January 7, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 723-review.doc).

Title: *Gastrointestinal side effects in children with Wilson´s disease treated with zinc sulphate.*

Author: *Anna Wiernicka, Wojciech Jańczyk, Maciej Dądalski, Yesim Avsar, Hartmut Schmidt, Piotr Socha*
Name of Journal: *World Journal of Gastroenterology*

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The format of the manuscript was updated and the content has been improved according to the suggestions of reviewers:

Reviewer 1

1. Word cohort is improperly used as it implies a large # of subjects of minimum 500 subjects. The study is restricted to 53 patients referred to as cohort then to 9 patients in abdelghaffar study as cohort.

Response: The term *cohort is nt being used in tis revised manuscript anymore.*

2. The study presumably has been approved by IRB . this needs to be mentioned under section on material and methods

Response: The study has been approved by the local ethic committee.

批注 **[WU1]:** Mamy zgodę? Widziałam że z Harmutem rozmawiałeś Piotrze na ten temat.

3. It was not clearly stated in any of the tables or under results what was the clinical presentations of the various patients: liver, neurological or mixed.

Response: Only patients with liver presentation have been presented in our study. Appropriate changes have been done in the manuscript, section material and methods.

4. Several studies have correlated specific homozygous mutations to specific liver presentations. It would be nice to add to the table the mutations identified in these subjects. Was there any correlation between the mutation and the side effect of the treatment in these patients? Was there any correlation with age? Though number of patients is small, what % of subjects under age of 10 or >10 yr had side effects?

Response: Baseline clinical and biochemical characteristics were similar in those who presented with gastrointestinal symptoms and those being free of symptoms. We added information about mutation analysis to the Table 1, Column 5. Correlation between the mutations and side effect have been described in the result and discussion sections and presented in Graphs/Tables (# 3. and # 4.). From all 53 investigated patients 21 were under the age of 10 and only 8 patients < 10yr had side effects. Most patients (62%) with side effects were > 10yr. This information has been added to the manuscript, section results.

批注 **[U2]:** Numbering of tables is wrong.

5. Table 1. Row 1 there is a typo, put rash instead of rush .there is no need for the column "Cause of conversion to zinc sulphate" that column may be disregarded. In addition the word no symptom under the column age of onset may be changed to asymptomatic. Were these

patients diagnosed by genetic screening or suspected because of positive family history this should be clarified

Response: Typo has been corrected. The column "cause of conversion to zinc sulphate" has been deleted. We changed *"no symptoms"* to *"asymptomatic"*and added additional information about asymptomatic patients in Table 1, column 3.

6. Endoscopic evaluations & images were for patients after receiving treatment, was there any variation/ulceration before treatment? How would the authors rule out this possibility? Barada et al have correlated homozygous mutations with early liver changes even in asymptomatic patients (<10 years old); there were liver changes in WD that varied between increased transaminasiss to ascites and liver cirrhosis .: Clin Gastroeneterology 2010, 44: 432-439 and JIMD Reports DOI 10.1007/8904_2011_91

批注 [WU3]: Piotrze nie wiem jak się odnieść do tego artykułu, co recenzent chciałby od nas?

Response: Prior to treatment patients did not have any clinical signs, nor symptoms, suggesting gastric disease. Although an interesting question, ethical concers prevented us from performing EDGs on asymptomatic children. However, in the discussion part we mention the lack of controlled endoscopic findings before and during zinc treatment.

Reviewer 2

Comments:
Introduction

1. In the line 6: It should be mentioned that liver transplantation is used in "acute liver failure" (instead of ...for severe cases...).

Response: Many thanks for improving this expression. Appropriate changes have been done in the manuscript.

2. In the 3rd paragraph, line 3: The number for citation by "Wiggelinkhuizen" should be given.

Materials and methods

Response: We included the citation in tis version. .

3. The method of Helicobacter pylori investigation should be given. If samples for histology were taken, the histology findings of mucosa could be of importance.

Response: H. pylori was tested during endoscopy by using the urea test assay. Histopathology of biopses showed mild or moderate lymphocyte infiltrations which are found in gastritis of any other origin. Moreover, recent papers indicate lack of correlation between histopathology and endoscopic findings [1]. Appropriate changes have been done in the manuscript in result and discussion sections.

4. The authors should give data regarding the abuse of another drugs than for the treatment of WD. Was the abuse of non-steroidal anti-inflammatory drugs excluded?

Response: All patients/parents were interviewed and asked for any additional therapy including non-steroid anti-inflammatory drugs. None of these children received non-steroid anti-inflammatory drugs. This is now appropriately stated in the result section.

批注 [WU4]: Piotrze, nie uwzględniłam tego w manuscriptcie.

Results

5. Line 2: It should be mentioned that the mean age 10 yrs is for the whole group of patients (probably – nor 2.5, neither 17 yrs age for diagnosis is not presented in any patient in Table 1).

[1] McKenna BJ, Appelman HD. Primer: histopathology for the clinician- how to interpret biopsy information for gastritis. Nat Clin Pract Gastroenterol Hepatol. 2006 Mar;3(3):165-71.

Response: Appropriate changes have been done in the manuscript in results section.

批注 [U5]: Please describe in more detail.

6. The authors mentioned that GIT symptoms resolved after proton pump inhibitors. How long was PPI used by these patients? Did the symptoms recurred or not after stopping PPI? Or, was the treatment by PPI continuous? Did the authors observed some adverse events of PPI treatment?

Response:

Seven patients complaint about persistent abdominal pain (Table 2). Four of those were treated with PPIs for six weeks. The patients remained symptomatic despite PPI treatment and needed to be switched from zinc sulphate to D-penicillamine. The other three patients experienced relief of symptoms with PPIs. One was treated for 3 months (patient #1)), another patient for 6 weeks (patient #2), for the third patient there is lack of data about timing. Recurrence of symptoms after discontinuation of PPIs or adverse effects while on PPIs were not observed. Appropriate changes have been done in the manuscript in result section.

An additional patient, that was suffering from GERD (Table 1- patient # 6) experienced relief of symptoms with a periodical use of PPIs during our observation.

7. In the section "Results" the authors describe the criteria for non-compliance. How many patients in the whole group fulfilled these criteria and what was the treatment regimen for these patients?

Response: Many pediatric patients do not take drugs, especially patient's with Wilson's disease. In many cases specialist psychological help in necessary. This problem is the subject of other our study.

批注 [WU6]: Nie wiem czy dobrze zrozumiałam pytanie ☺

8. The diagnosis of WD was confirmed by genetic testing in most patients. Was there any relationship between the genotype and frequency of zinc side effects?

Response: Correlation between the mutations and side effect have been described in the result and discussion sections and presented in Graphs/ Tables (# 3. and # 4.).

9. The data regarding the form of WD are not given (i.e. hepatic or neurologic form). Was there any relationship between the phenotypic manifestation of WD and side effect frequency?

Response: Only patients with liver presentation have been presented in our study. This data have been added to the material and method section.

10. Some patients with WD have liver cirrhosis even in childhood. It is known that liver cirrhosis is an independent risk factor for ulcer disease. Could the authors give the data regarding the presence of cirrhosis in examined patients?

Response: According to what we described in the discussion section zinc sulphate is the first line therapy in asymptomatic patients, in those with mild hepatic disease and as a maintenance therapy for patients, whose hepatic symptoms improved under D-penicillamine. In our study 50/53 cases zinc sulphate was used as first choice therapy, only in 3/53 cases treatment was initiated with d-penicillamine and then switched to zinc sulphate. Only one patient had features of liver failure, his therapy was initiated with D-penicillaminie and then changed to zinc sulphate. During maintenance therapy with zinc he didn't have any side effects.

11. In Table 1 in the 5th column the dosage of zinc in mg should be given.

Response: Appropriate changes have been done in Table 1.

Discussion

12. In discussion the author should try to explain the mechanism of mucosal changes in patients on zinc treatment. It could be also interesting to know, why the changes were exclusively in stomach and no in duodenum.

Response: Zinc sulphate may have a damaging effect on the intact mucous membrane, being capable of causing gastric erosion and haemorrhage. Zinc chloride is a very powerful caustic and is

likely to be formed by the action of gastric hydrochloric acid on zinc sulphate. The mechanism of mucosal changes has been described in discussion section.

References

13. Ref No.11: the diagnostic score of WD is usually attributed to Ferenci publication in Liver International (Ferenci P, Caca K, Loudianos G, et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003; 23:139-142).

Response: Appropriate changes have been done.

14. Ref 21: The names of authors are not cited correctly.

Response: Appropriate changes have been done.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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