Original Article

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Atomic Absorption Spectrometry in Wilson's Disease and Its Comparison with Other Laboratory Tests and Paraclinical Findings

Fatemeh Mahjoub*[1,2], MD; Rana Fereiduni[1,2], MD, Isa Jahanzad[1,2], MD; Fatemeh Farahmand[3,4], MD; Maryam Monajemzadeh[1,4], MD, and Mehri Najafi[3,4], MD

1. Department of Pathology, Tehran University of Medical Sciences, Tehran, Iran
2. Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Pediatrics, Tehran University of Medical Sciences, Tehran, Iran
4. Children's Medical Center, Pediatrics Center of Excellence, Tehran, Iran

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Abstract

Objective: Wilson's disease (WD) is an autosomal recessive disease with genetic abnormality on chromosome 13 causing defect in copper metabolism and increased copper concentration in liver, central nervous system and other organs, which causes different clinical manifestations. The aim of this study was to determine the sensitivity of different clinical and paraclinical tests for diagnosis of Wilson's disease.

Methods: Paraffin blocks of liver biopsy from 41 children suspicious of WD were collected. Hepatic copper concentrations were examined with atomic absorption spectrophotometry (Australian GBC, model: PAL 3000). Fifteen specimens had hepatic copper concentration (dry weight) more than 250µg/g. Clinical and laboratory data and histologic slides of liver biopsies of these 15 children were reviewed retrospectively. Liver tissue was examined for staging and grading of hepatic involvement and also stained with rubeonic acid method for copper.

Findings: Patients were 5-15 years old (mean age=9.3 years, standard deviation=2.6) with slight male predominance (9/15=60%). Five (33%) patients were 10 years old. Three (20%) of them were referred for icterus, 8 (54%) because of positive family history, 2 (13%) due to abdominal pain and 2 (13%) because of hepatosplenomegaly and ascites. Serum AST and ALT levels were elevated at the time of presentation in all patients. In liver biopsy, histological grade and stage was 0-8 and 0-6 respectively, 2 (13%) had cirrhosis, 1 (7%) had normal biopsy and 12 (80%) showed chronic hepatitis. Hepatic copper concentrations were between 250 and 1595 µg/g dry weight. The sensitivity of various tests were 85% for serum copper, 83% for serum ceruloplasmin, 53% for urinary copper excretion, 44% for presence of KF ring and 40% for rubeonic acid staining on liver biopsies.

Conclusion: None of the tests stated in the article were highly sensitive for diagnosis of WD, so we suggest that diagnosis should be based on combination of family history, physical examination and different tests.

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Key Words: Wilson's Disease; Atomic Absorption Spectrometry; Urinary Copper; Liver Biopsy

Introduction

Wilson's disease (WD) is an autosomal recessive disease with incidence of 1/30000 at birth [1]. The genetic abnormality is located on chromosome 13 involving one of the copper transporter proteins[2]. Defect in copper metabolism results in accumulation of copper in different organs, but

*** Corresponding Author;**
Address: Cancer Institute, Keshavarz Blvd, Tehran, Iran
E-mail: fmahjoub@sina.tums.ac.ir

definite pathogenesis of cell death is not known, free radical formation has been suggested [3]. Various different clinical manifestations have been reported with liver and central nervous system (CNS) being the most common sites involved [4,5]. The diagnostic tests include: eye examination for Kayser-Fleischer (KF) ring, measuring serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), serum ceruloplasmin, serum and urine copper concentration, liver histology with copper specific staining and liver copper concentration (dry tissue). The latter with a cut off more than 250µg/g is the gold standard for diagnosis [6].

The abundance of disease-specific mutations and their location at multiple sites across the genome had limited genetic testing to first-degree relatives of a patient when a specific mutation in the proband had been identified [8].

Accurate diagnosis can be made with high suspicion for the disease with combination of clinical and laboratory findings.

This is a disease of children and young adults. Early diagnosis and treatment is necessary because early medication can cause hepatic, ophthalmic and even neurologic manifestations to regress [9].

The aim of our study was to determine the sensitivity of different clinical tests (KF ring, AST, ALT, serum ceruloplasmin, urine copper concentration and liver histology with copper specific staining) in comparison with liver copper concentration (dry tissue) as gold standard for diagnosis of Wilson's disease.

Subjects and Methods

Paraffin blocks of liver biopsy from 41 children suspicious of WD during 1999-2008 were collected. The causes of clinical suspicion for WD were family history (35%), icterus (31%), elevated AST and ALT (5%), abdominal pain (12%) and hepatosplenomegaly (17%).

Hepatic copper concentrations were examined with atomic absorption spectrophotometry (AAS) (Australian GBC, model: PAL 3000). AAS is a procedure for quantitative determination of chemical elements employing the absorption of light by free atoms in the gaseous state. The electrons of atoms can be promoted to higher orbitals (excited state) by absorbing a defined wavelength of light. This wavelength is specific for a particular element. The wavelength for copper is 324 nanometer.

In our method, nitric acid and hydrogen peroxide were employed to digest liver tissue. Our AAS instrument uses electrothermal method (graphite tube) for atomizing. Then the absorbance of the atomized sample is calculated. The concentration of copper is proportional to the degree of absorbance. In 16 cases, liver tissue was inadequate for atomic absorption spectrometry.

Copper concentration (dry weight) of fifteen liver specimens exceeded 250µg/g. Clinical and laboratory data and histologic slides of liver biopsies of these 15 children were reviewed retrospectively. Clinical and laboratory data included serum copper and ceruloplasmin, urine (24hr) copper, AST, ALT and presence of KF ring in ophthalmologic examination.

Serum ceruloplasmin measurement methods are colorimetric, enzymatic and immuno-chemical[7]. In our center immuno-chemical method is used. For serum and urine copper concentration measurement several methods have been used such as colorimetric methods, flame spectrophotometry, atomic absorption spectro-metry, X-ray spectroscopy and neutron activation analysis [7]. In our center we use the colorimetric method.

In all patients, liver tissue was also stained with rubeonic acid method for copper. For grading and staging modified histological activity index (Modified HAI) was used [17].

The sensitivity of a test was calculated by dividing the number of the patients with positive results of that particular test by number of true patients (positive with gold standard AAS method).

Findings

Children were 5-15 years (mean=9.3 years, standard deviation=2.6) old with slight male predominance (9/15=60%). Five patients were 10 years old (33%). Three (20%) of the patients were

referred for icterus, 8 (54%) because of positive family history, 2 (13%) due to abdominal pain and 2 (13%) because of hepatosplenomegaly and ascites.

Serum AST and ALT levels were elevated at the time of presentation in all patients. In liver biopsy, histological grade and stage were between 0-8 and 0-6 respectively, 2 (13%) had cirrhosis, 1 (7%) had normal biopsy and 12 (80%) showed chronic hepatitis. Hepatic copper concentrations were between 250 and 1595 µg/g dry weight (Table 1).

The sensitivity of various tests were 85% for serum copper, 83% for serum ceruloplasmin, 53% for urinary copper excretion, 44% for presence of KF ring and 40% for rubeonic acid staining on liver biopsy (Table 2).

Table 1: Patient demographics and copper concentration by atomic absorption (in 25 out of 41 patients)

Number	Age	Sex	Liver copper
1	**12**	Male	561
2	**5**	Male	1198
3	**10**	Female	84
4	**9**	Female	-
5	**11**	Female	-
6	**5**	Male	105
7	**3**	Male	-
8	**7**	Male	59
9	**101**	Male	1323
10	**13**	Female	117
11	**9**	Male	250
12	**10**	Female	320
13	**7**	Male	166
14	**8**	Female	1476
15	**8**	Female	-
16	**10**	Female	-
17	**14**	Male	87
18	**7**	Male	716
19	**9**	Male	101
20	**9**	Female	-
21	**15**	Male	1333
22	**13**	Female	590
23	**9**	Male	-
24	**10**	Female	1595
25	**7**	Male	-
26	**12**	Male	-
27	**10**	Female	205
28	**9**	Male	116
29	**10**	Female	-
30	**6**	Female	1021
31	**10**	Female	-
32	**7**	Female	85
33	**4**	Male	-
34	**8**	Male	251
35	**7**	Female	389
36	**12**	Male	143
37	**10**	Male	980
38	**9**	Female	-
39	**9**	Female	172
40	**10**	Male	-
41	**10**	Male	343

Table 2: Sensitivity of various tests in our study

Test	Sensitivity (%)
Serum copper	85
Serum ceruloplasmin	81
Urine copper (24hrs)	58
KF ring	44
Copper staining (rubeonic acid stain) of liver tissue	40

Discussion

WD is an autosomal recessive disease of copper metabolism. The diagnosis is made by combination of clinical findings, laboratory results and family history. Gold standard for the diagnosis is hepatic copper content> 250µg/g dry weight[6].

Fragoso et al found slight male predominance (54%) with the most common manifestation of elevated aminotransferases and 100% sensitivity of urine copper excretion[10]. In our study male predominance (60%) was evident but the most common cause of referral was positive family history of WD (54%) and sensitivity of urine copper was only 53%.

In Merle et al series sensitivity of serum copper, ceruloplasmin, urinary copper and KF ring were 86%, 88%, 87% and 66% respectively[11].

In our study the sensitivity of above tests was 85%, 81%, 58% and 44%, respectively. So sensitivity of serum copper and ceruloplasmin seems to be near to that of Merle et al study. In Yuce et al study the sensitivity of serum ceruloplasmin, urinary copper, KF ring and Orcein staining of liver tissue was 82%, 100%, 63% and 88% respectively. So they suggested urinary copper for confirming diagnosis in patients with coagulopathy in whom liver biopsy cannot be done[12]. In our study the sensitivity of this test is 58% and is too low to be used as a confirmatory test[12]. Maybe it is due to genetic differences between patients or different method of measurement in different studies.

Serum ceruloplasmin level has been widely used to help in diagnosis of WD. In our study the sensitivity was 81% which is close to the results found in previous studies. It is normal in 16% of our patients. In previous studies some affected patients also had normal levels [13].

In the series of Ferenci et al [14] 42% and in the series of Yuce et al [13] 55% of patients had cirrhosis. In our study it was 13%. This difference may be due to earlier diagnosis in our patients.

KF ring is seen in 80% of patients with WD and in 98% of those with neurologic manifestations[16]. In our study it was seen in 44% of patients. We had no patient with neurologic manifestation.

Lecca et al in Italy showed that Timm's stain on liver tissue is more sensitive than rhodanine and orcein stains for demonstration of copper[16]. We used rubeonic acid on liver tissue for assessment of copper deposition. The sensitivity of this method was low (40%). Because of its time consuming property and low sensitivity we have omitted this test in evaluating WD in our center.

Our limitations were small number of cases and also inadequacy of tissue in some paraffin blocks for assessing with AAS method. Specifity of these tests should be evaluated with a group of non-affected individuals in future. In Asia few studies in Turkey have been done on Wilson disease. In Iran no study on determining the sensitivity of diagnostic tests of Wilson disease has been done till now.

No genetic studies were performed in our patients due to shortcomings of diagnosis of the disease with this method. Because WD is a disease of childhood and young adults and it will lead to severe complications without treatment, early diagnosis is essential.

Conclusion

None of the tests stated in the article were highly sensitive for diagnosis of WD, so we suggest that diagnosis should be based on combination of family history, physical examination and different laboratory tests.

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Conflict of Interest: None

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