Salivary anti-*Helicobacter pylori* positivity among endoscopy patients with chronic liver disease

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إيجابية الـمَلْويَّة البوابية اللعابية بين المصابين بمرض الكبد المزمن الذين أجري لهم تنظير هضمي

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الخلاصة: أجرى الباحثون في هذه الدراسة اختبارات لتحرِّي العدوى بالـمَلْويَّة البوابية، بكشف أضدادها في اللعاب أو المصل، لدى المرضى الذين أجري لهم تنظير هضمي، ممن كانَ لديهم مرض كبدي مزمن أو لم يكن لديهم. وقد توثق الباحثون من صحة قياساتهم بتحليل الكائن المشابه للـمَلْويَّة (وهو معيار ذهبي)، بعد إجرائه على مرضى تطلبت حالتهم إجراء خزعة من المعدة. ومن بين 114 مريضاً مصاباً بمرض كبدي مزمن، و50 مريضاً غير مصاب بمرض كبدي مزمن، كان التشخيص الأكثر شيوعاً بالتنظير الهضمي هو التهاب المعدة (27.2٪). وقد كان هناك ترابط يعتد به إحصائياً بين إيجابية الـمَلْويَّة البوابية في اللعاب وبين تقدم العمر. كما اتضح وجود حساسية منخفضة للإيجابية المصلية للأضداد المضادة للـمَلْويَّة البوابية (36.3٪)، ونوعية عالية (75.8) لدى المصابين بالمرض الكبدي المزمن.

ABSTRACT In this study, endoscopy patients with and without chronic liver disease (CLD) were examined and tested for *Helicobacter pylori* infection by detecting the presence of serum and salivary anti-*H. pylori* antibody. The validity of these measures was compared with *Campylobacter*-like organism analysis (gold standard) performed on patients requiring gastric biopsy. Among 114 patients with CLD and 50 without, the commonest endoscopy diagnosis was gastritis (27.2%). Salivary *H. pylori* positivity was significantly associated with older age. Salivary anti-*H. pylori* antibody positivity showed low sensitivity (36.6%) and high specificity (75.8%) in CLD patients.

Positivité des anticorps anti-*Helicobacter pylori* dans la salive de patients soumis à une endoscopie et souffrant d'une pathologie hépatique chronique

RÉSUMÉ Dans cette étude, des patients soumis à une endoscopie et souffrant ou non d'une pathologie hépatique chronique (PHC) ont fait l'objet d'un examen et d'une recherche d'infection à *Helicobacter pylori* par détection de la présence d'anticorps anti-*H. pylori* dans le sérum et la salive. La validité de ces mesures a été évaluée par comparaison avec l'analyse des organismes de type *Campylobacter* (méthode de référence) réalisée sur les patients nécessitant une biopsie gastrique. Sur 114 patients atteints de PHC et 50 patients non atteints par cette pathologie, le diagnostic endoscopique le plus courant était la gastrite (27,2 %). La positivité aux anticorps anti-*H. pylori* dans la salive était significativement associée à l'âge avancé. La sensibilité du test était faible (36,6 %) et sa spécificité élevée (75,8 %) chez les patients atteints d'une PHC.

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Introduction

Chronic liver disease (CLD) patients, including those with viral hepatitis, autoimmune hepatic disease and schistosomiasis, have an impact on dental practice. While 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. This is because patients with chronic liver disease may ultimately develop serious liver dysfunction and this poses problems in performing non-invasive and invasive dental treatment procedures. Furthermore, the altered immune reactions in such patients may result in them being susceptible to other infections [*1,2*].

Helicobacter pylori is a Gram-negative motile bacterium that can be transmitted orally; it has been detected in dental plaque, saliva and faeces [*3,4*]. *H. pylori* has been linked to Hepatitis A virus (HAV) because of their shared route of transmission through faecal–oral contamination. However, the association between the 2 organisms is possibly due to similar local conditions [*5*]. In addition to its link to HAV, *H. pylori* infection has been reported to be prevalent in patients with CLD [*6–9*], and has been identified as a possible cause of gastric ulcers seen in patients with CLD [*10,11*]. Furthermore, the high seroprevalence of antibodies to *H. pylori* in patients with hepatitis C virus (HCV)-positive liver diseases explains the elevated incidence of peptic ulcer in such patients [*6*].

H. pylori infection can be detected by several methods such as enzyme-linked immunosorbent assay (ELISA), *Campylobacter*-like organism (CLO) test, polymerase chain reaction and gastric biopsy [*12,13*]. Culture of endoscopic grasp biopsy samples constitutes the most specific way to establish the diagnosis of infection, but it is not easy. Hence, non-invasive techniques for detecting *H. pylori* infection, such as serum ELISA, rapid blood tests and salivary ELISA, can be used in patients where invasive procedures are contraindicated and also in children in whom these procedures are not easily tolerated.

Sensitivity and specificity of salivary tests have indicted that saliva could be useful as a non-invasive technique for detection of *H. pylori* infection [*14,15*]. 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 cases of treatment and eradication [*16,17*]. However, studies of salivary tests are few and have not been performed on endoscopy patients. The aim of this work was to determine the frequency of anti-*H. pylori* serum and salivary antibody positivity among endoscopy patients with (cases) and without (controls) CLD and to determine the validity of these measures compared with the CLO analysis (gold standard) for the same patients.

Methods

Patients and controls

This was a case–control study of *H. pylori* infection in endoscopy patients with and without CLD attending the Abdulaziz University Hospital endoscopy unit in Jeddah. Cases were CLD patients with hepatitis B virus (HBV) infection and/or HCV positive, having autoimmune hepatic disease or schistosomiasis.

The liver disease patients were attending the endoscopy unit for possible gastric or duodenal disease. Controls included patients attending the endoscopy unit because of gastric disease but who were free of hepatic pathology, as determined by a normal liver enzyme profile.

Based on previous research, the prevalence of antibodies to *H. pylori* in CLD patients was estimated at 89% and in controls at 59% [*18*]. The significance level was set at 0.05% and the power at 80%. Using *Medcalc* software, the minimum required sample size was calculated to be 30 subjects in each group. It was estimated that 40% of patients would refuse to participate in the additional tests required, therefore, the required sample size in each group was raised to 50. Consecutive patients admitted to the endoscopy unit were included in the study until the number of patients in each group was at least 50. Due to the high prevalence of CLD among endoscopy patients, their number reached 114 in contrast to 50 patients in the control group.

All participants were informed of the nature of the study and those who were willing to participate signed a consent form.

Data and sample collection

Patients' sociodemographic data were recorded, including age, sex and place of birth.

Unstimulated whole saliva was collected on the day of endoscopy by asking every participant to spit any accumulated saliva into a graded sterile tube. Saliva was then spun and stored at –20 ºC until analysed.

For the 35 patients who agreed to blood sampling, 3 mL of blood were drawn and centrifuged and the supernatant stored at –20 ºC until used.

The CLO test is based on the fact that mucosal biopsy specimens can be inoculated into a medium containing urea and phenol red, a dye that turns pink at pH 6.0 or higher. The pH rises above 6.0 when *H. pylori*, the CLO, metabolizes urea to ammonia via urease activity. The test was performed when gastric biopsy was indicated. Results were recorded for each patient after 1 hour if colouration denoted positivity.

Saliva and serum samples of patients with CLD and controls were tested for anti-*H. pylori* antibodies using an ELISA technique. HM-CAP kits (Enteric Products, Westbury, New York, USA) were calibrated for serum and saliva separately. Elevated lgG or IgA antibody titres were considered to indicate current *H. pylori* infection.

Statistical analysis

The outcome variables, CLO, and saliva and serum positivity were recorded for each participant in each group. The frequency of *H. pylori* infection among CLD patients and controls was stratified by the independent variable (age, sex and nationality). The chi-squared test was used to determine significance and association. Statistical analysis was performed using *SPSS*, version 10.

Results

There were 114 cases (CLD patients) and 50 controls. Gastric, salivary and blood sampling was not possible for every participant. Many patients did not consent to blood sampling and obtaining saliva caused discomfort and several samples were discarded because of the insufficient amount collected. In addition, not all patients were indicated for gastric biopsy; therefore CLO was performed for a total of 97 participants, 79 CLD patients and 18 controls. Usable salivary samples were obtained from 120 participants, 73 CLD patients and 47 controls. Only 35 participants agreed to provide blood samples, 32 CLD patients and 3 controls. In some cases only 1 or 2 of the 3 investigations (CLO, salivary and serum antibodies) were available for a particular patient, therefore the number of patients and controls in different tests and groupings was not identical.

Sociodemographic data of cases and controls are shown in Table 1. Place of

birth and age were significantly associated with the presence of CLD (Table 1), among which HCV infection was commonest followed by HBV then autoimmune liver disease. The endoscopy results showed that gastritis, oesophageal varices and duodenitis were the commonest diagnoses (Table 2). There were several patients with more than 1 endoscopy diagnosis.

Overall, anti-*H. pylori* antibody positivity in saliva was not associated with sex, age or place of birth (Table 3). On the other hand, it was significantly higher than among controls while serum and CLO tests were not significantly different between CLD patients and controls (Table 4).

When the CLO test was used as the gold standard, salivary anti-*H. pylori* antibody positivity showed low sensitivity and high specificity in all participants and also in the group with CLD (Table 5).

In this study, among CLD patients, serum anti-*H. pylori* antibody positivity was highest (87.5%), followed by CLO positivity (51.7%), then salivary antibody positivity (37.2%). In controls, CLO showed least positivity and salivary antibody positivity was significantly higher than in CLD patients. The 3 *H. pylori* tests were not significantly associated with endoscopy diagnosis.

Table 1 **Demographic characteristics of patients and controls**

Variable	CLD patients (*n* = 114) No. (%)	Controls (*n* = 50) No. (%)
Sex[a]		
Male	63 (55.3)	25 (50.0)
Female	51 (44.7)	25 (50.0)
Age (years)[*b]*		
Mean (SD)	49.6 (15.1)	37 (14.2)
Place of birth[*c]*		
Saudi Arabia	63 (55.3)	39 (78.0)
Egypt	27 (23.7)	4 (8.0)
Other	24 (21.1)	6 (12.0)

**Statistically significant at* P < *0.05.*
[a]P = *0.611;* [b]P = *0.001;* [c]P = *0.01.*
CLD = Chronic liver disease; SD = standard deviation.

Table 2 **Cause of chronic liver disease and endoscopy findings**

Finding	No.	%
Chronic liver disease (n = 114)		
Hepatitis B virus	25	21.9
Hepatitis C virus	78	68.4
Autoimmune	6	5.3
Other	48	42.1
Endoscopy diagnosis (n = 164)		
Hiatus hernia	14	8.5
Gastric polyps	4	2.4
Gastric dysplasia	5	3.0
Duodenal ulcer	15	9.2
Gastric reflux	5	3.0
Esophageal varices	32	19.5
Gastritis	50	30.5
Duodenitis	25	15.2
Gastric ulcer	6	3.7
Normal	40	24.4

Discussion

H. pylori infection has been intensely studied in patients with CLD and liver cirrhosis. The possibility of common routes of transmission as well as the frequent presence of gastric diseases in such patients has been reported [*19*]. Further, over-expression of HBV antigens (HBsAg and HBcAg) have been reported to coexist with the expression of *H. pylori* antigen in gastric mucosa, and difficult clearance of those antigens from gastric epithelial cells has been related to persistent *H. pylori* infection [*9*]. In addition, high levels of arterial blood ammonia in CLD patients correlated with severity of liver disease and *H. pylori* eradication was associated with a reduction in arterial am-

Table 3 **Comparison of salivary anti-*Helicobacter pylori* antibodies and selected variables in endoscopy patients**

Variable	Salivary *H. pylori*	
	Positive No. (%)	Negative No. (%)
Sex[a]		
Male	27 (43.5)	35 (56.5)
Female	28 (48.3)	30 (51.7)
Age (years)[b]		
Mean (SD)	46.5 (15.2)	48.5 (5.3)
Place of birth[c]		
Saudi Arabia	33 (48.5)	35 (51.5)
Egypt	12 (48.0)	13 (52.0)
Other	10 (37.0)	17 (63.0)

SD = standard deviation.
[a]*P = 0.737;* [b]*P = 0.323;* [c]*P = 0.581.*

monia levels in those patients [*20*]. Therefore, early treatment of *H. pylori* infection may be beneficial to the prognosis of patients with chronic liver disease.

The prevalence of *H. pylori* infection in the Middle East is higher than in Western Europe and North America [*19,21*]. The infectivity curve increases from 10% in childhood to 89% by the age of 60 years. Conversely, high infectivity rates (71%–92%) among endoscopy patients have been reported in Europe, the United States of America and Africa [*22–26*].

In this study, the high saliva antibody positivity among patients without liver diseases may be due to fact that most of these patients underwent endoscopy because of gastric disease and thus possible *H. pylori* infection, while in the CLD patients endoscopy was performed to explore hepatic-related disorders.

Residence of *H. pylori* in saliva, dental plaque and periodontal pockets is of major importance in order to achieve eradication. Furthermore, elimination of the infection has been found to overcome halitosis, even in positive, yet asymptomatic patients [*10*].

The value of salivary antibody detection as a non-invasive tool for diagnosing *H. pylori* infection has been increasingly explored, especially in wide screening and child management [*21,27*]. It was found that, when 4 cytotoxin genotypes were analysed by polymerase chain reaction in stomach and salivary isolates, there was 95% agreement between stomach *H. pylori* isolates and their corresponding saliva DNA in at least 1 genotype; 86% agreement with 2 genotypes; 59% agreement with 3 genotypes; and 27% agreement with all 4 genotypes. It has been suggested that more than 1 strain of *H. pylori* may exist in the stomach and saliva in the same patient. While salivary antibody tests are not indicated for the clinical diagnosis of *H. pylori* infection, they may be useful for large-scale prevalence surveys, provided they are validated locally and mathematical adjustment can be

Table 4 ***Helicobacter pylori* positivity among endoscopy patients**

Test	Patients with chronic liver disease No. (%)	Controls No. (%)
CLO[a]		
+ve	46 (58.2)	33 (41.8)
–ve	11 (61.1)	17 (38.9)
Salivary antibody[b]		
+ve	20 (36.4)	35 (63.6)
–ve	53 (81.5)	12 (18.5)
Serum antibody[c]		
+ve	7 (87.5)	1 (12.5)
–ve	25 (92.6)	2 (7.4)

[a]*P = 0.177;* [b]*P = 0.001;* [c]*P = 0.553.*
CLO = Campylobacter-*like organism test.*
CLO was performed for a total of 97 patients (57 cases and 40 controls). Salivary samples were obtained from 120 patients (73 cases and 47 controls); of the remaining 44, 75% did not want to provide saliva samples, and the samples of the remaining 25% were excluded due to small (unusable) amounts. Only 35 patients agreed to provide blood samples (32 cases and 3 controls)..

Table 5 **Parameters of serum and salivary anti-*Helicobacter pylori* antibody as screening tests among endoscopy patients**

Test	CLO test		Sensitivity	Specificity	Predictive value	
	+ve	–ve			+ve	–ve
	%	%	%	%	%	%
Serum antibody (all participants)			100.0	15.4	47.6	100.0
+ve	10	11				
–ve	0	2				
Saliva antibody (all participants)			41.7	61.0	55.6	47.2
+ve	20	16				
–ve	28	25				
Saliva antibody (CLD patients)			36.6	75.8	65.2	49.0
+ve	9	5				
–ve	16	15				

CLO = Campylobacter-*like organism.*
CLD = chronic liver disease.

made for misclassification. For example, 1 test, being inexpensive, non-invasive, and easily stored and handled, has been recommended as a valuable tool for studies of the epidemiology of *H. pylori* [*28*].

In this study, when CLO was considered the gold standard for *H. pylori* positivity, saliva showed low sensitivity among all participants as well as among CLD patients separately. On the other hand, specificity of saliva antibody test was particularly high among CLD patients (76%). *H. pylori* CLO positivity was higher among CLD patients while salivary antibody positivity was higher among controls. The study was under-powered to detect differences in prevalence of *H. pylori* between CLD patients and controls detected by the CLO test. Further study based on a larger sample size is needed.

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28. Cockburn M, Collett J, Cox B. Validation of the saliva-based *H. pylori* test, heliSAL, and its use in prevalence surveys. *Epidemiology and infection*, 2001, 126(2):191–6.Table 5 Parameters of serum and salivary anti-*Helicobacter pylori* antibody as screening tests among endoscopy patients.