Author's response to reviews

Title: Phenotypic and Genetic Characterization of a Cohort of Pediatric Wilson Disease Patients

Authors:

Tawhida Y Abdel Ghaffar (tyghaffar@gmail.com)
Solaf M Elsayed (elsayed683@yahoo.com)
Suzan Elnaghy (suzan_elnaghi@yahoo.com)
Ahmed Shadeed (Ahshadeed@hotmail.com)
Ezzat S Elsobky (elsobkyezzat@hotmail.com)
Hartmut Schmidt (hepar@ukmuenster.de)

Version: 2 Date: 7 April 2011

Author's response to reviews: see over
Dear Editors,

Please find enclosed the revised manuscript titled: “Phenotypic and Genetic Characterization of a Cohort of Pediatric Wilson Disease Patients”. We highly appreciate the constructive criticism and thank the reviewers for their helpful advice. Please find our ‘point-by-point’ responses to the reviewers’ comments below.

**Reviewer 1: Michael L. Schilsky:**

1. Minor essential revision: The authors should cite the work by Kalra V, Khurana D, Mittal R. Indian Pediatr. 2000 Jun;37(6):595-601 - Wilson's disease—early onset and lessons from a pediatric cohort in India. This cohort also had very early onset disease and a high percentage of young patients with KF rings.

   The reference was quoted (reference 17).

2. Discretionary point: For such early onset and frequency of KF rings, the authors should potentially stress environmental exposure as an exacerbating factor.

   A mention of the potential effect of environmental exposure to copper on the high frequency of KF rings was added in the discussion section.

3. Minor essential revision: With such a high incidence of non adherence to treatment yet such a regular routine monitoring schedule, did the authors note elevations in liver tests preceeding worsening disease as previously reported by Arnon et al? Could the patients that suffered from the non adherence been recognized earlier?
In this cohort, 13 patients were non compliant to treatment ≥ 50% of time, 6 of them were lost for follow up for periods of over one year. When they came back to us, they had both deterioration of their clinical condition and their liver enzymes.

For the rest of the instances, in order to give you an accurate reply, we need more time to analyze the data of the patients over the follow up period which was as long as 17 years in some of them.

4. Major point: The authors treated some patients with once daily zinc along with penicillamine. There is no basis for recommending only once daily zinc sulfate for treatment, even with a chelating agent. Data from Brewer has previously shown that at least twice daily treatment is needed. The authors should state why they chose this once daily treatment option and present evidence of its utility or state that it is uncertain in discussion.

We used once daily treatment with zinc only in patients receiving twice daily D-PCA (maintenance therapy). Our theory was that probably we could decrease the absorption of dietary copper and promote metallothioneine production thus maintain a negative copper balance during the day by using a midday zinc dose. Definitely using a twice daily dose is of evidenced benefit but increasing the number of doses per day would have compromised the compliance in a cohort where compliance is a major problem. We are uncertain that this regimen was of value for the patients as we used it in all our patients and so we do not have a comparative group. We want to draw your attention that in patients in whom zinc was used as a monotherapy three daily doses were given.
Reviewer 2: Anna Czlonkowska

Interesting is a high rate of consanguinity but authors did not give the definition of consanguinity.

Consanguineous marriage refer to marriage of couples or union of individuals related to each other as second cousins or closer.

Why authors are stating that disease is under diagnosed in Egypt. There is no information how many should be. They collected in 17 years 77 pediatric cases. The diagnosis is done only one year after symptoms onset which is not bad.

Because we are a tertiary center with a special interest in WD, we can do earlier diagnosis as stated in the paper. There are no reports on the prevalence of WD in Egyptian population but we think that the carrier frequency should be high as we previously reported in Abdelghaffar et al., "that several patients with non-consanguineous parents had homozygous mutations and two patients with consanguineous parents had heterozygous mutations" (Abdelghaffar TY, Elsayed SM, Elsobky E, Bochow B, Büttner J, Schmidt H: Mutational analysis of ATP7B gene in Egyptian children with Wilson disease: 12 novel mutations. *J Hum Genet* 2008,53: 681-687).

There are 4 different modes of therapy, so difficult to follow. Why they used D-PCA and zinc in those same persons.

We used once daily treatment with zinc only in patients receiving twice daily D-PCA (maintenance therapy). Our theory was that probably we could decrease the absorption of dietary copper and promote metallothionine production thus maintain a negative copper balance during the day by using a midday zinc dose. Definitely using a twice daily dose is of evidenced benefit
but increasing the number of doses per day would have compromised the compliance in a cohort where compliance is a major problem. We are uncertain that this regimen was of value for the patients as we used it in all our patients and so we do not have a comparative group. We want to draw your attention that in patients in whom zinc was used as a monotherapy three daily doses were given. Combination therapy was used by other groups as well (Dhawan et al, )

In that case measuring of copper in urine does not give information about effectiveness of treatment.

Measurement of copper in urine actually measures the effectiveness of treatment and compliance whether the patient is given D-PCA or zinc as recommended by “Brewer GJ and Askari FK. Wilson’s disease: clinical management and therapy. Journal of Hepatology 42 (2005) S13–S21”

How they could use urine copper as indicator of good treatment if at start they had data about copper level only from 25 cases.

Although we did measure copper in only 25 patients at diagnosis, repeated serial measurements during follow up visits (at least yearly) was done for all patients. Progressive decrease in urinary copper excretion was taken as one amongst other indicators of compliance as recommended by “Roberts EA, Schilsky ML, American Association for Study of Liver Diseases (AASLD): Diagnosis and treatment of Wilson disease: an update. Hepatology 2008, 47: 2089-2111”
I cannot understand many statements as “Of the 22 asymptomatic patients who were followed up, 19 had either a stable or an improving course”… What has improved in asymptomatic cases? Although these patients were asymptomatic, they either had elevation of liver enzymes (ALT and/or AST) or hepatomegaly as indicated in table (3). Improvement indicated a decrease or normalization of their liver enzymes.

Interesting is one year old baby, how it was diagnosed?

The one year old child was a sister of one of our WD patients. Close clinical and biochemical follow up showed elevation of liver enzymes and molecular testing confirmed the diagnosis.

Reviewer 3: George J Brewer:

1. (Major compulsory): Third paragraph of Methods: Using 2 of the 6 criteria for diagnosis is unacceptable, and I suspect the authors didn't actually do that. For example a heterozygote could have a positive family history and 20% of them have a low ceruloplasmin. Please correct to your actual criteria. These criteria were used only for patients who presented with neurological or hepatic disease as mentioned in the text. This was not used for asymptomatic patients. A heterozygous with low ceruloplasmin and positive family history should not have a clinical or biochemical evidence of liver or neurological disease.
Third part of Results: It says 69.2% positive KF rings, while the abstract says 97.6%. Please correct.

Corrected: 97.6% of the symptomatic patients while the 69.2% is of all studied patients (which included 27 asymptomatic patients, of whom only 4 had KF rings)

2. (Major essential) 5th par of Background: Zinc sulfate is mentioned as a treatment. Zinc acetate is used much more commonly around the world and is the FDA approved salt in the US as Galzin and is also approved in Europe as Wilzin. This should be pointed out. Zinc acetate is not available in Egypt and so we had to use zinc sulphate. In Egypt it is available as a pharmaceutical preparation.

The” unavailability of Zn acetate” has been added to text

Third part of Methods: A stain for copper is not very reliable for Wilson's diagnosis. Copper assay is the reliable method. The authors probably didn't have assay available, but they should clarify this difference.

Measurement of copper in dry liver is not available in Egypt (added to text)

3. (Discretionary) 2nd par of Treatment: A single daily dose of zinc is ineffective for copper control yet the authors used such a dose. Please explain why.
We used once daily treatment with zinc only in patients receiving twice daily D-PCA (maintenance therapy). Our theory was that probably we could decrease the absorption of dietary copper and promote metallothionein production thus maintain a negative copper balance during the day by using a midday zinc dose. Definitely using a twice daily dose is of evidenced benefit but increasing the number of doses per day would have compromised the compliance in a cohort where compliance is a major problem. We are uncertain that this regimen was of value for the patients as we used it in all our patients and so we do not have a comparative group. We want to draw your attention that in patients in whom zinc was used as a monotherapy three daily doses were given.

The authors referencing is sometimes inexplicable. For example ref 14 in 2nd par of Treatment Outcome doesn't seem to relate to the text. Similarly ref 7 in 5th par of Background is not a good ref for the topic. Much better would be Number XV of that series (Brewer et al J Lab Clin Med 132:264-278,1998).

Corrected.

Editorial request:

a) Ethics: Please name the specific body that gave approval for your study.

Done
b) Sample: we notice that you have included two adults in your cohort, as the rest of the sample is composed of children, please consider removing them, or please justify why you wish to retain them in your sample.

Both adult patients (sibs) were cousins of one of our children with WD and both presented to the pediatrics Hepatology clinic. Removal of these 2 patients will necessitate a change in the statistics which would be difficult to do given a limited time for the corrections.

Sincerely yours,

Tawhida Y. Abdel Ghaffar, Solaf M. Elsayed, Suzan Elnaghy, Ahmed Shedeed, Ezzat S. Elsobky, Hartmut Schmidt