Al-Kindy College Medical Journal (KCMJ)

Research Article

Association between gallbladder diseases and Helicobacter pylori infection

Ali Hamid Abd-Almahdi[1], Zuhair B. Kamal[2]*

1- Baghdad Teaching Hospital, Medical City Complex, Baghdad, Iraq
2- Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq

* Corresponding to zuhairkamal@kmc.uobaghdad.edu.iq

https://doi.org/10.47723/kcmj.v16i2.263

Article history:
Received 1st July 2020
Accepted 30th November 2020

Keywords:
Chronic cholecystitis, gallstones, H.pylori, Urease test

Abstract

Background: Helicobacter pylorus is one of the most harmful human pathogens & carcinogens. Of the world's population, more than 50% has H. pylori in their upper gastrointestinal tracts. It has been linked to a variety of extra gastric disorders. In correlation to hepatobiliary diseases; recently, the bacterium has been implicated as a risk factor for various diseases ranging from chronic cholecystitis and primary biliary sclerosing cholangitis to gall bladder cancer and primary hepatic carcinomas. However, the association between Helicobacter pylori (H. pylori) and gallbladder diseases is still vague and is controversial.

Aim of the study: To elucidate the association of H pylori and gallbladder diseases (calculus, acalculous, polyp), the feasibility of using rapid urease test in post-operative diagnosis, and many factors related to the bacterium.

Subjects & methods: This case series study was conducted in Al-Kindy Teaching hospital - surgical unit during a period extended for 2 years from September 2016 to September 2018, where patients who suffered from signs and symptoms of gallbladder disease were interviewed using a predesigned questionnaire including age, gender, occupation, residency and whether the drinking water was safe (purified) or not. Physical examination was done including weight status, BMI was calculated (BMI=wt. (kg)/ height (M). A provisional diagnosis of gallbladder disease was confirmed by examination, necessary laboratory investigations (Hematology, Biochemistry, and radiology). Cholecystectomy was done by using Laparoscopic cholecystectomy or open surgery.

The presence of H.pylori in the mucosa of the excised gallbladder was studied by using:
1) Rapid urease kit (HNAN C., LTD)
2) Histopathology & chemical analysis of associated gallstone

Results: Seventy-eight patients undergoing cholecystectomy for symptomatic gallbladder disease, the gallbladder mucosa of 30 patients were tested positive for H. pylori with any one of the tests used in this study. The rapid urease test was sensitive 57.1% and specific 58.3 % of the cholecystectomies performed in our study. The mean age of the studied patients was (34 ± 4 years). Females constitute 73.1% (57 out of the total 78 patients). Of the studied cases; 26 patients (33.3%) were obese. Employee patients constitute 43 (55.1%) of patients, and 53 patients (67.9%) lived in urban areas. Purified water was consumed by 61 (78.2%) of the studied population.

Gallstones were detected in 56 (71.8%) of studied cases, acalculus chronically inflamed gallbladder was found in 20 (25.64%), and 2 cases (2.56%). were found to have gallbladder polyp.

H. pylori infection was diagnosed in 30 (38.5%) of total cases (of the 56 calculus confirmed cases 48.2% were H.pylori positive, and of the 22 acalculus cases 13.6% H.pylori positive).

Conclusion:

A significant association is found between chronic calculus cholecystitis and H.pylori infection. While no significant association was found in correlation with acalculus cholecystitis and other gallbladder pathology. Regarding the feasibility of using the urease kit test, it is found that this test is sensitive 57.1%, and specific 58.3%, as such it is less accurate than histopathology study. A significant correlation was found between age, gender, weight status, and non-purified water *source with H.pylori infection.*

Introduction

Helicobacter pylori are one of the most successful human pathogens & carcinogens, over half of the world's population is colonized with this gram-negative bacterium [1].
Colonization usually persists lifelong if not treated. Members of genus Helicobacter are all microaerophilic, and in most cases are catalase and oxidase-positive, and many but not all species are also urease positive [1].
These species are subdivided into two major lineages, the gastric Helicobacter species and the enterohepatic (nongastric). Both groups demonstrate a high level of organ specificity, such that gastric helicobacter, in general, are unable to colonize the intestine or liver, and vice versa [2].
There is a strong association between H.pylori infection and the development of duodenal ulcers/stomach cancer. However, it is estimated that over 80% of individuals infected with the bacterium are asymptomatic, and it may play an important role in the natural stomach ecology [3].
Of the world's population, more than 50% has H. pylori in their upper gastrointestinal tracts [4]. It is more prevalent in developing countries than in Western countries [5].
It has been linked to a variety of extragastric disorders (coronary heart disease, dermatological disorders such as rosacea and idiopathic urticaria, autoimmune thyroid disease and thrombocytopenic purpura, iron deficiency anemia). The underlying hypothetical mechanisms include chronic low-grade activation of the coagulation cascade, accelerating atherosclerosis, and antigenic mimicry between H. pylori and host epitopes leading to autoimmune disorders [6].
In correlation to hepatobiliary diseases; recently, the bacterium has been implicated as a risk factor for various diseases ranging from chronic cholecystitis and primary biliary sclerosing cholangitis to gall bladder cancer and primary hepatic carcinomas [7].
Other species such as H. hepaticus and H.bilis have been associated with liver infections and even liver and biliary cancer. Many researchers have demonstrated the presence of Helicobacter DNA in the gall bladder of patients with gall stone disease, though none were able to isolate H. pylori [7].
The bacterium CagA protein has been found to have homology with aminopeptidase and hence can increase the gallstone formation [8].
Besides these, it has been proposed that H.pylori increase the lithogenicity by the production of soluble antigens that may bind to and inhibit key hepatobiliary genes like murine. This may lead to modulation of enterohepatic cycling of conjugated bile acids through genetic regulation of absorption at enterocyte level or modulation of the transit time through the gut [8]. Helicobacter antigens mediate host response in form of cytokines, and other inflammatory mediators are also proposed to play a role. However, the exact mechanism and exact role played by Helicobacter is still speculated [8]. As such, this research presents a prospective case series study for detection of H.pylori in the excised gallbladder:
• Histopathological study of the removed gallbladder (special Giemsa stain for H.pylori detection, and biochemical analysis of gallstones).
• Gallbladder mucosal biopsy and application of rapid urease kit test.

Aim of the study: To elucidate the association of H.pylori and gallbladder diseases, the feasibility of using rapid urease kit test in post-operative diagnosis, and many factors related to the bacterium.

Subjects and methods:

This prospective case series study was conducted in Al-Kindy Teaching hospital - surgical unit during a period extended for 2 years from September 2016 to September 2018, where patients who suffered from signs and symptoms of gallbladder disease were interviewed using a predesigned questionnaire including age, gender, occupation, residency and whether the drinking water was safe (purified) or not.

Physical examination was done including weight status, BMI was calculated (BMI=weight (kg)/ height (M) 2.

The provisional diagnosis was confirmed by examination, necessary laboratory investigations (Hematology, Biochemistry, radiology, and others).

Inclusion criteria
- Patients aged (12yr - 70yr) with history / symptoms and signs of gallbladder disease. (calculus, acalculus, and polyp)
- Patients with symptomatic gallstone.

Exclusion criteria
- Individuals on antibiotic/proton pump inhibitors 2 weeks prior to the commencement of the study.
- Patients ≤12 year of age (no pediatric department in Al-Kindy hospital)
- Alcoholic patients (due to altered liver function, decrease bile acid metabolism and excretion, possible associated cirrhosis).
- immune-compromised patient (Hepatitis B&C, HIV, Patients with cirrhosis & chronic renal disease. Due to different clinical course, debilitated patients, different prognosis, and different risk factors for gallbladder diseases)
- Patients with hemolytic diseases (risk for gallstone formation).
- Patients with a history of hepatobiliary or pancreatic surgery which changed the function and normal structure of the biliary system.

For all patients who were included in the study, cholecystectomy was done by using Laparoscopic cholecystectomy or open surgery.
The presence of H.pylori in the mucosa of the excised gallbladder was studied by using:

Rapid urease kit (HNAN C., LTD)
On the basis, that urease hydrolyses urea and produces ammonia, carbon dioxide. The reaction will decrease urea concentration, increase ammonia concentration and PH value. The indicator of the test card will have a color change (from a yellow color to cherry red color). So, the HP presence was noted under the color change reaction. (it is notable to know that this kit is not specific for hepatobiliary associated H.pylori)
The excised gallbladder was opened in the operating theatre, washed with normal saline, H.pylori kit (rapid urease kit) was applied to the mucosa of the gallbladder, looking for changes in kit indicator's color which occurred within few minutes. The result was determined as followed:
Negative: Minimal or No color change (Yellow color).
Positive: Color's change from yellow to cherry red.

Histopathology:
Removed gallbladder / gallstones of all cases were sent for histopathological study (histopathology, special Giemsa stain for H.pylori detection, biochemical analysis of gallstones).

Ethical Considerations
• The proposal of the study was fully discussed and approved by the scientific and ethical committee of Iraqi Board of Surgery.
• The agreement of the scientific committee in the Al-Kindy teaching hospital was taken before the start of the study.
• A written consent was taken from each patient after a full explanation of the aim of the study and ensures that collected data will be used for research purposes only and will be anonymous.

Statistical analysis
The collected data were introduced into Microsoft excel worksheet 16, and loaded into IBM – SPSS V24 to be used in statistical analysis.
Descriptive statistics were presented using tables (No. and frequency, means & standard deviations), while Chi-square & Fisher's exact tests were used for measurement of significance of associations between related variables.
The sensitivity and specificity of the screening test were calculated accordingly.
A P-value less than 0.05 was considered as the cut-off point for discrimination of significance.

Results

This study included 78 patients who suffered from signs and symptoms of gallbladder disease, operated on. Overall, in 30 (38.46%) of 78 patients undergoing cholecystectomy for symptomatic gallbladder disease; the gallbladder mucosa was tested positive for H.pylori with any one of the tests used in this study, and the final diagnosis was achieved through histopathological examinations.

In table 1 which showed an association between H.pylori infection and gallstone; 27 cases (48.2%) of the 56 patients with gallstones have positive H.pylori infection, in comparison with 3 cases (13.6%) of the 22 patients who have no stones found to be positive H.pylori infection. A significant association was recognized between H.pylori infection and gallstone, P-value 0.005.

In table 2 which showed an association between different studied variables and H.pylori infection; 22 cases (62.9%) among patients (>44yr) have H.pylori. A significant association was recorded between older age and getting H.pylori infection, P-value 0.001.

H.pylori positive patients showed 27 (47.4%) were females, as such significant association was recorded, P-value 0.008.

Of the 26 obese patients, 15 cases (57.7%) have H.pylori infection. A significant association was noticed between being obese and getting H.pylori infection. P-value 0.047.

No significant association was noticed between the job of the patient and getting H.pylori infection. H.pylori is positive in 17 (48.6%) of non-employed patients. P-value 0.098.

Residency of patients showed no significant association with H.pylori infection. P-value 0.420.

Eleven cases (64.7%) of non-purified water consumers have H.pylori infection, the association was significant. P-value 0.012.

In table 3 which showed an association between histopathology diagnosis and H.pylori infection; No significant association was found between the type of the stone and H.pylori infection. P-value 0.129.

In table 4, it shows no significant association was recorded between the type of acalculus disease/polyp and H.pylori infection. P-value 1

In table 5 which showed Distribution of H.Pylori results according to the Histopathology & Urease kit; to find out the possibility of usage of H.pylori urease kit as a diagnostic test for detection of H.pylori infection, Urease kit was used in 19 patients (logistic obstacles were encountered in the procurement of remaining kits), and compares the result of this test with the histopathological result of the same patients as the gold standard. As shown in Table 5; the sensitivity of the H.pylori kit in the detection of H.pylori in the mucosa of the removed gallbladder was 57.1%, and specificity was 58.3%.

Table 1.
Association between H.Pylori infection and Gallstone.

Variable		Histopathology				P-value
		Positive H.pylori		Negative H.pylori		
		Count	Row N %	Count	Row N %	
Gallstone	Stone	27	48.2%	29	51.8%	0.005
	No stone	3	13.6%	19	86.4%	

P-value less than 0.05 was considered as cut-off point for discrimination of significance

Table 2.
Association between different studied variables and H.Pylori infection.

Variables		Histopathology – H.Pylori infection				P-Value
		Positive		Negative		
		Count	Row N %	Count	Row N %	
Age	<25	2	10.0%	18	90.0%	0.001
	25-44	6	26.1%	17	73.9%	
	>44	22	62.9%	13	37.1%	
Gender	Male	3	14.3%	18	85.7%	0.008
	Female	27	47.4%	30	52.6%	
Weight status	Normal	6	30.0%	14	70.0%	0.047
	Overweight	9	28.1%	23	71.9%	
	Obese	15	57.7%	11	42.3%	
Occupation	Employed	13	30.2%	30	69.8%	0.098
	Non-Employed	17	48.6%	18	51.4%	
Residency	Urban	22	41.5%	31	58.5%	0.420
	Rural	8	32.0%	17	68.0%	
Water Source	Non purified	11	64.7%	6	35.3%	0.012
	Purified	19	31.1%	42	68.9%	

P-value less than 0.05 was considered as cut-off point for discrimination of significance

Table 3.
Association between Histopathology diagnosis and H.Pylori infection (Calculus & Acalculus cases).

Histopathology Result (Calculus cases)	Number	H.Pylori positive		H.Pylori negative		P value
		N	%	N	%	
Calculus-No biochemical analysis	7	1	14.3	6	85.7	0.129
Pure cholesterol	19	9	47.4	10	52.6	
Mixed stone	30	17	56.7	13	43.3	
Total	56	27		29		

**Fisher's Exact Test was applied instead of Chi-Square test, P-value less than 0.05 was considered as cut-off point for discrimination of significance*

Table 4.
Association between Histopathology diagnosis and H.pylori infection (Acalculus & polyp)

Histopathology Result (Acalculus & polyp)	Number	H.Pylori positive		H.Pylori negative		P value
		N	%	N	%	
Polyp	2	0	0	2	100	*1
Acalculus chronically inflamed	20	3	15	17	85	
Total	22	3		19		

**Fisher's Exact Test was applied instead of Chi-Square test. P-value less than 0.05 was considered as cut-off point for discrimination of significance*

Table 5.
Distribution of H.Pylori results according to Histopathology & Urease kit

		H.Pylori – Histopathology Result				Total
		Positive		Negative		
		No	%	No	%	
H. Pylori –	Positive	4	57.1	5	41.7	9
Urease kit	Negative	3	42.9	7	58.3	10
Total		7		12		19

Sensitivity=57.1%, Specify=58.3%, PPV=44.5%, NPV=70%, Accuracy of the test=58%.

Discussion

The presence of H. pylori in bile can be detected using different methods. Of which, the best way is to grow H. pylori in cultures. But owing to its microaerophilic properties, it is particularly hard to culture the bacterium that dies when they contact air.

Previous studies with different methods have revealed the presence of H. pylori in the biliary tract in 50–60% of patients studied [9-11]. H. pylori are found in 80% of the population in Turkey and similar countries [12].

H. pylori can resist bile salts and might survive and colonize within the biliary tract. The route of infection, however, is not clear.

It has been estimated that 30-40% of the US population is infected with H. pylori and 80% of people in the developing countries are infected during childhood [13-15].

The rapid urease test is easy to use and reliable [16]. This test was sensitive 57.1% and specific 58.3 % of the cholecystectomies performed in this study (gallbladder mucosal biopsy was applied to urease kit test). It is higher than the result was done by Wafi Attaallah et al studied gallstones and H.pylori infection; a study which showed positive rapid urease test in 22% of patients (21 patients out of a total of 95) [17]

While it is lower than the study which was demonstrated by Li Lin Lim et al which showed that the rapid urease test can offer a sensitivity of 80%-99% and a specificity of 92%-100% [18].

False-positives are rare and when present may be due to the presence of other urease containing organisms such as Proteus mirabilis, Citrobactor freundii, Klebsiella pneumonia, Enterobactor cloacae, and Staphylococcus aureus [19]

In the present study, 48.2% of patients with gallstones have positive H.pylori infection, in comparison with 13.6% of patients who have no stones found to be positive H.pylori infection. A significant association was recognized between H.pylori infection and gallstone, P-value 0.005. S.Y. Guraya et al showed 34.7% of patients (of a total of 95 patients) were positive with H.pylori antigens in bile [20].

Arshad Hussein et al demonstrated the presence of H.pylori in 55% of patients with calculus cholecystitis (out of 100 total patients) [21]

Wafi Attaallah et al demonstrate the presence of H.pylori in the gallbladders of 37% of patients with symptomatic gallstones [17].

While Yucebilgili and colleagues showed that despite 22% of samples were positive for bacteria; no significant association was found between H. pylori infection and cholelithiasis [22].

In this study, there is a significant association between older age and getting H.pylori in gallbladder disease (P-value 0.001).

Haitham I. Baqir in a similar study conducted in Iraq, Anbar university; showed higher prevalence among age groups 45-54 yr (86.6%) and >54 yr (88.7%) [23].

Ramakumar Manimaran's study showed Age-wise distribution showed prevalence rate progressively increased with age and the high prevalence was seen in the 41 to 50 years age group [24].

This study shows a significant association between female gender with H.pylori prevalence among patients with symptomatic calculus gallbladder disease.

Arshad Hussein et al demonstrated a significant association of H.pylori and female gender (67.3%, P-value 0.03). A study was conducted in Pakistan [21].

Many showed prevalence is independent of gender [25], some showed more prevalence in females [26, 27]. Other studies showed the opposite [28, 29].

Regarding increase BMI; this study share results with other studies which showed a significant association with obesity.

It is similar to the result by Ahmed Morad Hashim et al who showed H.pylori prevalence in obese 62% (p-value 0.052) than non-obese (30). Arsalan et al demonstrate a similar result of H.pylori prevalence in obese patients 66.2% [31].

Concerning socioeconomic status (un-employed 48.6% H.pylori positive, rural 32% H.pylori positive, 64.7% of non-purified water consumers have H.pylori infection with the significant association), results were similar to Haitham I. Baqir et al showed 80.3% prevalence in the low socio-economic group [27]. A similar result was demonstrated by Klein et al [26], Hopkins RJ. Et al who showed Seropositivity was > 60% in lower socioeconomic groups [27], as well.

Seyda T et al showed a significant association between the increased number of household members and low socioeconomic status, and H.pylori positivity (P-value <0.001) [32].

Significant association with H.pylori positivity in rural areas was highlighted by Seyda T et al [32] which is different from the result of the present study.

It is not unusual that transmission between infants and non-parental caretakers by contaminated food, water, or via intensive contact may have a greater influence than within-family transmission [33].

Close and careful monitoring of bottled mineral water production may reduce the risk of H. pylori transmission into the human population, as contaminated water may be the source of virulent and resistant strains of H. pylori [34].

Conclusion

A significant association is found between chronic calculus cholecystitis and H.pylori infection. While no significant association was found in correlation with acalculus cholecystitis and other gallbladder pathology., regarding the feasibility of using the urease kit test, it is found that this test is sensitive 57.1%, and specific 58.3%, as such it is less accurate than histopathology study. A significant correlation was found between age, gender, weight status, and non-purified water source with H.pylori infection.

Funding

This research did not receive any specific fund.

Conflicting Interest

No conflict of interest.

References

[1] Johannes G.Kusters, Arnoud H. M. van vliet, and Ernst J. Kuipers. "Pathogenesis of Helicobacter pylori infection. 2006. Clin. Microbiol. Rev. 19(3): 449-90 doi:10.1128/CMR.00054-05.

[2] Solnick, J. V., and D. B. Schauer. Emergence of diverse Helicobacter species in the pathogenesis of gastric and enterohepatic diseases. 2001. Clin. Microbiol. Rev. 14:59-97

[3] Blaser MJ , "Who are we? Indigenous microbes and the ecology of human diseases" (PDF). EMBO Reports. 2006; 7 (10): 956–60. doi:10.1038/sj.embor.7400812. PMC 1618379.

[4] Amieva, Manuel; Peek, Richard M, "Pathobiology of Helicobacter pylori–Induced Gastric Cancer". Gastroenterology. 2016; 150 (1): 64–78. doi:10.1053/j.gastro.2015.09.004. ISSN 0016-5085. PMC 4691563 .

[5] "Helicobacter pylori - Chapter 3 - 2016 Yellow Book | Travelers' Health | CDC". wwwnc.cdc.gov. 9 June 2015. Retrieved 25 April 2017.

[6] Gasbarrini, A., E. Carloni, G. Gasbarrini, and S. A. Chisholm. Helicobacter pylori and extragastric diseases—other Helicobacters. Helicobacter 2004; 9(Suppl. 1):57-66.

[7] Jafri Deeba, Singhal Sanjay, Malik Abida, Khan Athar. Helicobacter pylori in gall bladder disease. Biomedical Research 2010; 21(4).

[8] Kirk J. Maurer, Melanie M. Ihrig, Arlin B. Rogers, Vivian Ng, Guylaine Bouchard, Monika R. Leonard, et al. Identification of cholelithogenic enterohepatic Helicobacter species and their role in murine cholesterol gallstone formation. 2005; 128 (4), 1023–33.

[9] N. Figura, F. Cetta, M. Angelico et al., "Most Helicobacter pylori-infected patients have specific antibodies, and some also have H. pylori antigens and genomic material in bile: is it a risk factor for gallstone formation?" Digestive Diseases and Sciences. 1998; vol. 43, no. 4, pp. 854–62.

[10] J. G. Fox, F. E. Dewhirst, Z. Shen et al., "Hepatic Helicobacter species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis," Gastroenterology. 1998; vol. 114, no. 4 I, pp. 755–63.

[11] V. Neri, M. Margiotta, V. De Francesco et al., "DNA sequences and proteic antigens of H. pylori in cholecystic bile and tissue of patients with gallstones," Alimentary Pharmacology and Therapeutics, 2005; vol. 22, no. 8, pp. 715–20.

[12] World Gastroenterology Organisation, "World gastroenterology organisation global guideline: helicobacter pylori in developing countries," Journal of Digestive Diseases, 2011; vol. 12, pp. 319–26.

[13] 13. Dixon MF. Helicobacter pylori and peptic ulceration; histopathological aspects. J Gastroenterol Hepatol 1991; 6:125-30.

[14] 14. Williams D Chey, Benjamin CY Wong. American college of gateroenterology guideline on the management of Helicobacter pylori infection. Am Coll of Gastroenterology 2007 ;(102):1808-20.

[15] 15. Monstein HJ, Jonsson Y, Zdolsek J, Svanvik J. Identification of Helicobacter pylori DNA in human cholesterol gallstones. Scand J Gastroenterol 2002; 37: 1129.

[16] 16. Chen YK1, Godil A, Wat PJ. Comparison of two rapid urease tests for detection of Helicobacter pylori infection. Dig Dis Sci. 1998 Aug; 43(8):1636-40.

[17] Wafi Attaallah,, Nese Yener, M. Umit Ugurlu, Manuk Manukyan,, Ebru Asmaz,, A. Ozdemir Aktan. Gallstones and concomitant gastric helicobacter pylori infection. Gastroenterol Res Pract. 2013; 643109. Published online 2013 May 19. doi: [10.1155/2013/643109], PMCID: PMC3671525, PMID: 23762037

[18] Li Lin Lim, Khek Yu Ho, Bow Ho, and Manuel Salto-Tellez. Effect of biopsies on sensitivity and specificity of ultra-rapid urease test for detection of Helicobacter pylori infection: A prospective evaluation. World J Gastroenterol. 2004 Jul 1; 10(13): 1907–1910. Published online 2004 Jul 1. doi: 10.3748/wjg.v10.i13.1907

[19] Osaki T, Mabe K, Hanawa T, et al. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of Helicobacter pylori infection. J Med Microbiol 2008;57:814-9. [PubMed]

[20] 20. S.Y. Guraya, A.A. Ahmad, S.M. El-Ageery, H.A. Hemeg, H.A. Ozbak, K. Yousef, N.A. Abdel-Aziz. The correlation of Helicobacter Pylori with the development of cholelithiasis and cholecystitis: the results of a prospective clinical study in Saudi Arabia. Eur Rev Med Pharmacol Sci 2015; 19 (20): 3873-80

[21] 21. Arshad Hussain Abro, Irfan Zafar Haider*, Sarfraz Ahmad** Liaquat University of Medical and Health Sciences, Jamshoro, *Combined Military Hospital, Abbottabad, **Abbottabad International Medical College, Abbottabad, Pakistan. J Ayub Med Coll Abbottabad 2011;23(1)

[22] 22. Yucebilgili K, Mehmetoglu 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[PubMed]

[23] 23. Haitham I. Baqir, Assam M. Abdullah, Anton S. Al-Bana, Hadi M. Al-Aubaidi. Sere-prevalence of Helicobacter pylori infection in unselected adult population in Iraq (Anbar University). 2002, IJGE Issue 3 Vol 1

[24] 24. Ramakumar Manimaran, Ananthi Boj. Seroprevalence of Helicobacter pylori in patients with symptoms of upper gastro intestinal tract at a tertiary care hospital, Int J Res Med Sci. 2016; 4(11): 4945-8. doi: 10.18203/2320-6012.ijrms20163796

[25] 25. Macarthur C. Saunders N, and Feldman W.,Helicobacter pylori, gastroduedinal Disease, and recurrent abdominal pain in children-JAMA. 1995; 1; 273:729-34.

[26] 26. Klein PD, Gilman RH, Leon-Barua R. Diaz F, Smith EO, and Graham DY. The Epidemiology of Helicobacter pylori in Peruvain children between 6 and 30 months of age. Am J Gastroenterol; 1994; 89: 2196-200.

[27] 27. Hopkins RJ. Vial PA Ferriccio C, Ovalle J, Prado P, Sotomayor V, Russell RG, Wasserman SS. and Morris JG Jr. Seroprevalence of Helicobacter pylori in Chile: vegetables may serve as one route of transmission. J Infect Dis. 1993; 1068:222-6.

[28] 28. Goodman KJ, Correa P, Tengana-Aux HJ, Ramirez H. DeLany JP, Guerrero-Pepinosa O, Lopez-Quinones M, et al. Helicobacter pylori infection in the Colombian Andes: a population-based study of Transmission pathways. Am J Epidemiol; 1996; 144: 290-9.

[29] 29. Ma- L, You WC, Gail MR Zhang L. Blot WJ, Chang YS, Jiang J, Liu WD, Hu YR, Brown LM, Xu GW, and Fraumeni JF Jr. 1998. Helicobacter pylori infection and mode of transmission in a population at high risk of stomach cancer. Int J Epidemiol 1998; 27: 570-3.

[30] 30. Ahmed Morad Hashim, Abdulmenem Abualsel, Neveen Rashad Mostafa, Jenan Obaid, The Prevalence of Helicobacter Pylori Infection in Obese and Non-Obese Subjects Attending the Endoscopy Unit, Bahrain Med Bull 2015; 37(4): 234 - 6

[31] 31. Arslan E, Atilgan H, Yavaşoğlu I. The Prevalence of Helicobacter Pylori in Obese Subjects. Eur J Intern Med 2009; 20(7):695-7.

[32] 32. Seyda T, Derya C, Fusun A, Meliha K., The relationship of helicobacter pylori positivity with age, sex, and ABO/Rhesus blood groups in patients with gastrointestinal complaints in turkey. 2007 Jun; 12(3): 244-50.

[33] 33. Vale FF, Vítor JM , Transmission pathway of Helicobacter pylori: does food play a role in rural and urban areas?, Int J Food Microbiol. 2010 Mar 31;138(1-2):1-12.

[34] 34. Reza Ranjbar, Faham Khamesipour, Nematollah Jonaidi-Jafari,et al, Helicobacter pylori in bottled mineral water: genotyping and antimicrobial resistance properties, BMC Microbiol. 2016; 16:40.