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Detection of *Helicobacter pylori* antibodies in Patients with Chronic Liver Disease in Iraq

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ABSTRACT:

The study was carried out to determine the prevalence of *H. pylori* antibodies in patients with chronic liver disease (CLD), by detecting the presence of stool antigen (HpSA), serum, and salivary anti-*H. pylori* antibody using ELIZA and compared with Rapid Urease test (gold standard). A one hundred and seventy six endoscopy were carried out for patients with or without chronic liver disease. The result indicated that among 130 patients with (CLD), and 46 patients without CLD, the commonest endoscopy diagnosis by gastric biopsy was gastritis (22.72%), duodenal ulcer (17.61%), and Gastric ulcer (11.93%).

The serum antibody positivity was higher (83.0%), followed, HpSA (74.4%), salivary antibody positivity (71.0%) and rapid urease test (RU) positivity (70.6%). In controls salivary antibody and rapid urease test positivity was higher than in CLD patients, while HpSA and serum antibody was higher in CLD patients. On the other hand, it was associated with sex and age. The sensitivity and specificity of HpSA were 94.6% and 91.6%, respectively.

Key words: *H. pylori*, CLD, stool Ag, saliva Ab.

INTRODUCTION:

Infectious diseases are now being implicated frequently as the cause of the diseases that have been considered previously to be of non-infectious etiology. Infection by *Helecobacter pylori* is one of them. Many diseases, from short stature to hepatic diseases, have been related to this micro-organism. *H. pylori* results in a low grade chronic inflammatory process of gastric mucosa and it has been suggested that this may lead to elevation of IgG antibody for prolonged period. *H. pylori* is also associated with coronary artery disease, rosacea, cerebrovascular disease, growth retardation in children, and Raynaud's phenomenon (1, 2, 3).

H. pylori is a Gram negative spiral, motile bacterium lives in stomach, that can be transmitted oral-oral and fecal-oral, it has been detected in dental plaque, saliva and feces (4).

Chronic liver diseases (CLD) including (hepatitis, schistosomiasis and autoimmune hepatic disease) have an impact on dental

practice. The fear of hepatitis virus transmission in the dental clinic was a major concern, currently, managing chronically ill patients with hepatic disease is a challenge for dental practitioners (5). *H. pylori* have been linked to hepatitis A virus (HAV) because of their shared route of transmission through fecal –oral contamination. The association between the *H .pylori* and HAV is possibly due to similar local conditions (6, 9).The high seroprevalence of antibodies to *H. pylori* in patients with hepatitis C virus (HCV)-positive liver diseases explain the elevated incidence of peptic ulcer in such patients (10). *H. pylori* infection has been reported to be prevalent in patients with CLD. In addition to its link to HAV it has been identified as a possible cause of gastric wears seen in patients with hepatitis C virus (9, 11).

H. pylori can be detected by several methods such as culture of gastric biopsy samples which(is the most specific method for diagnosis of infection, but it is difficult), polymerase chain reaction (PCR),rapid urease (RU), and enzyme-linked immunosorbent assay ELISA (12-14).Hence non-invasive methods for detecting *H. pylori*, such as salivary ELISA, rapid blood test (agglutination test), serum and stool ELISA can be used in children in whom-those procedures are not easily tolerated(15,16).Salivary ELISA could be useful as a non-invasive technique for detection of *H. pylori* infection; there are several studies on the residence of the bacteria in the mouth and the possibility that dental plaque could be a source of reinfection in case of eradication (17, 18).

This study described the detection of *H pylori* in patients with chronic liver diseases and it was the first one in Iraq.

MATERAL &METHODS:

Cases were CLD patients with hepatitis B virus (HBV) infection, HCV positive, having autoimmune hepatic disease or cancer. The liver diseases patients were attending the endoscopy unit in gastrointestinal & hepatology teaching hospital and medical Baghdad city for possible gastric or duodenal disease during (10/2009-2/2011). Controls include patients attending the endoscopy unit because of gastric disease but free of hepatic disease, as determined by a normal liver enzyme profile.

Mucosal biopsy specimens were taken from observation areas and sent to the pathology laboratory for detection of *H. pylori.* Patient's data were recorded, including age and sex.

Whole saliva was collected on the day of endoscopy by accumulated saliva into a graded sterile tube, and stored at -20C until analyzed.

Stool samples for the HpSA test were taken from patients. Detection of *H. pylori* antigens in stool was carried out by ELISA polyclonal antibody test.

Serum and saliva samples of patients with CLD and controls were tested of anti-*H. pylori* antibodies using ELISA test. Elevated IgG or IgA antibody titers were considered to indicate current *H .pylori* infection.

The frequency of *H .pylori* infection among CLD patients and controls was stratified by independent variables (age and sex).The chi squared test was used to determine significance and association.

RESULTS & DISCUSSION:

The endoscopy results showed that, gastritis duodenal ulcer, and gastric reflux were the commonest diagnosis (Table 1).

ENDOSCOPY FINDING	NO.	%
Gastritis	40	22.72
Duodenal ulcer	31	17.61
Gastric ulcer	21	11.93
Gastric reflux	16	9.09
Gastric dysplasia	10	5.65
Duodenitis	10	5.65
Peptic ulcer	5	2.84
Normal	43	24.4
Total	176	100

The prevalence of *H. pylori* infection in patients with HBV-related, HCV-related, HBV and HCV-related, were 70.8%, 20%, and 3.1%, respectively (Table 2).

Table- 2: Percentage of endoscopy patients according to Cause of chronic liver diseases.

CAUSES	NO.	%
HCV	92	70.8
HBV	26	20
HCV+HBV	4	3.1
Autoimmune	4	3.1
Cancer	3	2.3
Unknown	1	0.8
Total	130	100

The anti-*H. pylori* positivity in serum revalas higher percentage (83.0%), followed by HpSA positivity (74.4%), then salivary positivity (71.0 %). In controls RU positivity was higher (29.4) than in CLD patients (Table 3). On the other hand, it was associated with sex and age (Table 4).

Table- 3: Percentage of *H. pylori* positivity among endoscopy patients.

TEST	CLD PATIENTS NO.	%	CONTROLS NO.	%
HpSA:				
+ve	99	74.4*	34	25.6
-ve	31	72.0	12	28.0
Rapid urease:				
+ve	72	70.6	30	29.4
-ve	58	78.4	16	21.2
Salivary Ab :				
+ve	88	71.0	36	29.0
-ve	42	80.8	10	19.2
Serum Ab:				
+ve	51	83.0*	10	16.4
-ve	79	68.7	36	31.3

*=statistically significant at P<0.05.

Table- 4: Percentage of *H. pylori* positivity with variables among endoscopy patients.

VARIABLE	*H.PYLORI*			
	Positive No.	%	Negative No.	%
Age (years) Mean(SD)	48.5	16.4	50.5	8.3
Sex				
Male	56	56.5	43	43.5
Female	38	49.4	39	50.6

SD=standard deviation. P=0.581.

The increased rate of *H. pylori* infection in patients with CLD has been explained in some studies, higher rates of gastric colonization with *H. pylori* by (16- 18).However, other studies have found lower *H. pylori* infection in CLD.A proper interpretation of the studies investigating the prevalence of *H. pylori* infection in patients with liver disease, several factors, such as diagnostic methods, geographic and racial differences in *H. pylori* prevalence should be considered (19).The high infectivity rates (70%-91%) among endoscopy patients have been reported in Europe, the United States of America and Africa (20- 22).

Helicobacter pylori infection has been intensely studied in patients with CLD. The possibility of common routes of transmission as well as the frequent presence of gastric disease in such patients has been reported (23). Most of the ammonia is of gut origin where it is produced by the bacterial flora. Stomach when infected with *H. pylori* is an alternative site of ammonia production. Normally the ammonia is extracted by the liver therefore, in liver failure large quantities reach the systemic circulation because of porto-systemic shunting and impaired ureagenesis. The role of *H. pylori* as a cause of hyperammonaemia in patients with liver diseases has still not been

fully clarified (24). The reduction in blood ammonia levels after *H. pylori* eradication found in some studies has been attributed to a nonspecific effect of antibiotic therapy on ammonia producing gut flora rather than *H. pylori* eradication (25).

The *H. pylori* stool test is a simple, non - invasive method for accurate diagnosis of *H. pylori* infection. Studies that use HpSA tests report higher prevalence than the studies that use the rapid urease test, histology as in our study. The sensitivity and specificity of HpSA were 94.6% and 91.6%, respectively; this result was in agreement with (26) which report the sensitivity of HpSA 93.1%, and specificity 95%.

The relatively high prevalence of *H. pylori* infection in CLD in our study can also be partially explained by the difference in the diagnostic methods used in the setting of *H. pylori* infection. Antibodies to *H hepaticus*, often cross reacts with *H pylori*, occur frequently in patients with chronic liver diseases, with no clear cut relation to specific diagnostic groups. The pathogenic significance of these findings is not known (18). In general, studies that use serologic tests (IgG to *H. pylori*) report higher prevalence than the studies that use the rapid urease test, histology, or urea breath test for detection of *H. pylori*, serologic determination of *H. pylori* infection may lead to confounding results (27). Residence of *H. pylori* in saliva, dental plaque and periodontal pockets is of major importance in order to achieve eradication(11).The value of salivary antibody detection as a non-invasive tool for diagnosis of *H. pylori* infection has been increasingly explored, especially in wide screening and child management (26,27). It was found that when 4 cytotoxin genotypes were analyzed by polymerase chain reaction (PCR) in stomach and salivary isolates, there was 27% agreement between stomach *H. pylori* isolates and their corresponding saliva DNA in all 4 genotype, 60% agreement with 3 genotypes, 85% agreement with 2 genotypes and 95% agreement with at least 1 genotype. It has been suggested that more than 1 strain of *H. pylori* may exist in the stomach and saliva in the same patient. So salivary antibody tests are not indicated for the clinical diagnosis of *H. pylori* infection, they may be useful for prevalence surveys, it has been recommended as a valuable tool for studies of the epidemiology of *H. pylori* (12, 28). The 2007 Maastricht Consensus Report on *H. pylori* diagnosis and treatment does not recommend serological determination of *H. pylori* infection in routine clinical practice. It also recommends that the primary diagnosis of *H. pylori* infection should be established by rapid urease test and/or histology (28).

This study showed that serum, HpSA and saliva positivity was higher among CLD patients, than control, while saliva antibody and (UR) positivity was higher among CLD patients group this may be due to fact that most of these patients underwent endoscopy because of gastric disease and thus possible *H. pylori* infection.

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الخلاصة:

تم التحري عن اضداد الملوية البوابية لدى المصابين بامراض الكبد المزمن والذين اجري لهم تنظير هظمي, وذلك بالكشف عن تلك الاضداد في مصل الدم واللعاب ومستضد الملوية البوابية في الخروج بطريقة الاليزا واستخدام اختباراليوريز(للخزعة) كمعيار ذهبي0 اجريت الاختبارات ل 176 مريض, تطلبت حالتهم اجراء تنظير هظمي واخذ خزعة من المعدة. ومن بين 130 مريض مصاب بمرض كبدي مزمن 46 مريضا غير مصاب بمرض كبدي مزمن , كان التشخيص الاكثر شيوعا بالتنظير الهظمي هو التهاب المعدة (22,72%), التهاب الاثني عشري (17,61%) وقرحة المعدة (11,93%) .وكانت ايجابية الاضداد عالية في مصل الدم (83%), واختبار الخروج (74,4%), واختباراللعاب (71,0%) ثم في اختبار اليوريز (70,6%). كانت الايجابية للملوية البوابية في اختبار اليوريز واختبار اللعاب عالية بين مجموعة السيطرة,بينما كانت ايجابية اختبارالخروج واختبار المصل اكثر بين مجموعة مرضى الكبد المزمن, مع وجود علاقة بين ايجابية الملوية البوابية في اللعاب وبين تقدم العمروالجنس.وكانت حساسية ونوعية اختبار اللعاب94,6% و91,6% على التوالي.