Wilson's Disease: Laboratory Evaluation of A Sample of Patients Attending Baghdad Teaching Hospital

Ahmed Kh. Mohammed*, Akram Ajeel Najeeb** ,
Wafaa Faeq Tawfeeq***

ABSTRACT:

BACKGROUND:

Wilson's disease (WD) is an inherited defect in copper metabolism that causes accumulation of copper in various body organs. It is a treatable; if it is diagnosed promptly and treated consistently.

OBJECTIVE:

Laboratory; hematological, biochemical, and diagnostic evaluation of a sample of Iraqi patients with WD, and study any association between clinical presentations and studied variables.

METHODS:

A case series study was conducted during 2011, from the 1st of February till the 10th of June. Sampling method was a convenient non-random one, through consecutive pooling of registered WD patients. A questionnaire-form paper had been developed for data collection, and required investigations were done in qualified laboratories.

RESULTS:

The study had enrolled 29 patients, with a male to female ratio of (1.07:1), The mean hemoglobin level was 10.64±2.53g/dl. Hemolysis was presented in 27.6% of patients, normochromic-normocytic RBCs in blood film were seen in 3.4% patient, platelet count was 243.97±89.35ccX10^9/L, WBC count was 5.70±2.84ccX10^9/L. ESR was 24.98±17.16mm/hr., serum bilirubin was 6.17±5.18mg/dl, serum cholesterol was 146.21±44.93mg/dl, serum triglycerides was 95.34±52.23mg/dl. Total serum protein was 72.86±11.15g/L, serum Albumin 39.17±7.88g/L and serum Globulin 31.07±7.89g/L. Penicillamine challenge test was positive in 96.6% patients. Serum ceruloplasmin level was 123.38±48.48mg/L, total serum copper was 72.48±25.11µg/dl, and 24h urinary copper excretion was 174.97±109.58µg/24hr.

CONCLUSION:

Most patients with WD are; anemic, with low serum levels of copper and cerulloplasmin, positive penicillamine challenge test, with abnormal liver function tests. With detected hemolysis in some of them; though no statistical difference or association was approved regarding laboratory values among patients with different presentations.

KEYWORDS: Wilson Disease , copper.

INTRODUCTION:

Wilson's disease is a rare autosomal-recessive disorder of copper metabolism. It is a hepatolenticular degeneration that characterized by the accumulation of copper in various body organs. It is fatal unless treated, it is a potentially treatable condition with the availability of effective pharmacologic therapy, while if not treated; it can cause severe brain damage, liver failure and death[1,2,3,4].

The prevalence of WD is about 1:30,000 and a frequency of heterozygotic carriers is about 1:90. WD resembles 6.9% of chronic liver diseases in Iraq[1,3,4,5,6,7,8].

The gene for WD is located on chromosome 13, mutations in this gene result in a production of a defective ATPase (ATP7B), a copper transport molecule, leading to impaired incorporation of copper into ceruloplasmin and copper retention; so in WD, intestinal copper absorption is normal but biliary excretion of copper is decreased, leading to excess copper accumulation, the later exerts its toxic effect by the generation of free radicals that result in lipid peroxidation [8,9,10].

The variation in the clinical presentation is tremendous. The five main categories of clinical presentation include hepatic, neurologic, psychiatric, hematologic, and ophthalmologic.

* Baghdad Teaching Hospital, Iraq.
**Baghdad Teaching Hospital, Medical City, Iraq.
***College of Health and Medical Technologies.

Symptoms may be vague and nonspecific, occasionally as a self-limited illness resembling acute hepatitis or resembling chronic liver disease. In hematologic presentation; patients present with hemolysis; so the constellation of young age, liver dysfunction and hemolysis should be assumed as WD until proved otherwise[1,5,11].

In WD; serum ceruloplasmin level is less than 200 mg/L in 95% of patients, the total serum copper is usually decreased (<70mg/dl"190-640µg/L",<11µmol/L"3-10"), and the level of daily urinary copper excretion of more than 100µg/d. (1.6mmol/d.); while on chelating therapy 24-hr. urinary copper excretion should be initially range between 200 and 500µg; such a value can also suggest that the patient is adherent to the drug. Penicillamine challenge test; which is the 24hr. urinary copper excretion stimulated by penicillamine administration; is usually have levels above 1600µg copper/24-hr. (25µmol/24-hr.) in WD patients[3,4,11].

The availability of effective treatment makes early diagnosis crucial. WD should be considered in any person younger than 30 years with liver disease. Nonalcoholic steatohepatitis, autoimmune hepatitis and viral hepatitis are the most frequently considered alternative diagnoses[2,11].

By eliminating copper-rich foods from their diet along with an effective lifelong treatment; a normal life-expectancy and liver function improvement can be offered within 6–12 months in most patients. Liver transplantation is curative as it corrects the metabolic defect[1,3,4,5,11].

So the objectives of this study is; Laboratory; hematological, biochemical, and diagnostic evaluation of a sample of Iraqi patients with WD, and study any association between clinical presentations and studied variables.

SUBJECTS AND METHODS:

This is a case series study with some analytic elements. The data was collected at the Department of Gastroenterology, Baghdad Teaching Hospital of Medical City, Baghdad, Iraq and its related authorized qualified teaching laboratories, in addition to toxicology center that related to study place. Arrangements for this study was approved by the local ethics committee and by the Iraqi MOH, Council of Arab Board of Medical Specialties and Baghdad Teaching Hospital/ Medical city. Data collection was extended from the 1st of February to the 10th of June 2011. The target population was all registered and newly diagnosed WD patients. The sampling method was a convenient non-random one, carried out through consecutive pooling of all WD patients. A data of 29 patients was collected by direct interviews, full clinical assessment, medical records analysis, and by doing some required laboratory investigations. Patients who accepted to participate in study, completed the required parameters and fit the inclusion criteria during the study period were included in study. The inclusion criteria were; history and clinical examination findings suggestive of WD as: hepatic manifestations, neuropsychiatric manifestations, family history of WD, serum ceruloplasmin level below 200 mg/L, presence of Kayser - Flischer ring, liver biopsy: if the liver copper concentration more than 250µg/g dry weight, serum copper level below 70 µg/dl "below 11µmol/L", 24-hr. urinary copper excretion more than 100µg/24-hr., and/or positive Penicillamine challenge test (if 24-hr. urinary Copper more than1600µg/24-hr., more than 25mmol/ L)[3,4]. The patient age should be fulfilling at least three of the above criteria and the last criterion is a definite diagnosis. These diagnostic standards had been proposed basing on ***Sternlieb's criteria***[11,12].

The exclusion criteria were; age above 60 years, evidence of coexisting liver diseases including; viral hepatitis A, B C or E, chronic liver disease with cholestatic component, Alpha one antitrypsin deficiency, Coomb's positive hemolysis, history of Alcohol intake, pregnancy, history of use of copper containing intrauterine devices (IUCD) or oral contraceptive[7], history of intake of medications that may cause extrapyramidal side effect such as antipsychotic drugs and Metoclopramide and/or history of chorea.

The Statistical Package for the Social Science (SPSS) version 17.0 software had been used for all computerized statistical analysis. Numerical; normally distributed variables was expressed as mean ± standard deviation; while categorical variables were expressed as frequency, range and percentage. Continuous variables were compared by ANOVA test for the variance analysis and categorical variables were compared by using Chi-square tests. P-value equal or less than 0.05 was considered as a statistically significant.

RESULTS:

In the current study; laboratory evaluation was based on several domains, including; hematological tests, bleeding studies, blood film

findings, biochemical tests, and toxicology tests.
The patients' total number was 29, males were 15 (51.7%) and females were 14 (48.3%), giving a male to female ratio of (1.07:1). Their mean age was 27.12±12.18 years(yr.). According to their main clinical presentation; patients had been classified into 4 clinical subgroups to facilitate studying them; hepatic in 8 (27.6%) of patients, neuro-psychiatric in 4 (13.8%), mixed hepato-neurologic presentation in 9 (31%), and other presentations in 8 (27.6%).
Regarding the laboratory hematological tests results and blood film findings of the study group; the mean hemoglobin level was 10.64±2.53 (5.3-14.1) g/dl, platelet count was 243.97±89.35 (115.0-451.0) cc $X10^9$/L and WBC count was 5.70±2.84 (2.3-12.0) cc $X10^9$/L. ESR level was 24.98±17.16 (1.5-64.0) mm/hr.. Evidence of hemolysis in blood film was found in 8 (27.6%) of patients and normochromic-normocytic RBCs in blood film were seen in only one (3.4%) patient. Regarding bleeding tests; PT was 16.76±4.66 (12.0-28.0) sec., PTT was 41.00±16.03 (20.0-80.0) sec. and INR was 1.39±0.28 (1.0-2.1) [Table (1)].
Table (2) shows that; the mean serum ceruloplasmin level was 123.38±48.48 (50.6-259.0) mg/L, the mean total serum copper was 72.48±25.11 (29.9-140.0) µg/dl, the mean basal 24h urinary copper excretion was 174.97±109.58 (61.0-480)µg/24hr., the mean 24h urinary copper excretion while patient was on treatment was 294.24±108.95 (128.0-497.0) µg/24hr..
Regarding liver function and other biochemical tests results; the mean total serum bilirubin was 6.17±5.18 (0.6-18.1) mg/dl, the mean serum AST (GOT) was 29.72±23.88 (5.0-83.0) IU/L, the mean serum ALT (GPT) was 31.24±24.33 (5.0-90.0) IU/L, the mean serum ALP was 106.52±82.19 (7.0-369.0) IU/L. The mean total serum cholesterol was 146.21±44.93 (65.0-235.0) mg/dl, the mean serum triglycerides level was 95.34±52.23 (45.0-245.0) mg/dl. Total serum protein was 72.86±11.15 (45.0-91.0) g/L, the mean serum Albumin 39.17±7.88 (20-51) g/L and the mean serum Globulin 31.07±7.89 (20.0-59.0) g/L. Penicillamine challenge test was positive in almost all 28 (96.6%) patients [Table (2)].
ANA, SMA, LKMI, Viral hepatitis markers, coomb's test, α1-antitrypsin and pregnancy test (blood test for female patients) were negative in all patients.
Table (3) and Table (4) show the laboratory hematological, biochemical tests results in different subtypes, though statistically there was no significant association between tests results and the main initial presentations (P value equal to or less than *0.05* is significant; while P value of more than *0.05* is not significant statistically).

DISCUSSION:

Comparing hematological tests results with another previous case series study (*Samiullah et al)* which was conducted on 24 patients at Hyderabad/Pakistan; showed similar results; hemoglobin level was 9.45±3.29g/dl, INR 1.34±.35[13].
Comparing serological and biochemical tests results in this study; which were within the diagnostic levels of WD; with other previous studies similar results of low serum ceruloplasmin, low total serum copper, and high urinary copper were reported;, like; (*Samiullah et al*) a descriptive series study was conducted by on 24 patients at Hyderabad/Pakistan[13], in (*Merle et al*) a retrospective cohort study by which was conducted in Heidelberg/Germany; low serum ceruloplasmin in 88.2% and high 24-hr. urinary copper in 87.1% of patients[14]. Another cohort study in Spain by (*Rodrigo Agudo et al*) was conducted on 29 patients; reported high transaminases levels in 50% of patients[15]. And other descriptive case series study (*Karim et al*) which was conducted on 32 WD children attending Pediatric Gastroenterology and Nutrition departments of B. Sh. Mujib Med. University (BSMMU)/Dhaka/ Bangladesh; low serum ceruloplasmin in 50% and elevated basal urinary copper in 100%[16]. In Italy (*Medici et al*) a prospective cohort study was conducted to follow up 35 WD patients at the Department of Gastroenterology of Padua University, a referral center for WD, it reported that ceruloplasmin levels were low in 87.5%. Urinary copper was 207±622µg/24-hr. and ALT was 126.6±100.89IU/L[17].
After classifying study's participants into four groups according to their main initial clinical presentations, the mean serum ceruloplasmin level was less than 200 mg/L in all four groups. The mean serum total copper level was the lowest among those with hepato-neurologic presentation and the highest among those with other presentations. Urinary copper excetion was high and diagnostic for WD in all presentations. Penicillamine challenge test was positive in all patients except a patient with a mixed hepato-

neurologic presentation. The mean values of liver functions tests results were abnormally high in all presentations except; neuro-psychiatric presentation. And hemolysis was the highest among those with other presentations (4 patients); a patient with pure and three with mixed hematologic presentations. Comparing these results with that of other previous studies: According to a retrospective cohort study by (*Merle et al*) was conducted in Germany; the mean values of serum ceruloplasmin, serum copper and urinary copper excretion levels were diagnostic for WD. However, there was no significant difference between patients with different presentations[14]. A cohort study (*Lowette et al*) which was conducted on 24 patients at Leuven university/Belgium; the mean serum ceruloplasmin (below 200mg/L) was seen in 80% of patients with neurologic symptoms and in 75% of the patients with liver disease. Urinary copper (> 100µg/24 hr.) was seen in 80% patients with neurologic symptoms and in all those with liver disease[18].

This can be explained by the fact that; results were within the diagnostic levels of WD; 95% of patients with WD have serum ceruloplasmin levels less than 200 mg/L, while serum copper level may be low, normal or increased but it usually (not always) decreased (<70µg/dl) in WD, however, it can be elevated in the setting of fulminant WD if ceruloplasmin level is reduced. The daily upper normal limit of urinary copper is 40µg/24hr. and the diagnostic level of WD is more than 100µg/24hr., this level represents the increase in circulating free copper, however; in about 20% of patients urinary copper excretion is between 40-100µg/24hr.. Penicillamine challenge test was negative in a patient, and this is because of the predictive value of this test in adults which is unknown. Liver function tests results were abnormal in all presentations except; neuro-psychiatric presentation; this may be due to fact that; patients included in this study were enrolled from the Department of Gastroenterology, so most of them presented with liver disease, except those with neuro-psychiatric presentation who were referred for management, so they reported normal results. In hepato-neurologic presentation hemolysis due to acute intravascular hemolysis is usually present in patients with hepatic involvement[3,4,5,11,12,19].

CONCLUSION:

Most of the patients with WD are; anemic, with abnormal liver function tests, low serum levels of total copper and cerulloplasmin, and positive penicillamine challenge test. With detected hemolysis in some of them; though no statistical difference or association was approved regarding laboratory values among patients with different clinical presentations..

Table 1: Laboratory hematological tests results and blood film findings of the study group (n=29).

Test result		Mean±SD	Range
Hb (g/dl)		10.64±2.53	5.3-14.1
Platelets count (cc X 10^9/L)		243.97±89.35	115.0-451.0
WBC (cc X 10^9/L)		5.70±2.84	2.3-12.0
ESR (mm/hr)		24.98±17.16	1.5-64.0
PT (sec.) (normal 11.5–14.5 sec.)		16.76±4.66	12.0-28.0
PTT (sec.) (normal 25–35 sec.)		41.00±16.03	20.0-80.0
INR		1.39±0.28	1.0-2.1
Blood film Finding		No	%
Hemolysis:Aniso-poikilocytosis, reticulocytes, fragmented and hypochromic-microcytic RBC	Positive	8	27.6
	Negative	21	72.4
Evidence of normochromic-normocytic RBC	Positive	1	3.4
	Negative	28	96.6
Total		29	100
SD; Standard Deviation, Hb: Hemoglobin level, WBC: White blood cell count, ESR; Erythrocyte sedimentation rate, PT; Prothrombin time, PTT; Partial throboplastin time, INR; International Neutralization Ratio, sec.; second, No; number, %; percent, RBC;Red Blood Cells.			

Table 2: Laboratory biochemical tests results of the study group (n=29).

Laboratory tests result		Mean±SD	Range
Serum Ceruloplasmin (mg/L)		123.38±48.48	50.6-259.0
Total serum copper (µg/dl)		72.48±25.11	29.9-140.0
Basal 24h urinary copper excretion (µg/24h)		174.97±109.58	61.0-480.0
24hr. urinary copper excretion during treatment (µg/24h)		294.24±108.95	128.0-497.0
Total serum bilirubin (mg/dl)		6.17±5.18	0.6-18.1
S. AST (GOT) (IU/L)		29.72±23.88	5.0- 83.0
S. ALT (GPT) (IU/L)		31.24±24.33	5.0-90.0
S. ALP (IU/L)		106.52±82.19	7.0-369.0
S.total cholesterol (TC) (mg/dl)		146.21±44.93	65.0-235.0
S. Triglycerides (mg/dl)		95.34±52.23	45.0-245.0
Total serum protein level(g/L)		72.86±11.15	45.0-91.0
S. Albumin(g/L)(g/L)		39.17±7.88	20.0-51.0
S. Globulin		31.07±7.89	20.0-59.0
		No	%
Pencillamine challenge test	Positive	28	96.6
	Negative	1	3.4
Total		29	100

SD; Standard Deviation,24h ; 24 hours, S.; serum, AST; Aspartatate transferase, ALT; Alanine transferase, ALP; Alkaline phosphatase, No; Number, %; Percent.

Table 3: Hematological, blood film and Pencillamine challenge test results among different clinical presentations (n=29).

The laboratory test	The main initial presentation								P value		
	Hepatic		Hepato-neurologic		Neuro-psychiatric		Other				
	Mean±SD		Mean±SD		Mean±SD		Mean±SD				
Hb (g/dl)	12.35±2.02		9.23±1.63		10.58±2.25		10.57±3.23		0.084		
Plt. count (ccX10^9/l)	248.13 ±44.48		231.11 ±91.23		293 ±134.12		229.75 ±104.4		0.680		
WBC (ccX0^9/l)	6.08±2.69		4.99±2.63		5.3±2.68		6.34±3.58		0.777		
ESR(mm/hr)	2.56±21.37		25.22±12.5		28.25±15.2		27.5±20.18		0.853		
PT(sec.)	16.25±4.33		17.0±4.97		19.75±5.68		15.5±4.31		0.524		
PTT(sec.)	41.88±12.03		41.78±19.65		50.75±21.87		34.38±11.45		0.427		
INR	1.28±0.29		1.44±0.31		1.5±0.27		1.39±0.27		0.548		
The test	Hepatic		Hepato-neurologic		Neuro-psychiatric		Other		Comparison of significance		
	No	%	No	%	No	%	No	%	Chi value	DF	P
Hemolysis in blood film	0	0	3	33.3	1	25.0	4	50.0	5.222	3	0.156
Positive pencillamine challenge test	8	100	8	88.9	4	100	8	100	2.302	3	0.512
Total	8	100	9	100	4	100	8	100			

No; number, %; percent, SD; standard deviation, Hb; Hemoglobin level, WBC; White blood cell count, ESR; Erythrocyte sedimentation rate, PT; Prothrombin time, PTT; Partial throboplastin time, INR; International Neutralization Ratio, sec.; second, RBC; Red Blood Cells, DF; degree of freedom, P; P value.

Note: P value equal or less than 0.05 is considered as a statistically significant; while P value of more than 0.05 is not significant.

Table 4: Laboratory biochemical tests results among patients with different clinical presentations (n=29).

The laboratory test	The main initial presentation				P
	Hepatic	Hepato-neurologic	Neuro-psychiatric	Other	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
S.ceruloplasmin (mg/L)	120.93±63.74	131.22±56.31	135.25±26.73	111.06±32.67	0.816
S.copper (µg/dl)	70.11±32.37	64.89±16.26	71.75±17.86	83.75±28.45	0.495
Basal u.copper(µg/24hr)	162.38±138.27	179.78±96.03	255.5±152.01	141.88±57.42	0.407
Urinary copper with treatment (µg/24hr)	334.88±110.99	282.78±83.94	185.00±87.9	321.13±118.74	0.121
Total serum bilirubin (mg/dl)	7.65±6.08	3.68±2.40	5.40±5.71	7.89±5.97	0.306
S.AST (IU/l)	38±29.72	31.78±23.16	15.75±9.64	26.13±22.87	0.480
S. ALT (IU/l)	45.88±31.9	28.89±14.48	17.25±11.32	26.25±25.77	0.202
S. ALP (IU/l)	155.13±120.48	89.89±57.03	50.75±30.26	104.50±59.66	0.170
S.cholesterol (mg/dl)	153.75±28.25	155.56±53.0	117.50±29.01	142.50±55.16	0.535
S.triglycerides (mg/dl)	91.00±45.94	98.44±59.87	88.25±50.55	99.75±59.38	0.978
Total serum protein	73.25±8.99	74.00±10.2	76.00±8.83	69.63±15.65	0.797
S. Albumin(g/l)	42.75±5.18	40.22±4.06	30.50±8.81	38.75±10.5	0.074
S. Globulin(g/l)	30.38±6.63	30.44±4.64	39.00±13.74	28.50±7.41	0.170
SD; standard deviation, P; P value, 24h; 24 hours, S.; serum, u.; urinary, AST; Aspartatate transferase, ALT; Alanine transferase, ALP; Alkaline phosphatase.					
Note: P value equal or less than 0.05 is considered as a statistically significant; while P value of more than 0.05 is not significant.					

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