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The Turkish Journal of Pediatrics 1999; 41: 151-160 Review

TRANSIENT HYPERPHOSPHATASEMIA – WHERE DO WE STAND?*

*Štěpán Kutílek MD**, Milan Bayer MD***

SUMMARY: Kutílek Š, Bayer M. (Department of Pediatrics, Charles University Faculty of Medicine, Prague, Czech Republic). Transient hyperphosphatasemia-where do we stand? Turk J Pediatr 1999; 41: 151-160.

Transient hyperphosphatasemia of infancy and early childhood (THI) is characterized by transiently increased serum activity of alkaline phosphatase (S-ALP), predominantly its bone or liver isoform, in children under five years of age. There are no signs of metabolic bone disease or hepatopathy corresponding with the increased S-ALP, nor is there a disease common to all children with THI. To date, THI has been reported in more than 400 children. Viral etiology of THI has been proposed; transiently increased bone turnover and impaired clearance of ALP from the serum were originally considered as its causes. The pathogenesis is most probably multifactorial. THI is a benign disorder, as prospective follow-up of children with a history of TH revealed normal growth and normal bone density. Children with TH should be spared from excessive diagnostic procedures. *Key words: alkaline phosphatase, hyperphosphatasemia.*

The serum activity of alkaline phosphatase (S-ALP) is a routine marker in the diagnosis of hepatic disorders and metabolic bone diseases. In healthy adults, the major activity of S-ALP is represented by liver and bone isoenzymes, while in healthy infants and children, as a result of growth, serum is rich in the bone isoenzyme of ALP[1,2]. Increased total S-ALP, termed as hyperphosphatasemia, is usually indicative of hepatopathy or is a marker of increased bone formation. Further determination of the ALP isoenzymes and isoforms is helpful in identifying the cause of hyperphosphatasemia.

Transient hyperphosphatasemia of infancy and early childhood (THI) is characterized by transiently increased S-ALP, predominantly its bone or liver isoform, in children under five years of age. There are no signs of metabolic bone disease or hepatopathy corresponding with the increased S-ALP, nor is there a disease common to all children with THI. To date, THI has been reported in more than 400 children. The etiology of the condition remains unclear, as does its mechanism. In this article, we focus on its clinical and biochemical features and management and the prognosis for children with TH.

History and Criteria of THI

Transient hyperphosphatasemia of infancy and early childhood (THI) was first observed by Bach et al.[3] in 1954. In 1969, Geudeke[4] performed an analysis of ALP isoenzymes and found that the hepatic isoform was responsible for the

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high ALP activity. In 1977, Posen et al.[5] observed increased activity of the bone isoform of ALP in children without metabolic bone disease. They considered this a new and insufficiently recognized syndrome and used the term "transient hyperphosphatasemia of infancy" for the first time. Wieme[6] performed an electrophoretic evaluation of the ALP isoenzymes in 1983 and discovered a new atypical pattern which consisted of cathodal and anodal migrating fractions, bearing some similarities to those isoforms of bone and liver origin. This pattern was considered a characteristic biochemical feature of THI[6, 7]. In 1983, Weiber et al.[8] documented a return to normal isoenzyme pattern which occurred simultaneously with the return of total S-ALP to normal values. The definition of THI remained vague until Kraut et al.[9] delineated criteria for its diagnosis in 1985. These include:

1. an age of less than five years.
2. variable, unrelated symptoms,
3. no bone or liver disease noted on physical examination or
4. from laboratory investigations,
5. isoenzyme and isoform analysis showing elevations in both bone and liver activity, and
6. a return to normal S-ALP values within four months.

However, these criteria may need some corrections, as THI has occasionally been observed in adults, and since the duration of THI has repeatedly exceeded the time limit of four months[7, 10-13].

Clinical and Biochemical Features of Children with THI

Transient hyperphosphatasemia of infancy and early childhood has been observed in healthy children, as well as in children with various chronic and acute diseases, in particular in children with respiratory, urinary and gastrointestinal tract infections, viral infections, hematological disorders (anemia, lymphoblastic leukemia), neurological affections (breath-holding spells, epilepsy, cerebral palsy), malabsorption syndrome, allergic disorders, Crohn's disease, metabolic bone diseases (vitamin D-dependent rickets type I, vitamin D-deficient rickets), in patients after liver transplantation and in children with congenital abnormalities[7, 11, 14-22]. In the above-mentioned patients with metabolic bone diseases and those with liver transplantation, no relationship was found between the severity of their state and S-ALP[16, 18, 21]. Transient hyperphosphatasemia had earlier THI been related to treatment with co-trimoxazol[7, 23], but this is unlikely, as THI has occurred in children who were never treated with this drug. A seasonal clustering of THI has been described, with the highest incidence reported September through December[19]. However, such clustering has not been observed by other authors[12].

The peak total S-ALP is typically five to 30 times the adult upper reference limit (URL), but it may exceed this limit up to 70 times[7]. The atypical isoenzyme pattern as revealed by the electrophoretic evaluation of ALP is a characteristic biochemical feature of THI. However, this is not reflected by inactivation-inhibition methods, where bone and hepatic isoforms prevail[7]. The serum concentrations of calcium and phosphate (S-Ca, S-P) and activity of transaminases (S-ALT, S-AST) in children with TH are within reference ranges and thus are not suggestive of either metabolic bone disease or hepatopathy[7]. However, there are reports of changes in markers of bone resorption during the course of THI, as increased serum activity of tartrate resistant acid phosphatase (S-TRAP) was observed in one child[24] and increased urinary excretion of hydroxyproline was observed in 15 children[25-21].

In a recent multicenter study by Griffiths et al.[28], electrofocusing technique was applied in the S-ALP analysis. S-ALP Serum activity of alkaline phosphatase was evaluated in 135 children aged three months to 12 years with hyperphosphatasemia, who were diagnosed as THI; numerous actively involved tissue sites were identified as the source of increased S-ALP[28]. Based on the results, the patients were sorted into three groups[28]:

1. Previously healthy patients, who showed additional laboratory evidence of viral and protozoal infection, in whom the ALP isoenzyme pattern reflected the primary target organ(s) of the infection;

2. Patients with clinical evidence of failure to thrive due to preexisting disease, along with a superimposed infection, where the ALP isoenzyme pattern reflected the specific infection and fractions associated with the primary disease; and

3. Patients exhibiting failure to thrive (nonorganic or caloric deficit) who did not show evidence of infection.

With adjustment of the children's caloric intakes, the S-ALP levels returned to reference ranges in the third group[28].

Transient hyperphoptasasemia is definitely not a rare condition, as its incidence varies between 0.3-1.5 percent[14, 19, 29]. There is no preponderance by sex[7].

Transient hyperphosphatasemia was initially termed as the "Ulysses syndrome", as the patients underwent an odyssey of investigations with no conclusive results[30].

Suggested Etiology and Mechanisms

The etiology of THI is currently believed to be an infectious one, as some of the children with THI experienced signs of viral disease, including fever and gastrointestinal irritation, two to three weeks prior to the diagnosis of THI[7, 28]. Furthermore, THI was observed in siblings and in children who were hospitalized together, further suggesting the infectious, most probably viral origin[31-34]. So

far, all attempts to find a common causative infectious agent have failed, as THI has been diagnosed in children with cytomegalovirus, rotavirus, adenovirus, herpes simplex virus, echovirus, rhinovirus, influenza A, B, C, respiratory syncytial virus and Epstein-Barr virus infections[7, 8, 28, 35-38]. The study using electrofocusing techniques further confirmed the viral origin of THI[28].

The question remains whether a small group of children will respond to infection with a high magnitude of S-ALP or, conversely, do all children respond, but only a small number undergo laboratory investigations that include evaluation of S-ALP[28].

To date, four mechanisms have been proposed as responsible for the elevated serum ALP activity:

1. Increased Production of ALP in its Tissue of Origin, Most Probably in Bone and Liver

Part of this hypothesis is based upon isolated findings of increased S-TRAP and urinary hydroxyproline excretion, which are markers of bone resorption, in patients with THI[24-27]. The temporary increase in bone resorption is known to be closely coupled to bone formation and followed by increased osteoblast activity[39]. Furthermore, the duration of THI (4 months) resembles the duration of the bone resorbing cycle. Therefore, it has been postulated that in THI, the increased ALP activity, reflecting high bone formation, is preceded by an increased bone resorption, as evidenced by the increased urinary hydroxyproline excretion and/or high S-TRAP[26, 38]. We have earlier speculated that putative transient increase in bone resorption might have been triggered by the viral infection[26, 38]. However, the heterogeneity of viral agents diagnosed in children with THI makes this hypothesis less likely. Furthermore, there has not been a similar increase observed in other markers of bone resorption in patients with THI, and there was no correlation between ALP activity and urinary hydroxyproline excretion[27].

The release of ALP into the circulation from the liver as a result of cell destruction is unlikely, as S-ALT, S-AST and hepatic biopsy samples did not indicate evidence of hepatic disease[7, 28].

2. Increased Production of ALP Triggered by the Increased Conversion of Vitamin 25-OH-D to 1,25 $(OH)_2$ Vitamin D in Children with Previous Failure to Thrive

This hypothesis is based upon seasonal clustering of THI described by some authors, and upon an observed relationship between changes in body weight and serum concentrations of vitamin D metabolites[19]. Weight loss in adults has been reported to be associated with decreases in concentrations of 1,25 $(OH)_2$ vitamin D (calcitriol) and conversely, weight gain is associated with increases

in serum calcitriol levels[40]. In addition, 1,25 $(OH)_2$ vitamin D stimulates the synthesis of ALP in bone and possibly also in the liver[19, 41]. Therefore, it has been speculated that a period of weight loss (for various reasons) during the summer, when 25-OH vitamin D levels are relatively high, might block conversion to 1,25 $(OH)_2$ vitamin D, leading to further accumulation of 25-OH vitamin D. Then, as catch-up growth occurs, the block may be lifted. The accumulated 25-OH vitamin D is converted to 1,25 $(OH)_2$ vitamin D, causing a surge in this metabolite that stimulates synthesis of ALP[19]. However, seasonal clustering of THI, which is one of the fundamental points of this hypothesis, has not been universally confirmed[12].

3. Increased Activation of Circulating ALP

The activation of circulating enzymes is quite improbable in view of the unusual isoenzyme pattern[7].

4. Impaired Clearence of ALP from the Circulation

This hypothesis is based upon experimental findings in mice infected with Riley virus, resulting in impaired clearance of certain enzymes from the circulation by the reticuloendothelial system[42]. Alkaline phosphatase, like other glycoproteins, is cleared from the circulation by hepatocyte uptake, and removal of the sialic acid residues is probably required prior to the clearance[19, 43]. Virus-induced sialylation of the liver and bone isoenzymes with a consequent decrease in uptake and clearance might lead to high concentrations of the ALP molecules in serum[7, 19]. The findings of atypical anodal-migrating ALP isoenzyme and signs of viral infection preceding the THI support this hypothesis. The impaired clearance of ALP from the circulation is still considered as the most likely mechanism of THI, which may be in combination with the mechanisms mentioned above in items 1 and 2[7, 19]. The pathogenesis of THI is thus most probably a multifactorial one.

Differential Diagnosis of THI

Once the grossly increased S-ALP is encountered, it is necessary to rule out other, more serious reasons of hyperphosphatasemia, in particular metabolic bone diseases, bone tumors or hepatopathy. It is important for the clinician to consider THI in the differential diagnosis and to avoid unnecessary investigation[44].

1. Metabolic Bone Disease

In either vitamin D-deficient rickets or vitamin D-dependent rickets type I or II, the increased S-ALP is accompanied by low-to-normal calcemia, hypophosphatemia, hyperphosphaturia and secondary hyperparathyroidism. In X-linked hypophosphatemia (vitamin D-resistant rickets), there is hypophosphatemia and

hyperphosphaturia, while serum concentrations of calcium and parathyroid hormone (S-PTH) are within reference ranges. In all types of rickets, rachitic changes are apparent on the radiograph of wrist.

Familial benign hyperphosphatasemia of autosomal dominant inheritance is characterized by increased S-ALP, predominantly of intestinal, bone and liver origin, with no biochemical nor skeletal abnormalities[45, 46].

Persistent non-familial asymptomatic hyperphosphatasemia occurs in both children and adults. The increased S-ALP is of skeletal origin and persists for six to 10 years. No other abnormalities are reported[47].

Primary idiopathic hyperphosphatasemia (juvenile Paget's disease) is an autosomal recessive disorder characterized by severe skeletal deformities, dwarfism, premature loss of teeth, high S-ALP and increased urinary hydroxyproline excretion[48, 49].

Jansen type of metaphysial dysplasia is caused by a constitutively active mutant receptor for parathyroid hormone (PTH), resulting in low-to-normal S-PTH, hypercalcemia, hypophosphatemia, hypercalciuria and high S-ALP. The children manifest with growth disturbance, short limbs and progressive metaphysial changes, initially reminiscent of rickets[50, 51].

Primary hyperparathyroidism is extremely rare in childhood, and is characterized by hypercalcemia, hypophosphatemia, hyperphosphatasemia, hyperphosphaturia, increased serum concentrations of PTH and subperiosteal lesions on the radiograph of the hand[52].

In renal osteodystrophy, hyperphosphatasemia is a result of secondary hyperparathyroidism and is present together with hypocalcemia, hyperphosphatemia and high serum creatinine levels[53].

Infantile cortical hyperostosis (Caffey's disease) occurs in infants under three months of age and is characterized by febrile course with marked swelling of soft tissues over the face and jaws together with progressive cortical thickening of long and flat bones. The S-ALP is midly elevated[54].

2. Bone Tumors

Increased S-ALP is a biochemical feature of osteosarcoma; however, this tumor occurs in patients over 10 years of age[55].

3. Hepatopathy

Increased total S-ALP with normal serum levels of bilirubin and normal transaminases (S-AST, ALT) activity is not indicative of hepatic disorders.

Management of Children with THI

Transient hyperphosphatasemia is generally considered a benign disorders. In children under five years of age with S-ALP exceeding five times the adult upper reference limit and no other apparent signs of bone or hepatic disease, the

diagnosis of THI should be taken into consideration. In 1988, Schonau et al.[56] proposed an algorithm for effective management of children with THI. One of the leading indices is electrophoretic evaluation of the ALP isoenzymes, as an unusual isoenzyme pattern is the most characteristic sign of THI. Unfortunately, electrophoretic evaluation of the ALP isoenzymes is not a universally available method. In such cases, the assessment of other relevant indices is necessary, together with the radiograph of the wrist. If basic biochemical markers (S-Ca, P, creatinine, ALT, AST) and radiograph of the wrist are normal, THI is the most likely diagnosis. Therefore, children with TH should be spared from further diagnostic procedures (such as ^{99}Tc bone scans, repeated radiographs and blood draws), and the S-ALP can be reassessed after two to three months.

On rare occassions THI might interfere with rickets. There is no causal relationship between those two conditions[18, 21]. Transient hyperphosphatasemia might occur or persist after the healing of rickets; it is the electrophoretic evaluation of S-ALP and the radiograph of the wrist which are helpful. A control x-ray of the wrist is required to confirm the healing of rickets. The abnormal isoform pattern obtained by the electrophoresis of ALP, or signs of healing or healed rickets in cases of THI following vitamin D-deficient rickets, can spare the children from unnecessary vitamin D overdosage[21].

Prognosis

As THI has been related to transiently increased bone resorption and formation[26, 27], questions have been raised about the further development and prognosis of children with a history of TH[7, 26]. Of particular concern was its postulated viral origin and thus a suggested parallel to Paget's disease[5, 7, 26]. Therefore, a follow-up of children with TH was recommended[7, 26]. It seems likely that any sharp increase in bone turnover during infancy and early childhood would influence bone density and somatic growth. However, in our recent work we did not observe any signs of stunted growth or failure to thrive during one to 102 months of follow-up of 40 children with a history of TH or with 29 children followed up for 12-102 months[57]. Neither was there a recurrence of THI: S-ALP was assessed in 12 of those children, six to 60 months after the last detection of THI, and was always found to be within reference ranges, together with normal S-AST and S-ALT values. Bone density, as reflected by velocity of ultrasound and broad band ultrasound attenuation of both heel bones, in seven children with a history of TH, was within reference ranges[57]. These data further suggest that THI is a benign state with good prognosis in a time horizon of at least eight years.

Conclusion

Transient hyperphosphatasemia of infancy and early childhood is a benign disorder, a biochemical rather than a clinical abnormality, of multifactorial

pathogenesis and transient character, with good prognosis. Children with TH should be spared from extensive investigations and unnecessary vitamin D applications.

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The Turkish Journal of Pediatrics 1999; 41: 161-172 *Original*

PRIMARY INTRACRANIAL GERM CELL TUMORS IN CHILDREN*

A Report of Eight Cases and Review of the Literature

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SUMMARY: Akyüz C, Köseoğlu V, Bertan V, Söylemezoğlu F, Kutluk MT, Büyükpamukçu M. (Department of Pediatric Oncology, Hacettepe University Institute of Oncology, Ankara, Turkey). Primary intracranial germ cell tumors in children: a report of eight cases and review of the literature. Turk J Pediatr 1999; 41: 161-172.

This study was conducted to evaluate the signs and symptoms on admission, diagnosis, localization, therapy, and survival of patients with primary intracranial germ cell tumors (PICGCT). Eight patients with surgically confirmed PICGCTs were treated and followed up at Hacettepe University's Department of Pediatric Oncology between 1974 and 1995.

While one patient was admitted with a second recurrence of her disease, the others were admitted or referred primarily to our institution. In this period, 357 germ cell tumor and 684 primary intracranial malignant tumors were diagnosed and treated at our institution. Thus, PICGCTs comprised 1.1 percent of the primary intracranial malignant tumors and 2.2 percent of the germ cell tumors. There were four females and four males and the median age was eight years (13 months to 12 years). On admission, the most common symptoms were diabetes insipidus (3/8) and vomiting (3/8). One patient also and Down's syndrome. Locations of the tumors were suprasellar in three, in the third ventricle in two, and in the cerebral parenchyma, and pineal and hypothalamic regions in the remainder. There were three germinomas, three malignant teratomas, and two mixed germ cell tumors. Only two patients could be treated with appropriate and adequate chemotherapy and radiotherapy. Three patients died: one in the postsurgical period, one after the third surgical approach and one 11 months after the diagnosis of progressive disease; three were lost to follow-up. The remaining two patients (with second recurrence and disseminated disease) are alive and without disease.

Our experience with these patients demonstrated that appropriate and adequate chemotherapy is as effective a treatment as radiotherapy, even with recurrence of the disease. *Key words: intracranial, germ cell, tumor, children.*

Primary intracranial germ cell tumors (PICGCTs) are very rare heterogeneous neoplasms which more often arise in the suprasellar and pineal regions of the brain, and account for less than five percent of all primary central nervous system tumors of childhood[1,2]. They are more frequently encountered in children than adults and generally become clinically apparent during the second decade of life[1,3]. They are histologically indistinguishable from germ cell tumors of gonads,

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so their histological subtypes such as gonadal germ cell tumors include: germinoma, teratoma, embryonal carcinoma, endodermal sinus tumor and choriocarcinoma. They may secrete substances such as alpha-fetoprotein (αFP) or a beta subunit of human chorionic gonadotropin (βHCG)[1,4].

It has been widely accepted that germinomas should be treated by radiotherapy, mature teratomas by surgical resection and the other malignant tumors by multimodality procedures including surgery, radiotherapy and chemotherapy[5]. However, central nervous system radiation in children has harmful side effects; consequently, other therapy modalities need to be evaluated[1]. A number of chemotherapeutic agents have been used for the treatment of intracranial germ cell tumors and chemotherapy has proved effective in recurrent germinomas[5-7]. The low incidence of PICGCTs makes it difficult for any center to acquire sufficient material for treatment modality and long-term follow-up evaluation[8]. For this reason, we report clinical features and treatment results for eight patients with PICGCTs.

Material and Methods

During the period 1974 to 1995, eight children with PICGCT were treated and followed up at Hacettepe University's Department of Pediatric Oncology in Ankara. Their clinical data, including patient age, sex, location of tumor, histopathological substype, surgical approach and treatment response were reviewed from the medical records.

Case Reports

Case 1

An eight-year-old girl was admitted with ptosis, mydriasis and poor vision in her left eye. Her medical records showed that she had been followed by the Pediatric Endocrinology and Genetic Units for diabetes insipidus and Down's syndrome. On physical examination, signs of Down's syndrome, left ptosis and pupillary dilation were found. Cranial computed tomography (CT) showed a suprasellar mass but other laboratory findings were normal. A biopsy was performed and histology revealed a malignant teratoma. Her parents did not accept any treatment and follow-up was discontinued.

Case 2

A 12-year-old boy was admitted with complaints of headache, vomiting, diplopia and gait abnormality. He was unconscious on his physical examination. Computed tomography of the head demonstrated a huge tumor in the third ventricle. An emergency surgical approach was carried out and partial resection was performed. Twenty-five days later, his cranial CT showed progression of the tumor, his disease progressed and he died on the 30th postoperative day. Histopathological examination of the resected tumor specimen revealed a mixed germ cell tumor.

Case 3

An eight-year-old boy who had been followed by the Pediatric Endocrinology Unit with the diagnosis of precocious puberty was referred to our department after a tumor in the third ventricle was demonstrated on his cranial CT. On admission, his physical findings showed signs of precocious puberty, bilateral grade I papilledema, 6^{th} and 7^{th} cranial nerve palsies, hyperreflexia, weakness of lower extremities, and unilateral Babinski positivity. The tumor was totally removed surgically and its histopathology revealed malignant teratoma. After the diagnosis, chemotherapy (vincristine 1 mg/m^2, procarbazine 50 mg/m^2, CCNU 100 mg/m^2) and radiotherapy were started. In the fifth month of his treatment, the tumor recurred and was again totally removed. Chemotherapy was then changed to cisplatin, vinblastine, and bleomycin (PVB) protocol. After three courses of this protocol, a good response was obtained, but at the end of the 5^{th} course, his disease progressed and he died in the eleventh month after the diagnosis.

Case 4

A seven-year-old boy was referred to our institution for evaluation of a mass located in the left hemisphere of his brain. Complaints were right hemiparesis, strabismus, diminished speech function, vomiting, headache, and seizures. Computed tomography showed a large mass located in the left hemisphere of the brain. On admission, he was unconscious. Craniotomy and total resection were done. Pathologic examination showed a malignant teratoma. His parents did not accept any treatment and the patient was discharged.

Case 5

An 11-year-old girl was referred to our institution for evaluation of her polyuria, polydipsia, diplopia and a suprasellar mass which had been demonstrated on her cranial CT. On admission her physical examination was normal. At that time, her computed tomography and magnetic resonance imaging (MRI) showed a huge suprasellar mass. Alpha-fetoprotein, βHCG and other laboratory findings were also normal. Subtotal resection was performed and histopathology revealed a germinoma. After the diagnosis, cranial and spinal radiotherapy was given at 4500 cGy and 3000 cGy, respectively. After six months, her MRI displayed residual disease. A chemotherapy protocol (BEP) was started and she was given two courses. She then refused the remaining chemotherapy courses.

Case 6

A 13-month-old girl was referred to our department with the diagnosis of intracranial hemorrhage. A pineal tumor with a hemorrhagic component was found on her cranial MRI. Cranial CT revealed the same tumor and hydrocephalus.

Alpha fetoprotein and βHCG of serum were normal. Craniotomy and partial resection of the tumor were performed and a shunt inserted. Histopathology of the tumor revealed a mixed germ cell tumor. On the 20th day following the first surgery, enlargement of the tumor was seen. A second surgical approach was done and the tumor was subtotaly resected again. While under observation in the intensive care unit, the patient became unconscious. She underwent a third surgical procedure, after which she died due to the progressive disease.

Case 7

A nine-year-old girl was admitted to another hospital in 1993 with complaints of left ptosis and mydriasis. At that time, her physical examination was normal except for left ptosis and mydriasis. Cranial MRI showed a suprasellar mass 3x2 cm in diameter (Fig. 1). She underwent surgery, and the tumor was totally

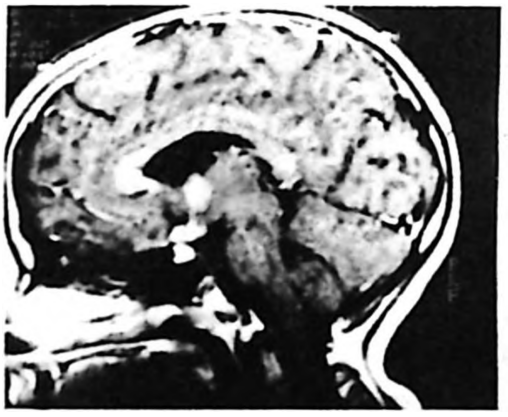

Fig. 1: MRI of the brain on admission demonstrating a suprasellar mass (Case 7).

removed. Histopathology of the tumor specimen revealed germinoma. Her disease relapsed after three months, a second operation was done and partial tumor resection was performed. Postoperatively, craniospinal (3000 cGy, 2500 cGy) radiotherapy was given, in addition to localization of the tumor (4920 cGy). Four months later, in May 1995, she was admitted to our institution with abdominal pain and vomiting. On admission, hepatomegaly, left ptosis and mydriasis were found on physical examination. Plain x-ray of the chest displayed nodular appearance on parenchyma of both lungs. Abdominal ultrasonography showed masses 8x7x8 cm and 11x10 cm in diameter in the right and left lobes of the liver, respectively, and abdominal tomography showed metastatic lesions in the parenchyma of the liver (Fig. 2). Cranial MRI showed a residual mass in the suprasellar region, and chest tomography displayed lymphadenopathy and diffuse parenchymal metastases. In the laboratory investigations, βHCG level was higher than the normal limits (730 mlu/ml). An ultrasonography-guided biopsy from the liver was performed. Histopathology revealed metastases of the germinoma. She was then treated with eleven courses of cisplatin, etoposide,

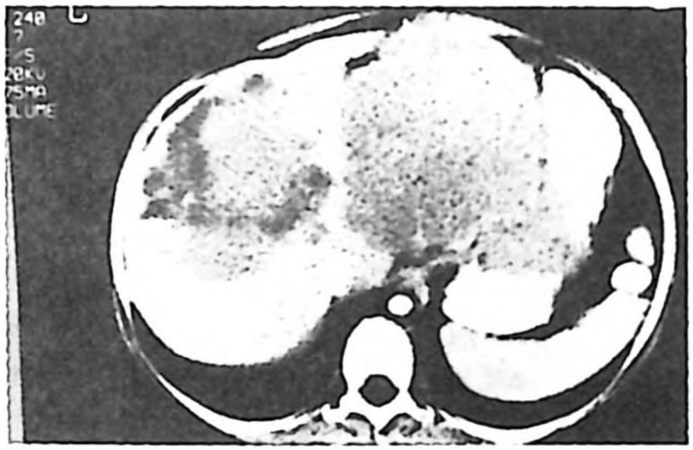

Fig. 2: An abdominal CT scan obtained on admission shows multiple metastatic lesions seen in the liver (Case 7).

and bleomycin (BEP) protocol. At the end of the treatment courses, no liver metastases were found (Fig. 3), and no residual mass was found on primary site (Fig. 4). There was regression of the parenchymal metastases of the lung.

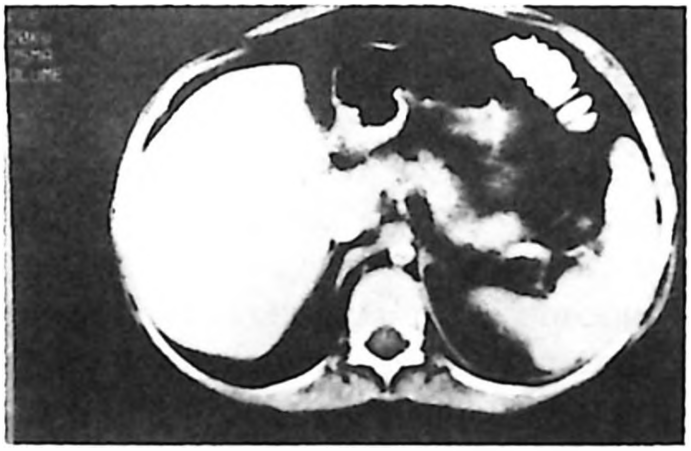

Fig. 3: A CT scan of the abdomen after chemotherapy showing no metastatic lesions of liver (Case 7).

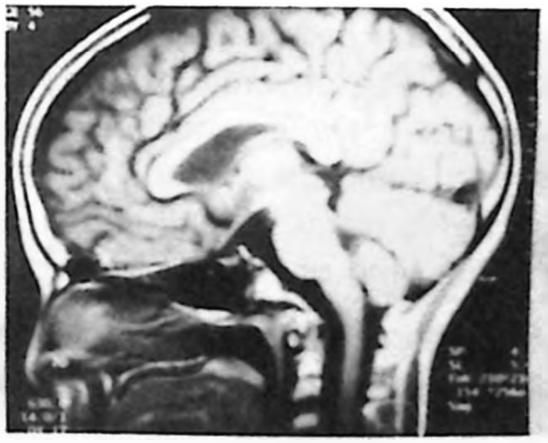

Fig. 4: MRI of the brain after chemotherapy shows no suprasellar mass (Case 7).

Thoracotomy was performed and one nodule was removed. Histopathology did not show any abnormality related to the primary disease. At this moment, she is alive without disease at after 20 months of relapse time.

Case 8

A ten-year-old boy was admitted to another hospital with polyuria and polydipsia. At that time, his physical examination was normal. His cranial MRI showed a mass in the hypothalamic region. He had been followed by the Pediatric Endocrinology Unit after the first admission. Nine months later, he underwent surgery and the mass was partially removed.

Histopathology of the resected specimens revealed a germinoma. After this diagnosis, cranial radiotherapy was given (4400 cGy). He was admitted to our institution following radiotherapy. On admission, his physical examination showed no abnormality. His cranial MRI revealed a mass in the hypothalamic region. BEP protocol was started and five courses were given. The decision was made to stop at the end of five courses of chemotherapy because the cisplatin was causing a decrease in the renal creatinine clearance. Eighteen months after diagnosis, he is alive and free of disease. He has no diminished renal function at this time.

Results

From 1974 to 1995, 357 children with germ cell tumors were treated at the Department of Pediatric Oncology, Hacettepe University. Eight of 357 germ cell tumor cases were diagnosed in an intracranial site. In the same period, 684 children with primary intracranial malignant tumors were diagnosed and treated. Thus, primary intracranial germ cell tumors comprised 2.2 percent of the germ cell tumors and 1.1 percent of the primary intracranial malignant tumors managed and treated in this single institution.

The age range of patients reviewed in this paper (Table I) was 13 months to 12 years, with a median age of eight years. Sex distribution was equal (4 male and 4 female). Of the cases in this group, three had been admitted with diabetes insipidus symptoms; one of these patients also had Down's syndrome. Three had vomiting, two had headache, two had multiple oculomotor palsies, one had visual disturbance, one had diminished speech function, one had a walking disorder, one had cranial nerve palsy, one had hemiplegia, and one had signs of precocious puberty at the time of diagnosis (Table II).

Alpa-fetoprotein and beta-human chorionic gonadotropin could not be measured in four patients. One of the remaining patients had an elevated serum beta-human chorionic gonadotropin level at the time of recurrence, which decreased gradually with chemotherapy and returned to normal limits (Case 7).

Table I: Clinical and Histopathological Findings of Patients with Primary Intracranial Germ Cell Tumor

Case No.	Age at Diagnosis	Sex	Localization	Surgery	Histopathology	Radiotherapy	Chemotherapy	Situation
1	8 years	F#	Suprasellar	Biopsy	Malignant teratoma	–	–	Lost to follow-up
2	12 years	M	Third ventricle	Partial resection	Mixed germ cell tumor	–	–	Died
3	8 years	M	Third ventricle	Total resection	Malignant teratoma	+	+	Relapsed Died
4	7 years	M	Left hemisphere	Total resection	Malignant teratoma	–	–	Lost to follow-up
5	11 years	F	Suprasellar	Partial resection	Germinoma	+	+	Lost to follow-up
6	13 months	F	Pineal	Partial resection*	Mixed germ cell tumor	–	–	Died
7	9 years	F	Suprasellar	Total resection*	Germinoma	+	+	Alive
8	10 years	M	Hypothalamic	Partial resection	Germinoma	+	+	Alive

* At first surgical approach.
\# Down's syndrome.

Table II: Demographic and Clinical Findings of Patients

Age (average)	8 Years (13 Months-12 Years)
Sex (M/F)	4/4 (1/1)
Complaints and physical examination findings	Number of cases
Cranial nerve palsies	4
Diabetes insipidus	3
Vomiting	3
Headache	2
Visual disturbance	2
Speech disorder	1
Precocious puberty	1
Walking disorder	1
Hemiparesis	1
Localization of the tumors	
Suprasellar	3
Third ventricle	2
Cerebral parenchyma	1
Pineal	1
Hypothalamic	1
Surgery	
Total resection	3
Partial resection	4
Biopsy	1
Histopathology	
Germinoma	3
Malignant teratoma	3
Mixed germ cell tumor	2

Locations of the tumors were different. Of the eight patients, three were suprasellar, two were in the third ventricle and the remainder were in the cerebral parenchyma, and pineal and hypothalamic regions. A tissue diagnosis of the tumor was made during surgery in these patients. Three total resections, four partial resections and one biopsy were performed in these cases. These tumors included three germinomas, three malignant teratomas and two mixed germ cell tumors.

Only two patients could be treated with appropriate and adequate treatment protocol in this group (Case 7 and 8). One patient received PVB protocol after the first recurrence of his disease. Two of eight patients are alive, three died, and the others were lost to follow-up.

Discussion

The incidence of primary intracranial germ cell tumors varies geographically and is distinctly higher in childhood than during adult life[9]. In a childhood series in Japan, it accounted for 4.8-15 percent of the primary intracranial neoplasms, which is much higher than the 0.3-3.4 percent reported in a western series[3,4]. Although the Japanase series showed increased incidence, the reason for this is not known[4,5,9]. The incidence was 1.1 percent in our series, lower than that of the Japanese series but similar to findings of the western series. Primary intracranial germ cell tumors tend to demonstrate male predominance in the literature[3,9]. In our series there was no distinction by sex (Table III). Reports of intracranial germ cell tumors have included newborn patients and elderly patients; 68 percent of the patients are between 10 and 21 years of age[9]. The age distribution of our cases agreed with previous reports; the median age was eight years[2,3,9]. To our knowledge there is no reported association between primary intracranial germ cell tumors and chromosomal disorders. However, two patients with primary intracranial germ cell tumors and Down's syndrome were reported separately by Fujita et al.[10] and Wada et al.[11] Our case with Down's syndrome (Case 1) is apparently only the third case in English literature with this association.

Clinical symptomatology varies and depends on the localization of the tumor. The common signs for patients with suprasellar germ cell tumors are chiefly endocrinologic manifestations including diabetes insipidus, hypopituitarism and visual deficit, while the symptoms for germcell tumors located in the pineal region are mainly due to increased intracranial pressure[12]. Growth retardation, precocious puberty and diabetes insipidus with no detectable etiology have been reported as initial symptoms[13-16]. It has been stated that follow-up of children with diabetes insipidus is important for determining whether or not it is idiopathic[17]. In our series, three cases were admitted with diabetes insipidus symptoms and one of these patients also had Down's syndrome. One boy had signs of precocious puberty at the time of diagnosis (Case 3). Precocious puberty is common in boys with hCG-secreting brain tumors, but it is extremely rare in

girls[15]. Signs and symptoms related to precocious puberty usually disappear with regression of the tumor following appropriate treatment[14]. In our case, we could not obtain any improvement because of treatment failure and recurrence of the disease. Vomiting, headache, diplopia and visual disturbance were the other frequent signs on admission.

Table III: Review of the Literature of Primary Intracranial Germ Cell Tumors

Authors	No of Cases	M/F Ratio	Age (Average)	Histopathology	Localization	Ref.
Ho et al.	51	2.6/1	13	30 germinoma 8 teratoma 5 EST 2 CC 6 MGCT 1P+S	17 pineal 14 suprasellar 10BG+T 5 lateral vent. 1 pituitary	9
Yoshida et al.	46	2.0/1	16	19 germinoma 13 EC 2 CC 4 teratoma 8 MGCT	28 pineal 11 suprasellar 4 BG 3 multiple	5
Hoffman et al.	51	1.8/1	10	34 germinoma 7 teratoma 4 EST 2 CC 4 MGCT	32 pineal 16 suprasellar	3
Ono et al.	79	3.0/1	14.2	50 germinoma 5 teratoma 7 extra-embryonic	–	21
Plantaz et al.	35	1.9/1	12	14 germinoma 6 NGGCT 5 P+S	15 pineal 15 suprasellar	22
Kiltie et al.	25	2.5/1	10	10 germinoma 9 NGGCT 6 no histology	14 pineal 9 S+Pt+3rd V 2 P+Pt+S	2
Wolden et al.	48	3.0/1	16	24 germinoma 3 MT 2 CC 1 EC 1 EST 3 MGCT		23
Present study	8	1/1	8	3 germimoma 3 MT 2 MGCT 1 pineal 1 hypothalamic	3 suprasellar 2 third ventricle 1 CP	–

Abb: MT: malignant teratoma, CC: choriocarcinoma, EC: embryonal carcinoma, EST: endodermal sinus tumor, MGCT: mixed germ cell tumor, NGGCT: non-germinomatous germ cell tumor, BG+T: basal ganglia+thalamus, P+S: pineal+suprasellar, BG: basal ganglia, S+Pt+3rd V: suprasellar+pituitary+third ventricle, P+Pt+S: pineal+pituitary+suprasellar, CP: cerebral parenchyma

Diagnosis of primary intracranial germ cell tumors is suggested by the findings on computed tomography and magnetic resonance imaging of the brain[12, 18]; however histopathological examination of the tumor tissue remains necessary for exact diagnosis. Alpha-fetoprotein and beta-human chorionic gonadotropin are useful tools when elevated both for diagnosis and monitoring the results of treatment[3, 8, 19]. They should be measured routinely in both serum and cerebrospinal fluid[3]. In our series, one patient had an elevated serum beta-human chorionic gonadotropin level at the time of recurrence, which decreased gradually and returned to normal limits at the end of therapy.

Primary intracranial germ cell tumors are complex lesions and little is known of their origin[4]. They are thought to derive from trapped germ cells which have failed to migrate. This migration defect of germ cells may explain the occurrence of the tumors in the midline of the brain[4]. Although basal ganlia, fourth and third ventricle, medulla oblongata and both suprasellar and pineal region occurrences are seen, they are generally situated in either the suprasellar or pineal region[9]. In our patients, location of the tumors were different. Of the eight patients, three were suprasellar, two were in the third ventricle and the remainder were in the cerebral parenchyma and pineal and hypothalamic regions. Although the locations of the germ cell tumors in our patients were similar to other studies, to our knowledge, this is the first reported case in English literature of a primary intracranial germ cell tumor located in the cerebral parenchyma.

Because of differences in radiosensitivity among primary intracranial germ cell tumors, histopathological confirmation of the diagnosis and identification of mixed components are essential for optimal treatment and determination of prognosis. For this reason, tissue diagnosis is of critical importance in dealing with primary intracranial germ cell tumors; a stereotactic biopsy or surgical resection should be performed before deciding to schedule treatment[8]. On the other hand, surgical removal may help to obtain better response to local radiotherapy[3]. In our cases, of the eight patients with primary intracranial germ cell tumors, three underwent total resection, four a partial resection and one a biopsy. Histopathological examination showed three germinomas, three malignant teratomas, and two mixed germ cell tumors. These findings were similar to previous reports.

Primary intracranial germ cell tumors are usually treated differently depending on their histopathological subtypes[2, 5]. For germinomas whole brain or whole CNS radiation therapy has been given after the diagnosis is made by surgical biopsy and by characteristic neuroradiological findings on CT and/or MRI. Recurrence rates at five years for completely resected tumors range from 10 to 40 percent in several published reports. Postoperative radiation therapy for all patients with large completely resected germinomas is recommended[1]. However, CNS radiation in children has devastating side effects. Consequently,

new approaches with high remission rate and without any sequelae are needed to treat these tumors. In the past, systemic administration of chemotherapeutic agents has played a limited role in the management of primary or metastatic brain tumors. A variety of agents have been used to treat primary intracranial germ cell tumors. Recently, chemotherapy regimens such as PVB, VP-16/CDDP, etc., which have been demonstrated to be effective in de novo and recurrent primary intracranial germ cell tumors, have been suggested for primary treatment instead of radiotherapy[1, 6, 7, 20]. In our series, two cases, one of them with recurrent disease, were treated effectively using the BEP protocol.

Although primary intracranial germ cell tumors are rare in childhood, they can be treated by appropriate treatment modalities. Our experiences and the data reported previously indicate that chemotherapy, especially BEP protocol, seems to be more effective, even in recurrent disease. It can be used as a first choice of treatment schedule, thus protecting patients from the harmful side effects of radiotherapy. Patients with Down's syndrome and diabetes insipidus with or without Down's syndrome need to be followed in order to determine whether or not they will develop intracranial germ cell tumors.

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ASSOCIATED BRAIN ABNORMALITIES IN PATIENTS WITH CORPUS CALLOSUM ANOMALIES*

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SUMMARY: Tekgül H, Dizdarer G, Yalman O, Şener N, Yünten N, Tütüncüoğlu S. (Neurology Unit, Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey). Associated brain abnormalities in patients with corpus callosum anomalies. Turk J Pediatr 1999; 41: 173-180.

Forty-nine patients with corpus callosum (CC) anomalies were evaluated in terms of the clinical features and magnetic resonance imaging (MRI) findings. CC anomalies were classified as CC agenesis: 6 (12%), CC hypogenesis: 5 (10%), and CC hypoplasia: 38 (78%). In the CC hypoplasia group the mean value of the genu thickness of the CC was 0.29 ± 0.1 cm, which was less than the normal value of the age-matched normal children (normal range: 0.6-1.2 cm). The associated brain abnormalities were in five distinct groups: gray matter abnormalities, white matter abnormalities, midline brain structure defects, cortical atrophy, and encephalomalacia. There was no uniformity for the clinical spectrum of CC anomalies. Microcephaly, developmental delay and seizures were the prominent findings in patients. The clinical features were more severe in cases with associated brain anomalies. *Key words: corpus callosum, magnetic resonance imaging, brain abnormalities.*

The corpus callosum (CC), a major associative pathway between the cerebral hemispheres, develops between 10-20 weeks of gestation. At the end of ninth months of infancy it has a similar appearance to that in adults. Any insult to the brain during this period of gestation results in an abnormality of the CC. Infectious agents, radiation, chemical agents, maternal hormones, nutritional deficiencies, hypoxia, and chromosomal and genetic factors have all been considered in the etiology of CC anomalies[1-5].

Cranial computed tomography (CT) and magnetic resonance imaging (MRI) have been used to evaluate the anomalies of the CC[6-15], but MRI provides better contrast resolution and the multiplanar imaging that facilitates the definition of these anomalies. Recently CC anomalies were classified by Jinkins et al.[8] as: 1) CC agenesis (a-initiative agenesis, b-obstructive agenesis), 2) CC hypogenesis-partial agenesis (a-interruptive agenesis, b-obstructive agenesis), and 3) CC hypoplasia.

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Although isolated anomalies of the CC may not cause significant functional deficits, severe neurological deficits have been reported in patients also having other cerebral anomalies[1, 8, 13]. In this study we evaluated the clinical features and associated brain abnormalities in patients with corpus callosum anomalies.

Material and Methods

The MRI scans of 328 patients followed up as Pediatric Neurology outpatients between 1991-1996 reevaluated. Each MRI image utilizing T1 and T2-weighted spin-echo technique in sagittal, axial and/or coronal planes was reviewed. Corpus callosum CC anomalies were classified as agenesis, hypogenesis or hypoplasia based on the presence or absence of the corpus callosum portions (genu, body, splenium, and rostrum). The corpus callosum CC was totally absent in patients with agenesis and was partially absent in patients with hypogenesis. In the CC hypoplasia group, the patients had a diminished width of the CC after its complete formation.

Morphometric measurements of the CC were also made on the midsagittal MRI scans of 38 patients with CC hypoplasia. The genu thickness was measured at a point which fits 1/3 of the anterioposterior of the CC (Fig. 1). Other orthogonal images were also evaluated for additional information about the rest of the brain. Magnetic resonance imaging MRI findings and clinical features of the patients were evaluated.

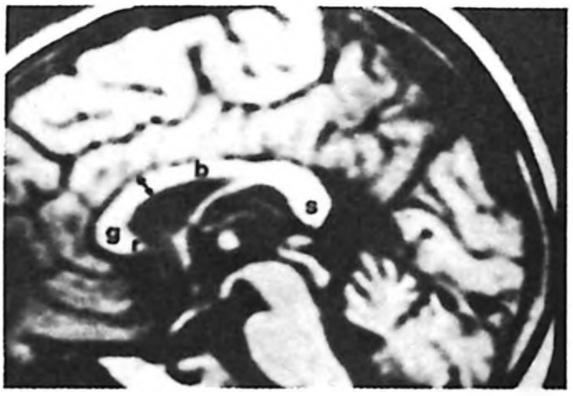

Fig. 1: Measurement of the thickness of genu at the anterioposterior length of the CC. T1-weighted sagittal image shows normal appearance of CC. r: rostrum, g: genu, b: body, s: splenium.

Results

According to MRI scans, CC anomalies were found in 49 of the 328 patients (15%). The corpus callosum was totally absent in six patients (12%) and partial agenesis was evident in five patients (10%) (Figs. 2, 3). Hypoplasia of the CC was found in 38 patients (78%) (Fig. 4) (Table I). Isolated agenesis of the CC was found in only three patients.

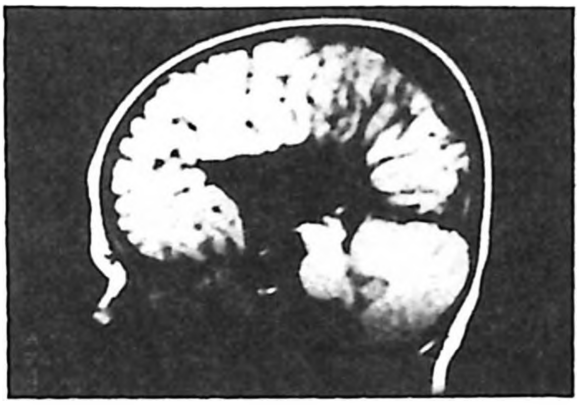

Fig. 2: T1-weighted sagittal image shows absence of the CC.

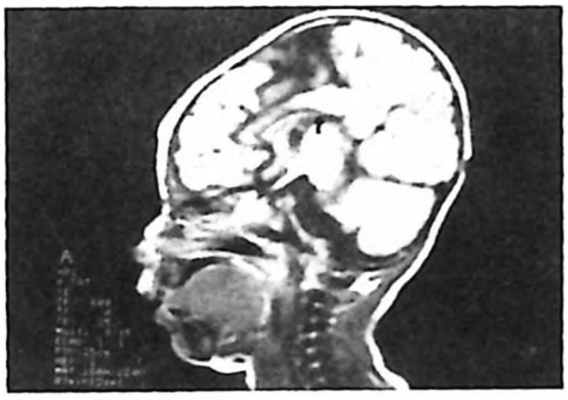

Fig. 3: T1-weighted sagittal image shows severe callosal thinning anteriorly and its absence posteriorly (hypogenesis). Note that the cingulate gyrus overlying the thin callosal structure, falsely appears as a normal corpus callosum.

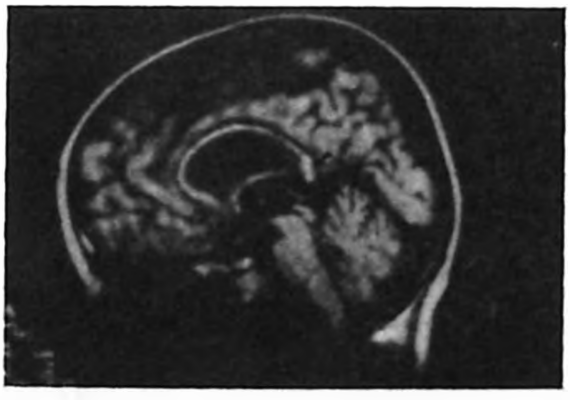

Fig. 4: T1-weighted midsagittal image shows diffusely thinned corpus callosum (hypoplasia). Note the junction of the body and the splenium shows an angle about 90°, creating an hammer appearence indicated by arrow.

In the CC hypoplasia group, genu thickness of the CC was measured at a point which fits 1/3 of the anterioposterior of the CC. The value for each patient was less than the normal range values obtained in the similar age group of Turkish

children, as reported by Oyar et al.[16] (normal range: 0.6-1.2 cm for 1-6 years of age). The mean value of the genu thickness of the CC was 0.29 ± 0.1 cm in patients with CC hypoplasia (Table I).

Table I: Clinical Features of Patients with CC Anomalies

	Agenesis of CC n = 6 (12%)	Hypogenesis of CC n = 5 (10%)	Hypoplasia of CC n = 38 (78%)
Genu thickness of CC (cm)			0.29 ± 0.1
Age (mean: month)	71 ± 29	26 ± 11	50 ± 18
Gender M, F	3, 3	4, 1	24, 14
Parental consanguinity	3 (50%)	4 (80%)	6 (16%)
Asphyxia	1 (17%)	1 (20%)	12 (32%)
Microcephaly	1 (17%)	2 (40%)	17 (45%)
Developmental delay	3 (50%)	4 (80%)	28 (74%)
Seizures	3 (50%)	2 (40%)	23 (61%)
Spasticity	2 (34%)	2 (40%)	11 (35%)
Hypotonia	1 (17%)	1 (20%)	7 (21%)
Optic atrophy	1 (17%)	–	4 (10%)

CC: corpus callosum.

Parental consanguinity was recorded in 80 percent 33 percent and 16 percent of the patients with CC hypogenesis, CC agenesis, and CC hypoplasia, respectively. Perinatal asphyxia, neonatal sepsis and maternal x-ray exposure were found in 43 percent of patients as an insult to the developing brain. Perinatal asphyxia was described in one-third of the patients with CC hypoplasia.

The associated abnormalities in CC anomalies were in five distinct groups: 1) gray matter abnormalities, 2) white matter abnormalities, 3) brain midline structure defects, 4) cortical atrophy and 5) encephalomalacia (Table II). In gray matter abnormalities, neuronal migrational anomalies were found in 10 patients with either CC agenesis or CC hypoplasia pachygyria: 8, schizencephaly: 1, gray matter heterotopia: 1) (Fig. 5). But these associated abnormalities were not found in the group with CC hypogenesis. White matter abnormalities were seen only in patients with CC hypoplasia (periventricular leukomalacia [PVL]: 10, delayed myelination: 6). The defects of the brain midline structures were septo-optic dysplasia (SOD), agenesis of cerebellar vermis, pons hypoplasia, aqueductus stenosis and Arnold-Chiari malformation. Septo-optic dysplasia SOD was found in only CC agenesis and hypogenesis groups. Cortical atrophy and encephalomalacia were frequently observed in patients with CC hypoplasia. Cortical atrophy and encephalomalacia showing diffuse cerebral insult were present in 20/38 (51%) patients with CC hypoplasia.

Microcephaly, developmental delay and seizures were the prominent clinical features in all three groups (Table I). Other clinical findings such as spasticity, hypotonia and optic atrophy were more frequent in patients with CC hypoplasia. Although patients with associated brain abnormalities had severe neurological disabilities, three children with isolated agenesis of the CC had minimal problems in hand skills.

Table II: Associated Brain Abnormalities in CC Anomalies

	Agenesis of CC n=6	Hypogenesis of CC n=5	Hypoplasia of CC n=38
(1) Gray matter abnormalities	pachygyria: 1 schizencephaly: 1 heterotopia: 1		pachygyria: 7
(2) White matter abnormalities			PVL: 10 dm: 6
(3) Midline brain structure defects	SOD: 2 pons hypoplasia: 1 callosal lipoma: 1	SOD: 1 stenosis of aqueductus: 1 agenesis of CV: 1	agenesis of CV: 2 pons hypoplasia: 1 Chiari: 1 malformation
(4) Cortical atrophy	2	1	15
(5) Encephalomalacia		1	5

SOD: septo-optic dysplasia, PVL: periventricular leukomalacia, dm: delayed myelinization, CV: cerebellar vermis.

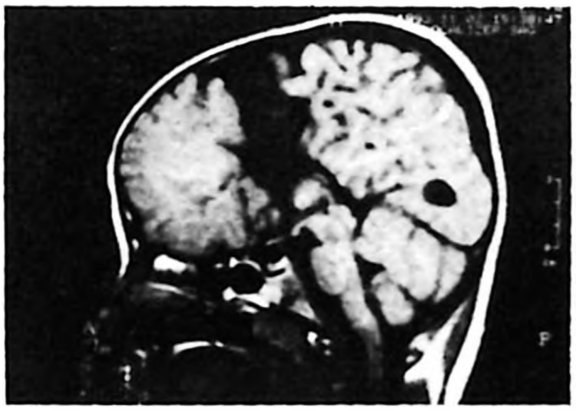

Fig. 5: T1-weighted sagittal image shows agenesis of CC and schizencephalic cleft. The presence of gray matter at the lip of the cleft differenciates the schizencephalic cleft from the interhemispheric cyst.

Discussion

During recent years CC anomalies have been reported as common congenital abnormalities of the central nervous system in childhood[1-15]. In this study we determined the rate of CC anomalies as 15 percent on the basis of MRI evaluation. This rate was higher than the rate detected by CT (2.3%)[1]. Because of the excellent sensitivity due to its superior contrast resolution and multiplanar scans, MRI is superior to CT in detecting these anomalies.

Agenesis and hypogenesis of the CC result from any insult to the developing brain between 10-20 weeks of gestation. Infectious agents, radiation, chemical agents, maternal hormones, and genetic factors have all been considered in the etiology of both primary initiative agenesis and interruptive hypogenesis[6, 8, 13]. The nature of the insult is far less important than the timing of the insult and the genetic susceptibility. In our study, parental consanguinity was more frequent in cases of patients with agenesis (50%) and hypogenesis (80%) of the CC than is present in the average Turkish population (20%). This finding indicates that genetic factors have an important role in agenesis and hypogenesis of the CC. On the other hand, perinatal asphyxia was determined as the most frequent etiologic factor in patients with CC hypoplasia (32%).

Morphometric analysis of the CC has been looked at in different studies[2, 15-19]. Hayakawa et al.[19] reported the mean thickness value (at a point which fits 1/3 of the anterior portion of the CC) as 0.62 ± 0.1 cm in the healthy population. In normal Turkish children the genu thickness was found between 0.6-1.2 cm for children one to six years of age[16]. In our study, we found that the thickness of the CC for each patient was less than the normal range.

Magnetic resonance imaging is preferred for detection of MRI CC anomalies and associated brain lesions. Barkovich and Norman[13] reported associated brain anomalies in half of the patients with CC anomalies. They also reported isolated agenesis of the corpus callosum in only one patient. In our series, isolated CC agenesis was found in three patients, with only minimal problems in hand skills. The remaining three patients in the CC agenesis group had associated brain anomalies with psychomotor retardation, seizures and tonus abnormality.

Neuronal migration anomalies are the most frequent associated gray matter abnormalities in patients with CC anomalies. Neuronal migration is a developmental period of the central nervous system that occurs between eight to 25 weeks of gestation, similar to development of the CC. A severe insult to the brain during this developmental period would explain the association of CC anomalies and neuronal migration anomalies[8, 13].

Septo-optic dysplasia (SOD) is a frequently encountered associated brain abnormality in patients with CC anomalies. Septo-optic dysplasia was SOD originally described as a pathologically distinct entity characterized by an absent or hypoplastic septum pellucidum and dysplasia of the optic chiasma and optic tracts[21]. Septo-optic dysplasia SOD can be associated with hypopituitary dwarfism. We found SOD in three patients with agenesis or hypogenesis of the CC. They had no finding suggesting pituitary insufficiency that would become overt in time.

There has been no general agreement on the clinical significance of hypoplastic CC[17, 20]. Schaefer et al.[20] reported that hypoplastic CC was not a normal variant and was usually associated with subnormal intellectual function in variable

degrees. In our study we found severe neurological findings such as microcephaly (45%), developmental delay (74%) and seizures (61%) in patients with hypoplastic CC. Spasticity, hypotonia and optic atrophy were also seen in those patients. These severe neurological disabilities may be due to the presence of associated brain abnormalities such as white matter abnormalities (PVL, delayed myelinization), cortical atrophy and encephalomalacia.

In conclusion, no characteristic clinical findings are seen in patients with CC anomalies, and the clinical features are closely related to the associated brain anomalies. The presence of a callosal anomaly strongly suggests an additional brain abnormality.

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The Turkish Journal of Pediatrics 1999; 41: 181-188 *Original*

HUMAN LEUKOCYTE ANTIGENS IN TURKISH PEDIATRIC CELIAC PATIENTS*

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SUMMARY: Erkan T, Kutlu T, Yılmaz E, Çullu F, Tümay GT. (Division of Gastroenterology, Department of Pediatrics, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey). Human leukocyte antigens in Turkish pediatric celiac patients. Turk J Pediatr 1999; 41: 181-188.

With the aim to determine the frequency of human leukocyte antigen phenotypes of celiac disease in Turkey, thirty celiac patients fulfilling the European Society of Pediatric Gastroenterology and Nutrition criteria were included in the study. The mean age of the study population was 5.8 ± 4.3 years and of the control subjects was 32.6 ± 6.7 years.

The human leukocyte antigens -A, -B, -DR and -DQ were studied serologically by micro lymphocytotoxic reaction.

It was found that human leukocyte antigens A-25(10), -B8, -DR18(3) and -DQ2 were more significantly frequent in the celiac population than in the control group. Children with antigen -B8 showed a five times higher risk for celiac disease and those with antigen -DQ2 showed a nine times higher risk. It was determined that human leukocyte antigen -B4 had a protective role in celiac disease.

The study suggests that the human leukocyte antigen -A25(10) is a phenotype particularly encountered in Turkish pediatric celiac patients. *Key words: human leukocyte antigen HLA types, celiac disease, Turkish children.*

Celiac disease (CD), an enteropathy caused by gluten-containing foods, is the most common cause of malabsorption in infants and children[1].

Genetic, environmental and immune system factors may play an important role in the pathogenesis of the disease[1]. It was reported for the first time in 1972 that relationship exists between celiac disease and human leukocyte antigen (HLA)-B8, and in 1983 between CD and HLA-DQ2 group[2,3]. The association with DR3 and DR7 is explained by the linkage disequilibrium of these alleles with the DQ2 allele[3]. Furthermore, HLA typing by molecular method showed a strong association of CD with the DQA1*0501 and dDQB1*0201 alleles combination at the DQ subregions[4]. The frequency of HLA subtypes in the ethnic populations varies significantly. In northern Europe HLA-DR3 was the most common antigen among celiac patients; in Spain and Italy, HLA-DR3 and -DR7 were the most common[5-7].

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In view of this, we studied the frequency of HLA types in Turkish pediatric celiac patients. To our knowledge, is the first such study.

Material and Methods

Thirty celiac patients fulfilling the European Society of Pediatric Gastroenterology and Nutrition criteria[8] were included in our study. After the establishment of the diagnosis by clinical presentation and hyperplastic villous atrophy in the first biopsy, patients recovered on a gluten-free diet. The mean age of the study population was 5.8 ± 4.3 (20 female, 10 male). Healthy control subjects were 30 randomly selected blood donors for renal transplant recipients. The mean age of the control subjects was 32.6 ± 6.7 years (15 female, 15 male).

The HLA-A, -B, -DR and -DQ were studied serologically by the standard microlymphocytotoxic method. Lymphocyte preparation was obtained from heparinized peripheral blood by ficoll[9]. These lymphocytes were purified by MN-Kwik (Lymphokwik MN cell/one lambda America). The remaining T and B lymphocytes were added to the HLA-ABC tray for determination of class I HLA.

For HLA class II typing, B lymphocytes were first separated from T lymphocytes using B1 and B2 kwik (lymphokwik B1 cell and lymphokwik B2 cell/one lambda America), then added to HLA class II tissue typing trays. The trays were scored using inverted light microscope[10].

The statistical significance of differences in the frequency of HLA types between celiac patients and controls was calculated by chi-square analysis. When the number of groups was below five, Fisher's exact test was utilized. In order to estimate relative risk, the odds ratio method was also used. The parents of the subjects were invited to give their consent after the purpose and requirements of the study were explained to them.

Results

The distribution of HLA in celiac patients and controls is shown in Tables I and II.

In celiac patients, HLA-A2 (43.3%), HLA-B6 (56.5%), HLA-DR53 (83.3%), and HLA-DQ7(3) (43.3%) were the most common HLA types observed in HLA groups -A, -B, -DR and -DQ respectively (Tables III and IV).

In the control group, HLA-A2 (46.6%), HLA-B4 (93.3%), HLA-DR53 (76.6%), and HLA-DQ4 (53.3%) were the most common HLA types obtained in HLA groups -A, -B, -DR and -DQ respectively (Tables III IV).

A significant positive association between CD and HLA-A25(10), -B8, -DR18(3) and -DQ2 ($p = 0.02$; $p = 0.01$; $p = 0.02$; $p = 0.002$, respectively) is evident

(Table V). But after the odds ratio test, only HLA-B8 and HLA-DQ2 demonstrated higher risk for celiac disease (five and nine times, respectively).

HLA-B4, -B7, -DR11(5), and -DQ6 antigens were significantly frequent in the control group (Table V). However, only HLA-B4 demonstrated a higher protective role (five times) (odds ratio: 0.5, interval: 0.01-0.31).

Table I: Distribution of HLA Types of the CD Patients

Name	HLA-AB	HLA-DR	HLA-DQ
S.Y	A3, A10, B6, B8	DR4, DR10, DR52, DR53	DQ2, DQ7(3)
G.Ç	A1, A2, B4, B13, B52(5)	DR7, DR11(5), DR52, DR53	DQ4, DQ7(3)
D.K.	A1, A9, B6, B8 B73	DR8, DR9, DR52, DR53	DQ4, DQ6(1)
Ş.K.	A25(10), B6, B8, B65(14)	DR7, DR15(2), DR52, DR53	DQ4
M.Y	A2, A25(10), B4, B6, B35, B51(5)	DR10, DR15(2), DR52, DR53	DQ2, DQ4
N.Ç	A1, A24(9), B4, B6, B8, B35	DR13(6), DR18(3), DR52	DQ2, DQ6(1)
S.K	A2, A11, B4, B6, B27, B35	DR1,DR4, DR52, DR53	DQ1, DQ3
N.B	A1, A19, B4, B17, B37	DR1, DR11(5), DR52, DR53	DQ1, DQ4
D.S	A2, A11, B4, B6, B40, B51(5)	DR8, DR14(6), DR52, DR53	DQ3, DQ4
Ş.E	A10, A11, B4, B6, B8, B44(12)	DR18(3), DR52	DQ6(1)
Z.O	A11, A24(9), B6, B8, B40	DR15(2), DR18(3), DR52, DR53	DQ1, DQ2
N.G	A1, A11, B6, B8, B18	DR7, DR11(5), DR52, DR53	DQ2, DQ4
G.A	A2, A3, B6, B22	DR1, DR12(5), DR52, DR53	DQ4, DQ7
H.Ş	A2, A66, B4, B27, B51(5)	DR4, DR15(2), DR52, DR53	DQ2, DQ7(3)
F.U	A1, A26(10), B4, B6, B35, B62(15)	DR11(5), DR13(6), DR52	DQ2, DQ7
U.B	A2, A24(9), B6, B8, B35	DR1, DR11(5), DR52, DR53	DQ7(3)
T.S	A1, A25(10), B6, B8	DR8, DR14(6), DR52	DQ1, DQ7(3)
M.Ö	A28, A36, B4, B6, B8, B12	DR4, DR18(3), DR52, DR53	DQ7(3)
E.M	A2, A3, B4, B6, B44(12), B51(5)	DR8, DR11(5), DR52, DR53	DQ6(1), DQ7(3)
D.E	A19, A28, B4, B6, B51(5), B78	DR1, DR11(5), DR52, DR53	DQ1, DQ2
A.K	A2, A30(19), B4, B51(5)	DR11(5), DR14(6), DR52	DQ6(1), DQ7(3)
C.K	A1, A3, B13, B35	DR7, DR15(2), DR52, DR53	DQ2, DQ7(3)
A.T	A2, A3, B14, B17	DR7, DR15(2), DR52, DR53	DQ6(1), DQ7(3)
Y.G	A2, A32(19), B5, B16	DR14(6), DR18(3), DR52, DR53	DQ4, DQ6(1)
K.E	A2, A10, B27, B60(40)	DR8, DR11(5), DR52, DR53	DQ6(1), DQ7(3)
C.G	A3, A25(10), B22 B27	DR10, DR15(2), DR52, DR53	DQ6(1), DQ7(3)
Z.Ç	A19, A24(9), B13, B51(5)	DR7, DR11(5), DR52, DR53	DQ3, DQ4
B.Y	A2, A25(10), B13, B60(40)	DR11(5), DR18(3), DR52, DR53	DQ2, DQ7(3)
M.S	A10, A11, B44(12), B62(15)	DR7, DR18(3), DR52, DR53	DQ2, DQ6(1)
E.Y	A9, A24, B8, B15, B62	DR3, DR4, DR52, DR53	DQ2

Table II: Distribution of HLA Types of the Control Group

Name	HLA-AB	HLA-DR	HLA-DQ
H.T.	A2, A3, B4, B6, B7, B44(12)	DR11(5), DR13(6), DR52	DQ3, DQ4
A.G	A1, A2, B4, B6, B7, B44(12)	DR4, DR11(5), DR52, DR53	DQ6(1), DQ7
D.G	A2, A3, B4, B13, B37	DR7, DR11(5), DR52, DR53	DQ1, DQ6
H.S	A3, A24(9), B4, B6, B17, B35	DR13(6), DR15(2), DR52	DQ1, DQ4
P.B	A2, B4, B6, B27, B35	DR10, DR13(6), DR52, DR53	DQ1, DQ3
A.S	A2, A24(9), B4, B6, B35, B44(12)	DR10, DR11(5), DR52, DR53	DQ4, DQ7
B.G	A2, A11, B4, B6, B51(5), B60(40)	DR11(5), DR13(6), DR52	DQ3, DQ7
İ.M	A10, A33(19), B6, B35	DR11(5), DR15(2), DR52, DR53	DQ1, DQ6
M.K	A2, A3, B4, B6, B7, B44(12)	DR8, DR11(5), DR52	DQ3, DQ6
H.A	A2, A19, B4, B27, B44(12)	DR10, DR11(5), DR52, DR53	DQ1, DQ3
F.B	A9, A11, B4, B6, B13, B35	DR11(5), DR13(6), DR52	DQ7(3), DQ4
H.T	A2, A19, B4, B27, B37	DR10, DR14(6), DR52, DR53	DQ6, DQ7
Ş.A	A2, A28, B4, B6, B44(12)	DR1, DR8, DR52	DQ1, DQ4
İ.T	A1, A30(19), B4, B5, B6, B7	DR11(5), DR15(2), DR52, DR53	DQ4, DQ6(1)
Z.S	A2, A24(9), B4, B6, B51(5), B60(40)	DR11(5), DR18(3), DR52	DQ4, DQ7(3)
B.Ş	A10, A11, B4, B6, B22, B44(12)	DR4, DR8, DR52, DR53	DQ6(1), DQ7(3)
M.G	A2, A24(9), B4, B6, B8 B44(12)	DR8, DR15(2), DR52, DR53	DQ6(1), DQ7(3)
F.A	A3, A19, B4, B13, B51(5)	DR7, DR11(5), DR52, DR53	DQ3, DQ4
Y.D	A10, A28, B4, B6, B13, B38(16)	DR10, DR11(5), DR52, DR53	DQ1, DQ4
A.P	A3, A23(9), B4, B37, B51(5)	DR11(5), DR15(2), DR52, DR53	DQ4, DQ7(3),
M.A	A3, A32(19), B4, B17, B51(5)	DR4, DR15(2), DR52, DR53	DQ6(1), DQ7(3)
M.K	A2, A10, B4, B51(5)	DR10, DR11(5), DR52, DR53	DQ4, DQ7(3)
Ü.K	A10, A30(19), B4, B17, B51(5)	DR10, DR14(6), DR52, DR53	DQ4, DQ3(7)
S.G	A24(9), A28, B4, B27, B37	DR8, DR11(5), DR52, DR53	DQ1, DQ7(3)
Ö.A	A9, A26(10), B4, B6, B27, B35	DR9, DR11(5), DR52, DR53	DQ2, DQ4
A.K	A1, A3, B6, B8, B35	DR11(5), DR15(2), DR52, DR53	DQ6(1), DQ7(3)
B.G	A10, A24(9), B4, B6, B8, B37	DR8, DR10, DR52, DR53	DQ2, DQ4
İ.A	A1, A3, B4, B17, B51(5)	DR1, DR15(2), DR52, DR53	DQ4, DQ6(1)
F.K	A2, A24(19, B4, B13, B51(5)	DR9, DR11(5), DR52, DR53	DQ4, DQ7(3)
S.Ü	A10, A30(19), B4, B6, B21, B35	DR8, DR14(6), DR52, DR53	DQ6(19), DQ7(3)

Table III: Frequency of HLA-A, -B in the Patient and Control Groups

HLA-A	Patient (%)	Control (%)	HLA-B	Patient (%)	Control (%)
A1	26.6	13.3	B4	43.3	93.3
A2	43.3	46.6	B5	3.3	6.6
A3	20	30	B6	56.6	0
A9	6.6	6.6	B7	0	13.3
A10	13.3	26.6	B8	36.6	10
A11	20	6.6	B12	3.3	0
A19	10	10	B13	13.3	16.6
A23(9)	0	3.3	B14	3.3	0
A24(9)	13.3	20	B15	3.3	0
A24	3.3	0	B16	3.3	0
A25(10)	16.6	0	B17	6.6	13.3
A26(10)	3.3	3.3	B18	3.3	0
A28	6.6	10	B21	0	3.3
A29(19)	0	3.3	B22	6.6	3.3
A30(19)	3.3	10	B27	13.3	16.6
A32(19)	3.3	3.3	B35	20	26.6
A33(19)	0	3.3	B37	3.3	16.6
A36	3.3	0	B38(16)	0	3.3
A66	3.3	0	B40	6.6	0
			B44(12)	10	26.6
			B51(5)	23.3	30
			B52	3.3	0
			B60(40)	6.6	6.6
			B62	3.3	0
			B65(14)	3.3	0
			B73	3.3	0
			B78	3.3	0

Table IV: Frequency of HLA-DR, -DQ in the Patient and Control Groups

HLA-DR	Patient (%)	Control (%)	HLA-DQ	Patient (%)	Control (%)
DRI	16.6	33.3	DQ1	16.6	26.6
DR3	3.3	0	DQ2	40	6.6
DR4	16.6	10	DQ3	10	20
DR7	23.3	6.6	DQ3(7)	0	3.3
DR8	16.6	23.3	DQ4	33.3	53.3
DR9	3.3	6.6	DQ6	0	13.3
DR10	10	26.6	DQ6(1)	33.3	26.6
DR11(5)	36.6	63.3	DQ7	6.6	13.3
DR12(5)	3.3	0	DQ7(3)	43.3	33.3
DR13(6)	6.6	16.6			
DR14(6)	13.3	10			
DR15(2)	23.3	26.6			
DR18(3)	23.3	3.3			
DR52	100	100			
DR53	83.3	76.6			

Table V: HLA Groups Associated with CD

HLA	Patient Group n	Control Group n	p	Odds Ratio*
B8	11	3	0.01	1.12-27.5
DR18(3)	7	1	0.02	0.96-204.9
DQ2	12	2	0.002	1.65-68.78
A25(10)	5	0	0.02	0.58-140.35
B4	13	28	0.00003	0.01-0.31
B7	0	4	0.03	0.01-2.39
DR11(5)	11	19	0.03	0.1-1.08
DQ6	0	4	0.03	0.01-2.39

*: Confidence interval: 95%.

Discussion

Human leukocyte antigen types have been used as a genetic marker of CD since 1972, and determining the frequency of specific HLA types in the population may suggest a predisposition to this disease[2].

Our results confirm a significant association of CD with HLA-A25(10), -B8, -DR18(3) and -DQ2. In different studies the frequency of HLA-B8 has been reported as 45-88 percent[11] in celiac patients. In the European Caucasian control group this ratio was 20 percent[12]. Our study result of 37 percent in celiac patients is similar to other reports.

In the present study, HLA-DQ2 was strongly associated with CD. Neverthless, the rate of DQ2+ patients was 40 percent, lower than reported in other series. The alleles DQA1*0501 and DQB1*0201, which encode for the HLA-DQ2 molecule, are found in over 90 percent of celiac patients from northern and southern Europe[13, 14]. At the same time, there is no increase in DQ1 and/or DQ8 (DR4+), which are expressed more in the -DQ2 population, as mentioned by Mantovani et al.[15] In the normal population, HLA-DQ is in strong linkage disequilibrium with HLA-DR3 and HLA-DR7 and, in view of this, it has been proposed that the significant association of CD with these latter antigens may be secondary to the association with the DQ2 antigen[16]. Recent studies have demonstrated that all gliadin specific T cells isolated from the small bowel only recognize gliadin antigen in the presence of HLA-DQ2 and not -DR and -DP class I molecules[17].

Because all reports about significant association between DR7 and CD concern pediatric patients, other works speculated that juvenile celiac patients are genetically different from adult celiac patients[7, 18, 19]. Our results are, however, not in keeping with this view, as we did not demonstrate an association between DR7 and CD. Meuli et al.[19] showed that there was a significant increase in the frequency of DR3 and DR7 in celiac patients.

The study of Betuel et al.[20], realized in adult patients, showed no difference between the HLA status of children and adults with CD, suggesting that the genetic heterogeneity appears to be more a result of different geographic origin than of patient's age. Thus, the strength of association is higher for DR3 and DQ2 in northern Europe, while DR7 is generally limited to southern Europe[14, 21].

The distribution of HLA in our population was between that of the Arabic and European populations. The absence of HLA-A1 and B8, which is very characteristic for these populations, did not exist in our group. In the Turkish population, HLA-A2, -DR2, -A9, -Cw4, -DR4, and -B35 were the most frequent antigens, and HLA-A29, -B39, -B15, -A23, and -A37 were the less frequent antigens. Thus, the antigens found to be in strong association with CD did not show a high frequency in the Turkish population. This observation suggests that the geographic difference alone may not be sufficient to explain the genetic heterogeneity of HLA distribution.

We could not find any report in the literature concerning the association of HLA-A25(10) and CD. This latter antigen may be a phenotype particularly encountered in Turkish pediatric celiac patients.

Our data indicate that HLA-B4 plays a protective role against the development of CD. Because more genes are less frequent in celiac patients than in healthy subjects, this finding supports the hypothesis that it compensates for the increased frequency of the other genes[18].

In conclusion, our study supports the hypothesis previously put forward by others that DQ2, B8, DR3 (the term HLA-DR18(3) indicates that -DR18 has split off HLA-DR3) are the phenotypes more strongly associated with CD, and also demonstrates that HLA-A25(10) may be an indicator particularly for Turkish pediatric celiac patients. Although linkage studies between HLA types and CD are now realized at the genetic level using molecular biology technology, serological studies are still of great value when molecular biology is not available.

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CRYPTOSPORIDIUM PARVUM PREVALENCE IN A GROUP OF TURKISH CHILDREN*

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SUMMARY: Akyön Y, Ergüven S, Arıkan S, Yurdakök K, Günalp A. (Departments of Microbiology and Clinical Microbiology, and Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Cryptosporidium parvum prevalence in a group of Turkish children. Turk J Pediatr 1999; 41: 189-196.

Stool samples from two hundred children with diarrhea and from 50 healthy children were examined, by modified Kinyoun's acid-fast staining (MAF), Giemsa staining and direct (DFA) and indirect immunofluorescence antibody (IFA) methods, in order to determine cryptosporidiosis prevalence under the age of 12 and to detect the most efficient identifying method for use in our country. Cryptosporidium oocysts were detected in seven (3.5%) of the cases. None of the samples from the control subjects was found to be positive for Cryptosporidium. Our results indicate that Cryptosporidial oocysts should be detected in children with diarrhea. Modified Kinyoun staining method is practical and reliable for this purpose. Immunofluorescence staining methods can be applied for conformation of the results, if available. *Key words: cryptosporidiosis, children.*

A small intracellular protozoon, Cryptosporidium parvum, which causes infection in humans and animals, belongs to the phylum Apicomplexa, subclass Coccidiasina[1]. The first case of human cryptosporidiosis was reported in an immunocompetent three-year-old girl, in 1976. Now, "cryptosporidiosis" is accepted as a zoonose, causing infection in humans[2]. Cryptosporidium parvum infects epithelial cells of the gastrointestinal tract in humans and animals. Transmission of cryptosporidiosis from person-to-person is well defined. Day-care centers and nosocomial outbreaks play an important role in the spread of Cryptosporidium parvum oocysts[3, 4].

Cryptosporidiosis is mostly prevalent in developing countries where malnutrition is a predisposing factor. It causes persistent diarrhea in children and the elderly and prolonged severe or fatal diarrhea in immunocompromised individuals.

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Infection is self-limited in immunocompetent individuals. It is the causative agent of diarrhea in children, especially under the age of two, during the summer and fall[5].

The prevalence of cryptosporidiosis is one to two percent and three to 20 percent in developed and developing countries, respectively[6]. In Turkey, the prevalence varies according to the geographic region[7,8]. In this study, our aim was to detect this protozoon in children under the age of 12 with diarrhea and to determine the most efficient identifying method for use in our country.

Material and Methods

Between July 1995 and January 1997, stool samples were collected from 200 children with diarrhea, who were referred to the Children's Diarrhea Unit, Hacettepe University İhsan Doğramacı Children's Hospital. They were aged between 13 days and 12 years. Fifty healthy children in the same age group were also included in the study as the control group. A questionnaire, requesting the age, gender, weight of the child, duration of diarrhea, daily number of defecations, form of the stool, presence of diarrhea in any other member of the family, usage of antibiotics, presence of fever, vomiting, nausea, dehydration, or underlying illness, and usage of breast-feeding, was presented to the patient and control groups.

Three stool samples were collected from each patient, separately preserved in 10 percent formalin and stored at -30 °C until examined. In order to provide the same conditions the stool samples were examined in groups of 20.

The stool preparations were stained with Giemsa and modified Kinyoun's acid-fast (MAF) methods (Fig. 1) and were examined under light microscope as described previously[9].

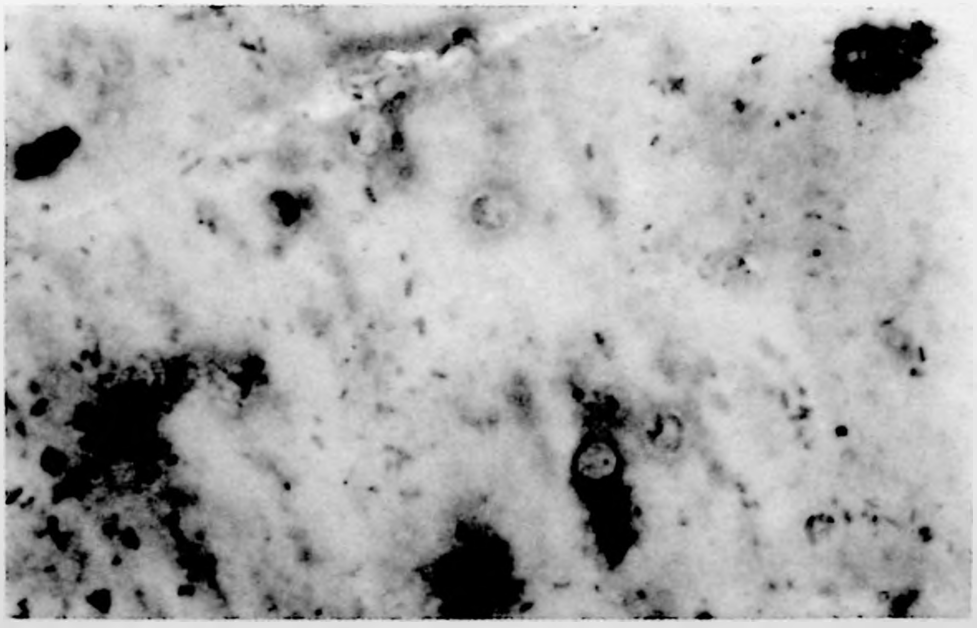

Fig. 1: Cryptosporidium oocysts by MAF (x 1000).

Direct (DFA) and indirect immunofluorescence antibody (IFA) staining methods were performed for each specimen. Merifluor Cryptosporidium/Giardia kit (Meridian Diagnostics Inc., Ohio 45244) was used for DFA and examined under the fluorescence microscope according to the manufacturer's instructions[10]. For the IFA test, mouse monoclonal antibodies (IgG and IgM culture supernatants) specific to Cryptosporidium oocysts, which were kindly provided from San Francisco General Hospital, Divison of Infectious Diseases, and the fluorescein isothiocyanate (FITC) conjugate "anti-mouse polyvalent immunoglobulins IgG, IgA, IgM (Sigma F1010) were used. The test was performed according to the instructions of Merifluor Cryptosporidium/Giardia kit and the results were interpreted under the fluorescence microscope, as described previously[11].

For routine parasitological examination stool samples of all of the children included in the study were examined by standard methods for the existence of egg and/or cyst forms of commonly encountered parasites other than Cryptosporidium[12].

Results

Stool specimens from 200 children with diarrhea were examined. In seven of the cases (3.5%) Cryptosporidium oocysts were detected. In 10 children, Giardia intestinalis cysts were detected (in 1 case together with Cryptosporidium oocysts). Taenia eggs, Hymenolepis nana eggs and Entamoeba histolytica trophozoites were detected, on each in the stool samples of three separate cases.

Cryptosporidium oocysts were not detected in any of the 50 children who were included in the study as the control group. Ascaris lumbricoides and Hymenolepis nana eggs were detected in one child and Giardia intestinalis cysts in four children.

Table I summarises the clinical features and results of the Cryptosporidial oocyst detection methods. As can be seen, there was an underlying disease in three of the patients whose stool samples yielded Cryptosporidium oocysts (hyper IgM syndrome, hypogammaglobulinemia, celiac disease). In these three cases the duration of diarrhea was 30 days, 10 years (intermittent) and 20 days, respectively. Cryptosporidium positivity could be detected using all the methods performed.

The Cryptosporidium oocysts were detected in May, June, July, September and October. Except for the seventh case, in whom there was also moderate dehydration, none were breast-fed. In the third case, Giardia intestinalis cysts, Hymenolepis nana eggs and Entamoeba histolytica trophozoites were detected in addition to the Cryptosporidium oocysts. In the second case, Cryptosporidium oocysts were detected for four months. While treated with Paromomycin, oocysts were absent in the stool samples, but upon cessation of treatment, diarrhea and oocyst excretion resumed. In the fifth and sixth cases, positivity was determined by staining and indirect IFA methods; in the 1st case it was shown only by staining.

Table I: Clinical Features and Detection Methods of Diarrheal Cases with Cryptosporidium Oocysts

Case	Age	Gender	Clinical Features	Staining Methods		Immunofluorescence	
				Giemsa	MAF	Direct	Indirect
1	2.5	F	Watery, mucus-containing diarrhea, for only one day 5-6 times/day	±*	+	–	–
2	4	M	Prolonged, yellowish, watery diarrhea, vomiting, hyper IgM syndrome	+	+	+	+
3	12	M	Watery intermittent diarrhea, for 10 years 4-5 times/day, hypogammaglobulinemia, malabsorption	+	+	+	+
4	2.5	F	Mucus-containing diarrhea, vomiting for 3 days 4-5 times/day	±*	+	+	+
5	16 mo**	M	Watery, mucus-containing diarrhea, vomiting, for 5 days 7-8 times/day	±*	+	–	+
6	3	M	Mucus-containing diarrhea, for only one day 3 times/day	±*	+	–	+
7	5	M	Watery diarrhea, for 20 days 14 times/day, celiac disease	+	+	+	+

* Structures resembling Cryptosporidial oocysts, but not exactly typical.
** months.

Discussion

In recent years Cryptosporidium species have become important protozoal infectious agents, especially in HIV (human immunodeficiency virus) infected patients. Person-to-person transmission and/or spread from water is well defined[9].

This protozoon is especially prevalent in developing countries where malnutrition is common. The agent is mostly detected in children; in adults it is mostly seen in immuncompromised patients as a causative agent of diarrhea[13].

Another water-spread agent is Giardia intestinalis. In most of the cases, these two protozoa can be detected together[14]. Giardia intestinalis prevalence is high in Turkey, therefore Cryptosporidium prevalence is also expected to be high[15]. As the routine parasitological methods are not sufficient for determining the oocysts of Cryptosporidium, this study was planned to determine the most effective method in view of our laboratory conditions[16].

In stool samples of seven of the 200 children (3.5%) with diarrhea who were admitted to Hacettepe University İhsan Doğramacı Children's Hospital, Cryptosporidium oocysts were detected. Based on the detection methods used, C. parvum is now accepted as one of the most common enteropathogens causing diarrhea in the world, mostly in developing countries. Prevalence has been reported in Europe as one to two percent and in North America as 0.6-4.3 percent. In Asia, Africa, Australia, and Central and South America it has been reported as between three to 20 percent. The studies show that the prevalence is higher in children two years old or younger when compared with adults[1]. In the United Kingdom, in acute or chronic diarrheal children, the prevalence has been reported as 3.2 percent. In Venezuela, in children under the age of two with diarrhea it was 10.8 percent. In Ghana in infants two to 12 months of age it was 21.6 percent, and in Haitian acute diarrheal children the rate was 16.7 percent[14, 17-20].

In Turkey, in a study performed in İzmir (West Anatolia) involving 600 children aged between zero to six years, Cryptosporidium oocysts were only detected in a 15-month-old child, together with Giardia intestinalis cysts[7]. In the Adana region (southeast), the percentage was 8.2 in diarrheal (n = 110) and 4.08 in non-diarrheal (n = 98) children[8]. In İstanbul (northwest) in 100 acute diarrheal cases it was two percent (17 months and 2 years of age)[21]. In Bursa region (northwest) the rate was 2.9 percent and in İstanbul (northwest) oocysts were detected in 1.36 percent of diarrheal children under five years of age. Investigators reported that this low percentage may be due to breast-feeding, good sanitary conditions and little animal contact[22, 23].

Modified Kinyoun's acid-fast staining method and the immunofluorescence technique are the most common diagnostic tools for Cryptosporidium oocyst detection in Turkey and other countries. Recently, enzyme-linked immunosorbent assay has also been used for detection of oocysts[24]. In addition, detection of oocysts from water samples by polymerase chain reaction can be used[25].

In our study, Cryptosporidium oocysts could only be detected in 3.5 percent of diarrheal children, which is similar to rates from other reports from Turkey. This result shows that Cryptosporidium can also be a causative agent of diarrhea in children.

Cryptosporidiosis can be seen in immunocompromised patients throughout the year, whereas in immunocompetent persons it is common during the summer and fall[24, 26].

Although the study was carried out for seven months, the seasonal distribution was found to be similar to the previous reports.

We defined Cryptosporidium oocysts in three immunocompromised patients (hyper IgM syndrome, hypogammaglobulinemia and malabsorption, and celiac disease). Some investigators have detected Cryptosporidium oocysts in diarrheal children with malnutrition, depressed cellular immunity, congenital hypogammaglobulinemia and primary immunoglobulin deficiency[27-30]. These findings emphasize that in chronic malabsorption cases and immunocompromised diarrheal children, Cryptosporidium oocysts should be investigated.

Laboratory diagnosis of this protozoon can be established by various techniques. Modified acid-fast (MAF) staining technique is superior to the Giemsa staining method in differentiating the oocysts from yeast cells[31]. In recent years with the discovery of the monoclonal antibodies specific to the oocyst wall, DFA and IFA methods are being used and are more sensitive.

In many studies the methods of detecting Cryptosporidium oocysts in stool samples have been compared. Some studies revealed that immunofluorescence methods are superior to the staining methods, as they can detect the oocysts even if the number is very low. Due to the prolonged application of the stain there may be false positive results; therefore, the sensitivity of the MAF staining method is low[11, 32-33].

Although immunofluorescence methods are rapid, some reports claim that there can be false positive results, because of the non-specific fluorescence[16, 34]. In our study, staining and immunofluorescence methods were positive in four cases. In one case, oocysts were detected only with the staining method, and this was considered a false positive result.

In conclusion, for the detection of Cryptosporidium oocysts, the MAF staining method is an easily performed and reliable method in immunocompromised children with chronic diarrhea. If possible, for conformation of the staining results, the immunofluorescence method should be performed.

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METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) C677T MUTATION IN TURKISH PATIENTS WITH THROMBOSIS*

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SUMMARY: Balta G, Gürgey A. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Methylenetetrahydrofolate reductase (MTHFR) C677T mutation in Turkish patients with thrombosis. Turk J Pediatr 1999; 41: 197-199.

Recently, the homozygote state for the thermolabile variant of the MTHFR gene (C677T) has been identified as a determinant of elevated homocysteine levels which are known to be a risk factor for arterial and thrombotic vascular disease. To determine whether this variant increases the risk of thrombosis, we analyzed the prevalence of the C677T substitution in the MTHFR gene in 94 patients with thrombosis and in 95 unmatched controls. Although homozygosiy for he mutation was found in 12 (12.8%) of the patients with thrombosis and in only six (6.3%) of the control subjects, the difference in the prevalence of the homozygous mutant genotype between patients and healthy subjects was not statistically significant. *Key words: methylenetetrahydrofolate reductase, MTHFR, C677T mutation, thrombosis, hyperhomocysteinemia, thermolabile enzyme.*

Elevated plasma levels of homocysteine appear to be a risk factor for arterial disease and for venous thrombosis. One of the metabolic pathways for homocysteine involves the enzyme methylenetetrahydrofolate reductase (MTHFR), which is responsible for the conversion of homocysteine to methionine. Frosst et al[1]. recently reported a C to T substitution at nucleotide 677 of the MTHFR gene that converts an alanine to a valine residue. Later, it was reported that homozygosity for the mutation was associated with a three-fold increase in the risk for premature cardiovascular disease[2]. Individuals homozygous for the thermolabile variant of the MTHFR gene due to this substitution have significantly elevated plasma homocysteine levels which may account for one of the genetic risk factors of arterial disease[1]. However, the risk for thrombotic vascular disease remains controversial. We have, therefore, evaluated the importance of the C6677T mutation in Turkish patients with thrombosis.

Material and Methods

Analysis of the MTHFR 677 C-T substitution was performed as previously described[1]. Genomic DNA was isolated from peripheral blood by standard methods[3]. A fragment of the MTHFR gene was amplified by polymerase chain

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reaction (PCR) as described[1]. Polymerase chain reaction products, 198 bp in size, were digested with restriction endonuclease Hinf I which recognizes C to T substitution at the nucleotide 677. The presence of 198 bp (normal) and 175 bp (mutant) DNA fragments was observed in a two percent agarose gel (Fig. 1). Statistical analysis was carried out by chi-square testing.

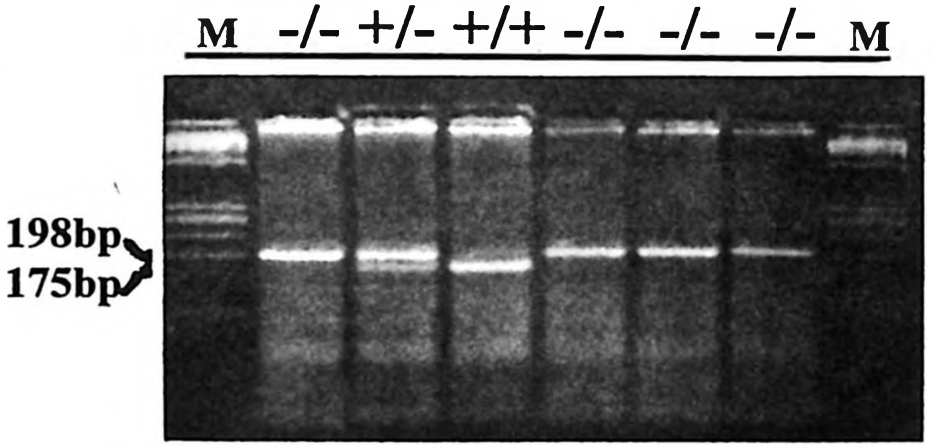

Fig. 1: Analysis of the C to T substitution at nucleotide 677 of the MTHFR gene by Hinf I digestion. The substitution creates a Hinf I recognition sequence which digests the 198 bp fragment into 175 and 23 bp fragments; the latter fragment has run off the gel. Heterozygous (+/-) and homozygous for mutated allele (+/+) or normal allele (-/-) patterns are shown in the figure. M: ϕX174/Bsu RI (Hae III) Marker.

Results

Among 94 patients with thrombosis, 12 (12.8%) were homozygous and 33 (35.1%) were heterozygous for the C677T mutation. In 95 controls, six subjects were homozygous (6.3%) and 50 subjects (42%) were heterozygous (Table I). Although the frequency of the mutation in thrombotic patients was twice that as in the control group, no significant difference for homozygosity was detected between thrombophilic and control subjects by chi-square testing (p = 013).

Table I: Distribution of Genotypes and Alleles of MTHFR C677T Substitution

		Genotype			Alleles	
Group	Number	-/-	-/+	+/+	-	+
Thrombotic patients	94	49 (52%)	33 (35.1%)	12 (12.8%)	131 (69.7%)	57 (30.3%)
Control subjects	95	49 (52%)	40 (42%)	6 (6.3%)	138 (72.6%)	52 (27.4%)

Discussion

Hyperhomocysteinemia is known to be associated with an increased risk of both arterial and venous thromboembolic disease[1,2]. Previous reports concerning the C677T mutation in the MTHFR gene suggest that this variant may be associated with an increased risk for coronary artery disease[1]. The prevalence of

homozygotes for the C6677T mutation may vary significantly in populations from different geographic areas (1.4% to 29.7%)[4,5]. However, its role in the pathogenesis of venous thrombosis remains controversial. Some authors reported that the mutation is higher in thrombotic patients than in the healthy control population[1-2]. Others have suggested that the MTHFR C677T mutation is not associated with an increased risk of thrombosis[6,7]. Recently, the occurrence of an interaction between the MTHFR genotype and folate status was shown, and when plasma folate concentrations were below the median, plasma homocysteine levels were significantly higher in homozygotes for the C677T mutation than in those with the normal genotype[8].

In the present study, although there was quite a difference the frequency of homozygosity for the C677T mutation in patients with thrombosis and in healthy controls, no difference in the prevalence of homozygosity for the mutation was found in patients and control subjects by statistical tests. Therefore, our data suggest that the MTHFR C667T mutation was not associated with an increased risk of thrombosis in our population. However, further studies in a larger number of patients are needed to determine whether it is an added risk factor in individuals with other thrombotic abnormalities.

Acknowledgement

We are indebted to Prof. Dr. Ç. Altay for her incredible support of this study and valuable comments.

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RHEUMATIC HEART DISEASE PREVALENCE AMONG SCHOOLCHILDREN IN ANKARA, TURKEY*

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SUMMARY: Olguntürk R, Aydın GB, Tunaoğlu FS, Akalın N. (Department of Pediatric Cardiology, Gazi University Faculty of Medicine, Ankara, Turkey). Rheumatic heart disease prevalence among schoolchildren in Ankara, Turkey. Turk J Pediatr 1999; 41: 201-206.

Rheumatic heart disease (RHD) continues to be a common health problem in the developing world. Although little longitudinal data are available, evidence suggests that there has been little if any decline in the occurrence of RHD over the past few decades. There are only a few population surveys available in Turkey for prevalence of RHD. This survey was undertaken to estimate its prevalence among schoolchildren and changes over the last 20 years in the capital, Ankara.

In Ankara, 4,086 schoolchildren aged between six and 17 years were screened over a period four months (March 1995-June 1995) by the same pediatrician. Forty-eight percent (n = 1,945) were female and 52 percent (n = 2,141) were male. Three children out of 4,086 (0.73 per 1,000) were noted to have findings consistent with RHD. Fifteen children had an episode of rheumatic fever (RF). Cumulative prevalence rate (prevalence rate for RF history) was 3.7 per 1,000. We concluded that RHD prevalence has decreased in Ankara over the last decades. *Key words: rheumatic heart disease, prevalence, Turkey.*

Although there has been some concern about the resurgence of rheumatic fever (RF) and rheumatic heart disease (RHD), the general trend is that of a declining prevalence, particularly in developed countries. However, in developing countries the exact magnitude of the problem remains largely unknown[1,2].

There are few available data on RF and RHD in Turkey. Some of them are based on hospital admission records and incidence rates that are not accurate and do not represent all the country[3]. Some studies are about RHD prevalence in different regions of Turkey. Those studies are about the prevalence of cardiac murmurs, and neither the RHD nor the cumulative prevalence rates were searched specifically[4-7]. One study, conducted in Ankara in 1975, was a survey of prevalence, which was found as 6.6 per 1,000 (range between 2.1 and 10.7 per 1,000 in higher and lower socioeconomic status) groups, respectively. Cumulative prevalence rate was 3.7 per 1,000[8].

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The specific objectives of this report were to carry out a case finding survey in order to determine the prevalence of RHD among schoolchildren in Ankara and to compare the results with previous studies.

Material and Methods

The study was a retrospective one, carried out by a specially trained pediatrician and a pediatric cardiology unit.

- The study involved schoolchildren at high risk for RHD (aged 6-17 years), between March and June 1995.
- Thirty-four schools were selected and from these schools 20 percent of the students were examined.
- Each child was given a questionnaire about RF history and the socioeconomic status of the famiy. They were asked about the age of onset of RF, the number and type of attacks, history of prophylactic regimen, and duration of valvular disease (if present), the number of individuals rooms in one household, and the total income of the family.
- All the students were examined by the same pediatrician. The students suspected of having heart disease were re-examined in a pediatric cardiology unit and electrocardiograms, chest x-rays anad echocardiograms were obtained if necessary.

Results

- In 34 schools, 4,086 students aged between six and 17 years were examined (47.6% female and 52.4% male) (Fig. 1).

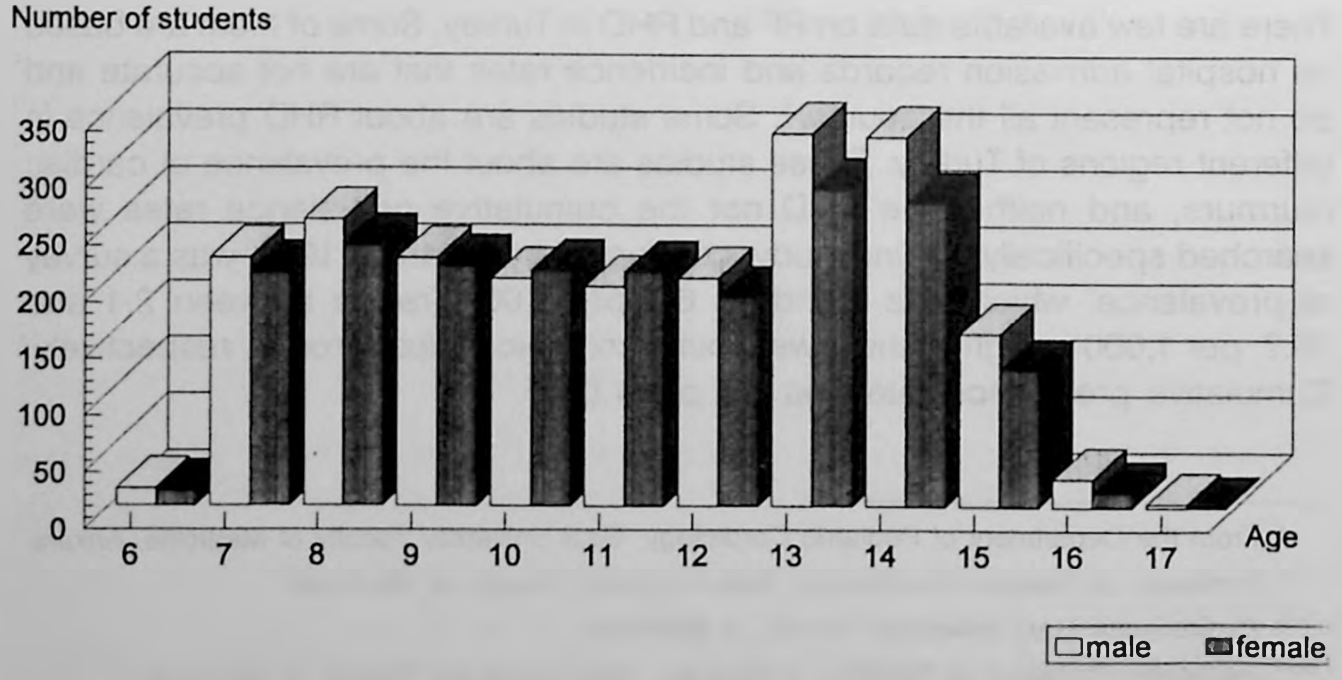

Fig. 1: Age and sex distribution of the schoolchildren in the survey.

• The average number of individuals per household was 4.9 (range 2-14). The average number of rooms per house was 2.8 (range 1-9). The average income per family was 13,200,000 TL (range 4,540,000-51,030,000).

• Fifteen children had an episode of RF attack. Thus, the cumulative prevalence rate (prevalence rate for RF history) was 3.7 per 1,000. All were on regular secondary prophylaxis.

• Three children were diagnosed as RHD. The prevalence rate for RHD was 0.73 per 1,000. The characteristics of each case with RHD are given in Table I. All had mitral valve insufficiency. One patient also had aortic valve insufficiency and one had tricuspid valve insufficiency and cardiac involvement (this patient underwent cardiac surgery for mitral valve replacement). Only one child was on regular secondary prophylaxis. The other two children were not aware of their disease.

Table I: Characteristics of the Students with Rheumatic Heart Disease

			Auscultatory Findings					
	Age	Sex	Area	Intensity	Character	EKG	X-ray	ECHO
1	12	boy	apical	II/VI	pansystolic	LA dilatation	cardiac enlargement	MR, TR, LA and LV dilatation
2	10	boy	apical	II/VI	pansystolic	normal	normal	MR
3	12	girl	aortic	II/VI	pansystolic	normal	normal	AR, MR (mild)

LA: left atrium; MR: mitral regurgitation; TR: tricuspid regurgitation; LV: left ventricle; AR: aortic regurgitation.

• The RHD prevalence rate was not statistically different in urban or rural regions (Figs. 2, 3).

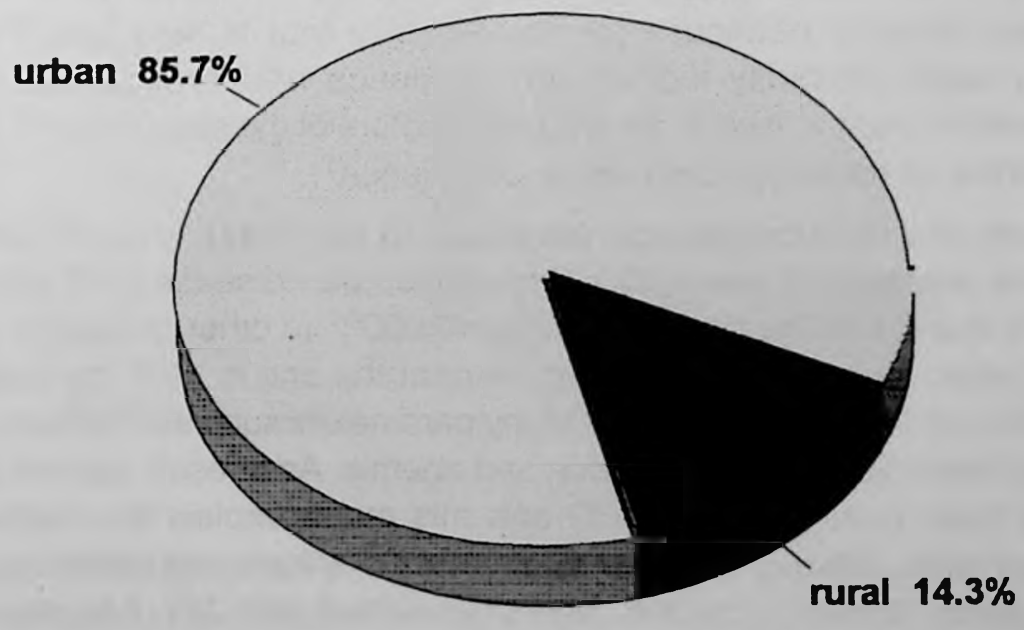

Fig. 2: Distribution of the Schools in Ankara.

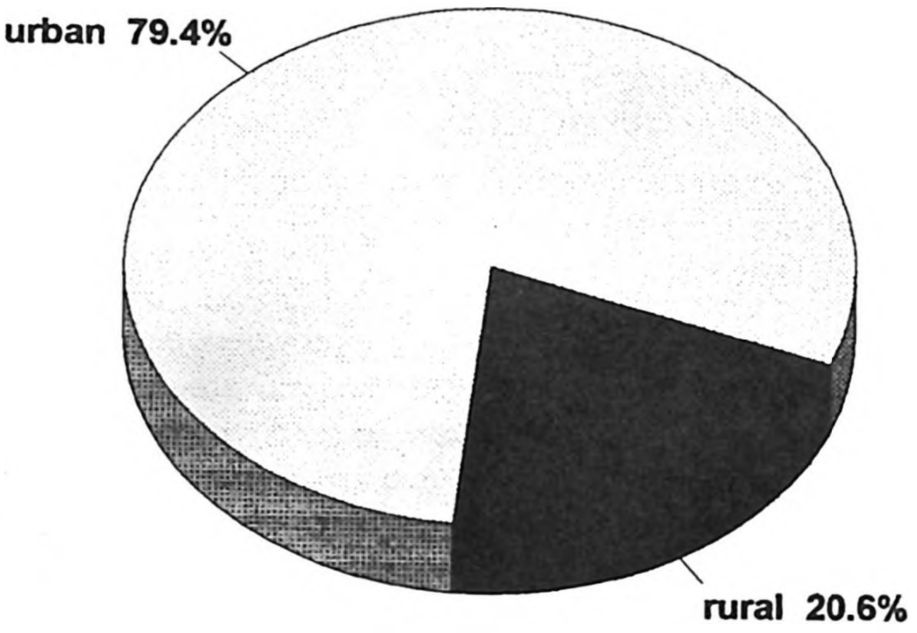

Figure 3: Distribution of the schools in the survey.

Discussion

Rheumatic fever continues to be a major cardiovascular health problem in developing countries. The continued presence of the disease in developing countries and the reappearance of RF in industrialized countries demands the renewed interest of researchers, clinicians and public health workers[9, 10].

Since mortality and morbidity as well as the cost of treatment are high in patients with RF and RHD, it is necessary to plan effective programs for the prevention and control of RHD. Thus, a precise knowledge of disease frequency is needed. The extent of an illness such as RF is better measured in terms of morbidity rather than mortality becacuse the case-fatality rate is very low. The most frequently used morbidity indices are incidence and prevalence. Another measure which may be used in developing a picture of the size of the RF problem is prevalence of history or cumulative prevalence[11].

In this study, the RHD prevalence rate was 0.73 per 1,000, and the cumulative prevalence rate was 3.7 per 1,000. Rheumatic heart disease RHD prevalence rates were found between 0.3 and 1.1 per 1,000[4-7] in other studies in different parts of Turkey (Fig. 4). These studies, except the one in 1975 by İmamoğlu[8], were performed for the prevalence of many parameters such as innocent murmur, congenital heart disease, hypertension and anemia, As a result, special attention might not have been given to RHD and this might explain the relatively low prevalence rates. Although the previous survey in Ankara was performed in only four elementary schools (children aged between 6 and 12), it is important to compare these two surveys to determine how RHD prevalence rates have

changed over the last 20 years. Rheumatic heart disease RHD prevalence decreased in Ankara nine-fold and cumulative prevalence by ten-fold (Fig. 5). This decrease might have been caused by continuous vigil, better socioeconomic conditions, prompt treatment of streptococcal pharyngitis and improvement in primary health care. Other surveys[4-8] also showed that RHD prevalence rate was decreasing.

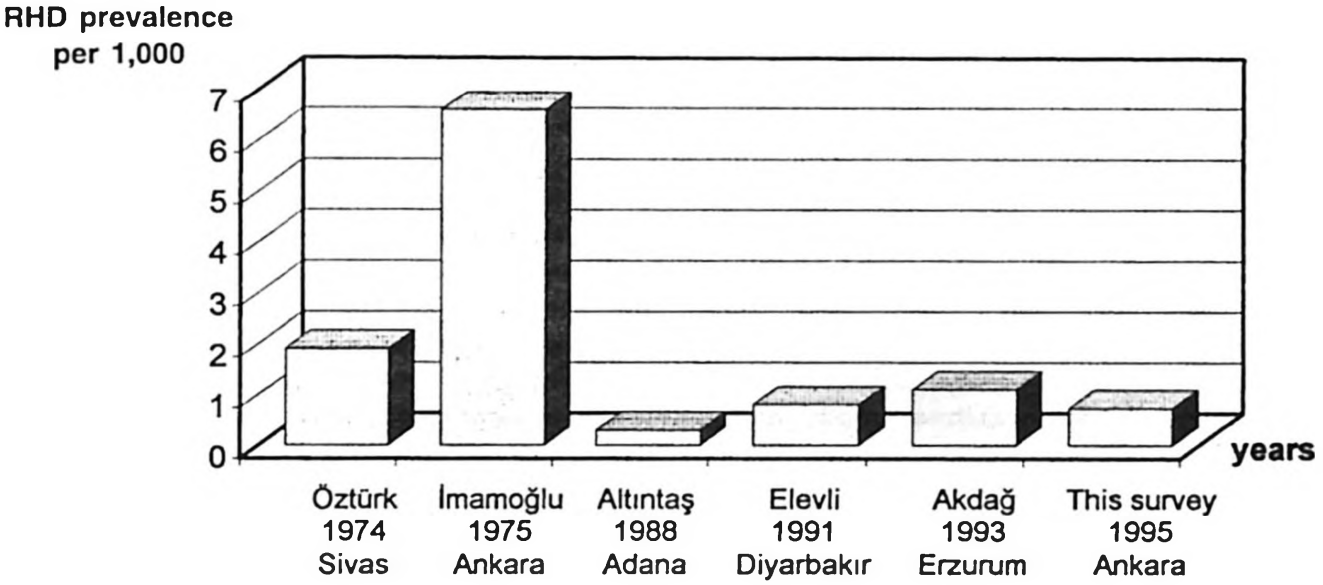

Figure 4: Rheumatic heart disease prevalence in Turkey.

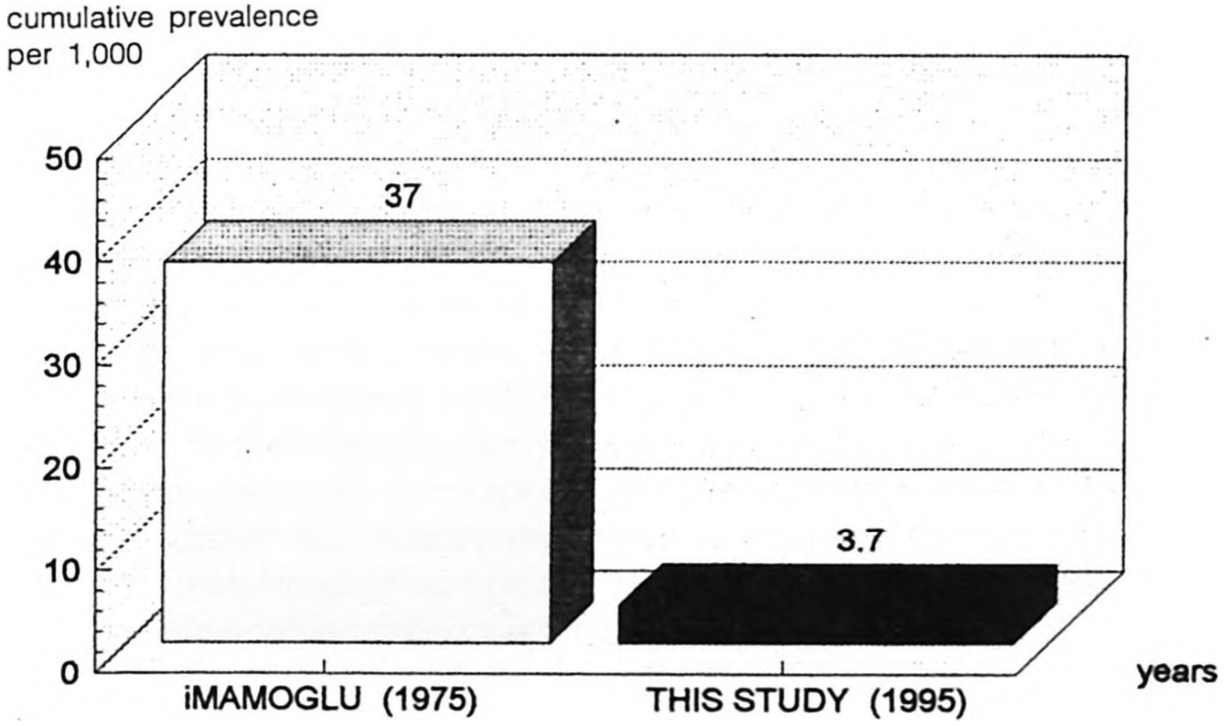

Fig. 5: Cumulative prevalence in Ankara.

Because of the large social and economic burdens imposed by RHD, a greater emphasis needs to be placed on the simple and cost-effective measures that are currently available to eradicate RHD in the world.

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The Turkish Journal of Pediatrics 1999; 41: 207-218

TROPISETRON IN THE PREVENTION OF CHEMOTHERAPY – INDUCED ACUTE EMESIS IN PEDIATRIC PATIENTS*

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SUMMARY: Mutafoğlu Uysal K, Olgun N, Sarıalioğlu F. (Department of Pediatric Oncology, Dokuz Eylül University, Institute of Oncology, İzmir, Turkey). Tropisetron in the prevention of chemotherapy-induced acute emesis in pediatric patients. Turk J Pediatr 1999; 41: 207-218.

We evaluated the antiemetic efficacy of tropisetron for control of acute emesis during grade 3 or 4 emetogenic chemotherapy in children. Tropisetron was administered as a single intravenous dose of 0.2 mg/kg an the first day and intravenously or orally with the same dose on subsequent days. A total of 125 courses of highly emetogenic chemotherapy was administered to 22 children with a median age of 14 years (range: 3-18 years). All 22 patients received tropisetron for at least two courses. Overall complete response on day I was observed in 80 out of 125 courses (64%). The response rates were consistent over multiple courses; a complete and major response rate on the first day of Course I (n: 22 courses) and Course II (n: 22 courses) was observed in 73 and 77 percent of cases, respectively. When the results were analyzed according to the daily schedules, overall complete response for grade 4, grade 3 and grade 1-2 emetogenic treatment days was 59, 85 and 75 percent, respectively. In this study, cost effectiveness for tropisetron was also determined; the cost per successfully controlled course was 162 USD. No side effects of tropisetron other than mild diarrhea and dry-mouth were documented in this study. In conclusion, the results of this study confirmed that tropisetron is a safe, well tolerated and effective antiemetic drug for the prevention of acute emesis in children and adolescents during highly emetogenic chemotherapy. *Key words: acute emesis, tropisetron, children, adolescent, antiemetic.*

Nausea and vomiting are among the most distressing and debilitating side effects of chemotherapy. Highly emetogenic drugs may result in dehydration and electrolyte imbalance which may potentiate the risk of toxicity related to anticancer drugs. Emesis and nausea are potentially more hazardous in children than in adults because of appetite loss and acute disturbances in nutritional status and electrolyte balance. Intensification of chemotherapy regimens administered to children with cancer usually results in highly emetogenic protocols which are frequently administered over several days- a trend which has considerably increased the risk of emesis.

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None of the traditional antiemetics is entirely effective either alone or in combination, and their side effects, such as extrapyramidal reactions and marked sedation, are frequently observed in children[1-3]. Over the past few years, 5-HT3 receptor antagonists have proved remarkably effective in the prevention of nausea and vomiting in children[4-9]. Tropisetron is a selective antagonist of the 5-HT3 receptor. In some previous studies, a high safety and efficacy profile for tropisetron in children was reported[6-9]. The aim of this study was to confirm the reported efficacy and safety of tropisetron in the prevention of nausea and vomiting in children receiving emetogenic chemotherapeutic regimens.

Material and Methods

Twenty-two children receiving chemotherapy for the treatment of malignant diseases were studied. The patients were recruited from the children routinely treated at our center. All the patients had histopathologically proven malignant diseases and were scheduled to receive at least two courses of highly emetogenic chemotherapy. No patient was excluded based on prior chemotherapy or radiotherapy.

In this study, a single daily dose of tropisetron of 0.2 mg/kg, with a maximum of 5 mg, was used. Tropisetron (Navoban® ampule 5 mg/5 ml; Sandoz, Basel, Switzerland) was given intravenously (i.v.) 15 minutes before the chemotherapy on the first day. On the subsequent days, patients received tropisetron with the same i.v. dose if i.v. route was maintained. Otherwise, it was given orally in capsule form (Navoban® capsule 5 mg; Sandoz, Basel, Switzerland) or as a drink solution immediately after diluting the appropriate amount of tropisetron from the ampule in orange juice one hour before food intake. Following the last dose of chemotherapy, tropisetron was continued for five days if the regimen contained cisplatin (CDDP), otherwise it was continued for three days. Corticosteroids were allowed only if they were part of the chemotherapy protocol or were given as a premedication to prevent hypersensitivity reactions. All the patients had adequate renal and hepatic functions prior to the therapy.

Chemotherapy regimens were classified by emetic grade[1-4] as adapted from Bleiberg et al.[10] (Table I). The grade of a course was determined by the highest emetic grade of each agent at the dose used. Chemotherapy of emetic grade 3 was upgraded when combined with grade 2 or 3 emetogenic agents. Regimens containing at least two drugs of grade 2 were considered as grade 3.

Chemotherapy protocos used during the study were heterogeneous, so we also classified the data according to the daily-administered emetogenic drugs using the same criteria as for emetogenic potential. Examples of this classification are given in Table II.

Table I: Criteria for Emetic Grade of Chemotherapeutic Agents* (dose in mg/m^2)[10]

Grade 1	Grade 2	Grade 3	Grade 4
Bleomycin	Dactinomycin < 0.3	Cisplatin ≥ 20	Cisplatin ≥ 60
Etoposide	Cyclophosphamide < 300	Carboplatin ≥ 150	Dactinomycin ≥ 0.45
Procarbazine	Doxorubicin < 45	Dactinomycin ≥ 0.3	Cyclophosphamide ≥ 1000
Mercaptopurine	Ifosfamide < 1000	Chlormethine ≥ 6	Cytarabine ≥ 1000
Thioguanine	Methotrexate < 3000	Cyclophosphamide ≥ 300	Ifosfamide ≥ 3000
Vinblastine		Cytarabine ≥ 150	
Vincristine		Dacarbazine ≥ 100	
		Daunorubicin ≥ 45	
		Doxorubicin ≥ 45	
		Ifosfamide ≥ 1000	
		Methotrexate ≥ 3000	

* Only the drugs used in this study are included.

Table II: Examples of Daily Schedule-Based Classification of Chemotherapy Regimens

Regimen	Drugs (mg/m^2)	Daily Schedule	Emetogenic Grade
ICE	Ifos (1500)	Day 1: Ifos/E	3
	Carbo (500)	Day 2: Ifos/E	3
	E (100)	Day 3: Ifos/E/Carbo	4
ABVD	Doxo (25)	Day 1: Doxo/Bleo/VBL/DTIC	4
	Bleo (10)		
	VBL (6)		
	DTIC (375)		
NHL-BFM 90	Dexa (10)	Day 1: Dexa/Ifos/VCR/MTX/*it	3
B-cell AA Block	Ifos (800)	Day 2: Dexa/Ifos	2
	E (100)	Day 3: Dexa/Ifos	2
	ARA-C (150x2)	Day 4: Dexa/Ifos/E/ARA-C	4
	VCR (1.5)	Day 5: Dexa/Ifos/E/ARA-C/*it	4
	MTX (1000)		

Ifos: Ifosfamide, Carbo: Carboplatin, E: Etoposide, Doxo: Doxorubicin, Bleo: Bleomycin, VBL: Vinblastine, DTIC: Dacarbazine, Dexa: Dexamethasone, ARA-C: Cytosine arabinoside, VCR: Vincristine, MTX: Methotrexate, Pred: Prednisolone, *it: Triple intrathecal therapy including MTX(6)+ARA-C(15)+Pred(5).

The antiemetic efficacy of tropisetron was assessed using a grading scale based on the combined measure of both emesis and nausea. The response to tropisetron per 24-hour period for each course was graded as:

Complete response : No vomiting, nor nausea.

Major response : 1-2 vomits or mild nausea.

Minor response : 3-5 vomits and/or less than 5 hours of moderate nausea interfering with daily activities.

Failure (no response) : More than 5 vomits and/or at least 5 hours of severe, bedridden nausea.

The cost effectiveness of tropisetron was also evaluated by determining its cost for the total 125 courses. Total cost was divided by the number of courses with complete control of acute emesis to calculate the cost per successfully controlled course. Results are given in US dollar (USD).

Results

A total of 22 children (11 boys and 11 girls) who received emetogenic chemotherapy were studied. Their median age was 14 years (range 3 to 18 years). Some characteristics of the patients are given in Table III. Four patients had received chemotherapy prior to the study and five had radiotherapy previously. There were two patients with a central nervous system tumor removed surgically without any trace, with no signs of increased intracranial pressure that might induce emesis.

Table III: Patient Characteristics

Total number of patients	: 22
Male/female	: 11/11
Median age (range) in years	: 14 (3-18)
Age groups	
< 10 years	: 3
≥ 10 years	: 19
Diagnosis	
Osteosarcoma	: 4
Hodgkin's disease	: 4
Ewing's sarcoma	: 4
Non-Hodgkin's lymphoma	: 3
Rhabdomyosarcoma	: 2
Malign mesenchymal tumor	: 2
Central nervous system tumor	: 2
Germ cell tumor	: 1

A total of 125 courses of grade 3 or 4 emetogenic chemotherapy were administered to 22 children (median:6, range: 2 to 15 courses) using 15 different regimens for the treatment of eight different types of tumors (Table IV). The duration of the regimens differed largely, from one to six days. These regimens contained different combinations of anticancer drugs. In some of the multiple-day courses, the emetogenic potential for each subsequent day was different. Some of the protocols like PNET III (UKCCSG 9102), NHL-BFM 90 B cell protocol, and CCG 94-7921 osteosarcoma protocol consisted of sequential blocks (courses), each of which included different emetogenic combinations (Table IV). Because of these variations, it was not possible to evaluate response rate in terms of control of daleyad emesis since some protocols were single-day

regimens and others had a duration of up to six days. Therefore, the efficacy results for tropisetron are given as "first day response during a course" which is consistent with control of acute emesis to provide uniformity between these heterogeneous chemotherapy courses. In this study, subgroup analyses could not be performed because of the small number of each unique course. Combination chemotherapy was administered on a single day for 28 courses (22%) and on multiple days for 97 courses (78%). All 22 patients received tropisetron at least during two courses of chemotherapy. Corticosteroids were part of the chemotherapy regimen in 13 courses (10%), and were administered as part of a premedication protocol with diphenhydramine to prevent allergic reactions in 10 courses (8%). Seventeen out of 125 courses contained CDDP; of these patients were under routine premedication with corticosteroid and diphenhydramine.

Antiemetic efficacy was also velauated using the daily schedule based responses in a total of 342 days of chemotherapy.

Table IV: Chemotherapeutic Regimens Used During the Study

Regimen	Tumor types	Emetic grade of the combination	Duration of a course	No. of courses	Corticosterold (cs) diphenhydramine (dph)
1. Single day regimens					
VAC	Soft tissue sarcoma	4	1	8	-
ABVD	Hodgkin's disease	4	1	15	-
MOPP	Hodgkin's disease	3	1	3	cs
COPP	Hodgkin's disease	3	1	2	cs
2. Multiple-day regimens (same drugs in each course)					
ICE	Resistant tumors	4	3	29	-
CEV/CE	Resistant tumors	4	3	15	-
VAI	Soft tissue sarcoma	4	5	3	-
CDDP+Paclitaxel	Resistant tumors	4	6	4	cs+dph
PVB	Germ cell tumors	4	3	6	cs+dph
3. Multiple-day regimens (different drugs in sequential courses)					
NHL-BFM 90-B cell	B cell NHL	3-4	5	6	cs
ALL-Rezidive BFM 90	Relapsed NHL	4	6	2	cs
PNET III	Medulloblastoma	4	3	3	-
EICESS 92	Soft tissue sarcoma	4	3	11	-
CCG 94-7921	Osteosarcoma	3-4	1-5	9	-
Osteosarcoma protocol	Osteosarcoma	4	2	9	-

Efficacy Results for the Courses: Overall complete response on day 1 was observed in 80 out of 125 courses (64%). A complete and major response rate on day 1 of Course I, Course II and overall 125 courses was observed in 73, 77 and 80 percent, respectively (Fig. 1). There were seven CDDP-containing courses given without corticosteroid. In this group, none of the patients was a complete responder on day 1: there were one major and five minor responses and one failure. On the other hand, CDDP courses given with corticosteroid and diphenhydramine (n: 10) resulted in five complete and four minor responses and one failure noted on the first day. When we excluded the courses with CDDP

and courses including diphenhydramine and/or corticosteroid, complete response was 68 percent on day 1 (n: 95 courses). There were 13 non-CDDP courses given with dexamethasone. In this latter group, 11 of the 13 courses resulted in a complete response on the first day. There were a limited number of patients in subgroups on different schedules. This study was not designed to test the hypothesis based on these smaller subgroups. Therefore, subgroup analyses could not be performed and statistics are purely descriptive.

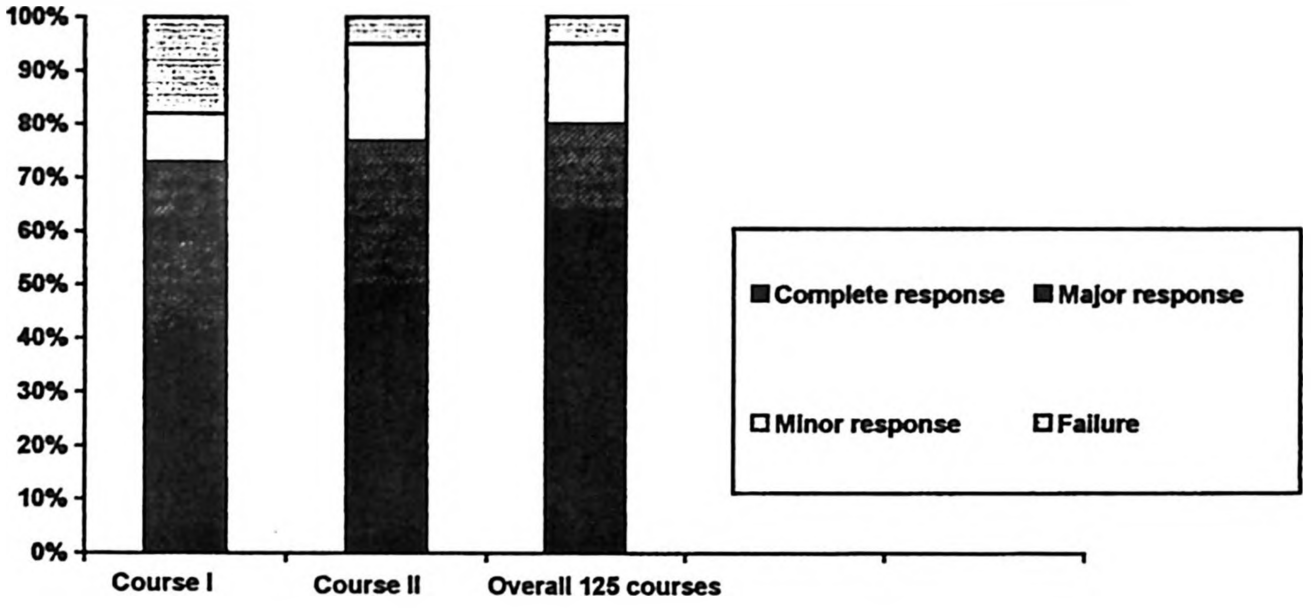


Fig. 1: Efficacy results for the first (Course I) and second (Course II) 22 courses and for the total 125 courses.

Efficacy Results for the Daily Schedules: A total of 342 days of emetogenic chemotherapy were administered during the study. Complete control of emesis was achieved in 246 (72%) days, while major and minor responses were observed in 42 (12%) and 39 (11%) days, respectively. Complete response rate was 59 percent for grade 4, 85 percent for grade 3 and 75 percent for grade 1-2 emetogenic daily schedules (Tables V-VI-VII). When CDDP-containing courses and courses administered with corticosteroids were excluded from the analyses, complete response rates for grade 4, 3 and 1-2 emetogenic daily schedules were 62, 84 and 82 percent, respectively. Response rates according to the daily emetic schedules are given in Figure 2.

Safety Results: A mild and self-limited diarrhea in two courses (1.6%) and dry-mouth in three courses (2.4%) were observed during the study. Routine microbiological examinations did not reveal any specific etiology for diarrhea. No other systemic or local side effect was observed.

Cost Effectiveness Analyses: A total of 302 ampules and 316 capsules were used during the study. Total cost of the antiemetic drug was 1,2950 USD. The cost per successfully controlled course was 162 USD.

Table V: Efficacy Results for Grade 4 Emetogenic Daily Schedules

Daily Emetogenic Regimen (mg/m²)	No of Days	Response (Days)				cs/dph
		Complete	Major	Minor	Failure	
Ifos (1500)/E(100)/Carbo (500)	35	30	3	2	0	-/-
Ifos (2000)/E (100)/Doxo (20)/±VCR (1.5)	18	6	7	2	3	-/-
Ifos (2000)/E (100)/Act-D (0.5)/VCR (1.5)	16	6	6	4	0	-/-
Doxo (25)/Bleo (10)/VBL(6)/DTIC (375)	15	9	2	3	1	-/-
Ifos (1800)/Act-D (0.15)/±VCR (1.5)	15	11	1	3	0	-/-
Ifos (1800)/Doxo (25)	10	7	3	0	0	-/-
CDDP (120)/Doxo (25)	8	0	1	5	2	-/-
CYC (600)/Doxo (60)/VCR (1.5)	8	7	1	0	0	-/-
CDDP (120)	6	1	0	5	0	+/+
Bleo (15)/CYC (600)/Act-D (0.6)	6	1	1	2	2	-/-
Dexa (20)/VCR (1.5)/ARA-C (2x2000)/*it	2	2	0	0	0	+/-
Dexa (20)/ARA-C (2x200)	2	2	0	0	0	+/-
Dexa (20)/6 TG (100)/DNR (50)/Ifos (400)	1	1	0	0	0	+/-
Dexa (20)/6 MP (100)/ARA-C (2x2000)	1	1	0	0	0	+/-
Total (%)	143 (100)	84 (59)	25 (17)	26 (18)	8 (6)	

cs: crticosteroid, dph: Diphenhydramine, Ifos: Ifosfamide, Carbo: Carboplatin, E: Etoposide, Doxo: Doxorubicin, VCR: Vincristine, Act-D: Actinomycine D, Bleo: Bleomycin, VBL: Vinblastine, DTIC: Dacarbazine, CDDP: Cisplatin, CYC: Cyclophosphamide, Dexa: Dexamethasone, ARA-C: Cytosine arabinoside, 6TG: 6 Thioguanine, DNR: Daunorubicin, 6MP: 6 Mercaptopurine *it: Triple intrathecaltherapy including MTX(6)+ARA-C(15)+Pred(5).

Table VI: Efficacy Results for Grade 3 Emetogenic Daily Schedules

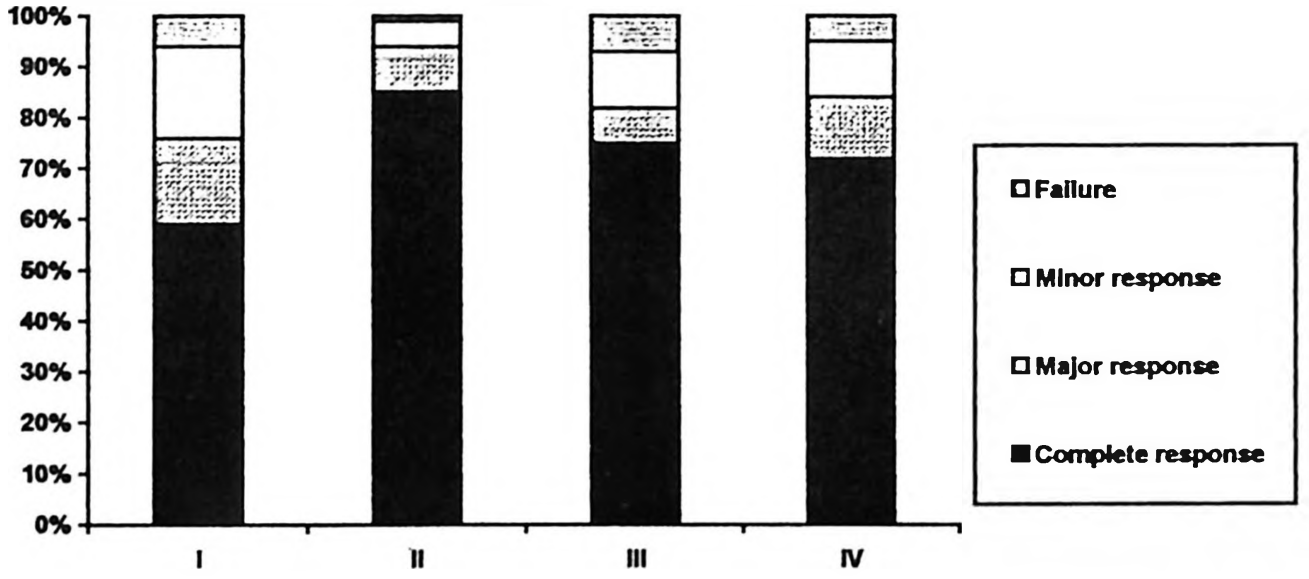
Daily Emetogenic Regimen (mg/m²)	No of Days	Response (Days)				cs/dph
		Complete	Major	Minor	Failure	
Ifos (1500)/E(100)	70	60	8	2	0	-/-
CDDP (20)	20	17	3	0	0	-/-
Carbo (500)/E (100)	7	5	0	1	1	-/-
Carbo (500)/E (100)/VCR (1.5)	6	4	0	1	1	-/-
MTX (18000)	6	5	1	0	0	-/-
Ifos (1800)	4	4	0	0	0	-/-
Meclor (6)/VCR (1.5)/Proc (100)/Pred (40)	3	1	0	2	0	+/-
Dexa (10)/CYC (200)/Doxo (25)	2	2	0	0	0	+/-
Dexa (10)/CYC (200)/Doxo (25)/*it	2	2	0	0	0	+/-
Dexa (10)/VCR (1.5)/MTX (1000)/Ifos(800)/*it	2	2	0	0	0	+/-
CYC (600)/VCR(1.5)/Proc(100)/Pred (40)	2	2	0	0	0	+/-
Dexa(10)/VCR(1.5)/MTX(1000)/CYC(200)/*it	2	2	0	0	0	+/-
Dexa(10)/Ifos(800)E(100)/ARA-C(150x2)	2	2	0	0	0	+/-
Dexa(10)/Ifos(800)E(100)/ARA-C(150x2)/*it	2	2	0	0	0	+/-
Dexa(20)/6TG(100)/VCR(1.5)/MTX(1000) /Ifos(400)/*it	1	1	0	0	0	+/-
Total (%)	131 (100)	111 (85)	12 (9)	6 (5)	2 (1)	

cs: crticosteroid, dph: Diphenhydramine, Ifos: Ifosfamide, E: Etoposide, CDDP: Cisplatin, Carbo: Carboplatin, VCR: Vincristine, MTX: Methotrexate, Mechor: Meclorethamine, Proc: Procarbazine, Pred: Prednisolone, Dexa: Dexamethasone, CYC: Cyclophosphamide, Doxo: Doxorubicine, ARA-C: Cytosine arabinoside, 6TG: 6 Thioguanine, *it: Triple intrathecal therapy including MTX(6)+ARA-C(15)+Pred(5).

Table VII: Efficacy Results for Grade 1-2 Emetogenic Daily Schedules

Daily Emetogenic Regimen (mg/m²)	No of Days	Response (Days)				cs/dph
		Complete	Major	Minor	Failure	
E (100)	22	17	1	2	2	-/-
VBL(6)	6	5	1	0	0	+/+
VBL(6)/Bleo(10)	6	0	3	2	1	+/+
Doxo(30)	6	1	0	3	2	-/-
Paclitaxel(135)	4	4	0	0	0	+/+
Dexa(20)/Ifos(800)	4	4	0	0	0	+/-
Dexa(10)/CYC(200)	4	4	0	0	0	+/-
Dexa(10)/E(100)	4	4	0	0	0	+/-
Dexa(20)/6TG(100)/Ifos(400)	3	3	0	0	0	+/-
Dexa(20)/6MP(100)	3	3	0	0	0	+/-
Dexa(20)/VCR(1.5)/L-Asp 25000 U/m²	2	2	0	0	0	+/-
Dexa(20)/VCR(1.5)/L-Asp 25000 U/m²	2	2	0	0	0	+/-
Dexa(10)/E(100)*it	2	2	0	0	0	+/-
Dexa(20)/VCR(1.5)MTX(1000)/6MP(100)/*it	2	2	0	0	0	+/-
Total (%)	68 (100)	51 (75)	5 (7)	7 (11)	5 (7)	

cs: crticosteroid, dph: Diphenhydramine, E: Etoposide, VBL: Vinbastine, Bleo: Bleomycin, Doxo: Doxorubicin, Dexa: Dexamethasone, Ifos: Ifosfamide, CYC: Cyclophosphamide, 6TG: 6 Thioguanine, 6MP: 6 Mercaptopurine, VCR: Vincristine, L-ASP: L-Asparaginase, MTX: Methotrexate, *it: Triple intrathecal therapy including MTX(6)-ARA-C(15)+Pred(5).



I. Efficacy for days with grade 4 emetogenic potential.
II. Efficacy for days with grade 3 emetogenic potential.
III. Efficacy for days with gradel-2 emetogenic potential.
IV. Efficacy for the total 342 days.

Fig. 2: Efficacy results according to the daily emetic schedules.

Discussion

Efficacy of an antiemetic drug used in multiple-day chemotherapy regimens depends on several factors. The duration of these regimens may differ largely from one protocol to another. Emetic grade of the drugs administered on each

day of the multiple-day regimen may be significantly different. For example, BFM 90 NHL B cell protocol consists of three different "five-day block" administered sequentially fortnightly. Each of these blocks includes a different combination of chemotherapeutics with the emetic grade of a given day changing from grade 2 to grade 4. It is not appropriate to classify a "five-day block" as a grade 4 emetic regimen since the patients receive only low emetogenic drugs on some of the days while high emetogenic combinations are given on the other days. Therefore, only first day responses were evaluated in our study, on each of which grade 3 or grade 4 emetogenic drugs were administered.

The results obtained in this study were mostly in agreement with the previous studies regarding tolerability and efficacy of tropisetron in the control of acute emesis in children and adolescents[5-9]. Side effects of tropisetron were almost totally absent as was the case in the previous studies performed in children. In terms of efficacy, complete response rate on day 1 was 64 percent for the total of 125 courses and failure rates were low (5% on day 1). Gershanovich et al.[13] reported a complete response of 69 percent on the first day of chemotherapy courses in children. In another study[6], 67 percent overall complete response was observed on day 1 in a total of 455 courses administered to pediatric patients Cefalo et al.[7] reported a good control (less than 2 emetic episodes per day) in 64 percent of the 184 treatment days in a pediatric population. The results of another study[8] showed a complete response rate of 77 percent in previously treated pediatric patients receiving non-CDDP regimens.

In the present study, the number of courses with CDDP was small, so a comparison between CDDP and non-CDDP courses was not possible. In seven courses with CDDP but without corticosteroid, there was no complete response on the first day. In a previous study[9], a complete response rate of 53 percent was reported on day 1 in children receiving CDDP-containing regimens. The control of emesis improved in 10 CDDP courses administered with dexamethasone and diphenhydramine (5 complete responses on day 1). We also observed better response rates for the control of acute emesis when corticosteroids were part of the protocol in non-CDDP courses; 85 percent complete response was achieved on the first day. These results confirmed the previously reported results about the increased efficacy of tropisetron with the addition of dexamethasone, as is true for all 5HT3 receptor antagonists[11, 13]. In this study, corticosteroid-containing CDDP courses also included diphenhydramine. Diphenhydramine is an effective antiemetic for motion sickness and may be used in combination with other antiemetics to potentiate effectiveness. This additional antiemetic effect probably played a role in the improved response rates observed in corticosteroid-containing courses. Control of acute emesis for the first and second courses was similar. This finding indicates a consistent response for tropisetron over courses based while on multiple chemotherapy.

In our study, emetogenic chemotherapy was given according to 15 different protocols resulting in heterogeneous data for the evaluation of the antiemetic response. Therefore, another evaluation based on the daily schedules was performed. Overall complete response rate for grade 3 and 4 emetogenic daily schedules was 85 and 59 percent, respectively, while low emetogenic (grade 1, 2) daily schedules resulted in a 75 percent compelete response. Because of the limited number of subgroups, statistical analyses could not be performed. In any case, several factors might have interfered with these results. There were some schedules given with diphenhydramine and/or corticosteroid (mainly dexamethasone). Administration of corticosteroids in 18 out of 131 treatment days probably improved the response rate for the grade 3 emetogenic daily schedules. Another important factor we had to overlook was the effect of the previous day's emetic schedule over a given day. For example, six days with vinblastine and bleomycin combination, which is known to be low emetogenic (grade 1), resulted in three major and two minor responses, and no complete response was noted. This combination is part of a protocol (PVB) containing CDDP on day 1, so this poor response can easily be explained by the delayed effect of CDDP. Additionally, our study population consisted mostly of adolescents (86% of the whole group), whose memory of previous episodes of emesis is a major problem interfering with the incidence and severity of nausea and vomiting[14].

In this study, the cost of tropisetron per successfully controlled course was 162 USD. In 45 courses (36%), nausea and vomiting could not be completely controlled. Our study was not designed to test the hypothesis based on subgroups; subgroup analyses could not be performed because of the limited number of each unique course. Therefore, the results are merely descriptive and can be used only to generate further hypotheses. We are planning a prospective trial using daily administered emetic drug based data for multiple-day regimens in order to design anantiemetic porotocol for children which is equally effective but lower in cost than the commonly used antiemetic regimens. Even though the advantages of the 5-HT3 receptor antagonists over traditional antiemetics are generally acknowledged, it is of interest to compare these drugs. A careful review of the literature by Roila et al.[15] revealed 22 comparative studies among the 5-HT3 receptor antagonists. The authors stated that several of these trials have some important shortcomings, especially in the study design, the size of the population studied and the type of antiemetic treatment selected, making their conclusions difficult to interpret. However, some randomized, double-blind studies have shown that the antiemetic activity and tolerability of ondansetron, granisetron and tropisetron are almost identical, at least in the prevention of cisplatin-induced emesis[16-20]. Therefore, from the efficacy and safety point of view, there is no reason to prefer one compound over the other. From the economic perspective, however, differences may exist and choice should be based on acquisition cost in each country, taking into account optimal dose and schedule.

In spite of the large number of studies investigating the efficacy of antiemetic agents in adults, only a few double-blind, randomized and multicentric studies have been reported for children and adolescents. Since the data from adult cancer patients cannot be extrapolated to pediatric oncology practice, multicentric trials in children will provide important clues to develop a consensus about the best practices and to encourage adherence to them.

In conclusion, the results of this study confirmed that tropisetron is a safe, well tolerated and effective antiemetic in prevention of acute emesis induced by high emetogenic regimens in children and adolescents. A single daily dose seems to be advantageous for both the patient and the physician.

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BIPHENOTYPIC CHARACTERISTICS, CELL SIZE AND PROGNOSIS IN CHILDHOOD ACUTE MYELOBLASTIC LEUKEMIA*

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SUMMARY: Olcay L, Hiçsönmez G, Ertem U, Okur H, Tuncer AM. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, and Division of Oncology, Dr. Sami Ulus Children's Hospital, Ankara, Turkey). Biphenotypic characteristics, cell size and prognosis in childhood acute myeloblastic leukemia. Turk J Pediatr 1999; 41: 219-224.

In order to determine the prognostic significance of cell size together with expression of biphenotypic markers in childhood acute myeloblastic leukemia (AML), we evaluated the cell size of children with AML, 12 with and 21 without biphenotypic markers. The patients were followed up for at least 12 months. The cells which were stained with FITC conjugated surface marker antibodies were divided into small, middle or large cell groups according to their mean channel number of forward scatter by flow cytometry. Nine of 12 biphenotypic and 15 of 21 non-biphenotypic children either died or relapsed within the first 12 months. The percentages of the small, middle and large cells were similar in children and in deceased patients, regardless of whether or not they expressed biphenotypic markers. We believe that biphenotypic marker expression is a poor prognostic factor regardless of cell size. *Key words: cell size, biphenotypic expression, childhood, acute myeloblastic leukemia.*

The known prognostic factors for acute myeloblastic leukemia (AML) are not as definite as they are for acute lymphoblastic leukemia (ALL)[1]. Although many factors have been examined for their prognostic role, the number of studies which have examined biologic and prognostic significance of myeloblast size is very few[2,3], (unpublished data), unlike with ALL[4].

Although we have shown that the percentage of large cells and their ratio to small cells were higher in nAML children with very short survival than in children who lived[3], the extensive form of this study, together with Kawada et al'.s[2] study, demonstrated no definite relationship between individual cell size and prognosis. Kawada et al.[2] established a relationship between myeloblast size and prognosis dependent on some surface markers. We established a relationship between blast cell size and prognosis dependent on high leukocyte count (unpublished data) in children with AML. Biphenotypic appearance of AML (presence of 2

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or more lymphoid markers) is known as a poor prognostic factor[1]. We carried out this study to evaluate the significance of cell size together with biphenotypic expression.

To our knowledge, this study is the first to investigate the prognostic significance of cell size together with biphenotypic expression in childhood AML.

Material and Methods

Patients: Children who were diagnosed as AML and underwent flow cytometric evaluation at admission between November 1993 and June 1996 were included in the study (median age: 11; mean age: 9.80; range: 1.5-16; 16 female, 17 male). Of these, 32 were de novo AML; one converted to AML from Fanconi's aplastic anemia [diepoxybutane (DEB) positive]. According to French-American-British (FAB) criteria, the patients had M1 (7 cases), M2 (14 cases), M3 (4 cases), M4 (5 cases), M6 (2 cases), M7 (1 cases) types.

Twenty-four of the patients died or relapsed from the 5^{th} day to the 12^{th} month of the treatment (median age: 10.5; mean age: 9.29; range: 2-15; 12 female, 12 male). Nine of the patients survived more than 12 months (median age: 13; mean age: 11.16, range: 15-16; female, 5 male): five for 13 months, two for two years and two for three years. Therapy of two patients was stopped two months before the completion of this study. Period of follow-up has been one to three years.

Of 33 patients, 12 did (median: 13; mean: 10.4; range: 2-16; 8 female, 4 male) and 21 did not (median: 10; mean: 9.59; range: 18 months-16 years; 8 female, 13 male) express biphenotypic markers. Of these, nine of the biphenotypic patients and 15 of the non-biphenotypic patients died or relapsed within the first 12 months. The others were alive and in remission when the study ended. According to French-American-British classification, the deceased biphenotypic patients had M4 (3 cases), M2 (5 cases, 1 being granular), and M1 (1 cases) types; the three living biphenotypic patients had M1 (one case), M2 (1 case), and M4 with eosinophilia (1 case).

Twenty-eight of these children were diagnosed and treated at Hacettepe Universiy, İhsan Doğramacı Children's Hospital, and five in Dr. Sami Ulus Children's Hospital. Thirty-two patients received the Hacettepe AML protocol[5] (11 received the 1993 version of this protocol and 21 the 1995 version) and one the Denver protocol[6].

Cytogenetic analysis was available in 10 of the 24 patients who died or relapsed within the first year, and eight of nine patients who lived for more than 12 months. In the former group abnormal cytogenetic results were as follows: 20 percent hypodiploidy, t(4;8) (q31,2;p23,1); trisomy 22; and trisomy 8 plus 22 in the same patient, dup (17)(q24), del (Y)(q11,23), t(8;21). For the latter group abnormal results were: del 13 (q12,1) and t(X;21)(q27,3;q21,1) in the same patient and trisomy 22 in another.

Flow Cytometric Analysis: Heparinized peripheral blood (PB) (n:10) or aspirated bone marrow (BM) (n: 23) samples were obtained before the treatment. The mean percentage of leukemic blasts which were determined morphologically was 66.47 percent for BM samples and 76 percent for PB samples. The size of blast cells were determined by flow cytometry (Becton Dickinson FAC Scan, Lysis I program) together with surface markers (CD2, CD3, CD5, CD7, CD22, CD10, CD20, CD19, HLA-DR) using fluorescein-isothiocyanate (FITC) conjugated antibodies[7]. Before analysis, the gated cells were examined on cytospin slides and the blast population was confirmed as more than 90 percent. The cells between 200-400, 400-600, 600-800 on forward scatter were considered as small, middle, or large cells, respectively, and cells were determined as a percentage. When more than 20 percent of blast cells expressed two or more lymphoid markers together with myeloid markers, these patients were considered biphenotypic. Two classifications were made to determine the significance of biphenotypic expression to cell size. In the first classification, the percentage of small, middle or large cells of the biphenotypic patients were compared with those of patients who were not biphenotypic. In the second classification, only the deceased-relapsed patients were evaluated. Among these, the percentages of small, middle or large cells of biphenotypic patients were compared with those of the patients who were not biphenotypic.

For statistical analysis, Mann-Whitney U test was used to compare the groups.

Results

In the first classification, the small, middle and large cells of the biphenotypic patients were compared with those of the patients who were not biphenotypic (Table I). It was striking that the percentages of small, middle and large cells of the patients were similar whether or not they expressed biphenotypic markers (34.19 ± 3.18 vs 39.39 ± 4.60; 41.67 ± 3.64 vs 38.78 ± 3.31; 24.12 ± 3.17 vs 22.03 ± 3.16, respectively) (p: 0.76; 0.82; 0.48).

Table I: Dispersion of Different-Sized Cells According to Biphenotypic Expression*

	Small		Middle		Large	
	B*Ψ	NB∂	B	NB	B	NB
N	12	21	12	21	12	21
Mean ± SE	34.19 ± 3.18	39.39 ± 4.60	41.67 ± 3.64	38.78 ± 3.31	24.12 ± 3.17	22.03 ± 3.16
Range	17.85-49.72	10.85-86.77	17.66-64.95	5.54-57.98	8.84-34.13	1.53-53.30
P		0.76		0.82		0.48

* Nine of the 12 (75%) biphenotypic patients died during the first year; three have been in remission for more than 12 months.

Ψ: Biphenotypic.
∂: NB: Non-biphenotypic.

In the second classification, only deceased patients were considered. The small, middle and large cells of the patients who were biphenotypic were compared with those of patients who were not biphenotypic (Table II). The percentages of the small, middle and large cells of patients who were biphenotypic were found similar to those of the patilents who were not biphenotypic (31.69 ± 3.19 vs 35.10 ± 4.19; 40.76 ± 4.81 vs 43.59 ± 2.60; 27.53 ± 3.24 vs 21.59 ± 3.53) (p: 0.70; 0.61; 0.20).

Table II: Dispersion of Different-Sized Cells in Deceased Patients According to Biphenotypic Expression

	Small		Middle		Large	
	B$^{\Psi}$	NB$^{\partial}$	B	NB	B	NB
N	9	15	9	15	9	15
Mean ± SE	31.69 ± 3.19	35.10 ± 3.19	40.76 ± 4.81	43.59 ± 2.60	27.53 ± 3.24	21.59 ± 3.53
Range	17.85-42.69	10.85-66.03	17.66-64.95	27.01-57.98	17.18-42.97	1.53-46.14
P		0.70		0.61		0.20

Ψ: Biphenotypic.
∂: Non-biphenotypic.

Discussion

In ALL, the size of the blasts are known to have prognostic significance. Presence of blasts with a large diameter (more than 2 times that of the erythrocyte diameter of the same patient established by peripheral blood smear examination) is a poor prognostic factor for survival independent of age and total leukocyte count[4]. In patients with lymphoma, cell size has been examined to determine any relationship with surface antigen expression[8,9]. In AML, the significance of blast cell size has been the subject of a few studies[2,3,10], (unpublished data). The first study was conducted by Kawada et al.[2], who planned to establish the role of cell size on heterogeneity of myeloblasts and to determine the relationship between cell size and surface markers in 23 adults with AML. It was demonstrated that the percentage and/or mean fluoresclence intensity (MFI) of surface markers (CD13, CD33, CD38, CD34, HLA-DR) were higher on the large cells than on the small cells. Furthermore, patients with a low ratio of MFI of antigen expression (CD13, CD38, CD33, HLA-DR) on the large cells to antigen expression on the small cells as a percentage exhibited longer remission durations or survival periods. The second study was ours[3]. In this preliminary study consisting of seven children with AML it was shown that the average percentage of large cells and the average ratio of large cell percentage to small cell percentage was higher in deceased patients than in patients who lived; surface markers were not taken into account[3]. And, in another study which was an extensive form of this study, we could not establish a definite relationship between cell

size at admission and prognosis (unpublished data). However, we determined that cell size of the blasts affected prognosis dependent on high leukocyte count (unpublished data). Kwong et al.[10] demonstrated near tetraploidy in large blasts of an adult AML patient.

The DNA synthesis rate and, therefore, cell proliferation rate, determined by tritiated thymidine uptake test in patients with infectious mononucleosis were demonstrated to be higher in the monocytes than in the large lymphocytes and higher in large lymphocytes than in small lymphocytes. In addition it was shown that a large mononuclear cell in infectious mononucleosis was equivalent to a myeloblast in the bone marrow[11]. DNA content in the band cells of normal people have been more than that found in the neutrophils[12]. Gavasto et al.[13], who examined the proliferation rate of blasts in connection with blast size in eight patients with different kinds of leukemia, demonstrated that thymidine uptake increased as blast size increased. Terasima et al.[14] demonstrated in synchronously dividing populations of HeLa cells that cell volume gradually increased during mitosis, reached maximum at the 18th hour and then gradually decreased. Although Minden et al.[15] showed in 1978 that the young progenitor cells were small in volume, contrary to general expectations, this has not been confirmed. In view of this, we consider the large cells determined by flow cytometry in our study as young cells with a high proliferation capacity.

In this study, death of nine of the 12 biphenotypic patients supports the theory that presence of biphenotypic expression is a poor prognostic factor. However, no difference could be established in the percentages of cells with different size, whether the patients were biphenotypic or not. Therefore, biphenotypy is a poor prognostic factor independent of cell size.

It has been reported that CD3 and CD7 were expressed most often on small (mature) cells, in patients both with good and poor prognosis (unpublished data). On the other hand, the CD3 percentage in small cells was found significantly higher than that of deceased-relapsed patients.

CD10 was found more often on the large (immature) rather than middle cells of the patients with a poor prognosis, while the percentages of CD10 on small, middle and large cells of patients with a good prognosis were similar. Moreover, the ratio of the percentage of CD20 expression on large and small cells was significantly higher in the deceased or relapsed patients compared to patients who lived. Therefore, we believe that CD10 and CD20 may be valuable parameters for poor prognosis.

On the other hand, the expression of CD10 more often on the large rather than middle cells in patients with a good prognosis and the similarity of CD19 expression on small, middle and large cells of patients with a poor prognosis (unpublished data) suggest that further studies in larger series are necessary to evaluate the prognostic role of expression of biphenotypy with each individual lymphoid marker.

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CYCLOSPORIN A PLUS PREDNISONE TREATMENT OF STEROID – SENSITIVE FREQUENTLY RELAPSING NEPHROTIC SYNDROME IN CHILDREN*

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SUMMARY: Aksu N, Türker M, Erdoğan H, Özinel S, Kansoy S. (Department of Pediatric Nephrology, Social Security Tepecik Teaching Hospital, Yenişehir-İzmir, Turkey). Cyclosporin A plus prednisone treatment of steroid-sensitive frequently relapsing nephrotic syndrome in children Turk J Pediatr 1999; 41: 225-230.

Recently, there have been numerous reports on the use of cyclosporin A (CyA) in children with nephrotic syndrome (NS). In this prospective study, we wanted to evaluate the efficacy of CyA together with prednisone therapy in children with steroid-sensitive frequently relapsing NS.

A total of 11 children (7 boys, 4 girls) with steroid-sensitive NS were included in this study. The patients ranged in age from 3.5 to 15 years (average 8.45 ± 4.26 years). Renal biopsy showed minimal change disease in five, mesangial proliferation in four, focal glomerulosclerosis in one and membranous glomerulonephritis in one. The NS had lasted from 13 to 113 months (average 50.27 ± 38.60 months). The number of relapses varied from three to 10 episodes with an average of 5.9 ± 3.3 episodes. Patients received 5 mg/kg CyA daily in two divided doses for five months and prednisone for a total of eight weeks (30 mg/m² daily for 4 weeks followed by 30 mg/m² on alternate days for 4 weeks). After the completion of the treatment protocol, no therapy was given unless a relapse was observed.

Mean follow-up period was 14.9 ± 5.99 months with a range from six to 26 months. Before this combined treatment, there was a mean relapse rate of 0.144 ± 0.05 relapses month with a range from 0.088 to 0.238. After discontinuation of therapy, the relapse rate dropped to a mean of 0.0179 ± 0.031 with a range of 0 to 0.083.

In conclusion, it would appear that a combination of CyA and prednisone is effective, sustaining the remission in steroid-sensitive NS. Corticosteroids in combination with CyA may be a better approach than conventional steroid treatment in such patients.
Key words: cyclosporin A, prednisone, frequently relapsing nephrotic syndrome, children.

Children with nephrotic syndrome (NS) are usually treated with corticosteroids alone or in combination with immunosuppressive agents[1-5]. It is well known that steroid-sensitive, frequently relapsing nephrotic syndrome is still an important clinical problem in pediatric renal clinics. Although conventional steroid treatment is effective, the steroid-sensitive frequently relapsing cases are complicated by steroid dependency and secondary complications with this treatment. These side effects of the drugs limit their use[6-8]. Recently, several papers have reported

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that cyclosporin A (CyA) might be beneficial in these situations[9, 10]. Although CyA could be used alone in treatment of NS, a combination of CyA and corticosteroids has also been reported[11, 12], with better results obtained in steroid-sensitive nephrotic syndrome[13, 14]. In this prospective study, we wanted to evaluate the efficacy of CyA together with prednisone therapy in children with steroid-sensitive frequently relapsing nephrotic syndrome.

Material and Methods

A total of 11 children (7 boys, 4 girls) under 15 years of age with steroid-sensitive nephrotic syndrome were included in this study. The patients ranged in age from 3.5 to 15 years (average 8.45 ± 4.26 years). The definitions of NS, remission, relapse and frequent relapsers were the same as used by the International Study of Kidney Disease in Children (ISKDC)[15]. Five had biopsy-proven minimal change disease, four had mesangial proliferation, one had focal glomerulosclerosis and one had membranous glomerulonephritis. The NS had lasted from 13 to 113 months (average 50.27 ± 38.60 months). The age at which the patients initially presented with NS varied from one to 12.5 years (mean 4.27 years) and the number of relapses varied from three to 10 episodes, with an average of 5.9 ± 3.3 episodes (Table I). All patients had previously received oral prednisone alone or incombination with cyclophosphamide upon each episode.

Table I: Clinical Data for Patients with Nephrotic Syndrome

No.	Patient	Age (y)	Sex	Age at Onset of NS (y)	Biopsy	Duration of NS (mo)	No of Episodes
1	K.A.	10	M	3.5	MCD	81	10
2	A.K.	4.5	F	2	MGN	28	3
3	E.Ö.	3.5	F	2.5	MCD	13	3
4	F.B.	8	M	7	MCD	13	3
5	O.Ç.	15	M	12.5	MCD	30	4
6	K.H.	5.5	F	3	Mes. Pro	29	3
7	E.E.	10.5	M	1	Mes. Pro	111	10
8	B.İ.	4	M	2.5	MCD	21	5
9	V.K.	14	M	4.5	FGS	113	10
10	C.T.	13	M	6	Mes. Pro	82	10
11	E.İ.	5	F	2.5	Mes. Pro	32	4
						X^2	5.9
						SD	± 3.3

MCD: minimal change disease; MGN: membranous glomerulonephritis; Mes. Pro: mesangial proliferation; FGS: focal glomerulosclerosis; y: year(s); mo: month(s); NS: nephrotic syndrome.

After informed consent was obtained, patients received 5 mg/kg CyA daily in two divided doses for five months and prednisone for a total of eight weeks (30 mg/m^2 daily for 4 weeks followed by 30 mg/m^2 on alternate days for 4 weeks). After the completion of the treatment protocol no therapy was given unless a relapse was observed. During the course of treatment no other drugs except diuretics were administered. During the course of the therapy, renal functions, liver functions, serum electrolytes, blood pressure and cyclosporin levels were monitored weekly in the first month, and monthly thereafter. Cyclosporin A CyA whole blood levels were measured 12 hours after the last CyA intake and determined by radioimmunoassay (RIA) method. The response to treatment was assessed in the conventional manner by clinical examination, determination of 24 hour urinary protein excretion andrenal function profiling which included serum albumin and cholesterol concentrations. Statistical evaluation was made using Chi-square and t test.

Results

All children completed the full trial. All patients came into full remission with a complete loss of proteinuria. The patients showed definite improvement and normalization of serum protein, albumin, triglyceride and cholesterol levels. Serum urea concentrations slightly increased during the treatment but returned to pre-treatment levels after CyA was discontinued. Serum creatinine concentrations remained normal (Table II). The mean blood levels of CyA were 136 ± 57.33 ng/ml. The relapse rates in the eleven patients were calculated as relapses per month. Mean follow-up period was 14.9 ± 5.99 months with a range from six to 26 months. Before the combination of CyA and prednisone treatment, there was a mean relapse rate of 0.144 ± 0.05 relapses/month with a range from 0.088 to 0.238. After discontinuation of therapy, the relapse rate dropped to a mean of 0.0179 ± 0.031 with a range of 0 to 0.083 (Table III).

Table II: Laboratory Parameters of Patients Before and After Treatment (Mean ± SD)

Parameters	Before	After	p
Serum protein (g/dl)	4.21 ± 0.36	7.03 ± 0.54	< 0.001
Serum albumin (g/dl)	1.69 ± 0.26	4.51 ± 0.45	< 0.001
Serum cholesterol (mg/dl)	486.6 ± 135.8	154.4 ± 28.7	< 0.001
Serum triglyceride (mg/dl)	389.7 ± 184.9	142.2 ± 75.3	< 0.001
Serum urea (mg/dl)	29.90 ± 6.52	34.18 ± 3.81	< 0.05
Serum creatinine (mg/dl)	0.62 ± 0.17	0.67 ± 0.20	> 0.05

Table III: Relapse Rates of Patients Before and After Treatment

	Pre-Treatment			Post-Treatment		
No.	No of Episodes	Relapse* Rate (rel/mo)	Follow-up Period (mo)	No of Episodes	Relapse*Rate (rel/mo)	Mean CyA Levels (ng/ml)
1	10	0.123	19	1	0.052	125.3
2	3	0.107	16	1	0.062	99
3	3	0.230	16	–	0.000	158.6
4	3	0.230	26	–	0.000	106.3
5	4	0.133	19	–	0.000	194.5
6	3	0.103	15	–	0.000	64.4
7	10	0.090	19	–	0.000	267.8
8	5	0.238	8	–	0.000	97
9	10	0.088	8	–	0.000	146.5
10	10	0.121	12	1	0.083	151.7
11	4	0.125	6	–	0.000	89.1
X^2	5.9	0.144	14.9	0.272	0.0179	136.38
SD	± 3.3	± 0.05	± 5.99	± 0.467	± 0.031	57.33

* Significance of relapse rates between pre- and post-treatment periods ($p < 0.005$).
rel: relapse; mo: month(s); CyA: cyclosporin A.

Hypertrichosis was recognized in five patients, but was not so disturbing. Gingival hyperplasia was seen in three patients. Chicken-pox occurred in one patient at the fifth month of treatment, but it is questionable whether or not this was related to the combined treatment.

Discussion

It is well known that steroid-sensitive frequently relapsing nephrotic sydrome is still a major unsolved clinical problem. These steroid-sensitive frequently relapsing nephrotic cases are complicated by steroid dependency and secondary complications by long-term use of corticosteroids[6, 7, 16].

The pathophysiology of nephrotic syndrome is still obscure. Recently, a variety of lymphokines have been identified in patients with nephrotic syndrome. It is known that on contact with a specific antigen, sensitized lymphocytes secrete these active lymphokines. It has been suggested that these lymphokines lead to the alteration of glomerular anionic sites resulting in increased capillary permeability to proteins[7, 10, 17]. This notion led to trials of CyA in the treatment of nephrotic syndrome. cyclosporin A CyA is a specific modulator of T cell function. The drug acts on T cells and specifically inhibits production of lymphokines (interleukin-2) from activated T helper cells, and reduces the release of interleukin-1 from the macrophages. Because of this specific action, it has

been suggested to extend the indications for CyA treatment to therapy of nephrotic syndrome which is thought to be T-cell mediated[18-20].

Cyclosporin A CyA in combination with corticosteroids has been proposed as a better approach in steroid-sensitive nephrotic patients. Cyclosporin A CyA duplicates the efficacy of steroids in nephrotic syndrome. This effect is possibly related to the similarities of action between these two drugs[11]. Our use of CyA together with corticosteroids in this study was an attempt to obtain a synergistic effect between the two drugs. Using this combined therapy, all patients showed a full remission with a complete loss of proteinuria. The patients showed definite improvement and normalization of nephrotic parameters. The duration of remission was significantly longer than that before initiation of combined treatment. There was a significant reduction in the relapse rate and the majority of patients did not experience any relapse with this treatment. Before the combination of CyA and prednisone therapy, there was a mean relapse rate of 0.144 ± 0.05 relapses/month with a range from 0.088 to 0.238. After discontinuation of this combined treatment, the relapse rate dropped to a mean of 0.0179 ± 0.031 with a range of 0 to 0.083 (Table III).

Clinical side effects of CyA treatment are reported as frequent, but of minor intensity. Side effects included renal insufficiency, gastrointestinal disturbances, hypertrichosis, mild hypertension, and gum hypertrophy in occasional cases[14, 21]. In our study, mean serum urea concentrations slightly increased during the treatment but returned to pre-treatment levels after CyA was discontinued. Mean serum creatinine concentrations remained normal. Slight hypertrichosis was observed in five patilents, but did not require any treatment. Gingival hyperplasia was seen in three patients. Chicken-pox occurred in one patient at the fifth month of treatment, but it is questionable whether this complication was primarily related to the combined treatment or to the natural course of the nephrotic syndrome.

In conclusion, it would appear that a combination of CyA and prednisone is effective in sustaining the remission in steroid-sensitive nephrotic syndrome. Corticosteroids in combination with CyA may be a better approach than conventional steroid treatment in such patients.

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The Turkish Journal of Pediatrics 1999; 41: 231-237 Case

GUANOSINE TRIPHOSPHATE CYCLOHYDROLASE I DEFICIENCY*
A Rare Cause of Hyperphenylalaninemia

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SUMMARY: Coşkun T, Karagöz T, Kalkanoğlu S, Tokatlı A, Özalp İ, Thöny B, Blau N. (Metabolism and Nutrition Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Guanosine triphosphate cyclohydrolase I deficiency: a rare cause of hyperphenylalaninemia. Turk J Pediatr 1999; 41: 231-237.

Tetrahydrobiopterin (BH_4) deficiencies are a heterogeneous group of disorders caused by a defect in two of the three enzymes involved in its biosynthesis or in the two recycling enzymes. Except for the deficiency of dehydratase, an enzyme catalyzing a reaction in the recycling pathway, all other variants of BH_4 deficiency are characterized by developmental delay, progressive neurological deterioration, hypokinesis, drooling, swallowing difficulty, truncal hypotonia, increased limb tone, myoclonus and brisk deep tendon reflexes.

A deficiency of guanosine triphosphate cyclohydrolase I (GTPCH), the first enzyme in the biosynthetic pathway of BH_4, is described in a 14-month-old male infant with hyperphenylalaninemia, developmental delay, hypertonia of the extremities, seizures, feeding difficulties, and vomiting. Urinary pteridine screening revealed very low levels of neopterin and biopterin which was highly suggestive of GTPCH deficiency. Low cerebrospinal fluid concentrations of 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid concentrations, together with no detectable neopterin and decreased concentrations of biopterin and folate, agreed with the diagnosis of GTPCH deficiency. Subsequently measured neopterin and biopterin synthesis in cytokine-stimulated skin fibroblasts confirmed GTPCH deficiency, albeit indirectly. The patient showed marked improvement on a low-protein low-phenylalanine diet with neurotransmitter precursor administration. The favorable outcome in this patient clearly shows that not only newborns with elevated phenylalanine levels but also older children with neurological signs and symptoms should be secreened for a BH_4 deficiency in order to have maximum benefit of the treatment. *Key words: hyperphenylalaninemia, guanosine triphosphate cyclohydrolase I deficiency, neopterin, biopterin, neurotransmitters, neonatal screening.*

Primary forms of hyperphenylalaninemia (HPA) result from a deficiency of either the apoenzyme, phenylalanine hydroxylase (PAH), or its cofactor, tetrahydrobiopterin (BH_4), which are required for the conversion of phenylalanine (Phe) to tyrosine (Tyr). BH_4 is the cofactor required not only by PAH but also

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by tyrosine and tryptophan hydroxylases, and its deficiency leads to defective catecholamines and serotonin synthesis in the central nervous system. Unlike patients with PAH deficiency due to neurotransmitter deficiency, BH_4-deficient patients usually do not experience a good neurological outcome on a low-Phe diet alone[1-3].

Variant forms of phenylketonuria (PKU) and HPA may be due to genetic defects in either the recycling or the de novo synthesis of BH_4. This cofactor is synthesized in vivo from purine nucleotide guanosine triphosphate (GTP). Conversion of GTP to a phosphorylated neopterin derivative, the first reaction in the BH_4 synthetic pathway, is catalyzed by guanosine triphosphate cyclohydrolase I (GTPCH)[1-3].

Compared to classical PKU (1:10,000), defects in BH_4 metabolism are rare, with an incidence of ≈1:500,000 to 1:1 million births; GTPCH deficiency is even more rare[1,3]. Considering its rare occurrence, we wish to present detailed clinical and biochemical features of a new case.

Case Report

A 14-month-old male infant was referred for evaluation of developmental delay, seizures, feeding difficulty, and vomiting. He was born at term to a 24-year-old gravida 1, para 1. The birth weight was 2,500 g. The parents were first cousins. His medical history was remarkable for noticeably increased muscle tone in the extremities and frequent seizure episodes starting at four months of age. He was treated with phenobarbital and valproic acid with almost no decrease in the frequency of seizures.

Physical examination revealed a spastic infant with normal vital signs. His weight was 6,150 g ($< 5^{th}$ percentile); length 68 cm ($< 5^{th}$ percentile), and head circumference, 44 cm ($< 5^{th}$ percentile). Neurological examination revealed spasticity of the extremities, hypotonicity of the trunk, and increased deep tendon reflexes. He was unable to control his head, to walk, or to speak. The remainder of the physical examinatilon results were unremarkable.

On laboratory evaluation complete blood count, routine urinalysis and blood chemistry were all within normal ranges. Hyperphenylalaninemia was detected by amino acid chromatography. Serum Phe concentration was 7.7 mg/dl (465 mol/L). The rather low urinary neopterin and biopterin concentrations, with a neopterin to biopterin ratio of approximately 1 as measured by HPLC on samples collected on filter paper[4], suggested a defect in GTPCH. After a loading test with BH_4 (20 mg/kg), serum Phe and Tyr concentrations were as follows: 0-hour Phe: 5.7 mg/dl (343 µmol/L), Tyr: 3.1 mg/dl (172 µmol/L); 4-hour Phe: 1.56 mg/dl (94 µmol/L), Tyr: 2.4 mg/dl (131 µmol/L): and 8-hour Phe: 1.0 mg/dl (61 µmol/L), Tyr: 2.7 mg/dl (147 µmol/L). Low cerebrospinal fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) concentrations, together with no detectable neopterin and decreased concentrations of biopterin and folate, agreed with the diagnosis of GTPCH deficiency

(Table I). Measurement of neopterin and biopterin synthesis in cytokine-stimulated skin fibroblasts, an indirect method of measuring GTPCH activity[5], confirmed the diagnosis (Fig. 1). Electroencephalography (EEG), cranial computerized tomography (CT), visual evoked potential (VEP), and brain-stem auditory evoked potential (BAEP) yielded normal findings.

Table I: Some of the Laboratory Data of the Patient

URINE	
Neopterin (mmol/mol creatinine) normal: 1.1-4.0	0.14
Biopterin (mmol/mol creatinine) normal: 0.5-3.0	0.17
PLASMA	
Phenylalanine (µmol/L) normal: < 120	465
RED BLOOD CELLS	
DHPR activity (mU/mg Hb) normal: 2-5	2.5
FIBROBLASTS*	
Biopterin (pmol/mg protein) controls: 160-350	22
Neopterin (pmol/mg protein) controls: 20-70	8
CSF	
5HIAA (nmol/L) normal: 224 (114-326)	127
HVA (nmol/L) normal: 660 (295-932)	79
3OMD (nmol/L) normal: < 50	112
5OHTrp (nmol/L) normal: < 10	8
L-dopa (nmol/L) normal. < 25	< 5
5MTHF (nmol/L) normal: 63-111	24
Neopterin (nmol/L) normal: 9-30	0
Biopterin (nmol/L) normal: 10-40	9

* stimulated with interferon-γ/tumor necrosis factor-α[5].
DHPR: dihydropteridine reductase, GTPCH: guanosine triphosphate cyclohydrolase I, CSF: cerebrospinal fluid, 5HIAA: 5-hydroxyindoleacetic acid, HVA: homovanillic acid, 3OMD: 3-O-methyldopa, 5OHTrp: 5-hydroxytryptophan, 5MTHF: 5-methyltetrahydrofolic acid.

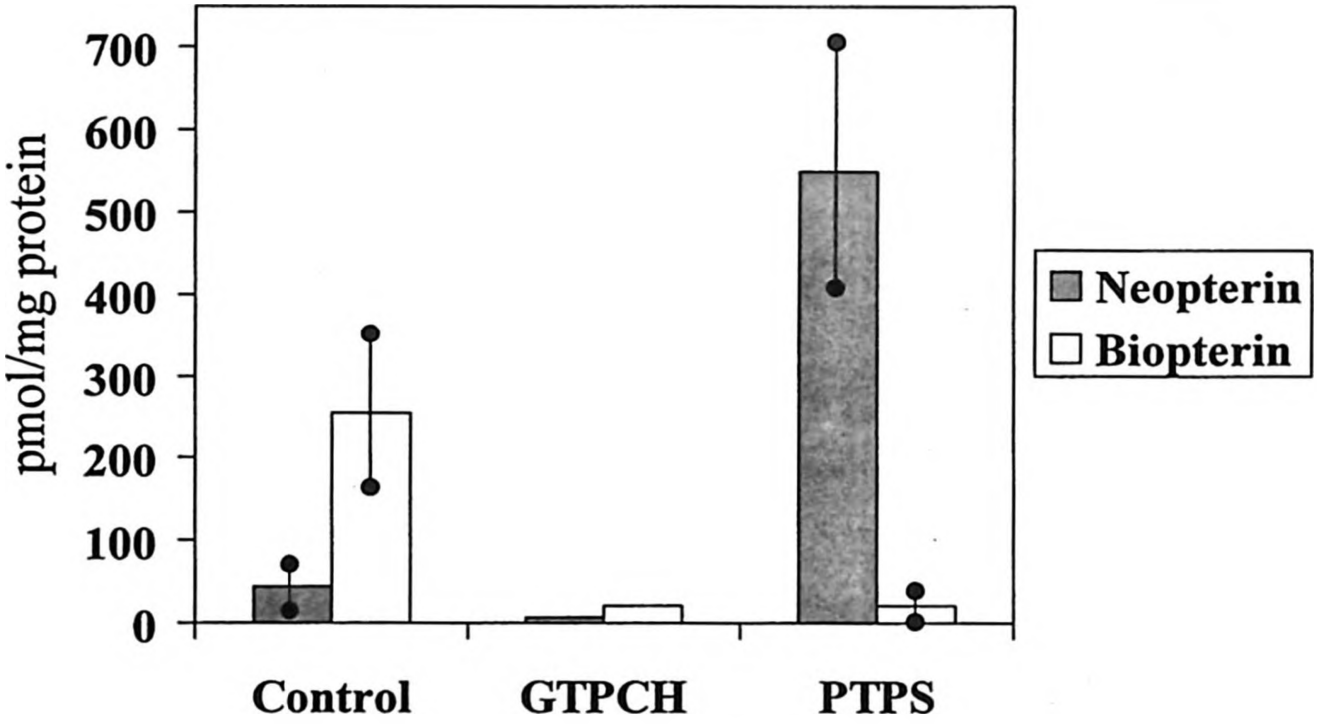

Fig. 1: Neopterin and biopterin concentrations in fibroblasts from controls, our patient with the GTPCH deficiency, and from PTPS-deficient patients after 24 hours stimulation with interferon-γ/tumor necrosis factor-α. The graph shows median values and the range. Neopterin and biopterin production is an indirect measure for the GTPCH activity[5].

A Phe-restricted diet was introduced along with neurotransmitter precursors replacement therapy consisting of L-dopa/carbidopa (7.5-10 mg/kg/day) and 5-hydroxytrytophan (7.5-10 mg/kg/day). Synthetic BH_4 was unfortunately not available. Doses of neurotransmitter precursors were adjusted according to serum prolactin levels (initial: 18.8 ng/ml; after substitution with 7.5 mg/kg/day L-dopa/carbidopa and 5-hydroxytryptophan: 9.3-15.0 ng/ml; after short time withdrawal: 21.0 ng/ml; normal 2.5-17.0 ng/ml). The patient is now $2^1/_2$ years old and is able to walk and speak a few words, and experiences almost no seizure activity.

Discussion

The frequency of various inborn errors of metabolism is quite high in Turkey, where the rate of consanguineous marriages continues to be high[6,7]. Aminoacidopathies, particularly PKU (1:6000) and other forms of HPAs (1:12,500), are the most common metabolic disorders[6,8]. Based on the internationally gathered data, BH_4 deficiencies account for one to three percent of all HPAs[9]. Although we do not routinely screen all hyperphenylalaninemic newbrons for BH_4 deficiencies, the data of the international registry points to a relatively high incidence (15%) in Turkey[9]. According to the international database most of the patients with BH_4 deficiency suffer from 6-pyruvoyl-tetrahydropterin synthase

(PTPS) deficiency (58%), followed by dihydropteridine reductase (DHPR) deficiency (35%), GTPCH deficiency (3%), and "primapterinuria" (4%)[2, 9]. Turkey, with a high number of cases with DHPR deficiency, has a different pattern of BH_4 deficiencies[10]. As can easily be seen from the international data, GTPCH deficiency is the rarest form. Since its first description in 1984 by Niederwieser et al.[11], there have been 16 cases recorded (including the present case) in the database (BIODEF, http://www.unizh.ch/~blau/bh4.html). To the best of our knowledge, this is the first documented case from Turkey.

Patients with BH_4 deficiency, except for those with pterin-4a-carbinolamine dehydratase (PCD) deficiency, exhibit a clinical picture that is characterized by developmental delay and progressive neurological deterioration, tremors, hypokinesis, drooling, drowsiness, irritability, swallowing difficulty, oculogyric crises, recurrent episodes of hyperthermia in the absence of infections, truncal hypotonia, increased limb tone, myoclonus or dystonic limb movements, and very brisk deep tendon reflexes. These symptoms, resulting from the biogenic amine deficiency, fluctuate diurnally in their intensity. Affected patients usually present with one or more of the signs and sympdoms listed above within the first few months of life (the median age at which clinical signs become evident is 4-5 months)[1-3, 9, 10]. Increased limb tone, seizures, developmental delay, and feeding difficulty and vomiting, most probably due to difficulty in swallowing, were noticed by our patient's parents at around four months of age. Most of the patients with BH_4 deficiency are born with a low birth weight as was the case in the presented patient[9]. Untreated patients develop progressive microcephaly with bioelectric activity abnormalities on EEG tracings and cerebral atrophy on CT scan or magnetic resonance imaging (MRI)[3]. Our patient was microcephalic with no EEG, CT, VEP, or BAEP abnormalities.

Defects involving the BH_4 metabolic pathway can be differentially diagnosed by measurement of the urinary neopterin and biopterin levels[2, 3, 9]. Guanosine triphosphate cyclohydrolase I deficiency is characterized by neopterin and biopterin deficiency with a neopterin to biopterin ratio of ≈ 1. In CSF, neopterin, biopterin, as well as the neurotransmitter metabolites such as HVA and 5HIAA are generally low[2, 3, 9, 12]. Our patient met all these laboratory criteria, and had responded to BH_4 loading with a decrease in serum Phe concentrations following cofactor administration, as is expected in BH_4 synthesis defects. Normal activity of dihydropteridine reductase in erythrocytes excluded the possibility of a regeneration defect. Based on the results of urinary pteridine measurements and of the BH_4 loading test, GTPCH deficiency was suspected in the present case and was confirmed by indirect measurement of enzyme activity in cultured fibroblasts (Table I; Fig. 1).

Untreated severe deficiency of neurotransmitters in the central nervous system may lead to death in some of the BH_4-deficient patients[1-3, 9]. Accurate and early diagnosis of such cases is, therefore, essential. Treatment is aimed at decreasing blood Phe levels by putting the patient on a low-protein and low-Phe diet or, even better, on BH_4 substitution, and normalizing low CSF levels of the neurotransmitters by oral administration of the neurotransmitter precursor, L-dopa and 5-hydroxytryptophan, together with a decarboxylase inhibitor (carbidopa). The efficiency of treatment should be monitored by regular analysis of neurotransmitter metabolites in CSF[3, 9]. Such an investigation, however, requires frequent lumbar punctures and thus cannot be easily repeated. Recently, the serum prolactin level was found to be a good indicator of L-dopa. If there is no effective inhibition on prolactin by L-dopa[13], serum prolactin levels increase[14]. Our patient's therapeutic regimen consisted of a low-protein and low-Phe diet, L-dopa/carbidopa, and 5-hydroxytryptophan. Doses of neurotransmitter precursors were increased to levels maintaining the serum prolactin concentration within normal limits. The dietary tolerance of Phe is higher in BH_4 deficiency (300-700 mg/day) than in classical PKU, and the dietary Phe tolerance increases markedly with age in these patients[3]. The present patient currently tolerates 680 mg Ph/day.

Treatment is much more effective when initiated as soon as possible after birth[3, 9]. Although in our patient treatment started at 14 months of age, he was doing well neurologically as evidenced by improvement in muscle tone, and being more active, able to walk and speak, and almost free of seizures. No doubt, the patient would have been doing better neurologically if his illness had been detected shortly after birth. Therefore, it is recommended that every newborn with even slight but persistent HPA be tested for a BH_4 deficiency[3, 9]. Recently, it has been shown that some variants of BH_4 deficiency may present without HPA and that some newborns may be missed by the PKU newborns screening program[15, 16]. Establishment of early and correct diagnosis is essential not only for effective treatment but also for giving parents the chance of prenatal diagnosis in a subsequent pregnancy. In GTPCH deficiency, affected families may benefit from prenatal diagnosis by the measurement of amniotic fluid neopterin and biopterin concentrations, though these are less reliable[2, 3, 9], and by the more accurate study of the GTPCH gene, which is mapped to chromosome 14q22.1-q22.2[17]. The family of the presented case will be offered this service since their only child thus far is affected by GTPCH deficiency.

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A WARM ANTIBODY MEDIATED ACUTE HEMOLYTIC ANEMIA WITH RETICULOCYTOPENIA IN A FOUR – MONTH – OLD GIRL REQUIRING IMMUNOSUPPRESSIVE THERAPY*

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SUMMARY: Olcay L, Düzova A, Gümrük F. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). A warm antibody mediated acute hemolytic anemia with reticulocytopenia in a four-month-old girl requiring immunosuppressive therapy. Turk J Pediatr 1999; 41: 239-244.

We present a four-month-old girl with severe hemolytic anemia and reticulocytopenia. This case is the youngest with hemolytic anemia encountered in our hospital. Findings of autoimmune hemolytic anemia were preceded by diphtheria-pertussis-tetanus (DPT) and oral polio vaccines which were given one month before. At admission, she had heart failure, her hemoglobin (Hb) was 27 gm/L, hematocrit (Hct) 8.5 percent, reticulocyte count 0.2 percent, and gamma and non-gamma Coombs tests were positive. Plasma Hb was 23 percent (N < 3%) and haptoglobin 0 mg/dl. Bone marrow aspiration smear revealed erythroid hyperplasia. No infection, immunodeficiency or malignancy could be established. She received multiple transfusions and did not respond to methyl prednisolone therapy of seven days' duration, but was successfully treated with a combination of immunosuppressive therapy (cyclophosphamide, 6-mercaptopurine, intravenous immunoglobulin and prednisolone, which was added later). This case is interesting in that the disease was preceded by DPT vaccination, was associated with reticulocytopenia and was resistant to steroids. *Key words: autoimmune hemolytic anemia, childhood, reticulocytopenia, warm antibody.*

Autoimmune hemolytic anemia (AIHA) of childhood is generally mediated by warm antibodies and is almost always extravascular; however, when hemolysis is severe intravascular hemolysis may coexist[1]. It generally pursues a chronic course, especially in children under two years of age, although this is not a rule[2]. There have been few patients having reticulocytopenia despite severe hemolytic anemia who responded to steroids[3-5]. We report herein a four-month-old girl with AIHA mediated by warm antibodies which pursued an acute course. She also had reticulocytopenia and signs of intravascular hemolysis. She did not respond to steroid therapy and was treated with immunosuppressive drugs.

Case Report

A four-month-old girl was referred to our hospital with jaundice and anemia. She had begun vomiting three days before and had no history of respiratory tract infection, or symptoms like diarrhea, skin lesions or fever. Two days prior to

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admission jaundice appeared. She had not been given any medication. She we breast-fed. She had been vaccinated regularly: BCG at the 2nd month diphtheria, pertussis, tetanus (DPT) and oral polio at the 2nd and 3rd months. Diphtheria-pertussis-tetanus and oral polio vaccines were the last administered one month prior to admission. She was the second child of healthy non-consanguineous parents. On admission, physical examination revealed a head circumference of 39.5 cm (50th percentile), weight of 5,300 g (25th-50th percentile), and length of 62 cm (75th-90th percentile). Skin and conjunctiva were icteric, there was no skin lesion and no lymphadenopathy. Heart rate was 162/min and the liver and spleen extended 4 and 2 cm below the right and left costal margins, respectively.

Hemoglobin (Hb) was 27 g/L (2.7 g/dl), hematocrit (Hct) 8.5 percent, mean corpuscular volume 76.9 fl, white blood cell count 16.4 x 10^9/L (16,400/mm^3) and platelet count 380 x 10^9/L (380,000/mm^3). Differential count revealed 44 percent neutrophils, 3 percent monocytes, 53 percent lymphocytes, and anisocytosis and poikilocytosis.

Reticulocyte count was 0.2 percent, total bilirubin 3.9 mg/dl, direct bilirubin 1.1 mg/dl, serum glutamic-oxaloacetic transaminase 66 IU/L, glutamic-pyruvic transaminase 16 IU/L, gamma Coombs test (+), non-gamma Coombs test (+), cold agglutinin (-), plasma Hb 23 percent (normal < 3%), and haptoglobine 0 mg/dl. The bone marrow aspiration smear revealed erythroid hyperplasia. Erythrocyte glucose-6-phosphate dehydrogenase, urinary and blood amino acid examination, and immunoglobulin G, A, M levels were normal. Epstein-Barr virus (EBV) IgM was (-), EBV IgG (+), cytomegalovirus (CMV) IgM (-), CMV IgG (+), Parvovirus (PV) B19 IgM (-), PV B19 IgG (+), hepatitis B surface antigen (-), and hepatitis B surface antibody (-).

Methyl prednisolone treatment was started with a dose of 30 mg/kg/day and was continued for seven days. However, blood transfusions had to be performed four times within the first week because of severe anemia. Plasmapheresis was attempted because of the intravascular component of the hemolysis, but was technically unsuccessful; therefore, intravenous immunoglobulin (400 mg/kg/day), cyclophosphamide (5 mg/kg/week), and 6-mercaptopurine (1.5 mg/kg/day) were started on the eighth day, when Hb was 38 g/L (3.8 g/dl). Cyclophosphamide was stopped at the third week when an Hb level of 125 g/L (125 g/dl) was maintained. Prednisolone (2 mg/kg/day) was then added to 6-mercaptopurine. Prednisolone was given for one month and was stopped after tapering the dose over 15 days. 6-mercaptopurine was continued for three months and was then stopped. The child has maintained an Hb level of 120 g/L (12 g/dl). Hemoglobin and Hct levels; reticulocyte counts; and gamma, non-gamma, and indirect Coombs tests during the follow-up are shown in Table I.

Table I: Hemoglobin and Hematocrit Levels, Reticulocyte Counts, and Direct and Indirect Coombs Tests During Follow-up

	Hb (gm/L)	Htc (%)	Ret (5)	Gamma Coombs Test	Non-Gamma Coombs Test	Indirect Coombs Test	Drugs*
1st week	27	8.5	0.2	+	+	+	HDMP
2nd week	38	12	0.2	+	+	+	Iv IgG Cyc 6MP
3rd week (discharge)	125	37	0.2	+	+	+	6MP continued Pred
5th week	99	32	0.4	+	+	+	6MP continued Pred continued
7th week	125	37	0.8	–	–	–	6MP continued Pred stopped
9th week	125	37	0.4	–	–	–	6MP continued
14th week	134	39	0.4	–	–	–	6MP stopped
16th week	126	36	0.2	–	–	–	
19th week	120.8	36	0.2	–	–	–	

High dose methylprednisolone (HDMP).. : 30 mg/kg/day x 7 days po.
Intravenous immunoglobulin G (Iv IgG) . : 400 mg/kg/day x 5 days iv.
Cyclophosphamide (Cyc)..... : 5 mg/kg/week iv.
6-mercaptopurine (6MP) ..... : 1,5 mg/day, po.
Pednisolone (Pred)..... : 2 mg/kg/day, po.
* Folic acid was given between 1st-4st weeks.

Discussion

In Childhood and adolescence the incidence of AIHA has been reported as 1/267,000 to 1/1,780,000. In the literature, patients' ages ranged from eight weeks to 10 years[4]. This patient is the youngest patient with hemolytic anemia encountered in our hospital.

Autoimmune hemolytic anemia can be associated with infections (respiratory infections, EBV, CMV, mycoplasma, human immunodeficiency virus); immunodeficiency syndromes (X-linked agammaglobulinemia, dysgammaglobulinemia, IgA deficiency, Wiskott-Aldrich syndrome, common variable hypogammaglobulinemia); malignancies (non-Hodgkin's lymphoma, Hodgkin's disese, acute lymphocytic leukemia, thymoma, ovarian cysts and tumors); and disorders associated with autoantibody production (systemic lupus erythematosus, neonatal lupus syndrome, rheumatoid arthritis, chronic active hepatitis, ulcerative colitis, thyroid disorders)[3, 1]. However, the detailed past history and laboratory examinations of our patient revealed none of these etiologic agents except the vaccines of DPT and oral polio which were done one month prior to illness. Among Zupanska et al.'s[6] cases of children with AIHA, in two out of 13 acute AIHA and one out of 12 subacute AIHA, immunization (against typhoid fever together with poliomyelitis, DPT and typhoid fever, respectively) preceded the

development of hemolysis. In all three patients AIHA was mediated by warm antibodies as it was in our patient. Symptoms of AIHA started one or two weeks after typhoid vaccine in the patient with subacute AIHA versus the four weeks in our case.

The onset of the illness may be acute with abdominal pain and vomiting (as in our patient) with or without fever, general weakness, anorexia, diarrhea, pallor, hemoglobinuria[6,7].

Constant physical findings such as pallor, tachycardia, jaundice, moderate splenomegaly and mild hepatomegaly, and mixed or unconjugated hyperbilirubinemia may be encountered[4], as in our patient. Leukopenia and thrombocytopenia, which were not features in our case, may accompany anemia in some patients.

Positive gamma and non-gamma Coombs tests which demonstrated the presence of IgG and C3, respectively, and the absence of cold agglutinins, confirmed that the hemolysis in our case was mediated by a warm antibody of IgG with C3. (In childhood, AIHA due to warm antibodies is more common than AIHA due to cold antibodies and generally pursues a chronic course[3,5]). However, in our patient the disease pursued an acute course. Moreover, it has been reported that AIHA in patients less than two years of age (and over 12 years of age) generally presents as chronic forms[2]. However, there are enough exceptions to these generalizations to preclude applying them in individual cases[8]. Signs of hemolysis in our patient disappeared within two weeks and the serologic tests were negative within a further six weeks. The disease has not relapsed for two years. Zupanska et al.'s[6] patients with AIHA mediated by warm antibodies and preceded by vaccination also did not have a chronic course. In warm antibody mediated AIHA, hemolysis is almost always extravascular; however, in severe cases intravascular hemolysis may occur[1], as in our patient.

This case is the first case of severe AIHA with reticulocytopenia we have encountered. Liesveld et al.[9] showed that median reticulocyte percentage of 109 cases with AIHA at diagnosis was 9%; 20% of the cases had an initial reticulocyte count < 4%, the lowest being 0.4%.These reticulocytopenic patients were nearly evenly distributed between warm and cold antibody mediated cases. Fifty-four percent of reticulocytopenic cases had a bone marrow examination. Three fourths of these marrows showed erythroid hyperplasia; erythroid hypoplasia was seen in only one case. The bone marrow of our patient also revealed erythroid hyperplasia.

Our patient differs from Liesveld et al.'s[9] reticulocytopenic patients, 76 percent of whom had increases in their reticulocyte index, the increase being more prominent among patients who received steroids. Among Liesveld et al.'s[9] reticulocytopenic patients, ony four out of 33 did not show any increase in reticulocyte counts as with our patient. Unlike Conley et al.'s[10] patients, who

displayed brisk reticulocytosis within a few days to a week after diagnosis and initiation of therapy, our patient's reticulocyte count never changed with therapy, in spite of recovery.

It has been suggested that reticulocytopenia in adults indicates poor prognosis, while in childhood reticulocytopenia this is not necessarily the case[5].

Delay in proliferation of red cell precursors[10] or preferential destruction of them[11], by a complement-dependent serum IgG inhibitor directed against erythroid colony and burst-forming units[12], are suggested as possible mechanisms for reticulocytopenia in AIHA.

Treatment of the underlying disorder brings the hemolytic anemia under control. Corticosteroids are the first agents to be used[5,7]. Patients with warm antibody induced AIHA generally respond to steroid therapy in dosages up to 2-10 mg/kg day of prednisolone. An increase in Hb can be accomplished within one to four days, and for reticulocytopenic patients within eight days[9].

Our experience shows that high dose methyl prednisolone is quite effective in patients with hemolytic anemias who are resistant to low dose prednisolone[13]. Thus, we immediately started high dose methyl prednisolone as the first choice of treatment rather than a low dose steroid to avoid a possible delay due to resistance to low dose prednisolone, in view of the severity of anemia in our case.

Transfusions should be given if AIHA is of life-threatening severity. As the response to high dose methyl prednisolone was inadequate, intravenous immunoglobulin, 6-mercaptopurine and cyclophosphamide were started. Plasmapheresis, exchange transfusion, other alkylating agents (chlorambucil), vitamins B_{12} and B_6, Vinca alkaloids, splenectomy, cyclosporin-A, monoclonal antibodies and danazol are other therapeutic strategies, although the roles of the last three have yet to be evaluated[3,1]. Improvement in AIHA with immunosuppressive drugs was reported in 60 percent of children[14].

The case was interesting as there was no history or finding of preceding infection only DPT and oral polio vaccines administered one month prior to illness, which is a rare cause of AIHA; reticulocytopenia occurred despite severe hemolysis; and there was no increase in reticulocyte count during steroid therapy. Moreover, forms of AIHA which are resistant to steroids and require additional immunosuppressive drugs are generally chronic forms and not acute types, as in our patient.

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RABDOMYOSARCOMA OF THE BILIARY TREE*

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SUMMARY: Balkan E, Kırıştıoğlu İ, Gürpınar A, Sınmaz K, Özkan T, Doğruyol H. (Departments of Pediatric Surgery and Pediatric Gastroenterology, Uludağ University Faculty of Medicine, Bursa, Turkey). Rhabdomyosarcoma of the biliary tree. Turk J Pediatr 1999; 41: 245-248.

Rabdomyosarcoma of the biliary tree is one of the rare causes of biliary tract obstruction in childhood. Nevertheless it is the most common cause of obstructive jaundice due to neoplastic biliary obstruction. We present a two-year-old child with obstructive jaundice secondary to an embryonal rhabdomyosarcoma of the biliary tree. She underwent surgery and, after total excision of the mass, a hepaticojejunostomy and Roux-en-Y anastomosis were performed. She was referred to the Pediatric Oncology Group for follow-up. Rhabdomyosarcoma of the bilary tree, although rare, must be considered in the etiology of obstructive jaundice in children.
Key words: rhabdomyosarcoma, biliary tree.

Rabdomyosarcoma of the biliary tree is an uncommon disorder of the bile ducts and is also a rare cause fo biliary tract obstruction in childhood[1,2]. However, it is the most common cause of neoplastic biliary tract obstruction in children[1]. The tumor, which originates from the biliary ducts, is often polypoid and embryonal botyroid types. It either obstructs the biliary lumen and causes obstructive jaundice or causes complications like cholangitis and hepatic abscess[3]. Herein we present a patient with rhabdomyosarcoma of the biliary tree whom we diagnosed and operated on in our department. Rhabdomyosarcoma of the biliary tree must be considered in the differential diagnosis of obstructive jaundice in children.

Case Report

A two-year-old female was admitted with a one-month history of jaundice, acholic defecation, loss of appetite and weight loss. On physical examination the patient was icteric with hepatomegaly and acholic stool.

Her liver function tests were as follows: aspartate aminotransferase 220 U/L (normal 2 to 35 U/L), alkaline phosphatase 4000 U/L (normal 70 to 350 U/L), and total bilirubin 7.6 mg/dl (direct 6.4 mg/dl). Tumor markers including alpha-fetoprotein, beta human chorionic gonadotropin and carcinoembryonic antigen were normal. Abdominal ultrasonography (USG) and computed tomography (CT)

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confirmed a cystic, lobular-shaped heterogneous mass 8 cm in diameter between the head of the pancreas and the porta hepatis (Fig. 1). Radiological and radioisotopic investigations revealed no metastases.

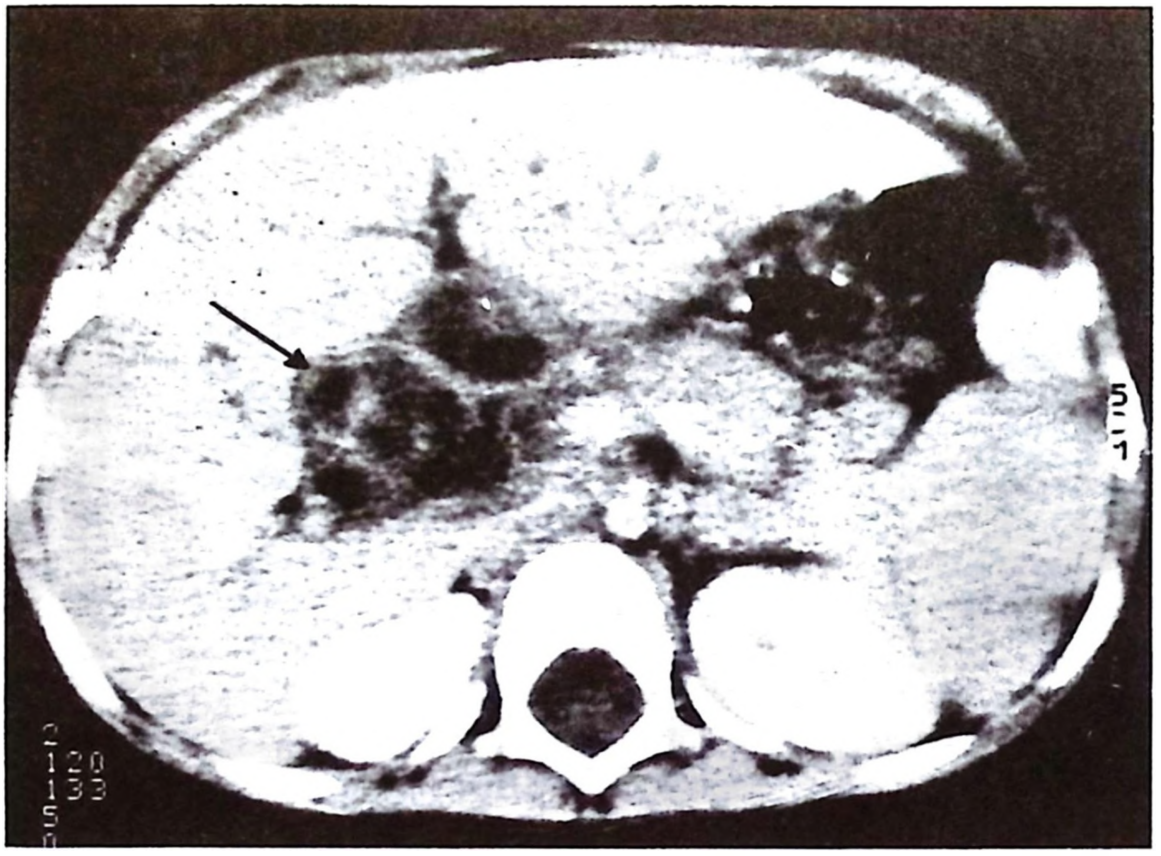

Fig. 1: Computed tomography CT scan of the abdomen at the level of T12-L1 levels the mass between the porta hepatis and the head of the pancreas.

The patient underwent an exploratory laparotomy which revealed a mass 8x3x2 cm in diameter arising from the choledochus. The mass extended to the ductus cysticus and common hepatic bile duct but did not penetrate the head of the pancreas or right-left hepatic ducts. The obstructed choledochus was opened and botryoid nodules and gelatinous material spontaneously extruded from the cyst cavity. Frozen section evaluation revealed rahbdomyosarcoma.

The tumor was considered surgically resectable and had no metastasis or vascular invasion. After total excision of the tumor, biliary draniage was achieved by hepaticojejunostomy and Roux-en-Y anastomosis. Final pathology on the specimen confirmed an embryonal rhabdomyosarcoma stemming from the choledochus. Microscopic residual tumor was found at the proximal and distal bile duct margins (Fig. 2). Lymph node from the porta hepatis was free of disease. Based on the above, the patient was given stage III protocol of the Intergroup Rhabdomyosarcoma Study Group, and was transferred to Pediatric Oncology

uneventfully on the 7th postoperative day. Two weeks later, a course of chemotherapy including vincristine, actinomycin D, cyclophosphamide and etoposide was started. Eleven courses of chemotherapy have been adiminstered to the patient to date. Radiation therapy was not applied. The patient is healthy and has had no relapse over the 18 months since the time of diagnosis.

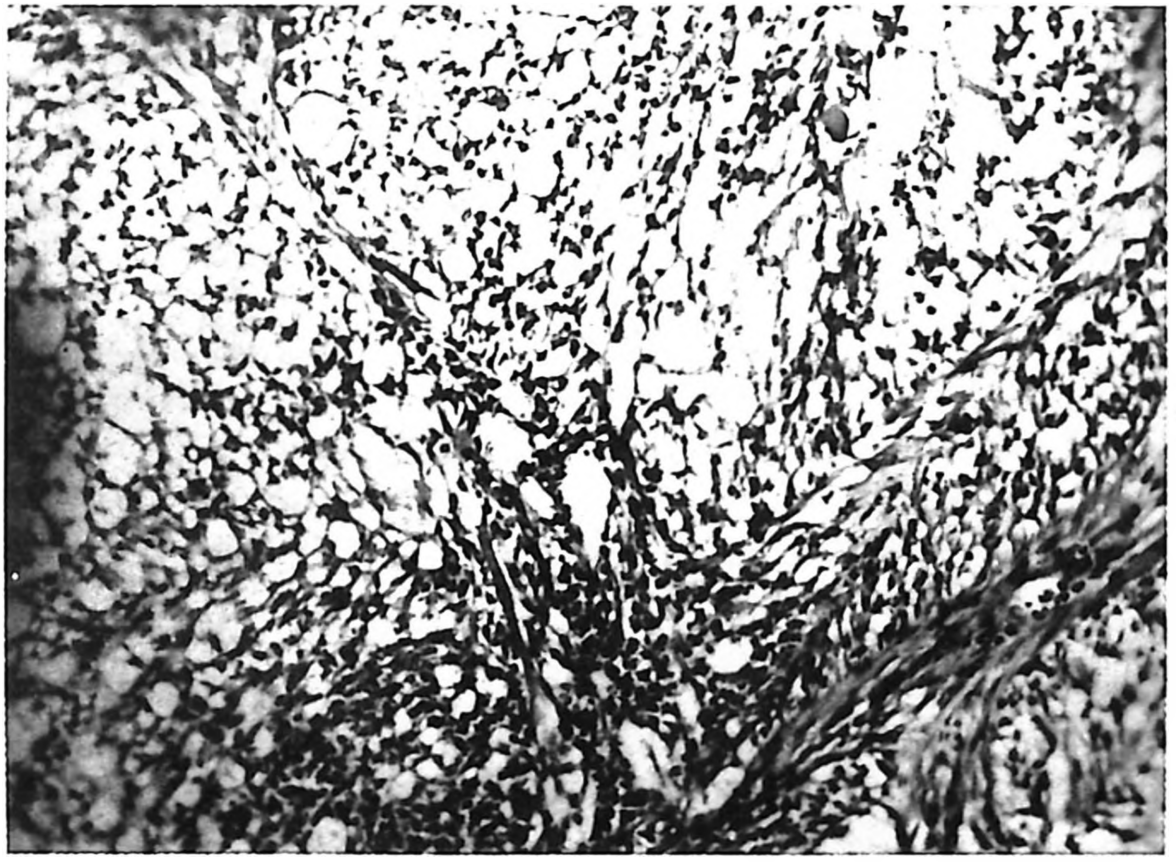

Fig. 2: Microscopic sections illustrate atypical striated rhabdomyoblasts in stroma (H&E, X 200).

Discussion

Although rhabdomyosarcoma is the most frequently seen soft tissue sarcoma in infants and children, its ratio in the biliary system is a reported three percent[5, 6]. rhabdomyosarcoma of the biliary tree usually stems from the choledochus, common hepatic bile duct or ampulla of Vater, but there are reports in the literature which present rhabdomysarcoma stemming from the intrahepatic bile ducts. The tumor as a rule extends into the liver and/or the ampulla of Vater. Because of this extension, total resection of the tumor is unfortunately impossible in most cases[3, 6]. Obstructive jaundice is the most common presenting symptom of this tumor[4]. But other symptoms such as abdominal pain, fever, itching and weight loss are also seen; cholangitis and liver abscess may complicate the

clinical presentation[3]. Most patients with rhabdomyosarcoma develop metastatic disease usually via the bloodstream and less commonly via the lymphatics to the regional lymph nodes, lung, bone, marrow, bone, liver and brain[5].

In our patient, the tumor did not extend to the junction of the right and left hepatic ducts or to the ampulla of Vater. The gallbladder was free of tumor. Obstructive jaundice and weight loss were the main symptoms of our patient; she was free of metastases after radiological and radioisotopic evaluation.

Ultrasonography, USG, CT and/or magnetic resonance imaging, endoscopic retrograde cholangio-pancreatography, or percutaneous transhepatic cholangiography can be used for diagnosis[6]. The diagnosis is unfortunately usually established during exploration. Ultrasonography and CT revealed the tumor between the head of the pancreas and the porta hepatis in our patient, but the diagnosis was established during exploration.

Currently, a multidisciplinary regimen is the recommended therapy for rhabdomyosarcoma of the bile ducts. Surgery, chemotherapy anad radiotherapy combination has been suggested. Chemotherapy must be multiagent. Radiotherapy, when used properly, should reduce the incidence of local recurrence at the primary tumor site[1,3]. Prognosis for rhabdomyosarcoma of the biliary tree is very poor, due to its strategic anatomical location, propensity for liver invasion and the frequent delay in diagnosis[1]. Patients with metastatic disease at the time of diagnosis also have a poor outcome[3].

In conclusion, rhabdomyosarcoma of the biliary tree, although rare, must be considered in the etiology of obstructive jaundice in children.

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CEREBROSPINAL FLUID PLEOCYTOSIS IN ACUTE LYMPHOBLASTIC LEUKEMIA WITHOUT CENTRAL NERVOUS SYSTEM RELAPSE*

A Report of Three Cases

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SUMMARY: Öner AF, Yetgin S, Uçkan D. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Cerebrospinal fluid pleocytosis in acute lymphoblastic leukemia without central nervous system relapse: a report of three cases. Turk J Pediatr 1999; 41: 249-251.

Three patients with acute lymphoblastic leukemia (ALL) developed mononuclear cells in the cerebrospinal fluid (CSF) after a flu-like histroy during maintenance treatment. None of the patients showed evidence of central nervous system (CNS) involvement by either clinical or laboratory follow-up. Although the presence of > 5 mononuclear cells/μl in the CSF is important, it may not necessarily indicate CNS disease. Clinical findings, history and cell morphology must be evaluated before deciding on further treatment.

Key words: acute lymphoblastic leukemia, central nervous system involvement, pleocytosis.

Meningeal relapse (MR) in patients with acute lymphoblastic leukemia (ALL) is defined as the presence of at least 5 mononuclear cells/μl of cerebrospinal fluid (CSF) with leukemic blasts apparent on cytocentrifuged sample[1]. However, cytomorphological evaluation and diagnosis of MR have remained controversial in patients with low CSF counts[1-6]. Flow cytometric analysis may help in the diagnosis, if there is an adequate number of cells in the CSF[2,3,7]. We report herein three patients with ALL from Hacettepe University İhsan Doğramacı Children's Hospital, who were noted to have mononuclear cells (MNC) in routine CSF samples obtained during maintenance phase intrathechal (IT) treatments on St Jude total XI protocol and who remained free of meningeal relapse.

Case Reports

Case 1

Twenty mononuclear cells/μl were detected in the routine CSF sample (20 mg/dl protein) obtained from a 12-year-old male with ALL during the first year of maintenance therapy. There was a history of a flu-like illness with headache three weeks earlier. Based on the mature morphology of the cells,

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maintenance therapy was continued according to the protocol without additional treatment. Follow-up CSF findings performed three times every two months were all within normal range.

Case 2

A 12-year-old male was diagnosed as ALL. The analysis of the CSF obtained at routine IT treatment at tenth month of maintenance therapy revealed 20 cells/μl and 32 mg/dl protein. No additional treatment was initiated because of the presence of morphologically mature MNC and absence of blasts in CSF cytology. This patient also had a history of an upper respiratory infection one week prior to this finding. Cerebrospinal fluid cytology was normal at two weeks and eight months follow-up.

Case 3

Cerebrospinal fluid pleocytosis was detected at ninth month of maintenance treatment in a 12-year-old male with ALL. Cytology revealed 20 MNC/μl with no blasts and CSF protelin was 28 mg/dl. There was a history of fever, headache and aphthous stomatitis three weeks earlier. No additional treatment was administered and follow-up CSF studies at one, three and five months were within normal limits.

Discussion

All three patients were free of neurological signs and symptoms and CSF examinations revealed > 5 MNC/μl without blastic appearance. Five-to-eight month follow-up CSF samples were within normal limits in all patients.

There have been conflicting reports in the evaluation of CSF findings in patients with ALL. In the presence of blasts, some investigators consider 10 cells/μl as significant, while others suggest that even < 5 cells/μl may indicate relapse[2,6]. In one study, 12 out of 23 patients treated for CNS involvement had < 5 cells/μl and later showed pleocytosis[2,3].

A common problem in the interpretation of cells in the CSF is the differentiation of normal and blastic cells[2,5]. Cerebrospinal fluid CSF cytocentrifugation is an important tool for differential diagnosis. Cerebrospinal fluid CSF involvement with leukemia, lymphoma, histiocytosis, multiple myeloma, or primary and metastatic CNS tumors may be detected by this technique[6]. Amo et al.[7] reported a patient with ALL with > 500/μl cells in CSF with some suspected blasts characterized by flow cytometry as a nonmalignant T-cell reaction. This finding was attributed to cytomegalovirus (CMV) infection and follow-up studies were normal. In general, flow cytometric analysis necessitates at least 3×10^6 cells per sample, which restricts the number of patients studied. Several other methods have been utilized for recognition of the leukemic cell. However, in clinical practice cytology has remained the most informative[7].

Based on the benign clinical course in our three cases with CSF pleocytosis, we emphasize that increased CSF MNC counts, in the absence of blasts, with normal CSF protein and without neurological symptoms does not necessarily indicate CNS involvement. Viral agents may cause signs and symptoms of intracranial hypertension, especially in immunocompromised patients (even with less virulent strains). History, physical examination and through morphological evaluation of the cytocentrifuged cells may eliminate the need for additional treatment.

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The Turkish Journal of Pediatrics 1999; 41: 253-257 *Case*

CONGENITAL PRIMARY HYPOPARATHYROIDISM PRESENTED WITH EXTENSIVE CUTANEOUS AND SUBCUTANEOUS CALCIFICATIONS*

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*Ayşe Aksoy MD****, Gülseren Çimşit MD*****, Nurcihan Ari MD*******

SUMMARY: Aslan Y, Gedik Y, Ökten A, Aksoy A, Çimşit G, Ari N. (Departments of Pediatrics, Dermatology and Pathology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey). Congenital primary hypoparathyroidism presented with extensive cutaneous and subcutaneous calcifications. Turk J Pediatr 1999; 41: 253-257.

Congenital primary hypoparathyroidism is very rare in infancy. It may be isolated or associated with other developmental defects, arising from the third and fourth pharyngeal pouches such as DiGeorge syndrome. Initial symptom of isolated primary hypoparathyroidism in an infant is usually generalized convulsion due to hypocalcemia. However, the clinical spectrum of DiGeorge's anomaly is highly variable. We report a two-hour-old neonate with congenital hypoparathyroidism presenting with extensive cutaneous and subcutaneous calcifications. To our knowledge, extensive calcification of the skin and subcutaneous tissue as a presenting feature of congenital primary hypoparathyroidism in an infant is reported for the first time. *Key words: congenital hypoparathyroidism, extensive calcifications.*

Hypoparathyroidism results from failure of parathyroid hormone (PTH) synthesis or secretion (true or primary hypoparathyroidism) or from resistance to the actions of PTH (pseudohypoparathyroidism)[1,2]. Hypoparathyroidism is very rare in infancy, but is an important disease in childhood[3]. The majority of cases with congenital hypoparathyroidism ar a component of DiGeorge's anomaly resulting from the defective development of third and fourth pharyngeal pouches; only a minority of the cases have isolated disease[2]. Isolated congenital hypoparathyroidism usually presents as a generalized neonatal convulsion due to hypocalcemia that relapses at each attempt to stop the treatment beyond six months of age. However, the clinical spectrum of DiGeorge's anomaly is highly variable[2].

Calcification is the result of deposition of calcium and phosphate in organic matrices of the tissue[4]. Cutaneous calcification may be divided into four categories: dystrophic, metastatic, idiopathic and iatrogenic. Metastatic calcification results from abnormal calcium and/or phosphate metabolism and occurs in a wide variety of unrelated diseases[4,5].

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This report describes a newborn baby with congenital hypoparathyroidism presented with extensive cutaneous and subcutaneous calcifications. As far as we know, extensive calcification of cutaneous and subcutaneous tissues as a presenting feature of congenital primary hypoparathyroidism in an infant has not previously been reported.

Case Report

A two-hour-old female infant was referred to our neonatal intensive care unit for evaluation of multiple cutaneous tumors. She was the fifth child of unrelated and healthy parents, and was born following a full-term uncomplicated gestation. Parental history revealed that two male siblings of the patient had died due to diseases presented with hypocalcemic convulsion during the early neonatal period. One female sibling had DiGeorge syndrome due to encephalitis at 11 years of age.

Physical examination revealed weight 3,950 g, length 51 cm, respiratory rate 42 breaths/min, heart rate 176 beats/min, blood pressure 78/46 mmHg, and temperature 36.8 °C. Her general condition was good. Deep tendon reflexes were brisk. Multiple hard and milky subcutaneous nodules were noticed on each side of the body. These nodules quickly became larger and many new nodules became visible. Tremors and convulsions lasting a few seconds to a few minutes developed, and Chvostek's sign was elicited during the second hour of admission. All other findings on physical examination were normal. On enquiry, the obstetrician reported the presence of metastatic calcifications on the placenta and umbilical cord.

Laboratory investigations included a leukocyte count of 8,200/μl, hemoglobin 14 g/dl, hematocrit 43.6 percent, platelet count 362,000/μl, glucose 78 mg/dl (normal 30 to 91), total calcium (tCa) 4.3 mg/dl (normal 7.3 to 9.2), ionized calcium (iCa) 1.8 mg/dl (normal 4.48 to 4.92), phosphorus (P) 12.1 mg/dl (normal 2.9 to 8.1), alkaline phosphatase 216 U/L (normal 150 to 400), total protein 6.2 g/dl (normal 5.8 to 8.2), albumin 3.1 g/dl (normal 3.3 to 4.5), magnesium 2.36 mg/dl (normal 1.20 to 2.50), C-terminal specific PTH 4.1 pmol/L (normal 22 to 66), 25 (OH) vitamin D 28 ng/ml (normal 20 to 60), and normal urea, creatinine, uric acid, electrolyte values and liver function tests. Urinalysis and initial arterial blood gases were within normal limits. The electroencephalogram showed a paroxysmal dysrhythmia, and electrocardiogram revealed marked lengthening of the QT segment and superimposition of T on P wave. Chest x-ray showed normal thymic shadow, and limb x-rays demonstrated multiple calcifications (Fig. 1). Ultrasonographies of the thymus, abdomen and cranium demonstrated normal findings. Results of studies for sepsis were negative and chromosomal analysis was normal. Immunological investigations revealed a lymphocyte count 3,444/μl (normal 2,000 to 11,000), T lymphocyte (CD3+ cell) percentage 73 (normal 60 to 75), CD3+ cell count 2,445/μl (normal 1,300 to 4,000), and CD4+ cell/CD8+ cell ratio 1.74 (normal 1.5 ± 2.2).

A skin biopsy adjacent to the nodular lesions was performed and submitted for histopathological study. Hematoxylin and eosin-stained sections of the skin biopsy material revealed numerous calcified deposits (Fig. 2a, b).

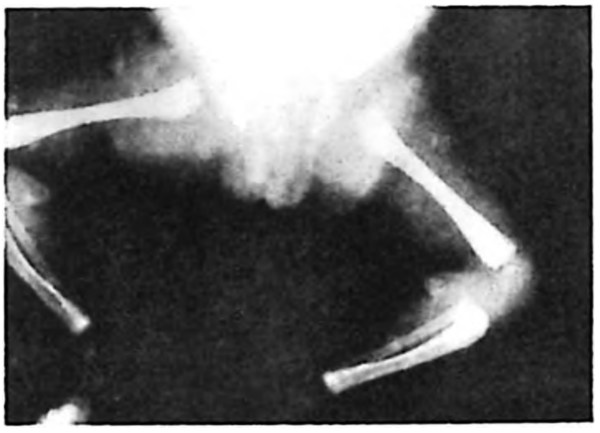
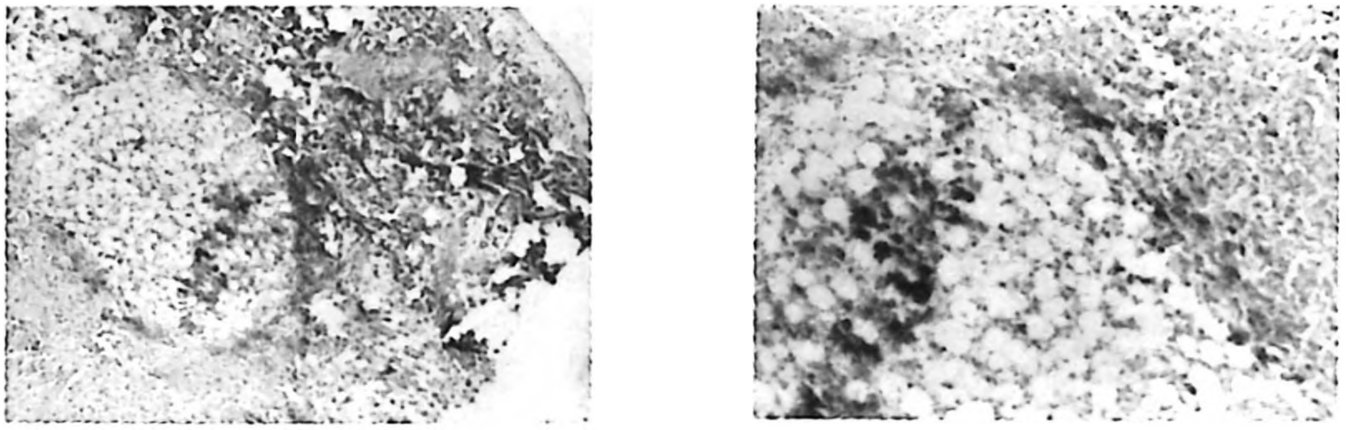


Fig. 1: X-ray film of the lower extremities showing multiple subcutaneous calcifications.



(a) (b)

Fig. 2a, b: Hematoxylin- [(a) H.E. x 40] and eosin-stained [(b) H.E. x 200] section of the skin biopsy showing numerous calcified deposits.

A diagnosis of idiopathic congenital hypoparathyroidism was made. She was given intravenous calcium gluconate (75 mg elemental Ca/kg/day), intravenous diazepam (0.5 mg/kg), 1,25 dihydroxyvitamin D (0.5 µg/kg/day), and aluminum hydroxide (100 mg/kg/day). Clinical and electrical seizures persisted; therefore, intravenous phenobarbital (20 mg/kg) and subsequently intravenous phenytoin (15 mg/kg) were added.

At the age of 24 hours, laboratory studies demonstrated tCa 5.1 mg/dl (normal 6.9 to 9.4), iCa 2.3 mg/dl, P 10.9 mg/dl (normal 2.9 to 8.1), total protein 6.5 g/dl (normal 5.8 to 8.2), albumin 3.4 g/dl, PTH 5.7 pmol/L, and magnesium 2.91 mg/dl. Electrolyte values were normal, and arterial blood gases demonstrated

mild hypoxemia. At the age of 45 hours, the patient, whose seizures repeated intermittently, developed ventricular arrhythmia secondary to hypocalcemia and died due to cardiorespiratory arrests that developed suddenly.

In order to determine a cause for the infant's hypocalcemia, the mother, father and sister of the patient were examined in our hospital. Their physical and laboratory findings, including Ca, P, and PTH levels, and chromosomal analyses were normal.

Discussion

Calcium is vital to many biologic processes. Serum Ca is tightly regulated by PTH and 1,25 dihydroxyvitamin D. Despite this careful regulation, calcification and ossification of cutaneous and subcutaneous tissues may occur[5]. Metastatic calcification occurs in diseases with chronically elevated serum Ca or P levels such as hyperparathyroidism, hypervitaminosis D, milk-alkali syndrome, chronic renal disease, sarcoidosis, pseudoxanthoma elasticum, destructive bone disease with excessive osteoclastic activity and others. All tissues may be affected, but skin involvement is rare[6].

Subcutaneous calcification and/or ossification may be the persisting feature of both pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism exhibiting characteristic phenotypic abnormalities[7]. Aberrant calcifications may also occur in patients with hypoparathyroidism, but they are usually intracranial, most often in the basal ganglia. Furthermore, these are usually seen in patients with acquired hypoparathyroidism resulting from alteration or injury to the parathyroid glands in the course of thyroid or other neck surgery[8]. Goldminz et al.[9] reported an adult patient with hypoparathyroidism who developed dermal calcification along the path of an infiltrated calcium chloride intravenous infusion.

Although the etiology remains obscure, X-linked recessive, autosomal dominant and autosomal recessive familial types of isolated primary hypoparathyroidism have been reported[1, 10]. Autosomal dominant form of congenital hypoparathyroidism with onset during the neonatal period is usually symptomatic, but some reports have noted the rare occurrence of autosomal dominant hypoparahyroidism with a benign course[1]. However, there is no known feature of DiGeorge syndrome that uniformly occurs[11]. The disorder is usually sporadic, but autosomal dominant and autosomal recessive inheritance have been reported[2, 11]. The most complete form includes aplasia of the parathyroid glands with complete hypoparathyroidism, thymus aplasia with severe T-cell immunodeficiency, cardiovascular malformations, and particular facial dysmorphies. In such cases, early diagnosis is mainly based on the association of hypocalcemia and the absence of the thymus on chest x-ray. However, there are milder forms of hypoparathyroidism with thymus hypoplasia without immunodeficiency or other features[2].

In the present case, there was no documented feature of DiGeorge's anomaly other than hypoparathyroidism. However, parental history revealed the presence of one sibling with DiGeorge's anomaly. Due to the lack of detailed genetical investigations and postmortem examination of the thymus, a diagnosis of isolated hypoparathyroidism or partial DiGeorge's anomaly could not be confirmed.

The initial symptom of hypoparathyroidism in the infantile period is usually generalized convulsion due to hypocalcemia[3]. In the peresent case, congenital hypoparathyroidism presented with excessive cutaneous and subcutaneous calcification at delivery.

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A TURKISH FAMILY WITH GREIG CEPHALOPOLYSYNDACTYLY SYNDROME*

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SUMMARY: Boduroğlu K, Balcı S, Topçu M. (Clinical Genetics and Neurology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). A Turkish family with Greig cephalopolysyndactyly syndrome. Turk J Pediatr 1999; 41: 259-265.

Greig cephalopolysyndactyly syndrome is a very rare autosomal dominant disease characterized by postaxial polysyndactyly of hands, preaxial polysyndactyly of feet and peculiar facial features, and has been shown to be due to mutations in the GLI3 gene. We present clinical findings of a 39-year-old man and his nine-day-old daughter with Greig cephalopolysyndactyly who showed variable expression with regard to syndactyly of fingers and toes. The role of obstetric ultrasonography in the prenatal diagnosis of the syndrome is also dicussed. *Key words: polydactyly, syndactyly, macrocephly, prenatal diagnosis.*

Greig cephalopolysyndactyly (GCPS) is a very rare autosomal dominant syndrome which is characterized by peculiar skull shape and postaxial (occasionally preaxial) polydactyly of hands, preaxial polydactyly of feet and syndactyly of toes and fingers[1,2]. Only a few dozen cases have been reported and, as far as we know, no case with GCPS syndrome has been published previously from Turkey.

We report a 39-year-old man and his nine-day-old daughter with GCPS syndrome. Interestingly, prenatal ultrasonographic examination showed the enlargement of the lateral ventricles in the fetus at the 28th week of gestation. However, since the father was not diagnosed prior to the pregnancy, this observation did not lead to a prenatal diagnosis of GCPS. If the father's illness had been recognized, it would have been possible to conclude that the fetus was affected with GCPS syndrome.

Case Reports

Case 1

This nine-day-old female patient was the second child of a non-consanguineous couple. The first child was a five-year-old healthy boy. The patient was referred to the Clinical Genetics Department soon after birth with complex polysyndactyly of the hands and feet. Enlargement of the lateral ventricles was observed at the 28th week of gestation by obstetric ultrasonography. The birth was at the 40th week by cesarean section. Her weight at birth was 4000 g (90-97th percentile), length 52 cm (90th percentile) and head circumference 39.2 cm (> 97th percentile).

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Family history revealed that the mother had been epileptic since five years of age and was treated with carbamazepine during pregnancy. We learned that the father had similar hand, foot and facial features of the baby.

On physical examination, the striking feature of the baby was the complex polysyndactyly of hands and feet. She also had a peculiar facial appearance characterized by a high forehead, broad nasal root and midly downslanting palpebral fissures (Fig. 1). The anterior fontanel was 6 x 6 cm. On both hands, thumbs were duplicated and postaxial polydactyly with complete syndactyly between second, third and fourth fingers was also noted (Fig. 2). On both feet there was preaxial polydactyly and both halluces were broad. There was complete cutaneous syndactyly between the hallux and second and third toes (Fig. 3).

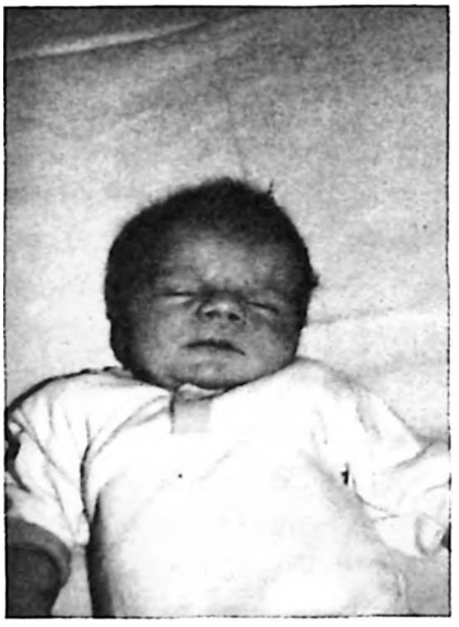


Fig. 1: Facial appearance of Case 1. Note prominent frontal region, hypertelorism and downslanting palpebral fissures.

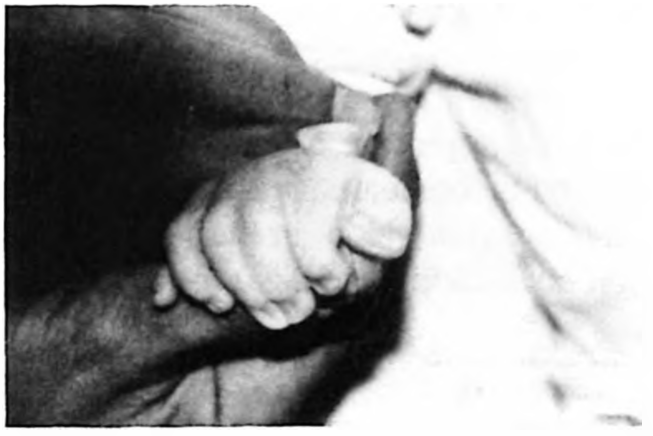


Fig. 2: Right hand of Case 1. Note bifid thumb, postaxial polydactyly and syndactyly betwen the second, third and fourth fingers.

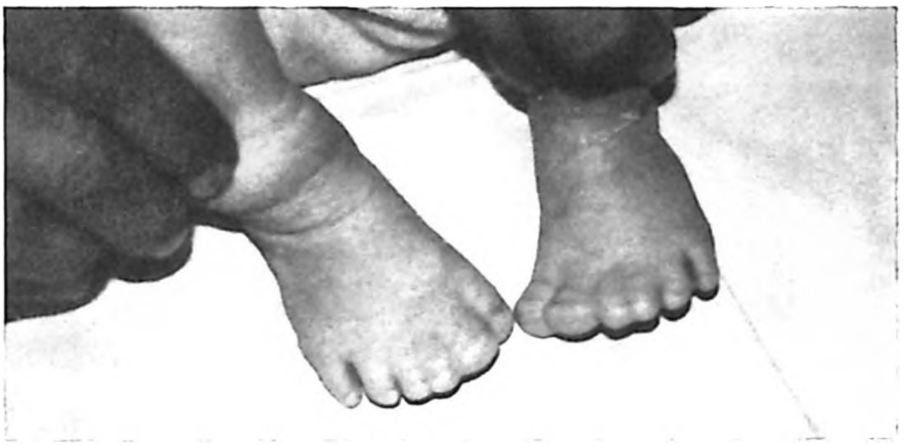


Fig. 3: Feet of Case 1. Note preaxial polydactyly on both feet. Halluces are broad and there is syndactyly between, the first, second and third toes.

Radiological examination, which was performed after removal of the supernumerary fingers in hands, showed duplication of te distal phalanges of both tumbs and fusion between the distal phalanges of the third and fourth fingers in both hands (Fig. 4). In feet, halluces were totally duplicated (Fig. 5). Cranial tomography showed mega cistema magna, enlargement of the lateral ventricles and variation of the cavum septi pellucidi (Fig. 6). Corpus callosum was present. High resolution chromosome analyses showed normal karyotype.

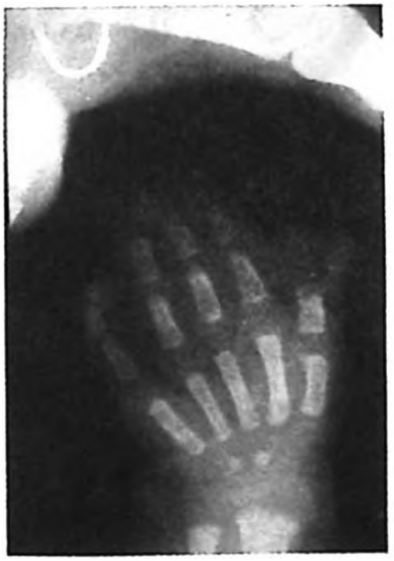


Fig. 4: X-ray of the left hand of Case 1. Note duplication of the distal phalanx of thumb and fusion of distal phalanx of third and fourth fingers.

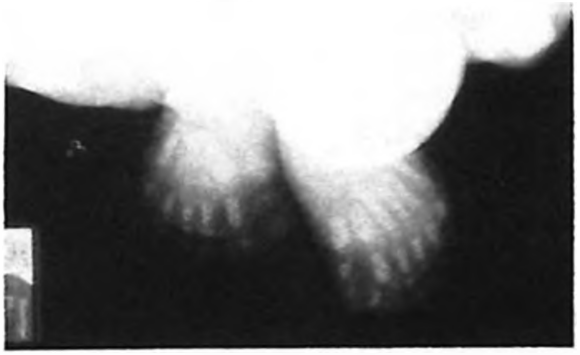
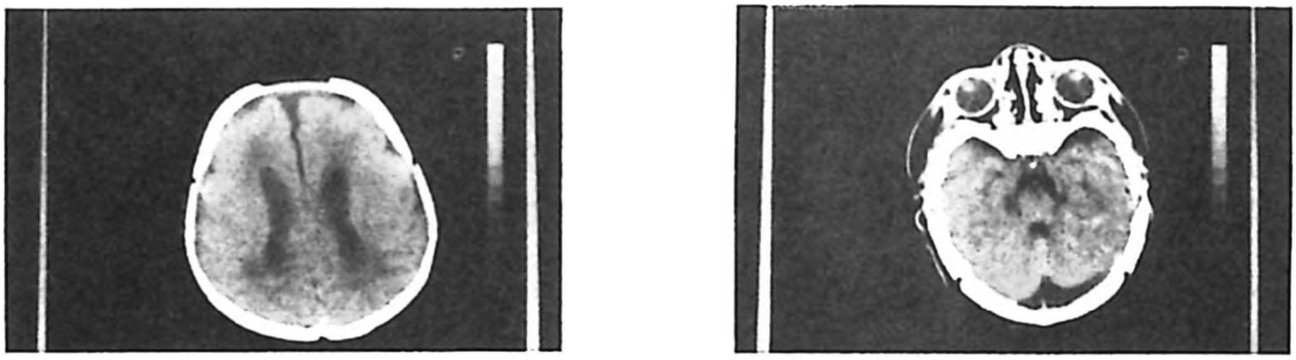


Fig. 5: X-ray of feet of Case 1. Note preaxial polydactyly in both feet.



(a) (b)

Fig. 6: Computerized tomography of the brain of Case 1. a) Note enlargement of the lateral ventricles and interhemispheric fissure b) Note mega cisterna magna.

Case 2

Case 2 was the 39-year-old father of Case 1. His facial and digital features were very similar to his daughter's. He mentioned that the postaxial polydactyly of his hands and preaxial polydactyly of his feet were excised in infancy and that did not have any other health problem. He was normal mentally.

On physical examination frontal bossing, high forehead and downslanting palpebral fissures were the characteristic facial features (Fig. 7). Head circumference was 58 cm. His right hand was normal but on the left hand the thumb was larger and shorter than normal and there was partial cutaneous syndactyly between the third and fourth fingers (Fig. 8). Scars due to excision of postaxial extra finger were visible on the ulnar side of both hands.

Fig. 7: Facial appearance of Case 2. Note prominent forehead and frontal region and downslanting palpebral fissures.

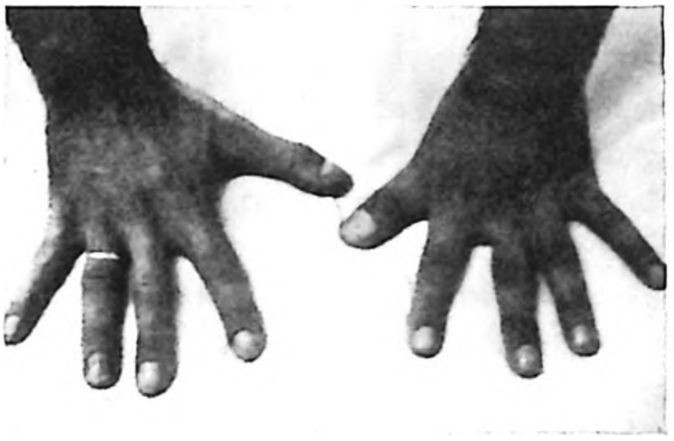


Fig. 8: Hands of Case 2. Note the short and broad thumb and partial syndactyly between the third and fourth fingers of the left hand.

The right hallux was larger than normal and there was a visible scar due to excision of preaxial extra finger. The left hallux and the preaxial extra toe were removed together. The other toes of both feet had normal appearance except for the syndactyly between the second and third toes on the left foot (Fig. 9). High resolution chromosome analyses showed normal karyotype.

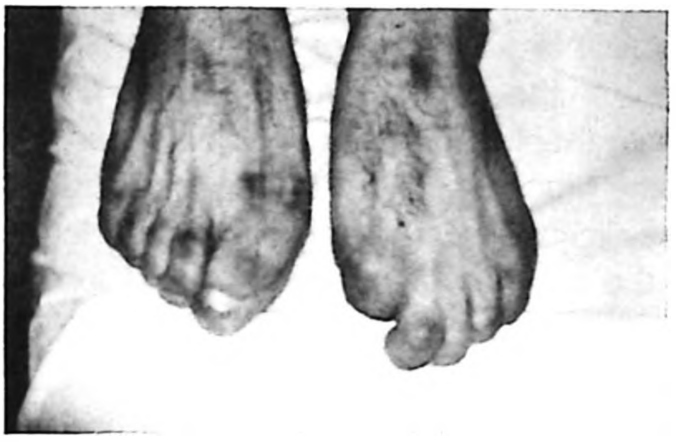


Fig. 9: Feet of Case 2. Preaxial polydactyly in both feet and hallux of the left foot were surgically removed in infancy. Note incision scar on the right hallux. There is an incomplete cutaneous syndactyly between the second and third toes.

Discussion

Greig cephalopolysyndactyly syndrome was first decsribed by Greig[3] in 1926. to date more than fifty cases have been published by several authors[4-7]. Major findings of GCPS are postaxial polydactyly (pedinculated postminimi) of hands, preaxial polydactyly of feet, cutaneous syndactyly of fingers and toes, and minor craniofacial abnormalities such as high forehead, macrocephaly, frontal bossing, broad nasal root and oblique palpebral fissures. Megalencephaly and enlargement of the lateral ventricles are also reported in patients with this

syndrome[1, 2]. Various authors have caled attention to the similarities between acrocallosal syndrome and GCPS and have proposed that they were same entity[4, 5, 8]. The main clinical distinction between these two syndromes is the presence of mental retardation in patients with acrocallosal syndrome, whereas patients with GCPS are usually normal mentally[4]. Linkage analysis has also confirmed that these two syndromes are not allelic[9]. Greig cephalopolysyndactyly syndrome, which has been shown to be due to mutations in the GLI3 gene[10], has an autosomal dominant mode of inheritance with high penetrance and variable expression[6]. Recognition of these findings and consideration of variable expression are very important for the diagnosis. The two patients in this report showed variable expression. Both of them had polydactyly of the hands and feet to a similar extent, but the daughter had more severe syndactyly of fingers and toes than her father. Mild craniofacial features and mild syndactyly of the father probably resulted in the diagnosis of uncomplicated polydactyly. Braitser et al.[4] (1983) proposed that some patients within the same family might have an indistinguishable phenotype from uncomplicated polysyndactyly (preaxial polydactyly type 4) due to very mild presentation of the facial features of GCPS syndrome. The father in this report is such a case with mild facial features. Since he had three corrective surgeries he never knew that he was a Greig cephalopolysyndactyly syndrome patient. If the father had been diagnosed correctly, it might have been possible to diagnose the baby prenatally in view of the ultrasonographic findings. Although intelligence is often normal in GCPS patients, some cases do have mild mentalretardation. For this reason, it might be important to diagnose this syndrome prenatally. Prenatal diagnosis of GCPS might be possible if there is a history of the disease in family members and there are specific prenatal ultrasound findings of the central nervous system and extremities.

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GASTRIC PERFORATION PRESENTING AS BILATERAL SCROTAL PNEUMATOCELES*

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SUMMARY: Aslan Y, Sarıhan H, Dinç H, Gedik Y, Aksoy A, Dereci S. (Departments of Pediatrics, Pediatric Surgery and Radiology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey). Gastric perforation presenting as bilateral scrotal pneumatoceles. Turk J Pediatr 1999; 41: 267-271.

Although processus vaginalis is patent in the majority of newborn infants, the expression of an intraabdominal pathology such as gastrointestinal perforation or bleeding in the scrotum is very rare. In a large percentage of neonates with the gastrointestinal perforation, pneumoperitoneum is absent. In any case, it may not be detected in early radiographs. We report a newborn baby who presented with bilateral scrotal pneumatoceles as a first sign of pneumoperitoneum due to gastric perforation. Plain x-ray of the abdomen was normal except for pneumoscrotum, but contrast study revealed gastric perforation. *Key words: gastric perforation, scrotal pneumatocele.*

Abdominal distention is a universal finding of gastrointestinal perforation. The presence of a blue discoloration of the abdominal wall and/or of disappearing bowel gas on abdominal x-ray should strongly suggest bowel perforation[1]. Most full-term newborns have an open processus vaginalis[2]. Therefore, one would expect an intraabdominal pathology to be expressed frequently in the scrotum. However, presentation of gastrointestinal perforation with a scrotal pneumatocele has been reported in only a few cases[3-6]. Here, we report a newborn baby with gastric perforation who presented with bilateral scrotal pneumatoceles.

Case Report

A 3,050 g boy was born vaginally at 42 weeks' gestation to a 30-year-old, healthy multigravida. Apgar scores three and six after one and five minutes, respectively. Umbilical cord blood gases revealed pH 6.09, PO_2 32 mmHg, and PCO_2 56 mmHg. Biochemical analyses yielded urea nitrogen 38 mg/dl, creatinine 1.2 mg/dl, aspartate aminotransferase 102 U/L, aminotransferase 94 U/L, creatinine kinase 1670 U/L, lactate dehydrogenase 2310 U/L, and electrolytes,

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calcium, phosphorus and uric acid levels within normal limits. Cranial ultrasound demonstrated a grade-II intracranial hemorrhage. Chest and abdominal roentgenograms were normal.

The infant was intubated for respiratory insufficiency due to hypoxic ischemic encephalopathy and intracranial hemorrhage. He was treated with synchronized intermittent positive pressure and subsequent intermittent mandatory ventilations. Dopamine, dexamethasone, mannitol and intravenous fluid were administered.

At the 34th hour, a sudden swelling of bilateral scrotums was noted. The swelling was translucent and compressible but could not be emptied into the abdomen, suggesting intrascrotal trapping of air (Fig. 1). Ten minutes later, the baby developed abdominal distention. Plain abdominal x-ray was normal except for bilateral scrotal pneumatoceles. Radiological contrast study of the upper gastrointestinal tract using iopamidol was performed. There was a prominent extravasation from the stomach immediately after the ingestion of the contrast agent and bilateral scrotal pneumatoceles (Fig. 2). neither peritoneal and gastric fluids nor blood and urine cultures grew any microorganism. Prothrombin time, thromboplastin time and platelet count were within normal limits.

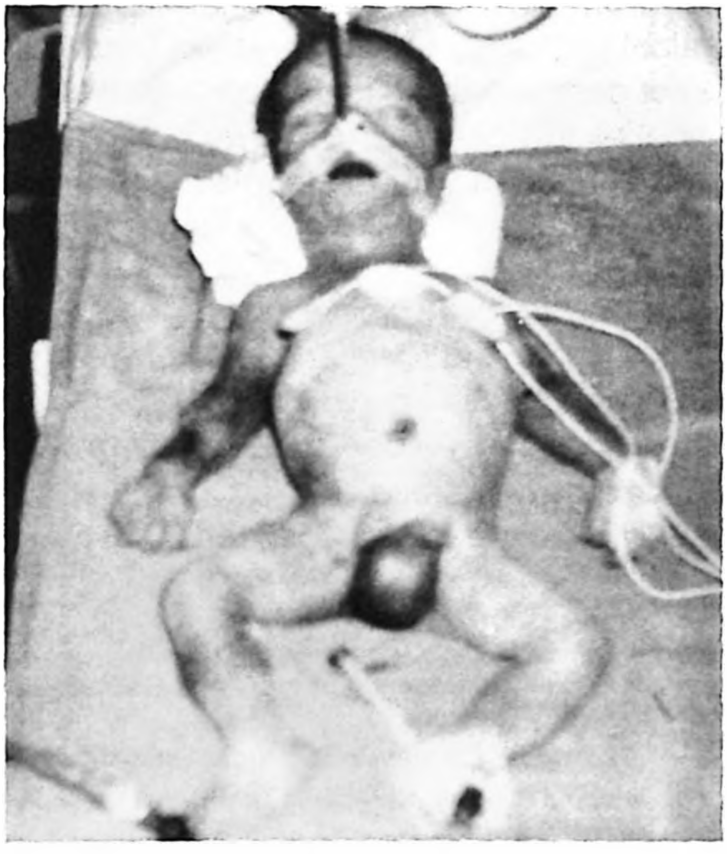

Fig. 1: The case, who developed sudden, translucent swelling of bilateral scrotums, suggesting intrascrotal trapping of air.

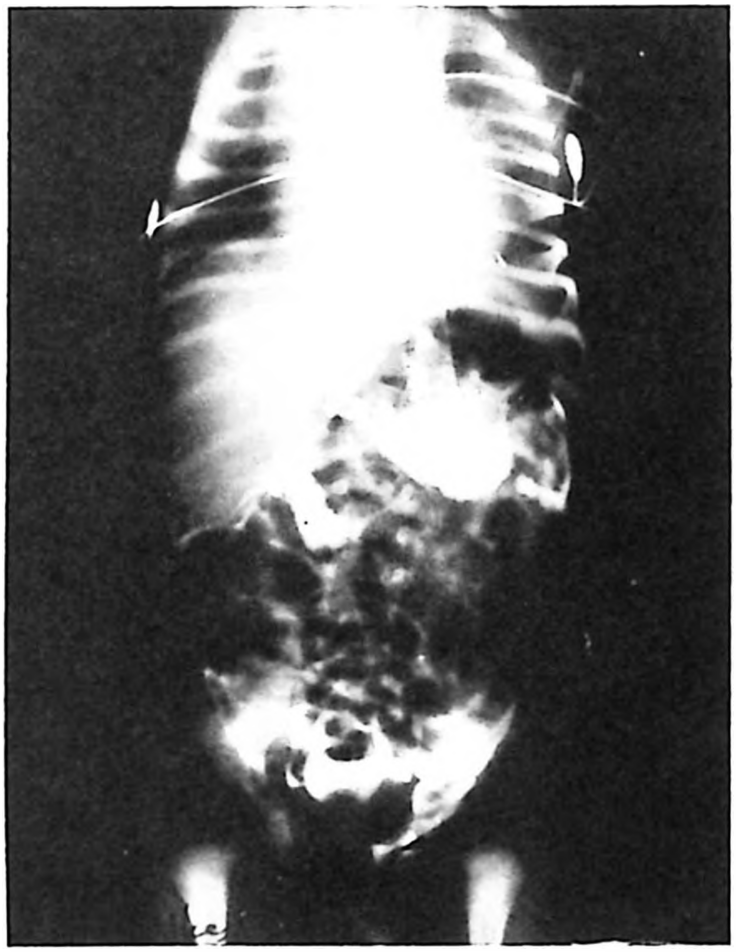

Fig. 2: Radiological contrast study of the upper gastrointestinal tract using iopamidol shows a prominent extravasation from the stomach immediately after the ingestion of the contrast agent and air shadows in bilateral inguinoscrotal regions (arrows).

A nasogastric tube was inserted. Bilateral scrotums were punctured and free air was removed by suction. Surgical intervention was performed in the neonatal intensive care unit. Stomach perforation was detected, and primary repair and drainage were performed. Intravenous antibiotics (ceftriaxone, netilmicin sulfate and metronidazole) were given. At the 46th hour, he developed bradycardia and died due to intracranial hemorrhage.

Discussion

Among the upper gastrointestinal tract perforations in infants and children, gastric perforations are predominant[7]. However, perforation of the stomach is rare during the newborn period and the first year of life. A wide spectrum of causes has to be considered[8]. Hypoxia is believed to be the most important datum in the pathogenesis of gastric perforation in childhood[9]. The present case had severe hypoxia.

The most common clinical presentation of gastrointestinal perforation is abdominal distention[1, 10]. Abdominal distention is frequently abrupt and rapidly progressive[11]. In our case, abdominal distention was not the first sign of the gastrointestinal

perforatilon. In a few cases reported previously, gastrointestinal perforation presented with scrotal signs such as pneumatocele or hydrocele, which require a patent processus vaginalis. Patency mainly depends on postnatal age. Eighty to 90 percent of full-term newborn infants have an open processus vaginalis[2]. Although the processus vaginalis is patent in the majority of newborn infants, expression of an intraabdominal pathology such as perforation, inflammation or bleeding in the scrotum is relatively rare. To our knowledge, scrotal pneumatocele reflecting the presence of pneumoperitoneum has been reported in only four cases prior to this one[3-6]. Pneumoperitoneum was the result of pulmonary barotrauma in one, abdominal surgery in two, and spontaneous gastrointestinal perforation in one. Hydrocele due to accumulation of blood and/or intestinal material and scrotal inflammation as a result of gastrointestinal perforation and/or meconium peritonitis have only been described in case reports[12, 13].

Pneumoperitoneum signals gastrointestinal perforation and, as a rule, requires a prompt laparatomy[14]. However, it is reported that patients with respiratory distress or patients ventilated artificially may develop pneumoperitoneum without any gastrointestinal leak[5, 14]. Critically ill infants with respiratory distress or hypoxia may not easily tolerate an unnecessary laparatomy; therefore, "medical" pneumoperitoneum should be distinguished from "surgical" pneumoperitoneum by clinical and radiographic findings[14]. However, early radiographs may not reveal pneumoperitoneum, which becomes obvious with time[11]. In addition, it is reported that in a large percentage of neonates with gastrointestinal perforation, pneumoperitoneum is absent[10]. Therefore, if the diagnosis is doubtful, radiological contrast study of the gastrointestinal tract using a water-soluble contrast agent has been advocated[15].

In our case, the presenting sign of the gastrointestinal perforation was bilateral scrotal pneumatoceles; abdominal distention developed later. Plain x-ray of the abdomen did not show pneumoperitoneum or an intraperitoneal air-fluid level suggesting gastrointestinal perforation, but it did demonstrate bilateral penumoscrotum. Radiological contrast examination of the gastrointestinal tract indicated gastric perforation in addition to pneumoscrotum.

In conclusion, we believe that awareness of scrotal pneumatoceles will enable early diagnosis of gastrointestinal perforation. Waiting for the typical clinical findings of gastrointestinal perforation such as abdominal distention or blue discoloration of the abdominal wall is likely to lead to a hazardous delay in diagnosis and treatment.

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TRACHEAL RUPTURE: A RARE COMPLICATION RELATED TO FOREIGN BODY ASPIRATION*

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SUMMARY: Çelebioğlu B, Tanyel FC, Altunkaya H. (Departments of Anesthesiology and Pediatric Surgery, Hacettepe University Faculty of Medicine, Ankara, Turkey). Tracheal rupture: a rare complication related to foreign body aspiration. Turk J Pediatr 1999; 41: 273-276.

A one-year-old patient admitted following foreign body aspiration and referred following cardiopulmonary resuscitation in a local hospital was diagnosed to have tracheobronchial rupture. We first assumed puncture of the mucous membrane of the left main bronchus by the tip of the tube. Later, we thought that the rupture might have been caused by rigid bronchoscopy. Etiology and treatment are discussed and recent literature reviewed. *Key words: trachea, rupture, aspiration, foreign bodies, rigid bronchoscopy, intubation complications.*

Perforation of the trachea and main stem bronchi are rarely encountered during surgical practice. Such injuries usually result from major trauma or iatrogenically. Intubation, suction and foreign body aspiration are other causes of tracheobronchial perforations[1].

Since tracheobronchial injuries may follow a rapidly lethal course, immediate diagnosis and surgical intervention are essential for a successful outcome[2].

A case of tracheobronchial rupture related to foreign body aspiration is reported to stress the importance of tracheobronchial evaluation following foreign body removal.

Case Report

A one-year-old girl was referred following foreign body aspiration. During dinner at 20:00 she had acute onset respiratory distress and cyanosis. She was admitted to a local hospital at 20:15 where she had cardiopulmonary arrest. Following intubation and resuscitation which included external cardiac massage but no intracardiac drugs, she was referred to our hospital at 21:00. She had spontaneous respiration through an endotracheal tube which was a 2.5 mm ID, rubber tube without cuff. Physical examination at admission revealed pulse rate of 145/min and a rate of respiration of 35-40/min. She weighed 10.4 kg. Plain chest radiograph at admission revealed consolidation in the right hemithorax. She was immediately taken to surgery, at 21:10.

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Anesthesia was induced with propofol 2 mg/kg intravenously (i.v.) and muscle relaxation was obtained with succinylcholine 1 mg/kg (i.v.) Additional incremental doses of succinylcholine were administered during the bronchoscopy.

Anesthesia was continued with an infusion of propofol and 100 percent oxygen. While the lungs were ventilated, airway pressures were normal throughout the procedure and oxygen saturation remained at approximately 96 percent.

A haricot bean was removed through rigid bronchoscope from right main bronchus. After foreign body removal the control bronchoscopy revealed a complete longitudinal tear of 2 cm in the posterior part from the distal trachea to the left main bronchus, which went unnoticed before the removal. Chest x-ray showed no pneumothorax. A surgical closure was decided and the patient underwent left thoracotomy. After diagnosis the selective right bronchus was intubated carefully and anesthesia was continued with isoflurane, N_2O, O_2 and vecuronium. The tear was exposed and sutured using 4-0 vicryl sutures.

Electrocardiogram (ECG), oxygen saturation and noninvasive arterial pressure which were monitored continuously throughout the procedure remained within normal limits.

The postoperative recovery was uneventful and the patient was discharged after a week. The patient was free of signs and symptoms after six months of follow-up.

Discussion

Foreign body aspiration into the tracheobronchial tree is preventable. It has been a leading cause of in-home accidental deaths in children under four years of age[3, 4].

Early diagnosis is imperative to ensure prompt and successful treatment. However, bronchoscopy in children is still hazardous and demands special skills[5].

Rupture of the trachea rarely complicates tracheal intubation. Only a few case histories dealing with this complication are found in the English language literature. In almost all cases reported to date, the cause has been obvious. Endotracheal tube-related ruptures may result from either over distended and/ or asymmetric cuff or from direct trauma caused by the tip of the tube[6].

In this case, the patient was intubated with a small red rubber tube without a cuff. But the tube was of small size for the patient and thus bronchial intubation might have occurred.

We first assumed that puncture of the mucous membrane of the left main bronchus by the tip of the tube was the primary cause and that the lesion was enlarged due to coughing. Tracheal rupture occurred in the left main bronchus but the foreign body was found in the right side. We believed, therefore, that the tube might have been pushed through the left main bronchus during resuscitation.

Our next thought was that the rupture may have resulted from the rigid bronchoscopy. Rigid bronchoscopy may induce bronchospasm and interfere with ventilation. Thus, the anesthetic technique should provide adequate analgesia and muscle relaxation to eliminate reflexes from stimulation of the respiratory tract[7]. In our case, we provided adequate analgesia with i.v. anesthetics. When the rupture was seen with bronchoscope, the patient had normal inspiration and there was no pneumothorax. Although the rupture was clearly iatrogenic in the reported case, the places of resuscitation, bronchoscopy and foreign body removal remains obscure.

Bronchoscopy is the diagnostic technique of choice for patients with tracheo-bronchial injuries but it sometimes fails and complications can occur. Rupture often occurs within 2.5 cm of the carina[8-10].

Optimal treatment for tracheal ruptures includes: endotracheal intubation past the injured area, immediate drainage of pneumothorax if this complication occurs, acceleration of the rate of absorption of the pneumomediastinum by administration of a high inspired oxygen concentration, and correction of metabolic acidosis[11]. Early diagnosis and prompt operative management can minimize the morbidity and mortality of tracheobronchial injures[12].

Especially in cases with increased risk, such as having undergone resuscitation, the tracheobronchial tear should be evaluated for injury following foreign body removal. Close cooperation between the anesthetist and surgeon regarding airway issues is mandatory. Our experience with this case indicates that both the anesthetist and surgeon must be cautious of this possibility.

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LEUKOCYTOCLASTIC VASCULITIS IN A CHILD WITH EPIDERMOLYSIS BULLOSA SIMPLEX*

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SUMMARY: Sezgin G, Ceyhan M, Seçmeer G, Bakkaloğlu A, Kanra G, Büyükkale G. (Infectious Diseases and Nephrology-Rheumatology Units, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Leukocytoclastic vasculitis in a child with epidermolysis bullosa simplex. Turk J Pediatr 1999; 41: 277-282.

A 10-year-old boy with epidermolysis bullosa simplex (Weber-Cockayne variant) together with leukocytoclastic vasculitis is presented. He was admitted to the hospital with the provisional diagnoses of infected epidermolysis bullosa simplex or drug eruption. On the sixth day of hospitalization he developed palpable purpura, abdominal pain and bloody diarrhea, together with hematuria and proteinuria. A generalized tonic-clonic convulsion, changes in mental status, fluctuations in arterial blood pressure and intractable pain in his extremities occurred during the course of hospitalization. Systemic pulse steroid therapy, antibiotics, and antihypertensive and anticonvulsive drugs were given. On the 30th day of hospitalization, a skin graft was performed to replace a large tissue defect on his left hand. Despite high dose steroid therapy, his hematuria, proteinuria and hypertension continued after his discharge, suggesting a steroid-resistant renal pathology, such as focal glomerulosclerosis, that occurred secondary to leukocytoclastic vasculitis. *Key words: epidermolysis bullosa simplex, leukocytoclastic vasculitis, childhood.*

Epidermolysis bullosa (EB) is a group of genetically determined diseases characterized by abnormal fragility of the skin and mucosa. It may be divided into three major inherited forms (simplex, junctional and dystrophic), based on the presence or absence of scarring, the mode of inheritance and the level of skin cleavage following minor trauma, and an acquired form (EB acquisita). Depending on the specific type of EB, prognosis varies considerably. At present there is no cure for any form of EB. Epidermolysis bullosa EB simplex is characterized by autosomal dominant inheritance. Blisters generally heal without scarring and mucosal involvement is mild. the commonest type of EB simplex is the Weber-Cockayne variant in which the blisters occur mainly on the hands and feet. Cytolysis of the epidermal cells is the essential histological feature of the EB simplex. Epidermal cleavage usually occurs in the midsquamous area but it may be noted anywhere from the suprabasal to the lower granular layers of the epidermis[2].

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Leukocytoclastic vasculitis is a necrotizing vasculitis affecting small blood vessels in which the polymorphonuclear leukocytes (PMNL) infiltrate the necrotic vessel wall, and scattered nuclear debris accumulates around the lesions. This is the predominant reaction in Henoch-Schönlein purpura (HSP), mixed cryoglobulinemia, hypersensitivity angiitis, hypocomplementemic urticarial vasculitis and other connective tissue diseases such as systemic lupus erythematosus (SLE). In the literature, there are a few reports of epidermolysis bulosa acquisita occurring in association with systemic lupus erythematosus[5, 6], but no report exists on the development of leukocytoclastic vasculitis in a patient with a hereditary form of epidermolysis bullosa.

Thus, we present herein a case of leukocytoclastic vasculitis in a child with epidermolysis bullosa simplex.

Case Report

A 10-year-old boy presented to Hacettepe University Children's Hospital Infectious Disease Unit, Ankara, in April 1996 with a diagnosis of infected EB simplex and a suspected drug eruption.

The diagnosis of EB simplex was first suspected when he was admitted to another hospital at 18 months of age with recurrent attacks since his neonatal period of bullous lesions confined to his hands and feet following minor trauma. One week before his admission at that time he developed erythema and papular lesions on his legs, and the bullous lesions that were present on his legs and feet increased. He was examined by a dermatologist and prescribed a topical steroid and an oral antihistaminic which were of no benefit.

He was the fourth child of healthy nonconsanguineous parents. He had one brother who had similar lesions beginning from birth who had died of a convulsion after vaccination with DPT at two months of age. There was no family history of autoimmune disease. He had no history of photosensitivity, malar rash, arthritis, serositis or renal, neurological or hematological disorders.

Clinical examination revealed multiple bullous lesions on his hands, feet, forearms, and legs; widespread erythema and maculopapular eruptions on thighs; and significant erythema on left ankle, lateral part of right foot, third finger of the left hand and right hand. Involvement of the soles made ambulation impossible and palmar lesions interfered with routine hand functions. Bullae were tense, measuring up to 2 cm in diameter and filled with clear yellow fluid. Physical examination was otherwise normal. Sulbactam-ampicillin was started at a dose of 100 mg/kg/day i.v. On the 6th day of his admission sulbactam-ampicillin was changed to ciprofloxacin because of the palpable purpura on his thighs and gluteal region and of the petechiae and aphthous lesions in his oral mucosa. At this time, the patient had intractable pain caused by his tense bullous lesions as well as from his palpable purpuric lesions, which required codeine preparation (Fig. 1).

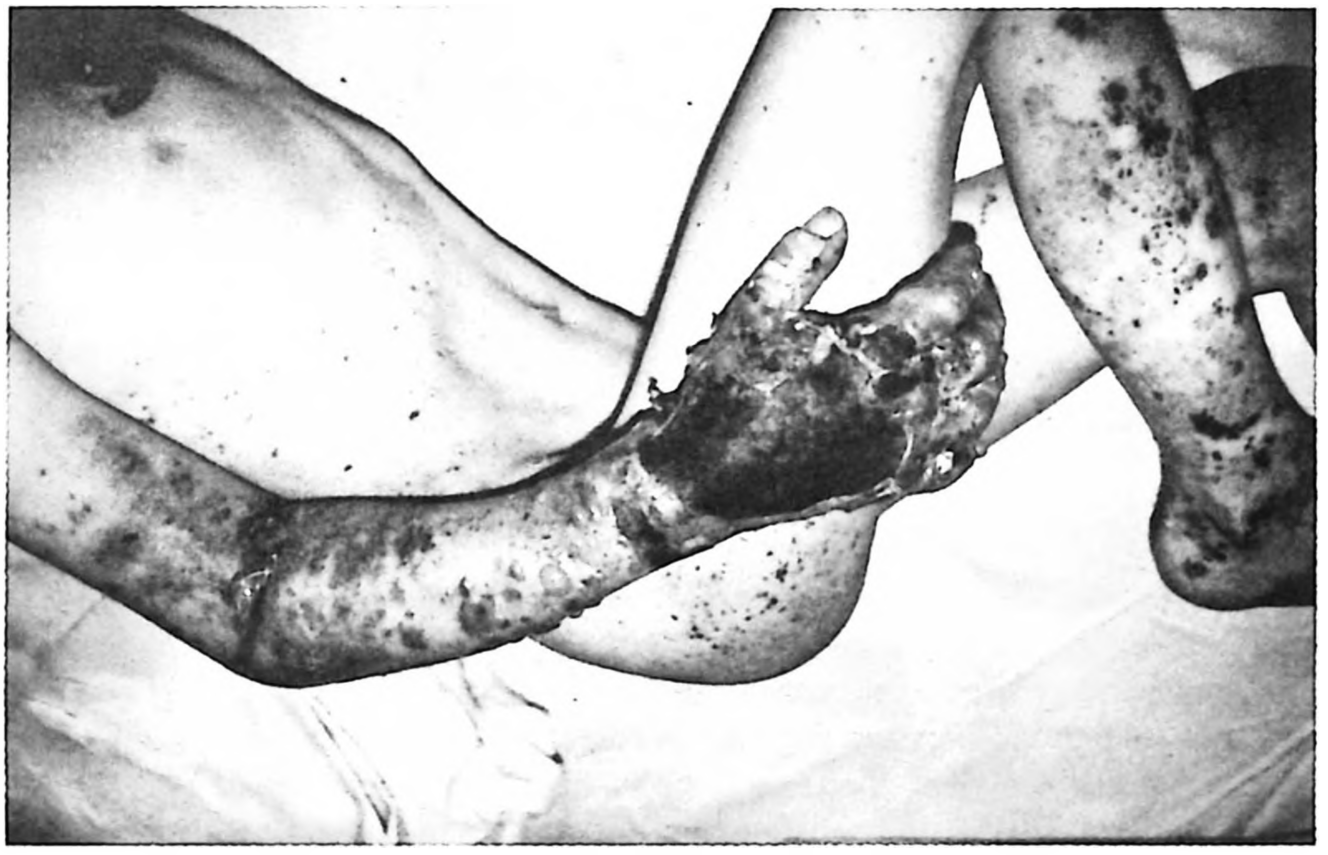

Fig. 1: Right hand and arm with blistering and scalded purpuric lesions.

Laboratory examination revealed normochromic normocytic anemia (HB: 11.5 g/dl, Htc: 32%, MCV: 84 fl), a white blood cell count of 18,600/mm^3 with predominance of PMNL on peripheral blood smear and normal platelet count. Erythrocyte sedimentation rate was 75 mm/hr. The urine was 3 (+) for protein and sediment contained 3-4 leukocytes and 3-4 erythrocytes per high power field. The values for urea nitrogen, creatinine, calcium, phosphorus, uric acid, total protein, and albumin were normal. Abdominal ultrasound showed increased echogenitcity in both kidneys such that the cortex and the medulla could not be distinguished and there was edema in the intestine, especially in the duodenum. Protein/creatinine ratio in spot urine specimen was 3.4 and creatinine clearance was 94 ml/min/1.73 m^2 (normal: 89-165 ml/min/1.73 m^2 for this age group). ASO titer was 244 U/ml (normal: 166-250 U/ml). Quantitative c-reactive protein (CRP) was 53 mg/L (Normal: 0-8 mg/L). C_3, C_4, CH_{50} values and serum immunoelectrophoresis for IgA, IgM, and IgG were normal.

Our preliminary clinical differential diagnosis favored HSP together with EB simplex.

On the fourth day following occurrence of his lesions the patient demonstrated edema and erythema of his eyelids, and prednisolone at 1 mg/kg/day was started. During the follow-up the patient also had a generalized tonic-clonic seizure. Magnetic resonance imaging revealed cortical and subcortical lesions in the posterior temporal, occipital, posterior parietal and frontal regions bilaterally (Fig. 2). These lesions were thought to be secondary to sinus thrombosis or vasculitis.

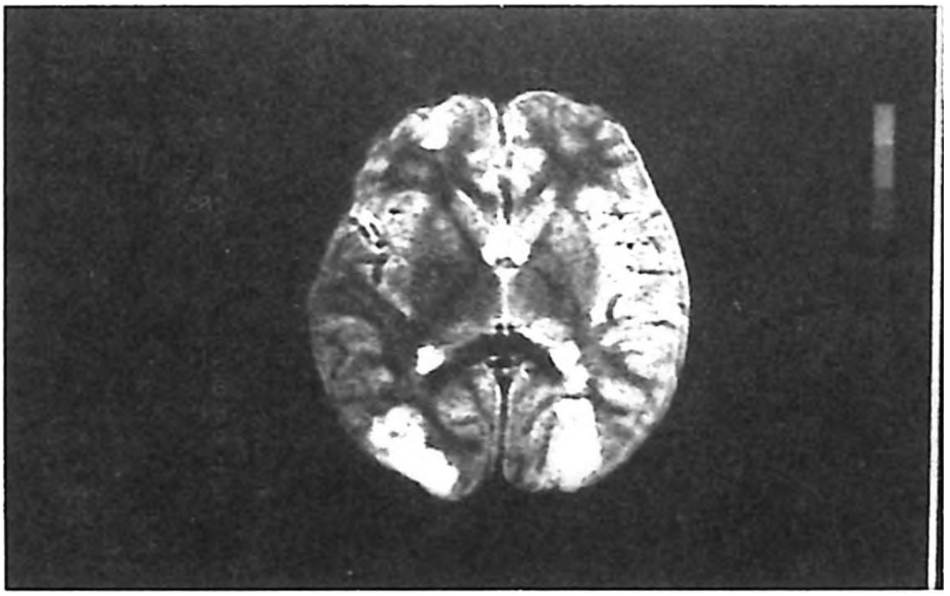

Fig. 2: Bilateral cortical and subcortical areas of increased density on cerebral magnetic resonance imaging (MRI).

As our patient's episodes of unconsciousness continued, we increased the dose of prednisolone first up to 2 mg/kg/day and then to 30 mg/kg/day. a punch biopsy specimen from the purpuric lesions revealed leukocytoclastic vasculitis. Direct immunofluorescence staining performed on the punch hiopsy specimen taken on the 7^{th} day of steroid therapy showed nonspecific C_4, IgA, IgM and fibrinogen positivity in the interstitial area of the upper dermis. Electron microscopic examination showed degeneration of the basal layer and cleavage of the epidermis above the basal lamina, both of which are correlated with the diagnosis of EB simplex.

Anti-nuclear antibodies, anti-DNA antibodies, anti-neutrophilic cytoplasmic antigen, anticardiolipin antibodies, lupus anticoagulant and rheumatoid factor were negative. Serology for CMV, EBV, Mycoplasma pneumoniae, Herpes simplex virus type I and type II, and markers for HBV and HCV were negative. Echocardiography was normal. Plasma proteins C and S and antithrombin III were normal. On the 30^{th} day of hospitalization, a skin graft taken from the right inguinal area was placed on his left hand where a tissue defect occurred. Pulse steroid therapy of 30 mg/kg/day was continued for four days and then gradually decreased to 2 mg/kg/day. All other bullous lesions and purpuric lesions healed without scarring and the inguinal area from which the skin graft was removed demonstrated no scarring. Before his discharge from the hospital his urea nitrogen, uric acid, creatinine, calcium and phosphorus were normal. The urine was still 3 (+) for protein and the sediment contained 2-3 erythrocytes per high power field. Creatinine clearance was 44 ml/min/1.73m^2 and protein/creatinine

ratio was 14 in 24-hour urine specimen.Although he has been on extended steroid therapy, the persistence of hematuria, proteinuria and hypertension may suggest a steroid-resistant renal pathology which occurred secondary to the vasculitic process that developed on the basis of a hereditary disease, EB simplex.

Discussion

The presence in this case of clinical symptoms such as non-thrombocytopenic palpable purpura, abdominal pain, bloody diarrhea, proteinuria, hematuria, generalized seizure, and mental status changes agreed with our diagnosis of HSP. Although our diagnosis was HSP together with EB simplex, we were not able to rule out hypersensitivity angiitis.

The diagnostic criteria for hypersensitivity angiitis was outlined in 1990 by the American College of Rheumatology (ACR)[1]. The presence of medication at disease onset, palpable purpura, maculopapular rash, and histological changes showing granulocytes in perivascular or extravascular locations suggested the diagnosis of Henoch-Schönlein nephritis, and hypersensitivity angiitis together with EB simplex. Belmen et al[3]. described three patients with HSP who developed prominent neurological smyptoms and signs during the course of their illness. Based on their experience and a review of the literature they suggested that nervous system involvement is more common than previously believed. Headaches and mental status changes are the most frequent complications of HSP followed by seizures, focal neurological deficits, mononeuropathies and polyradiculopathies. In our case the cause of the generalized seizure andperiods of mental status changes could also be attributed to fluctuations in blood pressure, but it is difficult to comment on whether it is a consequence of hypertension or secondary to the vasculitic process. Although we know from recent studies that glucocorticoids do not demonstrate any advantage over supportive therapy in gastrointestinal hemorrhage or renal disease[1], we administered with an increasing dose regimen because of the intractable pain and the possible central nervous system involvement.

Review of the literature gives no information related to EB simplex together with leukocytoclastic vasculitis. There are only reports regarding EB acquisita with clinical features of bullous lupus erythematosus[5,6]. Epidermolysis bullosa EB acquisita is thought to be an autoimmune disease in which patients have antibodies to the amino-terminal of type VII collagen[4]. It is a chronic progressive disease with periods of relapses and remissions and is resistant to systemic glucocorticoids. The diagnostic criteria of EB acquisita were originally outlined by Prussick et al.[5] and were subsequently modified. Patients with SLE, especially those with the bullous type, may produce antibodies to the EB acquisita antigen in addition to other autoantibodies[5,6]. In our patient, a diagnosis of EB acquisita

was ruled out in view of the family history of EB in a brother; the electron microscopic, light microscopic and direct immunofluorescence findings; and the clinical picture. In recent studies, it is suggested that autoantibodies to collagen types III, IV, V and laminin may be detectable in serum samples from patients with hereditary EB[4]. These serum antibodies would be of different molecular characteristics than those of EB acquisita autoantibodies. In our patient, death of one brother with EB at two months of age after vaccination and the development of hypersensitivity angiitis and Henoch-Schönlein nephritis suggest that some unknown autoimmune mechanisms based on EB simplex may also play a role.

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STENOTROPHOMONAS MALTOPHILIA PNEUMONIA IN A PREMATURE INFANT*

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SUMMARY: Özkan H, Paşaoğlu G, Olgaç N, Günel R, Yüce A, Gülay Z. (Departments of Pediatrics and Microbiology, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey). Stenotrophomonas maltophilia pneumonia in a premature infant. Turk J Pediatr 1999; 41: 283-286.

Stenotrophomonas (Xanthomonas) maltophilia is an aerobic, non-fermentative, Gram-negative bacillus that is generally considered an opportunistic pathogen. Infections due to S. maltophilia have become increasingly important in the hospital environment. Patients compromised by debilitating illnesses, surgical procedures or indwelling vascular catheters are most prone to S. maltophilia infections. To our knowledge, we report the first case of S. maltophilia pneumonia in a premature infant of 31 weeks gestational age. Although the therapy of choice for severe infections caused by S. maltophilia remains to be decided, this patient was successfully treated by amikacin.
Key words: newborn, nosocomial infection, Stenotrophomonas maltophilia.

Stenotrophomonas maltophilia (S. maltophilia) has emerged as a causative agent of serious nosocomial infections. S. maltophilia is an aerobic, nonfermentative, Gram-negative bacillus, originally referred to as Xanthomonas or Pseudomonas maltophilia[1,2]. This organism is mainly associated with postoperative wound infections, septicemia and urinary or respiratory tract infections. S. maltophilia is also a rare cause of endocarditis, ocular infections, meningoencephalitis and cholangitis in immunocompromised patients[3-10].

The rate of isolation is increasing and it is often resistant to antimicrobial agents that are commonly used initially to treat Gram-negative infections[3-5]. However, no well documented case of nosocomial pneumonia with this organism has been reported in newborn babies.

Case Report

The case was one of twins born at 31 weeks of gestation after spontaneous rupture of membranes. Her mother was 26 yers of age and healthy. The patient was admitted to our newbron unit at the 12th hour postnatally with Apgar scores

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of three at one minute and four at five minutes. Her mother had no infection in her pregnancy, but had used ritodrine as prescribed at the 12th week of pregnancy due to risk of abortion. On physical examination, her length was 41 cm (50th-75th percentile), weight was 1600 g (50th-75th percentile) and head circumference was 29.3 cm (50th percentile). She was hypothermic, tachycardic and tachypneic. Because her chest x-ray showed grade IV respiratory distress sydrome (RDS) and blood gases revealed acidosis, she received surfactant therapy followed by mechanical ventilation. Sulbactam-ampicillin and cefotaxime combination was started empirically. She recovered successfully and her chest x-ray findings became normal after three days of ventilatilon. Antimicrobial treatment was stopped at the fifth day because there was no growth in her blood culture. Total parenteral nutrition (TPN) which was started on the third day was gradually stopped by the 25th day. Grade III intracranial hemorrhage (ICH) was seen in the cranial ultrasonography (USG) on the third day. Because of the development of hydrocephalus in the serial USG and computed tomography controls, a postnatal ventriculo-peritoneal shunt was implanted on the 25th day. Vancomycin and ceftriaxone were given as prophylaxis. Respiratory distress symptoms appeared when she was 29 days old. Chest x-ray demonstrated interstitial pneumonia. S. maltophilia 10^5 CFU/ml (colony forming unit) was isolated from tracheal aspirate culture. As the oxidase test was negative and catalase and motility tests were positive, the isolate was identified as S. maltophilia. Disk diffusion test was performed according to National Committee for Clinical Laboratory Standards (NCCLS). According to culture results, S. maltophilia was sensitive to sulbactam-ampicillin, imipenem, and amikacin; was intermediately sensitive to ceftriaxone; and resistant to cefuroxime. Accordingly, ceftriaxone was stopped and amikacin was started. Vancomycin was continued because of the shunt operation. After 10 days, her respiratory distress signs and chest x-ray findings resolved. She was discharged when her weight reached 1780 g.

Discussion

S. maltophilia has emerged as a causative agent of serious nosocomial infections. It is isolated from a wide variety of clinical sources, including blood, the respiratory tract, urine, wounds, spinal fluid and from enviromental sources such as hospital water supplies, faucets, sink drains, respirators, prosthetic heart devices and disinfectant solutions[5-14].

Incidence of nosocomial infection is 20-33 percent in newborn intensive care units, increasing with length of hospital stay and degree of prematurity. Most common agents are: coagulase-negative staphylococcus, Gram-negative bacilli (Klebsiella pneumonia, E.coli, Salmonella, Campylobacter, Enterobacter, Citrobacter, Pseudomonas aeruginosa, Serratia), Enterococcus, S. aureus and

Candida spp.[15] Drews et al.[16] reported that among 229 neonates, the infection rate was 27.2, the infection proportion 20.2 and the incidence density 21.9 infections per 1,000 patient days in the neonatal intensive care unit. Major sites of infection were pneumonia (32.4%), blood stream infections (27.5%), infections of the skin and surgical site infections (11.4% each). The predominant pathogen was Staphylococcus aureus (24.7%) whilst Gram-negative bacteria accounted for 22.8 percent of the total. On the other hand, Gray et al.[17] reported that coagulase-negative staphylococcus was the most common pathogen noted in blood cultures in very low birth weight neonates. Recently, NICHD Neonatal Research Network[18] reported that the majority (73%) of late-onset infections in very low birth weight (VLBW) neonates were caused by Gram-positive organisms, with coagulase-negative staphylococci being the most common. Gram-negative pathogens accounted for 18 percent of late onset infections. Enterobacter species, Escherichia coli and Klebsiella were the most frequent Gram-negative pathogens. There was no case of S. maltophilia sepsis in series of 7,861 VLBW neonates[18].

When the neonatal literature was reviewed retrospectively, no case infected with this organism was reported. This case shows that S. maltophilia can be a causative agent of serious nosocomial infections in premature infants as in immunocompromised adult patients. The mode of transmission of this organism is not well known. Risk factors for colonization and infection by this organism in premature babies may include longer hospitalization, catheterization, administration of anti-pseudomonal antibiotics, and the use of hospital water supplies and disinfectant solutions contaminated with this organism.

Our case was successfully treated with amikacin, although S. maltophilia is usually considered resistant to multiple antibiotics, including beta-lactam agents, quinolones, aminoglycosides and carbapenems[19].

S. maltophilia is a causative agent of serious nosocomial infections. To our knowledge, we report the first case of neonatal pneumonia due to S. maltophilia. S. maltophilia may become one of the important opportunistic pathogens in premature babies in the near future.

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