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Research article

Evaluation of anti-*Helicobacter pylori* antibodies level in sera of patients with chronic hepatitis B

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ABSTRACT

The relationship between chronic hepatitis B virus and *Helicobacter pylori* infection was evaluated to determine, seventy five patients with chronic hepatitis B infection (8-70 years) were investigated. The results were compared with the results of 50 healthy volunteers. Anti-*H. pylori* antibodies IgA and IgG were measured by Indirect fluorescent antibody test (IFAT) in sera of patients and healthy groups. The percentage of anti-*H.pylori* IgA antibodies (26.67%) were significantly (P<0.01) higher than healthy control group. While, no significant difference was found between the percentages of anti-*H. pylori* IgG antibodies (48 %) in patient sera and these kind of antibodies in sera of healthy control group (P > 0.05). The present results indicated that the acute infection with *H. pylori* may be correlated with severity of chronic hepatitis B infection.

Keywords: Hepatitis B virus, chronic infection, Helicobacter pylori, antibodies.

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INTRODUCTION

Chronic liver disease and its complications are major health problems [1-3]. Peptic ulcer disease is one of the most frequently observed complications in patients with liver cirrhosis, although the incidence and prevalence of peptic ulcer disease appear to be increased in cirrhosis, the underlying mechanism of peptic ulcer disease in cirrhosis is unclear [4]. Several aspects of the relation between *Helicobacter* species (particularly *H. pylori*) and liver diseases have been assessed in human beings [5].

The higher prevalence of *Helicobacter* species associated with more advanced stages of liver disease supports the possibility of their role in the progression of chronic hepatitis towards cirrhosis and HCC [6]. Determinants of this evolution are not yet fully understood, including those occurring in HCV-positive patients [7]. Many studies have tried to explain the mechanisms by which *H. pylori* can affect the liver cell, suggesting that chronic hepatitis is an inflammatory disease and each inflammatory process is characterized

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by increased levels of pro-inflammatory cytokines such as interleukins 1, 6 (IL-1, IL-6), tumor necrosis factor (TNF) and presence of lymphocytic mononuclear infiltrate and lymphoid follicle formation [8].

Viruses, such as HCV are only capable of limit inflammation because of shedding of the IL-1 receptor in the circulation thereby limiting the possibility of IL-1 binding to the cellular receptor [9]. According to previous study of Abdel-Hady *et al.* (2008) a significant increase in fasting arterial blood ammonia and plasma end toxin measurement was associated with *H. pylori* infection in cirrhotic patients, and medical treatment of *H. pylori* infection led to a significant decrease in the severity of hepatic encephalopathy and fasting arterial blood ammonia levels [10] this evidence suggests that *H. pylori* infection might have a role in increasing the circulating levels of ammonia and end toxins of cirrhotic patients, thus facilitating the onset of hepatic enceph-alopathy and of hepatic inflammation by stimulating the secretion of pro inflammatory cytokines. The role of H. pylori in the pathogenesis of extragastroduodenal manifestations is still under investigation. Previous studies found that *H. pylori* could damage hepatocytes by a cytopathic effect and induce hepatitis [11]. Vacuolation cytotoxin of *H. pylori* could reach and damage the hepatocytes of patients with *H. pylori* infection without signs of known causes of liver disease [12].

On the other hand, Helicobacters are strong inducers of the inflammation cascade [13]. Infection with them leads to accumulate of extraordinary number of lymphocytes and polymorphnuclear cells in the infected tissue, IL-1 gene cluster polymorphisms enhance IL-Iβ production, confer an increased risk of inflammation, accelerated hepatic damage and cancer [14]. Expression of hepatitis B virus (HBV) antigens in *H. pylori* bearing gastric mucosa was shown by immunostaining methods [15].

The hypothesis that Helicobacter spp. might be a risk factor for human liver diseases has arisen after the discover of *H. hepaticus* and its association with murine hepatitis and hepatocellular carcinoma (HCC), *H. hepaticus*, an example of such species, was discovered in 1992 and was first isolated from a colony of A/JCr mice; it has been reported to be an agent that causes hepatic cancer in rodents.

The present aims to evaluate the presence of relationship between *H. pylori* infection and chronic liver disease (viral hepatitis).

MATERIALS AND METHODS

The study was carried out on seventy five patients infected with chronic hepatitis B who attended to hepatic and gastrointestinal tract hospital in Baghdad from first of November 2013 until February 2014.The ages of the total patients were ranged from 8 to 70 years. Fifty samples of healthy individuals, 23 female and 27 male were used as a control group of same ages and sex. Blood samples (5 ml) collected by disposable syringe were put into gel tubes and stand at room temperature until the coagulant formation. Then, the samples were centrifuged at 3000 rpm for 5 min. Sera were dispended on a seven Eppendorf tubes. All samples were stored at -20˚C until tested for immunological examinations.

Immunological examination

Anti-Helicobacter pylori antibodies IgA and IgG were measured in peripheral blood of patients and healthy control groups by IFAT test (Uroimmune, Germany) instructions of manufacture company was followed to evaluate the level of antibodies.

Statistical analysis

The statistical analysis system (SAS) was used to find the difference in several studied factors. Chi-square test and least significant difference were used to check the significant difference of presence of Anti-Helicobacter pylori antibodies IgA and IgG between control and test groups [16]. The results of present study were represented by mean.

Results and Discussion

In present study the immunofluracent technology was used to indentified the presence of anti- anti-H. pylori in patients and healthy control sera (**fig. 1**).

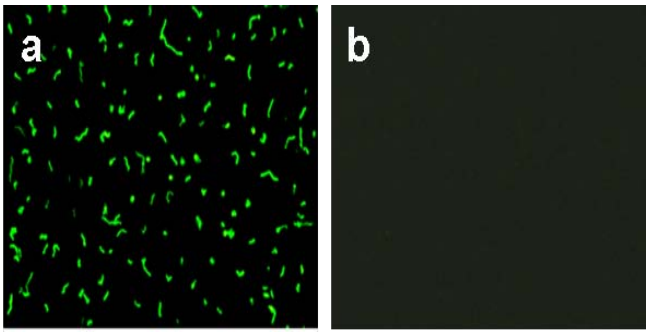

Fig. 1 munofluorescence technique for positive (a) and negative (b) sera group. The positive results appeared as green spark bacterial cells while, the negative results appeared as full black field.

The current study showed that the presence of anti-*H. pylori* IgA antibodies was found in 20 cases from 75 cases. Thus, the percentage of anti-*H. pylori* IgA antibodies in patients with chronic hepatitis B was found 26.67%. The results showed highly significant differences ($P<0.01$) when compared with healthy control groups. While, the percentage of anti-*H. pylori* IgG antibodies was 48% (36/75) and there was non significant differences ($P>0.05$) was found as compared with healthy control group (**table 1**). Immunoglobulin IgA considered as an indicator of recent (acute) infection and the titer of IgA was raced during the first two weeks of infection, while the titer of IgA decreased gradually and the titer of IgG was increased during the second and third week of infection for some time and

decreased to low level and stable for long life in serum of patients with chronic infection [17].

The results of current study agreed with another previous study that showed the percentage of chronic hepatitis B patients who has immunological markers of *H. pylori* infection was 43.4% [18], another study reported the percentage of *H. pylori* infection was 37.5% in CHB patients [19], while this study was disagreement with another study, it was found that the serorevalence of anti-*H. pylori* antibodies was 86.0% [20].

Table 1- The percentage of anti-*H. pylori* IgA and IgG antibodies in sera of CHB patients. * P < 0.01; NS, non significance.

Test	IgA		IgG	
	No.	%	No.	%
Positive	20	26.67	36	48.00
Negative	55	73.33	39	52.00
Total	75	100 %	75	100 %
Chi-square value	---	11.026 *	---	0.892 NS

The difference in results was found because of there were several methods used to investigate *H. pylori* infection. In our study, the indirect immunofluorescent test was used to detect *H. pylori* antibodies in sera of CHB patients, which was considered highly specific and sensitive method. Other studies were used ELISA test, urease test and fasting ammonium blood test. The real time reaction (PCR) for investigating the genomic sequences of *H. pylori* and there are other causes responsible for these differences, moreover period of time at which the samples was collected, type of infection, sample size under current studies and the place where the samples are taken may be played a certain role in presenting the different findings.

Many previous studies showed that the association between the *H. pylori* infection and hepatitis B and C infection [21]. The role of *H. pylori* in digestive diseases (gastritis, ulcer, gastric cancer, MALT lymphoma) is well known. It has been suggested relatively recently that infection with *H. pylori* can be involved in various extra-digestive conditions; *H. pylori* has been associated with the development of extragastric disorders. The postu-lated role of *H. pylori* in the pathogenesis of extra gastric disorders is based on the many facts; i: local inflammation with systemic effects, ii: *H. pylori* is a chronic process that lasts for several decades; iii: persistent infection induces a chronic inflammatory and immune response that is able to induce lesions both locally and remote to the primary site of infection [22]. *H. pylori* infection is commonly found in patients with chronic hepatitis B but clinical significance could not be identified in this study. Therefore a high prevalence of antibodies to *H. pylori* in patients with liver diseases has suggested an association between *Helicobacter* and liver disease. Furthermore, it has been assumed that environmental poor sanitation, unhygienic in conditions, overcrowding and lower socioeconomic status have role in altered level of consciousness in such patients, that is consistent with another previous study [23]. The old study suggested that the gastric mucosa in cirrhosis might provide a hospitable environment for the colonization of *H. pylori* especially when there is severe hemorrhagic congestion and edema of the mucosa. Factors like increased inducible nitric oxide synthase (iNOS) expression resulting in high reactive oxygen species, impairment of gastric mucosal defense due to portal hypertension. Furthermore, colonization of H. pylori strains results gastric inflammatory response, including interleukin (IL)-8, tumor necrosis factor-α, which may be associated with the sequence of events leading to gastropathy. The mechanism by which *H. pylori* colonizes the human liver is not totally enlightened. The *H. pylori* DNA detected in the liver tissue may result from bacterial translocation from the stomach into the blood through the portal system, especially in the later stages of chronic liver disease when portal hypertension occurs [24]. *H. pylori* is known to produce ammonia from urea that is rapidly absorbed from gastric lumen into circulation. Infection with these bacteria has been shown to be associated with elevated blood ammonia levels and recurrent attacks of overt hepatic encephalopathy [25]. These present study yielded a good indicator of the relationship between the infection of *H. pylori* and chronic hepatitis B infection in studied area.

Conflict of interest

The authors declare that they have no conflict of interests.

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