Journal of the Egyptian Society of Parasitology, Vol.44, No.1, April 2014
J. Egypt. Soc. Parasitol. (JESP), 44(1), 2014: 275 – 283

EVALUATION OF SALIVARY AND SERUM ANTI-HELICOBACTER PYLORI IN EGYPTIAN PATIENTS WITH H. PYLORI RELATED GASTRIC DISORDERS

By
EFFAT ABD EL-MONEM EL-FAKHFAKH[1], IMAN FAWZY MONTASSER[1*], and REHAM ALI AHMED KHALIFA[2]
Department of Tropical Medicine[1], and Department of Microbiology and Immunology[2], Faculty of Medicine, Ain Shams University, Cairo 11566, Egypt

Abstract

Helicobacter pylori is a common and important transmissible bacterial human pathogen. Although several diagnostic tests are available for the detection of *H. pylori* infection, all of them have both advantages and disadvantages, and none can be considered as a single gold standard. Serological methods analyzing (serum and saliva) by using enzyme immunoassays, which are simple, reproducible and inexpensive, can detect either antigen or antibody.

This study evaluated the frequency of anti- *H. pylori* serum and salivary antibodies positivity among Egyptian patients with gastric disorders and the validity of salivary, serum serological tests for diagnosis of *H. pylori*, comparing this with gold standard tests performed on endoscopy biopsy. This prospective, case-controlled study included 45 Egyptian patients who attended Ain Shams University Hospitals Cairo, Egypt between January 2013 and June 2013.

There were 29 males &16 females their mean age was 51.78±7 (range 18-60). Among the ulcerogenic drugs, Aspirin was the most common drug (46.7%).The evidence revealed the sensitivity of Rapid Urease Test (RUT) was 100%, specificity was 71.4%, Positive Predictive value (PPV) was 88.6% and Negative Predictive value (NPV) was 100%. The sensitivity of serum IgG was 68.97% and specificity was 42.86%; while the sensitivity of serum IgA was 89.6 % and the specificity was 50 %. Correlating the salivary IgG results with *H. pylori* status diagnosed by culture, salivary IgG succeeded to diagnose 19 cases from the 31 positive *H. pylori* patients with a sensitivity of 63.33% & specificity of 92.86% whereas the results of salivary IgA showed a sensitivity of 80% and specificity of 92.86% .

Keywords*:* Patients, *Helicobacter pylori*, salivary immunoglobulins, gastritis.

Introduction

Helicobacter pylori is the first formally recognized bacterial carcinogen and is one of the common human pathogens, as over half of the world's population is colonized with this gram-negative bacterium. Unless treated, colonization usually persist lifelong (Kusters *et al,* 2006). However, the prevalence of infection varies geographically ranging from 20 to 50% in industrialized countries to over 80% in developing countries (Dzierzanowska, 2006). Since *H. pylori* was first isolated from human gastric biopsy material in 1983, the gold standard diagnostic test has remained histological analysis and culture for antral biopsy specimen, these technique was invasive and may take 24-72 hours, and therefore there is a need for good serological test that would avoid need for endoscopy (Christie *et al,* 1996).

Serological testing is the most widely available test for detection of *H. pylori* with relatively high negative predictive value. Serology not affected by local changes in stomach that could lead to false negative results in other tests. Furthermore in patients treated with Proton pump inhibitors (PPIs), if it not possible to stop them for at least two weeks validated IgG serology test (ELISA) mught be used (Tonkic *et al,* 2012).

Sensitivity and specificity of salivary tests have indicated that saliva could be useful as none invasive technique for detection of *H. pylori* infection (Feteih *et al,* 2009).There are several methods available for diagnosis of *H. pylori* infection, but none of them is ideal and

therefore in many clinical situation several tests are needed (Veijola, 2007).

Evaluation of a new salivary tests for diagnosis of *H. pylori* comparing with gold standard evidence of *H. pylori* and with serum serological tests have advantages of being none invasive simple test that can easily performed in the general practice setting; collection is easy for patients and health care personnel and greatly reduces the risk of blood born infection (Christie *et al,* 1996).

The aim of the study was to evaluate the frequency of anti-*H. pylori* serum and salivary antibodies positivity among Egyptian patients with gastric disorders and the validity of the new salivary, serum serological tests for diagnosis of *H. pylori*, comparing this with gold standard tests performed on endoscopy biopsy.

Subjects, Materials and Methods

This prospective, case-controlled study included 45 Egyptian patients who attended Ain Shams University Hospitals Cairo, from January 2013 to June 2013. The study was approved by the Ethics Committee of Ain Shams University Hospitals, Cairo, Egypt in accordance with local research governance requirements. The principle of the study was explained to each patient, with particular highlighting on the steps of the work and patients sign of acceptance.

Inclusion Criteria: Adult Egyptian patients, 18-60 years old with symptoms suggestive of gastritis, peptic ulcer,

Exclusion Criteria: Recent use of medication (within month) for *H. pylori* related disorders, and ever co-morbidity as end stage renal disease and heart failure**.**

The primary outcome measures were 1- Assessment of the impact of different anthropometric measures on clinical, endoscopic and pathological severity of GERD in Egyptian patients, 2- The relation between serum leptin and clinical, endoscopic and pathological severity of GERD in Egyptian patients.

Sample size of 45 candidates was selected with study power of 80%, significance criterion of 0.05.

All patients were subjected to: 1- Full history taking and thorough clinical examination. 2- Laboratory investigations including **C**BC and Liver function tests (ALT, AST, total bilirubin, albumin, prothrombin time & concentration). 3- Upper endoscopy using fibro-optic endoscopy (Pentex A 11038). Thorough examination of the esophagus, stomach and duodenum any lesion was documented. 4- Endoscopic biopsies were taken by the standard biopsy forceps from the antrum of the stomach or any luscious lesions and transported in transport media prepared (Siu *et al,* 1998), sterile broth that contained brain heart infusion (Oxoid, Basingstoke, United Kingdom) broth with horse serum (5%), yeast extract (0.25%), 6mg of vancomycin per liter, 4mg of amphotericin B per liter, and 20mg of nalidixic acid per liter (BHI-VAN). Then specimens were taken for the following bacteriological & serological tests:

Rapid Urease Test (RUT): Biopsy of gastric antrum was used to detect urease activity of *H. Pylori* in gastric mucosal biopsies via color (PH) change resulting from the breakdown of urea by urease into ammonia. This was done using HelicotecUT Plus paper Rapid Urease test kit (Strong Biotech Corporation, Taiwan). Diagnosis by HelicotecUT Plus was interpreted within 5min up to one hour, yellow color represented negative result and change to pink or magenta represented positive result.

Culture and Sensitivity: Culture material was done on selective media, BD Helicobacter media (Becton Dickinson Diagnostic systems - Heidelberg/ Germany), and determination of antibiotic sensitivity pattern to first line *anti-Helicobacter* antibiotics clarithromycin and amoxicillin, second line antibiotics as tetracycline and third line antibiotics as levofloxacin by disc diffusion method using the following discs clarithromycin (CLR) (15µg), amoxicillin (AML) (10µg), tetracycline (TE) (30µg) and levofloxacin (LE) (5µg) supplied by Oxoid, England, and also using Hi-Comb test strips with concentrations CLR (240-0.001µg), AML (240-0.001 µg),

TE (240-0.01µg) and LE (240-0.005µg) supplied by Hi-media, India.

Test Procedure: Biopsy specimen's grinded or minced using sterile scalpel with a small amount of sterile physiological saline before they are applied to the medium. The homogenate placed immediately on the medium surface and should be caught with the sterile loop and then streaked over the surface using streak method.

The inoculated plates were incubated for 3 to 5 days at 35±2°C in a microaerobic atmosphere, e.g. in a GasPak jar with an atmosphere provided by using the Anaeogen GasPak, Oxoid, England. After incubation, the plates showed isolated colonies in the areas where the inoculum was appropriately diluted. *Helicobacter pylori* colonies are tiny to medium-sized and translucent. A Gram stain from respective colonies revealed Gram negative, slightly curved rods. Final identification was done using appropriate biochemical tests. A positive urease, oxidase, and catalase reaction were indicative of *H. pylori.*

Serum and salivary *H. pylori* IgG and IgA by using ELISA: Blood samples were collected by venipuncture in 10 ml evacuated tubes containing EDTA or heparin, with the usual precautions. Serum samples were stored at temperatures of -20ºC. Salivary samples saliva was collected on the day of endoscopy by asking every participant to spit any accumulated saliva into a graded sterile container. Saliva was then spun and stored at -20 ºC until analyzed.

Samples were diluted (1/100) for Serum: 1- 0.010ml (10µl) of each patient specimen was dispensed into 1 ml of serum diluent. Covered and mixed thoroughly by inversion, and stored at 2-8 ºC for up to 48 hrs. 2- Salivary samples not diluted. 3- The microplates' well was formatted for each serum reference, control and patient specimen to be assayed in duplicate. Any unused micro-well strips were back into the aluminum bag, sealed and stored at 2-8ºC. 4- 0.025 ml (25µl) of appropriate serum reference, control and patient specimen was pipetted into the assigned wells for IgG determination. For IgA 0.050 ml (50µl) of the appropriate serum reference, control or diluted patient specimen was pipetted into the assigned wells. 5- 0.100 ml (100 µl) of *H. pylori* Biotinylated conjugate solution was added. 6- Microplate gently swirled for 20-30 seconds to mixed, covered and incubated 60 minutes at room temperature. 7- The contents of microplates were discarded by decantation or aspiration. If decanting, the plate was dried with adsorbent paper. 8- 30µl of wash buffer was added, decanted or aspirated. Repeat 2 additional times for total of 3 washes. 9- 0.100 ml (100 µl) of enzyme antih-IgG, IgA Conjugate Solution was added to all wells. 10- Microplate swirled gently, covered and incubated for 30 minutes at room temperature. 11- Steps 5, 6 were repeated. 12- 0.100 ml (100µl) of working substrate solution was added to all wells and incubated for 15 minutes at room temperature. 13- 0.050 ml (50µl) of stop solution was added to each well and the microplate swirled gently for 15-20 seconds to mix. 14- Absorbance in each well was read at 450nm in microplate reader.

Interpretation: IgG and IgA antibodies to *H. pylori* was confirmed when the serum level exceeds 20U/ml.

Statistical analysis: Data was IBM computer using SPSS as follows: Description of quantitative variables in form of mean and standard deviation; Description of qualitative variables in form of frequency and percentages. Unpaired t test (t value) was used to compare a quantitative variable between two independent groups in parametric data. Mann Whitney test (Z value) was used instead of t test to compare a quantitative variable between 2 independent groups when data is non-parametric (SD > 25% of mean). Chi Square test (X2 value) was used to compare a qualitative variable between 2 independent groups. Spearman correlation test (rho value) was used to rank different non parametric variables against each other's either positively or inversely. P value (non-significant if >0.05, significant if < 0.05. Sensitivity (ability to detect +ve cases) = true +ve / (true +ve + false –ve). Specificity (ability to exclude -ve cases) = true –ve / (true –ve+false +ve). Positive predictive value (PPV) = percent of true +ve to all +ve cases. Negative predictive

value (NPV) = percent of true –ve to all –ve cases.

Results

The patients were 29 males &16 females with mean age of 51.78±7 (range 18-60). The patients (60%) live in rural areas, 31.1% were smokers and 22.2% were alcoholics. Aspirin was the most common drug (46.7%), followed by NSAIDs (26.7%). The endoscopy of patients showed more than one findings, which varied from reflux esophagitis in 25 (55.6%), congestive gastropathy in 20 (44.4%), esophageal varices in 14 (31.1%), gastritis in 23(51.11%), duodenitis in 17 (37.78%), gastric ulcer in 3 (6.7%) and duodenal ulcer in 10 (22.2%) patients (Tab. 1).

Culture positive (Tab. 2) was found in 31 (68.9%) patients and negative in 14 (31.1%). While sensitivity to clarithromycin was (54.84%), to amoxicillin was (38.71%), to tetracycline was (80.65%) and to levofloxacin was (70.97%).

Rapid urease test was positive in 35 patients (77.8%) and negative in 10 (22.2%). The serum and salivary *H. pylori* antibodies (Tab. 3) showed serum IgG positivity in 28patients (65.12%), serum IgA positivity in 33 (76.74%), while salivary IgG positivity in 20 patients (45.45%) and salivary IgA positivity in 25 (56.82%). Comparison between rapid urease test and culture showed sensitivity of RUT was 100%, specificity was 71.4%, PPV was 88.6% and NPV was 100%. Correlated serum IgG with *H. pylori* diagnosed by culture, serum IgG diagnosed 20 /31 positive patients with 68.97% sensitivity, 71.43 PPV, 42.86% specificity and 40% NPV. Serum IgA diagnosed 26/31 positive patients with 89.66% sensitivity & 78.79%, PPV also specificity was 50% & NPV 70%. Salivary IgG diagnosed 19 out of 31 positive patients with 63.33% sensitivity, 95% PPV, 92.86% specificity & NPV 54.17%. Salivary diagnosed 24/31 positive patients with 80% sensitivity, 96% PPV, 92.86% specificity and 68.42 % NPV (Tab. 4).

Table 1: Endoscopic finding in the studied patients. †

Variables	Number (N=45)	Percentage%
GERD	25	55.6
Gastritis	23	51.1
Duodenitis	17	37.7
Peptic ulcer disease:		
Gastric ulcer	3	6.7
Duodenal ulcer	10	22.2
Congestive gastropathy	20	44.4
Esophageal varices	14	31.1
No abnormality detected	3	6.6

GERD= Gastroesophageal Reflux disease, † Values = number (percentage).

Table 2: Culture and antibiotic sensitivity test in the study group†

Variables	Number (N=45)	Percentage %
Positive	31	68.9
Antibiotic sensitivity:		
Clarithromycin		
• Sensitive	17	54.84
• Resistance	14	45.16
Amoxicillin		
• Sensitive	12	38.71
• Resistance	19	61.29
Tetracyclin		
• Sensitive	25	80.65
• Resistance	6	19.35
Levofloxacin		
• Sensitive	22	70.97
• Resistance	9	29.03

Table 3: Rapid urease test, serum and salivary *H. pylori* antibodies (IgG, IgA) in patients. †

Variables	Number	Percentage %
RUT positive	35	77.8
Serum *H. pylori* antibodies (positivity)*		
• IgG	28	65.12
• IgA	33	76.74
Salivary *H. pylori* antibodies (positivity)**		
• IgG	20	45.45
• IgA	25	56.82

RUT= rapid urease test, *Serum (IgG, IgA) for 43 patients (2 patients refused). **Salivary (IgG, IgA) for 44 patients (1 patient refused).

Table 4: Validity of rapid urease test, serum and salivary of IgG & IgA tests in relation to culture.

Variables	Culture		Total*
	Negative	Positive	
RUT:			
Negative	10	0	10
Positive	4	31	35
Serum IgG*:			
Negative	6	9	15
Positive	8	20	28
Serum IgA*:			
Negative	7	3	10
Positive	7	26	23
Salivary IgG**:			
Negative	13	11	24
Positive	1	19	20
Salivary IgA**:			
Negative	13	6	19
Positive	1	24	25

*Serum (IgG, IgA) done for 43 patients (2 patients refused), **Salivary (IgG, IgA) done for 44 patients (1 patient refused).

Discussion

Helicobacter pylori is a common and important transmissible bacterial human pathogen. This infection varies worldwide being as low as 10% in developed western nations to more than 80% among many developing countries. The infection primarily involves the upper gastrointestinal tract causing progressive acute and chronic gastro-duodenal inflammation (Thirumurthi and Graham, 2012).

Although several diagnostic tests are available for the detection of *H. pylori* infection, all of them have both advantages and disadvantages, and none can be considered as a single gold standard. A combination of endoscopic biopsy-based methods (such as rapid urease testing, histologic examination, culture, and PCR) usually gives the most reliable diagnosis. These methods are invasive, expensive, and not always applicable. Thus, there is an increasing interest in non-invasive tests for *H. pylori* detection. Several tests have been developed and introduced into clinical practice: urea breath tests, stool antigen tests, and serologic tests (Dzieranowska *et al,* 2006). Serology of serum and saliva by using enzyme immunoassays are simple, reproducible and inexpensive and detect either antigen or antibody. Although serum-based enzyme immunoassay was used to detect *H. pylori* infection, but cannot distinguish between past and present infections as titers decline very slowly even after *H. pylori* treatment (Estakhri *et al,* 2008). Saliva offers advantages over serum as easy collected, noninvasive,

and less hazardous, and there is a greatly reduced risk of blood-borne infections and suitable for children (Krishnaswamy *et al,* 2012).

In the current study, the *H. pylori* infection in the presented patients reached up to 68.9%. This percentage is comparable to 68.4% reported in Oman (Al Balushi *et al,* 2013). But, in Canada from 7.1% up to 23.1% were reported (Chair *et al,* 2010), which may be due to the difference in environment and socioeconomic conditions.

Yucel *et al.* (2008) reported a slightly higher incidence of *H. pylori* infection in females, but Sasidharan *et al.* (2012) found that the prevalence rate among males was significantly higher than for females. On the other hand, Zhubi *et al.* (2010) and Alo *et al.* (2013) went with the present results that there was no gender predominance.

In this study, no significant difference was found between *H. pylori* positive and negative in terms of patients' symptomatology. This resukts agreed with Selgrad *et al.* (2008) who reported that *H. pylori* infection did show association with a specific symptom.

The optimal diagnostic approach in patients with dyspepsia is still controversial. Upper endoscopy is frequently performed as the primary diagnostic test, but being costly, not accepted by many patients and in most patients no underlying disease can be identified. The strategy based on the noninvasive testing for *H. pylori* could be more cost effective. Such a strategy either be imply the referral of only *H. pylori*-positive patients for endoscopy "test and scope" strategy or subjecting *H. pylori* positive patients to anti-*H. pylori* treatment "test and treat" strategy (Gisbert and Calvet*,* 2013). On the other hand, a new easy to perform non-invasive diagnostic tests have been introduced; the *H. pylori* serological tests on serum and saliva, highly promising outcome results. Besides, these sero-tests were claimed to be suitable for monitoring *H. pylori* infection, and could be competitive with other non-invasive tests (El-Mekki ***et al,*** 2011).

The present work was to evaluate the clinical significance of serum and salivary anti-*H. pylori* antibodies in the diagnosis of human infection, accomplished by correlating the results of serum and salivary antibodies with the results of culture and RUT as standard methods for diagnosis..

Most of studies used more than one diagnostic method as reference standard, as no single available test provides the definitive diagnosis of *H. pylori* by itself. The implication of this is that any infection missed by one test due to the patchy distribution of the infection and consequent sampling error could be easily picked by the other tests, thereby increasing the number of positive results by the reference standard (Jemilohun *et al,* 2011).

In the present study, the culture and rapid urease test were used as golden standard tests in diagnosing *H. pylori.* This agrees with Al-Ali *et al.* (2010) who also used these tests in diagnosing *H. pylori* and also Gosciniak *et al.* (2003) used culture and rapid urease test as golden standard tests.

Regarding culture and sensitivity examinations for diagnosing *H. pylori* infection, the results showed *H. pylori* positive culture using BD *Helicobacter* media in 31 patients (68.9%). This agreed with Asrat *et al.* (2007) who found *H. pylori* positive culture was (69%). But, Ibrahim *et al.* (2012) found that *H. pylori* positivity culture was (90%).

Regarding rapid urease test, it was positive in 35 patients (77.8%).This agreed with Menoni *et al.* (2013) who found that *H. pylori* positivity by RUT was 78%, and Ou *et al.* (2013) showed opposition to our results they found *H. pylori* positivity by RUT was only 32.6%.

On the other hand, in the present study the rapid urease test sensitivity gave 31/31 (100%) compared to culture results in the current study, while its specificity was 10/14 (71.4%) compared to culture results. Lustig (2010) reported that RUT sensitivity and specificity were 100% and 76% respectively. But, Foroutan *et al.* (2010) showed RUT sensitivity and specificity were 100%.

The low specificity of RUT test was explained by increases the possibility of interference from other urease positive bacteria in the gastric mucosa as *Klebsiella* or *Proteus mirabilis* (Johannessen *et al,* 2013). False positive results of RUT in the current study were 4 cases (12.9%) compared to culture results. Jemilohun *et al.* (2011) found false positive results was 11.62% and they explained these false results to be that thesalivate or have reflux alkaline bile into the stomach could have a weak positive reaction because the liquid may contaminate a small gastric biopsy specimen such that the resulting surface PH was > 6.0.

In current study serum *H. pylori* IgG was positive in 28 patients (65.12%) and 33 (76.74%) for IgA. These results more or less agreed with Keramati *et al.* (2007) who found the positivity rate of both IgG and IgA were (77.8%). She *et al.* (2009) showed that the positivity rate of *H. pylori* IgG was (35.6%) and IgA was (32.7%). Also Al-Windi *et al.* (2013) found IgG positivity rate was (32.3%) and IgA positivity rate was (58.2%).

In the present work the correlating of serum IgG test to culture results revealed a sensitivity of 68.97% and a specificity of 42.86%. On other hand, Khalilpour *et al.* (2013) found that sensitivity and specificity of serum IgG reach up to 100% for both. On the other hand, She *et al.* (2009) found IgG sensitivity of 88.6% and specificity of 46.2%.

The present results showed sensitivity of serum IgA (89.66%) and a specificity of (50%), which results are comparable to study of Sudraba *et al*. (2011) who found sensitivity of (96%) and specificity of (50%). She *et al*. (2009) found sensitivity of IgA (73.8%) and specificity (48.8%). On the other hand, Kienesberger *et al*. (2012) showed higher specificity (82%) and lower sensitivity (61%).

In the present study, anti-*H. pylori* IgG and anti-*H. pylori* IgA sensitivity were moderately higher than specificity. This agreed with Bolad *et al*. (2011) who found high sensitivity and low specificity making the accuracy of this test moderately satisfactory. Though ELISA was moderately high sensitive it might not be reliable for diagnostic purposes in some but not all patients due to its low specificity.

The current study showed salivary antibodies IgG positive in 20 patients (45.45%) and IgA positive in 25 patients (56.82 %) Estakhri *et al*. (2008) reported that salivary H. pylori IgG positivity was (44.75%). While, Golpak *et al*. (2013) reported that salivary H. pylori IgG positivity was (2.9%). In another study Guzik et al. (2004) reported salivary IgA positivity of 54%, but Feteih *et al*. (2009) reported 37.2%.

In the present work, the salivary IgG test revealed a sensitivity of (63.33%) and specificity of (92.86%). These results partially agred with Leal *et al*. (2008) who found also low sensitivity (69.1%) and high specificity (94.7%) of salivary IgG. On other study, El-Mekki *et al*. (2011) reported a high sensitivity (95%) and low specificity (70%) of salivary IgG. Also, Krishnaswamy *et al*. (2012) found that the salivary IgG sensitivity and specificity were (79.31%) and (63.64%) respectively.

In the present study, the salivary IgA test revealed a sensitivity of 80% and specificity of 92.86% ;but Kabir (2003) reported low sensitivity and specificity 76% and 61% respectively, and Feteih *et al*. (2009) reported low sensitivity and specificity (41.7%) and (61%) respectively.

In this study, serum and salivary IgA more correlated with culture and RUT than IgG this agreed with Al-Windi *et al*. (2013). On other hand She *et al.* (2009) found that IgG has better correlation with gold standard than IgA, while, Harris *et al*. (2005) found that IgA to be equal to IgG in performance.

In the study using salivary antibodies in diagnosis of *H. pylori* gave more sensitivity and specificity than serum antibodies. This agreed with Estakhri *et al.* (2008) who found sensitivity and specificity of serum was more than saliva, but Leal *et al.* (2008) found there was very slight difference between both tests in saliva and serum.

Conclusion

The use of *H. pylori* salivary antibodies test is safe and easily performed. Invasive diagnostic tests (culture and rapid urease) are still more sensitive and specific; however, salivary serological testing may have a role in epidemiological studies and in screening dyspeptic patients in general practice. Extensive study to evaluate salivary antibodies as screening test prior to endoscopy or for monitoring the response to therapy is ongoing and will be published later on.

References

Al-Ali, J, Al-Asfar, F, Dhar, R, *et al*, 2010: Diagnostic performance of gastric imprint smear for determination of *Helicobacter pylori* Infection. Can. J. Gastroenterol. 24, 10:603-6.

Al-Balushi, MS, Al-Busaidi, JZ, Al-Daihaniet, MS, *et al*, 2013: Sero-pre-valence of *Helicobacter pylori* infection among asymptomatic healthy Omani blood donors. Asian Pacific J. Trop. Dis. 3, 2:146-9.

Alo, MN, Alhassan, HM, Saidu, AY, *et al*, 2013: The prevalence of *Helicobacter pylori* infection in asymptomatic persons in Ethiopia East Local Government Area, Delta State, Nigeriamore. Int. J. Publ. Hlth. Pharm. 1, 1:115-9.

Al-Windi, A, Hussain, A, Hattem, A, Salih, N, 2013: Seroprevalence of anti-Helicobacter pylori antibodies in population of Sulaimani Governorate/ Kurdistan Region/Iraq. J. Zankoy Sulaimani 15, 3:175-85.

Asrat, D, Endale, K, Yohannes, M, *et al*, 2007: Comparison of diagnostic me-thods for detection of *Helicobacter pylori* infection in different clinical samples of Ethiopian dyspeptic patients. Austral-Asian J. Cancer 6, 4:231-7.

Bolad, A, Lutfi, M, Seif Eldein, S, *et al,* 2011: Conditional ratios of anti-*Helicobacter pylori* IgG and IgA in detection of chronic urticarial and other skin lesions. J. Sci. Tech. 12, 1:64-9.

Chair, RH, Xiao, SD, Megraud, F, *et al*, 2010: Helicobacter pylori in Developing Countries. Wld.Gastroenterol. Organ. 23:1-15.

Christie, JML, McNulty, CAM, Shepherd, NA, *et al*, 1996: Is saliva serology useful for diagnosis of *Helicobacter pylori*? GUT 39:27-30.

Dzieranowska, K, Philippe, L, Francis, M, *et al*, 2006: Diagnosis of *Helicobacter pylori* infection. Helicobacter 11, 1:6-13.

El-Mekki, A, Kumar, A, Alknawy, B, *et al*, 2011: Comparison of enzyme immunoassays detecting *Helicobacter pylori* specific igg in serum and saliva with endoscopic and biopsy findings in patients with dyspepsia. Indian J. Med. Microbiol. 2, 2:136-40.

Estakhri, R, Dolatkhah, H, Ghazan- chaei, A, *et al*, 2008: Saliva or Serum, which is better for the Diagnosis of Gastric Helicobacter pylori Infection? Iranian J. Clin. Infect. Dis. 3, 3:121-5.

Feteih, R, Abdel-Salam, M, Jamjoom, H, *et al*, 2009: Salivery Anti-*Heli-cobacter pylori* positivity among endoscopy patients with chronic liver disease. East. Mediterr. Hlth. J. 15, 6:1371-8.

Foroutan, M, Loloei, B, Irvani, S, *et al*, 2010: Accuracy of rapid urease test in diagnosing *Helicobacter pylori* infection in patients using NSAIDs. Saudi J. Gastroentrol. 16, 2:110-2.

Gisbert, J, Calvet, X, 2013: *Helicobacter pylori* ‘‘Test-and-Treat’’ strategy for management of dyspepsia: A comprehensive review. Clin. Translat. Gastroenterol. 4, 32:1-17.

Golpak, Y, Joshaia, N, Beatrice, S, *et al*, 2013: using enzyme immunoassy to assess the prevalence of *Helicobacter pylori* IgG in saliva and blood plasma**.** Pacific J. Med. Sci. 11, 2:40-50.

Gosciniak, G, Przondo-Mordarska, A, Iwanczak, B, *et al*, 2003: *Helicobacter pylori* antigens in stool specimens of gastritis children before and after treatment. J. Pediatr. Gastroenter. Nutr. 36: 376-80.

Guzik, M, Karczewska, E, Bielanski, W, *et al*, 2004: Association of the presence the *Helicobacter pylori* in the oral cavity and in the stomach. J. Physiol. Pharmacol. 55, 2:105-15.

Harris, P, Perez, G, Zylberberg, A, *et al*, 2005: Relevance of adjusted cut-off values in commercial serological immunoassays for *Helicobacter pylori* infection in children. Dig. Dis. Sci. 50**:**2103-9.

Ibrahim, NH, Gomaa, AA, Abu-Sief, MA, *et al*, 2012: The Use of different laboratory methods in diagnosis of *Helicobacter pylori* infection; a comparative study. Life Sci. J. 9, 4:249-59.

Jemilohun, AC, Otegbayo, JA, Ola, S O, *et al*, 2011: Diagnostic accuracy of rapid urease test for the diagnosis of *Helicobacter pylori* in gastric biopsies in the Nigerians with dyspepsia. African J. Clin. Exp. Microbiol.12, 2:62-6.

Johannessen, R, Kare, B, Constantin, J, *et al*, 2013: PCR versus culture in the diagnosis of *Helicobacter pylori* infection. Gastroenterol. Insights 5:1-6.

Kabir, S, 2003: Review article: Clinic-based testing for *Helicobacter pylori* infection by enzyme immunoassay of feces, urine and saliva. Alimen. Pharmacol. Thera. 17: 1345-54.

Keramati, MR, Siadat, Z, Mahmoudi, M, 2007: The Correlation between *H. pylori* infection with serum ferritin concentration and iron deficiency anemia. Int. J. Hematol. Oncol. 17, 1:16-20.

Khalilpour, A, Santhanam, A, Wei, C, *et al*, 2013: Antigenic proteins of *Helicobacter pylori* of potential diagnostic value. Asian Pacific J. Cancer Prevent. 14, 3:1635-42.

Kienesberger, S, Guillermo, P, Juan, RC, *et al*, 2012: Serologic Host Response to *Helicobacter pylori* and *Campylobacter jejuni* in socially housed Rhesus Macaques (*Macaca mulatta*). Gut Path. 4:1-9.

Krishnaswamy, R, Thirumala, C, Manoranjini, D, et al, 2012: Salivary IgG assay to detect Helicobacter pylori infection in an Indian adult population. Indian J. Dent. Res. 23, 5:694-5.

Kusters, JG, Arnoud, H, Vliet, M, Kupers, EJ, 2006: Pathogenesis of Helicobacter pylori Infection. Clin. Microbiol. Rev. 19, 3:449-90.

Daniel, L, 2010: GI Infections with an Initial. J. Pediatr. Gastroenterol. Nutr. 50, 4:400-3.

Menoni, S, Ferreira, M, Bonon, SH, Alves, ZJ, Murilo, R, *et al,* 2013: PCR-Based detection and genotyping of *Helicobacter pylori* in endoscopic biopsy samples from Brazilian Patients. Gastroenterol. Res. Pract. 13:1-8.

Ou, Z, Liya, X, Ding-You, L, et al, 2013: Evaluation of a new fluorescence quantitative PCR test for diagnosing *Helicobacter pylori* infection in children. BMC Gastroenterol.13:1-6.

Sasidharan, S, Batumanathan, G, Ma-nickam, R, *et al*, 2012: Prevalence of *Helicobacter pylori* infection among patients referred for endoscopy: Gender and ethnic differences in Kedah, Malaysia. Asian Pacific J. Trop. Dis. 12:55-9.

Selgrad, M, Kandulski, A, Malfer- theiner, P, 2008: Dyspepsia and Helicobacter pylori. J. Digest. Dis. 26, 3: 210-4.

She, RC, Andrew, WR, Litwin, CM, 2009: Evaluation of *Helicobacter pylori* immunoglobulin G (IgG), IgA, and IgM serologic testing compared to stool antigen testing. Clin. Vaccine Immunol. 16, 8:1253-5**.**

Siu, L, Leung, W, Cheng, A, *et al*, 1998: Evaluation of a Selective Transport Medium for Gastric Biopsy Specimen to be cultured for *Helicobacter pylori*. J. Clin. Microbiol. 36:3048-50.

Sudraba, A, Daugule, I, Rudzite, D, *et al*, 2011: Performance of routine *Helicobacter pylori* tests in patients with atrophic gastritis. J. Gastrointestin Liver Dis. 20, 4: 349-54.

Thirumurthi, S, Graham, D, 2012: *Helicobacter pylori* infection in India from a western perspective. Indian J. Med. Res. 136:549-62.

Tonkic, A, Tonkic, M, Lehours, P, *et al*, 2012: Epidemiology and diagnosis of *Helicobacter pylori* Infection. Helicobacter 17:1-8.

Veijola, L, 2007: New aspects of the diagnosis of *Helicobacter pylori* infection. Finland. 27-41

Yucel, T, Aygin, D, Sen, S, *et al,* 2008: Prevalence of *Helicobacter pylori* and related factors among university students in Turkey. Jpn. J. Infect. Dis. 61:179-83.

Zhubi, BC, Zana, BG, Ymer, MI, *et al,* 2010: *Helicobacter pylori* infection according to ABO group among blood donors in Kosovo. J. Hlth. Sci. 1, 2:83-9.