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Wilson Disease in Children: Analysis of 21 Patients

Çocuklarda Wilson Hastalığı: 21 Hastanın Analizi

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Abstract

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism. Affected children may be asymptomatic, makes the diagnosis more difficult. In this study, we aimed to evaluate the clinical, laboratory, histopathological and genetic characteristics, and outcomes of the patients with WD. Our study includes patients who were diagnosed with WD between January 2010 and December 2020. The presenting complaints, physical examination findings, consanguinity and family history, laboratory, genetic, histopathological evaluation results, treatment and outcomes were all recorded. A total of 21 patients from 18 families [median age 9.5 (1-14) years, 10 girls] were included. Kayser-Fleischer ring was detected in 11 (52.4%) patients. Serum ceruloplasmin (<20 mg/dl) was low in 15 patients. Urinary copper excretion was >100 µg/day in 17 patients. Copper was positively stained with rhodanine in 9 of the18 liver biopsies. Liver copper content was >50 µg/g dry weight in all patients, 50-250 µg/g in 3 patients and >250 µg/g in 15 patients. Genetic evaluation was available in 18 patients and revealed heterozygous mutations in the ATP7B gene in 4 patients, combined heterozygous mutations in 6, and homozygous mutations in 8. Except for two patients with neurological findings and three asymptomatic patients who were diagnosed by family screening, all were presented with liver findings. Neurological involvement was also detected in 2 patients during follow up. D-Penicillamine and zinc sulfate combined treatments were used in 16 patients, zinc sulfate monotherapy was given to a presymptomatic patient diagnosed with family screening, and trientine and zinc sulfate combined therapies were used in four patients with neurological involvement. Transaminase values returned to normal in a median of 8.3 (4-23) months in 15 patients. The Kayser-Fleischer ring disappeared in a median of 32.8 months (10-81) in seven out of eleven patients. While liver transplantation was performed in one of the two patients who presented with fulminant hepatic failure at admission, the other was followed up with plasmapheresis and chelation therapy without the need for transplantation. Wilson disease should be considered in the differential diagnosis of all kinds of liver diseases ranging from asymptomatic elevation of transaminases to acute liver failure. Since early diagnosis and treatment are very important, family screening should definitely be recommended in diagnosed patients.

Keywords: Ceruloplasmin; Copper; Kayser-Fleischer ring; Wilson disease

Özet

Wilson hastalığı (WH), bakır metabolizmasının otozomal resesif geçişli bir bozukluğudur. Etkilenen çocuklar asemptomatik olabilir ve bu tanı koymayı zorlaştırır. Bu çalışmada WH'li hastaların klinik, laboratuvar, histopatolojik ve genetik özellikleri ve izlem sonuçlarını değerlendirmeyi amaçladık. Çalışmamıza Ocak 2010-Aralık 2020 tarihleri arasında WH tanısı konulan hastalar dahil edilmiştir. Başvuru şikayetleri, fizik muayene bulguları, akrabalık ve aile öyküsü, laboratuvar, genetik, histopatolojik değerlendirme sonuçları, tedavi ve izlem sonuçları kayıt altına alındı.18 aileden toplam 21 hasta [ortanca yaş 9,5 (1-14) yıl, 10 kız] dahil edildi. 11 (%52,4) hastada Kayser-Fleischer halkası tespit edildi. 15 hastada serum seruloplazmin (<20 mg/dl) düşüktü. 17 hastada üriner bakır atılımı >100 µg/gün idi. Bakır, 18 karaciğer biyopsisinin 9'unda rhodanin ile pozitif olarak boyandı. Karaciğer bakır içeriği tüm hastalarda >50 µg/g olup, 3 hastada 50-250 µg/g ve 15 hastada > 250 µg/g idi. 18 hastada genetik değerlendirme yapıldı ve 4 hastada ATP7B geninde heterozigot mutasyonlar, 6 hastada kombine heterozigot mutasyonlar ve 8 hastada homozigot mutasyonlar saptandı. Nörolojik bulguları olan iki hasta ve aile taraması ile tanı konulan üç asemptomatik hasta dışında, tümü karaciğer bulguları ile başvurdu. Takiplerde 2 hastada nörolojik tutulum saptandı. 16 hastada D-penisilamin ve çinko sülfat kombine tedavileri, aile taraması ile tanı konan presemptomatik bir hastaya çinko sülfat monoterapisi, nörolojik tutulumu olan dört hastada trientin ve çinko sülfat kombine tedavileri uygulandı. 15 hastada ortalama 8,3 (4-23) ayda transaminaz değerleri normale döndü. Kayser-Fleischer halkası, on bir hastanın yedisinde medyan 32,8 ayda (10-81) kayboldu. Başvuru anında fulminan karaciğer yetmezliği ile başvuran iki hastadan birine karaciğer nakli yapılırken, diğerine transplantasyona gerek kalmadan plazmaferez ve şelasyon tedavisi uygulandı. Asemptomatik transaminaz yükselmesinden akut karaciğer yetmezliğine kadar her türlü karaciğer hastalığının ayırıcı tanısında Wilson hastalığı düşünülmelidir. Erken tanı ve tedavi çok önemli olduğundan tanı konulan hastalarda aile taraması mutlaka önerilmelidir.

Anahtar Kelimeler: Bakır; Kayser-Fleischer halkası; Seruloplazmin; Wilson hastalığı

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1. Introduction

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism caused by mutations in the ATP7B gene encoded in the long arm of the 13th (13q14.3) chromosome (1). As a result of the inability of copper to bind to ceruloplasmin and not be excreted in the bile, it accumulates first in the liver and then in other organs, especially in the cornea, brain and kidneys (2).

While the prevalence of the disease is 1/30,000 worldwide, the carrier frequency is 1/90 (3). Patients are frequently diagnosed between the ages of 5-35 (4). Patients may show a variety of clinical signs and symptoms, or they may be asymptomatic. While most of the cases present with hepatic findings in the first decade, 75% present with neuropsychiatric findings and 25% with hepatic and neuropsychiatric findings after the second decade (5).

Liver involvement may vary from asymptomatic liver enzyme elevation to acute hepatitis, chronic hepatitis, cirrhosis, and fulminant liver failure (6). Neurological involvement is manifested by movement disorders such as tremor, dystonia or parkinsonism (bradykinesia, rigidity, and resting tremor) and accompanying dysarthria, hypersalivation, and dysphagia (7). Common neuropsychiatric findings in children are decreased school success, inappropriate behaviors, and mood disorders (8).

There is no single diagnostic test that can exclude or confirm WD with certainty. Therefore, diagnosis is made by evaluating clinical, laboratory and genetic tests together (9). In addition, clinical symptoms may be absent and the Kayser-Fleischer (KF) ring can not be detected during early childhood and this makes the diagnosis even more difficult. It is one of the few genetic diseases which can be successfully treated with early diagnosis.

In this study, we aimed to evaluate the demographic, clinical, laboratory, histopathological and genetic characteristics and treatment responses of 21 pediatric WD patients.

2. Material and Methods

The study was conducted at Eskişehir Osmangazi University Faculty of Medicine, Department of Pediatric Gastroenterology and Hepatology. Patients who were followed up with a diagnosis of WD between January 2010 and December 2020 were included in the study.

The patients' presenting complaints, consanguinity and family history, physical examination findings, laboratory results and liver histopathological evaluation were recorded.

The serum ceruloplasmin level, the amount of 24-hour urinary copper excretion, the D-Penicillamine challenge test results, the presence of KF ring, the amount of liver copper content and the results of genetic analysis were recorded. The Ferenci score was calculated for all patients, and those who scored 4 and above were accepted as Wilson disease (10). Renal involvement was evaluated by complete urine analysis, urine amino acid study, and 24-hour urine calcium, phosphorus and protein excretion. Routine laboratory tests were measured by standard methods, serum ceruloplasmin level analized by nephelometric method and the 24-hour urine copper and liver copper content were measured by atomic absorption spectrophotometer method.

Portal system color Doppler ultrasonography (USG) and esophagogastroduodenoscopy (EGD) were used to evaluate the presence of portal hypertension and varicose veins. Complete neurological examination and cranial magnetic resonance imaging (MRI) data were used to determine the presence of neurological involvement. The dose, duration and adverse effects of drugs used for WD treatment, time to remission and liver transplantation requirements were also recorded.

3. Results

Four patients were excluded from the study due to lack of follow-up. A total of 21 patients from 18 families [median age 9.5 (1-14)

years, 10 girls, 11 boys] were included in the study. Six of the patients were siblings, and the parents of seven were consanguineous (Table 1).

The presenting manifestations of the disease were as follows: fatigue in 9 (42.9%) patients, jaundice in 4 (19%), epistaxis in 2 (9.5%), tremor in 1 (4.8%), and bradykinesia in 1 (4.8%). Two patients presented with fulminant hepatic failure. Seven of the patients were referred because of raised transaminases. Hepatomegaly was the most common clinical finding, in 11 (52.4%) patients, followed by splenomegaly in 8 (38.1%), jaundice in 6 (28.6%), ascites in 3 (14.3%), clubbing in 2 (9.5%), epistaxis in 2 (9.5%), fine tremor in 1 (4.8%), and bradykinesia in 1 (4.8%) (Table 1).

While the transaminase levels were high in 20 (90.5%) patients, one patient who was early diagnosed by family screening, had normal liver function tests. High international normalized ratio (INR) values were detected in 10 (47.6%) patients, hyperbilirubinemia in eight (38.1%), anemia in eight (38.1%), hypoalbuminemia in four (19%), and Coombs negative hemolytic anemia in one patient (4.8%) (Table 1). Renal involvement was present in three (14.3%) patients on admission. Two patients had proteinuria, impaired tubular phosphorus reabsorption, and mild to moderate amino aciduria, while one patient had hypercalciuria.

Table 1. Demographic features, physical examination findings and laboratory results of the patients

Patient characteristics	
Age at diagnosis, median (25-75p)	8.4 (6-11.1)
Sex (Girl/Boy)	10/11
Consanguinity, n (%)	7 (33.3)
Positive family history, n (%)	3 (14.2)
Hepatomegaly, n (%)	11 (52.4)
Splenomegaly, n (%)	8 (38.1)
Jaundice, n (%)	6 (28.6)
Aspartate aminotransferase, IU/L, median (25-75p)	126.5 (95.3-176.1)
Alanine aminotransferase, IU/L, median (25-75p)	136.1 (79.5-274.5)
γ-Glutamyltransferase, IU/L, median (25-75p)	116 (49-212)
Alkaline phosphatase, IU/L, median (25-75p)	322 (278-354)
Total protein, g/dL, median (25-75p)	7.1 (6.3-7.9)
Albumin, g/dL, median (25-75p)	3.8 (2.4-4.1)
Total bilirubin, mg/dL, median (25-75p)	0.58 (0.31-1.82)
Direct bilirubin, mg/dL, median (25-75p)	0.22 (0.083-0.81)
International normalized ratio, median (25-75p)	1.21 (1.16-2.01)

Hepatobiliary USG revealed hepatomegaly in 11 patients, splenomegaly in nine, liver parenchymal heterogeneity in eight, hepatosteatosis in six and, irregular liver contours and nodularity in three patients. Portal hypertension was detected in six patients on portal system color Doppler USG examination.

Kayser-Fleischer ring was present in 11 (52.4%) patients. The youngest patient with KF ring was 4 years old, and the others were older than 6 years old. Serum ceruloplasmin was low (<20 mg/dl) in 15 (71.4%) patients and lower than 10 mg/dl in eight (38.1%) of them. Urinary copper excretion was >100 µg/day in 17 patients. D-Penicillamine challenge test was performed in 2 patients with urinary copper excretion between 40 and 100 µg/day and more than fivefold increase was observed in both patients (Table 2). Eighteen (85.7%) patients underwent USG-guided tru-cut liver biopsy. Liver biopsies of nine (42.9%) patients were positively stained with rhodanine. According to the Ishak fibrosis staging, three patients had stage 1, two had stage 2, three had stage 3, two had stage 5 (precirrhosis), and eight had stage 6 fibrosis (cirrhosis). Steatosis was present histopathologically in eight (38.1%) patients. Liver copper content was >50 µg/g dry weight in all patients, 50-250 µg/g in 3 patients and > 250 µg/g in 15 patients. (Table 2).

Table 2. Specific laboratory finding of the patients with Wilson disease

Diagnostic tests	
Kayser-Fleischer ring, n (%)	11 (52,4)
Serum ceruloplasmin, mg/dl, median (25-75p)	14.1 (6.5-21.12)
<20 mg/dl, n (%)	15 (71.4)
Urinary copper excretion µg/day, median (25-75p)	262.5 (151.3-578)
>100 µg/day, n (%)	17 (81)
40-100 µg/day, n (%)	2 (10.5)
D-Penicillamine challenge test	
>fivefold increase, n (%)	2 (10.5)
Liver dry copper content, µg/g, median (25-75p)	538 (260-844.5)
>250 µg/g, n (%)	15 (71.4)
50-250 µg/g, n (%)	3 (14.2)
Histopathological findings	
Steatosis, n (%)	8 (38.1)
Chronic active hepatitis, n (%)	10 (47.6)
Cirrhosis, n (%)	8 (38.1)

In the ATP7B gene, heterozygous mutations were found in four patients, mixed heterozygous mutations in six patients, and homozygous mutations in eight patients. The most common mutation was p.H1069Q and its frequency was 19.1%.

Ferenci score was 4 and above in all patients.

Except for two patients with neurological findings and three asymptomatic patients who were diagnosed by family screening, all were presented with liver findings. Ten of the patients (47.6%) had chronic liver disease, two (9.5%) had findings of fulminant hepatic failure, and seven (26.3%) had elevated transaminases. Of the nine patients who underwent EGD for portal hypertension, stage 1 esophageal varices were found in three, stage 2 in two, and stage 3 in one. Endoscopic band ligation was performed in three patients for varice eradication.

Neurological involvement was detected in four (19%) patients. Two patients who presented with neurological findings had headache, amnesia, dysmetria, right bradykinesia in one, and dysarthria, hypersalivation and tremor in the other. Two patients with no neurological symptoms showed minor abnormalities on MRI at the time of diagnosis, and progress was detected in follow-up due to noncompliance with therapy. Kayser-Fleischer ring was present in all four patients with neurological involvement.

D-Penicillamine and zinc sulfate combined treatments were used in 16 patients, zinc sulfate monotherapy was given to a presymptomatic patient diagnosed with family screening, and trientine and zinc sulfate combined therapies were used in four patients with neurological involvement. No adverse drug reactions were observed in any of the patients. Penicillamine treatment was replaced by trientine in two patients due to the lack of improvement in transaminase values.

Transaminase values returned to normal in a median of 8.3 (4-23) months in 15 patients. D-Bilirubin levels returned to normal in a median of 2.5 (0.01-12) months, and the INR in a median of 8.5 (2-48) months. One of the two patients who were moved from D-penicillamine to Trientine owing to transaminase levels not normalized recovered to normal, while the other remained slightly higher. The Kayser-Fleischer ring disappeared in a median of 32.8 months (10-81) in seven patients. While liver transplantation was performed in one of the two patients who presented with fulminant hepatic failure on admission, the other was followed up with plasmapheresis and chelation therapy without the need for a liver transplant. Two of the patients with chronic liver disease also underwent liver transplant during follow-up.

4. Discussion

Because of its autosomal recessive inheritance and our country's high rate of consanguineous

marriage, taking a family history and performing family screening in WD is critical. In support of this, the consanguinity rate between the parents was 33% in our study, and three of our patients with a family history of WD were diagnosed with screening and started early treatment.

Although the presence of a KF ring and low ceruloplasmin together with clinical findings are sufficient for the diagnosis, this association is not always present especially in small children. The Kayser-Fleischer ring is found in practically all patients with neurological involvement, whereas only 40-50 percent of patients with hepatic involvement and 20-30% of presymptomatic individuals have it. (8). Since most of the pediatric patients present with hepatic involvement, the diagnostic power of the KF ring is low in children. In our study, KF ring was present in 11 (52.4%) patients, and this incidence in our hepatic presentation-weighted cohort was compatible with the literature.

Ceruloplasmin is a copper transport protein that binds 90% of circulating copper. Even in the case of genetically proven disease, ceruloplasmin levels can be in normal range approximately in 20% of patients. Its levels may increase in chronic active hepatitis and as an acute phase reactant. Furthermore, depending on the nephelometric approach, misleading highs may arise because it analyzes both ceruloplasmin and physiologically inactive apoform. (11,12). In our study, ceruloplasmin was found to be normal in five (23.8%) patients. This can be explained by the use of nephelometric method in the measurement of ceruloplasmin and the presence of chronic active hepatitis in histopathological evaluation in three of our patients. Low ceruloplasmin levels concurrent with KF ring was observed in nine (42.8%) patients, and only less than half of the pediatric cases could be detected with the positivity of these two tests.

The conventionally accepted diagnostic level for 24-hour urinary copper excretion in Wilson's disease is above 100 µg/day. However, it may be below 100 µg/day in 16-23% of children and in asymptomatic patients (8,13). In a study, the cut off value of 40 µg/g was shown to have a sensitivity of 78.9% and a specificity of 87.9% for the diagnosis of WD (14). In individuals with readings below 100 µg/day but who are clinically compatible with WD, a D-penicillamine challenge test is indicated. After the D-penicillamine challenge test, an increase of more than fivefold from the basal value is significant (15). Urine copper was below 100 µg/day in two of our 19 patients. In these two patients, a 5-fold increase in urinary copper from the basal values were obtained with the challenge test. On the other hand, there are also publications stating that there is no need for D-Penicillamine challenge test if the limit is taken as 40 µg/day (15). When the limit for urinary copper excretion was taken as 40 µg/day, it was observed that the patients in our study did not require the D-Penicillamine challenge test.

When the diagnosis cannot be confirmed with clinical findings and laboratory tests, liver biopsy is performed for histopathological evaluation and parenchymal copper concentration quantification. Histopathological findings are not pathognomonic for WD, and micro-macrovesicular steatosis, portal fibrosis and inflammation, fibrous bridging between portal areas and cirrhosis can be seen. Negative staining may occur because parenchymal copper accumulation is cytosolic in the early period and copper dyes can stain lysosomal copper (16). In our study, nine patients had positive staining with Rhodanin. Parenchymal copper concentration >250 µg/g in non-cholestatic patients has been shown to be diagnostic for WD. Lower values have been reported in about 20% of Wilson patients, but it has been reported that it may also be due to sampling error due to the inhomogeneous distribution of copper in the liver (8,17,18). In one of the few studies conducted with children, 28 of 30 WD patients were found to have a liver copper concentration of >250 µg/g, while two patients had a liver copper concetration less than 75 µg/g (15). In our study, tissue copper was measured above the normal value (50 µg/g) in all patients, and it was above 250 µg/g in 15 of them.

More than 700 mutations associated with WD have been reported so far (19). Although most

genetic studies begin with screening for common mutations in specific populations, gene sequencing is frequently required because no specific mutation is responsible for the majority of cases in our country. (20-22). With the new generation sequencing, 95% of the mutations can be detected in the affected person. However, this technique is not diagnostic in molecular defects outside the coding regions, intron-exon junctions, and deletions greater than 200 kd (23). In our study, 4 patients had heterozygous mutations. These patients, on the other hand, were diagnosed with WD according to the Ferenci score along with other laboratory and clinical findings. The most common mutation was p.H1069Q with a frequency of 19.1%, and it was found at similar rates (17.39%) with a previous study from our country (22).

The Ferenci scoring system was developed to solve the diagnostic confusion in WD (10). In a study evaluating its use in children, its sensitivity was found to be 98.14%, and its specificity was 96.59% (24). The absence of diagnostic methods such as “relative exchangeable copper, nonseruloplasmin bound copper”, electron microscopy evaluation and radioactive copper study, as well as the discussions about the thresholds of 24-hour urine copper excretion and liver parenchymal copper concentration can be considered as disadvantages of Ferenci scoring system. In addition, patients with idiopathic copper toxicosis and MDR3 deficiency can score 4 and above from the Ferenci score.

The treatment is based on the principle of removing excess copper from the body with chelators such as D-Penicillamine and trientine, and preventing its absorption from the intestine with zinc salts. All treatments have been shown to be effective in asymptomatic or mild hepatic involvement (25-27). In symptomatic patients, the use of chelators is recommended (8). D-Penicillamine was suggested to be used as the first choice in WD patients. Trientine was recommended as the second choice for those who developed adverse effects with D-penicillamine, but later it was also suggested to be used as the first choice in neurological involvement. However, Weiss et al. evaluated neurological deterioration after treatment and found the risk to be similar for all drugs (9.1% for D-Penicillamine, 8.8% for Trientine, and 7.3% for zinc) (28). In our center, trientine was given as first choice in patients with neurological involvement, zinc therapy was started in presymptomatic patients, and D-Penicillamine and zinc were given in other patients.

In the literature, early-stage side effects such as D-Penicillamine-induced hypersensitivity reactions and mid-late-term side effects such as nephrotoxicity and lupus-like syndrome have been reported up to 30% (8). However, interestingly, D-Penicillamine-related side effects were not observed in our patients. This may be due to the relatively small patient size in our study.

Follow-up should be done weekly in order to detect drug side effects at the beginning of the treatment, and then at intervals of 1-3 months until remission is achieved. Recovery of liver enzymes takes approximately 3-12 months, while recovery of INR takes approximately 3-12 months, with 1 month at the earliest (29). In our patients, transaminase values returned to normal in a median of 8.3 (4-23) months in 15 patients after medical treatment. Transaminase elevation continued throughout the follow-up period in two patients whose treatment compliance was not good. Kayser-Fleischer ring disappeared in seven patients in a median of 32.8 months (10-81).

Transplantation can be prevented with various supportive treatments in children with decompensated cirrhosis who have liver failure but do not have encephalopathy. It is recommended to use "King's Wilson Index" (KWI) to predict transplantation-free mortality in the follow-up of these patients (30). (31). One of the two patients in our study who presented with fulminant hepatic failure at admission received a liver transplant, while the other patient recovered without transplantation with plasmapheresis and chelation therapy, although the KWI was above 11. Liver transplantation was performed in another two patients with decompensated chronic liver disease during follow-up.

In conclusion, WD should be considered in the differential diagnosis of all liver diseases ranging from asymptomatic transaminases to acute liver failure. When the results of ceruloplasmin, KF ring and copper excretion in 24-hour urine are not sufficient for diagnosis, liver biopsy should be performed and histopathological evaluation of liver dry copper weight should be measured and the Ferenci score should be calculated with genetic testing to confirm the diagnosis. Family screening should be done in diagnosed patients because early diagnosis and treatment are critical.

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