Detection of *Helicobacter pylori* infection in Egyptian patients with chronic calcular cholecystitis

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ABSTRACT

Reports of *Helicobacter pylori* in biliary tract diseases in humans are very fragmentary, and therefore there is a need for further investigations. This study aims to detect *H. pylori* in the bile and gall bladder (GB) of patients with chronic calcular cholecystitis (CCC), and to determine the association of *H. pylori* infection with gallstone type. Thirty patients with CCC admitted for laparoscopic cholecystectomy were investigated, including upper gastro-endoscopy before cholecystectomy. Rapid urease test and histopathological examination were performed on gastric biopsies. The GB specimens were investigated for the presence of *H. pylori* by immunohistochemistry (IHC). *H. pylori* antigen in bile was detected by enzyme immunoassay. Chemical analysis of gallstones was performed to determine type. Immunohistochemistry testing showed 73.3 % and 66.7% positivity among GB neck and body biopsies, respectively, demonstrating high sensitivity and specificity. A significant association was found between gastric and GB *H. pylori* positivity ($P<0.01$). *H. pylori* antigen was detected in bile from three CCC cases. The greatest number of stones were of the calcium bilirubinate type. Gall bladder positivity for *H. pylori* was accompanied by chronic quiescent gastritis (40.9%). In conclusion, *H. pylori* infection may be an aetiological factor leading to cholecystitis. Gastric colonisation with *H. pylori* could be a source for GB infection, and the organism may act as a lithogenic component, especially in the context of pure pigmented gallstones.

KEY WORDS: Cholecystitis.
Gallbladder.
Helicobacter pylori.
Immunohistochemistry.

Introduction

Helicobacter pylori is a Gram-negative microaerophilic microorganism that can cause chronic gastritis, gastric and duodenal ulcers and also gastric adenocarcinoma.[1] Infection with *H. pylori* results in the development of gastritis in all infected humans.[2,3] *H. pylori* is one of the *Helicobacter* species that have so far been implicated as a cause of hepatobiliary disease.[4–7] Research in this area has been limited by the lack of a gold standard in the diagnosis of these organisms in bile.

Cholecystitis and cholelithiasis with its complications dominate disease of the biliary tract. It has been suggested that *H. pylori* is associated with the pathogenesis of human cholecystitis and cholelithiasis.[8,9] Bacteria play an important role in the formation of biliary stones;[10] however, the exact mechanisms involved in stone formation remain unclear. *H. pylori* could have a role in the formation of cholesterol gallstones.[11–13] As various types of gallstone exist, a correlation between *H. pylori* infection in bile and gallstone type has been recorded.[14]

Information about *H. pylori* contribution to the pathology of the biliary tract in humans is fragmentary, and the need for further investigations has been emphasised. This study aims to detect *H. pylori* in the bile and gall bladder (GB) of patients with chronic calcular cholecystitis (CCC), and to determine the association of *H. pylori* infection with gallstone type.

Materials and methods

Patients

A total of 30 patients (all female; mean age 41 years) were included in the study. Patients were admitted to the Department of Surgery, Medical Research Institute, Alexandria University, scheduled for laparoscopic cholecystectomy for CCC. They were subjected to upper gastro-endoscopy before cholecystectomy and biopsy specimens were obtained from the gastric antrum. Patients were instructed not to take antibiotics or bismuth salts for at least three weeks, or to ingest proton pump inhibitors in the two weeks prior to the upper gastrointestinal endoscopy. The study was carried out according to the principles of the Declaration of Helsinki and it was approved by the institutional ethical committee. Informed written consent was obtained from each patient.

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Specimens

The stomach was examined for the presence of gross pathology and gastric biopsies were taken. Two gastroscopy biopsies from the antrum were collected from each patient, one of which was tested by the rapid urease test (HelicotecUT Plus), following the manufacturer's instructions, immediately after gastroscopy. The other biopsy was fixed in 10% formalin.

Gall bladders were collected and bile was aspirated by a

Table 1. Urease and immunohistochemistry test results versus histological findings of *H. pylori* infection in the gall bladder neck and body biopsies.

	Histopathology						*P*	Sensitivity	Specificity	PPV	NPV
	Negative		Positive		Total						
	No	%	No	%	No	%					
GB neck (Urease Test)											
Negative	6	20.0	4	13.3	10	33.3	0.375	82.6	85.7	95.0	60.0
Positive	1	3.3	19	63.3	20	66.7					
Total	7	23.3	23	76.7	30	100.0					
GB neck (IHC)											
Negative	7	23.3	1	3.3	8	26.7	0.725	95.7	100	100	87.5
Positive	0	0.0	22	73.3	22	73.3					
Total	7	23.3	23	76.7	30	100.0					
GB body (Urease Test)											
Negative	8	26.7	5	16.7	13	43.3	0.415	75.0	80.0	88.2	61.5
Positive	2	6.7	15	50.0	17	56.7					
Total	10	33.3	20	66.7	30	100.0					
GB body (IHC)											
Negative	9	30.0	1	3.3	10	33.3	1.000	95.0	90.0	95.0	90.0
Positive	1	3.3	19	63.3	20	66.7					
Total	10	33.3	20	66.7	30	100.0					

sterile syringe. *H. pylori* antigen was detected in bile using a microwell enzyme-linked immunosorbent assay (ELISA) method. The gall bladders were opened fresh, the gallstones were extracted and imprint slides were performed and stained by haematoxylin and eosin (H&E) stain.

A fresh section from the GB neck and body was crushed in 1 mL saline and the homogenate was used for the urease test. Fresh sections from the GB body and neck were then obtained and fixed in 10% formalin.

The formalin-fixed gastroscopy biopsies were processed to paraffin blocks and stained by H&E to assess morphology and to detect *H. pylori*. Sections (5 µm) from the paraffin blocks of GB neck and body were stained by H&E and were also attached to coated slides for *H. pylori* immunostaining.

Chemical analysis was performed on the gallstones to determine type.[15]

Immunohistochemistry staining

Immunohistochemistry (IHC) was performed on sections (5 µm) from GB neck and body mounted on Superfrost slides (Menzel Glaeser, Germany) using an avidin-biotin peroxidase technique with anti-*H. pylori* rabbit polyclonal antibody (RG-9070; Thermo Scientific, Fremont, USA). Diaminobenzidine tetrahydrochloride (DAB; Dako Cytomation, Denmark) was used as a chromogen to detect the reaction product.

Statistical analysis

Data were assessed using SPSS version 16 software. All statistical analysis was performed using two-tailed tests. $P<0.05$ was considered significant. Mont Carlo exact and McNemar's tests were used. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for comparison with the gold standard.

Results

Table 1 shows urease and IHC results versus histological findings for *H. pylori* infection in the gall bladder neck and body biopsies from patients with chronic calcular cholecystitis. Histopathological examination revealed that *H. pylori* were present mainly on the surface of epithelial cells, in the intercellular zone or within the mucous glands. *H. pylori* were distributed in a dispersed or aggregated manner, similar to that seen in the stomach. At sites where *H. pylori* aggregated, the epithelial cells were degenerate, and eroded. In some parts, an eosinophil-rich inflammatory cell infiltrate was seen (Fig. 1). Figure 2 shows sections immunostained with anti-*H. pylori* antibody.

Out of the 23 histopathology-positive GB neck biopsies and seven negative GB neck biopsies, 15 (65.2%) and 2 (28.6%), respectively, were positive by direct imprint (Fig. 1C). Direct imprint examination showed a sensitivity of 65.2%, specificity 71.4%, PPV 88.2% and NPV 38.5 %. Of the 20 histopathology-positive GB body biopsies and 10 negative GB body biopsies, 15 (75%) and 3 (30%), respectively, were positive by direct imprint, with sensitivity and specificity of 71.4%, PPV 82.4% and NPV 53.8%.

Table 2 illustrates the significant associations between gastric and GB positivity for *H. pylori* infection as detected by IHC. Interestingly, in the histopathology-negative gastric biopsies, *H. pylori* was not detected in either GB neck or body biopsy.

H. pylori antigen was detected in bile in three out of the 30 CCC cases. The highest number of gallstones detected was of the calcium bilirubinate type (46.7%), followed by mixed gallstones (36.7%) and cholesterol stones (16.7%).

Table 3 summarises the histopathological findings from the gastric biopsies in relation to gall bladder neck and body positivity for *H. pylori* infection. The highest percentage of

GB positivity for *H. pylori* was accompanied by chronic quiescent gastritis (40%).

Discussion

Although *H. pylori* is recognised as a human pathogen associated with gastric lesions, up to now the literature ascribing a putative role for *H. pylori* in human hepatobiliary disease has been inconclusive.[16–19]

Currently, several diagnostic tests are used to detect *H. pylori* infection. Each has its own merits and disadvantages in terms of indication, sensitivity, specificity, cost and time.[20] Histopathology is one of the reliable methods for detection of *H. pylori* and in expert hands it has been noted to be as good as microbiology.[21,22] Histological examination permits the evaluation of cell damage and the detection of *H. pylori in situ*, and is currently regarded as the 'gold standard' test.[20 23,24]

In the current study, the infection rate of *H. pylori* among CCC patients was confirmed by IHC in about 95% of GB biopsies with high sensitivity and specificity. These results are supported by previous work;[25,26] however, relatively few were confirmed by IHC in work by Chen *et al*.[27]

In the present study, 11.1% histopathology-negative GB body biopsies were positive by IHC. This is supported by Orhan *et al*.[23] Using IHC with specific antibodies, it is possible to identify *H. pylori* in small numbers as well as in coccoid forms. Some 4–5% of GB biopsies positive by histopathology were negative by IHC, which could be attributed to the presence of *Helicobacter* species other than *H. pylori*.

Interestingly, *H. pylori* infection was negative by histopathology and IHC in two GB body biopsies, while the GB neck biopsies from the same patients were positive. In these cases, ascending infection from the duodenum is the most likely cause.[7]

In the present work, the localisation of bacterial site was not associated with specific pathomorphological GB changes, and these findings are consistent with previous reports.[17,25,28] However, it is possible that the bacterium colonised a previously damaged epithelium. Damage to the epithelial cells caused by *H. pylori* may relate to specific virulence factors in addition to urease enzyme, but an immunological rather than a direct role for *H. pylori* in cholecystitis should be considered. This is supported in the present study by cases positive for *H. pylori* showing intense mixed acute and chronic non-specific inflammatory infiltrate rich in eosinophils, which play a role in disorders such as allergic, immunological and malignant diseases.[29–32]

In the present study, imprint cytology had a sensitivity and specificity of approximately 70%, and is a relatively easy and quick method for detection of *H. pylori*. This finding was consistent with previous reports[33–35] pointing out that imprint cytology may be performed as an adjunct to histopathology as it provides a rapid diagnosis and does not require additional biopsies.

The urease test showed good correlation with the detection rates of other methods, with a sensitivity of 75–80% and specificity of 80–85%. However, a percentage of the urease-negative GB biopsies were positive by

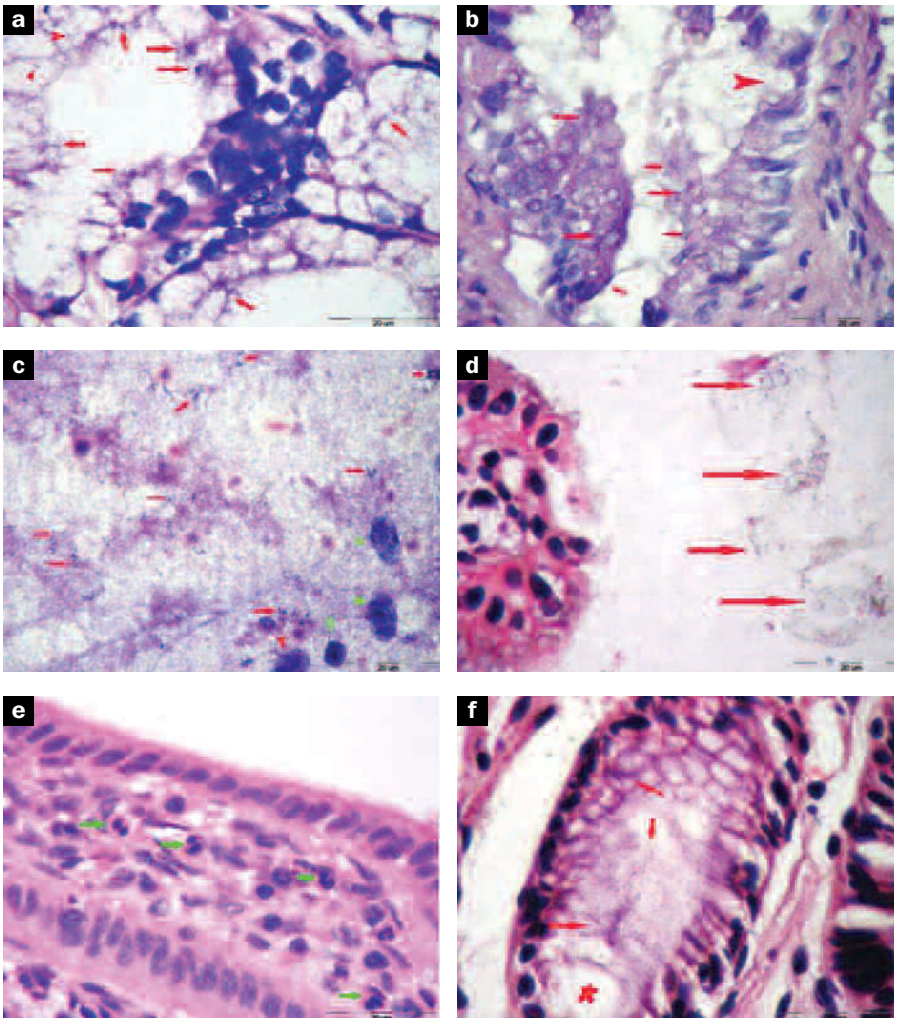

Fig. 1. a) Section in the gall bladder showing intense *H. pylori* colonisation of the mucosal gland lumen (bladder neck) and lining epithelium (arrow), The typical comma-shaped morphology can be appreciated focally (arrow heads). **b)** Photomicrograph of gall bladder mucosa colonised heavily by *H. pylori*, showing irregularity of the surface mucosa with cup-like erosion vacuoles (arrow head), together with adherent bacilli to cell surface (arrows). **c)** Imprint from a case of chronic calcular cholecystitis showing scattered and aggregated *H. pylori* in colonies (arrows) within the mucinous background and adherent to mucin-loaded cells (arrow heads). **d)** Gall bladder mucosa in a case of chronic calcular cholecystitis showing heavy colonisation of the surface mucus film by *H. pylori* (arrows). **e)** Surface mucosal fold from a gall bladder positive for *H. pylori*, showing eosinophil-rich inflammatory infiltrate of the core (arrows). **f)** Section from gastric mucosa showing intense *H. pylori* colonisation of the gastric pit (arrows). Note the cup-shaped erosion of the surface of lining epithelium (star) (H&E staining, original magnification x1000).

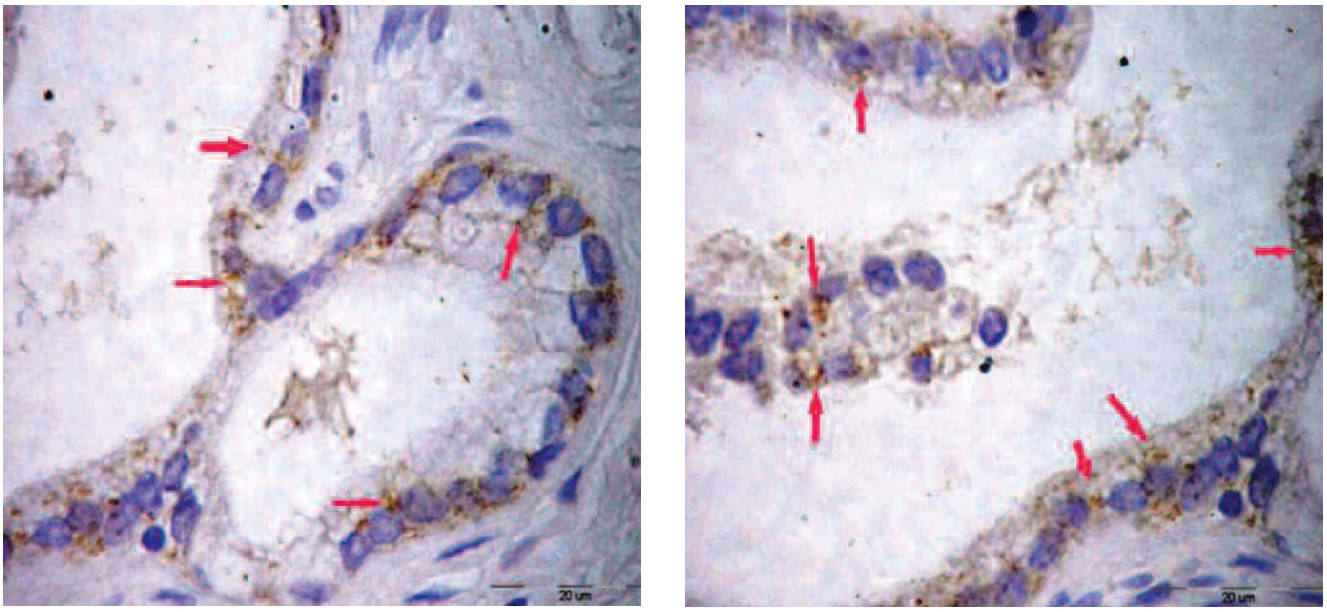

Fig. 2. Gall bladder sections immunostained with a polyclonal antibody specific to *H. pylori* antigens showing strong positivity for *H. pylori* on the surface and lumen with accentuation of the signal at intercellular junctions (original magnification x1000).

histopathology and IHC. These data are supported by previous work.[36,37]

A significant association was found between *H. pylori* detection in GB biopsies and the corresponding gastric antral biopsy specimens, and appears linked to ascending infection from the duodenum.[7] However, further studies are required in order to establish a significant correlation.

H. pylori has adaptive mechanisms that protect the organism from the hostile acidic environment of the stomach. If it survives in the biliary tract then it must have protective mechanisms directed against the adverse effects of an alkaline pH and bile acids. Expression of virulence factors may allow it to survive in different niches.[38]

In the present work, *H. pylori* antigen was present in only three cases. Detection of *H. pylori* antigens in cholecystic bile has been reported by Neri *et al*.[39] The pathways of penetration into bile have not been completely explained, but one possibility is the translocation from the duodenum via Oddi's sphincter. Furthermore, penetration of bacterial antigens into the portal circulation and lymphatic vessels is also possible.[40]

Data from the present study suggest that *H. pylori* could be a contributor to pure pigmented and mixed cholesterol gallstone formation. Calcium bilirubinate gallstones represent the major group associated with GB positivity for *H. pylori*. Colonisation of the GB mucosa by *H. pylori* has been proposed as a potential risk factor for gallstone formation.[41] Theory suggests that colonisation of the mucosa by *H. pylori* may cause chronic inflammation, impaired acid secretion, reduced solubility of calcium salts in the bile, and increased risk of precipitation in the lumen, hence favouring gallstone formation.[42] *H. pylori* may also interact with bile through the production of hydrolysing enzymes. However, other studies[43,44] report no association with gallstone formation. Abayli *et al*.[11] and Nafee *et al*.[45] report that *H. pylori* may be a potential initiator of cholesterol crystallisation or that its colonisation precipitates GB inflammation and subsequent cholesterol stone formation.

Most of the cases reported here showed chronic gastritis (either active or quiescent) on histopathological examination. However, *H. pylori* infection was not associated with certain gastric pathology. A study carried out by Stathopoulos *et al*.[46]

Table 2. Association between IHC detection of *H. pylori* in the gall bladder neck and body biopsies and histological finding of *H. pylori* infection in the corresponding gastric biopsies.

		Gastric biopsy (histopathology)						
		Negative		Positive		Total		
		No	%	No	%	No	%	*P*
GB neck (IHC)	Negative	1	3.3	7	23.3	8	26.7	0.008
	Positive	0	0.0	22	73.3	22	73.3	
	Total	1	3.3	29	96.7	30	100	
GB body (IHC)	Negative	1	3.3	9	30.0	10	33.3	0.002
	Positive	0	0.0	20	66.7	20	66.7	
	Total	1	3.3	29	96.7	30	100	

$P<0.05$ regarded as significant.

Table 3. Histopathological findings of the gastric biopsies in relation to gall bladder neck and body positivity for *H. pylori* infection.

	GB neck (IHC)					GB body (IHC)				
	Negative		Positive		*P*	Negative		Positive		*P*
Histopathology	No	%	No	%		No	%	No	%	
Chronic active gastritis	3	37.5	7	31.8	0.919	3	30	7	35	0.806
Chronic quiescent gastritis	3	37.5	9	40.9		4	40	8	40	
Atrophic gastritis	0	0.0	1	4.5		1	10	0	0	
Atrophic gastritis with gastric dysplasia	0	0.0	1	4.5		0	0	1	5	
Atrophic gastritis with intestinal metaplasia	2	25.0	4	18.2		2	20	4	20	

showed that GB function is not related to the degree of gastritis, and therefore it would appear that several factors and requirements have to be met in order to establish a definitive association between the presence of *H. pylori* and biliary disease.

In conclusion, *H. pylori* infection in the GB may be one of the aetiological factors leading to cholecystitis, and gastric colonisation could be a source for GB infection; however, the precise mechanism requires further verification. Immunohistochemistry staining is simple, sensitive, specific, reproducible and easy to perform for the detection of *H. pylori* infection in the GB biopsies, but further study on a larger number of patients and control groups is needed to ascertain its role in the pathogenesis of CCC. *H. pylori* may act as a lithogenic factor but future research focusing on the effect of bacterial eradication on the development of gallstone will be needed to ascertain whether *H. pylori* is just an innocent bystander or has a role in lithogenesis. □

References

1 Feldman RA, Eccersley AJ, Hardie JM. Epidemiology of *Helicobacter pylori*: acquisition, transmission, population prevalence and disease-to-infection ratio. *Br Med Bull* 1998; **54** (1): 39–53.

2 Bourke B, Jones N, Sherman P. *Helicobacter pylori* infection and peptic ulcer disease in children. *Pediatr Infect Dis J* 1996; **15** (1): 1–13.

3 Fennerty MB. Is the only good *H. pylori* a dead *H. pylori*? *Gastroenterology* 1996; **111** (6): 1773–4.

4 Solnick JV, Schaure DB. Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. *Clin Microbiol Rev* 2001; **14** (1): 59–97.

5 Lin TT, Yeh CT, Wu CS, Liaw YF. Detection and partial sequence analysis of *Helicobacter pylori* DNA in bile samples. *Dig Dis Sci* 1995; **40** (10): 2214–9.

6 Fox JG, Shen Z, Feng F *et al*. Enterohepatic *Helicobacter* spp. identified from humans with primary biliary cirrhosis. *Gut* 1998; *43* (Suppl 2): A3.

7 Fox JG, Dewhirst FE, Shen Z *et al*. Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. *Gastroenterology* 1998; **114** (4): 755–63.

8 Misra V, Misra SP, Dwivedi M, Shouche Y, Dharne M, Singh PA. *Helicobacter pylori* in areas of gastric metaplasia in the gallbladder and isolation of *H. pylori* DNA from gallstones. *Pathology* 2007; **39** (4): 419–24.

9 Nilsson HO, Taneera J, Castedal M, Glatz E, Olsson R, Wadstrom T. Identification of *Helicobacter pylori* and other *Helicobacter* species by PCR, hybridization, and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis. *J Clin Microbiol* 2000; **38** (3): 1072–6.

10 Swidsinski A, Lee SP. The role of bacteria in gallstone pathogenesis. *Front Biosci* 2001; **6**: E93–103.

11 Abayli B, Colakoglu S, Serin M *et al*. *Helicobacter pylori* in the etiology of cholesterol gallstones. *J Clin Gastroenterol* 2005; **39** (2): 134–7.

12 Lee JW, Lee DH, Lee JI *et al*. Identification of *Helicobacter pylori* in gallstone, bile, and other hepatobiliary tissues of patients with cholecystitis. *Gut Liver* 2010; **4** (1): 60–7.

13 Monstein HJ, Jonsson Y, Zdolsek J, Svanvik J. Identification of *Helicobacter pylori* DNA in human cholesterol gallstones. *Scand J Gastroenterol* 2002; **37** (1): 112–9.

14 Stewart L, Ponce R, Oesterle AL, Griffiss JM, Way LW. Pigment gallstones pathogenesis: slime production by biliary bacteria is more important than beta-glucuronidase production. *J Gastrointest Surg* 2000; **4** (5): 547–53.

15 Bauer JD, Ackermann PG, Toro G. Methods in clinical chemistry. In: Bauer JD, Ackermann PG, Toro G eds. *Clinical laboratory methods*. St Louis: Mosby, 1974.

16 Yucebilgili K, Mehmetolu T, Gucin Z, Salih BA. *Helicobacter pylori* DNA in gallbladder tissue of patients with cholelithiasis and cholecystitis. *J Infect Dev Ctries* 2009; **3** (11): 856–9.

17 Silva CP, Pereira-Lima JC, Oliveira AG *et al*. Association of the presence of *Helicobacter* in gallbladder tissue with cholelithiasis and cholecystitis. *J Clin Microbiol* 2003; **41** (12): 5615–8.

18 Bulajic M, Maisonneuve P, Schneider-Brachert W *et al*. *Helicobacter pylori* and the risk of benign and malignant biliary tract disease. *Cancer* 2002; **95** (9): 1946–53.

19 Mendez-Sanchez N, Pichardo R, Gonzalez J *et al*. Lack of association between *Helicobacter* sp. colonization and gallstone disease. *J Clin Gastroenterol* 2001; **32** (2): 138–41.

20 Misra SP, Dwivedi M, Misra V, Gupta SC. Imprint cytology – a cheap, rapid and effective method for diagnosing *Helicobacter pylori*. *Postgrad Med J* 1993; **69** (810): 291–5.

21 Deeba J, Sanjay S, Abida M *et al*. *Helicobacter pylori* in gallbladder disease. *Biomedical Research* 2010; **21**: 437–40.

22 Aktepe OC, Çiftçi IH, fiafak B, Uslan I, Dilek FH. Five methods for detection of *Helicobacter pylori* in the Turkish population. *World J Gastroenterol* 2011; **17** (47): 5172–6.

23 Orhan D, Kale G, Salt›k-Temizel IN *et al*. Immunohistochemical detection of *Helicobacter pylori* infection in gastric biopsies of urea breath test-positive and -negative pediatric patients. *Turk J Pediatr* 2008; **50** (1): 34–9.

24 Razaghi M, Boutorabi SM, Mirjalili A *et al*. Diagnosis of *Helicobacter pylori* infection by ELISA stool antigen and comparison with the other diagnostic methods. *HealthMED* 2010: **4**: 545–51.

25 Sheta E, Elfert A, Amin A *et al*. Molecular and immunohistochemical detection of *Helicobacter pylori* in patients with symptomatic gallbladder stones. *Arab J Gastroenterol* 2007; **8** (1): 5–9.

26 Apostolov E, Al-Soud WA, Nilsson I *et al*. *Helicobacter pylori* and other *Helicobacter* species in gallbladder and liver of patients with chronic cholecystitis detected by immunological and molecular methods. *Scand J Gastroenterol* 2005; **40** (1): 96–102.

27 Chen DF, Hu L, Yi P, Liu WW, Fang DC, Cao H. *H. pylori* exist in the gallbladder mucosa of patients with chronic cholecystitis. *World J Gastroenterol* 2007; **13** (10): 1608–11.

28 Chen DF, Hu L, Yi P, Liu WW, Fang DC, Cao H. *H. pylori* are associated with chronic cholecystitis. *World J Gastroenterol* 2007; **13** (7): 1119–22.

29 Aydemir SA, Tekin IO, Numanoglu G, Borazan A, Ustundag Y. Eosinophil infiltration, gastric juice and serum eosinophil cationic protein levels in *Helicobacter pylori*-associated chronic gastritis and gastric ulcer. *Mediators Inflamm* 2004; **13** (5–6): 369–72.

30 Saitoh O, Kojima K, Sugi K *et al*. Fecal eosinophil granule-derived proteins reflect disease activity in inflammatory bowel disease. *Am J Gastroenterol* 1999; **94** (12): 3513–20.

31 Rothenberg ME. Eosinophilia. *N Engl J Med* 1998; **338** (22): 1592–600.

32 Weller PF. The immunobiology of eosinophils. *N Engl J Med* 1991; **324** (16): 1110–8.

33 Knezević Stromar I, Jakić-Razumović, Knezević-Obad A. Imprint cytology of gastric mucosa biopsy – fast, simple and reliable method for detection of *Helicobacter pylori* infection. *Coll Antropol* 2008; **32** (1): 171–5.

34 Cubukçu A, Gönüllü NN, Erçin C *et al*. Imprint cytology in the diagnosis of *Helicobacter pylori*. Does imprinting damage the biopsy specimen? *Acta Cytol* 2000; **44** (2): 124–7.

35 Yasar N, Muharrem B, Ilyas OH *et al*. Imprint cytology: a cheap effective method for diagnosing *Helicobacter pylori*. *Turk J Gastroenterol* 2000; **1**: 30–3.

36 Abadi AT, Mobarez AM, Ajami A, Rafiee A, Taghwaii T. Evaluation on antibiotic resistance of *H. pylori* isolated from patients admitted to Tooba Medical Center, Sari. *J Mazandaran Univ Med Sci* 2009; **19**: 26–32.

37 Foroutan M, Loloei B, Irvani S, Azargashb E. Accuracy of rapid urease test in diagnosing *Helicobacter pylori* infection in patients using NSAIDs. *Saudi J Gastroenterol* 2010; **16** (2): 110–2.

38 Hynes SO, McGuire J, Wadstrom T. The effect of bile on protein expression by intestinal helicobacters, determined using Protein Chip Technology. *Gut* 2001; **49** (Suppl 2): A67 (Abstract).

39 Neri V, Margiotta M, de Francesco V *et al*. DNA sequences and proteic antigens of *H. pylori* in cholecystic bile and tissue of patients with gallstones. *Aliment Pharmacol Ther* 2005; **22** (8): 715–20.

40 Offner GD, Gong D, Afdhal NH. Identification of a 130-kilodalton human biliary concavalin A binding protein as aminopeptidase *N. Gastroenterology* 1994; **106** (3): 755–62.

41 Myung SJ, Kim MH, Shim KN *et al.* Detection of *Helicobacter pylori* DNA in human biliary tree and its association with hepatolithiasis. *Dig Dis Sci* 2000; **45** (7): 1405–12.

42 Griniatsos J, Sougioultzis S, Giaslakiotis K *et al.* Does *Helicobacter pylori* identification in the mucosa of the gallbladder correlate with cholesterol gallstone formation? *West Indian Med J* 2009; **58** (5): 428–32.

43 Bostanoglu E, Karahan ZC, Bostanolu A, Savas B, Erden E, Kiyan M. Evaluation of the presence of *Helicobacter* species in the biliary system of Turkish patients with cholelithiasis. *Turk J Gastroenterol* 2010; **21** (4): 421–7.

44 Rudi J, Rudy A, Maiwald M *et al*. *Helicobacter* sp. are not detectable in bile from German patients with biliary disease. *Gastroenterology* 1999; **116**: 1016–7.

45 Nafee AM, Hussien HIM; AbdEl Aal SM *et al.* Cholesterol gallstones. *Egyptian Liver J* 2012; **2**: 26–30.

46 Stathopoulos P, Zundt B, Spelsberg FW *et al*. Relation of gallbladder function and *Helicobacter pylori* infection to gastric mucosa inflammation in patients with symptomatic cholecystolithiasis. *Digestion* 2006; **73** (2–3): 69–74.