Wilson's Disease in Thai Children between 2000 and 2012 at King Chulalongkorn Memorial Hospital

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Objective: *Wilson's disease (WD) is a rare autosomal recessive disorder characterized by copper accumulation. Clinical presentations are extraordinarily diverse, and currently no single diagnostic test can confirm WD with high accuracy. A complete understanding of the presentations and improved diagnostic methods are important for disease management. The authors' aimed to examine disease characteristics, management, and treatment outcome of WD in children, especially when genetic analysis and liver copper measurements were limited.*

Material and Method: *Data was collected from 21 WD children who were treated at King Chulalongkorn Memorial Hospital between 2000 and 2012. Inclusion criteria followed the WD scoring system, where other liver diseases are ruled out systematically.*

Results: *The mean age at diagnosis was 13.5±3.36 years, with 19 symptomatic patients, and two asymptomatic individuals who were diagnosed through family screening. Presentations varied, jaundice (52%), ascites (52%), edema (52%), Coombs-negative hemolytic anemia (14%), neurological abnormalities (33%), renal involvement (19%), and fulminant hepatic failure (5%). Based on the key parameters in WD scoring system, 14 patients (66%) had Kayser-Fleischer (KF) rings. Seventeen (89%) had low serum ceruloplasmin, and 20 (95%) had increased urinary copper excretion. These positive findings made WD scoring system accurately diagnose 66% of patients. Chelation therapy was the first line of therapy for all patients except one, who underwent liver transplantation. After therapy, liver function test returned to normal in all patients. However, neurological symptoms did not improve with combined drug therapy using chelating and neuropsychiatric agents.*

Conclusion: *WD in children mostly affected the liver. WD was suspected in seven patients (34%), thus needed further investigation. Therefore, long-term follow-up in those with suspected WD is the appropriate method for diagnosis and management in limited diagnostic tests. We suggest further treatment, and use of clinical response to treatment, as a criterion for confirming the WD diagnosis.*

Keywords: *Liver disease, Cirrhosis, Wilson's disease, Copper, ATP7B*

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Wilson's disease (WD) is a rare autosomal recessive disease that occurs in approximately 1 in 30,000 people[1]. It is characterized by decreased biliary copper excretion, defective binding of ceruloplasmin to copper, and copper accumulation in the liver and other vital organs such as the brain, cornea, and kidneys[2]. When pre-symptomatic, copper accumulation phase may present as subclinical hepatitis or cirrhosis. In the advanced stage, copper accumulation in the brain may cause neuropsychiatric symptoms. Hepatic symptoms can be highly variable but ultimately may result in fatal acute liver failure. WD is clinically manageable if detected early.

Accurate determination of WD relies on both clinical and laboratory results. However, lack of proper facilities and resources occur in many areas. In developing countries, measuring liver copper content is difficult to perform although it is among the best diagnostics available. Furthermore, genetic testing of potential carriers by sequencing the relatively large ATP7B gene is limited due to cost, and only done occasionally for basic research[3-5]. Early diagnosis and pharmacological therapy compliance are necessary for good treatment outcome, as the lifelong medication is required[6].

The present study aimed to examine clinical data of 21 pediatric WD patients, spanning 12 years, and evaluated the clinical symptoms, available laboratory tests, treatments, and outcome of these individuals in situations where genetic analysis and

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liver copper measurement are limited. We also postulated that clinical response after chelation for WD would be helpful for diagnosis.

Material and Method

This study was conducted at The King Chulalongkorn Memorial Hospital (KCMH), a tertiary care institution in Bangkok, Thailand. The medical records of 21 pediatric Thai patients diagnosed with WD between 2000 and 2012 were reviewed. In particular, clinical manifestations, physical findings, diagnostic criteria (as per the WD scoring system[7]), and clinical test results were reviewed. Clinical tests included slit lamp examination for Kayser-Fleischer (KF) rings, measurement of serum ceruloplasmin levels, and baseline 24-hour urinary copper excretion before and after D-penicillamine challenge test (PCT) (administration of 500 mg D-penicillamine at the beginning, and then 12 hours later). Liver biopsies were performed in some patients. The liver copper content was not measured in any patient. Genetic analyses were unavailable at the hospital, and therefore not performed. Follow-up details on the course of the disease were collected for each patient. WD diagnosis, based on the WD scoring system, includes the neurological symptoms of WD, presence of KF rings, evidence of Coombs-negative hemolytic anemia, urine copper excretion (>100 μg/day or >500 μg/day after PCT), low serum ceruloplasmin (<20 mg/dL), and the presence of rhodanine stain in liver pathology. Scoring of WD diagnosis ranges from 0 to 4, where 4 and above is definitive WD, 2 to 3 is likely to be WD and 0 to 1 is unlikely to be WD.

This present study was approved by the ethic committee of KCMH. Statistical analysis was performed with SPSS statistical software version 11. Results are shown as median and percentage.

Results

Among 21 pediatric patients diagnosed with WD, 11 (52%) were male from 18 families, and two families had history of consanguinity. Affected individuals were not clustered in any particular geographical location as WD patients came from various parts of Thailand. The demographic data and laboratory results are shown in Table 1.

Clinical characteristics

Nineteen patients were symptomatic for WD, while two additional patients were diagnosed through family screening. The mean age (±SD) at diagnosis was 13.5 (±3.36) years, and the median age was 15 years. The youngest patient was 7-year-old. The manifestations of the disease were as follows: jaundice, edema, ascites, acute Coombs-negative hemolytic anemia, neurological abnormalities, renal involvement and fulminant hepatic failure at the first presentation. In addition, KF rings were present in 14 patients, while hepatomegaly and splenomegaly were present in three patients. Fifteen and seven children presented with hepatic and neurological manifestations respectively. One patient had both systems' involvement (jaundice and fine tremor). Children with acute Coombs-negative hemolytic anemia presented with jaundice and anemia in the meantime. One patient developed fulminant hepatic failure after a few days of jaundice. Interestingly, two children presented with limb edema due to proteinuria from renal loss, with abnormal liver function test, which instigated a WD investigation.

Laboratory investigations

Serum ceruloplasmin concentration was low (<20 mg/dL) in 17 of 19 (89%). Urinary copper excretion exceeded 100 μg/24 hours in 20 of 21 (95%) patients. PCT was performed in two patients with low urine copper excretion (<100 μg/dL in 24 hours). Copper levels after PCT increased by 1.5 and 14.3 times respectively, reaching the level above 1,000 μg/24 hours in one patient. Other additional laboratory values are presented in Table 1.

Diagnosis

According to theWD scoring system, 66% (14/21) were highly likely to have WD, with a score between 4 and 7. Thirty-four percent (7/21) had a probable diagnosis, with the score of 3. Six of seven patients (85%) presented with neurological symptoms that fulfilled the WD criteria, while 5/15 (33%) patients presented with hepatic problems which required further investigations, such as liver biopsy for copper measurement or genetic analysis. Similarly, the American Association for the Study of Liver Diseases (AASLD) guidelines 2008, WD was diagnosed in only 60% of patients with hepatic symptoms, in contrast to nearly 100% of patients with neurological presentation. The diagnostic details are shown in Table 2.

Treatment and outcome

D-penicillamine and zinc were selected as the first line therapy for 20 patients. One patient, who presented with fulminant hepatic failure, underwent liver transplant. In two patients, D-penicillamine was

Table 1. Characteristics of children diagnosed Wilson disease at King Chulalongkorn Memorial Hospital (n = 21) [+]

Characteristics	Values
Age at diagnosis (year)	13.5 (±3.36)
Male/female	11/10
Clinical manifestation	
Jaundice	11 (52%)
Edema	11 (52%)
Ascites	11 (52%)
Coombs positive hemolytic anemia	3 (14%)
Neurological symptoms	7 (33%)
Renal problems (hematuria, proteinuria, glucosuria)	4 (19%)
Fulminant liver failure	1 (5%)
Hepatomegaly	3 (14%)
Splenomegaly	3 (14%)
KF rings	14 (66%)
Laboratory investigation	
Serum ceruloplasmin, mg/dL	6.02±5.45
Urine copper excretion, µg/24 hour	764.67±610
Urine copper excretion after PCT more than 5 times UNL, µg/24 hour	1/2 (50%)
ALP, IU/L	111.95±69.78
SGOT, IU/L	95.15±93.38
SGPT, IU/L	74.25±83.50
GGT, IU/L	84.33±33.29
Negative viral hepatitis profile	21 (100%)
Positive ANA*	0/11 (0%)
Prolonged prothrombin time	24.87±11.87
Histologic finding*	
Steatosis	2/4 (50%)
Chronic hepatitis	2/4 (50%)
Fibrosis	2/4 (50%)
Cirrhosis	1/4 (25%)
Rhodanine stain positive	1/3 (33%)
Orcein stain positive	1/2 (50%)

KF = Kayser-Fleischer rings; PCT = penicillin challenge test; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; WD = Wilson disease

[+] All patients enrolment, * Patients undergoing the investigation enrolment

Table 2. Diagnosticcriteria for WD (n = 21) [+]

Diagnostic criteria for WD	Values
Scoring system for WD	
Highly	14 (66%)
Probable	7 (34%)
Diagnosis of WD by AASLD guideline 2008*	
(1) Hepatic involvement	9/15 (60%)
Positive KF rings, low serum ceruloplasmin and high urine copper (24 hour measurement)	
(2) Neuropsychiatric involvement	6/7 (86%)
Positive KF rings, low serum ceruloplasmin and high urine copper (24 hour measurement)	

AASLD = The American Association for the Study of Liver Disease

[+] All patients enrolment, * Results were calculated with the total number of patients with hepatic or neurological involvement

discontinued due to adverse drug reaction (rash with angioedema in one, and anaphylaxis in another), which occurred within a month of treatment. Both of these patients subsequently received zinc therapy, as no alternative drugs (trientine, ammonium tetrathiomolyb date) were available at the time. One patient had a satisfactory outcome, while the other patient with poor drug compliance eventually developed neurological symptoms. All patients survived and liver function test results returned to normal after a mean period of 6.29±3.24 months. In patients with neurological abnormalities (fine tremor, dysarthria and abnormal movement), treatment response was unsatisfactory despite treating these symptoms directly. On the other hand, treatment in seven patients with probable WD resulted in no jaundice, limb edema, or ascites in cases with these presentations. Liver function returned to baseline after the promising treatment with D-penicillamine and zinc in all. In the case of patients that presented with both jaundice and fine tremor, although liver function returned to normal, however the neurological problem did not subside after the treatment.

Discussion

Most patients in our study received a diagnosis of WD due to suspicious clinical findings that correlated with the diagnostic WD scoring system[7]. The mean age (13.5 years) of diagnosis was older than the previous study[8], however, most of the patients (71%) presented with hepatic manifestation. Although KF ring is useful for WD diagnosis, it was absent in up to 50% of patients with hepatic WD and most asymptomatic siblings[9]. On the other hand, KF rings were positive in 85% (6/7) of the neurological group. As a result, using AASLD diagnostic guideline (2008) in this present study, only 60% of patients with hepatic symptoms could be diagnosed. Consequently, WD scoring system may be more suitable for application in younger Wilsonian diagnoses.

Conversely, due to lack of information on the liver copper content and the ATP7B genetic test in this present study, it is possible that the scores calculated in this study using WD scoring system are more conservative. This implies that young WD patients, or pre-symptomatic WD patients, need further specific investigations or other criteria for a more definitive diagnosis such as liver content measurement and genetic mutation analysis[6-8]. Since laboratory liver content measurement and genetic mutation analysis may not be possible in some countries, treatment may be started immediately, and a clinical response may be an additional criteria for diagnosis.

At the same time, additional clinical tests may be useful in ruling out other liver diseases that mimic WD such as autoimmune hepatitis[10]. The present study revealed 47% of WD patients were positive for anti-smooth muscle antibody. Thus, awareness of misdiagnosing WD as autoimmune hepatitis is also necessary.

Urinary copper in excretion has shown to be highly positive in many studies in pediatric WD patients. We found positive results in 20/21 (95%) of patients. In a negative 24-hour urine copper excretion, the diagnosis yield may increase after PCT, a technique mainly helpful in children[11]. The intravenous radio copper loading test is also a useful diagnostic tool, especially in undefined cases[6]. Unfortunately, the test is complex and not routinely available. One study stressed the presence of gall bladder disease (gallstone) in younger patients, without underlying hemolysis, to be useful for diagnosis[12].

In the case of pre-symptomatic patients, family screening for siblings or first-degree relatives may be helpful. Several studies[13-15] developed a screening test for urine copper in this particular group because KF rings are uncommon in young children, and laboratory measurement for liver content and genetic analysis may not be available. In the present study, only two patients had WD at the time of family screening. Both had abnormal liver function test but did not have KF rings. Therefore, diagnosis was determined based on the WD scoring system[7]. To improve patient diagnosis rate, the urine copper excretion threshold should be decreased[16].

Limitations of diagnostic methods in some areas, such as liver content measurement and genetic analysis, continue to hinder accurate and timely diagnosis of WD. Some patients go undiagnosed due to vague clinical and laboratory findings. However, in some cases where the diagnosis of a cryptogenic liver disease does not confirm WD, and no genetic analysis is available, treatment should proceed with the assumption of a positive diagnosis, especially if there is a positive response to treatment. Although consensus data and cost-effectiveness studies need to be performed, we recommend proactive treatment in suspected cases with severe liver manifestation.

Conclusion

In this study, children with WD mostly presented with hepatic manifestation, with some

neurological manifestations. Diagnosis of WD using the triad criteria (positive KF rings, low serum ceruloplasmin, and high urine copper excretion) or the WD scoring system successfully diagnosed 66 to 86% children, especially with neurological manifestations. However, WD in children younger than 7-year-old may go undiagnosed due to a lack of KF-ring and vague laboratory findings, especially if first-degree relatives screening yields a negative result. Therefore, long-term follow-ups and re-evaluations are necessary. Even though mutation analysis can be helpful if the results of investigations are dubious, genetic testing poses certain limitations, including a wide variety of mutations possible on the causative gene. We suggest treatment and the clinical response as a criterion for confirming WD.

What is already known on this topic?

Wilson's disease (WD) diagnosis is not easy because clinical presentations are extraordinarily diverse. Excess liver copper content and genetic analysis are the major keys for WD diagnosis.

What this study adds?

This present study is the first retrospective study of WD children in Thai single center. In cases of unfulfilled criteria for definitive WD diagnosis, we showed the diagnostic treatment is a useful tool for confirming the WD diagnosis.

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Potential conflicts of interest

None.

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โรควิลสันในเด็กไทยในโรงพยาบาลจุฬาลงกรณ์ระหว่างปี พ.ศ. 2543-2555

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วัตถุประสงค์: โรควิลสันเป็นโรคทางพันธุกรรมชนิดจีนด้อย อาการแสดงทางคลินิคค่อนข้างหลากหลายและการวินิจฉัยโรค จำเป็นต้องอาศัยการตรวจยืนยันหลายอย่างประกอบกัน ดังนั้น การเข้าใจลักษณะเฉพาะของผู้ป่วยประกอบกับการพัฒนาการตรวจวินิจฉัยจึงเป็นปัจจัยที่สำคัญจึงเป็นที่มาของการศึกษานี้ เพื่อศึกษาอาการ อาการแสดงตลอดจนการตรวจวินิจฉัยที่สำคัญ การดูแลรักษาและผลการรักษาของผู้ป่วยเด็กโรควิลสัน

วัสดุและวิธีการ: เป็นการศึกษาแบบย้อนหลังในผู้ป่วยเด็กโรควิลสันที่เข้ารับการรักษาที่โรงพยาบาลจุฬาลงกรณ์ทั้งสิ้น 21 ราย ในช่วงเดือนมกราคม พ.ศ. 2543 ถึง ธันวาคม พ.ศ. 2555

ผลการศึกษา: ผู้ป่วยทั้งหมด 21 รายมีอายุเฉลี่ย 13.5±3.36 ปี ผู้ป่วยที่มีอาการจำนวน 19 ราย และ 2 ราย ตรวจพบจากประวัติครอบครัวมีญาติเป็นโรควิลสันโดยผู้ป่วยไม่มีอาการและอาการแสดง สำหรับอาการและอาการแสดงที่พบ ได้แก่ ตัวเหลือง (ร้อยละ 52), น้ำในช่องท้อง (ร้อยละ 52), บวม (ร้อยละ 52), ภาวะซีดแบบ Coomb's test negative (ร้อยละ 14), อาการแสดงทางระบบประสาท (ร้อยละ 33), อาการแสดงทางไต (ร้อยละ 19) และภาวะตับวายเฉียบพลัน (ร้อยละ 5) พบว่าผู้ป่วยจำนวน 14 ราย ตรวจพบ Kayser-Fleischer's ring ที่ตาผู้ป่วย 17 ราย (ร้อยละ 89) มีค่า ceruloplasmin ต่ำและผู้ป่วย 20 ราย (ร้อยละ 95) มีการเพิ่มของการขับสารทองแดงออกทางปัสสาวะผู้ป่วยจำนวน 14 ราย (ร้อยละ 66) เข้าได้กับเกณฑ์การวินิจฉัยโรควิลสัน ในแง่ของการรักษาในผู้ป่วยเกือบทั้งหมดได้รับการรักษาโดยยาขับทองแดง พบว่าผลของการรักษาตอบสนองดีโดยเฉพาะการทำงานของตับกลับมาเป็นปกติทุกรายมีเพียง 1 ราย ที่ได้รับการรักษาโดยการปลูกถ่ายตับ อย่างไรก็ตามในผู้ป่วยที่มีอาการทางระบบประสาทร่วมด้วยการรักษาด้วยยาขับทองแดงและยารักษาอาการทางระบบประสาทร่วมไม่ทำให้อาการดีขึ้น

สรุป: ผู้ป่วยเด็กโรควิลสันส่วนใหญ่มีอาการทางตับผู้ป่วยจำนวน 7 ราย (34%) ต้องการการตรวจพิเศษเพิ่มเติมเพื่อยืนยันการวินิจฉัย เนื่องจากการตรวจปริมาณสารทองแดงและการตรวจหาจีนที่ผิดปกติมีข้อจำกัด ดังนั้นควรมีการติดตามผู้ป่วยเด็กที่สงสัยโรควิลสันในระยะยาวการให้การรักษาและติดตามผลของการรักษาในผู้ป่วยบางรายที่สงสัยโรควิลสันแต่มีข้อจำกัดดังกล่าวอาจพิจารณาเป็นรายไป