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Clinical and Laboratory Findings in Patients with Wilson's disease Referred to Pediatric Gastroenterology Clinic in 2018-2019

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Abstract

Background: Early diagnosis and treatment of Wilson's disease in childhood can reduce long-term and life-threatening complications in these patients. Considering the lack of a database of Wilson's patients in Iranian patients, the present study was carried out with the primary objective of determining clinical and laboratory presentations in children with Wilson's disease referred to Akbar Hospital in Mashhad.

Methods: This cross-sectional descriptive study was conducted on children under 18 years of age with Wilson's disease who had presented to Akbar Children's Hospital in Mashhad during 2018-2019. The acquired information included demographic information, primary clinical symptoms (hepatic, cerebral, and psychological symptoms), and laboratory findings, including liver laboratory profile (AST, ALT, and ALP tests), coagulation tests, albumin, total serum protein, and direct and indirect bilirubin, and Wilson's diagnostic tests.

Results: In total, 25 patients with an average age of 15.88±4.54 years were included in this study. Hepatosplenomegaly, Kayser–Fleischer ring, and jaundice were observed in 72%, 68%, and 48% of patients, respectively. Gender of patients was not significantly correlated with the clinical and laboratory findings of Wilson's disease ($P<0.05$). 24-hour urine copper level was higher than 100 micrograms in 82.6% of patients. Serum ceruloplasmin level was lower than 200 mg/liter in 90% of patients. Serum ceruloplasmin levels in patients with ascites ($P=0.04$) and patients with lower limb edema ($P=0.02$) were higher than those in patients without these findings. Moreover, a lower 24-hour urinary copper level was detected in patients with seizures ($P=0.03$), and patients with depression ($P=0.005$) compared to patients without these conditions. The 24-hour urine copper levels were higher in patients with jaundice than in those without jaundice ($P=0.01$).

Conclusion: Hepatosplenomegaly, Kayser–Fleischer ring, and jaundice are common symptoms in under 18-year-old patients with Wilson's disease. Considering the findings regarding the high levels of serum ceruloplasmin and copper in 24-hour urine in a significant

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proportion of cases, these laboratory results can be used in diagnosing patients with Wilson's under 18 years of age.
Key Words: Copper, Jaundice, Pediatric diseases, Wilson's disease.

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1- INTRODUCTION

Wilson's disease is a clinical condition caused by a mutation on chromosome 1403q, first described in 1912 (1). A genetic disorder causes this disease in the excretion of copper, which leads to the toxic accumulation of copper in different body organs and damages them (2).

The prevalence of Wilson's disease is similar in most regions of the world. It is estimated to be approximately 0.5 cases per 100,000 population with a gene frequency of 0.56% and a carrier frequency of 1 in 90 patients (3). The prevalence of people carrying ATP7B mutations is estimated at one percent of the population. Accordingly, the risk of Wilson's disease in siblings is 1 in 4, and in children of the affected individual is 1 in 200. However, this disease is sporadic in certain areas. In this regard, more than 500 genetic mutations have been identified hitherto, and the smaller number of symptomatic clinical cases, due to the frequency of allele carriers in the population, may indicate a decrease in the penetration of mutations (3).

This disease is an autosomal recessive disorder caused by ATP7B gene mutations. This gene is related to the ATPase enzyme, a copper membrane transporter (4). Because many inactivating mutations in the ATP7B gene have been reported, screening for the disease has yet to be realized (2). DNA haplotype analysis can determine the genotype of the patient's siblings. Moreover, a rare multisystem disorder in copper metabolism with presentations similar to Wilson's disease has been reported. This disease is called MEDNIK syndrome and is caused by mutations in the APIST gene. This gene encodes an adapter protein necessary for the intracellular transport of copper pump proteins in Wilson's disease (5).

The main objective of the treatment for this disease is to reduce copper accumulated in the body and maintain physiologic copper levels. To achieve this, copper chelators such as zinc sulfate, d-penicillamine, and trientine can be used. Early diagnosis and treatment in childhood may reduce long-term and life-threatening complications; therefore, early diagnosis of this disease is essential. In this regard, it is vital for clinicians to be vigilant of the clinical presentation of copper toxicity, which is mainly caused by liver and brain involvement, thereby facilitating early diagnosis.

The diagnosis of this disease is based on the 24-hour urine copper level, serum ceruloplasmin, and ophthalmological examination to visualize the Kayser–Fleischer ring in the cornea. The diagnosis is confirmed by a liver biopsy and liver dry copper measurements (6-8). Other laboratory tests that can assist in diagnosis include liver aminotransferases, direct and indirect bilirubin, coagulation profile, serum albumin, and total protein levels. Identifying Wilson's disease mutation can also confirm the diagnosis and is utilized for screening other family members (7). As early diagnosis at young ages and treatment with copper chelators can effectively prevent complications, screening family members is vital (6).

Therefore, being vigilant of clinical manifestations, particularly in childhood, can lead to timely diagnosis and prevention of future complications. Various clinical manifestations have been described in Wilson's disease; however, most childhood presentations are related to the liver, and adulthood ones to the nervous system (9). The liver-related presentations of the disease are jaundice, hepatosplenomegaly, ascites, and signs of chronic liver failure, such as vascular telangiectasia, erythema of the palms, and bleeding disorders attributable to liver failure (10). Brain and psychological symptoms of the disease are seizures, pseudo-parkinsonism or slowness of movements and speech, depression, anxiety disorders, and psychosis (11). Other organs such as kidneys, eyes, heart, glands, hematological organs, and bones can be affected (6-8, 12).

As early diagnosis and treatment of Wilson's disease in childhood can minimize long-term and life-threatening complications, it is vital to detect the symptoms of this disease early in childhood. Considering that the presentations of Wilson's symptoms can be quite different, it is crucial to investigate and screen diverse populations in terms of the genetic indicators of the disease. Considering the lack of a database of Wilson's patients in Iranian populations, the present study was designed and conducted to determine clinical and laboratory presentations in children with Wilson's disease referred to Akbar Hospital in Mashhad, Iran.

2- METHODS

The current research was a cross-sectional descriptive study conducted on children under 18 years of age with Wilson's disease referred to Akbar Children's Hospital in Mashhad between 2019 and 2018.

2-1. Inclusion and exclusion criteria

The inclusion criteria in this study encompassed the age group under 18, having Wilson's disease. Incomplete files and non-cooperation of the patient to complete the information were considered as exclusion criteria. Moreover, if two or more family members were affected by Wilson's disease, only one was included, and the others were excluded from the study.

2-2. Study design

The participants included all patients under 18 years of age with Wilson's disease diagnosed according to the published criteria who were referred to the pediatric gastroenterology clinic of Akbar Hospital during the mentioned period. Since all eligible patients were included in the study through census sampling, there was no need to calculate the sample size.

The diagnosis of Wilson's disease was made by pediatric gastroenterology and liver specialists. The diagnostic criteria for Wilson's disease included visualization of the Kayser–Fleischer ring in the eye, 24-hour urine copper levels higher than 40 μg, and ceruloplasmin levels less than 20 mg/dL, or the presence of two or one criteria with liver biopsy and dry liver copper more than 250 micrograms per gram of dry liver weight. The required information about the patients was extracted from their files and recorded in a pre-prepared checklist. Besides new patients diagnosed with Wilson's disease investigated during the study period, the medical records of patients previously diagnosed with Wilson's disease were also reviewed, and the presenting symptoms were obtained from their history and documents. The collected information included demographic information, primary clinical symptoms (hepatic, cerebral, psychological symptoms), laboratory findings including liver laboratory profile (AST, ALT, ALP tests), coagulation tests, albumin, total serum

protein, direct and indirect bilirubin, and diagnostic tests of Wilson's disease.

2-3. Data analyses

The collected data were analyzed using SPSS software version 22. Descriptive statistical methods were used to describe the data, including central indices, dispersion, and frequency distribution. The relationship between quantitative variables was measured by independent t-test, ANOVA, or their non-parametric equivalents, and qualitative variables were measured by chi-square. A significance level of $p<0.05$ was used in all comparisons.

3- RESULTS

In total, 25 patients with an average age of 15.88±4.54 years were included in this study, among whom 60% (15 people) were male, and 40% (10 people) were female. The average age of the patients when Wilson's disease was diagnosed was 10.36±4.28 years with a median of 10 years (interquartile range 7.5 to 12 years). The frequency of clinical findings of the patients is tabulated in **Table 1**.

Table-1: Frequency of clinical presentations in patients with Wilson's disease

Clinical presentation	Number	Percent	Clinical presentation	Number	Percent
Jaundice	12	48	Pseudo parkinsonism	3	12
Hepatosplenomegaly	18	72	Depression	3	12
Ascites	11	44	Anxiety disorders	1	4
Vascular telangiectasia	2	8	Convulsion	3	12
Palmar erythema	1	4	Kayser–Fleischer ring in cornea	17	68
Evidence of bleeding disorders	3	2	Kidney presentations	2	8
Hepatic encephalopathy	1	4	Skeletal presentations	1	4
Leg edema	9	36	Cardiac presentations	0	0
Psychosis	0	0	-		

Regarding the results, hepatosplenomegaly, Kayser–Fleischer ring, jaundice, ascites, and lower limb edema were found in 72% (18 patients), 68% (17 patients), 48% (12 patients), 44% (11 patients), and 36% (9 patients), respectively. Psychosis and cardiovascular presentations were not reported in any of the patients. The results of the laboratory tests are demonstrated in **Table 2**. The findings indicated that among the 23 patients whose urine copper was measured, the 24-hour urine copper level was higher than 100 micrograms in 82.6% of them (19 patients). Moreover, in 90% of the patients, serum ceruloplasmin level was lower than 200 mg/liter (18 out of 20 patients whose ceruloplasmin level was measured). The amount of copper in the liver was calculated in only one patient, which was equal to 560 micrograms of copper per gram of dry liver.

The relationship between clinical and laboratory findings of the patients according to gender is shown in **Tables 3** and **4**, respectively. The findings indicated no significant relationship between the clinical and laboratory findings and the gender of the patients ($P<0.05$).

Table-2: Laboratory findings in patients with Wilson's disease

Laboratory finding	Mean ± SD	Median and quartile range	minimum	maximum

24-hours urine copper (microgram)	501/87 ± 638.43	280 (139-480)	56	2800
Serum ceruloplasmin (mg/L)	83.52 ± 79.69	51.5 (20.75-137.25)	0.09	2800
Alanine transaminase (U/L)	114.91 ± 131.13	77.5 (40.25-122.75)	20	639
Aspartat transaminase (U/L)	151.23 ± 160.42	97.5 (45.75-200.75)	27	655
Alkaline phosphatase (U/L)	465.78 ± 333.62	338 (213.5-730)	99	1082
Direct bilirubin (mg/dL)	1.61 ± 3.8	6 (0.2-1.5)	0.1	18.6
Indirect bilirubin (mg/dL)	16.29 ± 63.13	1.1 (3.7-6)	0.08	304
Prothrombin test (second)	18.47 ± 5.76	16.7 (13-23.9)	11.5	29
Prothrombin time test (second)	40.67 ± 9.25	40 (34.75-50)	24	64
INR	1.74 ± 0.84	1.5 (1-2.1)	1	3.9
Serum albumin (g/dL)	3.59 ± 1.02	3.4 (2.825-4.1)	2	6.5
Total serum protein (g/dL)	6.42 ± 1.076	6.55 (5.55-7.075)	4.4	8.4

Table-3: Frequency of clinical presentations in patients with Wilson's disease according to gender

Wilson's disease presentation		Female		Male		*P-value
		number	%	number	%	
Jaundice	No	6	60	7	46.7	0.68
	yes	4	40	8	53.3	
Hepatosplenomegaly	No	4	40	3	20	0.37
	yes	6	60	12	80	
Ascites	No	5	50	9	60	0.69
	yes	5	50	6	40	
Vascular telangiectasia	No	9	90	14	93.3	< 0.99
	yes	1	10	1	6.7	
Palmar erythema	No	9	90	15	100	0.4
	yes	1	10	0	0	
Evidence of bleeding disorders	No	7	70	15	100	0.052
	yes	3	30	0	0	
Hepatic encephalopathy	No	10	100	14	93.3	< 0.99
	yes	0	0	1	6.7	
Lower limb edema	No	4	40	12	80	0.08
	yes	6	60	3	20	
Convulsion	No	8	80	14	93.3	0.54
	yes	2	20	1	6.7	
Pseudo parkinsonism	No	9	90	13	86.7	< 0.99
	yes	1	10	2	13.3	
Depression	No	9	90	13	86.7	< 0.99
	yes	1	10	2	13.3	
Anxiety disorders	No	10	100	14	93.3	< 0.99
	yes	0	0	1	6.7	
Kayser–Fleischer ring in cornea	No	4	40	4	26.7	0.66
	yes	6	60	11	73.3	

*Chi-square test

Table-4: Laboratory findings in patients with Wilson's disease according to gender

Laboratory finding	female		male		P-value
	Mean or	SD or quartile	Mean or	SD or quartile	

	Median	range	Median	range	
24-hours urine copper (microgram)	280	221-730	262	105.25-475	0.51 **
Serum ceruloplasmin (mg/L)	90.62	86/89	78.79	78.59	0.75 *
Alanine transaminase (U/L)	128.5	33.26-186.75	73	40.25-110.5	0.29 **
Aspartat transaminase (U/L)	147	42.75-209.75	92.5	58-75.46	0.57 **
Alkaline phosphatase (U/L)	374.67	121.22	511.33	405.88	0.59 *
Direct bilirubin (mg/dL)	0.6	0.135-1.17	0.6	0.2-1.87	0.305 **
Indirect bilirubin (mg/dL)	1	0.495-3.35	1.1	0.55-4.12	0.82 **
Prothrombin test (second)	17.18	4.64	19.3	6.41	0.37 *
Prothrombin time test (second)	40.22	6.25	40.98	11	0.85 *
INR	1.65	0.77	1.79	0.91	0.7 *
Serum albumin (g/dL)	3.97	1.28	3.36	0.81	0.17 *
Total serum protein (g/dL)	6.95	0.99	6.11	1.03	0.08 *

* Independent T test

** mann-whitney U-test

The relationship between the age of the patients at the time of diagnosis and the clinical findings is illustrated in **Table 5**. Except for vascular telangiectasia, the results indicated no difference between the age of the patients and the clinical presentations (P>0.05). Nonetheless, in patients with vascular telangiectasia, the age of diagnosis was significantly lower than that in patients without this complication (P=0.04). The relationship between ceruloplasmin serum levels and 24-hour urinary copper of patients with clinical presentation is shown in **Table 6**. The findings demonstrated higher serum ceruloplasmin levels in patients with ascites (P=0.04) and lower limb edema (P=0.02) compared to patients without these clinical presentations. Moreover, compared to the others, a lower 24-hour urinary copper level was observed in patients with seizures (P=0.03) and depression (P=0.005). However, the 24-hour urine copper level was higher in patients with jaundice than in those without jaundice (P=0.01).

4- DISCUSSION

In sum, the results of the present study showed that the average age of the patients at the time of diagnosis was approximately ten years—the majority of patients presented with jaundice. The most common clinical presentation of the patients at the time of diagnosis was hepatosplenomegaly, observed in approximately three-quarters of the patients. Kayser–Fleischer ring, icterus, and ascites were reported in more than 40% of patients. The 24-hour urine copper level was above 100 micrograms in 82% of patients, and the serum ceruloplasmin level was below 200 mg/liter in 90%. There were no gender differences in the clinical and laboratory findings of the patients with Wilson's disease. The age of diagnosis in patients with vascular telangiectasia was lower than that in the other patients. Serum ceruloplasmin was higher in patients with ascites and lower

limb edema than in patients without these presentations, although it was expected that patients whose disease was more severe would have lower serum ceruloplasmin levels and liver failure signs such as early ascites and lower limb edema. The 24-hour urine copper level was higher in patients with jaundice than in the others. In comparison, this level was lower in patients with seizures and depression compared to patients without these clinical presentations.

In a study by Manolaki et al., who investigated the symptoms of 57 patients with Wilson's diagnosis, jaundice, epistaxis, and abdominal pain were reported in 24%, 27%, and 17% of patients, respectively. Moreover, leg edema, ascites, and Coombs-negative hemolytic anemia were observed in 12% of patients (13). However, in the present study, the frequency of ascites jaundice was reported in more than 40% of patients. Also, the frequency of lower limb edema was higher in the present study.

Table-5: Relationship between age at the time of diagnosis and clinical presentations in patients with Wilson's disease

Wilson's disease presentation		Mean	SD	P-value *
Jaundice	No	10.62	4.64	0.76
	yes	10.08	4.33	
Hepatosplenomegaly	No	11.57	3.30	0.38
	yes	9.89	4.60	
Ascites	no	10.21	4.00	0.85
	yes	10.55	4.80	
Vascular telangiectasia	no	10.87	4.00	0.04
	yes	4.50	3.53	
Palmar erythema	no	10.04	4.05	0.06
	yes	18.00	0.00	
Evidence of bleeding disorders	no	10.59	4.11	0.47
	yes	8.67	6.11	
Hepatic encephalopathy	no	10.38	4.37	0.93
	yes	10.00	-	
Lower limb edema	no	9.31	4.09	0.104
	yes	12.22	4.17	
Convulsion	no	10.50	4.49	0.66
	yes	9.33	2.51	
Pseudo parkinsonism	no	9.91	4.03	0.15
	yes	13.67	5.50	
Depression	no	10.50	4.49	0.66
	yes	9.33	2.51	
Anxiety disorders	no	10.29	4.35	0.704
	yes	12.00	-	
Kayser–Fleischer ring in cornea	no	10.50	5.45	0.16
	yes	10.29	3.80	

*independent T-test

Table-6: Relationship between serum ceruloplasmin levels, 24-hour copper, and clinical presentations in patients with Wilson's disease

Wilson's disease presentation		Serum ceruloplasmin (mg/L)		* P-value	24-hours urine copper (microgram)		** P-value
		Mean	SD		Median	quartile range	
Jaundice	No	80.96	97.29	0.89	221	95.5-295	0.01
	yes	86.09	63.37		480	225-1275	
Hepatosplenomegaly	No	47.67	64.72	0.32	158.5	88.5-606	0.15
	yes	92.49	82.65		289	191-638	
Ascites	no	54.87	65.85	0.04	232.5	96.5-381	0.053
	yes	126.51	83.87		300	251-1282	
Vascular telangiectasia	no	81.36	79.64	0.72	-	-	-
	yes	103	113.13		-	-	
Palmar erythema	no	80.39	80.88	0.46	-	-	-
	yes	143	-		-	-	
Evidence of bleeding disorders	no	89.44	84.47	0.44	280	115.75-702	0.89
	yes	50	40.85		280	220	
Hepatic encephalopathy	no	86.51	80.91	0.46	-	-	-
	yes	24.90	-		-	-	
Lower limb edema	no	54.66	61.07	0.02	280	97-367	0.29
	yes	137.12	87.34		380	175.5-934	
Convulsion	no	84.76	81.95	0.77	280	190-638	0.03
	yes	60.00	-		76	75	
Pseudo parkinsonism	no	91.13	80.66	0.21	280	144.25-717	0.404
	yes	15.04	21.14		220	69	
Depression	no	88.49	82.65	0.41	284	220-717	0.005
	yes	38.80	29.98		95	56	
Anxiety disorders	no	86.99	50.59	0.41	-	-	-
	yes	17.60	-		-	-	
Kayser–Fleischer ring in cornea	no	122.98	100.9	0.107	280	160-300	> 0.99
	yes	62.29	60.29		284.5	99.75-717	

* Independent T-test

** mann-whitney U-test

Additionally, similar to our study, the average age of the patients in Manolaki et al.'s study was low, the difference in the frequency of Wilson's manifestations in the two mentioned studies may be attributed to other demographic and genetic differences.

In the same line, a similar study on the under-18-year-old Iranian population by Dehghani et al. demonstrated that the most common symptoms of Wilson's disease were jaundice, abdominal distension, neurological presentations, and psychological disorder, which were reported in 40%, 15%, 17% and 13% of patients, respectively (14).

In the study by Choe et al., conducted in South Korea, ascites, liver cirrhosis, Parkinsonism, seizures, depression, and anxiety disorders were reported in 6.8%, 17.3%, 11.6%, 16.4%, 25%, and 30% of patients, respectively. Heart diseases and kidney disorders were reported in 13% and 4.4% of the patients, respectively. Moreover, 14% of the patients with Wilson's disease had tremors and dystonia. The findings of the present study demonstrated a higher frequency of ascites. Moreover, the prevalence of

pseudo-parkinsonism in the present study was 12%, which was lower than the prevalence of tremors and dystonia reported in the study by Choe et al. Furthermore, in our study, the rates of convulsions, depression, anxiety disorders, and heart diseases were lower than those in their study. These variations may be attributed to demographic differences, especially age differences. Naturally, more symptoms are generally observed in patients diagnosed at an older age. The emergence of more neurological and psychological manifestations in Choe's study, as compared to ours, can be explained in terms of its focus on the adult population. Evidence suggests that patients diagnosed in childhood usually have liver symptoms, and neurological symptoms are more commonly present in adults (15, 16). Also, the genetic differences between the populations of the two mentioned studies can partially justify the difference in observed symptoms.

In a study by Cheung et al., conducted on a Chinese population (both children and adults), liver involvement was reported in 89% of patients under 18 years of age. Cirrhosis and its complications, neurological presentations, and psychiatric symptoms were observed in 11%, 6%, 14%, and 9% of patients, respectively (17). Similarly, in the present study, most patients had hepatosplenomegaly and ascites, which may indicate liver involvement. Complications of cirrhosis, such as palmar erythema, bleeding disorders, and hepatic encephalopathy, had a prevalence between 4% and 12%, which is approximately similar to the results of Cheung's study. Neurological and psychiatric symptoms were similarly common in the two studies.

The study by Choe et al. showed that the prevalence of Wilson's disease was higher in males than in females (31). This finding was also observed in the present study, where 60% of the patients were male. The average age of disease onset in the present study was lower than that in the study mentioned above. This may be attributed to the difference in sample selection. The present study was conducted only in a pediatric clinic, and naturally, patients diagnosed in adulthood were not included. Choe et al.'s study showed that the age of diagnosis in male patients was lower than that in female patients. However, our findings did not confirm this. This may be due to the differences in the samples in the two mentioned studies. Based on the findings of some previous studies, the age of the presentation of the symptoms of the disease can vary between early childhood and the eighth decade of life (18, 19). Consistent with the findings of the present study, the study by Cheung et al., which was conducted on the Chinese population (both children and adults), showed that the detection rate of the disease is almost equal in males and females (17).

The presence of the Kayser–Fleischer ring and low serum ceruloplasmin is considered sufficient evidence for the diagnosis of Wilson's disease. However, this combination is not always present, and the diagnosis is controversial in some patients, especially young children (13). The presence of Kayser–Fleischer ring in patients with Wilson’s disease in different studies has been reported between 50% in patients with liver presentations and 98% in those with neurological symptoms (19-21).

In Manolaki et al.'s study, the Kayser–Fleischer ring was observed in 50% of patients aged eight years but only in 1 of 14 children younger than eight years (13). Other studies conducted on patients of different ages showed that Kayser–Fleischer ring and low serum ceruloplasmin were reported in 56% of patients (22, 23). However, this combination was rarely seen in young children. Therefore, a Kayser–Fleischer ring and low ceruloplasmin levels can

reliably identify almost half of the patients with Wilson's disease older than eight years. However, the present study's findings confirmed the presence of Kayser–Fleischer ring in the cornea of 68% of patients under 18 years of age, and no significant difference was observed in the incidence of Kayser–Fleischer ring in different age groups. The difference between the present study's findings and the study mentioned above may be due to genetic differences, which can alter clinical presentations. Generally, Kayser–Fleischer ring is more common in patients with neurological symptoms, and as mentioned, children are usually diagnosed with liver presentations. Therefore, the fact that the Kayser–Fleischer ring in children is less common is quite natural.

Another important indicator of this disease is the excretion of copper in the urine. Although this may occur in other liver diseases and is not present in some patients with Wilson's disease, the baseline measurement can provide useful diagnostic information. The usual diagnostic level for Wilson's disease is greater than 100 micrograms in symptomatic patients. The findings of a study by Lin et al., conducted over a period of 28 years, showed that 83.6% of the patients with Wilson's disease had a serum ceruloplasmin level of less than 200 mg/liter, and the 24-hour urine copper level in 88% was higher than 100 micrograms. Also, Kayser–Fleischer ring was observed in 93% of patients (24). Similarly, in the present study, abnormal levels of serum ceruloplasmin and 24-hour urinary copper were reported in more than 80% of the patients. However, only 68% of the study population detected the Kayser- Fleischer ring.

In the study of Manolaki et al., seven children had a low baseline 24-hour urinary copper excretion (13). Another study demonstrated that urinary copper excretion was less than 100 μg in 16% to 23% of patients (24). The diagnostic value of urinary copper excretion may improve after the penicillamine challenge, which is usually considered beneficial in children. In Manolakis study, the penicillamine challenge increased copper excretion to levels greater than 1,600 μg in 24 hours in 23 patients and facilitated the diagnosis in 13 patients who presented a combination of Kayser–Fleischer rings and low ceruloplasmin levels (13).

A liver biopsy may help assess the extent and severity of the liver involvement in Wilson's patients, and severe histological changes may occur in the early stages of the disease. If a liver biopsy is performed, copper should also be measured, besides histological examination. Measuring liver copper content is considered the gold standard and the most important diagnostic test in these patients. Genetic analysis is another important diagnostic method and may confirm the diagnosis in ambiguous cases. However, genetic testing is not always diagnostic due to the marked allelic heterogeneity reported in Wilson's disease. Genetic testing can be of great value in populations with only a few pathogenic mutations.

Several diagnostic criteria have been developed to overcome the difficulties involved in the diagnosis of Wilson's disease. By lowering the 24-hour urinary copper excretion threshold levels from 100 μg to 75 μg and from 1600 μg to 1000 μg after the penicillamine challenge, the sensitivity of the test for diagnosing Wilson's disease in children can be significantly increased. Ferenci et al. proposed a scoring system based on a combination of clinical symptoms, laboratory findings, and mutation assessment. This system is especially useful in patients with inconclusive standard diagnostic test results and may be useful in diagnosing Wilson's disease in children (25).

This study discussed the clinical presentation and laboratory findings of

Wilson's disease in children and highlights difficulties in diagnosing children, particularly in the pre-symptomatic stage. Since each test has its limitations, only a combination of clinical, biochemical, and genetic tests can assist in identifying most children with this disease. Generally, Wilson's disease diagnosis in children requires extensive investigations to achieve higher clarity and certainty.

4-1. Limitations, strengths, and weaknesses

The present study is the first to investigate the clinical and laboratory symptoms of a great number of patients with Wilson's disease in the east of the country and provided useful information to identify patients with Wilson's disease in the early stages. However, this study is not without limitations. The cross-sectional nature and lack of follow-up to investigate the changes in clinical presentations along with the increase in patients' age after treatment is among the study's limitations. Moreover, the single-centered and retrospective nature of the study makes it difficult to generalize the findings to other populations. Further studies are recommended to investigate the symptoms of patients with Wilson's at older ages. Moreover, conducting longitudinal studies to investigate the changes in the clinical symptoms of Wilson's patients over time is highly recommended.

5-ETHICAL CONSIDERATIONS

The present study was extracted from a PhD dissertation in medicine with the registration code of 990368, approved by the ethics committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1399.259).
The Declaration of Helsinki's ethical principles were followed in all stages of the study. To ensure the confidentiality of the information, the patients' information was confidentially coded and entered into the checklist.

6- FUNDING

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7- CONFLICTS OF INTEREST

None.

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