Wilson disease (WD) or hepato-lenticular degeneration is a rare autosomal recessive inborn error of copper metabolism that results from mutations of the \textit{ATP7b} gene on chromosome 13. The \textit{ATP7b} gene product, a P-type ATPase transporter, is responsible for incorporation of copper with apoceruloplasmin and its excretion into bile. Faulty P-type ATPase transporter leads to accumulation of free copper within hepatocytes, its slow release into the blood stream, and its accumulation in extra-hepatic tissues mainly the liver, brain, cornea, skin, joints, and kidneys.

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Wilson disease in 71 patients followed for over two decades in a tertiary center in Saudi Arabia: a retrospective review

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BACKGROUND AND OBJECTIVES: Wilson disease (WD) is a rare autosomal recessive disease. Our objective was to describe the diverse patterns, therapies, and outcomes of this disease.

DESIGN AND SETTING: A retrospective study over two decades on WD patients in a tertiary care center in Saudi Arabia.

PATIENTS AND METHODS: Clinical and laboratory findings of 71 patients with WD were retrieved from their charts, referral notes and our hospital electronic records and were analyzed.

RESULTS: The mean age and standard deviation was 16.8 (10.7) years and 56.5% were males. The main manifestations of WD were hepatic, neurological, and mixed in 39 (54.9%), 12 (16.9%), and 20 (28.2%) patients, respectively, and 11 (15.5%) were asymptomatic cases detected by family screening. A family history of WD was positive in 41 (57.7%) patients, and consanguinity of parents was found in 26 (36.6%) patients. The mean (SD) follow-up period was 92.2 (72.9) (range, 1-320) months. Ten (14.1%) patients died during follow up, while 45 (63.4%) and 16 (22.5%) were still on or lost from follow-up, respectively. The mean (SD) age at the end of follow-up was 25.3 (12) (range, 4-62) years. Hepatoma was discovered in 5 (7.0%) patients. Penicillamine therapy was used by 58 (81.7%) patients, while zinc and trientine were given to 32 (45.1%) and 11 (15.5%) patients, respectively. Sixteen (22.5%) patients underwent liver transplantation and one died (1.4%) on the waiting list. The liver condition remained stable or improved in 35 (49.3%), and the neurological status showed improvement in 11 (34.4%) of the 32 patients who had neurological involvement.

CONCLUSIONS: This is the biggest cohort to be reported from the Middle East. WD presentation and outcome of WD are very diverse, and its diagnosis still depends on clinical, laboratory, and radiological evidence of abnormal copper metabolism. WD should be considered in patients of any age with obscure hepatic and/or neurological abnormalities.

WD may present at any age and was described in every region of the world. Its incidence was estimated at 1 in 30 000 in countries like the United States, Germany, and Japan. However, WD incidence is more common (1 in 3 000 to 10 000) in communities where consanguineous marriages are prevalent such as the Druze, Yemenite, Iranian Jews, and Palestinians. Confirming the diagnosis of WD is usually straightforward late in the course of the disease if both the major clinical and laboratory features are present, namely, typical hepatic and/or neurological findings, Kayser-Fleischer (KF) rings, low serum levels of ceruloplasmin, and increased
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original article

urinary copper excretion. However, KF rings are often absent in patients who present with liver disease alone. In addition, ceruloplasmin levels may be normal, or even low together with high urinary copper excretion in patients with fulminant hepatic failure secondary to WD. Moreover, asymptomatic siblings of patients with WD, in whom the diagnosis needs to be confirmed or ruled out, may also present with diagnostic uncertainties.

A review revealed only a few studies, of small sample size, on WD in Saudi Arabia, where consanguineous marriages exceed 50%. Little is known about the long-term follow-up outcomes of patients with WD in our geographic locality especially with current advances in the diagnosis and management of liver disease including liver transplantation. Therefore, this study presents the experience of a tertiary care center in Saudi Arabia, with 71 patients with WD for over two decades illustrating the pattern of disease presentation, diagnosis, therapies, and outcomes.

PATIENTS AND METHODS

The medical records of 71 consecutive patients diagnosed as WD at a tertiary care center in Saudi Arabia between January 1987 and June 2008 were retrospectively reviewed. The center is a major tertiary care center in the central region of Saudi Arabia that receives referrals of patients with neurological and hepatic disorders of unknown etiology for advanced care. Liver transplantation began at this institution in 1996 for patients without KF rings or those with normal serum ceruloplasmin, confirmation of the diagnosis of WD was based mainly on: 1) high hepatic copper content (>250 µg/g dry weight of liver), 2) exclusion of other causes of chronic liver disease, 3) magnetic resonance findings in the basal ganglia compatible with WD, and 4) clinical and biochemical response to medical therapy. Liver function tests and other routine laboratory data were performed using standard methods. Serum ceruloplasmin was measured by immunoprecipitation, or oxidase activity. Liver copper concentration in dried liver tissue was measured by flame atomic absorption spectroscopy in a center in the United States.

Data were collected in a preformatted data collection form before being transferred to a Microsoft Excel Sheet, and underwent descriptive and analytical statistics using SPSS; version 15 (IBM Corp, Armonk, New York, United States). Data are presented as means (SD) or number (percentage) as appropriate. The means of continuous variables were compared using the t tests, non-parametric tests (Wilcoxon and Mann Whitney), or one-way analysis of variance (ANOVA) with posthoc test (Tukey) as appropriate. A Kaplan-Meier curve with the log rank test were used to test cumulative survival. A P value of <.05 was considered statistically significant.

RESULTS

The epidemiological features of the 71 patients with WD are shown in Table 1. Most were Saudis (93%). The other 5 patients were Yemenis (n=3) and Syrians (n=2). Regional distribution and the occupation at the time of presentation of the 71 cases are shown in Figures 1 and 2. As expected, most (n=26, 36.6%) were from the central region of Saudi Arabia. A positive family history of WD was found for in 41 (57.7%) patients, and consanguinity of parents was reported in 26 (36.6%) patients. Definite nonconsanguinity was reported by 29 (40.8%) patients, and in the remaining 16 (22.5%), parental consanguinity was not documented.
Table 1. Patient epidemiological characteristics (n=71).

| Variable                  | Results          |
|---------------------------|------------------|
| Age at onset (years)      |                  |
| Mean (SD)                 | 16.8 (10.7)      |
| Median (range)            | 15 (2-49)        |
| Age at end of follow up   |                  |
| Mean (SD)                 | 25.3 (12)        |
| Median (range)            | 23 (4-62)        |
| Male gender               | 40 (56.3)        |
| Body mass index           |                  |
| Mean (SD)                 | 20.9 (6.1)       |
| Median (range)            | 19 (12-42) kg/m |
| Saudi nationals           | 66 (93)          |
| Disease pattern, n (%)    |                  |
| Hepatic                   | 39 (54.9)        |
| Hepatic Hepatic and Neurological | 20 (28.2)    |
| Neurological              | 12 (16.9)        |
| Family history            | 41 (57.7)        |
| Consanguinity             | 26 (36.6)        |

Data expressed as number (percent) or mean (standard deviation) unless otherwise indicated.

Symptomatic disease at the time of presentation was the case in 60 (84.5%) patients, while 11 (15.5%) were asymptomatic subjects detected by family screening. The main manifestations of WD were hepatic, neurological, and mixed in 39 (54.9%), 12 (16.9%), and 20 (28.2%) patients respectively. Clinical findings at the time of presentation are shown in Table 2. The clinical, laboratory and pathological findings of the patients presented with hepatic WD and hepatic with neurological affection (n=59; 83.1%) are described in Table 3.

KF rings were absent, questionable, and present in 32 (45.1%), 3 (4.2%), and 36 (50.7%) patients, respectively. KF rings were detected at a significantly higher rate in patients who had neurological and mixed pictures; 9/12 (75.0%) and 13/20 (65.0%), respectively, compared to those with hepatic disease alone; 11/39 (28.2%), P<.01 for both (Figure 3). Liver biopsy in 37 patients found fibrosis stage from stage 2 to 4 (significant fibrosis) in 32 (86.5%) biopsies. Median copper dry weight in the liver biopsy was 298 µg. An abnormal level of dry copper in the liver tissue was seen in 20 (90.9%) of 22 biopsy specimen analyzed for dry copper.

Table 4 shows data on follow up, medical therapy and mortality. Hepatoma was discovered in 5 (7%) patients. Only 45 (63.4%) patients were still on regular follow-up, and 16 (22.5%) were lost from follow-up in our institution, 8 of whom had hepatic disease, 4 had neurological, and 4 had a mixed picture at presentation. These cases were residents of the Central (n=3), Eastern (n=5), Western (n=3), Northern (n=1), and
Southern (n=2) regions, and the remaining 2 cases were non-Saudis.

D-penicillamine therapy was the most commonly used medical therapy. Sixteen (22.5%) patients underwent liver transplantation; 15 (93.8%) were still alive in a good condition at the last follow up. The liver remained stable or improved in 35 (59.3%) of the 59 pa-

Table 3. Characteristics of patients with hepatic and hepatic with neurological Wilson disease (n=59).

| Variable | Result |
|----------|--------|
| Gender | |
| Male | 32 (54.2) |
| Female | 27 (45.8) |
| Presentation | |
| Screening | 10 (17.5) |
| FHF | 4 (6.8) |
| Chronic liver disease | 45 (75.7) |
| Anti-nuclear antibody (n=44) | |
| Positive | 15 (34.1) |
| Anti-smooth muscle antibody (n=37) | |
| Positive | 23 (62.2) |
| Liver kidney microsomal antibody (n=22) | |
| Positive | 1 (4.5) |
| Antimitochondrial antibody (n=26) | |
| Positive | 0 (0.0) |
| Hepatitis B surface antigen (n=55) | |
| Positive | 2 (3.6) |
| Hepatitis C antibody (n=55) | |
| Positive | 0 (0.0) |
| A-1 antitrypsin (n=22) | |
| Deficient | 0 (0.0) |
| Liver Biopsy (n=37) | |
| Fibrosis stage 2-4 | 32 (88.5) |
| Copper quantitation | 22 (59.5) |
| Level of liver copper | 20 (90.9)* |
| Liver transplantation, n(%) | 16 (27.1) |
| Mortality | |
| Yes | 10 (16.9) |
| No | 45 (63.4) |
| Lost-to follow up | 16 (22.5) |
| Brain magnetic resonance imaging (n=34) | |
| Abnormal | 24 (70.6) |

*90.9% of those whom liver copper was quantifies.

Data expressed as number (percent)
Table 4. Follow up, therapy, mortality of all patients (n=71).

| Variable                  | Result                  |
|---------------------------|-------------------------|
| Follow up (months)        |                         |
| Mean (SD)                 | 92.2 (72.9)             |
| Median (range)            | 96 (1-320)              |
| Medical therapy*          |                         |
| Penicillamine             | 58 (81.7)               |
| Trientin                  | 11 (15.5)               |
| Zinc                      | 32 (45.1)               |
| Liver transplantation     | 16 (22.5)               |
| Hepatoma                  | 5 (7)                   |
| Mortality (still alive), n(%) | 45 (63.4)         |
| Died during follow up     | 10 (14.1)               |

*Some patients were on more than one drug. Data expressed as number (percent). Or mean (standard deviation) unless otherwise indicated.

tients with hepatic involvement, while neurological disease showed improvement only in 11 (34.4%) of the 32 patients who had neurological involvement (P<.001).

Ten (14.1%) patients died after a median follow-up period of 64 (range, 1-229) months. The main causes of death were Castleman disease (n=1), hepatoma (n=2), post-transplant complications (n=1), and liver failure in 7 cases. Liver failure was associated with septicemia in 1, brain abscess and subarachnoid hemorrhage in 1, and cardiopulmonary complications in 2 cases. Seven of the deceased cases had hepatic disease and 3 had a mixed hepatic and neurological picture. All patients died pre-transplant apart from one who died 6 months post-liver transplant. Prognosis and mortality of 55 patients (excluded 16 cases who were lost to follow up) is shown as a Kaplan-Meier graph in Figure 4. None of the patients with pure neurological WD died; 5 patients each from hepatic and mixed (hepatic with neurological WD) died during follow up. Cumulative on-treatment survival of the whole cohort at 5, 10, and 20 years was 92.1%, 86.5%, and 57.0% respectively.

**DISCUSSION**

To our knowledge, this is the biggest cohort of patients with WD reported from the Middle East, and the second worldwide after the German study that describes 163 patients. The second biggest cohort from the Middle East was 56 cases reported from the National Guard Hospital (NGH), another tertiary care centre in Riyadh, Saudi Arabia. This necessitates the establishment of a Saudi National Registry of metabolic diseases, including WD, to be able to obtain more accurate nationwide epidemiological, genetic, and disease-related data.

When added to the cases reported by the NGH in our city, our data has shown that WD is a rather common disease in Saudi Arabia. This is probably related to the high rate of consanguineous marriages among the Saudi population. Indeed, the rate of consanguineous marriages in the present series was 36.6% (26/71), which is higher than that in the general Saudi population, despite the fact that consanguinity was clearly documented in the files of only 55 patients.

WD presentation and outcomes are very diverse. Diagnosis is easily made once patients present with low serum ceruloplasmin, KF rings with evidence of liver disease, with or without neuropsychiatric illness. The clinical presentation in our patients was similar to that described in both Saudi patients and in other ethnic groups and confirms the variable pattern of this disease, and the difficulty in its diagnosis especially at its earlier stages and in patients with atypical presentation.

Similar to any autosomal recessive disorder, it is important to screen family members and siblings of cases with WD not only for early detection that helps
in better long-term outcome, but also to prevent or avoid marriage to other undetected cases or carriers in the same or other families. In our cohort, 41 (57.7%) patients had a positive family history of WD, and 11 (15.5%) of our cases were asymptomatic and initially diagnosed by screening. Many of those family members who had WD were either not alive or on follow up at other hospitals.

Our patients did not undergo haplotype analysis to identify any specific mutations that may help in establishing the diagnosis of WD. If available such testing might have been helpful, particularly in determining the disease status in patients who were asymptomatic siblings of index cases and in those who did not have KF rings. It is possible that without haplotype analysis, clinicians may still miss asymptomatic WD cases with normal laboratory findings. We do not believe, however, that the risk of routine liver biopsy of siblings is justified if there is no clinical or biochemical evidence of WD. A recent genetic analysis in 56 Saudi patients with WD showed disease causing mutations in three exons (exons 8, 19 and 21) of the ATP7B gene in 28 patients (50%). Such mutations were not detected in 60 control Saudi subjects. Interestingly, mutations in exons 21 (18 cases) and 19 (one case) were reported to be unique for Saudis. These data need to be confirmed by another larger study.

The results of medical therapy for patients who presented in fulminant hepatic failure was rather disappointing as none of our patients with FHF survived while liver transplantation was being awaited. This (100%) mortality rate of patients with FHF in WD warrants an early consideration of liver transplantation and early referral to a transplant center. Sepsis was a leading cause of mortality in all patients.

HCC is a rare complication of WD, published mainly as case reports. This lead to the impression that copper and its receptors has a protective role against hepatic carcinogenesis. However, Long-Evans cinnamon (LEC) rats with partial deletion of the copper transporting ATPase gene homologous to the WD gene were found to develop HCC despite the presence of excess copper in their livers. Moreover, copper-chelating agents were found to inhibit the development of liver tumors in the same rat model. The incidence of HCC in patients with WD was attributed to the shortened life expectancy of untreated cases. The development of HCC in 5 (7%) of our cohort suggests that this complication is not uncommon in patients with WD. It was also argued that the HCC may develop in WD patients secondary to associated hepatitis B or C virus (HBV, HCV) infection. However, none of our patients had positive HCV and only 2 were positive for HBV; none of them developed HCC.

Patients with chronic or fulminant liver failure without urgent transplantation have a dismal prognosis. Many recent reports have demonstrated the beneficial role of liver transplantation in patients with predominantly hepatic WD. In our cohort, 16 patients underwent liver transplantation, and 15 (93.8%) of them were still alive and on regular follow up, a better outcome compared to medical treatment in one aspect, and in another aspect when compared to transplantation for other chronic liver disease. On-treatment survival of patients with WD has improved. Only 10 patients died in our cohort within 20 years from their initial diagnosis. When including patients lost to follow up (n=16), a cumulative 20-year survival of around 60% can be achieved.

The retrospective nature of any study is an inherent limitation. However, this is not the case in rare metabolic diseases such as WD. Obviously, prospective observational or interventional studies in WD are not feasible unless a worldwide study involving numerous high referral centers is organized. Also, our study still provides more information about disease presentation and outcomes in our locality, and provides the base for future genetic studies.

In conclusion, the diagnosis of WD still depends on clinical, laboratory, and radiological evidence of abnormal copper metabolism with no single test sufficient to make the diagnosis. WD should be considered in patients of any age with obscure hepatic and/or neurological abnormalities. Both a high index of suspicion and family screening are essential for early diagnosis and hence early effective therapy. Long-term treatment of patients with D-penicillamine/trientine along with dietary copper restriction is effective in resolving clinical and biochemical markers of the disease and improves prognosis and possibly survival. Patients who meet current criteria for liver transplantation should be transplanted especially if they present as fulminant liver failure, and if they deteriorate while on medical treatment. Further study on the genetic mutations that occurred in the patients who are still on follow-up as well as the impact of consanguinity is warranted. Moreover, a nationwide registry for metabolic liver disease including WD needs to be established.
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