DETECTION OF HELICOBACTER PYLORI IN HEPATIC TISSUE OF PATIENTS WITH CHRONIC HEPATITIS C AND HEPATOCellular CARCINOMA: (IMMUNOHISTOCHEMICAL STUDY)

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ABSTRACT

Background/Aim: Recent studies have suggested that bacterial confection with Helicobacter species in patients already infected with hepatitis C virus (HCV) could be involved in the development of cirrhosis and hepatocellular carcinoma (HCC). A retrospective study was performed in order to explore the association between Helicobacter pylori and HCV in hepatic tissue of Egyptian patients with chronic hepatitis C and hepatocellular carcinoma.

Methods: The presence of Helicobacter pylori was tested by immunohistochemistry on liver samples from two groups of patients; chronic hepatitis C (group I, n = 45) and hepatocellular carcinoma (group II, n = 15).

Results: Group I (chronic hepatitis C) involved 31 males and 14 females (male/female, 2.2:1). Their age ranged from 27 to 58 years with a mean of 45.8±7.0 years and HCC group involved 11 males and 4 females (male/female, 2.8:1). Their age ranged from 48 to 78 years with a mean of 60.2±10.0 years. Immunostaining revealed H. pylori microorganisms in 33/45 biopsies (73.3%) of chronic hepatitis C group and 5/15 (33.3 %) of HCC group (p = 0.005).

Conclusion: H. pylori organisms were present in liver tissues of HCV and HCC patients with significantly higher proportion in the former. Further studies are needed to ascertain its possible role, if ever, in the pathogenesis of cirrhosis and hepatic malignancy in Egyptian patients.

Keywords: H. pylori, chronic hepatitis C, hepatocellular carcinoma

INTRODUCTION

The role of H. pylori in the pathogenesis of extra-gastroduodenal manifestations is still under investigation. Previous studies found that H. pylori could damage hepatocytes by a cytopathic effect and induce hepatitis (1). Vacuolating cytotoxin of H. pylori could reach and damage the hepatocytes of patients with H. pylori infection without signs of known causes of liver disease (2). Chronic hepatitis is an inflammatory disease and is characterized by increased levels of the pro-inflammatory cytokines such as interleukins 1 and 6 (IL-1, IL-6), tumor necrosis factor and also by the presence of lympho-mono cellular infiltrate and lymphoid follicle formation (3). Viruses, such as hepatitis C virus (HCV), are only capable of inducing limited inflammation. On the other hand, Helicobacters are strong inducers of the inflammation cascade (4), infection with them could lead to the accumulation of extraordinary number of lymphocytes and polymorphonuclear cells in the infected tissue, IL-1 genecluster polymorphisms, thought to enhance IL-1b production, confer an increased risk of inflammation, accelerated hepatic damage and cancer (5).

Chronic hepatitis due to hepatitis C virus is the principal cause of end stage liver disease worldwide (6). Progression of the disease is variable and is governed by multiple factors. Though there are many factors known, still many remains to be identified. Incidence rate of progression of the disease, its decompensation and risk of carcinoma vary worldwide (7). This suggests that environmental factors such as infectious microorganisms, carcinogens, or nutrition play a role in pathogenesis. Bacterial co-infection with H. pylori in hepatitis C is another important factor in the development of cirrhosis and its decompensation (7). Helicobacter, a well recognized cause of duodenal and gastric carcinoma, induces a persistent infection and is thought to be a type-I carcinogen because of its role in the development of gastric carcinoma and gastric mucosal associated lymphoid tissue lymphoma (8). Recently, Helicobacter has been detected in bile and gallbladder tissue from patients with chronic cholecystitis and in liver tissue from patient with HCC (9-10). So, it is possible that H. pylori may also be risk factor for liver cancer (2).

However, the possible role of H. pylori in human liver disease, its progression and risk of hepatocellular carcinoma still needs to be
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evaluated. Verhoef et al. (13) suggested that gastric colonization with a specific subset of Helicobacter strains is associated with the induction of HCC, either directly via colonization in the liver or indirectly via secretion of specific toxins by helicobacter residing in the stomach. On the other side, Xuan et al. (12) reported that Helicobacter microorganisms were observed in the adjacent hepatocytes of HCC samples and the number of cocci forms was greater than that of bacilli. The aim of the study was to investigate whether Helicobacter pylori is present in the liver tissue of Egyptian patients with chronic hepatitis C and hepatocellular carcinoma.

MATERIALS AND METHODS

We conducted a revision of tissue blocks from the pathology archive of National Hepatology and Tropical Medicine Research Institute including formalin-fixed paraffin embedded liver biopsy specimens from 45 patients with chronic hepatitis C infection (group I) and 15 patients with hepatocellular carcinoma (group II). Samples were processed, where 4 μm thick sections were stained with H & E and immunohistochemistry for detection of H. pylori. Avidin biotin peroxidase complex technique (ABC) was applied.

Statistical analysis:

Data was analyzed using SPSS win statistical package version 15. Chi-square test (or Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test. A p-value less than 0.05 was considered significant. All tests were two-tailed.

RESULTS

Group I, chronic hepatitis C (n = 45) involved 31 males and 14 females (male to female ratio 2.2:1). Their age ranged from 27 to 58 years with a mean of 45.8±7.0 years. The histopathological findings of chronic HCV infected tissue including portal lymphoid infiltration, periportal interface hepatitis, lymphocyte infiltration of the lobules, hepatocellular necrosis, steatosis and fibrosis as shown in table (1.2) and figure (1A-D).

Portal lymphoid infiltrate was observed in all cases of the group I; it was mild in 11.2%, moderate in 44.4% and severe in 44.4% of cases. Periportal (piecemeal) or interface hepatitis was mild in 17.8%, moderate in 55.5%, and severe in 26.7% of biopsies. Sporadic hepatocellular necrosis (lytic foci) or minor hepatocellular damage was mild in 24.4%, moderate in 33.3%, and severe in 42.3% of patients. Confluent necrosis was absent in 9 cases (20%), unifocal in 57.8%, multifocal in 11.1% and panacinar in only 11.1% (table 1). Necroinflammatory activity (severity of liver injury including sum of portal, periportal inflammation, lytic, and confluent hepatocellular necrosis) was mild in 13.3%, moderate in 42.3%, and severe in 44.4% of patients. Immunostaining revealed that there are 33 biopsies of 45 HCV cases (73.3%) were positive for H. pylori (table 2 and fig 2A-D).

Group II (HCC) (n =15), involved 11 males and 4 females (male to female ratio 2.8:1). Their age ranged from 48 to 78 years with a mean of 60.2±10.0 years. All cases were classic HCC with well differentiated malignant cells arranged in a trabecular or trabeculo-glandular pattern with a sinusoidal network. Specific H. pylori immunohistochemical staining revealed slender, curved microorganisms in the hepatic sinus or intracellular of the hepatocytes in 5 specimens (33.3%) (table 2 and fig 3A-D).

Group I (HCV) had significantly higher proportion of H. pylori infection (73.3%) in comparison to (33.3%) in group II (HCC) with a p value of 0.005. Within the HCV group, [age, sex, stage of fibrosis, necroinflammation grade and steatosis] had no significant association with H. pylori infection (Table 3) and fig 4.

Table 1: Histopathological findings of the chronic hepatitis C group (n = 45)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Item</th>
<th>Portal inflammation</th>
<th>Periportal inflammation</th>
<th>Sporadic Lytic foci</th>
<th>Confluent necrosis*</th>
<th>NI grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>5 (11.2%)</td>
<td>8 (17.8%)</td>
<td>11 (24.4%)</td>
<td>26 (57.8%)</td>
<td>6 (13.3%)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>20 (44.4%)</td>
<td>25 (55.5%)</td>
<td>15 (33.3%)</td>
<td>5 (11.1%)</td>
<td>19 (42.3%)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>20 (44.4%)</td>
<td>12 (26.7%)</td>
<td>19 (42.3%)</td>
<td>5 (11.1%)</td>
<td>20 (44.4%)</td>
<td></td>
</tr>
</tbody>
</table>

NI = necroinflammatory
*There are 9 (20%) biopsies with no confluent necrosis

Table 2: H. pylori immunostaining in the two studied groups

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>H. pylori +ve</th>
<th>H. pylori –ve</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (HCV)</td>
<td>45</td>
<td>33 (73.3%)</td>
<td>12 (26.7%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Group II (HCC)</td>
<td>15</td>
<td>5 (33.3%)</td>
<td>10 (66.7%)</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3: H. pylori in the HCV group (n = 45)

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>H. pylori +ve (n = 33)</th>
<th>H. pylori –ve (n = 12)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD)</td>
<td></td>
<td>46.1±7.2</td>
<td>44.9±6.7</td>
<td>0.433</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>22 (71.0%)</td>
<td>9 (29.0%)</td>
<td>0.593</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>11 (78.6%)</td>
<td>3 (21.4%)</td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 3 or 4/6</td>
<td>35</td>
<td>25 (71.4%)</td>
<td>10 (28.6%)</td>
<td>0.589</td>
</tr>
<tr>
<td>Stage 5/6</td>
<td>10</td>
<td>8 (80.0%)</td>
<td>2 (20.0%)</td>
<td></td>
</tr>
<tr>
<td>Necroinflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>3</td>
<td>3 (100%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>20</td>
<td>12 (60.0%)</td>
<td>8 (40.0%)</td>
<td>*</td>
</tr>
<tr>
<td>Severe</td>
<td>22</td>
<td>18 (81.8%)</td>
<td>4 (18.2%)</td>
<td></td>
</tr>
<tr>
<td>Steatosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>5 (83.3%)</td>
<td>1 (6.7%)</td>
<td>*</td>
</tr>
<tr>
<td>Mild</td>
<td>29</td>
<td>20 (69.0%)</td>
<td>9 (31.0%)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>7</td>
<td>7 (100.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
<td>1 (33.3%)</td>
<td>2 (66.7%)</td>
<td></td>
</tr>
</tbody>
</table>

SD: standard deviation  
*p value due to the small number of cases within groups

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**Figure 1:**  
A) Chronic hepatitis C with Portal lymphoid aggregate and bile duct epithelial damage H&E stain X-100.  
B) Chronic hepatitis C with sporadic lytic foci H&E stain X-100.  
C) Chronic hepatitis C with Pan-acinar confluent necrosis H&E X40.  
D) Chronic hepatitis C with steatosis H&E X-100.
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Figure 2: A-D) Chronic hepatitis C with positive *H. pylori* Immunostaining ABC Technique X-200.

Figure 3: A-D) Hepatocellular carcinoma with positive *H. pylori*. Immunostaining ABC technique X-400.
DISCUSSION

In this study, *H. pylori* organisms were detected in 33.3% of HCC group compared to 73.3% of chronic hepatitis C group (p = 0.005). Our figures differ from previous studies, especially in the proportion of HCC cases having *H. pylori* in their liver tissue. Avenaude et al. detected *H. pylori* in 8 out of 8 (100%) specimens of primary liver cancer cases and in only 1 of 8 specimens of liver tissue without cancer. Rocha et al. reported that *Helicobacter* were detected in liver samples of 3.5% of patients with non-cirrhotic chronic hepatitis C and 68.0% of HCV positive cirrhosis and 61.3% of HCC patients. We did not find any association between the presence of *H. pylori* infection detected by immunohistochemical staining of liver tissue and stage of fibrosis, necroinflammation grade and steatosis within HCV group. Moon et al. reported that *H. pylori* infection promotes liver fibrosis in experimental animals, and immunohistochemical study against *H. pylori* showed positive antigen fragments in the liver and consequently *H. pylori* infection could be an important contributing infectious factor to the development of hepatitis and liver cirrhosis.

*H. pylori* is one of the most common bacteria worldwide found in more than 50% of human population. The incidence of infection is much higher in children than adults. *H. pylori* infection is endemic; it is primarily acquired in infancy, most probably via the oro-orogastric route, from other family member or close contacts. The prevalence of *H. pylori* infection varies between countries and is closely related to Growth Domestic Product. In developing countries the prevalence of infection is often more than 80% in young adults, in contrast to less than 10% for similar age groups in developed countries. Nilsson et al. found that *Helicobacter* genus-specific primers are positive in patients with primary biliary cirrhosis or primary sclerosing cholangitis. Furthermore, the gene sequence obtained from positive PCR of *Helicobacter* spp. 16S rRNA is usually analogous to *H. pylori*. Huang et al. reported that the microorganism was visualized in the liver tissue by means of specific immunostaining in HCC cases.

The presence of *H. pylori* organisms in liver tissues of patients with HCV and HCC needs further studies to ascertain its possible role, if ever, in the pathogenesis of cirrhosis and hepatic malignancy in Egyptian patients. Elmasry et al. reported that *H. pylori* infection was found to increase with the increase in both child and meld scores of cirrhosis and this may explain the frequent occurrence of gastro-duodenal ulcer in cirrhotic patients.

REFERENCES

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الملخص العربي
الكشف عن البكتريا الحلوئية في عينات الكبد المأخوذة من المرضى المصابة بالالتهاب الكبدى الفيروسى المزمن (سي) وأورام الكبد

أحسان حسن 1 - محمد اسماعيل 1 - سامح محمد سيف 1 - عزة حجازى 1 - شوكت شاكر جرجس 1 - منار محمد منير 1 - محمد عز العرب 1 - كمال الأتريبي 1

قسم الباثولوجي، والجراحة، والمنطقة والباطنة، المعهد القومي للأمراض المزمنة والكبد
وقسم الأبحاث الطبية، المعهد القومي للأورام

أثبتت الدراسات الحديثة أن هناك علاقة بين البكتريا الحلوئية والإصابة بليف الكبد وأورام الكبد وذلك كان لازماً أن نكشف عن
هذه العلاقة في المرضى المصريين المصابة بالتهابات مزمنة نتيجة الفيروس الكبدى سي وأورام الكبد الأولية.
تمت الدراسة على العينات الأشري حيث الموجودة في قسم الباثولوجي بالمعهد القومي لأبحاث الأمراض المزمنة والكبد وقسمت إلى

- المجموعة الأولى: بلوكات الشمع المحضرة من أنسجة المرضى المصابة بالتهاب فيروس البكتريا كبدى مزمن (سي) وعددهم
45 مريض منهم 31 رجلاً و14 سيدة تراوح أعمارهم بين 27 إلى 57 سنة.

- المجموعة الثانية: بلوكات الشمع المحضرة من أنسجة المرضى المصابة بأورام سرطانية أولية في الكبد وعددهم
15 مريض منهم 11 رجلاً و4 سيدة تراوح أعمارهم بين 48-59 سنة وكلهم مصابين بفيروس سي.

تم عمل فحص ميكروسكوبى للعينات المصبوغة بصبغات الهيماتوكسيلين والأوزينس وكذلك تم عمل الحماية النسبية المعنوية على
عينات الكبد المأخوذة من هؤلاء المرضى وأثبت النتائج أن 33 مريضاً من إجمالي 45 (نسبة 73.3%) المصابين بالتهاب
فيروس سي كان لديهم ميكروبات البكتريا الحلوئية في النسيج الكبدى وعدد 5 من إجمالي 15 (نسبة 33.3%) المصابين
بأورام في الكبد كان لديهم البكتريا الحلوئية في عينات الكبد.

ومن هذه النتائج يتبين وجود البكتريا الحلوئية في عينات النسيج الكبدى المأخوذة من المرضى المصريين المصابة بأمراض
الكبد المزمنة نتيجة فيروس سي والأورام مما يسبح إجراء دراسات مستقبلية لإثبات ما إذا كان لهذه البكتريا الحلوئية دور في
تطور مراحل المرض وصحة ك⊆لك معقل مساعد مع الفيروس (سي).