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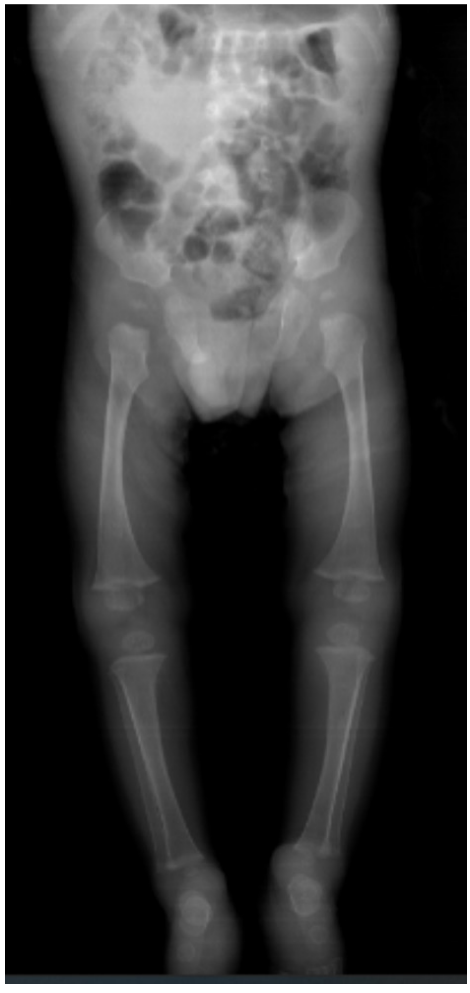

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Review

New Treatment Modalities in Hemophilia

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ABSTRACT

Hemophilia is a single gene disorder and as a genetical coagulation system problem it is a life-long bleeding disorder. Even though routine treatment modalities as plasma-derived and then recombinant factor concentrates available for last 50 years, unmet needs is continuing for hemophilia therapy. Gold standart treatment is regularly prophylactic FVIII/FIX infusions. However, life-long and frequent intra-venous infusions become medical burden for patients and families. New agents as enhanced half-life (EHL) factor concentrates and non-factor therapies which are able to be used subcutaneously are very hopeful. In this review, EHL factor concentrates, FVIII mimetic agents and re-balancing therapies will be discussed. Although celluler gene therapy is very hopeful and successful phase-3 studies are reported, gene therapy for hemophilia will not be mentioned in this review.

Keywords: Hemophilia, FVIII, FIX, EHL-products, emicizumab, fitusuran, concizumab

INTRODUCTION

Hemophilia-A is one of the rare hematological and genetical diseases. Prevalance rate is 1/10.000 and incidence is 1/5000 in male neonates. Hemophilia-B is also seen with a prevalance rate of 1/50.000. Both disorders are X-linked, recessive and genetically transmitted disorders.[1-3] Even though hemopohilia is a rare disorder; factor concentrates which are used in the treatment for many years are not orphan drugs. Because, in Western countries, approximately for 50 years, firstly plasma-derived FVIII and F-IX products and then the recombinant factor concentrates for the last 20 years have been widely used in routine practice. Gold standard of hemophilia therapy is prophylaxis.[1-3]

As Turkish hematologists and as Turkish patients with hemophilia as well; we are lucky that Turkey is one of the ten nations in the world where factor concentrates for prophylaxis of hemophilia patients are re-imbursed. Really, most of the severe hemophilia patients are in prophylaxis regimen. Hemophiliac artropathies are being significantly decreased for last 10 years thanks to prophylaxis.[4]

However, burden of disease is so heavy for hemophilia patients and families. Unmet needs are continuing. First of all, hemophilia is a life-long disorder. Prophylaxis must be given via intravenous route for a long-time. Venous accesss is a common problem for patients. Especially, during adolescent period, adherence problems to IV infusions are very well-known.[2,3]

All new therapeutic modalities will be discussed at the following.

Gene therapy is another treatment way for hemophilia due to the fact that hemophilia-A and hemophilia-B are single gene disorders. Phase-3 clinical studies are continuing nowadays. According to authorities, Food and Drug Administration (FDA) and European Medicines Agency (EMA) will give approval for HA and HB in 2022. Gene therapy is excluded from this review and will be discussed in another time.[5,6]

FVIII and FIX Products with Enhanced-half Life

Due to relatively short half-life of regular factor concetrates, frequent intravenous infusions are needed to maintain plasma FVIII levels. Technologies used for enhanced half-life (EHL)

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products are PEGylation, fusion protein technology (Fc part of immunoglobulin and albumin) and single-chain technology.[7,8]

In respect of new modalities, firstly enhanced-FVIII and IX products came into routine practice. For the last 5 years EHL-FVIII products were used for real-life conditions. These products were preferred for less frequent FVIII infusions and greater FVIII levels. However EHL-FVIII products did not bring definitive solution due to relatively low elevation (1.5 fold) in half-life. Some adult hemophilia-A patients are able to use once weekly programs. For children, twice weekly FVIII infusions are seen in the real-life conditions. Whereas, for hemophilia-B, EHL-FIX products are being used in every 7-10 days due to 4-5 fold increased half-life. In Western countries, EHL-FVIII and EHL-FIX products are now used for most of the patients in prophylactic regimens. In Turkey, some EHL-FVIII products received approval from the Ministry of Health, however re-imbursement agency (SGK) did not yet accept re-imbursement. Now, in Turkish market, all plasma-derived products and all regular recombinant FVIII/FIX products are available in re-imbursement list.[4]

The reason why for limited enhanced-FVIII half-life (maximum 1.5 fold) with the PEGylation and Fusion technologies is dependent on the half-life of von Willebrand factor. However, Sanofi has developed another technology called as "BVV-001 X-TEN Technology" which is not dependent on vWF half-life. With this technology following IV prophylaxis every 10 days, FVIII level is maintained more than 10% for the next 10 days. So, more than 10% FVIII level will provide better prevention against hemophiliac arthropaties in future. This regimens used with real FVIII products seem futural therapy for hemophilia-A (Table 1).[9]

Non-factor Therapies in Hemophilia

1. FVIII mimetic agents (Bi-specific antibodies),

2. Re-balancing therapies: Inhibitory monoclonal antibodies to natural anti-coagulant proteins in the blood stream as tissue factor pathway inhibitor (TFPI), antithrombin and/or protein-C.

Bispecific Antibodies for Hemophilia (FVIII Mimetic Agents)

Genetic defect in hemophilia-A is a mutation in the FVIII gene. In a patient with hemophilia-A, FVIII protein synthesis is not functional and not effective for hemostasis. In normal physiological conditions, For FXa, F-IX and FVIII should be available. In hemophilia-A patients, FVIII protein is not normal. In this new treatment regimen, bispesific antibodies are available. Both Fab arms of monoclonal antibody bind F-IXa and FX. The result of biological reaction is the development of FXa. So, new agent mimics of FVIII effect in coagulation process. Thrombin accumulation is final outcome for hemotasis.[10,11]

Emicizumab (Roche)

Emicizumab, a spesific monoclonal antibody that bridges FIXa and FX to allow the coagulation process to continue as a physiological hemostasis reaction. Emicizumab has been a life-transforming therapy for patients with or without inhibitors. This molecule is so important in history of hemophilia therapies. It is the first approved subcutaneous (SC) agent for hemophilia-A. Emicizumab was firstly licenced by FDA and EMA for patients with hemophilia-A with inhibitors.[10,11] Then it was approved in patients without inhibitors for HA. HAVEN studies were successfully completed before approvals.[12-15]

Table 1. Approved enhanced FVIII and F-IX products in Western countries

Generic name	Brand name and company	Technic for enhancing half-life	Practical using in Western countries
Efral-octocog	ELOCTATE/ELOCTA (Sanofi/Sobi)	Fusion protein (FVIII/Ig) Half-life: 19 h.	Approved 5 years ago by FDA and EMA. Not yet for Turkey as March, 2022
Damactocog	JIVI (Bayer)	PEGylated FVIII (60 kDa) Half-life: 19 h.	FDA approved two years ago. Turkish MoH approved in 2021 September
Octococ pegol	ADYNOVATE (Takeda)	PEGylated FVIII (2x20 kDa) Half-life: 14.3 h.	FDA approved 3 years ago.
Turoctococ pegol	ESPEROCT (Novo Nordisk)	PEGylated FVIII (40 kDa) Half-life: 19 h.	FDA approved last year.
rFIX-Fc	ALPROLIX (Sanofi/Sobi)	Fusion protein (F-IX/Ig) Half-life: 82 h.	It was approved 6 years ago. There is real-life experience in US and Europe. It is possible to use every 7 days
rF-IX- FP	IDELVION (CSL-Behring)	Fusion propein (F-IX/albumin) Half-life: 102 h.	Possible to use every 10 days for prophylaxis
Nonacoc pegol	REFIXIA (Novo Nordisk)	PEGylated protein F-IX Half-life: 96 h.	Possible to use every 7-10 days for prophylaxis
Efanes-octococ	BIVV-000-1 (Sanofi) rFVIIIc-vWF-X-TEN	X-TEN technology for EHL-FVIII	**Not yet approved**, phase-3 trial was completed. Possible to use every 10 days

FDA: Food and Drug Administration, EMA: European Medicines Agency

The HAVEN-1 study[12] was performed on adult patients with inhibitors. The first thrombotic events were observed in this study. Three patients with thrombo-microangiopathy and two patients with thrombo-embolism were reported. Mechanism of thrombotic events was evaluated as the combination of aPCC (FEIBA) and emicizumab. Today in the real-life conditions, combination with Emicizumab and aPCC is medically forbidden.[10,13] HAVEN-2 study[13] was performed for children with hemophilia-A with inhibitors. We also joined this internationally study with 8 Turkish patients from three expert centers including İzmir, İstanbul and Adana. We showed that Emicizumab was safe and effective for children with inhibitors. The MoH approved this molecule for every indications in HA in September 2019. However SGK has not yet approved it for re-imbursement. By the way, emicizumab (Hemlibra/Roche) has reached more than 10.000 hemophilia-A patients in Western countries.

Hemlibra is weekly loaded at a dose as 3 mg per kg SC. Then for weekly application, maintenance dose is 1.5 mg/kg. If you would like to use every two weeks, dose will be 3 mg/kg or for monthy dose the dose is 6 mg/kg.[14,15] The recommendation for children is SC injection every two weeks due to much less volume. Laboratory monitoring is not needed in routine practice. For breakthrough bleeds in non-inhibitory patients, FVIII treatment should be administered at a normal dose. Breakthrouh bleeds in patients with inhibitors are so different. First preferred by-pass agent must be rFVIIa (Novo Seven). Avoiding the use of aPCC (FEIBA) is important point and if required, lowest dose (50 IU/kg) of aPCC should be used.[10]

In hemophilia-B, unfortunately emicizumab is not effective. Because for it's effectivity, F-IX and F-X must be functionally available. Due to functional problem in F-IX in hemophilia-B, Hemlibra is ineffective (Table 2).[10]

Mim-8 (Novo Nordisk)

Mim-8 is another bispecific monoclonal antibody. However, it is not yet approved and phase-3 clinical study for adults has been initiated on 2022. We also studied with this agent in phase-2 study with success. The mechanism of action is similar to emicizumab. The molecule is given subcutaneosly. However, only weekly and monthly SC options are available.[16]

Definition of "next-generation" FVIII mimetics fits with Mim-8. Because Mim-8 is highly potent molecule bridging FIXa and FX in development for SC therapy. Mim-8 optimization process is aimed for efficient activation of FX by FIX in the presence of procoagulant membrane (as phospholipid membrane), minimal target binding, and low immugenecity risk. Mim-8 also has a high potency allowing for administration of small volume in a pen device.[16]

Re-Balancing Therapies

These type of therapies are interestingly effective in balancing coagulant/anticoagulant proteins. Another interesting point is FVIII or F-IX activities stay <1% during these treatments. Elevated thrombin levels are so important for gaining balance. The main characteristic of these therapeutic agents is inhibition of TFPI, antithrombin and protein-C. The most important point is efficacy in either HA or HB patients. So, patients with HB will be happy and hopeful with these agents. They were determined as official orphan drugs by FDA.[3]

Anti-TFPI Molecules

Concizumab (Novo Nordisk)

Mechanism of action is inhibitory effect to TFPI protein as a monoclonal antibody. This molecule is used daily with SC injections. However special insulin-like pens from Novo Nordisk are provided as an easy application. As a clinical center, we were in the phase-1[17,18] and phase-2 studies[19] and last year we initiatiated phase-3 study with success. Not only HA but also HB patients are nominates for this monoclonal antibody. FDA gave an orhan drug competence for HB patients with inhibitors.

Marstacimab (Pfizer)

This molecule can be used weekly or two weekly as SC infusions in HA and HB patients. Turkey is also one of the countries recruting most patients for phase-3 study and it is continuing.[20]

Antithrombin Agents

Fitusuran (Sanofi)

Fitusuran is designed to lower antithrombin, a protein that inhibitis blood coagulation, with the goal of promoting sufficient thrombin generation to re-balance hemostasis and prevent new bleeds. RNA-interference is a unique therapy for HA and HB patients with and without inhibitors. Interestingly, this molecule is used in every one month via SC route. Mechanism of action is antithrombin activity.

Table 2. Emicizumab and physiological FVIII comparison in coagulation balance

Comperative parameters	Physiological activated FVIII (FVIIIa)	Emicizumab (ACE910)
Interaction sites	Multiple sites of interaction	Single site of interaction
Affinity	High affinity for enzyme and substrate	Low affinity
Specifity	Specific for FIXa and FX (no binding to FIX and FXa)	FIX vs FIXa and FX vs FXa bindings possible
Co-factor activity	Full cofactor activity as phospholipid membrane binding/bridging from IX to Xa/FIXa stability	Partial cofactor activity only bridges FIXa to FX
On-off system	FVIIIa has a on-off system	EMI has no on-off system

However this effect reflects RNA inhibition in the hepatocyte level. Nowadays phase-3 studies are continuing.[3,21,22]

We are inside the ATLAS studies with 12 patients with HA and HB. Our experience with fitusuran has reached to four years. Now, protocol was changed after some thrombotic events. We use SC injections every 2 months now. Patients and families are so happy with this therapeutic modality. Interestingly, Turkey, India and Korea are the most recruiting countries for ATLAS studies.

In 2021 ASH congress, data from phase-3 studies showed that fitusuran significantly reduced bleeds in patients with hemophilia-A and hemophilia-B, with or without inhibitors.[21,22] Original portocol was 80 mg fitusuran SC in every month with control of alanine aminotransferase-aspartate aminotransferase levels. After protocol revision related with thrombo-embolic events in the study, dose was reduced to 50 mg in every two months. By the way, pediatric fitusuran phase-3 study was started in 2021, globally. Our center is one of the centers as always.

Anti-Protein C Agents

Serpin-PC (Apcintex/Centessa)

The last balancing molecule is Serpin-PC. Phase-1 and phase-2 studies[3] showed that it was a safe and efficient molecule when used SC. In 2022, phase-2B clinical trials will be started in Western countries and probably in Turkey[23].

CONCLUSION

Factor products with EHL mostly replaced regular factor products in Western countries. Much less IV infusion and very high trough factor levels are two biggest advantages for these products. However, they are used intravenously. So, future of SC agents seems more brightful for hemophilia therapies. SC application and very less frequency of injections (as weekly or as monthly) are very important unmet needs for currrent hemophilia therapy. In Western countries, 90% of patients with inhibitors and 50% of patients without inhibitors with HA have proceed to emicizumab. Unfortunately there is no SC agent approved for HB patients. Of course in future, fitusuran and concizumab are very hopeful and important options. Gene therapy wih AAV-based tecnology is being investigated in phase-3 studies. Approval time of FDA and EMA may be 2022.

Ethics

Peer-reviewed: Externally peer-reviewed.

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Original Article

Study of Liver Effect in Children with Celiac Disease

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ABSTRACT

Objective: It is well known that there are different rates of liver damage in celiac disease (CD). A wide spectrum of cases has been reported, ranging from asymptomatic transaminase elevation to cirrhosis. In this study, we investigated the frequency and clinical features of liver stress in children diagnosed with CD in our department.

Methods: Patients who were aged 1-18, serologically and histologically diagnosed as having CD and followed up were retrospectively included. Medical history, physical examination, serum anti-tissue transglutaminase and anti-endomysium antibodies, duodenal histology, liver function tests and imaging findings were evaluated.

Results: One hundred and eleven patients were included in the study. Of the patients, 74 were girls (64%) and 40 were boys (36%), and the mean age of admission was 7.1±4.3 years (1-18 years). The follow-up period was 3.5±4.4 (1-16) years. At diagnosis, alanine aminotransferase (ALT) was elevated in 5 (4.5%) of the 111 patients, and at follow-up, 3 patients were found to have lower levels that returned to the normal range. Elevation of gamma-glutamyl transferase (GGT) (3.7%) was found in 4 patients. Sclerosing cholangitis was diagnosed by liver biopsy in 2 patients with elevated GGT. Abdominal ultrasonography (USG) was performed in 50 (45%) patients. Hepatomegaly was found in 4 (3.6%) of these 50 patients and biliar dilatation in 2 (1.8%) patients. Abdominal USG also revealed hepatomegaly in 4 patients, without elevation in GGT and ALT levels.

Conclusion: We found 8% liver-related findings at the time of diagnosis in children with CD. No new liver effects were observed in 29% of patients followed up for five years.

Key words: Celiac disease, liver, child, transaminase levels

INTRODUCTION

Celiac disease (CD) is a disease that predominantly affects the proximal small intestine and is characterized by persistent intolerance to the gluten in wheat and other gluten-like grain proteins found in grains such as barley, rye and oats. Although CD is known as an enteropathy, it is a disease that affects many organs, and findings outside the gastrointestinal tract are common[1].

The association between CD and the liver was first demonstrated in 1977 by Hagander et al.[2] This association has been demonstrated in many studies over the past 40 years. Studies have reported that 9-42% of adults with CD and 24-40% of children with CD have elevated transaminase levels[3,4]. In addition, the prevalence in patients with unknown transaminase elevation is reported to be 4% in adults and 1.8% in children[5,6]. In CD, the only finding may be elevated liver enzymes; it may occur together with non-specific hepatitis, non-alcoholic fatty liver disease (NAFLD), autoimmune disease, and cholestatic liver disease. For this reason, it is recommended that liver functions be measured in children newly diagnosed with CD[7]. Elevated transaminases due to CD are defined as liver abnormalities caused by gluten that usually return to normal after 12 months of a strict gluten-free diet[8-10]. Changes in liver histology have been reported to improve with diet[11].

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The mechanisms of transaminase elevation associated with CD are not fully understood[12]. It is suggested that increased intestinal permeability in CD with elevated transaminase levels may facilitate the entry of toxins, microbial and other antigens, cytokines, and other mediators of liver injury into the portal circulation (and subsequently into the liver)[13,14]. However, in the presence of chronic liver disease and transaminase levels higher than five times the upper normal limit, detailed testing for other liver diseases should be performed at the time of diagnosis[13-15].

In addition to elevated transaminases due to CD, sclerosing cholangitis and autoimmune hepatitis may also be associated with CD. In studies of adults, the association of CD with sclerosing cholangitis has been reported at a rate of 0.1%-3%[16]. This association is an autoimmunity caused by a common genetic predisposition and resulting immune-related damage to the epithelium of the bile ducts and small intestine. Tissue HLA-DQ2 positivity poses a risk, especially in patients with primary sclerosing cholangitis and CD[16].

This study investigated liver disease in CD in childhood and clinical and laboratory findings that might be effective in patients diagnosed with CD and followed up in our department.

MATERIALS AND METHODS

The data of 111 patients, 71 girls and 40 boys, aged 1-18 years, diagnosed clinically, serologically and endoscopically with CD in Dokuz Eylül University Faculty of Medicine, Department of Pediatric Gastroenterology, Hepatology, and Nutrition, were retrospectively retrieved from the Hospital Information System database.

Ethical approval for this study was obtained from Dokuz Eylül University Non-Interventional Research Ethics Committee (approval no: 2019/28-14, date: 18.11.2019).

The diagnosis of CD is based on a combination of clinical, serological and histopathological data. Anti-endomysium immunoglobulin A antibody [Euroimmun, EUROPLUS liver (monkey)] and anti-tissue transglutaminase immunoglobulin A antibody (anti-TgA) (Euroimmune, ELISA method negative: <20 RU/mL, positive: ≥20 RU/mL) measurements were made. With regard to selective immunoglobulin A (IgA) deficiency, serum IgA levels (normal reference range: 70-400 mg/dL) were determined. The examination was performed with a Fujinon EG-590 WR (Japan) gastroscope device. In children with positive antibody, at least 4 biopsies were taken from the distal duodenum and at least 1 from the bulb. Histologic features of CD in the small intestine range from a mild change characterized only by increased intraepithelial lymphocytes to a severely atrophic mucosa with complete loss of villi, increased epithelial apoptosis, and hyperplasia of the crypt. The histologic severity of intestinal lesions in CD is graded using the Marsh-Oberhuber classification. The presence of Marsh type 2 and 3 lesions support the diagnosis[17]. Endoscopic duodenal biopsies of Marsh type-2 and Marsh type-3 were included in the study.

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase, alkaline phosphatase, albumin, total bilirubin, direct bilirubin, total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol of the patients were recorded. The upper normal limit for AST and ALT was 45 U/L. The abdominal ultrasonography (USG) records of the patients were examined. Abdominal USG was performed to evaluate for liver parenchyma as well as gallbladder stones and sludge that might accompany them. In 2 patients, the bile ducts were examined by using magnetic resonance cholangiopancreatography (MRCP).

Records of other causes (viral hepatitis, toxic causes) that might increase AST and ALT levels in the patients were reviewed. Body weight and height were recorded. Body mass index (BMI) and standard deviation score (SDS) were calculated.

Statistical Analysis

It was performed using SPSS 19.0 for Windows (SPSS, Inc.; Chicago, USA). Descriptive statistics were expressed as number (n), percentage (%), mean, SD. The Pearson chi-square test was used to search for the relationship between two categorical variables. A p-value of less than 0.05 was set for statistical significance.

RESULTS

One hundred and eleven patients that met the inclusion criteria were included in the study. Of the 111 patients, 71 (64%) were girls and 40 were boys (36%). The mean age at inclusion was 7.1±4.3 months (range, 1-18 years). The follow-up time of patients was 3.5 ±4.4 (range 1-16) years, and the number of patients followed up for 5 years or more was 32 (29%).

At the time of diagnosis, 5 (4.5%) patients were found to have elevated ALT levels. It was observed that ALT levels returned to the normal range in 3 (2.7%) of these patients with diet therapy. An elevated GGT level was observed in 4 patients (3.7%). Liver biopsy was performed in 2 patients in which ALT and GGT elevations were observed together. Liver biopsy was found to be compatible with sclerosing cholangitis. In these 2 patients, dilatation of the intrahepatic and extrahepatic bile ducts was observed on MRCP. Cholestasis was not observed in any of our patients. One of our patients was diagnosed with ulcerative colitis two months after the diagnosis of CD. Our patient was monitored in remission for 4 years with azathioprine therapy and a gluten-free diet. Elevations of ALT and GGT levels were never observed again. Another patient with CD and sclerosing cholangitis was not followed up after liver biopsy.

Abdominal USG was performed in 50 (45%) of the patients. Hepatomegaly was found in 4 (3.6%) of these patients and dilatation of the bile ducts in 2 (1.8%) patients. Hepatosteatosis was not detected in USG. In 4 patients with hepatomegaly, no elevation of ALT and GGT levels was detected in USG. In addition, gallbladder stones and sludge were examined by using USG.

Wool above BMI SDS 2 was detected in 4 of the 11 patients (3.6%). In these patients, elevated ALT and GGT levels were detected and no hepatosteatosis was detected in USG (Table 1). No liver involvement was observed in patients with a follow-up of 5 years or more.

DISCUSSION

In CD, the liver is affected to varying degrees. Because elevated liver enzymes are relatively common at the time of diagnosis of CD, it is recommended that all newly diagnosed patients with CD be tested for hypertransaminemia[18,19]. This approach was recently validated in the only population-based study of liver involvement in adults with CD in the USA[4].

In our study, ALT was found to be elevated in 5 (4.5%) of 111 patients. Compared with the literature, a lower rate of elevated ALT level was observed. It was observed that ALT levels returned to the normal range in 3 (2.7%) of these patients when treated with a gluten-free diet. When transaminases normalize on gluten-free diet therapy, annual follow-up is recommended[3]. In both patients, elevations in both ALT and GGT levels were observed, which were evaluated as sclerosing cholangitis after liver biopsy. The frequency of sclerosing cholangitis in patients with CD was 0.1-3% in the literature[16] and our frequency (1.88%) was in accordance with the literature. Patients with CD and sclerosing cholangitis should be evaluated in terms of inflammatory bowel disease (IBD), which may accompany them. Recognition of the coexistence of sclerosing cholangitis and CD is also important in terms of treatment options. In one of our patients, CD, sclerosing cholangitis, and ulcerative colitis were observed together. We were unable to complete the study of our other patient on this issue. In our study, coexistence of CD, PSC, and IBD was noted in 1 (0.9%) of 111 patients. IBD was significantly more common (3.2%) in the adults with CD than in the general population according to the literature[20]. There was an association between CD and gallstones, but gallstones were not found in this study using USG.

Table 1. Clinical and laboratory findings of the patients

Gender	
Female	74 (64%)
Male	40 (36%)
Age (mean)	7.1±4.3 years (1-18 years)
Follow-up time (mean)	3.5±4.4 (1-16) years
Reason for admission (n)	
Growth retardation	27
Diarrhea	19
Abdominal pain	25
Constipation	11
Screening (type I diabetes, family history of celiac disease)	29
Laboratory	
ALT (10-46 U/L)	5 (4.5%)
GGT (10-20 U/L)	4 (3.7%)
ALT and GGT (combined elevation)	2 (1.8%)
Radiology (USG) (n)	
Hepatomegaly	4
Hepatosteatosis	0
Bile duct anomalies	2
Liver biopsy	2
Sclerosing cholangitis	2 (1.8%)

ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, USG: Ultrasonography

Liver biopsy is not required in newly diagnosed CD with isolated hypertransaminasemia. However, it is recommended in selected patients with suspected chronic cholestatic liver disease that cannot be diagnosed by using non-invasive methods. There are no pathognomonic findings in liver histopathology and mild or non-specific changes are observed. Severe fibrosis or cirrhosis is rare. Liver histology is preserved, and mild mononuclear infiltrates in portal and lobular areas, and mild hyperplasia of Kupffer cells may be observed[21].

Other causes of transaminase elevation (viral hepatitis, toxic causes) were investigated in the patients. Hepatitis B and C were not detected in our pediatric patients.

NAFLD is the most common cause of chronic liver disease in children and adolescents in Western countries and is estimated to occur in 20% of the population[22,23]. Obesity and metabolic syndrome are the main causes of NAFLD[24]. However, not all patients with NAFLD are obese[25]. The prevalence of CD in adult NAFLD patients was reported to be 2.2-7.9%, and BMI was often within the normal range in these patients[26,27]. In the study by Reilly et al.[23], individuals diagnosed with CD in childhood were found to have a relative risk of NAFLD of 4.6%. In their studies of adults, Bakhshipour et al.[25] found that fatty liver was more common in CD, and CD was more common in patients with fatty liver.

In our study, 4 patients (3.6%) out of 111 patients with BMI SDS of 2 and >2 were observed. In these 4 patients, no hepatosteatosis was detected in USG, and no elevation in ALT and GGT levels were observed in the laboratory.

Moreover, in patients in whom CD and NAFLD coexist, a low gluten diet also reduces liver fat in these patients[28]. However, in the study by Reilly et al.[23], the increase in hepatosteatosis in patients with CD was higher than in the normal population, and this increase was not only in the first year but also continued for 15 years after diagnosis.

In our study, the number of patients with more than 5 years of follow-up was 32 (29%). During the follow-up period, NAFLD and non-alcoholic steatohepatitis were not encountered.

Study Limitations

The major limitation of our study was that it was a retrospective study and we primarily attempted to search for liver pathologies using ALT and USG based on the literature in the screening program. Although the use of USG in routine screening programs for NAFLD was not supported, it was preferred because it was non-invasive, simple, and inexpensive. Studies in adults observed in the literature were evaluated with the gold standard liver biopsy in suspicious patients after screening with USG and with ALT measure.[29,30]

CONCLUSION

As a result, elevation of transaminase levels in newly diagnosed celiac patients, apart from other specific liver diseases, may be due to CD and is common. Patients respond to a gluten-free diet. Intestinal barrier dysfunction, dysbiosis, and bacterial translocation are characteristic of CD and liver disease. Early diagnosis and treatment of CD is critical because a gluten-free diet can both relieve symptoms and prevent more serious celiac-related liver damage. If elevation of liver enzymes persists for 1 year despite a strict diet, further investigation for the etiology should be planned. Although autoimmune liver diseases are less associated with CD, their diagnosis is important in treatment of patients with severe clinical findings. Therefore, liver enzymes should be checked in newly diagnosed celiac patients. As the relationship between gluten-related immunity and liver damage becomes better understood, new opportunities for both prevention and treatment will emerge.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from Dokuz Eylül University Non-Interventional Research Ethics Committee (approval no: 2019/28-14, date: 18.11.2019).

Informed Consent: Retrospective study.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Concept: Y.Ö., Design: Y.Ö., Data Collection or Processing: G.Ş., Analysis or Interpretation: G.Ş., S.K.Ç., Literature Search: G.Ş., S.K.Ç., Y.Ö., Writing: G.Ş.

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Original Article

Retrospective Analysis of the Pediatric Intoxication Cases

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ABSTRACT

Objective: Various pharmaceuticals may be involved in pediatric intoxications, and treatment can be challenging for physicians. However knowledge of the clinical manifestations and prognosis of intoxication will be of assistance to physicians in conducting an appropriate clinical evaluation. The purpose of this study was to analyze the patient characteristics, outcomes and clinical features of pediatric intoxication.

Methods: One hundred eighty five children aged between 1 and 17 years with pharmaceutical intoxication (135 mild, 18 moderate, and 32 severe cases) were included in the study. Demographic characteristics, clinical features, and outcomes were compared between the subgroups of clinical severity and in terms of reasons for exposure.

Results: Suicidal behaviour was responsible for 61.1% and accidental exposure for 38.9% of intoxications. The drug group most frequently responsible for intoxication was analgesic-antipyretic medications. Clinical severity, length of hospitalization, and multiple drug intoxication rates were higher in the suicide group than in the accidental group ($p=0.037$, $p=0.016$, and $p<0.001$ respectively). Mortality occurred in one patient.

Conclusion: Analgesics and neurological system agents were responsible for the majority of intoxications. Intoxication for purposes of suicide resulted in longer hospital length of stay, and greater clinical severity than accidental poisoning. Understanding the differences between intentional and accidental intoxication may be assistance to physicians in performing appropriate assessments.

Keywords: Pediatric poisoning, pharmaceutical, accidental, suicidal

INTRODUCTION

Acute intoxications head the list of global preventable health problems in the pediatric age group. Pharmaceutical intoxications are one of the principal causes of presentations to pediatric emergency departments.[1]

Although toxic substances frequently result in a moderate illness course, they may occasionally cause morbidity and/or mortality, depending on the dose and the substance involved. Pediatric intoxications differ from those seen in other age groups because children are more susceptible to poisoning and suffer greater damage from it.[1] The causes of intoxication, the type of agent, and the age groups and genders involved may vary between different countries, between different parts of the same country, and even among communities in the same region.[2] Understanding the causes of intoxication and taking appropriate measures against the potential risks may therefore help to prevent such poisonings.

Despite the fact that numerous studies on intoxications have been published previously, the number of studies dealing only with pharmacological intoxications is limited. Each region should determine and update its own epidemiological data in order to design suitable prevention and treatment approaches, train health personnel, and increase public awareness.

This study was performed to determine the patient characteristics, outcomes, and clinical features of pediatric pharmaceutical intoxications in the emergency department.

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MATERIALS AND METHODS

Study Population

This retrospective study was conducted at the pediatric emergency department of a tertiary referral hospital in Turkey between December 2020 and 2021. One hundred eighty five children aged between 1 and 17 years with histories of pharmaceutical intoxications were included.

Definitions

Pharmaceutical intoxication was defined as the ingestion, either accidentally or for purposes of suicidal behaviour of chemical substances at doses eliciting toxic responses.

Clinical presentations were classified based on the Poisoning Severity Score:[3]

0: Asymptomatic: with no distressing symptoms, and with no specific findings following physical examinations in the pediatric emergency department

1: Mild, transient, spontaneously resolving symptoms

2: Prolonged symptoms and findings

3: Life-threatening symptom and findings

Patients were divided into three subgroups based on the severity of the clinical findings as mild (scores 0 or 1) moderate (score 2) and severe (score 3).

A flow chart for patient selection and the subgroups of clinical severity are shown in Figure 1.

Heart rate and systolic and diastolic blood pressures were defined according to normal values for age groups.[4]

All patients were consulted with the National Poison Advice Center. Gastric lavage and/or activated charcoal were applied as indicated depending on the type of substance involved.

The reasons for intoxication in children were classified as accidental or suicidal behaviour.

Exclusion Criteria

Patients with missing information and data in the medical records, with exposure to pharmaceuticals which could not be identified, with non-pharmaceutical intoxications, with food or plant poisoning, or patients intoxicated by insecticides or pesticides, insect bites and stings, cleaning materials, carbon monoxide, or hydrocarbons were excluded from the study.

Study Design

The patients' age and gender, the drug/drugs causing intoxication, the reason for poison exposure (accidental or suicidal behaviour), presenting complaints on admission, vital signs, gastric lavage and activated charcoal administration during hospitalization, length of hospital stay, place of hospitalization, and outcome of intoxication (discharge/mortality) were recorded retrospectively from patients medical files. Reasons for exposure were reported by family members, the patients themselves, or witnesses.

Patients were classified based on clinical severity following evaluation of the initial history and physical examination findings.

Approval for the study was granted by the Aydın Adnan Menderes University Local Ethics Committee (decision no: 04-2022/17, date: 27.01.2022).

Statistical Analysis

Statistical analyses were performed on Statistical Package for the Social Sciences for Windows (SPSS Inc., Chicago, IL, USA) version 22 software. Variables were expressed as median, minimum and maximum count (n) and percentage (%). The normality of the distribution of numerical variables was assessed using descriptive statistics, histogram charts, and the Kolmogorov-Smirnov test. Student's t-test and one-way analysis of variance were applied for normally distributed parameters, and the Mann-Whitney U test and Kruskal-Wallis tests for non-normally distributed parameters. The chi-square test was used for categorical variables. P values <0.05 were regarded as statistically significant.

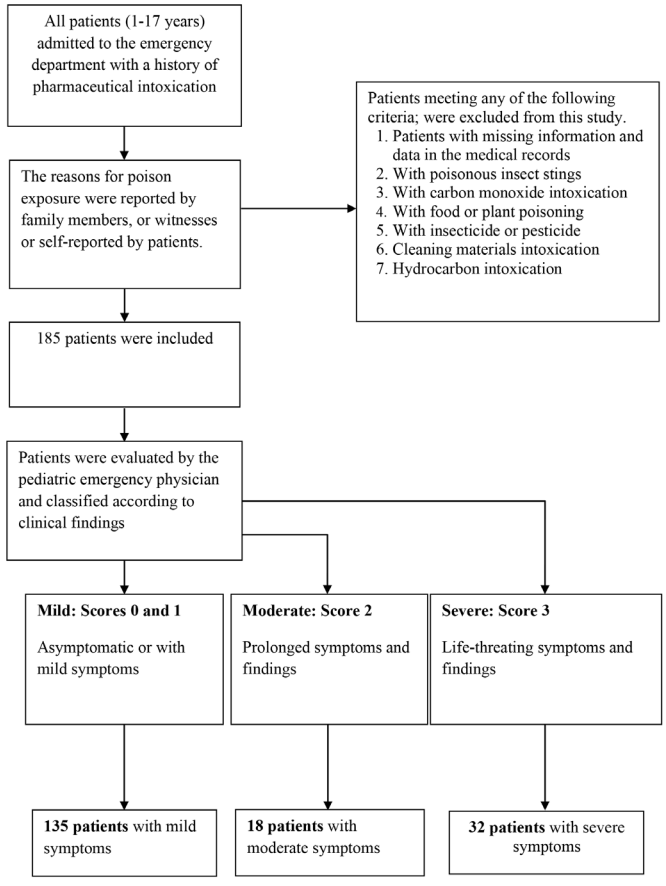

Figure 1. A flow chart for patient selection and the clinical severity subgroups

RESULTS

Patient Characteristics

One hundred eighty five children with pharmaceutical intoxication (135 mild, 18 moderate, and 32 severe case) were included in the study (Figure 1).

The patients' demographic characteristics and clinical presentations are shown in Table 1 and Figure 2.

Intoxication was the result of suicidal behaviour in 61.1% of patients and it was accidental in 38.9%.

The mean age of the patients with suicidal behaviour was 15.0 (11.0-17.0) years and 89.4% were girls, while the mean age in the accidental intoxication group was 3.0 (1.0-7.0) years and 68.1% were boys.

One hundred nine patients (58.9%) were intoxicated by a single drug, 37 (20%) by two, and 39 (21.1%) by multiple drugs. Gastric lavage was performed on 13 (%7) patients, activated charcoal was administrated to 21 (11.4%), while 105 (56.8%) received both gastric lavage and activated charcoal. Seventy-three percent of patients were asymptomatic at the time of presentation, while nausea and vomiting (8.1%) were the most common presenting symptoms.

The patients' demographic characteristics by severity subgroups are shown in Table 2.

The majority of patients (73%) had mild intoxication. There were no significant gender differences between the patient subgroups (p=0.981). The median age of the mild intoxication group was significantly lower than the moderate group (p=0.029). All patients in the severe intoxication group and 33.3% of the moderate severity group were admitted to the pediatric intensive care unit (p<0.001). Length of hospital stay was significantly longer in the severe intoxication group (p<0.001). Respiratory support in the form of mechanical ventilation was required in one patient.

A comparison of patients' demographic characteristics between the two causes (accidental or for purpose of suicide) is presented in Table 3 and Figure 2.

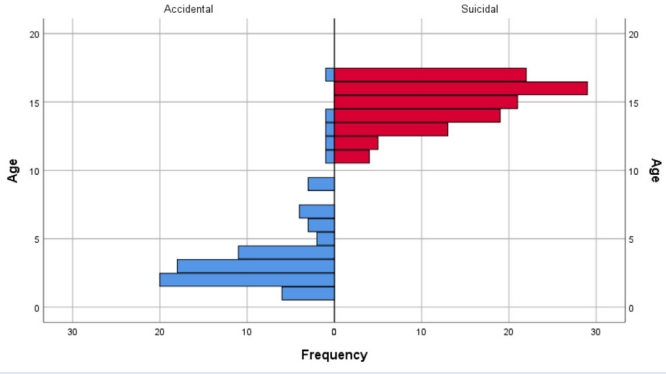

Figure 2. Patients' age distribution between the two causes (accidental or for purposes of suicide)

Table 1. Patient characteristics and clinical presentations

Parameter	Patient group (n=185)	Percentage (%)
Gender		
Male	61	33.0
Female	124	67.0
Reason for poison		
Accidental	72	38.9
Suicidal behaviour	113	61.1
Number of agents		
One	109	58.9
Two	37	20.0
More than two	39	21.1
Procedure administered		
Observation	46	24.9
Gastric lavage	13	7.0
Activated charcoal	21	11.4
Gastric lavage+activated charcoal	105	56.8
Place of hospitalization		
Emergency care	49	26.5
Pediatric ward	98	53.0
Intensive care	38	20.5
Clinical features		
Asymptomatic	135	73.0
Nausea-vomiting	15	8.1
Somnolence	6	3.2
Hypotension	6	3.2
Tachycardia	4	2.2
Double vision	4	2.2
Seizure	3	1.6
Toxic hepatitis	2	1.1
Abdominal pain	1	0.5
Pancreatitis	1	0.5
Hypokalemia	1	0.5
Bradycardia	1	0.5
Headache	1	0.5
Hypertension	1	0.5
Dysmetria	1	0.5
Dystonia	1	0.5
Hypokalemia	1	0.5
Exitus	1	0.5

Table 2. Demographic characteristics by severity subgroups

Parameter	Mild		Moderate		Severe		p-value
	n	%	n	%	n	%	
Gender							
Male	4	2.6	6	3.3	1	4.4	0.981
Female	1	7.4	2	6.7	1	65.6	
Age (years)*	13.0 (1.0-17.0)		15.0 (3.0-17.0)		14.0 (1.0-17.0)		**0.029**[a]
Place of hospitalization							
Emergency care	9	6.3	0	0	0	0	**<0.001**[b]
Pediatric ward	6	3.7	2	6.7	0	0	
Intensive care	0	0	6	3.3	2	100	
Number of agents							
One	5	3.0	8	4.4	6	50.0	0.058
Two	8	0.7	2	1.1	7	21.9	
More than two	2	6.3	8	4.4	9	28.1	
Length of hospitalization (days)*	2.0 (1.0-5.0)		3.0 (1.0-6.0)		4.0 (1.0-14.0)		**<0.001**[c]

*Median (minimum-maximum). Chi-square test was used for comparison of categorical data, Kruskal Wallis test was used for comparison of numerical data (Dunn's test was used for pairwise analysis).

[a]Mild vs moderate, p<0.05.

[b]Mild vs moderate, mild vs severe, moderate vs severe, p<0.05.

[c]Mild vs moderate, mild vs severe, p<0.05

Table 3. A comparison of demographic characteristics between the accidental and suicidal behaviour groups

Parameter	Suicidal behaviour		Accidental		p-value
	n	%	n	%	
Gender					
Male	12	10.6	49	68.1	**<0.001**
Female	101	89.4	23	31.9	
Age (years)	15.0 (11.0-17.0)		3.0 (1.0-7.0)		**<0.001**
Place of hospitalization					
Emergency care	28	24.8	21	29.2	**0.014**
Pediatric ward	54	47.8	44	61.1	
Intensive care	31	27.4	7	9.7	
Number of agents					
One	51	45.1	58	80.6	**<0.001**
Two	26	23.0	11	15.3	
More than two	36	31.9	3	4.2	
Length of hospitalization (days)	3.0 (1.0-14.0)		2.0 (1.0-9.0)		**0.016**
Severity					
Mild	75	66.4	60	83.3	**0.037**
Moderate	13	11.5	5	6.9	
Severe	25	22.1	7	9.7	

*Median (minimum-maximum)

Chi-square test was used for comparison of categorical data, Mann Whitney U test was used for comparison of numerical data

Table 4. Pharmaceuticals causing poisoning in children			
Pharmaceuticals	**Patient group (n = 185)**	**Suicidal (n=113)**	**Accidental (n=72)**
Analgesic-antipyretic			
Paracetamol	49 (26.5)	30 (26.5)	19 (26.4)
Proven derivative	27 (14.6)	18 (15.9)	9 (12.5)
Phenylacetic acid derivative	11 (5.9)	2 (1.7)	9 (12.5)
Metamizole	3 (1.6)	3 (2.6)	0 (0.0)
Acetyl salicylic acid	2 (0.5)	1 (0.8)	1 (1.3)
Neurological system agents			
SSRI	21 (11.3)	18 (15.9)	3 (4.1)
CNS stimulant	21 (11.3)	17 (15)	4 (5.5)
Antipsychotic	18 (9.7)	11 (9.7)	7 (9.7)
SNRI	12 (6.5)	8 (7)	4 (5.5)
Antiepileptic	6 (3.2)	5 (4.4)	1 (1.3)
Tricyclic antidepressant	5 (2.7)	4 (3.5)	1 (1.3)
Anxiolytic	2 (1.1)	1 (0.8)	1 (1.3)
Anti-Parkinson	2 (1.1)	1 (0.8)	1 (1.3)
Cardiovascular system drugs			
Beta-blocker	7 (3.8)	6 (5.3)	2 (2.7)
Calcium channel blocker	5 (2.7)	3 (2.6)	1 (1.3)
Other drugs			
Antihistaminic	19 (10.2)	17 (15)	2 (2.7)
Antibyotic	17 (9.2)	16 (14.1)	1 (1.3)
Antispasmodic	15 (8.1)	12 (10.6)	3 (4.1)
Hormone and vitamin	8 (4.3)	6 (5.3)	2 (2.7)
Proton pump inhibitor	8 (4.3)	7 (6.1)	1 (1.3)
Antidiabetic	6 (3.2)	5 (4.4)	1 (1.3)
Iron	6 (3.2)	5 (4.4)	1 (1.3)
Colchicine	5 (2.7)	4 (3.5)	1 (1.3)
Antiemetic	3 (1.6)	2 (1.7)	1 (1.3)

The male gender was predominantly associated with accidental poisoning, while the female gender was more associated with suicidal behaviour ($p<0.001$). The median ages of the two groups also differed significantly ($p<0.001$). Children treated for suicidal poisoning were significantly older than those treated due to accidental poisoning [15.0 (11.0-17.0) vs. 3.0 (1.0-7.0) years, respectively].

Clinical severity and the rate of intoxication resulting from multiple drug ingestion were significantly higher in the suicide group than in the accidental group ($p=0.037$, and $p<0.001$, respectively). Length of hospital stay was longer among the children in the suicidal group [3.0 (1.0-14.0) days] compared to those in the accidental intoxication group [2.0 (1.0-9.0) days].

Psychiatric consultation was requested for all the patients intoxicated as a result of suicidal behaviour.

The drugs responsible for intoxication are shown in Table 4.

The drug most frequently responsible for intoxication was paracetamol (26.5%) as an analgesic-antipyretic medication. This was followed by the central nervous system (CNS) drug group, selective serotonin reuptake inhibitors (11.3%), CNS stimulants (11.3%), and antipsychotics (9.7%).

The comparison of drugs causing intoxication between children with suicidal behaviour and those with accidental intoxication is shown in Table 4. Paracetamol was the most common causative agent in both groups.

Age distribution of accidental intoxications is shown in Table 5.

A comparison of patients' age distribution and demographic features revealed that accidental poisoning mostly occured in the age group of 3-6 years (47%). Males were more prone to accidental poisoning than females in our study with a male to female ratio of 2:1. Accidental intoxications were mostly caused by a single agent in all age groups (80%).

Table 5. Demographic characteristics of accidental intoxications by age distribution							
Parameter	**0-2 years**		**3-6 years**		**>6 years**		**p-value**
	n	**%**	**n**	**%**	**n**	**%**	
Gender							
Male	18	69.2	22	64.7	9	75.0	0.795
Female	8	30.8	12	35.3	3	25.0	
Place of hospitalization							
Emergency care	6	23.1	14	41.2	3	25.0	0.142
Pediatric ward	18	69.2	20	58.8	7	58.3	
Intensive care	2	7.7	0	0.0	2	16.7	
Number of agents							
One	22	84.6	27	79.4	9	75.0	0.791
Two	3	11.5	5	14.7	3	25.0	
More than two	1	3.9	2	5.9	0	0.0	
Length of hospitalization (days)*	2.0 (1.0-9.0)		2.0 (1.0-4.0)		2.5 (1.0-4.0)		0.324
Severity							
Mild	23	88.5	30	88.2	7	58.3	0.052
Moderate	0	0.0	3	8.8	2	16.7	
Severe	3	11.5	1	2.9	3	25.0	

*Median (minimum-maximum)

Chi-square test was used for comparison of categorical data (exact statistics was used if needed, Kruskal -Wallis test was used for comparison of numerical data (Dunn´s test was used for pairwise analysis)

DISCUSSION

Due to the high rates of morbidity and mortality in pharmaceutical intoxications when interventions are delayed, early diagnosis and treatment are of very great importance.[5]

The reasons for intoxication may vary, depending on factors such as age and sex. Accidental intoxications are more common among boys at the ages of 1-5 years.[6]

Eighty-one per cent of all intoxications below the age of eight years were accidental in a two-year prospective study.[7] Singh et al.[8] described one-third of all poisonings as unintentional. In the present study, in agreement with the previous literature, accidental intoxication was more frequent in boys during the first seven years of life.

The effect of factors of growth and development on accidental intoxication becomes particularly significant during the toddler and preschool periods.[9] As young children grow up and start to become independent, they are drawn to investigate new and fascinating objects and places. Young children may experience accidental intoxications since their ability to protect themselves and self-regulate are less developed than other ages. Drugs must therefore be kept out of the reach of children, who must also be closely supervised by their parents.

Case of suicide is reported to be more frequent in girls, particularly those aged over 10.[10,11]

Adolescent girls are thought to be more susceptible to suicidal behaviour since they are more emotional and experience greater mental conflicts than boys.

The reasons for intoxication may also play an important role in patient outcomes. In the present study, the effective agent was ingested accidentally by 38.9% of the patients, and for suicidal purposes by 61.1%.

Children with suicidal intoxication had significantly longer hospital stays than those with accidental intoxication, and the majority of children admitted to the pediatric intensive care unit were from the suicidal group. It is therefore of particular importance to understand the differences between intentional and accidental intoxication. Identifying the reason for intoxication may therefore be of assistance to physicians in performing appropriate assessments.

Intoxication for purposes of suicide is more common among girls (89.4%), while intoxication due to accidental ingestion of an agent occurs more frequently among boys.

However, in the present study intoxication was more common in girls than in boys, which might be attributable to the predominance of girls among the cases of suicidal poisoning.

The majority of children exposed to toxic agents were shown to expose to only a single substance.[12] Karcıoğlu et al.[13] reported that 53.6% of pharmaceutical intoxications involved a single drug, while Kaygusuz et al.[14] reported that 52.5% of intoxications involved multiple drugs.

In the present study, 58.9% of intoxications involved single drug, 20% involved two, and 21.1% involved multiple pharmaceutical agents. The rate of multiple drug intoxication was higher in the suicidal group than in the accidental group. In addition, and consistent with the previous literature, clinical severity and rate of admission to the pediatric intensive care unit were both higher in the suicidal group, and length of hospital stay was longer (p=0.037, p=0.014 and p=0.016, respectively).[15,16]

Many patients exposed to intoxication (48.3-70%) are clinically asymptomatic.[17-19]

Ağın et al.[20] reported that 41% of patients were asymptomatic at the time of presentation, while nausea-vomiting was observed in 18%. Yorulmaz et al.[21] reported that approximately one-third of patients were asymptomatic, the most common presentation symptoms also being nausea-vomiting. Binay et al.[22] also described vomiting as the most common finding.

In the present study, asymptomatic patients accounted for 73.0% of patients, and the most common symptoms were also nausea-vomiting (8.1%). This might suggest that the effect of the toxic substance concerned was low, or that full exposure did not occur.

Pediatric intoxication may involve a broad course spectrum, from mild course to mortality. This may depend on the patient's age, sex, the type and dosage of pharmaceutical agent, the form of exposure, and individual patient characteristics.[23,24]

Clinical severity, length of hospital stay, and rate of admission to pediatric intensive care unit were all higher in the suicide group than in the accidental intoxication group (p=0.037, p=0.016, and p=0.014 respectively). The rate of multiple drug intoxication was also higher than in the accidental group. This finding may indicate the importance of identifying the type of drug ingested, and measuring the drug levels should be seriously considered for early diagnosis

Analgesics and drugs affecting the CNS, such as antidepressants, are the most frequently implicated agents in pediatric poisoning cases.[19,25,26] Moon et al.[27] reported that cardiovascular drugs were more frequently involved in accidental poisonings, while acetaminophen and psychotropic drugs were more frequently involved in suicide attempts. Analgesics are reported to be responsible for 23.7-40% of all drug-related intoxications with paracetamol being involved in 30-45% of such cases.[17,25,28]

Analgesics, followed by neurological system agents, were most frequently associated with pediatric pharmaceutical intoxication in both study groups in the present study.

The incidence of paracetamol-related intoxications among analgesics was higher in both groups. We think that this may be due to analgesics and antipyretics, most of which are packaged in bottles with child safety caps, being available over the counter in Turkey, with no prescription required, making them potentially accessible in the home.

The next most common agents in pediatric intoxications in both groups in this study were drugs affecting the CNS. The most common of these were antidepressants. We attribute this to the increasing use of antipsychotics and antidepressants in recent years creating a tendency for these drugs also to be used for a suicidal purpose.

Intoxication mortality rates range between 0.4% and 7.6%.[1] Unintentional poisoning in children is rarely fatal and is preventable in most of the patients.[29]

Mortality occurred in only one female patient due to calcium channel blocker intoxication as a result of suicide attempt. Our low mortality rate may be attributed to a higher level of parental awareness in terms of the importance of early intervention and treatment in cases of intoxication.

Study Limitations

There are several limitations of this study. In particular, due to its retrospective nature, information concerning the time elapsing before presentation to hospital, whether patients had previously presented to another health institution and, if so, the treatment administered there could not be investigated. Other limitations of this study were limited data and relatively limited number of patients.

CONCLUSION

This study investigated detailed categories of pharmaceutical pediatric intoxications. Analgesics and neurological system agents were found to be involved in the majority of patients. Intoxications due to purpose of suicide resulted in longer hospital stay, and higher clinical severity than accidental intoxication.

Parents should be educated to store these agents appropriately to avoid accidents involving young children. Greater attention is also required to ensure that pharmaceuticals are not made easily available to adolescent children to prevent them from being used in suicide attempts.

Ethics

Ethics Committee Approval: Approval for the study was granted by the Aydın Adnan Menderes University Local Ethics Committee (decision no: 04-2022/17, date: 27.01.2022).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.O., Design: S.Ö., Data Collection or Processing: E.O., Analysis or Interpretation: S.Ö., A.Ç., Literature Search: E.O., Writing: E.O., S.Ö.

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Original Article

Is Plasma C-Type Natriuretic Peptide Level Suitable for Diagnosing and Typing Skeletal Dysplasia?

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ABSTRACT

Objective: Skeletal dysplasia is a heterogeneous group of diseases that lead to abnormal enchondral ossification and typing of the disease is quite complex. C-type natriuretic peptide (CNP), one of the members of the natriuretic peptide family, has been implicated in bone development, and CNP levels are high in some types of skeletal dysplasia. The aim of this study was to evaluate the use of CNP as a marker for skeletal dysplasia types and to investigate its role in typing.

Methods: Thirty-seven patients aged six months to 18 years [26 (70.3%) girls] were included in this cross-sectional study from among 75 skeletal dysplasia patients. All subjects were physically examined; anthropometric measurements were obtained, and bone surveys were evaluated. ELISA was used to assess CNP plasma levels. Forty-nine healthy children aged six months to 18 years [24 girls (49%)] comprised the control group.

Results: The CNP concentration of the patient group (n=37) was 1.31±1.40 ng/mL which was similar to the control group (n=49) at 1.04±1.40 ng/mL (p=0.207). However, the CNP concentration of patients with achondroplasia (n=17) was significantly higher (1.79±1.64 ng/mL) than the control group (p=0.032).

Conclusion: Our study contributes evidence concerning CNP values of both healthy children and children with skeletal dysplasia. Compared with healthy children, those with achondroplasia have elevated plasma levels of CNP. Further larger studies are necessary to assess the use CNP as a marker for the diagnosis and typing of skeletal dysplasia.

Keywords: Achondroplasia, c-type natriuretic peptide, short stature, skeletal dysplasia

INTRODUCTION

C-type natriuretic peptide (CNP) is a member of the natriuretic peptide family and plays a key role in regulating endochondral bone development. Although CNP can be expressed in many tissues, such as cartilage, bone, brain, endothelium, smooth muscle, and heart, it is mostly synthesized in the hypertrophic zone of the growth plate.[1,2]

The CNP is a powerful positive regulator of linear growth.[3,4] CNP released from the endothelium initiates cartilage matrix synthesis and stimulates chondrocyte proliferation and differentiation.[1,2] Furthermore, CNP plays a role in bone development. Experimental

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studies have shown that changes in the CNP gene lead to short stature in knockout mice.[3] Plasma CNP levels were found to be high in adult patients with achondroplasia, thanatophoric dysplasia, and hypochondroplasia.[4]

Skeletal dysplasia is a heterogeneous group of syndromes accompanied by malformations of the skeleton with enchondral ossification. Skeletal dysplasia comprises 461 different diseases that are classified into 42 groups.[5] A detailed history, physical examination, advanced laboratory studies, and a multidisciplinary approach are crucial for the differential diagnosis of skeletal dysplasia. The aims of this study were to evaluate the possibility of using CNP as a marker for skeletal dysplasia types.

MATERIALS AND METHODS

This study was a case-control study. It was approved by the Ege University Faculty of Medicine Ethics Committee (decision dated: 18.01.2017, with approval number 16-8/9). The study was supported by Scientific Research Projects, with grant no. 2018-TIP- 031. Written consent was obtained from all the children and their parents who agreed to participate in the study.

Study Subjects

Subjects were recruited from among 75 patients with skeletal dysplasia followed up in our pediatric endocrinology outpatient unit. The subjects included were between the ages of 6 months and 18 years. The 38 subjects that were excluded either declined to participate, had other systemic diseases such as congenital cardiac defects, were being treated with growth hormones, were on other medical therapy, or were out of the age range of 6 months and 18 years. Thirty-seven of the 75 patients agreed to participate and were included in the study. Fifty healthy children and their parents who attended our general pediatrics outpatient unit were informed about the study, and 49 healthy children were randomly included in the study as a control group. All followed up patients and controls who met the inclusion criteria were included in the study. Post-hoc power was calculated from the mean of the concentration in healthy subjects and patients with skeletal dysplasia. Accordingly, the power of the study was calculated as 0.612.

Study Procedure

A physical examination was performed, and anthropometric measurements were obtained for all subjects. Weight measurements were evaluated by a Turkish Standards Institution (TSI)-approved Baster weighing instrument, with 0.1 kg intervals, and height measurements were made using a Baster-brand measuring instrument with TSI-approved 0.1 cm intervals. Head circumference and chest circumference were measured with an elastic measuring instrument with TSI-approved 1 mm intervals on a precision tape measure. Sitting height was measured using a sit-down Harpenden sitting height table. Standard deviation values of weight, height, head circumference, and body mass index (BMI) were calculated according to the norms of Turkish children developed by Neyzi et al.[6]. The standard deviation of the sitting height and the standard deviation of the sitting height/aspect ratio were evaluated using the Tanner-Whitehouse method.[7]

The puberty stage was classified according to the Tanner scale. Bone surveys were evaluated in the Department of Pediatric Radiology. Bone age was evaluated using the Greulich and Pyle atlas.[8] All subjects who were clinically suspected of skeletal dysplasia underwent radiographic evaluation after whole-body assessment of anterior/posterior and lateral radiographs. The diagnosis of skeletal dysplasia was confirmed by the pediatric radiologist.

Venous blood samples (1 mL) were drawn into EDTA tubes for the measurement of CNP in both the skeletal dysplasia group and the control group. Human CNP (2',3'-cyclic-nucleotide 3'-phosphodiesterase) enzyme-linked immunosorbent assay for quantitative detection of CNP in serum or plasma (Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China) was used according to the manufacturer's protocol. The CNP kit we used was sensitive to values of 0.188 ng/mL and could detect values in the range 0.313-20 ng/mL. Plasma samples were isolated by using the method of centrifugation of the blood at 1000xg for 10 minutes and then stored at -80 °C until assayed. The supernatant was collected, and each sample was tested at least twice. We excluded laboratory errors, as shown in the figures below. The healthy group and the patient group perfectly fitted polynomial curves (Figure 1).

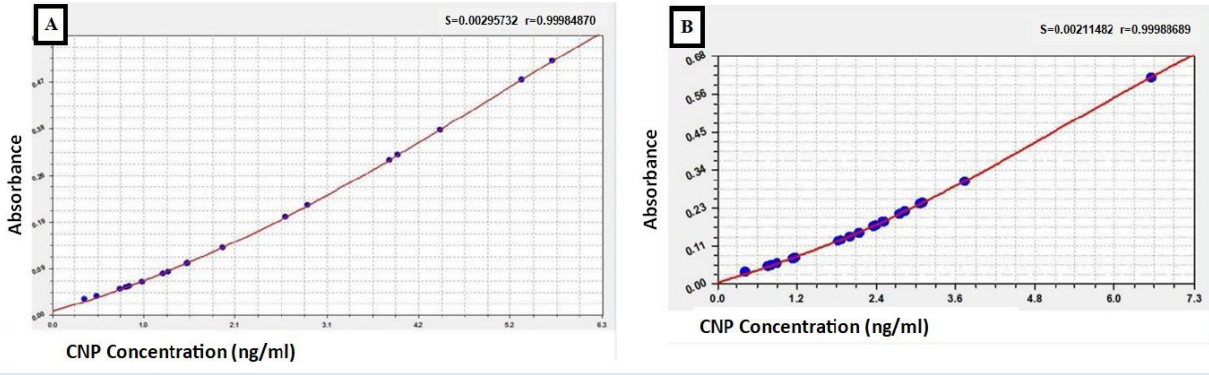

Figure 1. A) Healthy group polynomial curve fit (<0.313 ng/mL excluded), B) Patient group polynomial curve fit (<0.313 ng/mL excluded)

Statistical Analysis

The SPSS, version 21.0 (IBM Corp., Armonk, NY, USA) was used to obtain descriptive statistics of continuous data with mean, standard deviation, median, and lowest and highest values, and of categorical data using frequency and percentage values. The distribution of the data sets was assessed using the Shapiro-Wilk test.

All patients with skeletal dysplasia were compared with the control group, and the subgroup of patients with achondroplasia was also compared with the control group. Age groups were classified as: 1) 0-1 year; 2) 1-5 years; 3) 5-9 years; and 4) >9 years, based on age-dependent changes in height velocity.

The Mann-Whitney U test was used for independent group comparisons, and the Kruskal-Wallis test was used for comparison between independent multiplex groups with continuous variables. Spearman's correlation coefficient was used to determine the direction and degree of linear relationships between CNP concentration and other continuous variables, which included body weight, height, sitting height, BMI, and bone age.

Since CNP concentration did not show a normal distribution according to gender and groups, "sex effect," "group effect," and "gender x group interaction" were analyzed by using non-parametric methods using factorial design * SAS software (Version 9.3; procedure: mixed; SAS Institute, Cary, NC, USA). This approach was also used when the age effect was grouped and analyzed. The significance level was determined as 0.05 in all analyses, and the Bonferroni correction was used in binary comparisons.[9]

RESULTS

The study consisted of 86 participants, including 37 (43%) patients with skeletal dysplasia and 49 (57%) healthy subjects. Of these participants, 59% (n=51) were male, 41% (n=35) were female, and 36% (n=31) were younger than 4 years , 35% (n=30) were between 5 and 9 years , and 29% (n=25) were older than 9 years.

The skeletal dysplasia group consisted of achondroplasia (45.9%, n=17), spondyloepiphyseal dysplasia (16.2%, n=6), metaphyseal dysplasia (13.5%, n=5), epiphysial dysplasia (5.4%, n=2), hypochondroplasia (5.4%, n=2), acromesomelic dysplasia (2.7%, n=1), and unclassified skeletal dysplasia (10.8%, n=4).

In all participants consisted of controls and skeletal dysplasia groups, no significant difference was found between the median CNP and the genders (p=0.082). The median CNP in the skeletal dysplasia group (n=37) was 0.423 (0.31-6.59) ng/mL. The median CNP in the control group (n=49) was 0.313 (0.31-5.725) ng/mL (p=0.207) (shown in Table 1).

The median CNP was significantly higher in the skeletal dysplasia group (p=0.039). When the CNP was compared by gender, the median CNP in boys was significantly lower (p=0.015). Group *sex interaction was not statistically significant (p=0.103) (shown in Table 2).

The median CNP between the skeletal dysplasia group and the healthy group (p=0.187) and between age groups was not different (p=0.456), and the age interaction between the groups *was not statistically significant (p=0.256) (shown in Table 3).

The median CNP was significantly different between the patients with achondroplasia and the control group (p=0.038), whereas no difference was found in terms of CNP between age groups (p=0.814). Group * age interaction was not statistically significant (p=0.319) (shown in Table 3).

In the achondroplasia group (n=17), the median CNP was 1.87 (0.31- 6.59) ng/mL and this was significantly higher than the healthy control group (p= 0.032) (shown in Table 4).

Anthropometric measurements were significantly lower in the skeletal dysplasia group and in the achondroplasia group than in the healthy group (p<0.001) (shown in Table 5).

There was no correlation between CNP and groups' anthropometric measurements, except for sitting height SDS (shown in Table 5).

DISCUSSION

The C-type natriuretic peptide is an essential regulator of skeletal development. Mutations that cause loss of function of CNP receptor, natriuretic peptide receptor-B (*NPR-B*, gene *NPR2*), cause short stature, autosomal recessive skeletal dysplasia, acromesomelic dysplasia Maroteaux type (AMDM).[1,10,11] Considering that 1 in 700 people carries the NPR2 mutation, the reason for the idiopathic short stature phenotype seen in 1 in 30 people might be carrying the *NPR2* mutation.[10,12] Similar phenotypic features were observed in some individuals that overexpressed CNP due to balanced translocations.[1]

Achondroplasia is the most common form of skeletal dysplasia ranging from 1 in 10,000 to 40,000 newborns worldwide.[13] Fibroblast Growth Factor Receptor 3 (*FGFR3*) mutations cause achondroplasia.[13,14] FGFR-3 activates many cascades in the growth plate, such as the signal transducers and activators of the transcription (STAT1) pathway and the MAPK kinase (MEK)/ERK MAPK pathway. These two pathways inhibit CNP intracellular signaling, blocking chondrocyte proliferation and differentiation.

Table 1. Comparison of gender related CNP concentration (ng/mL) values in all population and comparison of CNP Concentrations (ng/mL) in patient, achondroplasia and control groups

Variables		$\tilde{X}$ [Min; Max]
CNP (ng/mL)	Female (n=35)	0.42 [0.31; 6.59][a]
	Male (n=51)	0.31 [0.31; 3.76][a]
	Patient (n=37)	0.42 [0.31; 6.59][b]
	Achondroplasia (n=17)	1.79 [0.31; 6.59][c]
	Control (n=49)	0.31 [0.31; 5.72]

$\tilde{X}$: Median, CNP: C-type natriuretic peptide, Min: Minimum, Max: Maximum

*p<0.05: Level of significance *Mann-Whitney U Test

[a]p= 0.082[mw] : compared with gender groups

[b]p= 0.207[mw] : compared with control group

[c]p= 0.032[mw] : compared with control group

Table 2. Comparison of gender related CNP concentration (ng/mL) values

	Patient	Control	p-value*		
Gender	X̃ (n) [Min; Max]	X̃ (n) [Min; Max]	Gender Effect	Group Effect	Interaction
Female	1.87 (11) [0.31; 6.59]	0.31 (24) [0.31; 5.72]	0.015	0.039	0.103
Male	0.31 (26) [0.31; 3.76]	0.31 (25) [0.31; 2.93]			

X̃: Median, CNP: C-type natriuretic peptide, Min: Minimum, Max: Maximum

*p<0.05: Level of significance *Non-parametric method in factorial design

Table 3. Comparison of distribution of CNP plasma levels in different age groups

	Patient	Achondroplasia	Control	Patient-Control p-value*			Achondroplasia-Control p-value*		
Age	X̃ (n) [Min; Max]	X̃ (n) [Min; Max]	X̃ (n) [Min; Max]	Gender Effect	Group Effect	Interaction	Gender Effect	Group Effect	Interaction
0-1 y.	- (0)	- (0)	3.28 (2) [1.54; 4.44]	0.456	0.187	0.256	0.814	0.038	0.319
1-5 y.	0.42 (10) [0.31; 2.85]	1.17 (6) [0.31; 2.85]	0.31 (19) [0.31; 2.93]						
5-9 y.	1.51 (14) [0.31; 6.59]	2.14 (6) [0.31; 6.59]	0.78 (16) [0.31; 5.38]						
>9 y.	0.31 (13) [0.31; 3.1]	0.81 (5) [0.31; 3.11]	0.31 (12) [0.31; 5.7]						

X̃: Median, CNP: C-type natriuretic peptide, Min: Minimum, Max: Maximum

*p<0.05: Level of Significance *Non-parametric method in factorial design

Overactivity of FGFR-3 inhibits NPR-B. Without an effective receptor, CNP can not initiate intracellular CNP/cyclic guanosine monophosphate (cGMP) signalization. As a result, the plasma CNP levels will be elevated.[12,15] We thought that this well-known mechanism could help typing and diagnosing skeletal dysplasia according to plasma CNP level degrees. Our study found that the level of CNP was higher in patients with achondroplasia than in the reference population (p=0.032).

In the literature, few studies have evaluatedthe CNP plasma level in skeletal dysplasias. Most of them are experimental animal studies.[12,16-19] To our knowledge, CNP level in children with skeletal dysplasia is not known. In the literature, a few studies investigated CNP levels of humans with skeletal dysplasia, which was consisted of patients in adulthood and childhood.[4]

Olney et al.[4] investigated CNP resistance in a group consisting of 63 participants, which consisted of 20 adults with achondroplasia, six children with hypochondroplasia, two children with tanatophoric dysplasia, and four children and one adult with acromesomelic dysplasia, Maroteaux type (AMDM). CNP and NTproCNP were higher in patients with achondroplasia. Similarly, CNP and NTproCNP levels were increased in adult patients with achondroplasia. The NTproCNP/CNP ratio was a measure of CNP degradation, and no difference was found from the reference population. They also showed elevated CNP levels in two children with thanatophoric dysplasia, but their sample size was too small for statistical evaluation. That study showed a positive correlation between NTproCNP level and growth rate in children with achondroplasia and in the reference population. NTproCNP and CNP levels were higher in patients with hypochondroplasia and achondroplasia than in the reference population. They showed increased proCNP products in skeletal dysplasias and CNP resistance in tissues.[4] The results in the achondroplasia group were similar to our study. We observed an apparent elevation in CNP levels in the achondroplasia group (p=0.032). However, since the number of patients with other skeletal dysplasias was insufficient to make reliable statistical analysis, no comparison between CNP level in these small sub-groups and the reference population was attempted (shown in Table 4). The other types of skeletal dysplasia were infrequent. The other studies could not obtain statistical confirmation in the literature. When we compared the patient group with the reference population, we found no difference in CNP levels (p=0.42). Indirectly, we can say that CNP levels are not elevated in all types of skeletal dysplasia. Elevated CNP level is significant for achondroplasia.

Although experimental animal studies showed that CNP levels were potential biomarkers of long bone growth,[12,16-19] it was hard

to use CNP level in daily clinical practice. There are few suitable assays, and there are no reference ranges in CNP measurements. We believe that our study contributed to the literature the measurement of CNP level in skeletal dysplasia.

To understand the other factors that affected CNP levels, we discussed the studies about CNP levels in healthy subjects. CNP levels vary with age, and there is no significant difference between the genders.[20] Del Ry et al.[20] recommended that at least five different reference ranges should be used when evaluating CNP. Therefore, we classified CNP levels according to age groups. We did not include newborns because the CNP level in the newborn period is variable. In a study by Olney et al.[21], both CNP and NTproCNP levels showed an evident variation based on age. CNP and NTproCNP showed very high levels in newborns in the first week of life, followed by a downward trend after six weeks. CNP levels did not change with pubertal status, and there was no difference between boys and girls, as we also found in our study.[21] Our findings supported a study that found no significant difference between male and female populations.[20] However, CNP levels were higher in female patients. We found no interaction with gender and skeletal dysplasia effect (p=0.103). While the gender distribution was similar in the whole group and in the healthy control group, the skeletal dysplasia group had male dominance. Although the age distribution was homogeneous in both groups, we found no correlation between CNP plasma level and age. Del Ry et al.[20] had a lot of newborn subjects in their study. Bone development and height velocity were the fastest in the newborn period. We had no newborns so that we might not find difference. There was a need for studies with a more significant number of healthy participants to evaluate the reference range.

In 2014 Topçu et al.[22] published a study that established a reference value for Turkish children in Ankara and Denizli and compared the relationship between NT-proCNP level and height velocity. In this study, plasma NTproCNP concentration negatively correlated with age, body weight, and height in children. Gender was not a factor affecting age-related plasma NTproCNP concentration until puberty. Contrary to other studies, the plasma NTproCNP concentration in overweight/obese children in the Turkish population was significantly lower than in normal-weight children. In our study, we excluded obese individuals as they could affect the results in the reference group, and there was no clear evidence concerning CNP concentrations in obese children compared to normal-weight peers in the literature. We did not find any significant relationship between body weight, height, age, gender, and CNP levels in either the skeletal dysplasia group or the healthy group.

Our study was more comprehensive than other studies because it included skeletal dysplasia patients and healthy subjects. Thus, we compared many auxological parameters, such as body weight, height, head circumference, sitting height, BMI, and bone age, gender, and age. While there was no clear consensus on the CNP level reference range and the factors affecting its outcome, our study contributed to the literature by providing the CNP values of healthy children and children with skeletal dysplasia.

Table 4. Types of skeletal dysplasia and CNP (ng/mL)

Types of skeletal dysplasia	N (%)	X̃ [Min; Max]
Achondroplasia	17(45.9%)	1.87 [0.31; 6.59]
Spondyloepiphyseal dysplasia	6 (16.2%)	0.61 [0.31; 2.78]
Metaphyseal dysplasia	5(13.5%)	0.31 [0.31; 3.76]
Epiphysial dysplasia	2(5.4%)	0.31 [0.31; 0.31]
Hypochondroplasia	2(5.4%)	0.54 [0.31; 0.76]
Chromosomal dysplasia	1(2.7%)	0.31 [0.31; 0.31]
Other	4(10.8%)	0.73 [0.31; 0.76]

X̃: Median, CNP: C-type natriuretic peptide, Min: Minimum, Max: Maximum

Table 5. Correlation of CNP concentration and anthropometric measurements of designated groups

Variables	Group (n)	X̃ [Min; Max]	p	ρ*
Weight SDS	Patient (37)	-1.45 [-14.5; 2.29]	0.95	0.11
	Ach. (17)	-1.57 [-14.5; 1.60]	0.96	-0.01
	Control (49)	0.15 [-1.67; 1.27]	0.55	0.09
Height SDS	Patient (37)	-4.57 [-14.73; 1.93]	0.72	-0.06
	Ach. (17)	-5.05 [-14.73; 1.93]	0.37	0.23
	Control (49)	0.04 [-1.21; 1.44]	0.17	0.2
Sitting Height SDS	Patient (37)	-2.115 [-9.72; 0.47]	0.99	0.001
	Ach. (17)	-1.40 [-6.76; 0.39]	0.66	-0.12
	Control (49)	-0.21 [-1.75; 1.21]	0.05	0.29
BMI SDS	Patient (37)	1.08 [-5.06; 4.23]	0.47	0.12
	Ach. (17)	1.58 [-5.06; 4.23]	0.49	-0.18
	Control (49)	0.12 [-1.95; 1.06]	0.32	0.15

X̃: Median, CNP: C-type natriuretic peptide Ach.: Patients with achondroplasia, SDS: Standard deviation score, BMI: Body mass index, Min: Minimum, Max: Maximum
p<0.05: level of significance; ρ correlation coefficient; *Bonferroni correction

Study Limitations

This study had some limitations. Since the different skeletal dysplasia groups, with the exception of the achondroplasia group, did not have sufficient numbers the utility of CNP as a biomarker for other skeletal dysplasia types could not be evaluated. Our study was designed as a pioneer study. We hope that this study will act as the spur and a pilot for a nationwide study. We showed that the small group of achondroplasia patients was discriminated from the other types of skeletal dysplasias by considering CNP concentration alone.

CONCLUSION

In our study, we investigated the effects of body weight, height, sitting height, BMI, bone age, gender and age on CNP levels in osteochondroplasia. There was no clear consensus on the CNP level reference range or the factors affecting its outcome. In this context, our study only contributed to the formation of reference values in achondroplasia patients. To establish a definite relationship, studies involving large numbers of different types of osteochondroplasia are required.

Our study showed that plasma CNP levels were higher in patients with achondroplasia. We believe that our study has produced some promising findings and may provide the basis for large samples derived from multicenter or national studies. To conclude that CNP can be used in the typing of all skeletal dysplasias, more clinical studies with molecular genetic analyses are needed.

Acknowledgments

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Ethics

Ethics Committee Approval: It was approved by the Ege University Faculty of Medicine Ethics Committee (decision dated: 18.01.2017, with approval number 16-8/9).

Informed Consent: Published written consent was obtained from parents and their children.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.K.Ç., D.G., S.Ö., H.A., Ş.D., Concept: D.G., S.Ö., Ş.D., Design: D.G., Ş.D., Data Collection or Processing: S.K.Ç., D.G., S.Ö., H.A., E.I., Ş.D., Su.Ö., Analysis or Interpretation: S.K.Ç., Ş.D., Su.Ö., Literature Search: S.K.Ç., E.I., Ş.D., Writing: S.K.Ç., Ş.D., Su.Ö.

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Case Report

Doctors Need Different Doctors to Treat Their Relatives: A Subject That Does Not Receive Enough Attention in Medical Education

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ABSTRACT

Due to having medical knowledge, sometimes doctors may not need to refer to other doctors in case of illness of themselves or their relatives. Therefore, the correct diagnosis may be delayed. This paper discussed this issue from the perspective of a daughter whose father, a doctor, caused the diagnosis of her diseases to be delayed. However, delay in diagnosis is difficult to measure, reasons for the delay can originate from the system or can be caused by the course of the disease, by the patient, and sometimes by physicians, as in our patient.

Keywords: Child, physicians, delayed diagnosis, algorithms, trust

INTRODUCTION

Sometimes diagnosis of diseases may be delayed. However, delay in diagnosis is difficult to measure, reasons for the delay can originate from the system or can be caused by the course of the disease, by the patient, and sometimes by physicians, as in our patient. Physicians generally underestimate the possibility of their diagnosis being wrong and that this tendency to over self-confidence is related to both internal and systemically reinforced factors. The incidence and impact of physician-induced diagnostic errors or delays has not been a subject frequently studied and emphasized in the literature. [1] This may result in an over-diagnosis, or under-diagnosis. This paper discussed this issue through a doctor who delayed the diagnosis of his daughter. Through this case, new studies can be conducted on strategies to increase the accuracy of diagnostic decision making.

CASE REPORT

A healthy baby girl, who was born by spontaneous vaginal birth at term to a healthy 27-year-old mother and weighed 3,200 g, had no health problems until the age of nine years. However, from the ages of nine to 15, she had perennial rhinorrhea with varying severities of serous characteristics, which was not accompanied by fever but caused frequent bouts of sneezing, slight wateriness of the eyes, and itching. The patient, whose growth and development were in accordance with those of her peers, had no history of cough, headache, or repeated infections accompanying these complaints. The patient's father, a practicing doctor, never referred her to another doctor until she was 16 years of age. When the complaints became severe, he recommended using antihistamines, which did provide some relief. However, the patient's complaints increased in the following years with intermittent headache, severe itching in her nose and palate, and intermittent shortness of breath. The patient stated that she was tired of experiencing these complaints. She was even prepared to have a nasal operation if necessary, and she was referred to our outpatient clinic. The patient's general health was good, although she appeared to be somewhat tired. There was slight redness under her eyes (allergic shiner), and her nasal examination showed bilateral hyperemic and hypertrophic concha. There was also a postnasal serous drip. The

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patient had no deformity in the thorax, and both hemithoraces contributed equally to respiration. On pulmonary auscultation, her expiration was found to be slightly lengthened, and occasional sonorous rhonchus was heard. Examination of systems other than respiratory system were normal. Laboratory results were within normal ranges including complete blood counting (Hb, Eos), biochemistry and total serum IgE levels. Posterior-anterior pulmonary x-rays showed no increases in aeration, with slight peribronchial thickening. The patient's forced expiratory volume in one second /forced vital capacity ratio was within normal limits, and a skin-prick test showed sensitivity to house dust mites. Based on all these findings, the patient was asked questions concerning allergic rhinitis and related criteria and was diagnosed with slight intermittent asthma. The treatments of nasal steroids, montelukast, and salbutamol if needed were recommended and improvement of clinical symptoms was observed in follow-up. Parents of the patient provided informed consent to publish the report.

DISCUSSION

Doctors often behave neglectfully about their own and family members' health. Due to having medical knowledge, doctors and their families do not visit other doctors. It seems that doctors trust themselves and are only admitted to other doctors if symptoms progress.[2] Furthermore, the literature does not emphasize the necessity for doctors or their families to visit other doctors for correct evaluation. Especially, to prevent the progression of disease and complications, this topic has not received the attention that it deserves in medical training. Due to the intense and lengthy working hours that doctors devote to their profession, they are frequently very tired at the end of their workdays and may also get bored with listening medical complaints.

As a result, even though they become sick, unless their illnesses are severe they let things ride, and this may cause delays in diagnosis.[1,3] Therefore, trying to solve their medical problems by themselves may lead to delays in diagnosis. Additionally, doctors may refrain from having a check-up due to being afraid of facing medical problems. Furthermore, neglecting to admit to another doctor may be due to the lack of time.

However, doctors who are parents may treat their children's diseases themselves and might simply assume that the illnesses are easy to treat. Indeed, doctors sometimes diagnose their relatives as having a certain disease without physical examinations, and they may often try to eliminate the symptoms rather than making clear diagnoses and recommending the correct treatments.[4,5] Thus, an accurate diagnosis might be delayed. This report addresses the topic of under-diagnosis. As a result, an accurate diagnosis might be made later than it would be in other patients who have the same symptoms and findings.

In conclusion, sometimes having medical knowledge and over self-confidence may become disadvantages for doctors' and their families' health. Including this topic in medical training may help prevent this kind of problems.

Ethics

Informed Consent: Parents of the patient provided informed consent to publish the report.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.Y., F.B., Concept: N.K., Ş.Y., H.A., Design: N.K., Ş.Y., H.A., Data Collection or Processing: Ş.Y., H.A., Analysis or Interpretation: N.K., F.B., Literature Search: N.K., Ş.Y., F.B., Writing: Ş.Y., H.A.

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Case Report

The Difficult Differential Diagnosis for A Pediatric Patient with Shwachman-Diamond Syndrome; A Case Report and Literature Review

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ABSTRACT

Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive disease characterized by bone marrow dysfunction, exocrine pancreatic insufficiency and skeletal abnormalities. Persistent or intermittent neutropenia caused by bone marrow hypoplasia is the most common hematological abnormality in SDS. It can be difficult to diagnose the disease that usually occurs in early childhood. SDS should be kept in mind in the differential diagnosis of neutropenic patients. If the signs of pancreatic insufficiency are not observed, the diagnosis may be missed. The article wanted to present a patient with pancreatic insufficiency and SDS with the biallelic mutation who presented with neutropenia in a newborn.

Keywords: Shwachman-Diamond syndrome, Neutropenia, Biallelic mutation, Bone marrow transplantation

INTRODUCTION

Shwachman-Diamond syndrome (SDS), a rare autosomal recessive disorder first defined in 1964, is a multi-system syndrome characterized by exocrine pancreatic insufficiency, neutropenia, and skeletal changes.[1-3] Permanent or intermittent neutropenia caused by bone marrow hypoplasia is the most common hematologic abnormality in SDS.[1,4] The diagnosis of SDS is based on a clinical phenotype, but it is difficult to diagnose in children with general symptoms such as bronchiolitis, diarrhea, and anemia. Therefore, SDS should be considered in the differential diagnosis in children with diarrhea, low weight gain, skeletal anomalies, and neutropenia.[4]

The purpose of this report was to present a differential diagnosis for SDS in a patient with diarrhea, neutropenia, exocrine pancreatic insufficiency, and skeletal changes, and discuss the follow-up condition of this patient after bone marrow transplantation.

CASE REPORT

A two-month-old male patient was referred to our hospital with the symptoms of cough, respiratory distress, and diarrhea. The patient presented with neutropenia and anemia, as well as acute bronchiolitis. The physical examination of the patient revealed that his body weight (BW) was 4200 g (<3 P), height 58 cm (25 P), and head circumference 38.5 cm (3-10 P). The patient had abdominal swelling and proportional shortness in the extremities. In a lung examination, bilateral crepitant rales were detected. There was no organomegaly.

The patient weighing 3200 g at the 38th week of gestation was born from a 24-year-old mother. He had neutropenia, recurrent infection attacks, and diarrhea in the neonatal period. In his family history, there was second-degree consanguinity between the parents, but there were no similar diseases. The results of the laboratory tests were as follows: hemoglobin (HGB) 7.6 g/dL, hematocrit (HCT) 23%,

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mean corpuscular hemoglobin (MCH) 32 pg, mean corpuscular volume (MCV) 95 fL, white blood cell count 4000/µL, the total number of neutrophils 400/µL, platelet count 217,000/µL, and reticulocyte percentage 1.5%. The biochemical test results were normal. Fat was found +2 in the stool. The percentages of lymphocytes, neutrophils, and monocytes in the blood smear were 80, 10, and 10, respectively, and platelets were sufficiently clustered. Bone marrow aspiration was heterogeneous normocellular. Normal myeloid and erythroid serial development pressures were seen. The myeloid series was decreased compared with the erythroid series. No atypical cells or bone marrow dysplasia was observed. The fecal elastase test result was 117 µg/g, and the sweat test was normal. The extremity and chest radiographs revealed irregularity and enlargement at the level of the costochondral junction, increased sclerosis in both the radius and distal ulnar metaphyses, and an ovoid-shaped vertebral corpus (Figure 1, 2). Ultrasonography and computed tomography of the abdomen showed that the pancreas body and tail section were hypoplastic. Serum amylase was detected as 12 U/L (25-125 U/L), serum lipase 4/L (8-78 U/L), vitamin D level 10.9 ng/mL, vitamin E 3.7 mg/L (6.6-14 mg/L), and vitamin A 115 mcg/L (200-430 mcg/L). Cystic fibrosis mutation analysis was normal. In the mutation analysis of SDS, biallelic heterozygous mutations were detected in the 7q11.21 gene (SBDS):c.258+2T>C. The SDS diagnosis was made, and 5 µ/kg of granulocyte colony-stimulating factor (G-CSF) was started. An adequate response was achieved when the dose of G-CSF was increased to 10 µ/kg. Enzyme replacement therapy was added to the treatment plan due to the presence of a pancreatic exocrine disorder. During the follow-up, diarrhea decreased, but the total neutrophil count was still about 420/mm^3. When the patient was aged 11 months, he was followed up with persistent severe neutropenia, recurrent infection attacks, and thrombocytopenia that required platelet transfusion. Therefore, hematopoietic stem cell transplantation (HSCT) was performed from the bone marrow of his fully compatible healthy sister. A conditioning regimen including fludarabine 30 mg/m^2 (-8-3 days), busulfan 2.5 mg/kg (-5-3 days) and thymoglobulin 10 mg/kg (-5-3 days) were used. Cyclosporine 3 mg/kg and mycophenolate mofetil (MMF) 1200 mg/m^2 were given for graft versus host disease (GVHD) prophylaxis (Table 1). CD34+ cells (10.9 x10^6/kg) were infused. Neutrophil and thrombocyte engraftment was achieved on +15th and +20th days. On the 16th day, the full donor myeloid chimerism was recorded using polymerase chain reaction/short tandem repeat loci analysis. No signs of acute or chronic GVHD and other transplant-related complications were observed in the follow-up in the 4 years after HSCT with full donor chimerism. The pancreatic insufficiency improved and he did not need pancreatic enzyme replacement after age 4 years. In the final physical examination, BW was 13.4 kg (<3P) and height was 100 cm (<3P) and the laboratory test results were 13.6 g/dL for HGB, 40% for HCT, 9020/µL for the white blood cell count, 2680/µL for the total neutrophil count, and 215,000/µL for the platelet count.

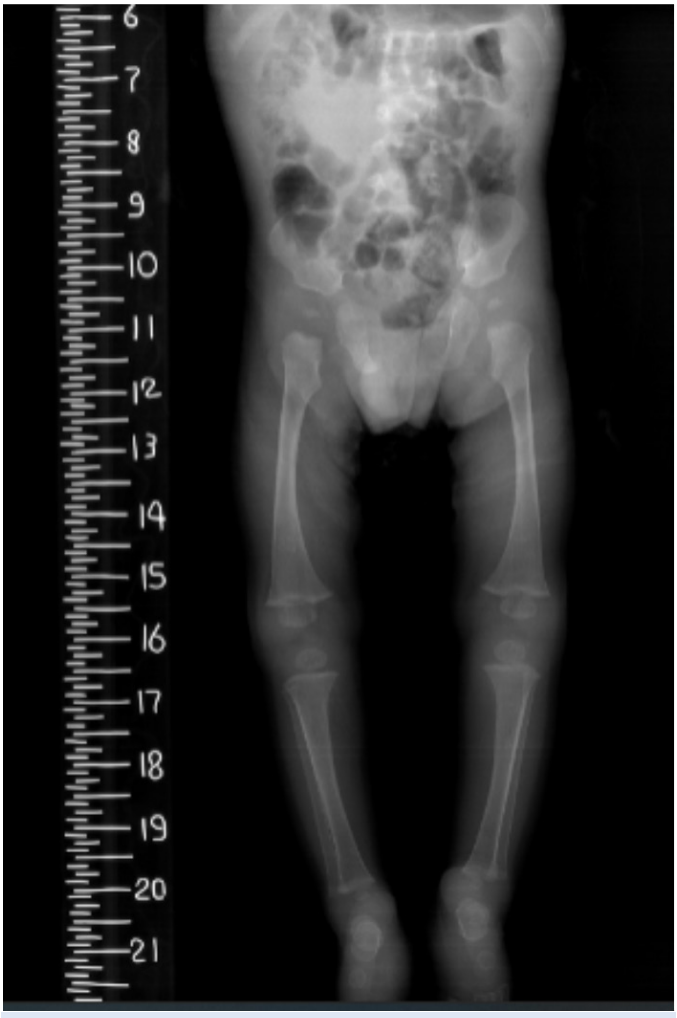

Figure 1. Limb shortening and epiphysis enlargement

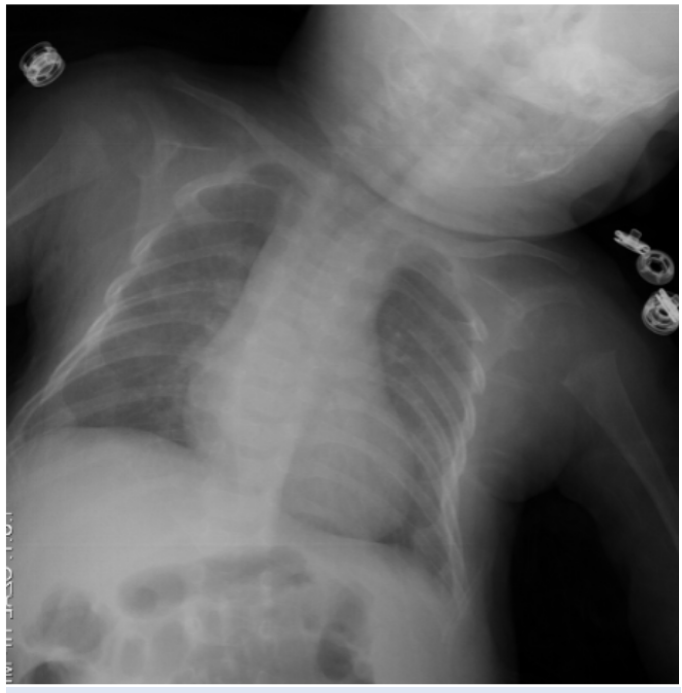

Figure 2. Irregularity and enlargement of the costochondral junction were detected on chest radiographs

Table 1. Transplantation characteristics	
Age at transplantation	**11 months old**
HLA matched sibling	Bone marrow
Myeloablative	Fudarabine 30 mg/m^2 (-8-3 days); Busulfan 2.5 mg/kg (-5-3 days); Thymoglobulin 10 mg/kg (-5-3 days)
CD 34+ cells	10.9x10^6/kg
GVHD prophylaxis	Cyclosporine 3 mg/kg Mycophenolate mofetil 1200 mg/m^2
Post transplant follow-up age	5 years old
The pancreatic insufficiency	No
Cytopenia	No
Recurrent infection	No
Growth retardation	Yes
Acute and chronic GVHD	No
AML and MDS transformation	No

HLA: Human leukocyte antigens, MDS: Myelodysplastic syndrome, AML: Acute myeloid leukemia, GVHD: Graft versus host disease

DISCUSSION

Shwachman-Diamond syndrome is a rare hereditary disease with serious clinical consequences, such as pancreas and bone marrow failure. Neutropenia is a common hematologic abnormality seen in patients affected by SDS, and chemotaxis defects also occur in neutrophils with reduced numbers. Apart from neutropenia, normochromic normocytic anemia is the second most common cytopenia. Increased fetal hemoglobin levels are accompanied by thrombocytopenia.[5,6] Although the treatment of patients with anemia and thrombocytopenia can be supported by red cell and platelet transfusions, very severe neutropenia (ANC <200/µL) remains an adverse prognostic factor because it is associated with a high mortality rate and risk of life-threatening infections.[7,8] The hematologic abnormality of our patient was severe neutropenia (ANC: 200/µL), and although there was initially no thrombocytopenia, mild thrombocytopenia and aplasia occurred three months later. Our patient had recurrent episodes of infection due to neutropenia. Erythrocyte suspension occurred due to anemia, resulting in the requirement of transfusion.

The clinical symptoms of pancreatic insufficiency appear after more than 98% of the exocrine acinar capacity of the pancreas has been lost. Steatorrhea is seen in 90% of affected children with SDS in the first year of life. Fat-soluble vitamin deficiency (vitamins A, D, E, and K) is caused by steatorrhea and malabsorption.[6] Our patient presented with diarrhea, growth retardation, and therefore we considered the possibility of cystic fibrosis, which was the most common cause of pancreatic insufficiency. Stool elastase level and fat-soluble vitamins were low, and genetic evaluation for cystic fibrosis was negative. After the diagnosis of cystic fibrosis was excluded and anemia was added to neutropenia, the patient was evaluated with suspicion of SDS. The patient's extremity radiographs were evaluated and limb shortening was detected. Genetic testing was planned for SDS and pancreatic enzyme and vitamin supports were given for diarrhea.

The detection of cytogenetic abnormalities in the bone marrow is a marker of clonal evolution and malignant transformation.[5] It was stated that c.258+2T>C heterozygous mutations might be associated with severe cytopenia.[9] Our patient had these biallelic heterozygous mutations in the *SDS* gene located on chromosome 7q. Therefore, when the patient was aged 1 year, an allogeneic hematopoietic stem cell transplant was performed from his sister with full human leukocyte antigen compatibility. After the transplant, the patient was followed up for 4 years, during which his pancytopenia improved and he did not require transfusion. The patient had received neutropenic fever treatment four times before the transplant, and over the 4-year follow-up period, he was referred to the hospital twice with symptoms of fever. A decrease in the frequency of infections was observed after transplantation, which was linked to the resolution of neutropenia.

Patients with SDS may have malabsorption, recurrent infections, metaphyseal dysostosis, low birth weight, delayed bone age, delayed puberty, mucositis and periodontal infections, dental dysplasia, hepatomegaly, elevated liver enzymes, severe eczema, and cognitive and attention impairment. The clinical phenotype of SDS is extremely heterogeneous showing a wide range of abnormalities and symptoms; therefore, a definitive diagnosis still presents a challenge.[7,10-12] Although our patient had neutropenia and recurrent infection attacks in the neonatal period, the diagnosis of SDS was made late. Therefore, it is important to consider SDS in the differential diagnosis in patients with neutropenia, diarrhea, and recurrent infections.

In a literature review from 1988 to 2016, Cesaro et al.[13] evaluated the results of a total of 91 patients. They found that the prognosis was poor in non-transplant patients who transformed to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML).[13] Myers et al.[14] determined that 33 of 36 patients presented with MDS or AML had previously been diagnosed as

having SDS. In another retrospective study, the same author in the same year reported the results of HSCT in 52 patients with SDS. Myers et al.[15] emphasized that transplant success declined after AML or MDS transformation. They found a 5-year survival of 72% in patients with bone marrow failure transplanted before MDS or ALL developed.[15] Our patient presented with neutropenia and recurrent infection attacks in the neonatal period. When it was learned that he had diarrhea, the patient was thought to have cystic fibrosis and the relevant tests were performed. Further tests were requested in the differential diagnosis of SDS when the patient's clinical status progressed and anemia developed.

In conclusion, if there is growth retardation in a child with diarrhea, steatorrhea, and neutropenia; skeletal abnormalities for SDS should be investigated and exocrine pancreatic insufficiency and bone marrow failure should be evaluated. SDS should be kept in mind in the differential diagnosis of patients with neutropenia. If a compatible donor is found, it will be appropriate to plan a bone marrow transplant before the development of MDS or leukemia.

Ethics

Informed Consent: Informed consent was obtained.

Peer-reviewed: Externally peer-reviewed.

Authorship Contributions

Concept: D.Ç.A., A.A., Z.Ş.H., Ü.Ö., Design: D.Ç.A., A.A., Z.Ş.H., Ü.Ö., Data Collection or Processing: D.Ç.A., A.A., Z.Ş.H., Ü.Ö., Analysis or Interpretation: D.Ç.A., A.A., Z.Ş.H., Ü.Ö., Literature Search: D.Ç.A., A.A., Z.Ş.H., Ü.Ö., Writing: D.Ç.A., A.A., Z.Ş.H., Ü.Ö.

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