prescribed antimicrobial agents were evaluated in hope of producing a guide to the clinician.

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Material and Methods

Gram-negative organisms which were isolated from consecutive urine cultures with significant growth (10^5 cfu/ml) were collected over a three-month period in 1996. One isolate from each patient was included in the study. The isolates were identified by Sceptor ID (Becton Dickinson, USA). Microbroth dilution tests were employed to determine the in vitro efficacy of the antimicrobial agents. These were supplied from manufacturers in their powder form. The antibiotics and their suppliers were as follows: amikacin and gentamicin (FAKO İlaçları A.Ş.); netilmicin and cefixime (Eczacıbaşı A.Ş.); tobramycin (Nobel A.Ş.); ampicillin sulbactam/ampicllin, cefoperazone and sulbactam/cefoperazone (Pfizer İlaçları A.Ş.); cefuroxime and ceftazidime (Glaxo Wellcome İlaçları A.Ş.); ceftriaxone and TMP/SMX (ROCHE A.Ş.); imipenem (Merck Sharp ve Dohme İlaçları A.Ş.) and ciprofloxacin (Bayer A.Ş.).

Two-fold dilutions of the antibiotics were prepared so that the concentration in the first microwell was 256 mg/L. For TMP/SMX and ciprofloxacin only, the concentration in the first microwell was 16 mg/L. Mueller-Hinton broth (Oxoid) was used in the tests and the results were evaluated according to the NCCLS break points[1].

Results

A total of 209 Gram-negative bacteria were isolated during the study period. The frequencies of these bacteria are shown in Table I. E. coli was the most frequently isolated organism followed by Klebsiella spp. While most of the E. coli were isolated from community-acquired infections with only 17 nosocomial isolates (12%), 18 (46%) of the Klebsiella spp. were isolated from hospitalized patients. Similarly, half of the Pseudomonas spp., six of the Proteus spp. and one of the Enterobacter spp. were nosocomial isolates.

Table I: Gram-Negative Urinary Tract Pathogens (n: 209)

Organism	Number	%
E. coli	141	67
Klebsiella spp.	39	18
Proteus spp.	19	9
Pseudomonas spp.	8	3
Enterobacter spp.	2	< 1

In vitro efficacy of the 15 antibiotics for all the organisms is shown in Table II. Imipenem, ciprofloxacin and amikacin were the most effective antibiotics in vitro against urinary tract isolates. However, the level of resistance varies according to the pathogen.

Comparative in vitro activity of the antibiotics against E. coli and Klebsiella spp. is shown in Tables III and IV, respectively.

Table II: In Vitro Susceptibility of UTI Isolates to Antimicrobial Agents (mg/L)

Antibiotic	Range	MIC_{50}	MIC_{90}	Resistance (%)
Amikacin	≤ 0.125-64	1	16	7
Netilmicin	≤ 0.125-> 256	0.50	64	16
Gentamicin	≤ 0.125-> 256	0.50	64	21
Tobramycin	≤ 0.125-> 256	0.50	32	20
Ampicillin	0.50-> 256	> 256	> 256	75
Sulbactam/Ampicillin	0.25-> 256	32	> 256	64
Cefuroxime (oral)	≤ 0.125-> 256	4	> 256	25
Ceftriaxone	≤ 0.125-> 256	≤ 0.125	64	19
Ceftazidime	≤ 0.125-> 256	≤ 0.125	32	12
Cefoperazone	≤ 0.125-> 256	4	256	43
Sulbactam/Cefoperazone	≤ 0.125-> 256	1	32	12
Cefixime	≤ 0.125-256	0.25	32	19
Imipenem	≤ 0.125-8	≤ 0.125	2	1
TMP/SMX	≤ 0.008-> 16	> 16	> 16	52
Ciprofloxacin	≤ 0.008-> 16	0.01	0.06	3

Table III: In Vitro Susceptibility of E. coli to Antimicrobial Agents (mg/L)

Antibiotic	Range	MIC_{50}	MIC_{90}	Resistance (%)
Amikacin	≤ 0.125-32	1	4	2
Netilmicin	≤ 0.125-128	0.25	1	4
Gentamicin	< 0.125-256	0.50	2	4
Tobramycin	≤ 0.125-64	0.50	2	6
Ampicillin	0.50-> 256	> 256	> 256	65
Sulbactam/Ampicillin	0.25-> 256	16	> 256	58
Cefuroxime (oral)	0.25-> 256	4	16	14
Ceftriaxone	≤ 0.125-> 256	≤ 0.125	2	8
Ceftazidime	≤ 0.125-> 256	≤ 0.125	0.50	2
Cefoperazone	≤ 0.125-> 256	1	256	32
Sulbactam/Cefoperazone	≤ 0.125-> 256	1	16	8
Cefixime	≤ 0.125-256	0.25	1	7
Imipenem	≤ 0.125-8	≤ 0.125	0.25	< 1
TMP/SMX	≤ 0.008-> 16	4	> 16	49
Ciprofloxacin	≤ 0.008-16	≤ 0.008	0.03	3

Table IV: In Vitro Susceptibility of Klebsiella spp. to Antimicrobial Agents (mg/L)

Antibiotic	Range	MIC_{50}	MIC_{90}	Resistance (%)
Amikacin	050-64	2	32	17
Netilmicin	≤ 0.125-128	2	128	43
Gentamicin	≤ 0.125-> 256	8	128	51
Tobramycin	≤ 0.125-> 64	32	256	53
Ampicillin	4-> 256	> 256	> 256	97
Sulbactam/Ampicillin	2-> 256	64	> 256	74
Cefuroxime (oral)	≤ 0.125-> 256	4	256	46
Ceftriaxone	≤ 0.125-> 256	1	> 256	41
Ceftazidime	≤ 0.125-> 256	2	256	41
Cefoperazone	≤ 0.125-> 256	64	> 256	66
Sulbactam/Cefoperazone	≤ 0.125-> 256	8	128	25
Cefixime	≤ 0.125-256	1	> 256	43
Imipenem	≤ 0.125-8	0.25	0.50	2
TMP/SMX	≤ 0.01-> 16	0.25	> 16	38
Ciprofloxacin	≤ 0.008-16	0.03	0.25	3

Discussion

Several studies point to E. coli as the most frequent causative agent in both community and hospital-acquired infections[2]. According to the results of our study, E. coli was the predominant Gram-negative organism in our patients with urinary tract infections, with 67 percent of the cultures yielding this organism. Klebsiella spp. was the second most frequent pathogen and was isolated in 18 percent of the cultures. The most striking difference between these organisms is that Klebsiella strains are mostly nosocomial isolates, whereas most of the E. coli strains were isolated from outpatients. This is in accordance with other studies[2,3]. However, its rate of isolation is reported to be higher in patients with community-acquired infections[3,4]. In some reports, Proteus spp. are the second most frequent organism, but recent studies indicate that there have been changes in the urinary pathogens and that Proteus spp. have been overtaken by Klebsiella spp. and Enterobacter spp. in the hospital. This has been attributed to the use of antibiotics, as well changes in hospital hygiene[2].

Overall resistance to aminoglycosides in these strains is quite high. Amikacin is the most effective agent among this group of antibiotics. The level of resistance is higher in Klebsiella spp., with 17 percent resistance to amikacin and approximately 50 percent to gentamicin and tobramycin. The resistance rate for netilmicin is also high in these isolates (Table IV). Resistance to aminoglycoside antibiotics is related to development of the strains producing aminoglycoside-modifying enzymes active against these agents[5]. It is evident that AAC-6, the

enzyme which is active against amikacin, is rarer than the enzymes modifying netilmicin, gentamicin and tobramycin. This has been reported from most of the centers in Turkey[5, 6]. However, extensive use of this antibiotic may cause an increase in resistance in the near future and therefore must be used with caution.

In general, there is a very high rate of resistance against ampicillin. Nearly all Klebsiella spp. and 65 percent of E. coli are resistant to this agent. Addition of sulbactam had no significant effect on resistance in this in vitro study, suggesting that resistance may be due to inhibitor-resistant β-lactamases[7]. These isolates are reported frequently in community-acquired infections especially among organisms responsible for urinary tract infections[8]. However, a definite conclusion can only be drawn by further studies investigating the mechanisms of resistance in these isolates. Resistance to new β-lactams is not an unexpected result in Klebsiella spp., as these agents are widely used in our hospital. Although broad-spectrum β-lactamases were not investigated in this study, high resistance rates for the cephalosporins suggest that these enzymes were present in our isolates. However, the definite proportion of these enzymes can only be determined by studies using specific methods[9].

TPM-SMX resistance is reported to be higher in developing countries. In a study by Murray et al.[10], 44 percent resistance was observed among E. coli isolated at a pediatric hospital in Chile and 40 percent in Thailand. Most isolates in that study were from urinary tract infections. Overall resistance to TMP/SMX was 52 percent in urinary tract isolates in our study and the rate of resistance was higher in E. coli than in Klebsiella spp., reflecting the extensive use of this antimicrobial agent in urinary tract infections in Turkey. TMP/SMX resistance was nine percent in a U.S.A. study in 1991 and 19 percent in E. coli in a study from the U.K. in 1992[2, 11].

Our results indicate that imipenem is the most effective β-lactam agent against urinary tract isolates, followed by ciprofloxacin. However, these agents should only be used in severe cases as their overuse may lead to a resistance problem sooner than expected. This has been reported by Thomson et al.[11], who point out that noncritical use of the fluoroquinolones over a period of four years in the United States has resulted in the emergence of fluoroquinolone resistance at a greater rate than was originally anticipated. Empirical therapy, when needed, must be initiated according to the resistance pattern of the etiological agents until the susceptibility test results are reported to the clinicians. For safe and effective therapy, broad-spectrum agents are not usually necessary.

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THE EVALUATION OF OCULAR TRAUMA IN CHILDREN BETWEEN AGES 0-12*

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SUMMARY: Arıtürk N, Şahin M, Öge İ, Erkan D, Süllü Y. (Department of Ophthalmology, Ondokuz Mayıs University Faculty of Medicine, Samsun, Turkey). The evaluation of ocular trauma in children between ages 0-12. Turk J Pediatr 1999; 41: 43-52.

Ocular trauma is the leading cause of noncongenital unilateral blindness in children under 20 years old. In this study, 138 patients (36 female, 102 male) with ocular trauma between November 1983 and October 1996 were reviewed retrospectively at the Department of Ophthalmology, Ondokuz Mayıs University Faculty of Medicine. Twenty-four of these patients were blunt trauma and 114 were perforating eye injury. The mean age of the patients was 6.96 ± 3.01 years. Mean post-treatment follow-up was 10.7 months (range 1 to 121 months). Forty-five patients were admitted to the eye clinic within the first 24 hours after trauma. The most frequent finding was hyphema in blunt injury, and corneal laceration in perforating injury. The most frequent cause of injury was wood and stone in blunt trauma and glass and knife in perforating trauma. While the ratio of visual acuities equal to or better than finger counting was 37.5 percent (9 eyes) in blunt trauma cases and 20.2 percent (23 eyes) in perforating trauma cases prior to treatment, it was 79.2 percent (19 eyes) and 55.3 percent (63 eyes), respectively, at last visit examination post-treatment. The most frequent complication was traumatic cataract in blunt trauma and corneal leukoma and anterior synechia in perforating trauma. The results obtained suggested that socioeconomic and sociocultural status and family negligence are important factors in eye injuries in children that occur during games. *Key words: trauma, children, ocular injury.*

Eye injury in childhood is an important cause of unilateral blindness and may result in significant visual impairment. Ocular trauma is second only to cataract as the most common cause of visual impairment[1]; it is estimated that 29-52 percent of all eye injuries occurs in children[1-6]. In the United States, it has been reported that the incidence of eye injuries requiring inpatient hospitalization for children younger than 16 years was 15.2 per 100,000 per year[7]. Ocular injuries in childhood have some different characteristics from those in adults. Children under eight years of age are at particular risk of perforation leading to amblyopia. In addition, pretreatment evaluation is often hindered by inadequate history and poor cooperation during physical and ophthalmologic examination. These factors may have a negative effect on treatment methods and visual outcome. Eye injuries

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in children usually occur accidentally: injuries by the hand or foot of another child or during play and sport. Many of these eye injuries could be prevented, with an increased awareness and subsequent removal of the common risk factors[8, 9]. The best method of prevention of blindness or visual impairment in ocular trauma is not through successful operation, but rather through the prevention of eye injuries.

The aim of this study was to analyze the causes and prognosis of all eye injuries in children between ages 0-12.

Material and Methods

The records of all children between 0-12 years of age who were treated for serious ocular injuries at the Ondokuz Mayıs University Faculty of Medicine, Department of Ophthalmology, from November 1983 to October 1996 were reviewed retrospectively. The following data were recorded for each patient: age, sex, date of injury, type of injury, cause of injury, length of time from injury to hospitalization, diagnosis, treatment, visual outcome and late complications.

Results

A total of 601 patients were admitted to our clinic for the treatment of serious ocular injuries. Of these, 138 were children between 0-12 years of age, thus representing 23 percent of all patients with ocular injuries. These 138 children were included in this study.

Of the 138 children 102 (74%) were boys and 36 (26%) were girls. The mean age was 6.96 ± 3.01 (range 1 to 12 years). The ratio of boys to girls was 3:1. The age and sex distribution of these children with ocular trauma is shown in Table I and Fig. 1. As shown in Fig. 1, the accidents occurred nearly equally at all ages among the girls, while among the boys the frequency increased considerably from the age of eight years.

Of the 138 ocular injuries, 68 (49.3%) were right eye and 70 (50.7%) were left eye. All were monocular injuries.

Table I: Age and Sex Distribution

Age (Year)	Males No. (%)	Females No. (%)	Total No. (%)
0-5 y	31 (22.5)	15 (10.9)	46 (33.3)
6-12 y	71 (51.4)	21 (15.2)	92 (66.7)
Total	102 (73.9)	36 (26.1)	138 (100)

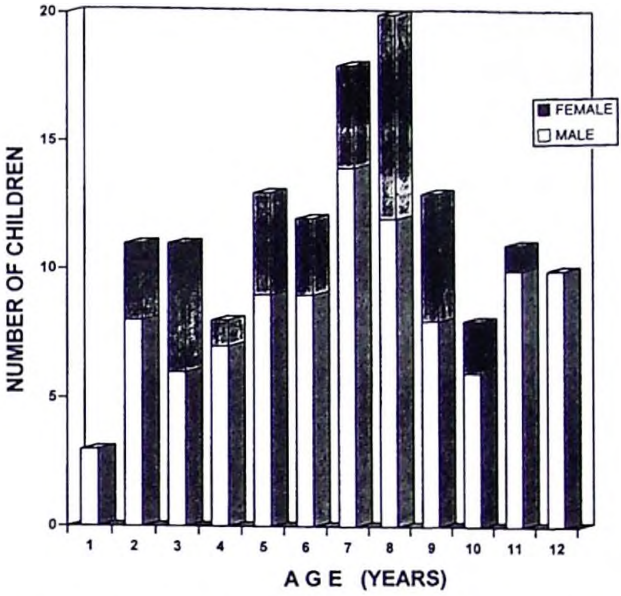

Fig. 1: Age and sex distribution of patients with ocular injuries.

Types of injury are presented in Table II. As can be seen, blunt injury was in 24 patients (17.4%) and perforating injury was in 114 patients (82.6%). The length of time from injury to hospitalization was within the first 24 hours in 45 patients (32.6%), and from 96 hours and over in 12 patients (9.0%). Among admissions within the first 24 hours, blunt injury was seen in 8 and perforating injury in 37 (Table III).

Table II: Types of Injury According to Age Groups

	0-5 y n(%)	6-12 y n(%)	Total n(%)
Blunt	3 (6.5)	21 (22.8)	24 (17.4)
Perforating	43 (93.5)	71 (77.2)	114 (82.6)
Total	46 (100)	92 (100)	138 (100)

Table III: Distribution of Patients by Length of Time from Injury to Hospitalization According to Types of Injury

	Types of Injuries		
Time	Blunt Trauma	Perforating Trauma	Total
First 24 hours	8	37	45
24-48 hours	3	57	60
72 hours	11	10	21
96 hours and over	6	6	12

The injured children were divided into the following age groups: zero to five years of age (infants and preschool) and six to 12 years of age (elementary school). Of those zero to five years of age, blunt injury was in three patients and perforating injury in 43 patients. Of those six to 12 years of age, blunt injury was in 21 patients and perforating injury in 71 patients. The ratio of perforating to blunt injury was 14:1 in the zero to five years of age group and 3:1 in the six to 12 years of age group. Perforating injuries are more frequent than blunt injuries and are more frequent in the zero of five years of age group than in the six to 12 years of age group. Most of the blunt and perforating injuries occurred in children ages five to nine, especially in elementary school children aged seven to eight.

The prevalence of injuries occurring in each month of the year is presented in Figure 2. The largest number of accidents took place in the October to December and May to August periods.

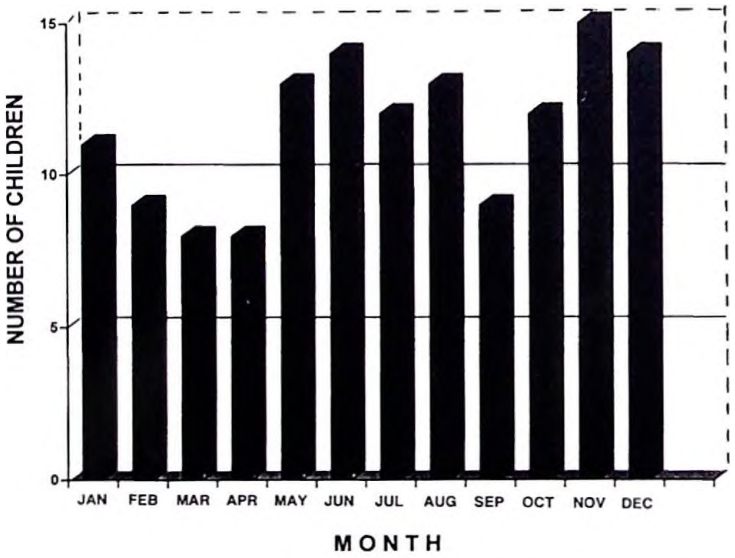

Fig. 2: Distribution of patients with ocular injuries according to month.

The causes of injuries are shown in Table IV. The most common causes of injury were sharp objects (31 patients, 22.45%), wood (19 patients, 13.8%), fall (14 patilents, 10.1%) and toys (12 patients, 8.7%). The most common causes of blunt injuries were wood (37.5%) and stone (25%) and of perforating injuries were sharp objects (such as glass and knife, 27.2%) and fall (10.5%).

The diagnosis of the traumatic eye injuries are presented in Table V. The most frequent primary admitting diagnoses were hyphema (54.3%), corneal laceration (52.9%), traumatic cataract (22.5%), and traumatic iridocyclitis (12.3%). The most frequent primary admitting diagnosis was hyphema in blunt injury and corneal

laceration in perforating injury. The mean post-treatment follow-up was 10.7 months (range 1 to 121 months). Visual outcomes for all patients are presented in Table VI. Analyzing the visual outcome in 118 children with eye injuries (20 children with unknown acuity were excluded), more than half of the children (69.5%) had very good visual acuity in the long term. While the initial visual acuity was > 0.1 in 32 patients, in final visual acuity this number increased to 82. The initial visual acuity was no light perception in 12 patients, and final visual acuity was no light perception in 16 patients. Of these, 14 were perforating injury and two were blunt injury. There was a correlation between the type of injury and the final visual outcome.. Anterior segment perforating injuries, whether alone or in combination with other ocular injuries, demonstrated an even worse prognosis for final visual outcome. In 16 eyes with no light perception, phthisis bulbi occurred in two, enucleation was required in two, and evisceration was required in nine eyes. Enucleation and evisceration during hospitalization were performed in 11 eyes.

Of the 138 children admitted for ocular trauma, 12 were treated medically and 126 required surgical treatment. The late complications observed are presented in Table VII. The most frequently recorded late complications of eye injuries were leukoma in 58 (42%), anterior and posterior synechia in 48 (34.8%), traumatic cataract in 33 (23.9%) and secondary glaucoma in 9 (6.5%) eyes.

Table IV: Causes of Eye Injury According to Types of Injury

Causes	Types of Injury		Total
	Blunt Trauma no (%)	Perforating Trauma no (%)	
Sharp objects (knife, glass)	–	31 (27.2)	31 (22.5)
Wood	9 (37.5)	10 (8.8)	19 (13.8)
Branches	2 (8.3)	9 (7.9)	11 (8)
Toys	3 (12.5)	9 (7.9)	12 (8.7)
Bush	–	10 (8.8)	10 (7.2)
Rod	1 (4.2)	9 (7.9)	10 (7.2)
Fall	2 (8.3)	12 (10.5)	14 (10.1)
Stone	6 (25)	2 (1.8)	8 (5.8)
Wire	–	6 (5.3)	6 (4.4)
Gun powder	1 (4.2)	–	1 (0.7)
Gun shot (bullet)	–	1 (0.9)	1 (0.7)
Paper	–	1 (0.9)	1 (0.7)
Nail	–	1 (0.9)	1 (0.7)
Pencil	–	1 (0.9)	1 (0.7)
Unknown	–	12 (10.5)	12 (8.7)
Total	24 (100)	114 (100)	138 (100)

Table V: Ocular Findings in Initial Ocular Examination

Ocular Findings	Types of Injury: Blunt Trauma no (%)	Types of Injury: Perforating Trauma no (%)	Total
Hyphema	12 (50)	63 (55.3)	75 (54.3)
Corneal erosion	2 (8.3)	–	2 (1.5)
Perforation of cornea	–	73 (64)	73 (52.9)
Perforation of sclera	1 (4.2)	5 (4.4)	6 (4.4)
Combination	–	37 (32.5)	37 (26.8)
Retinal and macular edema	1 (4.2)	4 (3.5)	5 (3.6)
Traumatic iridocyclitis	9 (37.5)	8 (7)	17 (12.3)
Traumatic cataract	3 (12.5)	28 (24.6)	31 (22.5)
Retinal and vitreous hemorrhage	–	5 (4.4)	5 (3.6)
Intraocular foreign body	–	2 (1.8)	2 (1.5)
Retinal detachment	1 (4.2)	–	1 (0.4)

Table VI: Initial and Final Visual Acuity in Children with Ocular Trauma According to Types of Injury

	Initial Visual Acuity						
Types of injury	NLP	P+P-	P+P+	CF-0.1	0.2-0.5	0.6-1.0	Undetermined
Blunt	3	5	4	4	3	2	3
Perforating	9	26	26	11	10	2	30
	Final Visual Acuity						
Types of Injury	NLP	P+P-	P+P+	CF-0.1	0.2-0.5	0.6-1.0	Undetermined
Blunt	2	–	1	2	8	9	2
Perforating	14	10	9	21	19	23	18

* Last visit examination.
NLP: No light perception, P+P-: Light perception, no projection, P+P+: Light perception and projection, CF: Counting fingers.

Table VII: Post-Treatment Complications

Complications	No.	%
Corneal leukoma	58	42
Anterior and posterior synechia	48	34.8
Narrowing anterior chamber	5	3.6
Traumatic cataract	33	23.9
Lens dislocation	7	5.1
Secondary glaucoma	9	6.5
Phthisis bulbi	2	1.5
Endophthalmitis	1	0.7
Retinal detachment	5	3.6

Discussion

Different rates of occurrence of ocular trauma in children have been reported in various studies. Canavan et al.[4], reporting on 2,032 traumatic eye injuries, found that 38.4 percent were sustained by children. Eye injury in childhood was reported as 34.4 percent by Niiranen[10], 47 percent by Rapoport[3], 29 percent by Maltzman[6] and 22 percent by Grin[11]. In our series, 23 percent of ocular traumas at Ondokuz Mayıs University Hospital occurred in children between the ages 0-12. These results were similar to those in the other series.

The rate of ocular trauma among boys was much higher than that among girls. In our study, the ratio of boys to girls was 3:1, similar to other surveys[3, 10, 12]. However, Nelson et al.[9] reported a ratio of 2:1 in children who were evaluated as outpatient in the emergency room. Cascairo et al.[13] reported a ratio of 2.6:1 in a study combining both inpatient and outpatient injuries.

As for seasonal increases in prevalence in other series, a higher prevalence in the second quarter of the year was noted[9, 11]. Usually, the distribution of ocular injury is equal for each month. In our study, the distribution of ocular injuries showed higher prevalence during May to August and October to December, the most obvious reasons being the warm weather and prolonged daylight during school holiday months and snow-related injury and winter sports-related injuries during the winter months. Turkey's increase in eye injuries during October to December may also be due to the pick-up of wood and bush for winter preparation. Grin[11] reported that the largest number of accidents took place during April to June and the smallest number during January to March. Cascairo and coworkers[13] reported a higher prevalence in March, April and August. The 0-12 years of age group includes preschool and elementary school children.

In this study, the incidence of eye injury occurred most commonly among children seven to eight years of age. Among girls, ocular injury occurred equally at all ages, while among boys it increased from the age of eight, with the largest number of injuries occurring in children eight to 10 years of age[10, 11, 14]. This is presumably due to the high physical contact and aggressive nature of play among boys. Most of the injuries occurred outdoors and during play or sport[3, 7, 10, 13].

In evaluating types of injury, we found that blunt injury accounted for 17.4 and perforating injury for 82.6 percent. In other studies, the rate of blunt injury was higher than perforating injury. The rate of perforating injury was reported as 30.5 percent by Rapoport[3], 27 percent by Rudd[15], 19 percent by Takvam[16], 8.9 percent by Niiranen[10], and 34.5 percent by Grin[11]. In our study, percentage of perforating injury obtained was much higher than in other studies. Also, we found that perforating injury in the zero to five years of age group was higher than in childen six to 12 years of age. Despite visual outcome being directly

related to the severity of ocular injury, children under eight years of age are at particular risk of perforation leading to amblyopia. In addition, the eye may become disfigured from corneal scarring and a squint may develop. For this reason, preventive measures should be taken to avoid or lessen the number of eye injuries among children.

Geographical location, climate, population, and sociocultural and socioeconomic status affect the type of injury and cause of injury. According to statistical studies undertaken in 1993, 33 percent of the general population in our country are between 0 to 15 years of age. The number of children per family is about 3.3 in rural areas and 2.5 in urban areas[17]. As the population of children and the number of children per family increases, the attention and supervision of parents over children tends to decrease. This situation results in an increase in the number of accidents and cases of eye trauma. The cause of an increased prevalence of perforating eye traumas among male children is probably due to the aggressive nature of toys, the inclusion of dangerous parts in manufactured toys, and the high interest of male children in perforating and cutting devices. We suspect that the high level of perforating trauma observed in our study is a result of the reference nature of our clinic as a university hospital in north and northeast Anatolia. Blunt traumas result from extraocular injury and generally do not require hospitalization. These patients are usually outpatient and they apply to the state hospital emergency service. All cases in our study were severe enough to require hospitalization. Furthermore, the cases admitted to our clinic were perforating traumas referred from other clinics which were not able to manage the patients.

The most frequent causes in our study were perforating injuries by glass or sharp objects (22.5%), wood (13.8%), toys (8.7%), and falls (10.1%). The most frequent causes as reported in other studies are stone (19.6%) and sharp objects (16.6%) by Rapoport[3], sport accidents (15%) and motor vehicle accidents (12%) by Cascairo[13], and accidental injuries by the hand or foot of another child (12%) by Nelson[9]. Niiranen and Raivio[10] reported that 21 percent resulted from thrown missiles (a snowball being the most common) and that 16 percent resulted from being shot with arrows. Takvam[16] reported that the most common cause of injury was projectiles (21.5%) followed by sticks. These results show that climate variations, sociocultural specifications and customs in each country are all effective as underlying factors for eye traumas.

Eye traumas can adversely affect the psychologic growth of children. The worst result is loss of light perception and blindness. In this study, the rate of cases who lost their light perception was 11.6 percent. This was 9.1 percent (2/22) of cases with blunt trauma, and 14.5 percent (14/96) of those with perforating trauma. Rudd et al.[15] reported the ratio of blindness in traumatic globe perforation of children under 16 years of age as 7.1 percent (3/42). This ratio was reported as 1.6 percent of blunt traumas and 9.3 percent of perforating traumas by

Rapoport et al.[3], two percent of all of cases by Cascairo[13], 29 percent of corneoscleral perforations by Barr[18], and 23.4 percent of cases of perforating injuries with no intraocular foreign body by Elder et al.[19] Our ratio was also high, as seen in other studies. In this study, the biggest proportion of eye injuries (87 cases) happened in or before 1990. This ratio has declined within the last few years. We believe that progression in diagnostic and therapeutic modalities in our clinic was the major factor in improving the results.

The most effective way to prevent blindness from trauma is not a successful therapy, but is prevention of the injury. For this purpose the most important requirement is education. Parents, teachers, and children must be educated about eye injuries and their consequences. A better supervision of parents over their children and more care in selecting toys for children seem to be appropriate. It is also very important for the significance of eye traumas to be stressed frequently by the media. In addition, movies on television and computer games that contain violence should be strictly inspected because they could adversely affect the psychologic status of children and lean them toward aggressiveness.

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INTERMITTENT CHEMOTHERAPY FOR MILIARY TUBERCULOSIS IN CHILDREN*

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SUMMARY: Anadol D, Kiper N, Göçmen A, Özçelik U. (Chest Disease Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Intermittent chemotherapy for miliary tuberculosis in children. Turk J Pediatr 1999; 41: 53-59.

Miliary tuberculosis is a severe manifestation of tuberculosis. Six children aged between two months and 10 years with the diagnosis of miliary tuberculosis were treated with intermittent antituberculous therapy for six, nine or 12 months. All the patients showed clearance of both clinical and radiological symptoms; there was no drug toxicity or resistance and no relapses were seen in the follow-up period ranging from nine months to nine years. Intermittent therapy is safe and effective in miliary tuberculosis and it may be an alternative therapy because of its minimal toxicity and lower cost. *Key words: miliary tuberculosis, intermittent chemotherapy, children.*

Tuberculosis remains a major problem for the world because of its high morbidity and mortality. Although significant progress in the control of this disease has been made in Turkey it is still one of the most important public health problems[1].

Miliary tuberculosis is a severe manifestation of tuberculosis, especially in children[2]. It is characterized by marked variation in clinical presentation and often significant delay in diagnosis[3]. During the prechemotherapy era, it was a common complication of primary tuberculosis with a very high mortality rate, but with the advent of treatment mortality declined[2]. The official recommendation in miliary tuberculosis is 12 months of treatment, with 10 months of isoniazid (INH) and rifampin (RIF) following the initial two months of daily multidrug therapy[4]; However, the therapy for tuberculosis has undergone major changes in the past 10 years[5, 6]. We hearby present our data with intermittent therapy for miliary tuberculosis.

Material and Methods

Patients with the diagnosis of miliary tuberculosis between January 1982 and December 1995 were included in this retrospective study. A presumptive diagnosis of miliary tuberculosis was made by typical miliary pattern on chest radiogram and clinical findings like fever and weight loss[7].

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The age and sex of the patients, their contact with another case of tuberculosis, results of their Mantoux test with 5 TU purified protein derivative (ppd) skin tests, and whether or not they had a BCG vaccine were recorded. An induration of ≥ 15 mm on the Mantoux test in vaccinated patients and > 10 mm in patients without a BCG vaccine was considered positive. The presenting symptoms, the duration of the symptoms and the clinical findings were noted.

Wellcome classification was used for the diagnosis of protein-energy malnutrition[8]. Hepatomegaly was defined as palpation of the lower edge of the liver 2 cm below the inferior costal margin in the midclavicular line. Splenomegaly was defined as the palpation of the splenic margin > 0.5 cm below the inferior costal margin.

The results of the mycobacterial culture from the gastric aspirates of all children were noted. Chest x-ray findings, microbiological findings, drug regimens, drug toxicity, time for complete clinical remission and radiological clearance, and the results of the treatment were also recorded. Clinical and radiological evaluations were made on the 15th day of the treatment and every three months during the therapy. Improvement in appetite and weight gain, normalization of body temperature, and cessation of sweating were accepted as criteria for recovery during the course of treatment. Resolution of infiltrates and miliary pattern on chest radiograph were accepted as criteria of radiological recovery. In order to estimate the compliance of the patients, the drugs were counted and the color of the urine was examined on every visit. Liver function tests were made if indicated in this period. After the treatment was discontinued, clinical and radiological outcome was assessed every three months in the first year, every six months in the second year and every 12 months in the following years.

Results

From January 1982 to December 1995, 19 cases were diagnosed with miliary tuberculosis. Only the six who we could be sure would be closely followed up, come regularly to hospital visits, and have a good compliance were treated with intermittent chemotherapy.

There were four boys and two girls and the ages of the patients ranged between two months and 10 years with a mean age of three years. The majority of patients (67%) were younger than six years, while half of the children were less than two years old.

Three patients (50%) had a history of contact with a tuberculous patient, with all infections occurring from household contact (one from mother, one from uncles, and the other from a distant relative).

The most common presenting symptoms were cough and fever, each noted in four and three patients respectively. One patient complained of vomiting, headache and convulsions. Weight loss, hemoptysis and reduced range of hip

motion were other presenting symptoms. The duration of time between the initiation of symptoms and diagnosis ranged from two weeks to seven months; the mean duration of time was seven weeks.

As for the predisposing conditions, two of our cases already had malnutrition. Only one patient was vaccinated with the BCG vaccine.

On clinical evaluation, five patients were found to have crackles and all but two had hepatosplenomegaly. One patient had reduced range of hip motion with pain.

Tuberculin test was positive in only two (33%) patients -both were unvaccinated. Four patients- one vaccinated- had a negative test.

On chest radiograms, all patients had miliary lesions; two had consolidation and one had pulmonary infiltration in addition to miliary opacifications.

One or more gastric aspirates were submitted for culture from all the children evaluated. A positive culture of Mycobacterium tuberculosis was obtained from gastric aspirates of four children (67%); there was no resistance to drugs. The characteristics, and clinical, radiological and bacterial findings of the patients are shown in Table I.

Table I: Characteristics and Clinical, Radiological and Bacteriologic Findings of the Patients

Case	Age*	Sex	Contact with tbc	BCG	ppd	Predisposing Factor	Clinical Findings	Radiological Findings	Culture
1	8 m	Male	+	–	>15 mm	–	Crackles, hepatosplenomegaly	Miliary lobar consolidation	+
2	2.5 y	Male	+	–	negative	–	Crackles, hepatosplenomegaly	Miliary	–
3	2.5 m	Female	–	–	>15 mm	–	Crackles, hepatosplenomegaly	Miliary, lobar consolidation	+
4	10 y	Male	–	–	negative	–	Hip pain	Miliary	+
5	6 y	Female	+	–	5 mm	malnutrition	Crackles	Miliary	–
6	2 m	Male	–	+	negative	malnutrition	Crackles hepatosplenomegaly	Miliary, Lobar infiltration	+

*m: months, y: years.

The dose for INH was 10 mg/kg/day to a maximum of 300 mg, for RIF was 10-15 mg/kg/day to a maximum of 600 mg and for streptomycin (SM) was 30 mg/kg/day to a maximum of 1 g. Medication was given once daily, preferably in the morning before breakfast. The drug regimen and the prognosis of the patients are shown in Table II. The difference in the regimens is a result of changes in protocols used in our department.

Table II: Drug Regimens and the Prognosis of the Patients

Case	Drug Regimen	Clinical Recovery	Radiological Recovery	Time of Follow-up
1	INH, RIF daily for 15 days, twice a week for the next 8.5 months	5 months	8 months	7 years
2	INH, RIF daily for 15 days, twice a week for the next 8.5 months	2 months	8 months	9 years
3	INH, RIF daily for 15 days, INH, RIF twice a week for 5.5 months	3 months	3 months	16 months
4	INH, RIF, SM daily for 15 days, INH, RIF twice a week for the next 8.5 months	3 months	6 months	9 years
5	INH, RIF, SM daily for 15 days, INH, RIF twice a week for the next 8.5 months	4 months	8 months	9 months
6	INH, RIF, SM daily for 15 days, INH, RIF twice a week for the next 11.5 months	5 months	10 months	13 months

Results of therapy were judged on the basis of elimination of symptoms, disappearance of extrapulmonary findings and clearing of chest roentgenogram abnormalities. Clearance of symptoms occurred between two and five months. The miliary findings on the chest radiograms were cleared between three and ten months (Figs. 1 and 2).

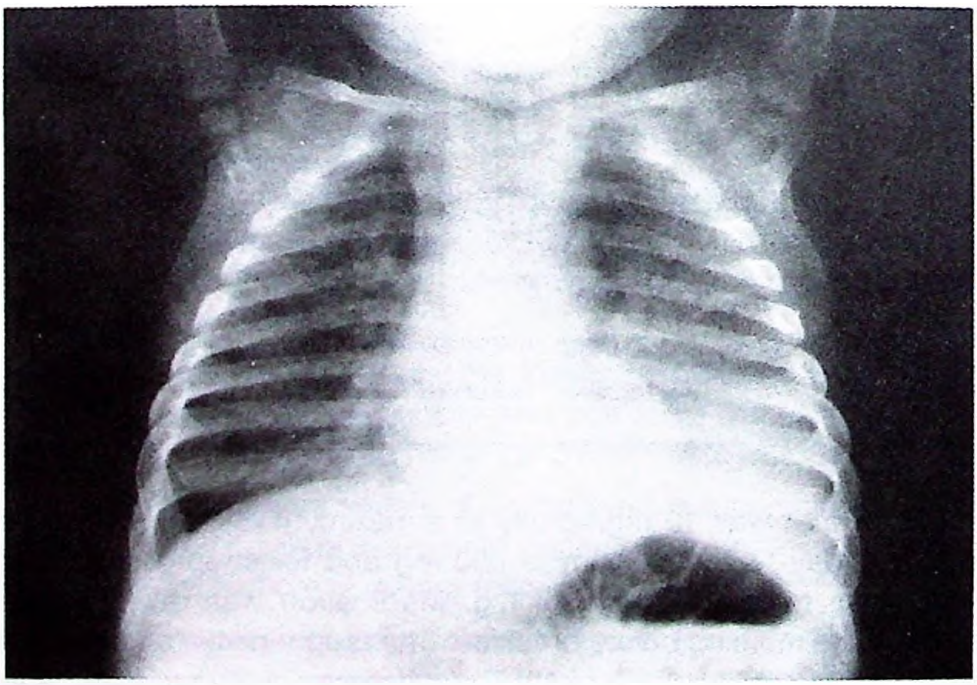

Fig. I: Miliary lesions on the chest radiogram of one of the patients before treatment.

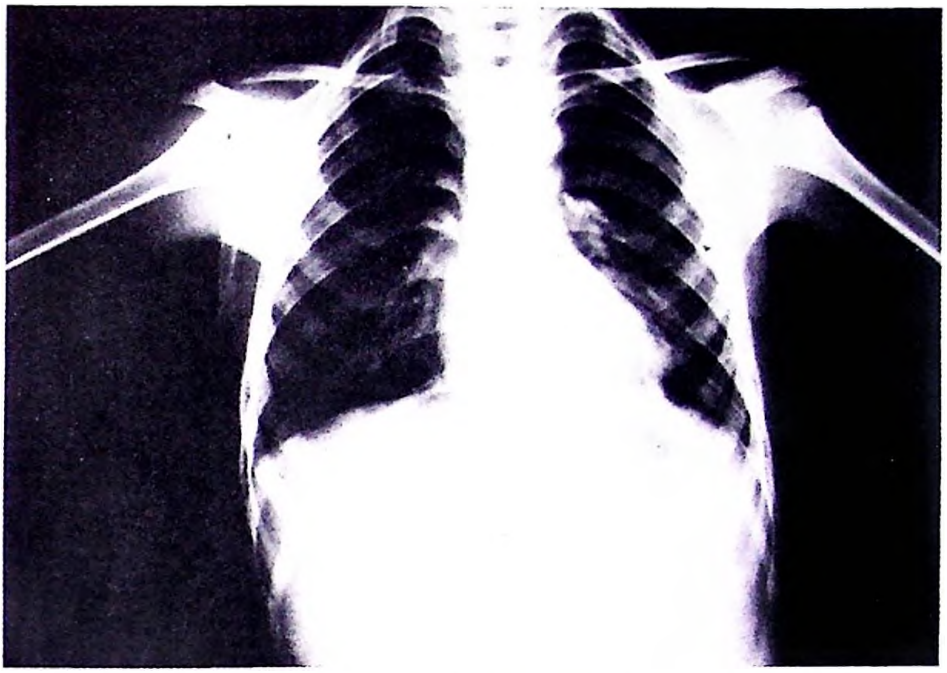

Fig. 2: Chest radiogram of the same patient after the treatment; miliary lesions are cleared and calcifications are seen.

As a result of therapy, all the patients diagnosed with miliary tuberculosis showed clearance of both clinical and radiological symptoms. They were followed up for a period ranging from nine months to nine years (mean, 4 years); no relapses were seen in this period.

No adverse reactions were noted among any patients except the elevation of SGPT in one patient up to 91 IU/L as a mild side effect.

Discussion

Miliary tuberculosis is the most commonly recognized form of disseminated infection. It occurs when a large number of bacilli invade the blood stream from a caseating focus, often a lymph node which ruptures into a blood vessel. It differs from other forms of tuberculosis in some ways: the clinical manifestations of miliary disease are generally nonspecific, including low-grade fever, anorexia, weight loss and night sweats[7]. The most common presenting symptoms of our patients were cough and fever. In this disease, hepatomegaly, splenomegaly and lymphadenopathy are common, as was seen in our patients. It is also well known that preexisting malnutrition is a predisposing factor to miliary tuberculosis.

Most of our patients were less than six years old, while half were less than two years old. These results emphasize the importance of paying much more attention to infants and younger children with tuberculosis in aspects of complications

like hematogenous dissemination. Results reveal that the diagnosis of miliary tuberculosis was made after as long as approximately seven months after the initiation of symptoms. Therefore, symptoms like fever, cough and weight loss must be taken into consideration for tuberculosis in children.

Many patients with miliary tuberulosis are anergic to tuberculin. Tuberculin skin tests are positive in about three quarters of patients with acute miliary tuberculosis[7]. In our study the test was positive in only two out of six patients (33%).

It is known that BCG does not prevent infection but it does decrease the incidence of serious disease and prevent severe complications such as tuberculous meningitis and miliary tuberculosis[2, 4]. However, considering the fact that only one of our patients was vaccinated with BCG, it was nevertheless disturbing to note the failure of the vaccine to prevent the disease. On the other hand, it is striking to see that five out of six children in our study were unvaccinated, as the policy of Turkey is to administer the BCG vaccination to every infant. This situation demonstrates that there is a great public health problem in controlling this disease in our country.

The radiological hallmark of acute disseminated tuberculosis is the miliary pattern on chest radiogram[7]. Our patients' diagnoses were also based primarily on this finding, and all of them had the miliary appearance on their chest x-ray.

The most definitive laboratory test for the diagnosis of tuberculosis is the mycobacterial cluture. However, sputum smears and culture for acid-fast bacilli are often negative, because relatively few organisms are involved despite widespread dissemination[7]. In our study, a positive culture for Mycobacterium tuberculosis was obtained from gastric aspirates of four children (67%); In our study only mycobacterial cultures were used. Newer culture methods like the BACTEC radiometric system, which can grow mycobacteria from sputum specimens in seven to 10 days, or polymerase chain reaction (PCR), which is a very rapid but not sufficiently reliable method, were not used for the diagnosis[4].

The official recommendation is that children who have miliary tuberculosis receive a total of 12 months of INH and RIF following an initial two months of daily multidrug therapy[4]. However, we gave our patients an alternative therapy, an intermittent regimen with two or three drugs daily for 15 days followed by two drugs twice weekly completed in up to six, nine or 12 months, which appears to have been successful in all the patients. The drug regimens differed from each other depending on the protocols we used in our department. There was no drug resistance reported in our study. In addition, there were no significant clinical or biochemical adverse effects except mild and transient elevation of liver enzymes in one patient. No relapses within our follow-up time of up to nine years suggests the effectiveness of this therapy.

Short-course intermittent chemotherapy has also been shown to be effective in controlling tuberculosis in young infants aged less than six months[9]. Compared to conventional regimens, an intermittent regimen has several advantages: shorter treatment time, fewer doses of medication, lower cost and minimal toxicity[10]. But, compliance is the most important factor in this type of therapy. It remains the single biggest problem in treating children with tuberculosis, many of whom live in a social environment not conducive to consistency or completeness of care. So, the ability to administer twice-weekly antituberculosis medications by a health care professional using directly observed therapy (DOT) is a necessary part of treatment programs. The adoption of DOT has been associated with a reduced rate of treatment failure, relapse an drug resistance[11]. Even though noncompliance with DOT was reported in an urban tuberculosis control program, it was found to be closely associated with alcoholism and homelessness[11].

To the best of our knowledge, this is the first report on intermittent therapy in miliary tuberculosis in children. Intermittent therapy seems to be safe and effective in miliary tuberculosis in children and it may be an alternative therapy, especially in developing countries, because of its minimal toxicity and lower cost.

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Rh DISEASE: INTRAUTERINE INTRAVASCULAR FETAL BLOOD TRANSFUSION BY CORDOCENTESIS*

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SUMMARY: Önderoğlu L, Öncüloğlu C. (Department of Obstetrics and Gynecology, Hacettepe University Faculty of Medicine, Ankara, Turkey). Rh disease: intrauterine intravascular fetal blood transfusion by cordocentesis. Turk J Pediatr 1999; 41: 61-65.

A total of 49 cordocenteses, including 40 intrauterine intravascular fetal blood tranasfusions, were performed in 30 pregnancies complicated by red cell isoimmunization. Transfusions were started at 19-33 weeks' gestation and repeated up to five times, at one-to-four week intervals. The volumes of transfused blood were 20-110 ml, hematocrits were 58-82 percent and the rate of transfusions was 1-15 ml/min. The pretransfusion fetal hemoglobins were 3.5-11.6 g/dl and the posttransfusion fetal hemoglobins were 7.5-15.6 g/dl. There were three intrauterine deaths and two neonatal deaths. The overall survival rate was 83.3 percent including all cordocenteses. The survival rate for the intrauterine transfusions was 81 percent. *Key words: Rh disease, fetal blood transfusion, cordocentesis.*

Severe fetal anemia and fetal hydrops caused by blood incompatibilities are generally associated with poor perinatal outcome, especially if hydropic signs develop during the second trimester. Although intraperitoneal transfusion has been used efficiently to treat fetuses without hydrops, its results for treatment of hydropic fetuses have been disappointing[1,2].

Since first reported by Daffos et al.[3] in 1983, ultrasonographically guided percutaneous access to fetal umbilical circulation has become a popular technique. The potential applications of percutaneous umbilical blood sampling or funipuncture, previously called cordocentesis, have increased.

Here we report our experience in fetal intravascular blood transfusion by cordocentesis in the management of 30 pregnancies complicated by Rh isoimmunization between 1993 and 1996 in Hacettepe University Hospital.

Material and Methods

Nine patients had only fetal blood sampling. In the remaining 21 patients, 40 intrauterine intravascular transfusions were performed in total. Before cordocentesis, no maternal sedation or fetal paralysis was done. The site and direction of the umbilical cord at its placental insertion was defined by a high resolution real time ultrasound scanning with a curvilinear probe (3.5 MHz). The site of entry on the

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abdominal wall was cleaned with antiseptic solution (povidone iodine). With the transducer in one hand, a 22 gauge needle 15 cm long held in the other hand was guided sonographically. The tip of the needle emitted a clearly visible echo, which was followed on the scope toward the insertion of the cord.

Briefly, three situations were encountered: a) when the cord insertion was anterior, the needle was introduced by transplacental access, avoiding introduction into the amniotic cavity, and the cord was punctured at its base; b) when the cord insertion was lateral, the needle was introduced through the placenta and then through the amniotic cavity before penetrating the cord at about 1 cm from its insertion. c) when the cord insertion was posterior, the needle was introduced through the amniotic fluid and penetrated the cord at about 1 cm from its insertion. By certain experience the resistance of penetration of the cord was easily felt. It was necessary to push firmly but gently to penetrate Wharton's jelly.

After penetration of the cord the needle's stylet was removed, a syringe was attached to the hub of the needle and 2-3 ml of pure fetal blood was obtained. This fetal blood was used for the determination of fetal hematocrit and hemoglobin level and for blood group, Rh factor and direct Coombs. If the hemoglobin level was less than 2 SD below the normal mean for gestation (Table I)[4], the tip of the needle was kept in the lumen of the umbilical cord and a connecting tube, 10-15 cm long, was attached to the hub of the needle for intravascular transfusion. 0-Rh negative fresh, packed erythrocytes with at least a 60 percent hematocrit level were infused manually through a 20 ml syringe into the fetal circulation. The rate of transfusions was 1-15 ml/min. The sonographically detectable turbulence and echogenicity in the umbilical cord produced by the infused blood allowed identification of the punctured vessel whether it was artery or vein. The fetal heart rate and the flow of the infused blood were monitored continuously throughout the procedure by ultrasonography. At the end of the transfusion a further sample (2-3 ml) was aspirated for posttransfusion levels.

Table I: Normal Fetal Hemoglobin and Hematocrit Values

Weeks of Gestation	Hematocrit (%)	Hemoglobin (g/dl)
18-20	35.8 ± 7.3	11.4 ± 2.6
21-22	38.5 ± 8.1	12.2 ± 2.8
23-25	38.6 ± 6.5	12.4 ± 2.3
26-30	41.5 ± 7.1	13.3 ± 2.3

Results

Thirty patients with Rh isoimmunization had a total of 49 cordocenteses. On 19 occasions the umbilical cord was entered transplacentally and on 30 it was punctured transamniotically. In 40 of 49 cordocenteses, the fetal hematocrit was

below the normal range for gestation and fetal intravascular blood transfusion was given. At the time of the first transfusion, 12 (40%) fetuses were hydropic.

The mean gestational age at initial transfusion was 27 weeks (range 19-34 weeks) (Table II). The mean pretransfusion fetal hemoglobin was 6.23 g/dl (range 3.5-11.6 g/dl); postransfusion it was 12.5 g/dl (range 7.5-15.6 g/dl). The mean donor hematocrit was 68 percent (range 60-82%). The transfused blood volume was 20-110 ml (mean 64 ml). The mean interval between transfusions was 19 days (range 7-33 days). The rate of decrease of fetal hemoglobin following a transfusion was 0.12-0.42 g/dl/day (mean 0.30 g/dl/day). In four of 12 hydropic fetuses reversal of hydrops was observed after multiple consecutive transfusions. In eight patients severe hydrops did not resolve despite treatment.

Table II: Cordocentesis and Intravascular Transfusions (IVT) According to the Gestational Ages

Weeks of Gestation	FBS (N)	IVT (N)
18-20	–	1
21-24	–	8
25-28	–	10
29-32	2	14
> 33	7	7

There were three intrauterine fetal losses and two postpartum infant losses. The gestational weeks of the intrauterine losses were 22, 25 and 28 weeks, respectively, and all were hydropic at the time of admittance. One of the losses was directly related to the procedure. This case terminated by immature labor because of chorioamnionitis. The remaning two cases ended by intrauterine death at one and two weeks following the procedures. These two losses were probably related to inadequate transfusion volumes to correct the fetal anemia because of technical difficulties.

There were two postpartum losses. The first infant, to whom only fetal blood sampling was performed, was delivered at 33 weeks, was 1270 g and had intrauterine growth retardation. The mother had class II cardiac disease and preeclampsia. The infant died 19 days after the delivery because of pulmonary infection not related to the isoimmunization. The second infant, who had two successful transfusions at 23 and 28 weeks of gestation, was delivered at the 32nd week. He died eight days after delivery because of prematurity and acute renal failure.

Discussion

The results of this preliminary study show that intrauterine intravascular blood transfusion can be performed as early as 19 weeks of gestation and until the third trimester. It is a relatively safe and effective method of fetal therapy. The

overall fetal survival rate in our study was 83.3 percent including all fetal blood samplings. If only fetal intrauterine transfusions are taken into account this rate was 81 percent, which is comparable with those in the literature (Table III).

Table III: Comparison of Survival Rates After Straight Intravascular Transfusion in Seven Studies

	No. of Cases	Overall Survival (%)	Survival of Hydrops (N)
Rodeck et al.[5]	19	84	11/13
de Crespigny et al.[6]	4	75	1/4
Grannum et al.[7]	26	82	16/20
Berkowitz et al.[8]	16	76	–
Brass et al.[9]	23	85.7	5/6
Önderoğlu et al.	30	83.3	7/12

Most of our blood transfusions were done into the umbilical vein. The advantages of transfusing into the vein are: a) the sonographic observation of the intravascular flow of blood provides constant reassurrance that the tip of the needle has not slipped into the Wharton's jelly, where injection of 0.5 ml of blood could lead to cord tamponade and fetal death, and b) fetal bradycardias occur more often when transfusing into an artery than a vein, presumably as a result of a procedure-related spasm of the more muscular umbilical artery.

Transplacental rather than transamniotic entry to the umbilical cord reduces the risk of displacement of the needle by fetal movements. Furthermore, since the amniotic membrane is not punctured, transplacental cordocentesis avoids both leakage of amniotic fluid and intra-amniotic fetal bleeding. However, with this route, fetomaternal hemorrhage is more than in the transamniotic route and the severity of the disease can be increased.

One of the theoretical risks of the procedure is the possibility of fetal exsanguination from the puncture point on the umbilical cord. However, the duration of the bleeding from the puncture point after withdrawal of the needle was clearly visible on the scope and noted in each case. In our series there was not significant bleeding from the puncture site and no complication related to the puncture was observed.

A recent review of the literature suggested that 33 percent of fetal mortality was associated with isoimmune-induced hydrops[10]. In addition, most losses following intrauterine intravascular transfusion in hydropic fetuses did not appear to be procedure related[8, 11, 12]. Consistent with those reports, we observed that all the fetuses who died in utero were hydropic at the administration; only one in utero death was related to the procedure itself.

In conclusion, intrauterine intravascular fetal blood transfusion is the procedure of choice for Rh disease of the fetus. Especially it is the only procedure capable of treating the most severely affected fetuses, namely those with hydrops fetalis. It enables anemia to be corrected efficiently, hydrops to be reversed and in most cases, leads to a mature healthy newborn requiring minimal treatment during the neonatal period. As opposed to the intraperitoneal approach, it is possible with the intravascular route to obtain information about the hematologic and acid-base status and biochemical data of the affected fetus.

Cordocentesis and intravascular transfusion require an experienced team and a laboratory capable of performing a variety of tests on a small volume of specimen. It is not practical from the standpoint of personnel, costs, and experience for every hospital to offer a similar service. These facilities must be regionalized to maximize both safety and efficacy.

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FREQUENCY OF ANTINUCLEAR ANTIBODIES AND REHUMATOID FACTOR IN HEALTHY TURKISH CHILDREN*

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SUMMARY: Kasapçopur Ö, Özbakır F, Arısoy N, İngöl H, Yazıcı H, Özdoğan H. (Departments of Pediatrics and Rheumatology, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, and Air Force Hospital, Etimesgut-Ankara, Turkey) Frequency of antinuclear antibodies and rheumatoid factor in healthy Turkish children. Turk J Pediatr 1999; 41: 67-71.

The frequency of antinuclear antibodies (ANA) and rheumatoid factor (RF) was investigated in 118 apparently healthy children (56 male, 62 female). The mean age was 9.8 ± 2.3 years. Antinuclear antibodies (ANA) were detected by indirect immunofluorescence, using a Hep-2 cell substrate. Nephelometry was used to quantify RF in 116 children. Five serum samples (4%, 3M, 2F) were ANA-positive in low titers and all had a speckled pattern. None of the ANA-positive children had other extractable antinuclear antibodies. Rheumatoid factor (RF) was over 25 IU/ml in four children (3%, 3F, 1M). None of these was positive for both antibodies.

Our results suggest a similar frequency of ANA in healthy Turkish children even with a Hep-2 cell substrate, when compared to results of other reports. On the other hand, RF was more frequent than in other reported series. *Key words. antinuclear antibodies, rheumatoid factor, Turkish children.*

Positive antinuclear antibody (ANA) and rheumatoid factor (RF) test results are hallmarks of some autoimmune diseases like systemic lupus erythematosus, dermatomyositis, and rheumatoid arthritis[1-3]. These autoantibodies have been reported in healthy adults and children, also in relation to various infections and drugs[4-6].

The significance of a positive ANA test in an individual without an apparent autoimmune disorder is a topic of controversy[7, 8]. Using tissue culture cell substrates, usually Hep-2 cells, for ANA has increased sensitivity but decreased specificity, leaving us with a group of individuals with low titer, positive ANA tests[6, 9].

The effect of geographical distribution in the frequency of autoantibodies has also been discussed[4, 10]. We have shown previously that ANAs were less frequent in Turkish patients with juvenile chronic arthritis (JCA) when compared to series from the United States and the United Kingdom[11]. The present study was designed to determine the prevalence of ANA and RF positivity in a group of apparently healthy Turkish children.

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Material and Methods

This study was conducted in the Nurettin Ersin Primary School in Etimesgut, Ankara. The students attending this school included in this study were the children of military personnel, all of whom had a medium socioeconomic status. The school consisted of 12 classes and a total of 535 students (age range of 5 to 12 years). Ten children from each class were randomly selected. After the exclusion of two children with a recent history of infection, a total of 118 students (56 boys, 62 girls) were included in the study. The mean age was 9.8 ± 7.3 years (range 6-15 years).

The parents were asked to answer a standard questionnaire. No child with either a previous diagnosis of a disorder known to be associated with ANA positivity or with a recent history of infection within the last three months was included. All the children found eligible for the study underwent a physical examination by one of the authors (ÖK). Informed consent was obtained from the parents.

Sera taken from 118 children were stored at -20 °C until studied Antinuclear antibody (ANA) titer was determined by indirect immunofluorescence techniques on commercially prepared Hep 2 tissue slides (Kallestad Quantaflour). Specimens were diluted in phosphate-buffered saline (PBS), initially to 1:20. If positive, they were retested at subsequent dilutions. The diluted sera were incubated on the Hep-2 tissue slides for 10 minutes, at room temperature. They were then rinsed and washed in PBS for 10 minutes. Twenty-five 24 µl FITC conjugate were applied into the wells which were then incubated at room temperature for 20 minutes. This was followed by 10 minutes of further rinsing and washing with PBS containing Evans bule. The preparation was ready for examination after the application of buffered mounting media to the slides. They were examined at a magnification of 500x using a flourescent microscope (Leicca).

All sera positive for ANA in titers 1:20 and over were analyzed for antibodies to DNA, DNP, Ro, La, Sm, RNP and Scl-70. Rheumatoid factor (RF) was determined by nepholometry (Orion, Finland), as described elsewhere[12]. Results of 20 IU/ml and over were considered positive.

Children with positive ANA and/or RF were reexamined and tested for whole blood count, urinalysis, and erythrocyte sedimentation rate (ESR). Only the ANA-positive children underwent ophthalmological examination with biomicroscope.

Results

Antinuclear antibody (ANA) positivity at a dilution of 1:20 was detected in nine children (7.9%, 5M, 4F). Five of these were positive at a dilution of 1:40 (4.2%, 3M, 2F), and only one at 1:80 (0.8%). Nuclear flourescence was speckled in all. None of the nine children positive for ANA at a titer of 1:20 had antibodies directed against DNA, RNP, Sm, SS-A/Ro, SS-B/La or Scl-70 antigens.

Rheumatoid factor (RF) was positive in four children (3.3%, 1M, 3F). There were no children positive for both ANA and RF.

The initial physical examinations of the 118 children were normal. Thirteen children with a positive test result were reexamined and tested for whole blood count, urinalysis and ESR, all of which were found within normal limits. Children with ANA positivity were evaluated with biomicroscope by an ophthalmologist. All except one were normal. Examination of the girl who had a positive ANA titer of 1:80 revealed a sequela of chronic anterior uveitis. The family and the child denied any symptomatology related to this finding.

Discussion

We determined the frequency of ANA and RF in a total of 118 healthy children and found that four percent were positive for ANA at a titer of 1:40 and three percent for RF. There are a number of studies investigating the frequency of ANA in healthy children. The study population in the majority of these reports consists of children attending hospitals because of minor trauma or for simple surgical procedures[2, 4, 5, 9, 13]. Our study was a field survey and the study population consisted of primary school children with no recent history of infection.

Another important difference from the other reports was that all children were physically examined and the ones with ANA positivity underwent an ophthalmological examination[4, 5, 13]. With this approach we were able to detect a girl with a sequela of chronic anterior uveitis. She was also the child who had the highest ANA titer (1:80) in the study group. Her parents denied any joint manifestations since birth. She is being followed regularly by the ophthalmologist.

The positive ANA and RF results we detected in healthy school children were in general comparable with previous studies (Table I)[1-6, 10, 13-15]. The wide range of ANA positivity (0.8-18%) in healthy children might be the result of the different methods used and geographical factors[4, 5, 10].

Table I: Reported Series of Antinuclear Antibodies and Rheumatoid Factor Positivity in Healthy Children

Author	Year	ANA Researh Method	ANA Positivity (%) (n)	RF Research Method	RF Positivity (%) (n)
Petty et al.[1]	1973	Mouse liver	3 (3/90)	NI	NI
Goel et al.[2]	1975	IIF	0 (0/134)	Latex fixation	4 (5/134)
Osborn et al.[14]	1984	Hep-2 cell	8.5 (3/35)	NI	NI
Haynes et al.[15]	1986	Hep-2 cell	5.5 (1/18)	NI	NI
Arroyave et al.[9]	1988	Hep-2 cell Mouse kidney	0.8 (2/241) 0.4 (1/241)	NI	NI
Martini et al.[13]	1989	Rat liver	3 (8/268)	Latex fixation	05 (1/168)
Allen et al.[4]	1991	Hep-2 cell	18 (18/100)	NI	NI
Siamopolou-Mavridou et al.[10]	1991	Hep-2 cell	3 (2/66)	NI	NI
Forslid et al.[6]	1994	Hep-2 cell	1.3 (3/219)	NI	NI
Kanakoudi et al.[5]	1995	Hep-2 cell	2.2 (33/1500)	Nephelometry	1.2 (18/1500)
Present study	1997	Hep-2 cell	4.2 (5/118)	Nephelometry	3.3 (4/118)

NI: not investigated.

Allen et al.[4] reported an 18 percent ANA positivity in 100 healthy children at a titer of 1:40, which persisted at nine percent at a titer of 1:60. This finding has led to questions regarding the Hep-2 cell method. Contrary to Allen et al.'s report, in other studies utilizing the same method, the ANA positivity rate was similar to our findings[5,6,9].

In our children, 4.3 percent positivity rate at a titer of 1:40 declined to 0.8 percent at a titer of 1:80. A similar observation was reported by Forslid et al.[6]. Thus, attaining a positive result at a high or low titer is important for the evaluation of ANA positivity. Osborn et al.[14] detected a positivity rate of nine percent at a titer of 1:40; this rate decreased to null at a titer of 1:80. In the same study, the percentage of ANA positivity in children with juvenile rheumatoid arthritis (JRA) at a titer of 1:40 was 60 percent; the positivity rate declined to 31 percent at a titer of 1:80. Therefore, we suggest that a titer of 1:40 is useful in the evaluation of ANA positivity.

As Allen et al.[4] reported, in the postinfectious period, transient ANA positivity may be observed. However, none of the children in this study had a history of infection during the previous three months. In previous studies it has been reported that a higher ANA positivity rate was detected in girls, but in the present study there were no such differences[4,13]. It has also been reported that ANA positivity increases with age[13], but again, we did not observe such an increase.

In the Hep-2 cell method, ANA can be seen in four different forms: homogeneous, speckled, peripheral and nucleolar. The speckled view particularly shows the presence of other autoantibodies[6]. All five ANA-positive children had ANA positivity of the speckled type, but in none of them was a positive ENA detected. Although it has been reported that ANA positivity in an asymptomatic patient does not imply an uderlying autoimmune disorder, we detected an uveitis sequela in one of the ANA-positive patients[7,8].

Positive RF rates detected in this study were considerably higher than those detected in Italian and Greek children. This may reflect an increased sensitivity of the quantitative nephelometric method[5,13].

In conclusion, in Turkish children with JCA, RF and ANA positivity rates were lower than those in the British and North American series[11]. However, the results obtained in the healthy children were comparable to healthy populations from the above countries. We may conclude that the role of frequent infections and infestations in the positivity of ANA and RF is not as expected among Turkish children.

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IDENTITY CONFUSION AND DEPRESSION IN GROUPS OF ADOLESCENTS HAVING PSYCHIATRIC AND PHYSICAL SYMPTOMS*

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SUMMARY: Çuhadaroğlu F. (Department of Child and Adolescent Psychiatry, Hacettepe University Faculty of Medicine, Ankara, Turkey). Identity confusion and depression in groups of adolescents with psychiatric and physical symptoms. Turk J Pediatr 1999; 41: 73-79.

The aim of this study was to investigate the identity status of adolescents having psychiatric and physical symptoms and the relation of depression with identity problems in adolescence. Three groups of university students were given a sociodemographic questionnaire, Beck Depression Inventory (BDI) and Sense of Identity Assessment Form (SIAF). The first group consisted of 31 students who were seen by the consultant psychiatrist at the Student Health Center of a university in Ankara. The second group included 37 students who applied to the same center with various physical complaints but did not need to be consulted by the psychiatrist. The third group was a group of 50 healthy students at the same university. The analysis revealed that only those with psychiatric complaints had identity confusion and that for the males in this group depressive symptoms are significant predictors of identity confusion. *Key words: adolescence, depression, identity.*

The major developmental task of adolescence is identity formation. As Erikson[1] has stated, the adolescent gains a sense of identity by going through a crisis during which all past identifications and perceptions about oneself are mixed to form a unique integrated sense of identity. If an integrated sense of identity cannot be established by the end of adolescence, it leads to a state of identity confusion which is characterized by undecisiveness, giving up easily, inability to concentrate on tasks, self-consciousness, loss of self-esteem, avoidance of intimate relations, uncertainty about future aims, and inability to make a clear description of self. Marcia et al.[2] was the first to try measuring what Erikson has put forward about identity. As the result of their studies with adolescents they discriminated four identity status groups:

1. Achievers: Those who survived the identity crisis and developed an identity of their own with their own value systems.
2. Those in moratorium: Adolescents who are still living the identity crisis.
3. Foreclosures: Those who formed their identity by the values of their parents without going through an identity crisis.
4. Identity confusion: Those who could not finish the identity crisis and are having problems at a pathological level.

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The studies done using Marcia et al.'s identity status groups showed no gender difference in distribution of each identity status[3-5]; however, males were more often achievers and females, foreclosures[6].

Later Benion and Adams[7] developed the Scale for the Objective Measure of Ego Identity Status Validity and reliability studies of this scale have been done for Turkish adolescents[8], but is not used widely. In 1994 Dereboy and colleagues[9] developed the Sense of Identity Assessment Form (SIAF). Validity and reliability studies of this scale showed that it is efficient for clinical purposes[9].

Erikson[10] states that identity confusion is not a descriptive diagnosis but a psychodynamic condition; however, it may lead to different kinds of psychiatric disorders. The clinical importance of this subject is at the point of discriminating the underlying causes of psychiatric disorders seen in adolescents. Whether or not the psychiatric disorder is the manifestation of an underlying identity confusion is an important factor in determining the treatment plan for the youth. It was shown that adolescents with various psychiatric disorders treated only with medication had a recurrence of the symptoms in short period of time. Only when they were reexamined for underlying identity confusions and treated with both medication and psychotherapy for the resolution of the identity confusion were they cured[11].

Another important point in evaluating adolescent development from the aspect of identity status is in determining the psychiatric risk factors for adolescents. It was shown that adolescents with identity confusion are vulnerable to various psychiatric disorders[12]. In two studies with university students, it was found that 70-80 percent of university students with different psychiatric diagnoses were suffering from identity problems psychodynamically[13, 14]. Ten percent of those suffering from identity problems visited physicians with different kinds of somatic symptoms before they were consulted by the psychiatrist[13].

The aim of this study was to investigate the identity status and its relationship to psychopathology among adolescents. It is hypothesized that the psychiatric states seen among youth are related to underlying identity confusion.

Material and Methods

The sample consisted of three groups of adolescents: The research group consisted of 31 consecutive students (16 females, 15 males) who came to see the psychiatrist in the Health Center of Bilkent University in Ankara. The first control group was formed by 37 students (10 females, 27 males) who applied to the same center with only physical complaints and did not need to be consulted by the psychiatrist. The second control group was a group of 50 healthy students (27 females, 23 males) selected randomly in one of the faculties of the same university.

Each group was given a sociodemographical questionnaire, Beck Depression Inventory (BDI) and Sense of Identity Assesment Form (SIAF). Beck Depression Inventory (BDI) is made up of 21 items related to depression, each rated on a zero-to-three point scale. The reliability and validity of BDI was tested among Turkish university students and the cut-off point for depression was found to be 17[15]. The Sense of Identity Assessment Form (SIAF), developed in Turkey, consists of 28 items, each questioning different aspects of identity development. Its reliability and validity have been tested among university students using the Rosenberg Self-Esteem Scale[16] and the Offer Self-Image Questionnaire[17], which have been widely used in studies in this country[18-21]. The cut-off point for identity confusion is 70 on the SIAF[9].

All analyses were done on a personel computer using SPSS. Sociodemographical factors were analyzed by chi-square test. One way ANOVA was used in evaluating BDI and SIAF scores between groups, and post-hoc analyses were done by t-tests. T-tests were also applied for comparisons between genders in the same group. Correlation analysis was done to see the relation between BDI and SIAF scores.

Results

Sociodemographical factors

The average age of the two patient groups was 21 and of the control group was 20. School achievement was average for all groups. The majority of the two patient groups were living in the dormitory while most of the healthy students were with their families ($\chi^2 = 14.62$, $p = 0.02$).

The questions on family relations were evaluated as "no problems", "some problems" and "frequent problems". Those with psychiatric complaints considered their family relations more conflicting when compared to the other two groups ($\chi^2 = 10.28$, $p = 0.035$). Mother's educational level was lower in the research group ($\chi^2 = 26.54$, $p = 0.002$).

Factors such as socioeconomic status, number of siblings, birth order, structure of the family, age and profession of the parents, and father's educational level did not show any significant difference between groups.

BDI Results

Depressive symptom scores were above the cut-off point in the group with psychiatric complaints ($F = 19.20$, $p = 0.04$) (Table I). There was no difference between the females of the two patient groups. However, the males in the first group were differentiated from the other two groups of males by their high BDI scores ($F = 15.22$, $p = 0.00$). Within the two control groups, females had a

significantly higher score than males (t = 3.49, p = 0.001 and t = 3.36, p = 0.001). This gender difference disappeared in the first group due to the elevated BDI scores of the males (t = 61, p = 0.55).

Table I: BDI Scores Among Groups and Genders

Gender	Group 1	Group 2	Group 3	F	p
Females	21.43	18.11	12.64	8.66	0.001
Males	19.33	8.80	7.95	15.22	0.00
Total	20.42	11.36	10.62	19.20	0.04
t	0.61	3.49	3.36		
p	0.55	0.001	0.001		

SIAF Results

The distribution of SIAF scores among groups and genders is given in Table II. Sense of identity assessment form (SIAF) scores were above the cut-off point only in the research group; the difference of scores between the groups was found significant by variance analysis (F = 21.38, p = 0.000). This difference is reflected both in comparison of girls among groups (F = 8.30, p = 0.001) and in comparison of boys among groups (F = 15.28, p = 0.000).

Table II: SIAF Scores Among Groups and Genders

Gender	Group 1	Group 2	Group 3	F	p
Females	74	59	53	8.30	0.001
Males	76	50	48	15.28	0.00
Total	75	52	51	21.38	0.00
t	25	1.50	1.39		
p	0.80	0.142	0.16		

Correlations of SIAF with age and gender are given in Table III. No significant correlations were found between SIAF scores and either age or gender in either of the groups.

Table III: Correlations of SIAF with Age and Gender

Variable		Group I	Group 2	Group 3
Age	r	0.055	0.221	-0.036
	p	0.770	0.200	0.130
Gender	r	0.046	-0.253	-0.140
	p	0.800	0.140	0.170

Because SIAF scores were found to discriminate the psychiatric patients from the other two groups, the correlations of SIAF with sociodemographical factors discriminitive for the same group (educational level of the mother and family relations) were analyzed. The results revealed that SIAF is not correlated with these two variables ($r = 0.146$ and $r = 0.153$).

The only sociodemographical factor which showed a significant correlation with SIAF scores was family structure in the research group ($r = 0.378$, $p = 0.036$).

In all groups SIAF and BDI scores were significantly correlated.

Discussion

Sense of identity assessment form (SIAF) scores were found above the cut-off point for identity confusion only in the group of students having psychiatric complaints. This result confirms the hypothesis we made, that is, adolescents with psychiatric symptoms are suffering from identity confusion significantly more than those having only physical symptoms and healthy adolescents. It can be concluded that the state of identity development, or identity confusion in this research, is an important factor underlying the psychiatric symptoms of adolescents.

Sense of identity, measured by SIAF, is not related to age or gender differences. This result is compatible with the results of some other studies in the literature[8-10, 14]. This again shows that adolescents having psychiatric symptoms are discriminated from the other two groups by their state of identity confusion which is independent of age or gender.

The only sociodemographical factor correlated with SIAF in this group was the structure of the family (intact or broken). The anxiety provoked by separations within the family is thought to have an important impact on young people's perception of parental and self roles and on the development of a sense of identity, thus resulting in an increase of identity problems.

In all groups sense of identity is affected by depressive symptoms. This effect is more prominent among males with psychiatric problems. The ratio of SIAF scores between the two sexes was altered in this group. The SIAF scores of females were higher in the two control groups, whereas there was a significant increase in the scores of males with psychiatric problems. Also, these males were differentiated from the other two groups of males by their high depressive symptom scores; such a significant difference was not found for females. In most of the studies done with adolescents, females are found to be more depressive than males. However, in this study, when the genders were evaluated for depression in terms of the state of identity confusion, no difference of depressive symptoms between males and females having identity problems was found. The females in all three groups were alike in their depressiveness and there was not an increase

of depressive symptoms among girls having identity problems. However, in males with identity confusion, depressive symptoms increased to the level of clinical depression (only this group of males had BDI scores above the cut-off point for clinical depression). Thus, depression discriminates males having identity confusion both from the other groups of males and also from the females having identity problems. It can be concluded that the depression seen in male adolescents is an important factor in predicting the underlying identity diffusion. This result has clinical importance for pediatricians working with adolescents, showing a more severe indication of psychiatric consultation when there is a depressed boy. For psychiatrists working with adolescents, the results of this study point to the importance of identity problems underlying psychiatric symptoms in adolescents. These adolescents should be evaluated psychodynamically and treated by psychotherapy in addition to drug treatment. The second point is the predictive value of depression for identity problems in male adolescents. Since there is not much research data on the depressive symptoms of male adolescents, it can be suggested that future research involve surveys of depression and identity problems in normal male adolescents and the factors related to them. Another subject for future research research would be investigating predictive factors of identity problems among female adolescents.

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PREVALENCE OF GB VIRUS C / HEPATITIS G VIRUS INFECTION IN PEDIATRIC PATIENTS RECEIVING MULTIPLE TRANSFUSIONS IN SOUTHERN TURKEY*

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SUMMARY: Koçabaş E, Antmen B, Yarkın F, Serin M, Aksungur P, Tanyeli A, Kılınç Y, Aksaray N. (Departments of Pediatric Infectious Diseases, Pediatric Hematology-Oncology, and Microbiology, Çukurova University Faculty of medicine, Adana, Turkey). Prevalence of GB virus C/hepatitis G virus infection in pediatric patients receiving multiple transfusions in Southern Turkey. Turk J Pediatr 1999; 41: 81-90.

The aim of this study was to determine the prevalence of GB virus C (GBV-C) infection in pediatric patients receiving multiple blood transfusions in Turkey where HBV and HCV infections are common. Sera of a total of 148 children, of whom 85 had cancer and 63 hemoglobinopathies, were tested for GBV-C RNA and HCV RNA by RT-PCR and for antibodies to HBV and HCV. Demographic and clinical information as well as laboratory results were recorded for the patients (81 boys, 67 girls, aged 1-19 years). HBsAg positivity was found in 23 (15.5%) patients, HBV DNA positivity in 12 (8.1%), HCV RNA positivity in 9 (6.7%), and GBV-C RNA positivity in 4 (2.7%). There was no significant difference in the GBV-C RNA positivity between patients with cancer (3.2%) and patients with hemoglobinopathies (2.4%) ($p > 0.05$). GBV-C RNA was found in 4 (3.1%) out of 127 patients who had received transfusions, but it was not found in any of 21 patients who had not received transfusions. However, there was no relationship between GBV-C RNA positivity and the number of transfusions. Two of the patients with GBV-C RNA had high levels of ALT (ALT > 40 IU). In these two patients, neither HBV DNA nor HCV RNA were detected by PCR, and serological tests were also negative for these agents. We concluded that pediatric patients who had multiple transfusions in Turkey are at risk of being infected with GBV-C, in addition to HBV and HCV. Investigation of GBV-C RNA in patients with high ALT levels in the absence of other viral markers may be useful. *Key words: GBV-C, pediatric cancer, hemoglobinopathy.*

Screening of blood donors for hepatitis C virus (HCV) using sensitive serological methods has greatly reduced the incidence of non-A, non-B hepatitis that occurs after transfusions. On the other hand, the fact that post-transfusional hepatitis not related to viral hepatitis A-E is still seen in two to three percent of cases has led to studies concentrating on other hepatitis viruses[1,2].

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In 1967, a viral GB agent which could cause hepatitis was reproduced in tamarins (small primates)[3]. In 1995, the presence of two genomes resembling Flaviviruses was detected in the serum of tamarins infected with the GB agent using a new PCR method called representational difference analysis (RDA). These two agents were called GB virus A (GBV-A) and GB virus B (GBV-B). During the same year, a new virus, GB virus C (GBV-C), which showed a 59 percent nucleic acid and a 64 percent amino acid homology with GBV-A, was isolated from the serum of a patient with non A-E hepatitis using reverse transcriptase polymerase chain reaction (RT-PCR). Other studies have reported that GBV-C has a 29 percent amino acid homology with HCV and a 28 percent amino acid homology with GBV-B and that this is the first in a series of viruses which have so far escaped the notice of researchers[4-7]. In 1996, a new RNA viral genome, 9393-nucleotide in length, was found in the serum of a patient with chronic non-A, non-B hepatitis using the RT-PCR; this virus was named hepatitis G virus (HGV)[5-8]. Because of a 90 and 95 percent similarity in nucleotide and amino acid levels of the HGV and GBV-C prototypes, respectively, it has been suggested that these are two different isolates of the same virus. While GBV-A and GBV-B are viruses of the tamarin, it has been reported that GBV-C is a human virus belonging to the Flaviviridae family due to its genomic organization[4-9].

HGV/GBV-C infection is frequently seen in patients with hemophilia and thalassemia who have received multiple transfusions of blood and blood products, in those who use intravenous drugs, and in patients with acute and chronic non A-E hepatitis resulting from blood transfusions[8, 10]. The transmission of HGV/GBV-C occurs parenterally (in those receiving blood and blood products, drug addicts and those undergoing hemodialysis) and vertically from mother to infant[11, 12]. At this time, there are no serological tests which can detect carriers of HGV/GBV-C. Currently, the only reliable method for detection of viral RNA in serum or other infected fluids or tissues is specific reverse transcriptase polymerase chain reaction (RT-PCR).

Previously, we have shown that children with hemophilia and cancer are at higher risk for hepatitis B virus (HBV) and hepatitis C virus (HCV) infection in Turkey, where HBV and HCV infections are common in the general population and screening of blood procedures for these agents is not effective[13, 14]. Because GBV-C is transmitted by transfusion as HBV and HCV are, it can be expected that pediatric patients receiving multiple transfusions in Turkey are at a higher risk for GBV-C infection too. Our aim in this study was to investigate the prevalence of GBV-C infection in Turkish pediatric patients with hemoglobinopathy and cancer who had received multiple blood transfusions.

Material and Methods

This study was carried out between October and December 1996 in the Pediatric Hematology-Oncology Department of Çukurova University Faculty of Medicine

located in southern Turkey. A total of 148 children, of whom 85 had cancer (53 with acute leukemia; 12 lymphoma; and 20 solidtumors) and 63 had hemoglobinopathies (46 with thalassemia and 17 sickle cell anemia), were investigated. All the patients with cancer were under maintenance therapy. Demographic (age and sex) and clinical information (time elapsing since diagnosis, number of transfusions, and history of operation, jaundice, and HBV vaccination) as well as laboratory results were recorded for the patients (81 boys, 67 girls, aged between 1-19 years). Serum samples which were taken for the detection of serum alanine aminotransferase (ALT) and hepatitis markers and for PCR studies were kept at -20 °C until testing was done. The ELISA method was used for the detection of HBsAg, anti-HBs and anti-HBc (Murex, England) and the III generation ELISA method for the detection of anti-HCV antibodies (UBI, New York, USA).

Detection of GBV-C RNA with Nested RT-PCR

Extraction of RNA

The extraction of the RNA was carried out as follows: 100 µl of the serum of each patient was mixed with 400 µl lysis buffer containing 4M guanidine thiocyanate, 25 mM Na citrate, 0.5 percent sarcosyl, and 3 µl DDT. The mixture was incubated at 60 °C for 10 minutes. After 500 µl of isopropyl alcohol was added to each tube, they were centrifuged at 13,000 rpm for 15 minutes. After removal of the upper phase, 1 µl of 70 percent ethanol was added to the pellet and the tubes were placed in dry ice for 10 minutes. The tubes were than again centrifuged at 13,000 rpm for 10 minutes and the upper phase was discarded. The precipitated RNA was suspended in 500 µl of 1xPCR buffer without gelatin.

Reverse transcriptase (RT) and Amplification of cDNA

In order to produce the complementary DNA (cDNA) of the extracted RNA, 2 µl of the RNA extract was mixed with the lower master mix (0.5 µl RNase block, 0.5 µl containing 10 U reverse transcriptase, 4 µl dNTP, 1 µl primer G9, 2 µl of 25 mM $MgCl_2$, and 1 µl RT buffer). This reaction mixture was incubated for one hour at 43 °C in a M.J. Research (PTC-150) type thermal cycler. Later, in order to inactivate the enzymes, the mixture was kept at 99 °C for five minutes and then cooled in dry ice. At the end of this reaction, the upper master mix containing 32.5 µl sterile distilled water, 1 µl 10xPCR buffer, 3 µl $MgCl_2$ (25 mM), 0.5 µl Taq polymerase (2.5 U), 1 µl primer G8 and 1 µl primer G9 was added to the lower master mix. The first round of PCR was performed for 35 cycles (94 °C, 30 sec denaturation; 55 °C, 30 sec annealing; 72 °C, 60 sec elongation) using 50 µl of this reaction mixture. The final elongation was carried out at 72 °C for eight minutes.

The second round of PCR for HGV was carried out with 5 µl of the amplification product, 5 µl 10xPCR buffer, 3 µl (25 mM) $MgCl_2$, 0.5 µl dNTP, 0.5 µl Taq

polymerase, 1 µl primer G10, 1 µl primer G11 and 34 µl sterile distilled water. Poymerase chain reaction (PCR) was performed for 30 cycles with each cycle consisting of the same schedule as the first-round PCR, except for the primer extension at 72 °C which was carried out for 45 sec. The final elongation was then carried out at 72 °C for eight minutes[15].

Analysis of the Amplification Products

The amplification products were analyzed using electrophoresis (6 volts/hour) in two percent agarose gel. All of the amplification products were separated throughout the agarose and stained with etidium bromide. The bands were seen on UV-transilluminator (Vilber Lourmat). A ϕx174/HeaII DNA marker was used for the molecular weight measurements of the viral DNA. Expected lengths for products of the first and second rounds of the PCR were 158 bp and 83 bp, respectively. The samples in which the 83 bp DNA fragments were detected were accepted as being positive for GBV-C RNA. A positive patient serum control and distilled water for a negative control were included in the cDNA transcription and the PCR procedures.

The primers used for the alpha helicase region of GBV-C are as follows:

Primer G9: 5'-TCYTTGATGATDGAACTGTC-3'
Primer G8: 5'-TATGGGCATGGHATHCCYC-3'
Primer G10: sense, 5'-CATTCVAAGGCGGAGTGYGA-3'
Primer G11: antisense, 5'-TCYTTACCCCTRTAATAGGC-3'

(Y, mixture of T and C; D, mixture of A, G and T; H, mixture of A, C, and T; V, mixture of A, C and G; R, mixture of A and G).

The amplification of HBV DNA in serum was carried out using PCR with the primers for the core region of the HBV genome which are HBV CA1:GCT TTG GGG CAT GGA CAT TGA CCC (1893-1916) and HBV CA2B. Biotin-TGA TAA GAT AGG GGC ATT TGG TGG (2302-2325). The amplification products were made visible using microwells coated with streptoavidin and RNA probes conjugated with biotin (Digene Sharp Signal System, Cat. no. 4601-110).

The HCV RNA in serum was amplified with primers aimed at the conservative 5'-untranslated region of the viral genome using RT-PCR according to the method of Okamato for HCV[16].

The data analyses were carried out using the SPSS packet statistical program including chi-square, Student's t test, Fisher's exact test and nonparametric Mann-Whitney U test. Values $p < 0.05$ were considered to be significant.

Results

A total of 148 pediatric patients including 85 with pediatric cancer and 63 with hemoglobinopathies were investigated in this study. The clinical and laboratory

findings of the patients are shown in Table I. HBsAg positivity was found in 23 (15.5%) patients, HBV DNA positivity in 12 (8.1%), anti-HCV positivity in 17 (11.5%), HCV-RNA positivity in 9 (6.7%), and GBV-C RNA positivity in 4 (2.7%). The mean age and the mean number of transfusions and the prevalence of HBV vaccination in the hemoglobinopathy group were higher than in the cancer group ($p < 0.01$). However, the prevalence of a history of surgery was higher in the cancer group as compared to the hemoglobinopathy group ($p < 0.01$). In contrast to this, there was no significant difference in the positivity of HBV DNA, HCV RNA and GBV RNA between the two groups ($p > 0.05$).

Table I: Clinical and Laboratory Findings in Patients with Hemoglobinopathies and Cancer

	Patients			
Clinical and Laboratory Characteristics	Total Patients (n=148)	Patients with Hemoglobinopathy (n=63)	Patients with Cancer (n=85)	(p)
Mean age (± SD) (years)	8.6 ± 4.3	9.8 ± 4.7	7.8 ± 3.8	<0.01
Male/Female ratio	81/67	34/29	47/38	NS
Mean duration of diagnosis (± SD) (months)	49.2 ± 51.5	48.7 ± 51.1	49.7 ± 52.2	NS
Mean number of transfusion (± SD)	33.5 ± 59.9	72.1 ± 76.6	4.9 ± 4.7	<0.001
Mean level of ALT (± SD) (IU)	49.9 ± 72.5	42.4 ± 51.4	55.4 ± 84.8	NS
ALT > 40 IU (+) (no, %)	50 (33.8)	18 (28.6)	32 (37.6)	NS
History of surgery (+) (no, %)	30 (20.3)	6 (9.5)	24 (28.2)	<0.01
History of jaundice (+) (no, %)	2 (1.4)	0 (0)	2 (2.4)	NS
HBV vaccination (+) (no, %)	5 (3.4)	5 (7.9)	0 (0)	<0.01
HBsAg (+) (no, %)	23 (15.5)	8 (12.7)	15 (17.6)	NS
Anti-HBs (+) (no, %)	34 (23.0)	14 (22.2)	20 (23.5)	NS
Anti-HBc (+) (no, %)	15 (10.1)	8 (12.7)	7 (8.2)	NS
HBV DNA (+) (no, %)	12 (8.1)	5 (7.9)	7 (8.2)	NS
Anti-HCV (+) (no, %)	17 (11.5)	8 (12.7)	9 (10.6)	NS
HCV RNA (+) (no, %)	9 (6.1)	4 (6.3)	5. (5.9)	NS
GBV-C RNA (+) (no, %)	4 (2.7)	2 (3.2)	2 (2.4)	NS

The clinical and laboratory findings of the patients according to the number of transfusions received are shown in Table II. The mean duration of time which elapsed after diagnosis was longer in the group who had received transfusions (52.9 ± 53.9 months) in comparison to those who had not received transfusions (26.7 ± 24.3 months) ($p < 0.05$) (Table II). However, the frequency of a history of surgery was higher ($p < 0.05$) in patients who had not received transfusions (52.4%) in comparison to those who had received transfusions (15%). GBV-C RNA was found in four (3.1%) out of 127 patients who had received transfusions, but it was not found in any of the 21 patients who had not received transfusions.

The clinical and laboratory findings in GBV-C RNA positive patients are shown in Table III. Two of these patients had thalassemia; 1, acute lymphoblastic leukemia (ALL); and 1, non-Hodgkin's lymphoma (NHL). The time after diagnosis

for these patients ranged from seven months to 16 years. None of the patients had HBV or HCV markers. Two patients with GBV-C RNA seropositivity had high ALT levels (ALT > 40 IU).

Table II: Clinical and Laboratory Findings of Patients According to the Number of Transfusion

Clinical and Laboratory Characteristics	Those with no Transfusions (n=21)	Patients with 1-10 Transfusions (n=76)	Patients with 11 or more Transfusions (n=51)	(p)
Mean age (± SD) (years)	8.7 ± 3.5	2.4 ± 3.6	10.7 ± 4.7	p<0.001
Male/Female ratio	6/15	39/37	36/15	p<0.05
Mean duration of diagnosis (± SD) (months)	26.7 ± 24.3	44.7 ± 45.8	65.1 ± 62.6	p<0.05
Mean number of transfusion (± SD)	0	5.0 ± 2.75	89.8 ± 75.0	p<0.05
Mean level of ALT (± SD) (IU)	47.1 ± 59.7	49.5 ± 85.4	51.4 ± 55.5	NS
ALT > 40 IU (+) (no, %)	7 (33.3)	24 (31.6)	19 (37.3)	NS
History of surgery (+) (no, %)	11 (52.4)	13 (17.1)	6 (11.8)	p<0.05
History of jaundice (+) (no, %)	0	1 (1.3)	1 (2.0)	NS
Hepatitis B vaccination	0	0	5 (9.8)	NS
HBsAg (+) (no, %)	3 (14.3)	15 (19.7)	5 (9.8)	NS
Anti-HBs (+) (no, %)	1 (4.8)	21 (27.5)	12 (23.5)	NS
Anti-HBc (+) (no, %)	1 (4.8)	8 (10.5)	6 (11.8)	NS
HBV DNA (+) (no, %)	2 (9.5)	5 (6.6)	5 (9.8)	NS
Anti-HCV (+) (no, %)	2 (9.5)	8 (10.5)	7 (13.7)	NS
HCV RNA (+) (no, %)	1 (4.8)	5 (6.6)	3 (5.9)	NS
GBV-C RNA (+) (no, %)	0	2 (2.6)	2 (3.9)	NS

Table III: Clinical and Laboratory Findings of GBV-C RNA (+) Patients

Clinical and Laboratory Characterics	Patient I	Patient II	Patient III	Patient IV
Sex/Age (year)	F/17	M/3	M/5	M/16
Diagnosis of illness	Thalassemia	ALL	NHL	Thalassemia
Duration of diagnosis	16 years	2 years	7 months	15 years
Number of transfusions	36	1	9	260
ALT level (IU)	81	21	58	21
History of surgery	(-)	(-)	(-)	(+)
History of jaundice	(-)	(-)	(-)	(-)
Hepatitis B vaccination	(+)	(-)	(-)	(-)
HBsAg	(-)	(-)	(-)	(-)
Anti HBs	(+)	(-)	(-)	(+)
Anti HBc	(-)	(-)	(-)	(-)
Anti HCV	(-)	(-)	(-)	(-)
HCV RNA	(-)	(-)	(-)	(-)
GBV-C RNA	(+)	(+)	(+)	(+)
Elapsed time since last transfusion (months)	12	24	6	1

Discussion

Hepatitis G virus is clearly a transmissible agent that may be spread in the same manner as other blood-borne viral agents such as the hepatitis viruses and retroviruses[17]. Studies in the Centers for Disease Control and Prevention have found that among patients in the United States with newly diagnosed non-A, non-B hepatitis, approximately 18 percent were positive for HGV RNA. Most of these patients (approximately 80%) were also infected with HCV[6]. The seroprevalence of HGV is found in 18 percent of patients with hemophilia and thalassemia who are frequently given blood transfusions, as well as in 33 percent of those using intravenous drugs and in 3.1-20 percent of patients undergoing renal dialysis[5, 8, 10].

Limited data regarding the epidemiology of HGV is available. It has been reported that the seroprevalence of HGV/GBV-C in West Africa is 25.9 percent; in Japan, four percent and in Taiwan, one percent[5, 18-20]. A study carried out on voluntary blood donors in the United States reported that the prevalence of GBV-C in donors with normal levels of ALT was 1.7 percent and that the prevalence in those with levels of ALT over 45 IU was 1.5 percent. In various countries, the prevalence of HGV/GBV-C in voluntary blood donors ranges from one to two percent[8].

In studies carried out in Turkey, the rate of GBV-C RNA positivity in adult patients given frequent blood transfusions is reported as 15 percent[21]; in patients with chronic liver disease, 4.6-20.2 percent[22, 23]; and in patients undergoing hemodialysis, 21-36.4 percent[24, 25]. The prevalence of GBV-C in the 148 pediatric cancer and hemoglobinopathy patients investigated in this study was 2.7 percent. There was no significant difference in the prevalence of GBV-C between the patients with cancer (3.2%) and those with hemoglobinopathy (2.4%).

HGV/GBV-C may be associated with acute hepatitis. Chronic hepatitis characterized by elevated serum ALT levels may occur, but chronic infection with no evidence of hepatitis is also common. Hepatitis due to GBV-C infection is often milder than that seen with HCV infection alone. Seventy-five to 80 percent of those who have been infected with GBV-C as a result of transfusions have normal levels of ALT. These with viremia who have normal levels of ALT are thought to be normal carriers. A high level of ALT is usually due to a co-infection with HCV, and the ALT activity is related more to the level of HCV RNA than to HGV/GBV-C RNA[5-7, 17]. While 73 percent of patients with only a HBV/GBV-C infection have no evidence of hepatocellular damage, about 16 percent of them show slight increases in ALT levels[7, 17, 20, 26]. For these reasons, it has been suggested that HGV/GBV-C may not be a primary hepatotrophic agent and that the presence of HGV/GBV-C RNA in the lymphocytes of patients with viremia may lead to the production of hepatitis only under certain circumstances[5, 27]. In this study, it was found that in two (50%) out of four GBV-C RNA positive patients, the level of the serum ALT was 1.5-2 times higher than normal. Even

though a high level of serum ALT is usually due to a co-infection by HCV and HBV[11], in these patients neither HBV DNA nor HCV RNA was detected with PCR, and serological tests were also negative for these agents. Just as chronic hepatitis could be the reason for the higher level of ALT found in these two patients, it may also have been due to the drugs given for non-Hodgkin's lymphoma (NHL) and/or other reasons.

HGV/GBV-C infection is characterized by the presence of persistent viremia and it has been reported in the literature that this may continue for as long as 17 years[5, 17]. In a study of patients who had received a massive blood transfusion because of liver transplantation, GBV-C RNA was detected six to 14 days after the transfusion. The period of viremia lasted for five months to four years in these patients. The most important source for transmission of GBV-C are transfusions of plasma, blood, and erythrocyte and thrombocyte concentrates[28]. In our study, the length of time after diagnosis in the four GBV-C RNA positive patients ranged from seven months to 16 years, and the time that passed between their last transfusion and the time this research was carried out ranged between one months to 2 two years. The GBV-C RNA positivity detected in this study may have been due either to a persistent viremia or to a passive transfer of the agent from a donor with viremia. The presence of a significant length of time since diagnosis in the GBV-C RNA positive patients supports the view that GBV-C may be a cause of a persistent chronic infection.

The facts that patients who were negative for HGV/GBV-C RNA before transfusion developed HGV/GBV-C RNA positivity within such a short a time as two weeks, that in patients who had not received transfusions the incidence of HGV/GBV-C was significantly low, and that after transfusion 10 percent of the patients were infected with HGV/GBV-C clearly indicate that HGV/GBV-C is transmitted by transfusion[6, 7, 26]. This view is supported by the fact that in our study GBV-C RNA positivity was detected in four (3.1%) out of 127 pediatric patients with cancer and hemoglobinopathy who had transfusions, where as all of those who had not had transfusions were negative. The presence of GBV-C RNA was detected in two (4.3%) of 46 thalassemia patients, in one (2.1%) of 48 ALL patients and in one (16.6%) of six NHL patients. In previous studies, no relationship had been found between the number of transfusions and GBV-C viremia[26]. In the present study, no statistically significant relationship was found to exist between viremia and the number of transfusions.

Even though HGV/GBV-C usually causes chronic persistent infections, it may also be eliminated by the immune system within three years from one-third of patients with a normal immune system. On the other hand, in patients with immunosuppression, this period may be longer and persistent infections may be more common[26, 29]. In one study, in which a seven year follow-up was made of seven pediatric patients, three of whom had thalassemia and four sickle cell

anemia it was found that there was a persistent viremia but no sign of liver disease. This situation may be explained by a persistent viremia in patients with a normal immune system and thereby a chronic carrier state[30]. The GBV-C RNA positivity found in the patients in our study may be due to immunosuppression in the cancer group or to multiple blood transfusions in all the patients.

Turkey, which is a developing country, is regarded as intermediate in endemicity for HBV infection. Prevalence of HBsAg reactivity among the general population ranges between 3.9-12.5 percent in different regions of Turkey. The prevalence of HCV specific antibody ranges from 0.3 to 1.8 percent among blood donors[31]. As seen in this study, pediatric patients with cancer and hemoglobinopathies have high HBsAg seropositivity (15.5%) and anti HCV reactivity (11.5%). However, in this study patients with GBV-C RNA positivity had no co-infection with HCV or HBV. In addition, the rate of seroprevalence of GBV-C in Turkish patients receiving multiple transfusions was not higher than that in patients in western countries, where HBV and HCV infection are not common. We think that transmission of GBV-C may not be directly related to the transmission of HBV or HCV infection, or else the presence of GBV-C infection may be related to the other unknown factor.

In conclusion, it may be considered that pediatric patients with cancer and hemoglobinopathies who have had multiple transfusions in Turkey are at risk of being infected with GBV-C, in addition to HBV and HCV. In these patients, GBV-C infection may cause chronic hepatitis characterized by elevated serum ALT without co-infection with HBV or HCV. For this reason, the development of simple and reliable tests to detect HGV infection are needed.

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URSODEOXYCHOLIC ACID THERAPY IN CHILDREN WITH CHOLESTATIC LIVER DISEASE*

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SUMMARY: Dinler G, Koçak N, Yüce A, Gürakan F, Özen H. (Gastroenterology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Ursodeoxycholic acid therapy in children with cholestatic liver disease. Turk J Pediatr 1999; 41: 91-98.

The beneficial effect of ursodeoxycholic acid have been documented in adults but experience with this agent is limited in the pediatric population. The objective of this study was to evaluate ursodeoxycholic acid treatment in children with cholestatic liver disease.

Twenty-four patients with intrahepatic cholestasis (neonatal hepatitis 7, Byler disease 7, idiopathic intrahepatic cholestasis 10) whose ages ranged from 1.5 months to 15 years were treated with ursodeoxycholic acid (15-20 mg/kg/day) for 12 months. Liver biopsy was performed initially on all patients and on 17 at the end of the twelve months. The outcome was evaluated by monitoring clinical and biochemical markers of cholestasis, including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, cholesterol, total serum fasting bile acids and total and conjugated bilirubin at entry and every three months of treatment.

Pruritus was ameliorated in all patients; there was complete disappearance of itching in 16.7 percent. There were significant decreases in mean serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin and gamma-glutamyl transpeptidase. Liver biopsy specimens showed a significant improvement in the cholestasis but not in fibrosis. No adverse effects of therapy were noted.

The improvements in the clinical and biochemical parameters and tolerability of the drug suggest that ursodeoxycholic acid is a safe and effective treatment in children with intrahepatic cholestasis. *Key words: children, ursodeoxycholic acid, cholestatic liver disease.*

Over the last few years, several clinical studies proposed that ursodeoxycholic acid (UDCA) was beneficial in the treatment of a variety of cholestatic liver diseases both in adults and children[1-6]. Parenchymal damage in cholestatic liver disease is thought to be due to intrahepatic accumulation of toxic bile acids[4]. At least 50 percent of these toxic bile acids are hydrophobic. If untreated, patients may eventually progress to severe biliary cirrhosis with portal hypertension and liver failure. Therefore, changes in the hydrophobic-hydrophilic balance of the bile acid pool, with the aim of increasing its hydrophilicity by exogenous administration of

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hydrophilic bile acids like UDCA, may be beneficial[3-5]. Ursodeoxycholic acid, (UDCA), a tertiary bile acid, is more efficiently absorbed from the ileum than the other bile acids. It is less toxic than the other bile acids for hepatocytes and is also a choleretic agent. Direct hepatoprotective effect, cholehepatic shunt mechanism and immune modulating effects may also be important[7-9]. With the exogenous UDCA therapy, intestinal absorption of endogenous bile acids decreases, and concentrations of these endogenous bile acids in the bile acid pool decline. Ursodeoxycholic acid (UDCA) replaces these bile acids and increases total bile acid pool[7-9]. Therefore, enrichment of the circulating bile acid pool with hydrophilic UDCA may improve cholestatic liver disease.

The beneficial effects of UDCA have been documented in adults with cholestatic liver diseases such as primary biliary cirrhosis and sclerosing cholangitis, but experience with this drug in pediatric patients is limited. We prospectively evaluated the efficacy and tolerance of a long-term administration of UDCA in pediatric cholestatic liver diseases.

Material and Methods

Children with cholestatic liver disease evaluated between January 1994 and December 1995 were enrolled in the study. Patients with extrahepatic biliary abnormalities were excluded. The diagnosis was based on typical clinical symptoms such as pruritus, jaundice and laboratory findings of elevated conjugated bilirubin (≥ 1 mg/dl), total serum bile acids (≥ 15 μmol/L), alkaline phosphatase (AP) (≥ 50 IU/L) and histological features of cholestasis. Twenty-seven patients fulfilled the above criteria, 24 of whom completed a 12-month course of treatment. Their ages were between 1.5 months and 15 years (mean 39.7 ± 48.0 months, median 21 months); 11 were below 12 months of age. Two were lost to follow-up and one died at the third month of treatment. Seven of the patients were diagnosed as neonatal cholestatic hepatitis (3 cytomegalovirus hepatitis, and 4 idiopathic neonatal hepatitis). Seven patients were diagnosed with Byler disease because of the presence of positive family history, chronic cholestasis, severe pruritus and normal serum gamma-glutamyl transpeptidase (GGT) levels with elevated total serum bile acids and bilirubin[10]. No cause could be found in the remaining 10 patients (3 cirrhosis, 1 chronic active hepatitis, 6 chronic cholestatic hepatitis histologically), and they were regarded as idiopathic intrahepatic cholestasis (IIHC). Parents were given a description of the study and informed consent was obtained.

Prior to starting the treatment, the conventional liver function tests [alanine transaminase (ALT), aspartate transaminase (AST), total and conjugated bilirubin, GGT, AP, cholesterol, and prothrombin time] were all carried out by routine laboratory methods. Serum was tested for markers of viral infections (hepatitis

A, B, C, CMV, rubella) and autoimmunity. Serum α-1 antitrypsin level and sweat chloride concentration were also measured. Abdominal ultrasonography was done on all patients and hepatobiliary scintigraphy, when indicated. Total serum bile acid concentrations were measured by the 3α-hydroxysteroid dehydrogenase method[11].

A liver biopsy was performed on all patients within one month prior to therapy. Ursodeoxycholic acid (UDCA) (Ursofalk, Falk Co, Germany) was administered orally at doses of 15-20 mg/kg/day, divided into two doses, for 12 months in 24 patients, Physical examination and conventional liver function tests were carried out every three months. Follow-up liver biopsy was possible in 17 patients after 12 months of treatment. Other parents refused the second biopsy.

Friedman two-way ANOVA was used for repeated measures to analyze the changes in serum liver enzyme levels and changes in bile acid concentrations[12]. Values are expressed as mean ± standard deviation (SD) and a two-tailed p value < 0.05 was considered significant.

Results

At presentation jaundice was seen in 21 patients (87.5%) (8 IIHC, 7 neonatal hepatitis, 6 Byler disease) and a history of recent jaundice was obtained in the remaining three patients. Eighteen patients (75.0%) (4 neonatal hepatitis, 7 Byler disease and 7 IIHC) suffered from pruritus. Fourteen patients (58.3%) had acholic stools and 10 patients (41.7%) had abdominal distention. All patients had hepatomegaly and 19 (79.2%) also had splenomegaly.

Pruritus was ameliorated in all patients, and disappeared completely in three (7 IIHC, 1 Byler disease). Jaundice resolved in 71.4 percent of the patients. Three patients with IIHC, two with Byler disease and one with neonatal hepatitis were still icteric at the end of treatment. Stool color became normal in all patients with acholic stools. Hepatomegaly disappeared in four (16.7%) and reduced in size in 12 (50%). Splenomegaly disappeared in four (21.1%) patients and decreased in size in six (31.6%). Reduction of hepatomegaly and splenomegaly was confirmed by physical examination and ultrasound scanning.

Serum AST and ALT levels were high in 22 (91.7%), total bilirubin levels in 21 (87.5%), AP phosphatase in 16 (66.7%), GGT in 13 (54.2%), cholesterol in 12 (50.0%), and prothrombin time in six (25.0%) patients before the treatment. Prothrombin time became normal after vitamin K administration in all patients.

Effects of UDCA therapy on routine laboratory tests are summarized in Table I. The mean serum concentrations of ALT, AST, AP, GGT, and bilirubin fell significantly. Cholesterol values decreased slightly. There were no significant changes in total serum bile acids. However, at the end of treatment ALT, AST, AP, GGT and bilirubin levels were still abnormal in 10, 10, 12, eight and six

patients, respectively. Biochemical parameters continued to improve during the second six months of therapy. There were no improvements in clinical and biochemical findings in patients with cirrhosis.

Table I: Mean Concentration Values of Liver Function Tests Before and After Six and Twelve Months of UDCA Therapy (mean ± SD)

Tests (Normal Range)	Before UDCA	After 6 Months	After 12 Months	p Values
ALT (< 50 IU/L)	174.2 ± 148.4	137.1 ± 148.4	93.3 ± 115.5	0.0038
AST (< 50 UI/L)	247.0 ± 233.8	128.9 ± 125.2	96.2 ± 82.8	0.0006
AP (< 400 IU/L)	805.2 ± 703.9	397.0 ± 186.3	493.8 ± 256	0.0008
GGT (< 35 IU/L)	319.1 ± 727.6	83.6 ± 126.1	67.0 ± 98.8	0.0129
Total bilirubin (< 1.2 mg/dl)	7.1 ± 7.8	1.8 ± 2.7	1.6 ± 2.7	0.0000
Bile acids (< 15 µmol/L)	238.3 ± 201.3	185.9 ± 279.6	206.7 ± 248.1	0.8883
Cholesterol (< 200 mg/L)	223.8 ± 91.7	157.3 ± 60.2	165.2 ± 87.0	0.0709

If neonatal hepatitis, Byler disease and IIHC were considered separately, patients with neonatal hepatitis showed improvements in all biochemical parameters, but a significant decrease was present in AP and bilirubin values (Table II). ALT and AST levels were high in all seven patients at the beginning and normalized in four of them at the end of 12 months of therapy. Bilirubin levels were high in all patients at the beginning and normalized in six. Cholesterol, AP and GGT levels were high in five, five and six patients, respectively, before therapy, and high levels continued in three, two and one patients, respectively, at the end.

Table II: Biochemical Parameters Before and After Six and Twelve Months of UDCA Therapy in Patients with Neonatal Hepatitis (men ± SD)

Tests (Normal Range)	Before UDCA	After 6 Months	After 12 Months	p Values
ALT (< 50 IU/L)	293.8 ± 175.5	252.4 ± 402.2	118.1 ± 153.4	0.3679
AST (< 50 UI/L)	449.2 ± 305.3	196.5 ± 198.1	110.2 ± 87.2	0.1801
AP (< 400 IU/L)	1145 ± 1136	538.4 ± 266.4	416.4 ± 184.0	0.0498
GGT (< 35 IU/L)	765.1 ± 123.5	150.0 ± 229.2	87.3 ± 160.9	0.4493
Total bilirubin (< 1.2 mg/dl)	11.9 ± 10.7	2.7 ± 4.4	1.9 ± 3.8	0.0388
Bile acids (< 15 µmol/L)	226.8 ± 115.9	269.6 ± 316.8	205.6 ± 252.4	1.000
Cholesterol (< 200 mg/L)	232.0 ± 61.4	158.7 ± 91.7	173.2 ± 103.7	0.1738

In patients with Byler disease, only AST concentrations decreased significanty, whereas ALT, bilirubin, AP, GGT and cholesterol concentrations only slightly decreased (Table III). All patients had high levels of ALT and AST, which persisted in only two at the end of therapy. While bilirubin and AP levels were abnormal in six and five patients, respectively, at the beginning, they remained high in two and four at the end. Cholesterol levels normalized in both patients with high levels.

Table III: Biochemical Parameters Before and After Six and Twelve Months of UDCA Therapy in Patients with Byler Disease (men ± SD)

Tests (Normal Range)	Before UDCA	After 6 Months	After 12 Months	p Values
ALT (< 50 IU/L)	126.7 ± 136.2	83.5 ± 72.3	85.2 ± 138.6	0.1561
AST (< 50 UI/L)	166.1 ± 172.2	87.2 ± 46.3	79.1 ± 88.8	0.0595
AP (< 400 IU/L)	551.8 ± 145.1	389.8 ± 69.5	527.0 ± 136.0	0.1738
GGT (< 35 IU/L)	20.8 ± 11.5	15.5 ± 6.5	14.0 ± 4.1	0.5134
Total bilirubin (< 1.2 mg/dl)	7.7 ± 8.2	2.1 ± 2.3	2.2 ± 3.2	0.1738
Bile acids (< 15 µmol/L)	462.3 ± 307.2	261.0 ± 444.9	355.0 ± 371.9	0.8187
Cholesterol (< 200 mg/L)	181.1 ± 71.3	126.8 ± 26.2	136.5 ± 40.4	0.2122

In patients with IIHC, biochemical values improved significantly, with the exception of serum bile acid and cholesterol levels (Table IV). Among 10 IIHC patients, ALT, AST and bilirubin levels were high in eight initially and were still high in five, five and three of them, respectively, at the end. Alkaline phosphatase GGT and cholesterol levels were abnormal in six, seven and five patients respectively, at presentation, and were still high in five, six and one at the end of treatment.

Table IV: Biochemical Parameters Before and After Six and Twelve Months of UDCA Therapy in Patients with Idiopathic Cholestasis (men ± SD)

Tests (Normal Range)	Before UDCA	After 6 Months	After 12 Months	p Values
ALT (< 50 IU/L)	161.7 ± 134.6	93.9 ± 65.7	81.7 ± 70.1	0.0450
AST (< 50 UI/L)	162.0 ± 113.6	110.7 ± 84.8	98.4 ± 82.5	0.0450
AP (< 400 IU/L)	744.4 ± 497.0	303.0 ± 112.8	524.9 ± 355.9	0.0017
GGT (< 35 IU/L)	192.5 ± 102.3	99.6 ± 79.8	95.1 ± 59.7	0.0313
Total bilirubin (< 1.2 mg/dl)	3.4 ± 1.9	0.9 ± 0.6	1.0 ± 0.8	0.0006
Bile acids (< 15 µmol/L)	121.6 ± 103.1	106.5 ± 135.8	125.1 ± 126.6	0.9726
Cholesterol (< 200 mg/L)	257.8 ± 124.6	206.3 ± 33.5	200.6 ± 119.2	0.0970

None of the patients with normal biochemical values initially showed abnormalities during the treatment period, nor did biochemical values deteriorate in any of the patients after reaching normal levels at six months.

Before the treatment all but one biopsy material showed fibrosis. Cellular and canalicular cholestasis was evident in 21 specimens. After 12 months of treatment there was a complete disappearance of cholestasis in 70 percent and diminution in 20 percent of 17 specimens evaluated. There was no change in fibrosis. Ursodeoxycholic acid (UDCA) was well tolerated and compliance was good in all patients. No adverse effects were noted.

Discussion

There is no effective medical treatment for intrahepatic cholestasis. Recently, UDCA has been proposed as a possible treatment modality for a variety of cholestatic liver diseases. The mechanism by which UDCA affects liver functilon tests has

not been clearly established. Parenchymal damage in cholestatic liver disease is thought to be due to the intrahepatic retention and accumulation of toxic bile acids. Ursodeoxycholic acid (UDCA) may reduce the toxicity of endogenous bile acids by competitively inhibiting their absorption from the intestine, thereby reducing their concentration and increasing hydrophilic bile acid concentrations[1-4]. The UDCA supplementation markedly increases the relative proportions of UDCA in the bile acid pool[3, 4]. The intake of hydrophilic bile acid reduced the proportion of the hepatotoxic hydrophobic compound. In our study, initial serum bile acid levels mildly decreased at the sixth month of therapy and increased at the end. As only total bile acid levels could be measured, we could not determine the composition of the bile and proportion of UDCA.

Most of the studies have involved adult patients with various chronic liver diseases such as primary biliary cirrhosis, chronic active hepatitis, primary sclerosing cholangitis, and benign recurrent intrahepatic cholestasis[1, 8]. Studies with UDCA in pediatric patients are limited and the majority of them are related to children with cystic fibrosis[3, 13, 14]. Our study was planned to determine the effects of UDCA in children with intrahepatic cholestasis of different etiologies.

Jaundice, a prominent symptom, disappeared in the majority of the patients. The most devastating symptom of cholestasis is pruritus, for which UDCA is one of the administered medications. Pruritus completely disappeared in 16.7 percent and was ameliorated in all our patients. In their preliminary report, Balistreri et al.[15] also found a marked decrease in pruritus in 60 percent of patients with intrahepatic cholestasis, but they only studied five patients. Similar results were reported in adult patients with primary biliary cirrhosis[2, 4].

The majority of the studies have focused on the effect of UDCA on symptoms and biochemical parameters, but reports on the effects on liver and splen size are scarce. In our study, hepatomegaly disappeared in 16.7 percent and reduced in size in half of the patients; changes in splenomegaly were also seen. In a recent report by Spagnuolo et al.[16], UDCA (30 mg/kg/day, divided into 3 doses) was administered to seven children with total parenteral nutrition-related cholestatic liver disease. All patients had hepatomegaly and six had splenomegaly. Reduction of hepatomegaly and splenomegaly was observed within two weeks of the onset of UDCA administration. In our cirrhotic patients no improvement was noted. This effect seems to depend on the degree of liver damage, as no change in liver and spleen size was observed in patients with primary biliary cirrhosis in another study[2]. Ursodeoxycholic acid (UDCA) may have a beneficial effect in the early stages of the liver disease.

The most predominant effect of UDCA therapy was shown on biochemical parameters. This might reflect an amelioration of liver function. Liver function tests, including AST, ALT, AP, GGT and bilirubin concentrations, improved significantly

after UDCA administration in patients with primary biliary cirrhosis[2, 4], cystic fibrosis with concomitant chronic liver disease[3, 13, 14], chronic active hepatitis caused by hepatitis B and C viruses[5], and with extrahepatic biliary atresia and chronic intrahepatic cholestasis[15, 17]. UDCA-related improvement in liver function tests is dose dependent, at least in cystic fibrosis[14]. We did not evaluate the effect of dose on liver function tests. It has also been shown that improvement continues with treatment and that rebound effects may be seen after discontinuation of therapy[2, 5, 16]. In our study, the mean serum concentrations of ALT, AST, bilirubin, AP, and GGT fell significantly. Although it was not statistically significant, the mean concentrations of cholesterol also decreased. Decreases in beochemical parameters occurred during the sixth month of therapy and persisted throughout the study. Treatment with UDCA for one year led to marked improvement in serum liver tests and, once normal values were obtained, no deteroriation was noted. Although the number of the patients was small, we observed the positive effect of UDCA in different diseases. More parameters were found to improve in patients with IIHC. Our results show that continuation of UDCA therapy after six months increases improvement in parameters. It was also shown that the proportion of patients recovering clinically increased with prolonged therapy[2, 4]. We did not observe any adverse effects and all patients tolerated the drug well.

Another question is whether UDCA treatment may alter the liver histology. After 12 months of treatment, cellular and canalicular cholestasis disappeared or decreased in 90 percent of our patients. We did not observe any change in fibrosis. None of the patients showed complete recovery in liver histology. The duration of follow-up in our study may not have been enough to see improvement in hepatic fibrosis, but Leuschner et al.[2] showed histological improvement in only three of 22 patients after four to 12 years of treatment. Poupon et al.[4] also showed significant improvements in the mean histologic features, except for fibrosis, similar to our study. The patient groups in the last two studies were primary biliary cirrhosis. Although Ikeda et al.[18] observed beneficial clinical effects of additional colchicine administration in UDCA-treated patients with primary biliary cirrhosis, they did not mention histologic changes. We had previously shown that long-term colchicine treatment alone did not affect hepatic fibrosis[19]. We did not try it together with UDCA.

Although our study represent only a preliminary and uncontrolled investigation, the results suggest that administration of UDCA leads to clinical and biochemical improvement in cholestatic liver disease in children. Ursodeoxycholic acid (UDCA) also improves cholestasis, but not fibrosis, histologically. A larger-scale controlled study is needed to show the effects on liver function, histology and survival in patients with cholestatic liver disease. This improvement in clinical and laboratory parameters and absence of side effects suggest that UDCA is a safe and effective drug for childhood cholestatic liver disease. It improves the quality of life of the patients and may be given as long as the disease continues.

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WILSON'S DISEASE PATIENTS WITH NORMAL CERULOPLASMIN LEVELS*

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SUMMARY: Yüce A, Koçak N, Özen H, Gürakan F. (Gastroenterology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Wilson's disease patients with normal ceruloplasmin levels. Turk J Pediatr 1999; 41: 99-102.

Wilson's disease, an inborn defect of copper metabolism, is a fatal disease unless specific treatment is given. Hepatic presentation mimics almost all kinds of liver disease and the diagnosis is sometimes problematic. The diagnosis is based on clinical findings, family history, presence of Kayser-Fleischer rings, and results of key laboratory tests such as low serum ceruloplasmin level, increased urinary copper excretion and hepatic copper content.

We report four patients with Wilson's disease with hepatic manifestations with unknown there were difficulties in making the diagnosis because of normal serum ceruloplasmin levels. Inspite of normal ceruloplasmin levels and absence of Kayser-Fleischer rings, strong family history suggested Wilson's disease and the diagnosis was confirmed by increased urinary and hepatic copper amounts. *Key words: Wilson's disease, ceruloplasmin.*

Wilson's disease (WD) is an inborn defect of copper (Cu) metabolism and is inherited in an autosomal recessive pattern. Therapeutic success with oral chelating agents have made this disease one of the treatable metabolic liver diseases[1]. The patients usually present with either hepatic involvement or neurological manifestations; some exhibit findings in both organ systems[1]. Progressive accumulation of Cu in the liver due to impaired Cu excretion and its subsequent deposition in other organs cause the varied clinicopathologic features of WD[1]. Although a low ceruloplasmin level had been considered as a pathogenic factor in WD, normal ceruloplasmin levels have been reported in up to 18 percent of patients with WD[2].

Because of the high rate of consanguineous marriages in Turkey, WD is not rare. We followed 152 patients with WD and here in report four of them with whom there were diffuculties in making the diagnosis because of normal ceruloplasmin levels.

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Case Reports

Case 1

An eight-year-old girl presented with a one-month history of abdominal distension and jaundice. She had two siblings with WD, one of whom had died; the other had a low serum ceruloplasmin level (4 mg/dl). On admission she had icterus and hepatomegaly. Kayser-Fleischer ring (KF) was absent. Laboratory evaluation showed anemia with a hemoglobin of 7.4 g/dl, alanine aminotransferase (ALT) 429 IU/L, aspartate aminotransferase (AST) 182 IU/L, total bilirubin 2.2 mg/dl, conjugated bilirubin 1.3 mg/dl, total protein 8.4 g/dl, albumin 3.2 g/dl, and serum ceruloplasmin 38 mg/dl (normal range 20-40 mg/dl). Copper excretion in 24-hour urine was increased (320 μg/day, normal < 100 μg/day). Liver biopsy was consistent with cirrhosis, and copper content of the liver was 1200 μg/g dry weight (normal < 50)[1]. She was treated with a low-Cu diet, D-penicillamine and zinc sulphate and has been followed for one year without any complication.

Case 2

A 14-year-old girl of consanguineous parents was referred with abdominal distension. Her brother had died of cirrhosis. Physical examination revealed hepatosplenomegaly and icterus. Kayser-Fleischer ring was absent. On laboratory evaluation the hemoglobin level was 8.1 g/dl, ALT 104 IU/L, AST 34 IU/L, total bilirubin 19.3 mg/dl, conjugated bilirubin 12.9 mg/dl, serum ceruloplasmin concentration 40 mg/dl, and urinary copper 520 μg/day. Liver histology showed cirrhosis and liver Cu concentration was 300 μg/dry weight. Splenoportography indicated portal hyertension and spontaneous splenorenal shunts. She has been followed with the treatment of a low-Cu diet, D-penicillamine and zinc sulphate for six months.

Case 3

An eight-year-old girl was admitted with abdominal distension and jaundice. Her parents were first-degree relatives and two brothers had died of cirrhosis at six and eight years of age. She had hepatosplenomegaly and icterus; KF ring was absent. Laboratory tests showed anemia (hemoglobin 8.9 g/dl), and elevated ALT (252 IU/L), AST (409 IU/L), and total and conjugated bilirubin (4.2 and 2.2 mg/dl, respectively). Serum ceruloplasmin level was normal (50 mg/dl); urinary Cu was increased (480 μg/day). Liver biopsy was compatible with cirrhosis, and liver Cu content was raised at 450 μg/g dry weight. A low-Cu diet, D-penicillamine and zinc sulphate treatment was given. She has been followed for three months without any complaints.

Case 4

A six-year-old girl, sister of Case 3, presented with abdominal distension. She had hepatosplenomegaly; KF ring was absent. Laboratory evaluation revealed a normal complete blood count and bilirubin levels, and elevated ALT (185 IU/L) and AST (151 IU/L), serum ceruloplasmin 55 mg/dl, urinary Cu excretion 144 µg/g day, and liver Cu content 680 µg/g dry weight. She has been followed for three months with the treatment of a low-Cu diet, D-penicillamine and zinc sulphate.

Discussion

In patients with WD, hepatic dysfunction is the leading symptom during childhood and adolescence and it mimics various forms of liver disease ranging from asymptomatic transaminasemia to cirrhosis[1,3,4]. Therefore, there is often a serious delay before the correct diagnosis. Most patients have cirrhosis at presentation similar to our patients. It is important to start the treatment before irreversible damage occurs.

No single test can be used for the diagnosis of WD; clinical findings, family history, and key laboratory tests establish the diagnosis. Criteria for the dignosis are low serum ceruloplasmin levels, presence of KF rings, increased 24-hour urinary Cu excretion, and hepatic Cu content. Among them, the last two are valuable diagnostic tests[1,3]. Kayser-Fleischer (KF) rings are almost always present in patients with neurological involvement, but may be absent in patients with hepatic disease[5]; therefore, the absence of KF ring does not exclude WD in a patient with liver disease. It means that extrahepatic accumulation of Cu has not become extensive. Although the majority of patients with WD have low ceruloplasmin levels, some have normal levels due to hepatic inflammation[2,3,5]. If WD is not considered in the differential diagnosis in a patient with liver disease and a normal ceruloplasmin level, further evaluation is generally not done. There was a strong family history in our patients who had exclusively hepatic manifestations. Although their ceruloplasmin levels were normal and KF rings were absent, tests to determine urinary Cu excretion and liver Cu content were performed and diagnosis of WD was confirmed.

In fact it is difficult to make a differential diagnosis among causes of hepatic Cu overload, other than WD, such as Indian childhood cirrhosis and Cu-associated childhood cirrhosis. Presentation age is younger in patients with Indian childhood cirrhosis and there are specific histological manifestations[6]. D-penicillamine is also used for the treatment of both diseases[1,6].

Medical management in WD patients is reduction of accumulated Cu by therapy with several Cu chelating agents, zinc sulphate and a low-Cu diet[1,3]. Penicillamine therapy early in the course of the disease has resulted in reduction in mortality and improvement in hepatic histology[1,7]. Wilson's disease (WD) needs to be

considered in almost all patients with liver disease and a family history, like in our patients. Normal ceruloplasmin levels do not exclude WD even if the siblings had low serum ceruloplasmin levels. Without treatment, WD is uniformly fatal, therefore, urinary excretion of Cu and liver Cu assays should be done for the diagnosis, and treatment should be started as early as possible in the course of the disease. Early diagnosis is also important for screening of other family members to detect asymptomatic patients[8].

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CONGENITAL MALARIA*

A Case Report

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SUMMARY: Kuyucu N, Yaralı N, Sönmezışık G, Yılmaz S, Teziç T. (Dr. Sami Ulus Children's Hospital, Ankara, Turkey). Congenital malaria: a case report. Turk J Pediatr 1999; 41: 103-106.

Congenital malaria is an uncommon disease even in endemic areas. A 19-day-old female infant with congenital malaria is presented. The mother of the patient was diagnosed to have malaria at the seventh month of gestation and was treated with chloroquine orally for three days. No malarial prophylaxis was given. The infant developed fever, hyperbilirubinemia, anemia and hepatosplenomegaly postnatally. Thin blood smears revealed many Plasmodium vivax parasites. She was treated with oral chloroquine for three days. We emphasize the importance of adequate antenatal medical therapy and prophylaxis during pregnancy. *Key words: congenital malaria, newborn, pregnancy.*

Congenital clinical malaria is rare, even in endemic areas, although the presence of parasites in cord blood is not infrequently reported. It occurs in the offsprings of 0.3 percent of immune and 10 percent of nonimmune mothers with malaria; placental infection occurs in more than 30 percent in endemic areas[1,2].

Vertical transmission of malaria from the symptomatic or asymptomatic mother to the fetus, attributable to the failure of the barrier action of the placenta, is generally believed to be uncommon[1,3].

We report a case of congenital Plasmodium vivax malaria to emphasize the importance of antenatal antimalarial therapy.

Case Report

A 19-day-old female neonate was admitted to our hospital with a history of fever and poor feeding for two days. She was born to a 19-year-old mother. At the seventh month of gestation the mother had a febrile illness that was dignosed as malaria by blood smear and was treated with chloroquine orally or three days. No malaria prophylaxis was given. She had fever and chills one week before delivery. Unfortunately, she did not seek medical care at that time and thus was not given any treatment. Pregnancy and delivery were otherwise uncomplicated.

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Physical examination revealed an icteric, but otherwise well-appearing neonate with mild dehydration, heart rate 176/minute, respiratory rate 60/minute, axillary temperature 38.5 °C, length 52 cm, weight 2670 g and head circumference 36 cm. Significant pathologic findings included a palpable liver four cm below the right costal margin and a palpable spleen four cm below the left costal margin.

Laboratory findings revealed: hemoglobin 8.5 g/dl; mean corpuscular volume 95 fl; white blood cell count $7.6x10^9$/L with 72 percent lymphocytes, 28 percent neutrophils; platelets $50,000x10^9$/L; and unconjugated bilirubin 9.22 mg/dl. Other biochemical tests and urinalysis were normal. Peripheral blood smear revealed many Plasmodium vivax parasites.

She was treated with oral chloroquine phosphate, 10 mg/kg of base as an initial dose, followed in six hours by 5 mg/kg. Subsequent doses of 5 mg/kg of chloroquine were given at 24 and 48 hours after the first dose. She was afebrile on the first day, and on the third day of treatment no parasite was noted on peripheral blood smear. Her mother was treated with chloroquine and primaquine.

Discussion

Congenital malaria can develop with any of the species of Plasmodium but most commonly is due to falciparum and vivax[3].

In pregnancy, immunity acquired in the past against malaria is often lost and susceptibility to malaria increases. Besides, pregnancy is associated with heightened risk for relapse or for an increase in parasitemia[4,5], possibly due to a reduction in the rate of gamma-globulin synthesis[6]. The exact mechanism and time of transmisison of the malarial parasites from the mother to the fetus are not known. The placenta normally forms an effective barrier against the parasite. However, when the placenta is damaged, either during delivery or owing to placental abnormalities, infected red blood cells are transferred into the fetal circulation[1,3,7,8]. Nonetheless, infants of mothers who have had malaria in the past are probably less affected because of maternal IgG transfer, but they are not completely protected[9]. Congenital malaria is more common among infants of women who had clinical attacks of malaria during pregnancy than of those with chronic subclinical infections. However, congenital malaria may occur in infants of mothers who are asymptomatic throughout their pregnancy[5,10].

In addition to congenital transmission, the newborn may acquire malaria by transfusion of blood products, as a simple transfusion or exchange transfusion, postnatally[3,5].

Symptomatic congenital malaria should be distinguished from cordblood parasitemia and transfusion-induced malaria. In cord blood parasitemia, parasites are cleared without involving the peripheral circulation and it is not associated with clinical disease[3,5].

The diagnosis of congenital malaria can be easily missed if it is not considered, especially in infants of asymptomatic women[10]. The examination of a single peripheral blood smear may not yield the diagnosis.

Clinical signs and symptoms similar to other congenital infections, such as fever, anorexia, hepatosplenomegaly, hemolytic anemia and hyperbilirubinemia present days or weeks after birth. Fever pattern is generally not synchronized[1, 3, 11]. Our patient had several features of congenital malaria such as fever, poor feeding, hepatosplenomegaly, jaundice and thrombocytopenia.

Treatment of congenital malaria, in a patient who is not very sick consists of oral administratilon of chloroquine (10 mg/kg of base followed by 5 mg/kg of base at 6, 24 and 48 hours). Primaquine is not needed for the newborn infant, because no persistent liver phase exists in congenitally acquired infections[3, 8, 9]. In severely ill infants parenteral chloroquine is effective and safe[12]. If chloroquine-resistant P. falciparum is suspected, mefloquine and quinine are the drugs of choice. In addition to quinine, pyrimethamine in combination with a sulfonamide (Fansidar) should be used[3, 5, 8].

As malaria often results in abortion, prematurity, low birth weight, still birth and neonatal death, adequate antenatal treatment is essential. Chloroquine is known to be safe in pregnancy, including use as a prophylactic agent in endemic areas[13]. Primaquine should not be given during pregnancy because of unknown effects on the fetus. In P.vivax and P.ovale infections, it is recommended to give chloroquine therapy and then to continue with the prophylactic treatment once weekly until after delivery. Afterwards, primaquine can be given for prophylaxis[10, 13]. The mother of our patient was prescribed chloroquine therapy at seven months of gestational age. As chloroquine prophylaxis was not given, relapse and subsequent congenital malaria occurred.

We recommend that once malaria is diagnosed in pregnancy, especially in nonimmune women, chloroquine chemoprophylaxis should be continued until delivery.

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TWO SIBLINGS WITH BLOOM'S SYNDROME EXHIBIT DIFFERENT CLINICAL FEATURES: POSSIBLE EFFECT OF SEX*

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SUMMARY: Boduroğlu K, Tunçbilek E. (Clinical Genetics Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Two siblings with Bloom's syndrome exhibit different clinical features: possible effect of sex. Turk J Pediatr 1999; 41: 107-111.

Bloom's syndrome is a rare autosomal recessive disease. All patients with Bloom's syndrome have prenatally onset growth retardation and an increased tendency to develop various types of cancer. Features other than these are not constant and may not be present in some of the patients. Reason for the phenotypic heterogeneity is not clear. Different mutations in the same locus may explain the heterogeneous phenotypes in different ethnic groups. Here we present a seven-year-old boy and his four-year-old sister, both with Bloom's syndrome, who exhibit different clinical features with respect to sun-sensitive skin lesions. The sister has severe facial sun-sensitive skin lesions whereas her brother has none. It is expected that two siblings who are supposed to have the same mutation should also have similar clinical features. Possible role of environmental effects and sex are discussed. *Key words. Bloom's syndrome, sun-sensitive lesions, clinical heterogeneity, sex effect.*

Bloom's syndrome (BS) is a rare genetic disorder, with characteristic clinical features of proportionately small body size, unusual face, sun-sensitive skin lesions and predisposition to various types of cancer[1]. It was first described by a dermatologist in 1954[2]. The basic biochemical defect has not yet been identified.

Small body size and predisposition to cancer of all cell types and at all sites are constant features of Bloom's syndrome. Clinical features other than the growth failure and predisposition to cancer, such as hypersensitivity to sunlight, patchy areas of hypo and hyperpigmentation, diabetes mellitus and immunodeficiency, may or may not be present. If present, they may be in various degrees of severity[1, 3-5]. Variety in several clinical features in different ethnic groups is reported[5, 6]; However, complementation studies suggest that BS is due to a mutation at a single locus in all patients, even in different ethnic groups[7]. Bloom's syndrome gene (BLM) has recently been mapped to chromosome band 15q26.1[8]. Here we present two patients who have been added to the Bloom's Syndrome Registry. They exhibit different phenotypes with respect to sun-sensitive skin lesions.

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Case Reports

Case 1

This seven-year-old boy was the first child of second-degree consanguineous parents. His birth weight was 1200 g and he suffered feeding difficulties and vomiting during infancy. No history of serious infection was present. He had not experienced any sun-sensitive skin lesions until the time of his admission. Family had no complaint about his mental status.

On physical examination head circumference was 43.7 cm (< 5th percentile), height 96 cm (< 5th percentile) and weight 10.7 kg (< 5th percentile). There were numerous hyperpigmented and hypopigmented macular skin lesions in various dimension, disseminated over the body, but especially clustered over the back. Triangular face, malar hypoplasia, prominent nose and protuberant ears were the characteristic facial features (Fig. 1). Echocardiography showed normal cardiac structure. Chromosome analysis revealed an increased sister chromatid exchange (SCE) ratio, average 100 per metaphase.

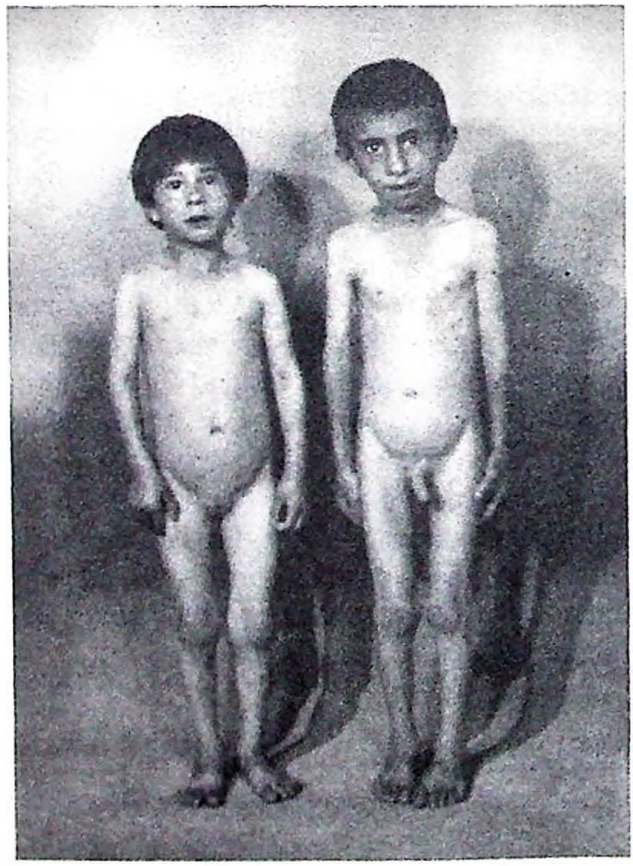

Fig. 1: General appearance of the two siblings with Bloom's syndrome.

Case 2

She is the four-year-old sister of Case 1, with a similar medical history except for having sunlight sensitivity since the age of one year. Her birth weight was 2000 g. She also was a poor sucker and failed to thrive. Prior to admission, she had experienced. three episodes of otitis media infections. At one year of age, after a significant exposure to sunlight, an erythema appeared on her face which continued with exacerbation in summers and remission in winters. Last year a dermatologist performed an incisional biopsy and it was reported as poikiloderma congenitale.

On physical examination head circumference was 42 cm (< 5th percentile), height 86 cm (< 5th percentile) and weight 9.8 kg (< 5th percentile). Telangiectases and erythema were present over the nose and cheeks. There were multiple hypo and hyperpigmented skin lesions over the trunk and proximal part of the lower extremities. Facial appearance was similar to her brother's (Fig. 1). Peripheral blood chromosome analysis revealed a mean of 98 SCE's per metaphase.

The third child of the family was normal except for a bifid thumb on the right hand. She had a normal growth pattern and never experienced a sun sensitive skin lesion. She had 9 SCEs per metaphase.

Discussion

Histological study of the sun-sensitive skin lesion has been undertaken in a few cases with Bloom's syndrome, but made no contribution to further understanding the pathogenesis[1]. Biopsy is not indicated for diagnostic purposes. Before receiving the definite diagnosis of Bloom's syndrome in Case 2, a dermatologist took a skin biopsy. We learned that the histopathological findings on light microscopy were consistent with poikiloderma congenitale. Our efforts to reevaluate the specimen failed because paraffin blocks were not found. Poikiloderma congenitale (Rothmund-Thomson syndrome) is an autosomal recessive disease, in which the characteristic features are erythema, dwarfism, hypogonadism and cataracts. Erythema begins on the face and subsequently extends to the dorsa of hands and feet, arms, legs and buttocks. Later, silght atrophy develops with telangiectases and mottled hyper and hypopigmentation. Exposure to sunlight aggravates the lesions. Histopathological examination shows hydropic degeneration of the basal layer leading to pigmentary incontinence, flattened epidermis, capillary dilatation and chronic inflammatory infiltration in the upper dermis. With the exception of pigmentary incontinence, all these findings are common for Bloom's syndrome and poikiloderma congenitale. Aside from these histopathological findings, Bloom's syndrome resembles poikiloderma by demonstrating telangiectatic erythema of the face starting in infancy, sensitivity to sunlight and growth retardation. It differs from poikiloderma congenitale by the lack of mottled hyper and hypopigmentation, absence of hypogonadism and

cataracts, high incidence of sister chromatid exchanges, and an increased risk for malignant disease. Differentiation is better made on clinical rather than histopathological grounds[9].

It is reported that physical features other than small body size and predisposition to cancer may present in varying degrees of severity in patients with Bloom's syndrome. Phenotype may differ due to ethnic origin. Complementation studies have confirmed that all patients have the same mutated locus. The phenotypic difference between different populations may be due to different mutations in the same locus[10, 13]. Although cells from all cases with Bloom's syndrome exhibit the diagnostic high SCE rate, in some, a minor population of low SCE lymphocytes exists in the blood[11, 12]. Because recombination events also occur within the BLM locus, these low SCE lymphocytes arise in patients who inherited paternally and maternally derived BLM alleles mutated at different sites[10]. But there is no evidence that these compound heterozygotes have a different phenotype. Even if there is evidence, it does not explain the difference in our patients. Since their parents are related they have to have the same mutation on both BLM alleles. These two sibs, however, exhibit different clinical features although they have the same mutation.

Environmental factors may affect the clinical presentation. Two sibs are different with respect to sun-sensitive skin lesions over the face. In this case, the environmental factor responsible for the difference may be exposure to sunlight. There are reports of sibships with multiple affected children, in which the rigorous protection from the sun of a later-born affected child is frequently associated with mild or absent skin lesions[1]. Our patients live in the same house and play together. Because they were diagnosed at the same time, the parents did not protect one of the children from sunlight, as with an experience of a previously diagnosed child. The amount of exposure to sunlight is same for these two patients or even longer in the case of the brother due to his older age.

The explanation for the difference between them, then, may be their sex. The sex ratio among all persons with Bloom's syndrome recognized to date is distorted: 94 males and 71 females. There is a male preponderance that is unexpected because of the autosomal recessive mode of transmission. Studies from different countries confirm the deficit in females. A possible explanation for the relatively low incidence in females is thought to be the high death rate among females during fetal or early postnatal life, before the pathognomonic skin lesion appears. Underdiagnosis is a second possible explanation for the low incidence Bloom's syndrome among females; their skin lesions are usually less severe than that of males. Observations have shown that one-fifth of all known affected females have had minimal skin lesions and only one-fifth had severe lesions. In contrast, among all known males only two had minimal lesions, and four-fifths were severely

affected[3, 4-6]. In this report the older male patient has no sun-sensitive skin lesions, whereas the younger female patient has severe facial lesions. This is in contrast to the previous information provided by various reports.

Bloom's syndrome gene (BLM) has recently been assigned to chromosomal locus 15q26.[18]. There are reports of various mutations in different ethnic groups. We think that effects of sex on the clinical presentation need further explanation. Mutation analyses of a greater number of BS patients may construct a phenotype/genotype correlation and the role of sex on phenotype.

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NEONATAL SUBLINGUAL TRAUMATIC ULCERATION (RIGA – FEDE DISEASE)*

A Case Report

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SUMMARY: Uzamış M, Turgut M, Ölmez S. (Department of Pediatric Dentistry, Hacettepe University Faculty of Dentistry, Ankara, Turkey). Neonatal sublingual traumatic ulceration (Riga-Fede Disease): a case report. Turk J Pediatr 1999; 41: 113-116.

Early eruption of primary teeth rarely occurs and is referred to as "natal or neonatal teeth". These teeth may cause some complications, including ulceration of the sublingual area, pain during suckling and future nutritional problems. A two-month-old infant suffering from sublingual area ulceration due to two neonatal teeth was examined. His teeth were extracted and healing of the ulcerated area was observed within the first week. *Key words: neonatal teeth, Riga-Fede disease, sublingual ulceration.*

Normal eruption of primary teeth begins with the eruption of mandibular incisors at about six months of age[1]. Prematurely erupted primary teeth, referred to as "natal or neonatal teeth", were first introduced by Massler and Savara[2]. "Natal teeth" are defined as teeth which are present in the oral cavity at birth; those that erupt within the first month of life are defined as "neonatal teeth"[2].

The incidence of natal and neonatal teeth reported in the literature has varied from 1 in 1,000 to 3,500, with females affected more frequently[2, 3]. The occurrence of neonatal teeth is undoubtedly less than that of natal teeth[2].

According to scanning electron microscopic (SEM) studies, neonatal teeth exhibit enamel anomalies. It has been concluded that these anomalies may be related to injury of the ameloblasts due to an early closure of the mandibular suture. This early closure may be responsible for premature eruption[4].

In polarized light and microradiographic studies, these teeth showed enamel hypoplasia and dentinal disturbances, including the formation of osteodentin and irregular dentin in the cervical portions and interglobular dentin in the coronal region[5].

Although mandibular primary central incisors are most often involved, there are a few cases of natal canine[5] and molars in the literature[6, 7].

Natal and neonatal teeth may create a problem during breast feeding, lacerate breasts and create a risk for future nutritional problems. Since these teeth may be loose and movable, there is a danger of swallowing or aspirating them[2].

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Additionally; they may cause an ulceration of the tongue and sublingual area. Ulceration of the tongue and sublingual area caused by natal or neonatal teeth is referred to as Riga-Fede disease. Elzay[8] has referred to this lesion as traumatic ulcerative granuloma with stromal eosinophilia. Other authors have termed it "Riga's disease", "sublingual growth in infants", "sublingual ulcer", "sublingual granuloma" and "reparative lesion of the tongue"[9-11]. Recently, a more appropriate descriptive term is defined by Goho[12] as "neonatal sublingual traumatic ulceration".

The following case documents neonatal sublingual traumatic ulceration caused by two neonatal teeth.

Case Report

A two-month-old male infant was referred to Hacettepe Universty, Faculty of Dentistry, Department of Pediatric Dentistry suffering from two prematurely erupted primary teeth. The mother reported that these teeth had erupted within the first month after birth. She was complaining of the baby's lack of weight gain and of pain during breast-feeding.

Clinical examination revealed two neonatal teeth on his mandibular central incisor region. There was also a 2x2 cm. sublingual ulceration extending from the anterior border of the tongue to the lingual frenulum (Fig. 1).

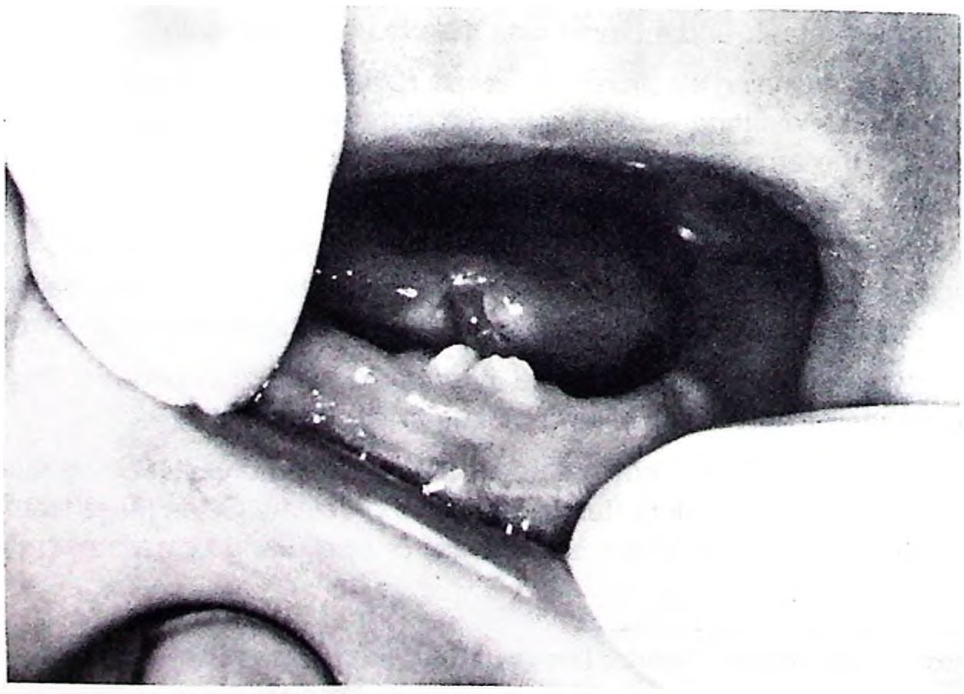

Fig. 1: Two neonatal teeth and tongue-sublingual ulceration.

The neonatal teeth showed moderate mobility and palpation of the ulcerated area elicited a response from the baby indicating pain.

The periapical radiograph confirmed that these teeth were supernumeraries with well-formed crowns and partially developed roots.

The ulcerated area was large and required a rapid healing to prevent future nutritional problems. After consultation with his pediatrician, it was decided to extract the teeth. Local anesthesia (Ultracaine) was given after application of topical anesthesia containing 10 percent lidocaine (Xylocaine) and the teeth were extracted with forceps.

At the first recall appointment one week later, the extraction area had healed rapidly and the ulceration had nearly resolved (Fig. 2). The patient is being followed for future complications.

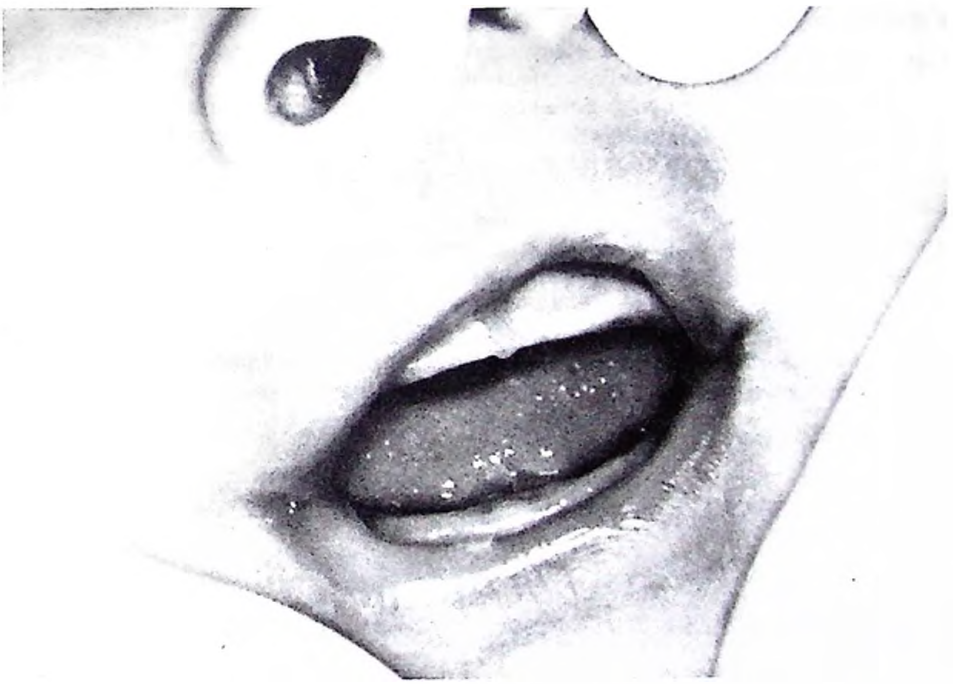

Fig. 2: One week later, the ulcerated area nearly resolved.

Discussion

to date, there are few reports about ulceration of the tongue and sublingual area caused by natal or neonatal teeth (Riga-Fede disease)[1, 2, 8, 12].

Treatment of Riga-Fede disease consists of either smoothing the rough incisal edges of natal or neonatal teeth or placing composite (a restroative material) over them, or extraction[12-15].

In cases of mild to moderate trauma, conservative treatment choices may be preferred. However, if the ulceration area is large and a rapid healing is required to relieve pain and restore proper suckling, the more appropriate teratment choice is extraction[12].

Since the ulcerated area on the tongue and sublingual area was large in our case and the baby exhibited pain during suckling, we preferred to extract the neonatal teeth.

The baby is being followed to observe any changes in the next erupting teeth and dentition.

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FAILURE OF GRANULOCYTE COLONY – STIMULATING FACTOR AND GRANULOCYTE – MACROPHAGE COLONY – STIMULATING FACTOR IN A PATIENT WITH KOSTMANN SYNDROME*

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SUMMARY: Hazar V, Ongun H, Yeşilipek MA,Yeğin O. (Department of Pediatrics, Akdeniz University Faculty of Medicine, Antalya, Turkey). Failure of granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in a patient with Kostmann syndrome. Turk J Pediatr 1999; 41: 117-120.

We present a seven-month-old boy referred to our hospital with a history of recurrent suppurative infections starting in his neonatal period. Anemia, absolute neutropenia absolute neutrophil count (ANC: 500 cells/μl), pneumonia, purulent otitis media and maturational arrest of granulocytes at promyelocyte-myelocyte level in bone marrow were detected on his admission. He was diagnosed as Kostmann syndrome and recombinant human granulocyte colony-stimulating factor (rhG-CSF) therapy was started at a dose of 10 μg/kg/d, gradually increasing up to 120 μg/kg/d in sequential seven-day courses. As there was no response, rhG-CSF was stopped and recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) was started subcutaneously with 2.5 μg/kg/d and was escalated by doubling the dose every seven days to 20 mg/kg/d. By this therapy absolute neutrophil count (ANC) transiently reached above 500 cells/μl, but eosinophilia developed with a total white cell count of 88.200 cells/μl, and a differential count showing 86 percent eosinophils. Since eosinophilia of this magnitude has deleterious effects, and neutrophil production did not significantly increase, we tried combined therapy with rhG-CSF and rhGM-CSF at doses of 10-20 μg/kg/d and 5-10 μg/kg/d, respectively, without any effect on absolute neutrophil count. The patient succumbed from sepsis eight months after the diagnosis.
Key words: eosinophilia, Kostmann syndrome, rhG-CSF, rhGM-CSF.

Kostmann syndrome (KS) (severe congenital neutropenia) is an autosomal recessive syndrome characterized by profound absolute neutropenia and maturation arrest of marrow progenitor cells at the promyelocyte-myelocyte stage, probably resulting from a defect in granulocyte colony-stimulating factor (G-CSF) binding receptor which transduces signals critical for the proliferation and maturation of granulocytic progenitor cells[1]. Patients with KS suffer from frequent episodes of severe bacterial infection starting in the first months of life. To stimulate differentiation of myeloid progenitor cells, clinical trials with recombinant human granulocyte colony-stimulating factor (rhG-CSF) and recombinant human

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granulocyte-macrophage colony-stimulating factor (rhGM-CSF) have been reported[2-4]. In this report a patient with KS suffering from severe infections since the neonatal period is presented.

Case Report

A seven-month-old boy who experienced several episodes of purulent otitis media and suffered from frequent pneumonia and omphalitis since his second day of life was admitted to our hospital with a history of cough, fever, and purulent discharge from the ears. On admission, physical examination revealed bilateral perforated tympanic membranes, fine and crackling rales on both sides of lungs and minimal hepatomegaly. He had a normal total white blood cell count of 7,800/μl, with a differential count showing four percent neutrophils, two percent eosinophils, four percent monocytes and 90 percent lymphocytes. This profound neutropenia was accompanied by a hypochromic and microcytic anemia with a hemoglobin level of 7.1 g/dl and a normal platelet count of 309,000/μl. Immunoglobulin determination and immunoelectrophoresis showed polyclonal increase in all isotypes (IgA: 874 mg/dl, IgM: 527 mg/dl, IgG: 3712 mg/dl). Bone marrow aspiration showed maturation block between promyelocytes and myelocytes. A few bands and mature neutrophils were present. Anti-neutrophil antibodies were negative. Liver and renal function tests were within normal limits. Chest x-rays showed bronchopneumonic consolidation. The diagnosis of KS was established on the basis of profound neutropenia, maturation arrest of neutrophil lineage in bone marrow and frequent bacterial infections since his neonatal period. The patient was started on antibiotics and rhG-CSF (Neupogen, Roche) beginning with 10 mg/kg/d subcutaneously (SC) which was escalated by a dose of 10 mg/kg/d every seven days. When a dose of 120 mg/kg/d was reached, absolute neutrophil count (ANC) was still under 500/μl. Thereafter rhGM-CSF (Leucomax, Sandoz) was given initially at a dose of 2.5 mg/kg/d SC and doses were increased to 5, 10 and 20 mg/kg/d in sequential seven-day courses. A transient response to therapy was achieved at a dose of 20 mg/kg/d with an increase in ANC to greater than 1,000 cells/μl; however, at this level eosinophilia developed with a total white cell count of 88,200 cells/μl with a differential count showing 86 percent eosinophils and four percent neutrophils. Since eosinophilia of this magnitude has deleterious effects and neutrophil production did not significantly increase, we tried combined therapy with rhG-CSF and rhGM-CSF at doses of 10-20 mg/kg/d and 5-10 mg/kg/d, respectively, without any effect on ANC. Nevertheless, eosinophilia continued and echocardiography showed signs of hypertrophic cardiomyopathy. He was given rhG-CSF alone again at a dose of 20 mg/kg/d SC and the eosinophilia subsided. His parents did not accept suggestion of allogeneic bone marrow transplantation. He died from sepsis eight months after the diagnosis.

Discussion

Patients with KS experience frequent episodes of fever, pneumonitis, and skin infections, usually beginning in early infancy and often leading to fatal infections despite antibiotics. Several therapeutical approaches, such as white cell transfusions, and administration of steroids, lithium and androgens have been attempted in the past[5-7]. Therapeutic alternatives today are either allogeneic bone marrow transplantation (BMT) or rhG-CSF. Both have resulted in correction of the neutropenia[2-4, 7]. As several difficulties limit application of BMT, rhG-CSF seems to be the most applicable therapeutic approach. Therefore, our patient was first started on rhG-CSF. Because a majority of patients with KS need higher doses of G-CSF to promote neutrophil formation compared with the doses used in patients with chemotherapy-induced neutropenia, we reached high doses[2-4, 8]. However, could not obtain an ANC above 500 cells/µl even at a dose of 120 mg/kg/d. This failure may be due to reduced responsiveness of neutrophil progenitor cells to G-CSF as reported before[9].

In contrast to rhG-CSF, rhGM-CSF induced an increase of blood granulocytes. However, this increase was due to eosinophilia. During this treatment period the eosinophils increased up to nearly 76,000 cells/µl, demonstrating the potent biologic activities of rhGM-CSF, yet there was still no increase in the ANC. Welte et al.[3] showed that an increase in absolute granulocyte count secondary to eosinophilia developed in four out of five patients with KS treated with rhGM-CSF. In addition, they commented that the high number of eosinophils activated by rhGM-CSF might be of clinical benefit because of no severe bacterial infections occurring in the patients during eosinophilia. But our patient had no clinical improvement during eosinophilia and on echocardiography we determined hypertrophic cardiomyopathy. This was due, we believed, to eosinophilia as reported before because his telecardiography on admission was normal and he had no prior symptoms of the cardiovascular system[10, 11]. These results were in contrast to those in rhGM-CSF therapy in other neutropenic conditions such as myelodysplastic syndrome after BMT and idiopathic neutropenia, in which substantial increases in ANC have been noted[9, 12-14]. This indicates that KS might have a different pathophysiology as compared with these conditions. The G-CSF receptor abnormality could explain the lack of response to this cytokine, but it remains to be seen if this abnormality is applicable to all patients with KS.

The patient presented here does not allow us to make definitive statements regarding the percentage of patients who may respond to rhG-CSF and/or rhGM-CSF therapy. It is possible that there are different mutations responsible for KS. In case there is no response to rhG-CSF and rhGM-CSF, allogeneic BMT should be planned.

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LATE INFANTILE ACID MALTASE DEFICIENCY*
A Case Report

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*Nur Aydınlı MD****, Meral Özmen MD******

SUMMARY: Çalışkan M, Yılmaz Y, Serdaroğlu P, Aydınlı N, Özmen M. (Division of Pediatric Neurology, Department of Pediatrics, and Department of Neurology, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey). Late infantile acid maltase deficiency: a case report. Turk J Pediatr 1999; 41: 121-125.

A five-year-old boy with late-infantile (juvenile) form of acid maltase deficiency is presented. His symptoms were restricted to skeletal muscle. There is commonly a correlation between the amount of residual acid maltase activity and the severity of the clinical picture. Although the residual enzyme level was very low in our patient, no progression of his neurological findings have been observed during the follow-up period of two years. *Key words: acid maltase deficiency, glycogen storage diseases, late-infantile form.*

Glycogenosis type II is an autosomal recessively inherited disorder caused by defects in lysosomal acid maltase (acid alpha-glucosidase). Acid maltase deficiency (AMD), a clinically heterogeneous disorder, can be divided into three clinical forms: infantile, late-infantile (childhood/juvenile) and adult, based on the extent of organ involvement, age of onset and rate of progression. The fatal infantile-onset form is characterized by massive accumulation of glycogen in all tissues, including cardiac and skeletal muscle. In the late-onset forms, symptoms begin in childhood or adult life, the course is usually slow and the clinical picture is mostly restricted to skeletal muscle[1-3].

We present a five-year-old boy with delayed motor development and muscle weakness who was diagnosed as AMD by muscle biopsy. The dignosis was confirmed by enzyme deficiency in skin fibroblast culture.

Case Report

A five-year-old boy was admitted to the Pediatric Neurology Unit because of difficulties in walking and muscular weakness. He was the only child of healthy first-degree consanguineous parents; there was no history of neuromuscular disease in the family. His pre-, peri- and postnatal histories were unremarkable with the exception of his premature birth weight of 1900 g. He had head control at four months, sat without support at nine months and walked unaided at 18 months. He had been suffering from recurrent upper respiratory infections.

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Clinical examination revealed a mentally normal boy with a body weight of 14 kg (10^{th} percentile), height of 100 cm (20^{th} percentile), and head circumference of 48.5 cm (10^{th} percentile) (Fig. 1). He had generalized hypotonia. Muscle strength was diminished more in the proximal than distal extremities. When lying in a supine position, he could not raise his head; however, most muscles moved against gravity. He rose from the floor manifesting a prominent Gowers' sign and walked unaided. There was marked lordosis of the lumbar spine and bilateral contractures of the Achilles tendons. Tendon reflexes were hypoactive. Examination of other systems including heart did not reveal any abnormal findings. During the follow-up period of two years, there was no progression in his neurological findings.

Laboratory findings: The serum creatine kinase (CK) was elevated to 2409 U/L (N: 30-200), SGOT was 352 U/L and SGPT 224 U/L. The electrocardiogram, chest x-ray and cardiac ECHO were normal. Pulmonary function tests could not be performed due to lack of cooperation.

Fig. 1: Five-year-old boy with acide maltase deficiency.

Motor nerve conduction velocity was normal. On the needle electromyography the motor unit potentials were of short duration, mostly polyphasic and low voltage. Occasional myotonic-like bursts of activity were recorded. Cranial MRI performed at age of seven years was normal.

Muscle biopsy was characterized by vacuolar myopathy. Most of the fibers contained large and usually multiple vacuoles. Most vacuoles contained periodic acid-Schiff (PAS) positive material, some of which persisted after diastase digestion, as well as acid phosphatase reactivity (Figs. 2, 3).

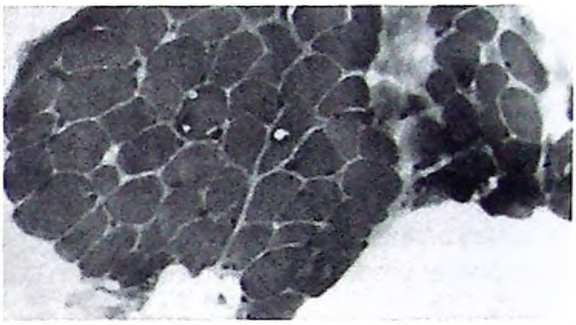
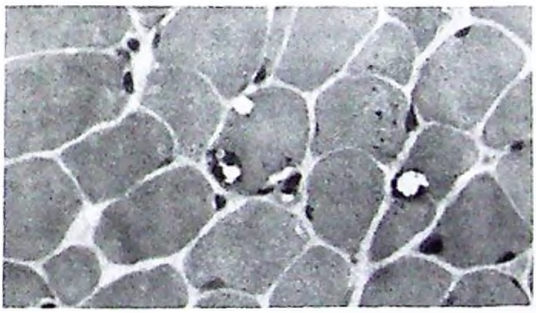


Fig. 2. Hematoxylin and eosin preparation (44X) demonstrating the variability in fiber size and vacuolation in two fibers.



(a)

(b)

Fig. 3: Modified Gomori trichrome preparation [88 X (a) and 176 X (b)] demonstrating vacuolation of some fibers.

Skin fibroblast culture (Mainz-Germany) revealed a markedly decreased alpha-glucosidase level of 0.059 mU/mg (N: 1.41-5.83).

Discussion

In 1965 Zellweger et al.[4] reported a mild "abortive" form of AMD in two brothers aged 15 and $4^1/_2$ years with a mild myopathy. Since then clinically heterogeneous forms of the disease have been described, often resembling a limb girdle or Duchenne muscular dystrophy. In these patients symptoms usually become apparent in the first decade of life. Although in some cases there has been evidence of associated cardiac involvement, other systems do not appear to have been significantly involved[3, 5, 6]. The symptoms of our patient were also restricted to skeletal muscle. This type of case stresses once again the importance of muscle biopsy in apparently typical "muscular dystrophy" with a raised CK level and myopathic electromyographic changes.

A definitive diagnosis is based on the biochemical demonstration of decreased alpha-glucosidase activity in peripheral lymphocytes, in muscle or in cultured fibroblasts[1, 2].

The cause of the clinical heterogeneity remains obscure. Reuser et al.[2] showed a logical correlation between the level of residual activity and the course of the disease in 25 adult, four juvenile and 46 infantile forms. But the relatively mild clinical phenotype of some adult patients with an exceptionally low residual activity remained unexplained. The very low level of acid maltase in our patient correlated with the moderate-severe form of the disease. However, during the two year follow-up period, we have not observed any progression in the neurological findings. The family has also been informed about the availability of prenatal diagnosis. A second pregnancy was terminated because of low level of alpha-glucosidase in the amniotic cell culture. Although the course of the late-infantile form is usually slowly progressive, the sudden onset of respiratory failure might be life threatening and is usually fatal before the third decade. In many cases, nightly ventilatory support is ultimately needed, and was also planned for in our case[2, 3].

In some rare cases, storage of glycogen in vascular smooth muscle cells leading to an aneurysm of the basilar artery has been described. This was complicated by fatal rupture in two patients[11]. Cranial MRI performed at the age of seven years was normal in our case.

The gene for acid maltase has been mapped to chromosome 17 q 21-23. In most reported families all affected members are afflicted with the same disease variant. However, there are some reports about intrafamilial clinical heterogeneity. In one of them, three siblings had infantile AMD and their paternal grandfather had adult-onset AMD. Allelic diversity with various combinations of homo- ("severe" allele) and heteroallelic (a "severe" and a "mild" allele) mutant genotypes has been suggested as the basis for the clinical and biochemical heterogeneity of AMD[8-10].

Early trials of enzyme therapy failed because of insufficient quality and quantity of the administered enzyme. In a more realistic animal model, preparations of human placental and bovine testis alpha-glucosidase were administered to healthy mice and found to be taken up by heart and skeletal muscle, the major target organs. Although these results are promising, the ultimate effect of enzyme therapy in AMD can only be tested in clinical trails in humans[12].

Acknowledgement

The authors wish to thank Dr. Beck (Mainz-Germany) for his analysis of alpha-glucosidase activity in the skin fibroblast culture.

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ECHOCARDIOGRAPHIC DIAGNOSIS OF SINUS VALSALVA ANEURYSM RUPTURE IN TWO PEDIATRIC PATIENTS*

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SUMMARY: Öztunç F, Akalın F, Polat B, Bilal MS. (Institute of Cardiology, İstanbul University, İstanbul, Turkey). Echocardiographic diagnosis of sinus Valsalva aneurysm rupture in two pediatric patients. Turk J Pediatr 1999; 41: 127-132.

Sinus Valsalva aneurysm rupture (SVAR) is a rare cardiac abnormality that requires surgical correction when diagnosed. Previously, cardiac catheterization and angiography were thought to be necessary for its diagnosis. We present two pediatric cases of SVAR with subarterial ventricular septal defect (VSD) diagnosed noninvasively by echocardiography; surgical findings confirmed the diagnosis. In both of our cases the origin of SVAR was the right coronary sinus. The first case was ruptured into the right ventricular cavity; the second was ruptured into the right ventricular outflow tract.

Continuous murmurs heard during follow-up of children with VSD must alert the physician to this pathology. Combined two-dimensional, Doppler and color-Doppler echocardiography is an accurate, noninvasive method for diagnosis of SVAR. *Key words: sinus of Valsava aneurysm rupture, echocardiography, subarterial ventricular septal defect, children.*

Congenital aneurysms of the sinuses of Valsalva are thin-walled tubular sacs nearly always in the right sinus or the adjacent half of the noncoronary sinus[1]. Separation of the aortic wall media from the valve ring tissue causes this relatively rare lesion[2]. Rupture of sinus Valsalva aneurysm into right or, in rare cases, left heart chambers results in aortocardiac fistula[3]. Sinus Valsalva aneurysm rupture (SVAR) is rare in infants and children (0.1-3.5% of all congenital cardiac abnormalities)[4].

Although cardiac catheterization was considered necessary for accurate diagnosis of SVAR, recent reports indicate that SVAR can be diagnosed noninvasively by Doppler and color-Doppler echocardiography[5,6].

We herein present two cases of SVAR in the pediatric age group diagnosed echocardiographically and confirmed by surgery.

Case Reports

Case 1

A.K. was an asymptomatic 13-year-old boy with a known history of a small ventricular septal defect diagnosed by echocardiography in another center. On physical examination the left side of the precordium was prominent and

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hyperdynamic, and peripheral pulses were bounding. A loud continuous machinery murmur associated with a thrill was heard at the third and fourth intercostal spaces. Blood pressure was 110/70 mmHg. Electrocardiography showed normal for age sinus rhythm and QRS axis and left ventricular hypertrophy. Cardiothoracic ratio was 0.5 on chest x-ray and pulmonary vascular markings were normal.

Echocardiographic examination showed that all the heart chambers were slightly enlarged and ventricular contractions were normal (shortening fraction was 36%). A small ventricular septal defect on the subpulmonic region of the interventricular septum was present. The direction of the shunt through the ventricular septal defect was from left to right and a systolic gradient of 75 mmHg was measured between the two ventricles. Parasternal long and short axis and apical four chamber views demonstrated that the right coronary cusp of the aortic valve was prolapsed over the ventricular septal defect. A windsock-shaped tubular aneurysm arising from the right sinus of Valsalva and protruding into the right ventricle was detected (Figs. 1 and 2). Color flow mapping showed turbulent continuous flow originating from the aneurysm. Continuous wave Doppler interrogation of this flow revealed a continuous flow signal with a systolic peak of approximately 4 m/sec and a diastolic peak of 3.8 m/sec between aorta and right ventricle. Neither aortic regurgitation nor right ventricular outflow tract obstruction was found.

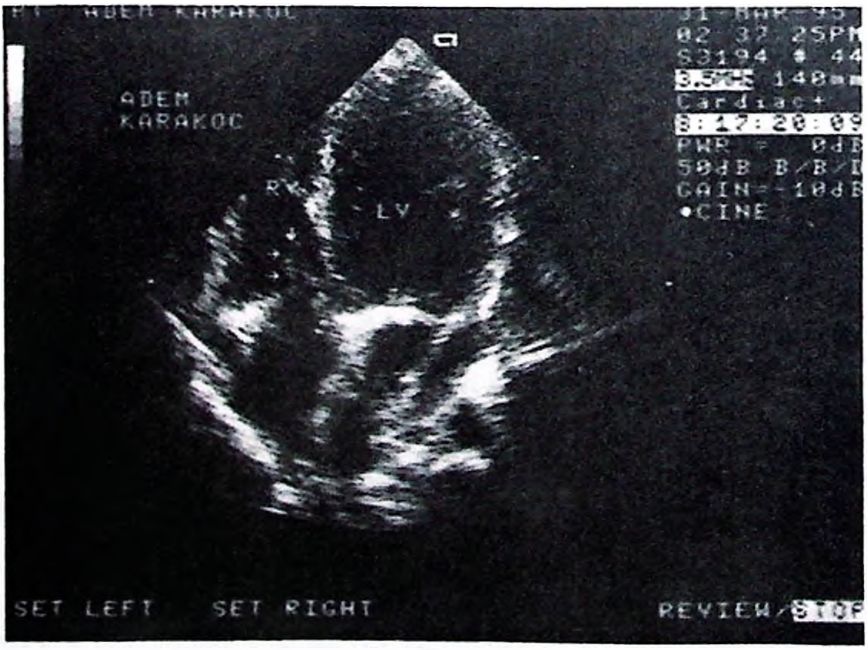

Fig. 1: Two-dimensional four chamber view of the first patient demonstrates the ruptured sinus Valsalva aneurysm protruding into the right ventricular cavity. LV: left ventricle, RV: right ventricle, Arrows: windsock-shaped aneurysm of sinus Valsalva protruding into the right ventricle.

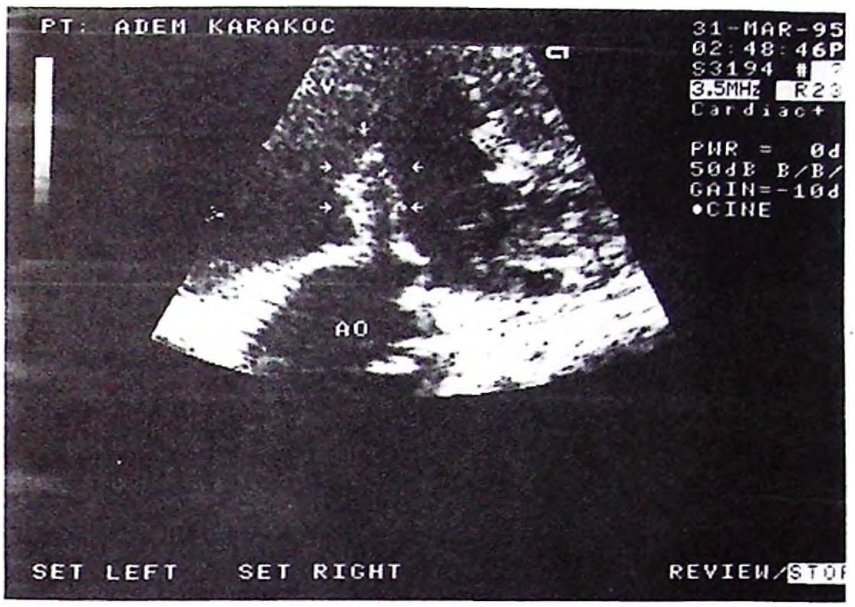

Fig. 2: Sinus Valsalva aneurysm protruding into the right ventricular cavity is seen in the parasternal short axis view of the first patient.

The ventricular septal defect was closed surgically with a dacron patch, and resection of the aneurysm pouch and primary repair of the rupture were performed. Echocardiographic findings were confirmed by surgery.

Case 2

A.T. was another asymptomatic eight-year-old boy without exercise intolerance. He was referred to our hospital for evaluation of a cardiac murmur heard during a routine examination. Physical examination revealed a hyperdynamic precordium and a 4/6th grade pansystolic murmur at the left sternal border associated with an early diastolic decrescendo type murmur; a thrill was palpable on the same region. Peripheral pulses were bounding. Cardiac silhouette was enlarged, pulmonary vascular markings were increased on chest x-ray and electrocardiography showed left ventricular hypertrophy.

Left atrium, left ventricle and aortic root were enlarged on echocardiographic examination. Contractility of the left ventricle was normal (shortening fraction 40%). Two dimensional echocardiography demonstrated a large subarterial ventricular septal defect. Left to right shunt was detected through the ventricular septal defect and the gradient between the left and right ventricles was 70 mmHg. A ruptured aneurysm of sinus Valsalva was protruding into the right ventricular outflow tract originating from the right coronary sinus of the aortic valve; a turbulent flow was detected in this region (Fig. 3). The leaflets of the aortic valve were deformed. Color Doppler echocardiography showed moderate aortic regurgitation. A mild obstruction with a 27 mmHg gradient at right ventricular outflow tract was present.

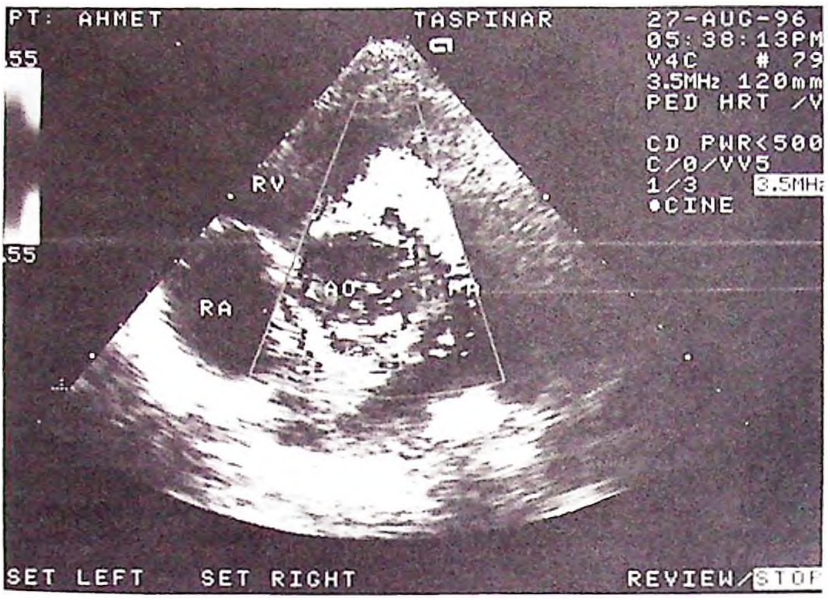

Fig. 3: Color flow imaging of the turbulent flow in the right ventricular outflow tract originating from the sinus Valsalva. Aneurysm is seen in the parasternal short axis view of the second patient. RA: right atrium, RV: right ventricle, Ao: aorta, PA: pulmonary artery.

At surgery, patch closure of the ventricular septal defect, repair of the SVAR and aortoplasty were performed. Anatomic features observed during surgery were consistent with the echocardiographic findings.

Discussion

Sinus Valsalva aneurysm rupture is a rare cardiac lesion. Its incidence among all patients who have undergone cardiopulmonary bypass is reported to range from 0.14-1.5%[7]. Though it has been reported in children as young as three years old, SVAR is more common in adults[1,2,8,9]. It is also more common in oriental populations in which subarterial ventricular septal defect is more prevalent[1]. Our patients are the fourth and fifth patients in our center to undergo open heart surgery with the same diagnosis in ten years (0.15% of all patients having open heart surgery). However, these are the first two patients to undergo surgery after echocardiographic examination without angiography.

Some of the patients may be asymptomatic, while in some, acute symptoms such as severe cardiac pain, dyspnea, tachycardia, exercise intolerance and congestive heart failure may occur[1]. It is thought that acute symptoms at the time of rupture may be less frequent when a subarterial VSD is also present[1]. There is little correlation between the size of the fistulous opening and the history of acute symptoms. Neither of our patients had acute symptoms but both had associated subarterial VSD which may explain the absence of symptoms.

Systemic diseases such as syphilis, infective endocarditis, Marfan's syndrome, cystic medial necrosis, atherosclerosis and trauma may cause SVAR[1, 7, 10-12]. No such disease was found in our patients.

Van Son et al.[1] found ventricular septal defect in 16 of their 37 patients with SVAR, and 15 of them were subarterial type ventricular septal defects. Both of our cases also had this type of ventricular septal defect.

Association of VSD with SVAR is reported in 30-50 percent of the cases in various studies[2, 4, 10]. Although presence of aortic valve prolapse associated with VSD and SVAR is reported rarely, it may be more common than indicates[3]. In both of our patients aortic valve prolapse was observed: aortic valve was competent in the first patient while moderate aortic regurgitation was observed in the second one.

Sinus Valsalva aneurysm rupture SVAR most frequently originates from the right coronary sinus (67-90%). Non-coronary sinus (8-25%) and left coronary sinus (0-8%) may be involved less frequently[2, 4, 10]. In both of our cases the origin of SVAR was the right coronary sinus. Aneurysms may rupture into the right ventricular outflow tract (60-85%), right ventricular cavity (20-25%), left ventricle (0-5.5%), or right atrium (9-66%)[2, 4, 10]. Our first case was an example of rupture into the right ventricular cavity and the second was ruptured into the right ventricular outflow tract. For SVAR in association with VSD, rupture into the right ventricular outflow tract is reported to be more common in childhood[2].

Sinus Valsalva aneurysm SVA may cause right ventricular outflow obstruction by mechanical compression. Aortic regurgitation is also detected in patients with VSD and aortic valve prolapse[13, 14]. Our second patient had both right ventricular outflow tract obstruction and aortic regurgitation.

Cardiac catheterization and angiography were considered to be necessary for diagnosis of SVAR in earlier reports[15]. Contrast echocardiography in combination with two-dimensional echocardiography was also used successfully in these patients[15]. Intracardiac ultrasonography is another method used for this purpose[16]. But with the development of Doppler and color-Doppler echocardiography and the improved resolution of two-dimensional echocardiography, presence of the aneurysm, location and size of the ventricular septal defect, origin of the aneurysm and cavity in which the rupture occurred can be demonstrated noninvasively[2, 5, 6].

In patients in which the sinus Valsalva aneurysm ruptures into the right ventricular cavity, the loud continuous signal in this area may cause difficulty for identification of right ventricular outflow tract obstruction. Similarly, small ventricular septal defects may not be detected because of this continuous flow. However, angiography may also miss some of these cases[2, 5, 6]. Color flow echocardiography is more valuable in detecting abnormal blood flow. In our cases we could define all anatomic features noninvasively by combining two-dimensional, Doppler and color-Doppler echocardiography, and our findings were confirmed by surgery.

In addition, our first case highlights the importance of follow-up in patients with small ventricular septal defects.

In conclusion, SVAR is a rare abnormality that requires surgical correction when diagnosed. Continuous murmurs heard during follow-up of children with VSD must alert the physician to this pathology and an echocardiographic examination must be performed. Combined two-dimensional, Doppler and color-Doppler echocardiography is an accurate, noninvasive method for diagnosis of SVAR.

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VASCULAR HAMARTOMA OF THE MEDIASTINUM*

A Case Report

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*Can İlyas MD*****, İlhan Paşaoğlu MD****

SUMMARY: Güvener M, Doğan R, Demircin M, İlyas C, Paşaoğlu İ. (Departments of Thoracic and Cardiovascular Surgery and Pathology, Hacettepe University, Faculty of Medicine, Ankara, Turkey). Vascular hamartoma of the mediastinum: a case report. Turk J Pediatr 1999; 41: 133-137.

Vascular hamartoma of the mediastinum is a rare benign vascular tumor. A 13-year-old girl presented with back pain, persistent coughing, palpitation, and angina pectoris. Preoperative investigations demonstrated an enlarging mass involving the superior mediastinum extending posteriorly (T6-T8). An encapsulated, 6x5x3 cm dark purplish mass adherent to the aortic wall was found. The main mediastinal mass was totally excised but limited resection was carried out in the paravertebral region. Microscopic examination revealed a vascular hamartoma. *Key words: hemangioma, mediastinum, vascular hamartoma.*

Vascular hamartomas have been reported as a rare pathological entity. They consist of hemangiomas that fall in a gray area between hamartomatous malformations and true neoplasms. Hemangiomas are bingn vascular tumors and are rare developmental vascular hamartomas that, by definition, do not group by mitotic activity[1, 2]. They occur more commonly in women between 30 and 50 years[2] of age[1]. Localization of vascular hamartomas can involve different parts of the body such as mediastinal, retinal, dermal, epidural, and testicular tissue[3-6].

Various vascular tumors, both benign and malignant, occur in the mediastinum. Many mediastinal tumors in infants and children are vascular. Only one-third of 38 mediastinal vascular tumors reviewed by Shields, Attar and Cowley[7, 8]. (1964) were found in persons younger than 20 years. The other two-thirds occurred in older persons mostly in their fourth and fifth decades of life. The incidence in men and women is approximately equal.

The tumor is generally solid and discrete, although diffuse infiltrative lesions have been reported. Calcification (phlebolith) occasionally occurs. Bony erosion or enlargement of an intervertebral foramen may also occur.

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Although various histological types are seen, most of these tumors are benign. The benign lesions have been classified as hemangioendotheliomas or capillary hemangiomas. The malignant lesions are designated as angiosarcomas or hemangiosarcomas. Although malignant lesions occur in all age groups, they are most common in the fourth decade of life.

A case of vascular hamartoma involving the superior and posterior mediastinum is here in reported.

Case Report

A 13-year-old girl was admitted to our hospital with complaints of back pain in the subscapular region, persistent coughing, palpitation, and angina pectoris. The severity of pain increased when lying or coughing. Routine physical examination was normal. The chest roentgenogram revealed a double contour in the aortic arch (Fig. 1). Echocardiographic examination suggested aortic aneurysm. On angiocardiographic examination no true aortic aneurysm was found. Magnetic resonance imaging (MRI) examination of the lesion revealed a round mass extending along the aortic wall from the superior mediastinum posteriorly to the sixth and eighth thoracic vertebral bodies; septation was present in the solid hemorrhagic mass (Figs. 2a, 2b). The abdominal ultrasonography (USG) was normal. Complete blood count, biochemical analysis and electrocardiogram were found within normal limits.

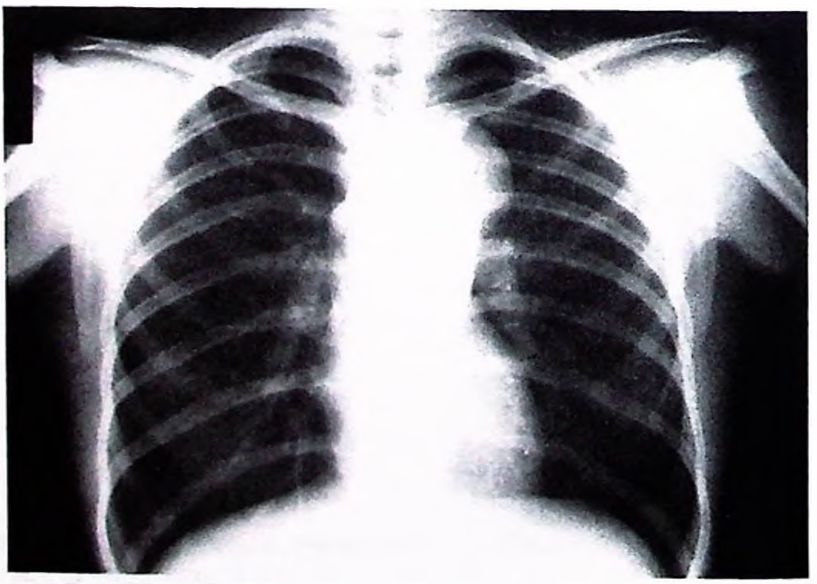
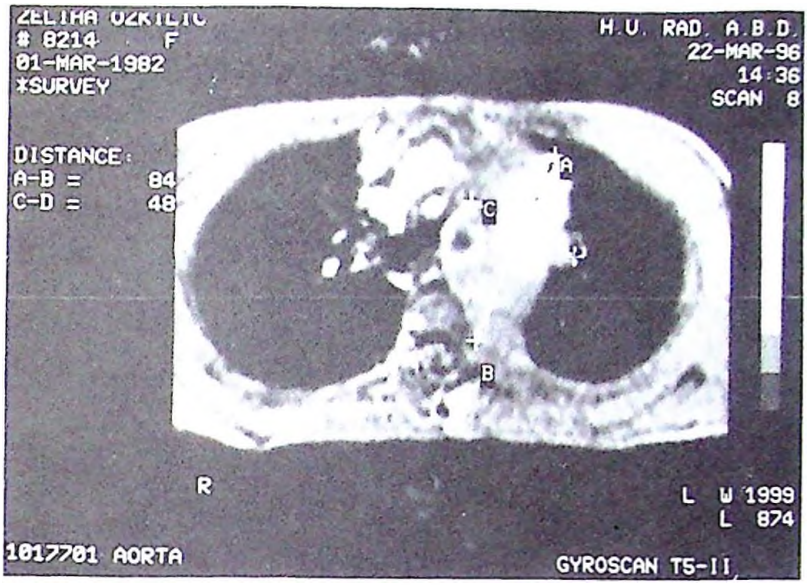
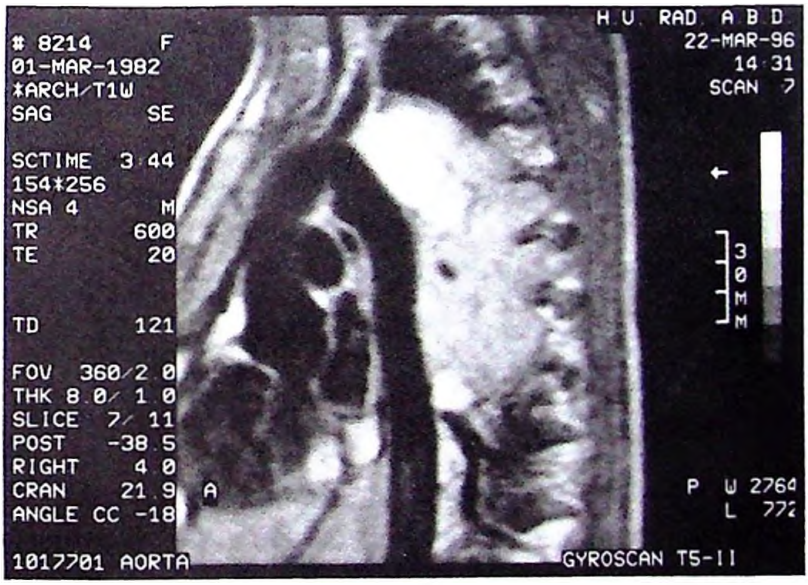


Fig. 1: Preoperative posteroanterior chest roentgenogram showing a double counter in the aortic arch.



(a)



(b)

Fig. 2: Preoperative MRI examination. (a) Transverse section showing a round mass extending posteriorly along the aortic wall from the superior mediastinum. (b) Sagittal section showing the relation between the mass and paravertebral region.

Left posterolateral thoracotomy was performed. A 6x5x3 cm dark purplish encapsulated mass adherent to the aortic wall was found. On exploration, the tail of the mass was found to be extending to the paravertebral region (T6-T8). The main mediastinal mass was totally excised but limited resection was performed in the paravertebral region.

Microscopic examination revealed a vascular hamartoma characterized by an irregularly lobulated cystic structure with coagulated blood in the cysts (Fig. 3). Postoperative irradiation was not applied. Postoperative course was uneventful and the patient was discharged on the sixth postoperative day.

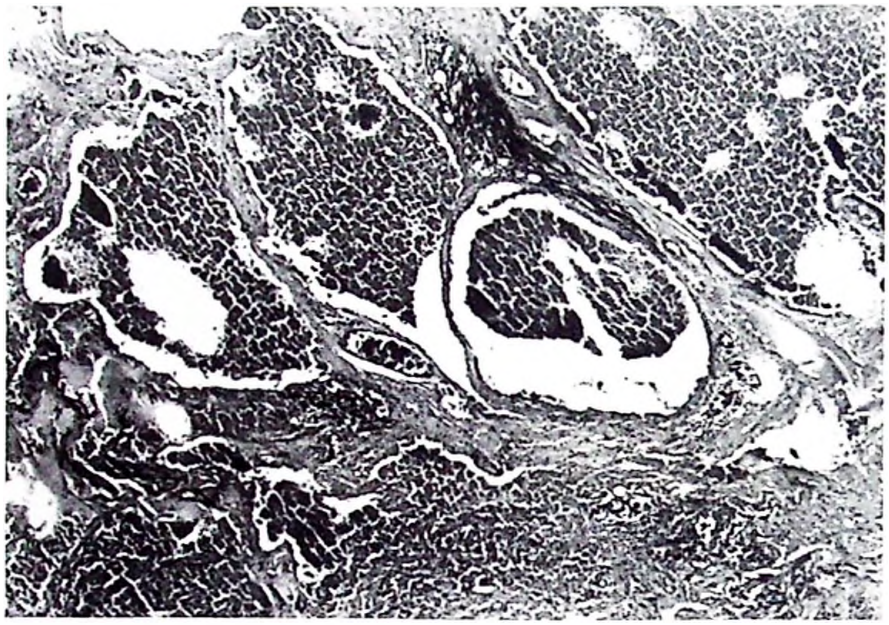


Fig. 3: Histological sections of the mass. The lesion is composed of large dilated blood-filled vessels lined by flattened endothelium (hematoxylin-eosin; original magnificantion x 100).

In the control MRI examination on the 24th day, the appearance of the excision area was evaluated as a hematoma. In the follow-up examinations, six months after the operation, the patient was well.

Discussion

Vascular hamartoma of the mediastinum is a benign tumor rarely found in the mediastinum. Thirty-eight mediastinal vascular tumors (benign or malignant) were reviewed by Shields, Attar and Cowley (1964)[7,8], and 18 benign mediastinal hemangiomas were reported by Moran and Suster (1995)[9]. The others in the literature are case reports[10,11].

Most vascular masses occur in the anterior mediastinum and adjacent visceral compartments. The remaining ones are present in the posterior aspect of the visceral compartment and paravertebral sulci. In our case the mass in the superior mediastinum was extending like a tail to the paravertebral sulci. Approximately 30 percent of these masses produce no symptoms; large and malignant lesions are more likely to be symptomatic. In this case, back pain, angina pectoris and coughing were the major symptoms. Concurrent vascular

masses may be present in the neck or elsewhere; however, in our patient there were not any accompanying lesions. Magnetic resonance imaging (MRI) is very useful in establishing a diagnosis of mediastinal vascular hamartomas[12].

At thoracotomy, the vascular masses are often soft, encapsulated, and dark purplish in color, and may or may not be pulsatile[7,8]. Both benign and malignant tumors may infiltrate adjacent structures[7,8]. In our patient, the mass had infiltrated the aortic adventitia and the epidural layer of the spinal cord. The choice of surgical treatment is enucleation when possible; however, sometimes only a partial resection can be accomplished because of infiltration of vital structures. Even with a benign mass such as this one, limited excision could be carried out in the paravertebral region. Blood loss may be extensive. In our patient blood drainage was less than expected. Postoperative irradiation appears to have little value. When the mass is benign, as in our patient, the prognosis is favorable.

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CARTILAGINOUS HAMARTOMA OF THE CHEST WALL WITH SECONDARY ANEURYSMAL CYST – LIKE AREAS IN AN INFANT*

A Case Report

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*Erdener Özer MD**, Tanju Aktuğ MD*****

SUMMARY: Göre O, Kılıçalp A, Başdemir G, Özer E, Aktuğ T. (Department of Pathology, Dokuz Eylül University Faculty of Medicine; Department of Pathology, Ege University Faculty of Medicine; and Department of Pediatric Surgery, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Cartilaginous hamartoma of the chest wall with secondary aneurysmal cyst-like areas in an infant. Tur J Pediatr 1999; 41: 139-142.

A case of a four-month-old infant diagnosed as cartilaginous hamartoma of the rib is presented. This rare tumor usually presents at birth. The patient had respiratory distress syndrome. Swelling of the ribs was palpable on physical examination and the infant underwent surgery for excision of the ribs. Histopathologically, the tumor showed benign focal overgrowth of normal skeletal elements with cartilaginous, vascular and primitive-appearing mesenchymal elements. Additionally, secondary aneurysmal cyst formation coexisted with the tumor. The diagnosis was infantile cartilaginous hamartoma of the rib. In this entity, primitive-appearing mesenchymal stroma may be mistaken for a malignant condition. Usually a benign clinical course is expected and treatment is by block excision. *Key words: cartilaginous hamartoma, benign mesenchymoma, rib.*

Infantile cartilaginous hamartoma (ICH) of the rib is an extremely rare neoplasm characterized by cartilaginous, vascular and primitive-appearing mesenchymal elements. It presents in newborns and infants. The tumor is associated with a benign clinical course and cure is by block excision. Mirra[1] pointed out that this entity might be mistaken for a malignant condition because of the primitive appearance of the mesenchymal component. The rarity of the tumor has been reported by McCarthy et al.[2] and McLeod et al.[3] under the designations infantile ICH or benign mesenchymoma of the rib. Since the entity never metastasizes and is curable with local resection, it is now clear that it is a congenital benign hamartomatous lesion of the rib.

Case Report

A four-month-old female infant was admitted to Dokuz Eylül University Hospital with the complaint of respiratory distress. The initial chest radiogram and thoracal computed tomography revealed a large multicystic intrathoracic mass occupying

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almost the whole of the left hemithorax (Fig. 1). There was erosion on two ribs. At surgery, the entire mass along with portions of the left sixth and seventh ribs were completely, removed. On gross examination, the tumor measured 85x40x35 mm and showed two large nodules (Fig. 2). The inner aspect of the ribs was destroyed by the mass. The cut section showed firm whitish glistening cartilaginous tissue admixed with multicystic congested tissue. Microscopic sections of the tumor revealed a mixture of mesenchymal elements, including chondroid tissue with large endothelium-lined blood spaces resembling aneurysmal bone cyst with osteoclast-like giant cells and osteoid, and foci of calcification (Fig. 3). The cartilaginous component was composed of mature hyaline cartilage and prechondrocytes resembling chondroblasts. Some cartilaginous areas adjacent to the bone showed vacuolization and primitive formation of colums resembling that seen in epiphyseal cartilage (Fig. 4). The pathological diagnosis was infantile cartilaginous hamartoma. In the 20 months since diagnosis, the patient has grown normally with no evidence of recurrence.

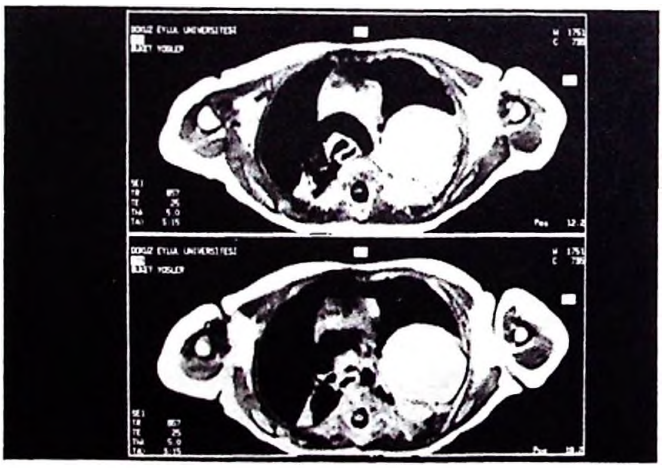

Fig. 1. Computed tomography of the thorax showing multicystic mass occupying the whole of the left hemithorax

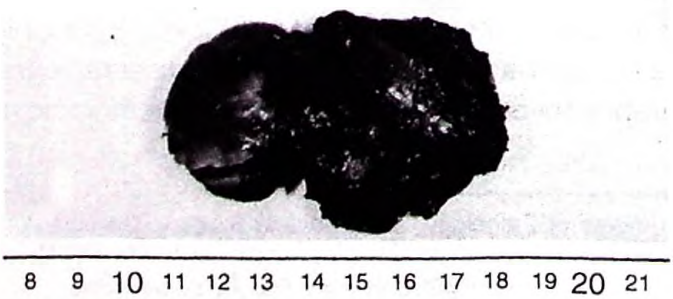

Fig. 2: Macroscopic appearance of the lesion. The lesion is composed of two nodules. The rib is partially destroyed.

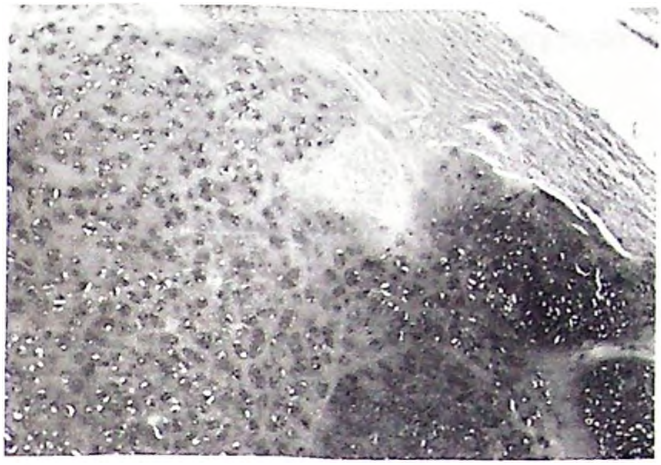

Fig. 3: Microscopic appearance of the lesion showing areas of large masses of hyaline cartilage, (H and E X40).

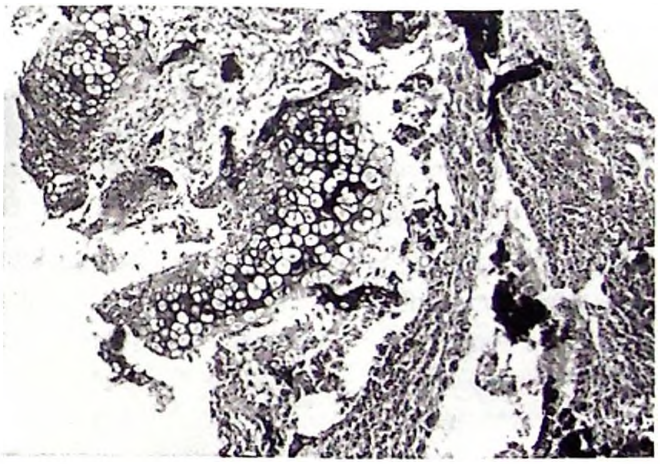

Fig. 4: Microscopic appearance of the lesion: Foci of woven bone and cartilage showing vacuolization and primitive column formation (H and E X40).

Discussion

The report by McLeod et al.[3] reveals that infantile cartilaginous hamartoma, commonly known as benign mesenchymoma, is very rare in infants and usually presents at birth. The incidence is about 0.03 percent of primary bone tumors. About 25 cases have been reported in English literature[4-6]. As seen in our case, this is a solitary lesion of the rib with a size varying from 2 to 15 cm. Because the character of the lesion is benign, Mirra[1] and Cohen[7] noted that excision of the rib is the recommended procedure. Immunohistochemical localization of various collagen types is consistent with the non-neoplastic notion of character[6]. This lesion should be distinguished from primary aneurysmal bone cyst, chondroma, chondrosarcoma and other mesenchymal neoplasms.

This lesion is presented with either rib swelling or respiratory distress symptoms. In our case, respiratory distress symptoms were predominant. Rarely, incidental chest radiograms may reveal a large expansive mass of the rib, with or without spotty calcification characteristic of cartilaginous tumors. More than one rib may be involved and destroyed by the lobulated mass. The report by Blumenthal et al.[8] suggests that ribs adjoining the main lesion can be deformed or eroded by the extrinsic mass. The external surface of the rib is well circumscribed and smoothly expanded. The cut section shows variable-sized foci of chondroid to cartilaginous tissues with foci of calcification. In many cases, dilated blood-filled spaces similar to that seen in aneurysmal bone cysts may be observed[1].

Constant histological features in ICH are sheets of chondroblast-like mesenchymal cells without atypical mitoses or frank anaplasia, hyaline cartilage derived from hamartomatous chondroblast-like stroma which can be mistaken for a chondrosarcoma, and prominent vascularity for aneurysmal bone cyst. The variable histological findings are foci of calcification, woven bone production, reactive osteoclast-like giant cells, enchondral ossification similar to growth plate cartilage and fibroblastic hyperplasia, particularly in aneurysmal bone cyst-like areas. In 1972 two cases with atypical mitoses, frank anaplasia and prominent hypercellularity were reported as intrathoracic malignant mesenchymoma[8]. However, no evidence of malignancy was observed in the present case.

To conclude, we have reported a case of infantile cartilaginous hamartoma because of its rarity and constant histological features. There has been no evidence of recurrence, during the 20 months since the excision, consistent with its benign clinical course.

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AN UNCOMMON ASSOCIATION OF H – TYPE TRACHEOESOPHAGEAL FISTULA WITH INFANTILE HYPERTROPHIC PYLORIC STENOSIS*

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*Akgün Hiçsönmez MD****

SUMMARY: Oğuzkurt P, Tanyel FC, Haliloğlu M, Hiçsönmez A. (Departments of Pediatric Surgery and Radiology, Hacettepe University Faculty of Medicine, Ankara, Turkey). An uncommon association of H-type tracheoesophageal fistula with infantile hypertrophic pyloric stenosis. Turk J Pediatr 1999; 41: 143-146.

Although infantile hypertrophic pyloric stenosis following esophageal atresia repair is known, infantile hypertrophic pyloric stenosis following H-type tracheoesophageal fistula has not been encountered previously. A case of H-type tracheoesophageal fistula and infantile hypertrophic pyloric stenosis is presented. The patient, operated on for H-type fistula, a rare congenital anomaly of the esophagus, on the tenth day of life was readmitted 19 days later because of continous vomiting after every feeding. The clinical findings and physical and radiological examinations revealed infantile hypertrophic pyloric stenosis which required surgical treatment. It is suggested that the association of H-type tracheoesophageal fistula with infantile hypertrophic pyloric stenosis is coincidental, given the estimated incidence of one in every 84,375,000 males and 337,500,000 females. *Key words: hypertrophic pyloric stenosis, H-type tracheoesophageal fistula.*

Esophageal atresia (EA) with tracheoesophageal fistulas (TEF) and infantile hypertrophic pyloric stenosis (IHPS) are among surgical problems frequently encountered in the newborn period. Although the occurrence of infantile hypertrophic pyloric stenosis is reported in patients with esophageal atresia and tracheoesophageal fistula, this entity has not been previously reported in patients with H-type tracheoesophageal fistula in English language literature. To the best of our knowledge this is the first described case.

Case Report

A four-day-old male was admitted to the hospital because of coughing, choking and cyanosis during and after feeding. He was the product of the fifth pregnancy of a 32-year-old mother and had a birth weight of 3000 g. Physical examination revealed severe icterus and coarse rales in both hemithoraces. Laboratory examinations including complete blood count, urinalysis and blood chemistry

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were within normal limits except for indirect hyperbilirubinemia with 24.2 mg/dl. After exchange transfusions were repeated twice, his indirect bilirubin level returned to normal limits. On posteroanterior chest x-ray there was consolidation at the right upper lobe. Cineesophagography was performed and revealed H-type tracheoesophageal fistula at the level of the second thoracic vertebra (Fig. 1). On his tenth day of life, he was operated on through a left cervical transverse incision, and a large fistula was isolated, transected and sutured. He was fed orally on the fourth day following the operation and was discharged without any complaints. When he was 29-days-old, he was readmitted because of vomiting and coughing for 15 to 30 minutes following feeding. Barium meal performed with the suspicion of refistulization revealed the previous fistula region to be normal. Gastroesophageal reflux was observed on fluoroscopic examination. On erect abdominal x-ray taken the next day, the stomach air was dilated (Fig. 2). On physical examination a small mass (olive) was palpable above the umbilicus and the abdomen was scaphoid. Laboratory investigations revealed alkalosis and mild hypochloremia. Abdominal ultrasonography was performed and supported the clinical diagnosis of hypertrophic pyloric stenosis (Fig. 3). The patient was operated on through a small right upper quadrant transverse incision. A hypertrophic pyloric muscle was found and Ramstedt pyloromyotomy was performed. The patient was fed on the first day of the operation and he had an uneventful recovery. He had no problems during the six month follow-up period.

Fig. 1: Cineesophagography of the patient demonstrating the H-type tracheoesophageal fistula at the level of the second thoracic vertebra.

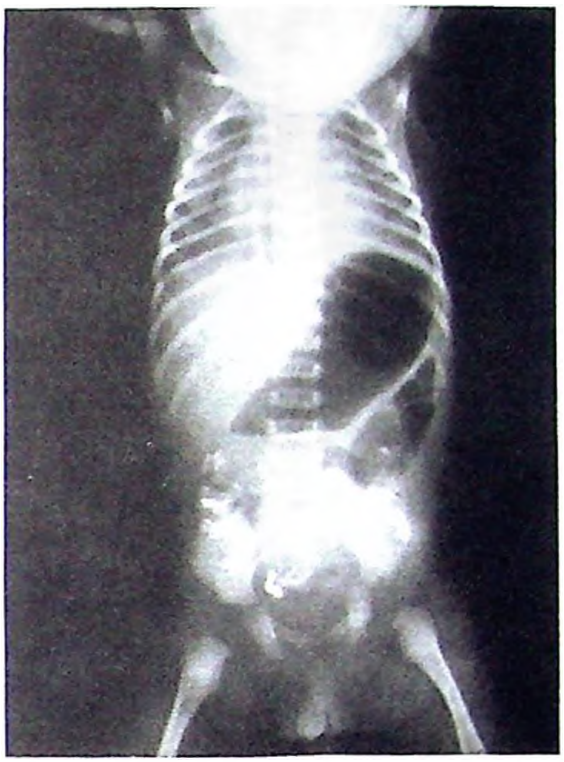

Fig. 2. Plain abdominal x-ray with the dilated gastric air.

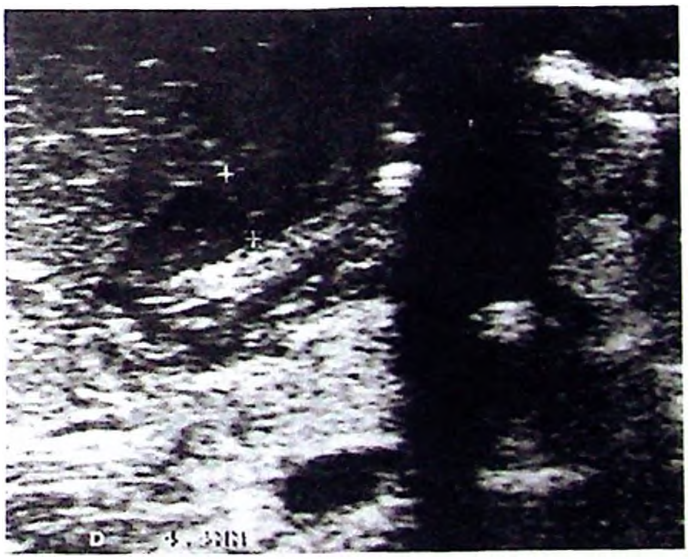

Fig. 3: Ultrasonography showing the thickness of the pyloric muscle.

Discussion

Nearly 50 percent of patients with EA have additional congenital anomalies[1,2]. The frequency of other gastrointestinal anomalies in patients with EA is 18 percent[2], the most commonly encountered being imperforate anus, duodenal atresia (with or without annular pancreas) and pyloric stenosis[1,3]. In patients with EA the frequency

of IHPS has been reported to be between one and 10 percent[4]. On the other hand, only six percent of patients with IHPS had additional anomalies, 13 percent of which are associated with the gastrointestinal system[4].

There are nearly 40 cases in the literature where IHPS developed following operations for EA[1]. However, IHPS associated with H-type fistula has not been reported perviously. Since their etiologies are different, it is suggested the occurrence is coincidental. Since EA is encountered once in 4,500 live births with males and females equally affected and since four percent of the cases are H-type TEF[5], the estimated incidence of H-type TEF becomes one in 112,500 males or females.

On the other hand, IHPS is encountered once in 300 live births, with a 4:1 male to female ratio[6]. These ratios make the estimated incidence of both IHPS and H-type TEF one in 84,375,000 males and 337,500,000 females.

Since no common etiologic factor is known for H-type fistula and IHPS, this suggests a coincidence. Although this is the first reported case of this association, it should be estimated as one in 84,375,000 males and 337,500,000 females.

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THE SPLIT NOTOCHORD SYNDROME WITH DORSAL ENTERIC FISTULA, MENINGOMYELOCELE AND IMPERFORATE ANUS*

*Hüseyin Dindar MD**, Turan Kanmaz MD***, Murat Çakmak MD****
*Çağrı Savaş MD***, Selçuk Yücesan MD*****

SUMMARY: Dindar H, Kanmaz T, Çakmak M, Savaş Ç, Yücesan S. (Department of Pediatric Surgery, Ankara University Faculty of Medicine, Ankara, Turkey). The split notochord syndrome with dorsal enteric fistula, meningomyelocele and imperforate anus. Turk J Pediatr 1999; 41: 147-150.

A male infant was referred to our department because of lumbosacral meningomyelocele, dorsal enteric fistula and imperforate anus. The mother had received a parenteral drug containing estradiol benzoate and progesterone for inducing abortion in the first trimester. She also used an anal pomade containing triamcinolone and lidocaine-HCl during the pregnancy for hemorrhoids.

Sigmoid end colostomy was performed after meningomyelocele repair. On abdominal exploration a wandering spleen was detected but no other anomalies. Two months later, an abdominoperineal pullthrough was performed, and the patient was discharged well after three weeks.

Our case is the sixth that had split notochord syndrome associated with dorsal enteric fistula and imperforate anus. Additionally, penoscrotal transposition and wandering spleen were present in this case. To our knowledge, these associated anomalies have been extremely rare. *Key words: split notochord syndrome, dorsal enteric fistula, anal atresia.*

Split notochord syndrome (SNS) is rare, There are vertebral, central nervous system, and visceral anomalies in various combinations. In most of the reported cases, the cervicothoracic spine is involved. Split notochord syndrome (SNS) of the lumbosacral area is less common. Associated meningomyelocele with a dorsal enteric fistula is interesting in this whole group. The following is a description of the clinical, radiographical and surgical findings of such a case.

Case Report

A male newborn was referred to the Department of Pediatric Surgery at the University of Ankara on the day of birth because of gross anomalies overlying the spine. The gestational age of the newborn had been estimated as 41 weeks and the birth weight 3100 g. The 35-year-old mother received a parenteral drug containing estradiol benzoate and progesterone for inducing abortion in the first

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trimester. She also used an anal pomade containing triamcinolone and lidocaine-HCl during the pregnancy for hemorrhoids. She had no x-ray examinations. Previously, the mother had had one normal child, who was 15 years of age. There was consanguinity between the parents, but no family history of similar anomalies.

Physical examination revealed a large head with wide sutures and large, full fontanels. A meningomyelocele was present in the thoracolumbar region. Caudal to the meningomyelocele, intestinal mucosa protruded and meconium was excreted from the enteric opening (Fig. 1). There was a palpable defect in the lumbar spine and sacrum. Penoscrotal transposition with a small phallus was present. Testes were palpable within the bifid scrotum. The anus was imperforate. There was active movement of the lower extremities.

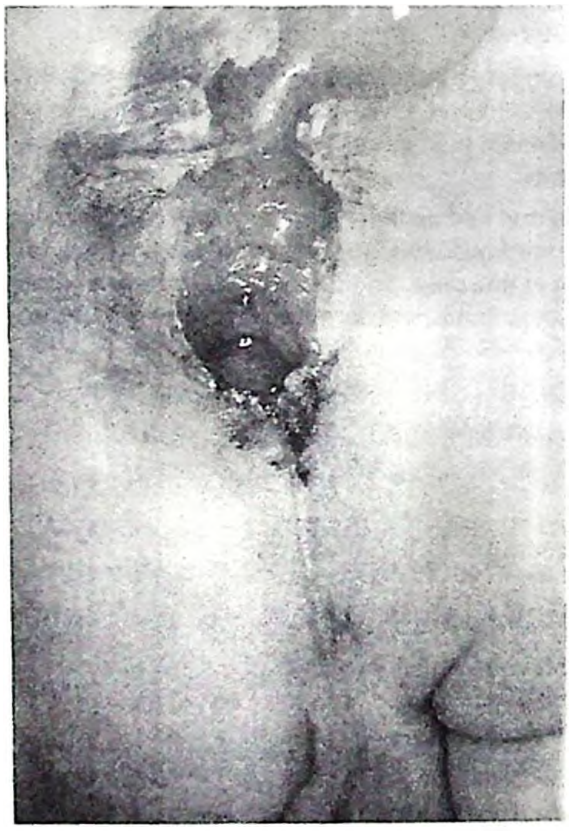

Fig. 1: Intestinal mucosa is protruded and meconium excreted from the enteric opening caudal to the meningomyelocele in the thoracolumbar region.

An x-ray of the spine showed a complete split of the lower lumbar spine and absence of sacrum (Fig. 2). Magnetic resonance images of the spine demonstrated two separate division of the spinal chord at the L2-L4 levels. A 5x3.5x1.5 cm meningomyelocele was seen posterior to the L2 vertebral body. Computed tomographic images showed posterior spina bifida at all levels, thoracolumbar rotoscoliosis and sacral dysgenesis.

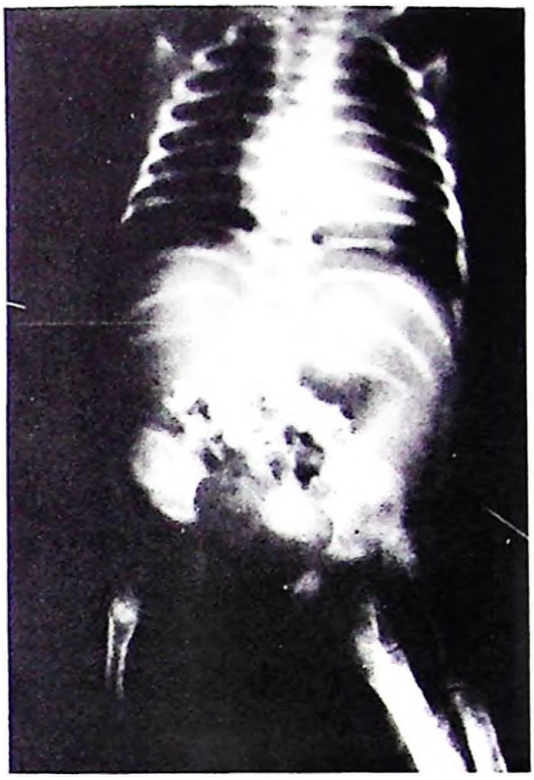

Fig. 2: An x-ray of the spine showed a complete split of the lower lumbar spine and absence of sacrum.

The meningomyelocele was repaired on the first day of life. It was found to communicate with the spinodural canal by means of a small narrow sac. After excision of the neural lesion, the protruded bowel opening was dissected, ligated and placed into the abdomen. After closing the dura, the defect was reinforced with paraspinal fascia and the skin edges approximated over the defect. A laparotomy was then performed, and a wandering spleen (movable spleen because of a lengthened pedicle) was detected on surgical abdominal exploration. The colon ended at the sigmoid level and the rectum was not found. The fistula was between the sigmoid end and neural canal. Sigmoid end colostomy was performed and no other anomalies were found in the abdomen. The postoperative period was uneventful. He was discharged well after one week. Two months later, an abdominoperineal pullthrough was performed for anal atresia. The patient was discharged well after three weeks.

Discussion

Split notochord syndrome (SNS) is an extremely rare anomaly that may be associated with a wide spectrum of developmental anomalies involving skin of the back, spine, central nervous system, and gut. The majority of cases of SNS have involved the spine above the level of the diaphragm. Bentley and Smith[1] were the first to note that an abnormal splitting of the notochord could cause a wide variety of malformations of not only the vertebral bodies and neural tissue but also enteric viscera.

Split notochord syndrome (SNS) of the lumbosacral spine in association with dorsal enteric fistula is a rare phenomenon. To date, only 15 human cases have been reported in the literature[2]. Hoffman et al.[2] described such a case and reviewed the previous cases: of the 15 cases, eight were associated with meningomyelocele, five had an imperforate anus, two were associated with bladder extrophy, and one had a rudimentary extremity extending from the spinal cleft. Another case of SNS was associated with dorsal sinus lipoma (dorsal mesodermal sinus), colonic duplication, annular pancreas, and meconium peritonitis[3].

Several etiologic theories have been proposed, including the persistence of the neuroenteric canal, the occurrence of an ectopic or accessory neuroenteric canal, a division or local redundancy of the notochord, an endodermal-ectodermal adhesion, neural tube rupture caused by oversecretion of fluid, and failure or aberrance of dorsal aortic distribution to the region of the neural folds resulting in prevention of timely neural tube closure[4]. It was shown that a large malformation spectra, especially abdominal wall defects, occur following intraamniotic administration of glucocorticoids in the chick embryo[5]. The mother of this case used an anal pomade containing triamcinolone. This drug might have been absorbed from the rectal veins and in might have affected the fetus. The mother also used a parenteral drug containing estradiol and progesterone for inducing an abortion. This drug might also have affected the patient. Despite these theories, the split notochord syndrome remains complex and poorly understood.

Our case is the sixth one that had SNS associated with dorsal enteric fistula and imperforate anus. Additionally, penoscrotal transposition and wandering spleen were present in this case. To our knowledge, these associated anomalies have not been reported previously. Gupta et al.[6], after reviewing the literature, found very few cases involving the lumbosacral area with very few survivals. The presented patient also had lumbosacral cleft, but was nevertheless alive and healthy. We recommend repair of both meningomyelocele and dorsal enteric fistula as fast as possible to prevent the contamination of the meningocele sac by enteric bacteria.

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