Reviewer's report

Title: Diffuse duodenal nodular lymphoid hyperplasia: A large cohort of patients etiologically related to helicobacter pylori infection.

Version: 3 Date: 4 March 2011

Reviewer: vatsala misra

Reviewer's report:

Thanks for sending the manuscript by Khuroo et al for re review. I regret the delay in review process due to difficulty in downloading the mainfile of the manuscript. For the concern expressed by the authors, I would like to state that any comment by the reviewers are for the modification and improvement of the manuscript so that all the aspects become more clear to the unbiased reader and the manuscript may suite to the stature of the journal. But, in present case instead of understanding the questions and answering and incorporating the changes in the manuscript, authors have felt offended and showed concern for the knowledge of the reviewer and tried to prove that every query is irrelevant. It should not be encouraged.

I'm again sending the point wise comment for many answers and how those details would have helped in improving the manuscript.

Q 2 Which classification was used to classify gastritis and duodenitis on histology?

Please give method of doing RUT.

Ans: Both these have been addressed in Material and methods. We have used Modified Sydney classification for grading of gastritis and H pylori infection. Relevant references are included. (For RUT, 2 forceps punch biopsies were taken from gastric incisura and embedded in agar gel urea-rich medium (HP test™, Allied Marketing Corporation, Kolkata, India) and read as per manufacturer’s instructions. Multiple gastric biopsies (two from antrum; two from body and additional specimens from any visible endoscopic visible lesions, if needed) were taken and stained with Hematoxylin & Eosin to type and grade gastritis; Alcian blue to detect intestinal metaplasia and Giemsa stain for H. pylori detection and density.25,26)

My comments –

At no place in the material and methods they have mentioned the name of Sydney System, neither the reference for it has been quoted. The reference for Sydney system is by Mesiwicz J J et al,1990. This system describes 4-5 parameters Viz.Inflammation, atrophy, activity, intestinal metaplasia and H,Pylori
Separately. Lymphoid aggregates and Lymphoid proliferation if present should also be mentioned. It does not take superficial or full thickness in account but the area involved like antrum predominant, corpus predominant or Pan gastritis. By the terminology used by them it appears they have classified according to whitehead classification.

Q3. It appears to be quite unusual that despite 100% biopsies showing presence of H. pylori (heavy in 34 patients), only 2 patients had MALToma and rest others had no evidence of lymphoid hyperplasia in stomach but all the cases had nodules in the duodenum.

Ans: The reviewer is mixing up the entity of low grade MALT lymphoma and Mucosa Associated lymphoid tissue. Low grade MALT lymphoma is a malignant disease of stomach related to H. pylori infection and potentially reversible disease after H. pylori eradication as seen in 2 of our patients. Low grade MALT lymphoma can evolve into high grade gastric lymphoma with local and distant spread. Mucosa Associated Lymphoid Tissue (MALT) is a very common association of H. pylori gastritis. In fact most of patients with H. pylori gastritis (in our study as well) have lymphoid follicles in the gastric mucosa and this is a hallmark of gastritis caused by H. pylori.

My comments:

I'm not at all mixing the two entities. Lymphoid follicles (MALT) are not seen in normal gastric mucosa. (Owen et al, 1986 and Genta et al human Pathology 1993), and commonly seen with the H.pylori infection. When the histology of the gastric biopsies was described in detail they should have mentioned about the lymphoid proliferation which may vary from Chronic infiltrate to few lymphoid aggregatess to well-formed lymphoid follicles without any lymphoepithelial lesions. I just wanted to give them the no (%) of biopsies showing the L. aggregate/L. follicles out of 38 (If they had observed it), as the study is mainly concerned with the lymphoid proliferation and correlation with duodenal changes would have helped in explaining the pathogenesis. In a scientific paper, we need to have exact numbers for any measurable parameter and terms like ‘most’, ‘sparse’ and ‘infrequent’ should not be used.

Q4. How many duodenal biopsies had evidence of gastric metaplasia and Evidence of H.pylori?

Ans: This has been dealt with in the Results section. In fact duodenal mucosal changes have been dealt with in detail in this section. H. pylori was rarely seen in The section of duodenal biopsies and was very sparse.

My comments:
In result section they have not mentioned about the areas of gastric metaplasia with the description of duodenal histology. These could have been easily identified in the sections stained with the PAS stain (which they have done according to the material and method). H. pylori is highly selective for its microenvironment and is not seen in duodenal mucosa except in areas of gastric metaplasia. They are not even seen in the areas of atrophy and intestinal mucosa in gastric mucosa (very rarely reported in low density). Authors are reporting them in duodenal mucosa without metaplasia and 100% positivity for H. pylori in gastric mucosa in moderate and high density despite 14 cases showing chronic atrophic gastritis with intestinal metaplasia (?). Here also I just wanted them to give the no(%) of duodenal biopsies showing gastric metaplasia and how many of them had H. pylori that would have helped them in establishing the H. pylori as etiological agent for DDNLH which they are trying to prove and publish.

Q5. How the H. pylori present in the stomach led to nodular lymphoid hyperplasia in the duodenum without producing any change in the gastric mucosa – please explain.
Ans: H. pylori can cause lot of extragastric manifestations such as Idiopathic thrombocytopenic purpura (ITP), iron deficiency anemia etc. The reviewers comments that there were no changes in the stomach is incorrect. Refer to endoscopic and histologic findings of gastric mucosa in results section which reads as follows:<Examination of stomach showed no endoscopic abnormality in 8 patients; linear erythematous antral gastritis in 20 patients; exudative fundic gastritis in 6 patients and atrophic gastritis in 4 patients. Two patients had diffuse ulcerative nodular lesions limited to the antrum. None of the patient had pyloric or duodenal ulcer. RUT was positive in all patients and H. pylori were seen in gastric biopsies in all patients. Density of H. pylori was moderate in 6 patients and heavy in 34 patients. Histology of gastric biopsies revealed chronic superficial gastritis in 24 patients, and chronic atrophic gastritis with intestinal metaplasia in 14 patients. Two patients with diffuse ulcerative nodular disease of antrum showed histologic features of low grade MALT lymphoma>.

My comments:
If they would have noted the lymphoid hyperplasia in the gastric biopsies and correlated it with the corresponding duodenal biopsies, they could have easily explained it as follows ‘though the lymphoid hyperplasia was present in both the gastric and duodenal biopsies the immune response was more marked in the duodenum leading to focal oedema (as can be seen from the picture in lowpower) and nodularity”

Q6. Diagnosis of H. pylori was made by RUT and histology but after treatment
Only Breath test was used
It seems that reviewer is not aware of diagnostic protocol of H. pylori infection. Prior to antibiotic therapy H. pylori infection uniformly affects antrum (not patchy) and grows up to body, and fundus. In such a situation endoscopic forceps biopsies are very useful for diagnosis of H. pylori (histology and RUT). Both tests need to be done for diagnosis and we have meticulously followed the protocol for taking correct site and number of biopsies. However, after antibiotic therapy H. pylori infection persists (in resistant cases) in small pockets and is scattered as small islands of infection in gastric epithelium. Thus forceps biopsies cannot be used to diagnose H. pylori eradication. In such a situation we need a global test for H. pylori eradication and these are either 14C-UBT or fecal H. pylori antigen. In this study we have employed 14C-UBT to check for H. pylori eradication.

Q7. How can you compare the three different methods for showing eradication. A pre and post Urea breath test should have been done to show the eradication. Ans: It seems that reviewer is not aware of diagnostic protocol of H. pylori infection. Prior to antibiotic therapy H. pylori infection affects antrum and grows up to body, and fundus. In such a situation endoscopic forceps biopsies are very useful for diagnosis of H. pylori (histology and RUT). Both tests need to be done for diagnosis and we have meticulously followed the protocol for taking correct site and number of biopsies. However, after antibiotic therapy H. pylori infection persists (in resistant cases) in small pockets and islands in gastric epithelium. Thus forceps biopsies cannot be used to diagnose H. pylori eradication. In such a situation we need a global test for H. pylori eradication and these are either 14C-UBT or fecal H. pylori antigen. In this study we have employed 14C-UBT to check for H. pylori eradication. The reviewers contention of comparing diagnostic tests for H.pylori infection and eradication is grossly misconceived as she needs to review proper protocol for diagnosis of H.pylori infection and for checking eradication of H pylori infection.

My comments:
I'm very well aware of the diagnostic protocol for diagnosis and showing eradication while treating an individual patient. In that case 14CUBT is done post therapy to show eradication and avoid unnecessary endoscopy and biopsy, where chances of finding the organism are less even in expert hands but while doing the scientific projects, sometimes you have to deviate or add some extra tests to existing protocol to prove your hypothesis by comparing pre and post
treatment findings by statistical methods,(as you have done in the present study. Do you routinely perform all these tests on a patient coming for UGI endoscopy including IHC for T and B lymphocyte or serological estimation of immunoglobulin?)

Therefore adding either pre-treatment 14CUBT or post treatment biopsy examination (patients already had upper G.I. endoscopy) along with comparison of results and demonstration of disappearance of lymphoid hyperplasia along with disappearance of nodularity would have further helped in proving your hypothesis and supporting the manuscript that DDNLH is associated with H.pylori.

Q8. Discussion –
Authors try to state that the nodules in the duodenum were probably due to immune stimulation secondary to heavy H.pylori infection. If it was so, then how the stomach, small intestine and colon were spared

Ans: Pathogenesis of H. pylori induced nodular lymphoid hyperplasia of the duodenum need further studies. In fact selective involvement of the duodenum is characteristic and pathognomonic of this entity. This can only be answered by doing studies similar to those done on H. pylori induced ITP. This we are convinced will define the cause of selective involvement of the duodenum.

My comments:
Lymphoid proliferation was seen in both stomach and duodenum but the response was more exaggerated in duodenum which was associated with other changes like oedema leading to nodularity. Besides due to lesser thickness of duodenal mucosa as compared to gastric mucosa, even single large I.follicle lead to nodularity,(as seen in figure).

Q9. References – Most of the references are quite old. some new references should be added.
Ans: We have added 2 references of the reviewer in the list.

Q10. Photographs
Histology photograph is not good. Getting a single lymphoid follicle is not unusual. A scanner or low magnification (10x) showing multiple large follicles should be given
Ans: Reviewer is confusing this entity with nodular gastritis induced by H. pylori. In H. pylori induced gastritis numerous lymphoid follicles are seen in the gastric mucosa in the low power view. However, in DDNLH we have striking nodular lesions of 2 to 5 mm size and endoscopic view of these lesions is the basis of
this entity. Forceps biopsy with a size of 2 to 3 mm can pick up one nodule of same or larger size. Characteristic of these biopsies is the large lymphoid follicles which cause these characteristic elevated lesions. It is possible to see numerous nodular lymphoid lesions only in surgically resected specimens and we have not surgically resected any of these lesions. We are enclosing a low power view of the duodenal biopsy to address this issue. In the low power view of the duodenal biopsy (around 3 mm in size) only one lymphoid follicle can be seen, the cause of the elevated nodular lesion. I hope this point is clear.

My comments:
Again there is no question of confusion. The size of the biopsy forceps used and the tissue obtained is same for the stomach and duodenum and therefore if a gastric biopsy can show multiple lymphoid follicles (as told by the authors in their answer), a duodenal biopsy can also show if it is there. Even in the figure provided two more follicles of the same size can easily come if present. Anyway that was not an issue. I just wanted a better photograph to improve the manuscript. If authors did'nt had it they would have simply stated that ‘none of the biopsies obtained had more than one lymphoid follicle per section” rather than explaining the size of the biopsy and labelling me confused.

My recommendations:
Re write the material and methods and results with separate headings for endoscopic and histological changes in gastric mucosa and duodenal mucosa along with the changes suggested to make it more clear for the readers.
Modify the discussion as suggested.