

Original Research Article

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Screening for *Helicobacter pylori* Infection among Asymptomatic University Students in Alexandria, Egypt, Using Non Invasive Laboratory Techniques

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ABSTRACT

Globally, *Helicobacter pylori* (*H. pylori*) is becoming an increasingly troublesome economic and public health problem. Over 50% of the world's population was estimated to harbor *H. pylori* in the upper gastrointestinal tract (GIT) and over 80% of them reside in developing nations. Unless treated, colonization persists lifelong where *H. pylori* presents a key factor in the etiology of GIT diseases; ranging from asymptomatic chronic gastritis up to gastric carcinoma. Therefore, accurate and timely diagnosis is the first step to address this burdensome problem. The aim of this descriptive-cross sectional study was to compare two non-invasive techniques for the diagnosis of *H. pylori* infection among university students. Stool and serum samples were collected from one hundred asymptomatic students to detect *H. pylori* antigens (Ags) in stool using chromatographic immunoassay and IgG antibodies (Abs) in serum by enzyme linked immune sorbent assay (ELISA). Fifty five (55%) of tested students were positive for each of stool Ags and serum Abs. The recorded sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the serum Ab test in relation to stool Ag test were 98.18%, 97.78%, 98.18 % and 97.78, respectively. There was very good agreement (98%) between the results of the two tests.

Keywords

Helicobacter pylori, *H. pylori* stool antigen, *H. pylori* antibodies, chromatographic immunoassay, ELISA

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Introduction

Helicobacter pylori (*H. pylori*) discovered, as a human pathogen, by Marshall and Warren in 1982 (Marshall and Warren, 1984) is a gram-negative bacterium that colonizes the human stomach and can lead to chronic gastritis, peptic ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma besides causing other extra-digestive infections (McCull, 2010). A systematic review from 73 countries in 6 continents in

2018 revealed an overall prevalence of 44.3% worldwide. This rate ranged from 50.8 in developing countries to 34.7% in developed countries (Zamani *et al.*, 2018). The prevalence rate differs considerably from one country to another and even within the same country, but overall, it is less frequent in developed countries than in developing ones in which prevalence rates of >80% were reported previously (Yan *et al.*, 2013, Hussain and Hamid, 2014, Whalen and Massidda, 2015, Perez-Perez *et al.*, 2004). Childhood was identified as the critical time for

acquisition of *H. pylori* infection and mothers probably play a key role in transmission (Taneike *et al.*, 2001).

The exact mode of contracting *H. pylori* infection is not absolutely known, but person-to-person (fecal-oral or oral-oral routes) is regarded as the main route of transmission of infection, followed by contaminated water and food (Sethi *et al.*, 2013, Vale and Vitor, 2010). Iatrogenic transmission is another important mode of transmission, where tubes or endoscopes that have been in contact with gastric mucosa of an infected person are used for another one. Occupationally acquired infections were reported especially among endoscopists and gastroenterologists (Rastogi, *et al.*, 2014).

The risk of acquiring *H. pylori* infection is related to socioeconomic status and living conditions early in life (Ravelomanana *et al.*, 2013). In developing nations the majority of children are infected before the age of 10 (Malaty *et al.*, 2002, Kivi *et al.*, 2005) and adults probably harbor the same strain for several decades (Nourai *et al.*, 2009). Around the world, infection rates reported among children ranged from approximately 35% in Russia, 20% in China and Poland, 12% in Korea and America to <10% in France, Belgium and Finland (Li *et al.*, 2004).

In Egypt, prevalence of *H. pylori* infection is alarmingly high (Mohammad *et al.*, 2008). Prevalence rates of 33% and 72.38% were reported among children less than 6 years and among school children, respectively (Frenck *et al.*, 2006).

The prevalence of *H. pylori* infection is inversely related to standards of living, hygiene and sanitation (Hanafi and Mohamed 2013, Wangda *et al.*, 2017). *H. pylori* is known for its intrafamilial clustering and is associated with crowded conditions (Krueger

et al., 2015). Factors such as density of housing, overcrowding, number of siblings, sharing beds and lack of running water have all been linked to a higher acquisition of *H. pylori* infection. Within a particular country, the decline in prevalence of *H. pylori* tends to parallel the economic improvement and the active elimination of carrier ship by efficient antimicrobial therapy (Omosor *et al.*, 2017).

Over 80% of the infected cases remain asymptomatic for decades (Alo *et al.*, 2013), and only 30% of those infected are clinically symptomatic (Kusters *et al.*, 2006). Presence of *H. pylori* is associated with an increased risk of various gastric pathologies including gastritis, duodenal and gastric ulcers, non-ulcer dyspepsia, gastric adenocarcinoma and lymphoma (Zhong *et al.*, 2016). According to Kusters *et al.*, (Kusters *et al.*, 2006), subjects infected with *H. pylori* develop peptic ulcer (10 – 20% as lifetime risk) and stomach cancer (1-2%). *H. pylori* was defined as a Class I carcinogen by the World Health Organization and the International Agency for Research on Cancer (Hu *et al.*, 2016). Extra-digestive diseases associated with *H. pylori* include autoimmune diseases, bronchiectasis, cardiovascular diseases, colonic and pancreatic diseases, diabetes mellitus, hepatobiliary system diseases, neurological diseases, skin diseases, infertility and hematological diseases (Pastukh *et al.*, 2018).

There is no single test (other than histology from gastric biopsy) that can be set as a Gold Standard and optimal diagnostic technique for *H. pylori* infection. The only way to improve reliability of diagnosis is to apply for a multi-diagnostic tool (Islam *et al.*, 2010, Sethi *et al.*, 2013).

The diagnostic tests for *H. pylori* infection can be classified into two categories: invasive and non-invasive tests. Invasive (biopsy-based) tests require upper gastrointestinal (GIT)

endoscopy and include: histological examination, culture, rapid urease test (RUT), polymerase chain reaction (PCR) and fluorescent in situ hybridization (FISH). Invasive tests require use of specialized equipment by experienced personnel and a long time to get results (El Khadir *et al.*, 2016).

Non-invasive assays are cheaper, more comfortable and safer in comparison to invasive methods. Non-invasive techniques are categorized into two groups of direct and indirect tests. Stool antigen test (SAT) is a direct non-invasive test where the presence of *H. pylori* antigens is directly evaluated. Testing for the presence of antibodies (Abs) against *H. pylori* in serum, saliva and urine samples or other elements like CO₂ that result from *H. pylori* infection is classified as an indirect non-invasive technique. Those tests include 13C- urea breath test (UBT) and serologic assays (Ranjbar *et al.*, 2017). In spite of the high sensitivity (88-95%) and specificity of UBT (95-100%) it has many limitations including its high cost, need of trained staff and equipped laboratory equipment, patients must fast before testing and proton pump inhibitor (PPI) administration before testing results in false negative results as it alters the gastric pH and thus lowers urease activity of *H. pylori* (Shimoyama, 2013).

Serological tests were designed for detecting specific Abs such as anti-*H. pylori* immunoglobulin (Ig) G or anti-CagA and anti-VacA Abs (Ranjbar *et al.*, 2017). According to the type of serological tests, their recorded sensitivity was up to 90%–97% and their specificity varied from 50% to 96% (Bytzer *et al.*, 2011). Serological tests are in fact not able to distinguish active infection from previous contact; with a 30% false positive rate, as Ig G detection indicates both previous (treated) and current infections. To avoid detection of past

H. pylori infection, the UBT and SAT are preferred (Sethi *et al.*, 2013).

H. pylori SAT is used on a wide scale as a simple, cheap and feasible noninvasive technique to diagnose and follow-up *H. pylori* infection (Lario *et al.*, 2016, Osman *et al.*, 2014). Two types of SATs; one based on enzyme immunoassay (EIA) and another on chromatography (ICA) ARE PRESENT (Shimoyama, 2013). A sensitivity and a specificity of up to 95% have been recorded in several reports (Malfertheiner *et al.*, 2012, Ranjbar *et al.*, 2017) and positive and negative predictive values of 100% and 96.5% have also been reported (Gulcan *et al.*, 2005). There are two methods for antigen detection based on monoclonal and polyclonal antibodies. In polyclonal antibody method, cross reactivity of the *H. pylori* SA with non-viable or coccoid forms of the *H. pylori* may result in false-positive results (Elitsur *et al.*, 2004).

The monoclonal antibody test was reported to have higher sensitivity than the polyclonal antibody (98% vs. 93.8%) (Sharbatdaran *et al.*, 2013). This test is suitable to monitor the success of anti- *H. pylori* therapy and in screening of asymptomatic subjects (El-Nasr *et al.*, 2003). The test was approved by the United States Food and Drug Administration (FDA) as a pre-endoscopic diagnostic test for *H. pylori* infection in adults. A new generation of rapid monoclonal antibody based *H. pylori* SAT that works with lateral flow immunochromatography technique in 5 minutes is now available (Yang *et al.*, 2008). They were reported as the most effective screening tests both in populations of high and low prevalence of *H. pylori* infection (Queiroz *et al.*, 2013, Cardenas *et al.*, 2008).

The accuracy of SATs is diminished when stool samples are watery or unformed due to dilution of *H. pylori* antigens. Temperature and time gap between sample collection and

testing also influences results (Shimoyama, 2013).

Regarding the broad spectrum of available *H. pylori* diagnostic tests and since a reliable diagnosis is mandatory both before and after eradication therapy; it is crucial to evaluate the broad spectrum available *H. pylori* diagnostic methods to be able to select the most accurate diagnostic laboratory tests that could be used for efficient diagnosis of *H. pylori* infection (Leal *et al.*, 2008, Tameshkel *et al.*, 2018, Shimoyama, 2013). Diagnostic and predictive values besides the cost-effectiveness of tests help to choose the optimal methods

From the public health aspect, determination of prevalence rates, identification of high-risk population and confirmation of risk factors for *H. pylori* infection are crucial to establish health policies and treatment strategies to prevent *H. pylori* related diseases. This is even more important for people who are harbouring *H. pylori* but are asymptomatic (Rastogi *et al.*, 2014).

The current study was carried out to evaluate two non-invasive techniques (*H. pylori* SAT using rapid chromatographic immunoassay and *H. pylori* IgG in serum by ELISA technique) in detection of *H. pylori* prevalence rate among a sector of asymptomatic university students. The study also aimed to find out the relationship between *H. pylori* infection and some personal and environmental risk factors.

Materials and Methods

Study design, sample size and study setting

A cross-sectional descriptive study was conducted at the Medical Laboratory Technology Department of the Faculty of Allied Medical Sciences, Pharos University (PUA), Alexandria, Egypt in the period from

September to December 2017. One hundred university students were recruited in this case-control study. They comprised 60 males and 40 females; within the age range of 17 to 24 years. The enrolled students admitted they didn't suffer any GIT symptoms suggestive of *H. pylori* infection (anorexia, nausea, vomiting, diarrhea, heart burn, epigastric pain, indigestion, bloating, bleeding, weight loss), persistent for one month at least within the past three years. The exclusion criteria included previous diagnosis for *H. pylori* infection, gastritis, gastric or duodenal ulcers and/or treatment with H2 blockers, PPI, antibiotics (commonly used in treatment of *H. pylori*, including metronidazole), steroids or non-steroidal anti-inflammatory drugs (NSAIDs) during the past three months. Written consents were signed by volunteer students and they filled in questionnaires that included questions covering demographic data (age, sex, and residence), socioeconomic data (monthly income, crowding index), lifestyle, dietary habits, past history of *H. pylori* infection and eradication therapy. This study received ethical approval from the High Institute of Public Health (HIPH) Ethics Committee.

Samples collection and processing

Fresh stool samples provided in sterile containers were immediately sent to the microbiology laboratory of the Medical Laboratory Technology department at Faculty of Allied Medical Sciences. Exclusion criteria of the provided samples were non formed stool, inadequate amount and delayed delivery after collection. Examination of the stool samples to detect *H. pylori* antigen was performed using rapid chromatographic Immunoassay (ABON one step test code no: 1155976703) (ABON Biopharm Co 2012). Small portions; collected from three different sites of every stool sample (adding up to 50 mg, approximately) were transferred to the

sample collection tube containing extraction buffer. After two minutes of vigorous shaking and after two other minutes of resting the tube, two full drops were dropped into the round window of the test cassette. Reading followed 10 minutes of incubation at room temperature.

Interpretation of the results

The appearance of two colored lines across the central window of the cassette, C (control) and T (test) zones or appearance of a pale colored line in T zone indicated a positive test. Only one line in C zone indicated a negative result. Test result was considered invalid in cases where no line appeared in C line zone.

The collected sera were stored frozen at -20°C until used for detection of serum *H. pylori* IgG using the commercially available Immunospec Helicobacter pylori IgG ELISA kit (Catalog No.E30-145) (Immunospec Co 2006). ELISA was performed following the manufacturer's instructions.

Qualitative results

The cut-off control corresponds to calibrator one. If the absorbance of the sample was higher than that of the cut-off, the sample was considered positive for the presence of specific IgG. The ratio between optical density (OD) value of the sample and that of the cut-off was calculated. Repeated freezing and thawing of sera was avoided.

Statistical Analysis

Data were analyzed using IBM SPSS software package version 20.0 (Kirkpatrick and Feeney, 2013). Qualitative data were described using number and percent. Comparison between various groups regards categorical variables was tested using Chi-square test. When more than 20% of the cells had expected count below 5, correction for chi-square was

conducted using Fisher's exact test or Monte Carlo tests. Significance of the obtained results was judged at the 5% level. Calculation of sensitivity and specificity of tests were also chosen at the optimal cut off which has a highest positive likelihood ratio (+LR)

$$\text{Where } *LR = \frac{\text{Sensitivity}}{1 - \text{Specificity}} = \frac{\text{TP rate}}{\text{FP rate}}$$

The agreement between the tests was analyzed using Kappa coefficient test.

Results and Discussion

The discovery of *H. pylori* by Warren and Marshall in 1982 was preceded by nearly a hundred years of inconspicuous publications in regard to spiral bacteria, achlorhydria, gastritis, gastric urease, and antimicrobial therapy for peptic ulcers. The infection has now been involved in the etiopathogenesis of chronic gastritis, peptic ulcer, gastric cancer and gastric MALT lymphoma. (Jemilohun and Otegbayo, 2016)

Epidemiologic studies have shown that 50% of adults in the developed countries and nearly 90% of adults in the developing countries carry *H. pylori* in their upper GIT; making it the most widespread infection in the world (Silva Rossi, *et al.*, 2009) Although over 80% of cases present with asymptomatic infection, *H. pylori* infection can cause gastric and peptic ulcer disease and is a cofactor in gastric cancer; therefore, an accurate and reliable method for diagnosis is mandatory (Ahmed and Shammari, 2015).

Previous studies on the prevalence of *H. pylori* published worldwide reported variable infection rates. The main reason for this variation was the socioeconomic difference between such populations. Lack of proper sanitation, safe drinking water and basic hygiene, besides; poor diet and overcrowding,

all play a role in determining the overall prevalence of infection (Azevedo *et al.*, 2009, Ibtihal, 2010). It also seems that prevalence rates depend not only on the rate of acquisition but also on the rate of loss of infection and the duration between acquisition and loss (Muhammad *et al.*, 2012).

In the current study, a total of 100 university students were recruited, out of whom 60% were males and 40% were females. The age of the participants ranged from 17 to 26 years old. The overall prevalence of *H. pylori* infection recorded was 55%.

Results of the present study revealed a prevalence rate of 55% for *H. pylori* infection among asymptomatic university students. Different results were reported among Egyptians as those reported by Hassanein *et al.*, (Hassanein *et al.*, 2017): 24% and by Sabah *et al.*, (Sabah *et al.*, 2015): 69.4%. The present prevalence rate was in line with results reported in previous studies carried out in African countries as that in Nigeria :52.5% (Omosor *et al.*, 2017) and these carried out in Libya : 54.4% (Almehdawi, 2016) and 56.5% (Almehdawi and Ali, 2016). Higher percentages were reported in Ethiopia: 70% (Alebie and Kaba, 2016), Libya: 76% (Bakka and Saleh, 2002), Nigeria: 80% (Oluwasola, 2002), Tunis: 83% (Ben Ammar *et al.*, 2003), Morocco: 92.65% (Bouder *et al.*, 2017) and in a public survey carried out also in Nigeria 93.6% (Olokoba *et al.*, 2013).

The recorded percentages in studies carried out in Asian countries, for screening for *H. pylori* infection among asymptomatic subjects, varied from as low as 13.1% in Iran (Namakin and Nejad, 2014), Lebanon (21%) (Naous *et al.*, 2007), India (46%) (Rastogi *et al.*, 2014), Saudi Arabia (51%) (Mubashir and Ghazi, 2007), Korea (54.4%) (Lim *et al.*, 2013), China (63.4%) (Zhu *et al.*, 2014), Oman (69.5%) (Al-Balushi *et al.*, 2013) to as high as

82.5% in Turkey (Ozaydin *et al.*, 2013).

Even in developed countries variable rates of *H. pylori* infection were reported among asymptomatic subjects as in Portugal: 84.2% (Bastos *et al.*, 2013), Mexico: 52.2% (Alvarado-Esquivel, 2015), Brazil: 41.1% (Pacheco, *et al.*, 2013), Canada: 37.9% (Sethi *et al.*, 2013), Netherlands: 32% (van Blankenstein, *et al.*, 2013), USA: 25.4% (Krueger *et al.*, 2015) and Belgium: 11% (Mana *et al.*, 2013). In Japan, the prevalence recorded was near 90% among individuals born before 1950s, with a subsequent decreasing trend, reaching less than 2% among subjects born after 2000s (Inoue, 2017). The decline in prevalence is an indication of improvement of socio-economic status, hygiene and sanitation and the active eradication of carrier ship by proper antibiotic treatment (Kingsley *et al.*, 2017).

It is actually difficult to compare the prevalence rates indifferent studies due to variations in age and the sector of population studied. Comparing the results of the present study to those of previous studies performed to screen for *H. pylori* infection specifically among asymptomatic university students; nearly similar percentages were reported among Nigerian university students: 54% (Ishaleku and Ihiabe, 2010) and among Irish ones: 59% (Sheehan *et al.*, 2004). Lower prevalence rates were reported among the same sector in Brazil: 23.4% (Melo *et al.*, 2003) and Saudi Arabia: 35% (Almadhi *et al.*, 2007). A higher prevalence rate of 71% was reported among Ethiopian university students (Alebie and Kaba, 2016). Nevertheless, the prevalence rate variations observed between the current report and others may be attributed to differences in methodology and technical factors as well as level of sanitation and social economic status of individual subjects screened.

The high prevalence of this bacterial infection

among university students indicates that the major public health problem of peptic ulcer disease and gastric cancer in the society will be difficult to be eradicated since this age group is engaged in high interpersonal social activities; which enhances the transmission of *H. pylori* infection.

In the current study although the prevalence rate was higher among females (70%) than among males (45%); yet no significant difference between both sexes was reported ($\chi^2=6.061$; $p=0.014$). [Table 1]

The relationship between gender and *H. pylori* infection has been controversial in other studies (Yordanov *et al.*, 2017, Yu *et al.*, 2017). A systematic review with meta-analysis carried out in 2018 reported that no significant difference was observed between the two genders in worldwide *H. pylori* prevalence (Zamani *et al.*, 2018). The role of sex to put males at significantly higher risk of *H. pylori* infection compared to females was observed in many previous studies (Ibrahim *et al.*, 2017, Valliani *et al.*, 2015, Omosor *et al.*, 2017, Jeong *et al.*, 2007, Kaore *et al.*, 2012). Nevertheless, such trend contradicted the present and other studies' findings; where gender was not significantly associated with *H. pylori* infection (Seyda *et al.*, 2007, Mathewos *et al.*, 2017, Tadege *et al.*, 2005). Non-significant higher prevalence rates among females were similarly previously reported in many studies (Munish *et al.*, 2014, Montazer-Saheb *et al.*, 2011, Almehdawi and Ali 2016, Alemayehu 2011, van Blankenstein *et al.*, 2013, Adlekha *et al.*, 2013, Vilaichone *et al.*, 2013, Dorji *et al.*, 2013, Benajah *et al.*, 2013, Mathewos *et al.*, 2013, Omosor *et al.*, 2017)

Thirty five out of the 100 students screened in the current work (35 %) came from urban areas while 65 (65%) came from rural areas. Prevalence rate of *H. pylori* infection among urban dwellers was 54.3% (19/35) compared

to 55.4% (36/65) in rural dwellers. Residence was not significantly associated with prevalence of *H. pylori*. This finding is in line with previous studies carried out in Egypt, Mexico and Lybia (Mohamed *et al.*, 2016, Laszewicz *et al.*, 2014, Almehdawi *et al.*, 2016). On the other hand, several researchers reported a positive correlation between rural life and *H. pylori* infection (Abdallah *et al.*, 2014, Lim *et al.*, 2013, Vilaichone *et al.*, 2013, Hanafi and Mohamed 2013). This could be attributed to inadequate sanitary conditions and to absence or poor personal hygiene

Eighty seven (87%) of the current participants were classified as middle socioeconomic class and only 13 (13%) belonged to the high socioeconomic class; according to modified score for social leveling of families (Fahmy *et al.*, 2015). There was a high significant association between the socioeconomic standard of the students and their parents and the prevalence of *H. pylori* among them. The prevalence rate was higher in medium than in high socioeconomic groups (84.65 and 39.1%, respectively).

This finding is consistent with previous studies carried out in Egypt and other countries which have demonstrated that the prevalence of *H. pylori* was higher in those who come from large families, had poor hygiene, limited living standards, poor sanitary practices and overcrowded living conditions (Hassanein *et al.*, 2017, Rastogi *et al.*, 2014, Mohammad *et al.*, 2011, Dattoli *et al.*, 2010). Salih *et al.*, (2009), noted that overcrowded conditions entailing closer contact between individuals sharing the same bed is important factor for the acquisition of such infection. In contrast, McLaughlin *et al.*, (2003) found no association between the prevalence of *H. pylori* and socioeconomic standard in Zambia. Socioeconomic status is not restricted to income and social class but also other factors such as living standards,

urbanization and educational level are major determinants of *H. pylori* prevalence (Khalifa *et al.*, 2010, Naous *et al.*, 2007, Eshraghian, 2014).

Regarding family history of *H. pylori* infection and related gastritis, it was mentioned by 30 (30%) of the students screened in the present study and denied by 70 (70%) of whom. *H. pylori* prevalence among those with family history of *H. pylori* (especially infection of the mother) was 70% (21/30) compared to 48.6% (34/70) among others.

The difference between both groups was statistically significant ($\chi^2=3.896$; $p=0.048$). It is worth mentioning that in the 21 cases with positive family history; it was the mother who had *H. pylori* infection.

Fathers tend to have less contact with their siblings than mothers, so they are less involved in the transmission (Fujimoto *et al.*, 2007). It was reported that the relative risk of a person becoming infected with *H. pylori* is approximately four or eight times greater; when the father or the mother is infected, respectively (Manfredi *et al.*, 2016). Molecular studies carried out to trace intrafamilial transmission confirmed the mother-to-child transmission in most cases and further reported a grandmother-to-child transmission. (Didelot *et al.*, 2013, Osaki *et al.*, 2013, Urita *et al.*, 2013). It seems that mothers could transmit the infection through mouth secretions; using common spoons or tasting the food.

Furthermore, interfamilial transmission may be also responsible for re-infection with *H. pylori* as its presence among asymptomatic family members may facilitate the transmission among households (Manfredi *et al.*, 2013, Ryu *et al.*, 2010). Several previous studies consistently supported infected

siblings as a risk factor for *H. pylori* infection among families (Dattoli *et al.*, 2010, Fialho *et al.*, 2010, Muhsen *et al.*, 2010, Cervantes *et al.*, 2010, Nam *et al.*, 2011).

Regarding unhealthy eating habits, 53 (53%) of the students shared articles like: spoons, glasses and plates with their family members and/or roommates compared to 47 (47%) who didn't share articles. Sharing articles with family members or room-mates was not significantly associated with a higher prevalence rate of *H. pylori* infection among the screened students in the present work. This finding is consistent with those reported in Egypt, Libya and Turkey (Mohamed *et al.*, 2016, Almehdawi and Ali 2016, Kaya *et al.*, 2014).

An inverse association between the level of the parents' (mother's) education and *H. pylori* infection among the studied students was reported in the current work. *H. pylori* prevalence recorded among students whose mothers had only primary school education was 89.5% (17/19) compared to 65.1% (28/43) among those whose mothers had high school education and to only 26.3% (10/38) among those whose mothers achieved university education or higher. This variance was highly statistically significant ($\chi^2=25.534$; $p<0.001$).

The same association was reported in other similar studies (Bastos *et al.*, 2013, Mana *et al.*, 2013).

Among the lifestyle habits common among university students; smoking showed a high significant association with *H. pylori* infection among the students screened in this work. The prevalence of *H. pylori* recorded among smokers was 80.4% (37/46) compared to 33.3% (18/54) among non-smokers. ($\chi^2=22.266$; $p<0.001$).

Table.1 Relation between *H. Pylori* prevalence and different parameters

	N	H. Pylori				χ^2	P
		+ve (n = 55)		-ve (n = 45)			
		No.	%	No.	%		
Gender							
Male	60	27	45.0	33	55.0	6.061*	0.014*
Female	40	28	70.0	12	30.0		
Residence							
Urban	35	19	54.3	16	45.7	0.011	0.916
Rural	65	36	55.4	29	44.6		
Socio-economic standard							
High	13	2	15.4	11	84.6	9.475*	0.002*
Middle	87	53	60.9	34	39.1		
Low	0	0	0.0	0	0.0		
Family history of H. Pylori related gastritis							
Yes	30	21	70.0	9	30.0	3.896*	0.048*
No	70	34	48.6	36	51.4		
Smoking							
Yes	46	37	80.4	9	19.6	22.266*	<0.001*
No	54	18	33.3	36	66.7		
Dietary Habits							
1. Drinking coffee							
Yes	79	51	64.6	28	35.4	13.883*	<0.001*
No	21	4	19.0	17	81.0		
2. Drinking tea							
Yes	89	53	59.6	36	41.4	6.769*	^{FE} p=0.011*
No	11	2	18.2	9	81.8		
3. Eating spicy food							
Yes	82	47	57.3	35	42.7	0.988	0.320
No	18	8	44.4	10	55.6		
4. Eating raw vegetables							
Yes	88	51	58.0	37	42.0	2.586	0.108
No	12	4	33.3	8	66.7		
5. Protein rich diet							
Yes	74	48	64.9	26	35.1	11.191*	0.001*
No	26	7	26.9	19	73.1		
6. Skipping meals							
Yes	91	54	59.3	37	40.7	7.697*	^{FE} p=0.010*
No	9	1	11.1	8	88.9		
Awareness of transmission routes							
Yes	90	47	52.2	43	47.8	2.806	^{FE} p=0.178
No	10	8	80.0	2	20.0		
Sharing articles							
Yes	53	29	54.7	24	45.3	0.004	0.952
No	47	26	55.3	21	44.7		
Mother's education							
Illiterate	0	0	0.0	0	0.0	23.534*	<0.001*
Primary school	19	17	89.5	2	10.5		
High school	43	28	65.1	15	34.9		
University	38	10	26.3	28	73.7		

χ^2 : Chi square test for comparing between the two categories

^{FE}p: p value for Fisher Exact for Chi square test for comparing between the two categories

*: Statistically significant at $p \leq 0.05$

Table.2 Relationship between results of *H. Pylori* stool Ag test and serum test among the 100 studied students

Stool Ag results Serum test results	Stool Ag		Sensitivity	Specificity	PPV	NPV
	-ve	+ve				
Serum test -ve	44	1	98.18	97.78	98.18	97.78
Serum test +ve	1	54				
Total	45	55				
κ (p)	0.960* (<0.001*)					
Level of agreement	Very good					
% of agreement	(44+54)/100%=98%					

κ: kappa test

Value of K	Strength of agreement
< 0.20	Poor
0.21 - 0.40	Fair
0.41 - 0.60	Moderate
0.61 - 0.80	Good
0.81 - 1.00	Very good

Other authors also reported that smokers were at higher risk of acquiring *H. pylori* infection. (Hanafi and Mohamed, 2013, Ozaydin *et al.*, 2013, Alebie and Kaba 2016). On the other hand, in most studies, there was no significant association between smoking and *H. pylori* infection (den Hollander *et al.*, 2013, Zhu *et al.*, 2014, Sodhi *et al.*, 2013, Almehdawi, 2016, Almehdawi and Ali, 2016, Camargo *et al.*, 2004).

The absence of association in such studies may be due to less number of smokers screened, besides the type of tobacco and the frequency of smoking. Smoking was implicated to promote virulent infection in individuals by inducing the expression of virulent genes, including cag A, E and T (Ghosh and Bodhankar, 2012).

As regards the dietary habits of the participants in the current work; drinking coffee and tea, intake of high protein diet and skipping meals were significantly implicated to increase the risk of *H. pylori* infection.

($p < 0.05$) This finding coincided exactly with the results of screening university students in Ethiopia (Alebie and Kaba, 2016).

Skipping meals was previously reported to have a significant association with *H. pylori* infection rates. As for protein rich food stuffs, it was postulated that *H. pylori* could survive in some animal products rich in protein, including meat and dairy products at temperature below 30°C. Moreover, such foods could serve as source of amino acids which support the growth of this bacterium in the stomach (Abu Farsakh, 2002).

Drinking coffee supports the growth of *H. pylori* by suppressing acid production in the stomach. Coffee drinking was also claimed to be involved in hyper stimulation and increased levels of stress related hormones such as cortisol, adrenaline and norepinephrine (Almehdawi, 2016); which in turn could negatively influence the activity of the immune system supposed to combat *H. pylori*. On the other hand, Rana (2007)

reported tea consumption as a protective factor against *H. pylori* infection.

On the other hand, neither eating spicy food nor raw vegetables was significantly associated with a higher *H. pylori* prevalence in the present work. This is in line with the findings of a Libyan study (Almehdawi, 2016) and disagrees with those of another Libyan study; which reported a higher prevalence rate of *H. pylori* among the groups eating raw vegetables (Bakka *et al.*, 2009).

Although infection rate among those students who were not aware of the transmission routes of *H. pylori* was far beyond that among those who were aware of such routes (80% (8/10) and 52.2% (47/90), respectively); yet the difference was not statistically significant.

This contradicted the findings of a similar study applied on Ethiopian university students.(Alebie and Kaba, 2016) Awareness creation in the community about good personal hygiene and environmental sanitation is the first step towards the control of *H. pylori* contamination of food and water sources (Salih, 2009).

H. pylori infection remains the most frequent and persistent bacterial infection worldwide; thus the need for an accurate diagnosis of infection is imperative. The ideal test for detection of *H. pylori* infection should be noninvasive, highly accurate, widely available and inexpensive (Jafar *et al.*, 2013).

The invasive techniques for diagnosis of *H. pylori* are difficult, expensive and not preferred by the patients (Ni *et al.*, 2000). So, a rapid and cost-effective detection method for diagnosis of *H. pylori* infection is required. Therefore, non-invasive testing for *H. pylori* has been strongly recommended as it is cheaper, more patient friendly than invasive methods and does not require very

complicated laboratory facilities (Osman *et al.*, 2014).

Stool Ag test is one of the non-invasive methods that is broadly used in the diagnosis of *H. pylori* infection and had been known for the accuracy of its results and comparability to invasive methods (Ni *et al.*, 2000). In the current research, *H. pylori* stool Ag test was considered the gold standard method for diagnosis of *H. pylori* infection. This is attributed to its previously reported high sensitivity and specificity (up to 97%) (Bakri 2012, Pourakbari *et al.*, 2013, Garza-González *et al.*, 2014, Jekarl *et al.*, 2013,) and its excellent positive and negative predictive values regardless of *H. pylori* prevalence (Bakri 2012).

Compared to UBT, stool Ag test was reported by Frenck *et al.*, (Frenck *et al.*, 2006) at Cairo University to be equivalent to its sensitivity and specificity. They concluded that UBT and stool Ag test had comparable high sensitivity (98 and 94%, respectively) and specificity (89% and 81%, respectively) and thus the stool Ag test has been evaluated as equivalent to the UBT.

In addition to stool Ag test, serological tests are also useful non-invasive methods for the diagnosis of *H. pylori* infection. They are acceptable by patients because of their non-invasiveness; quick results, less liability to be affected by colloidal bismuth, PPIs, or antibiotics (Shah *et al.*, 2014).

In comparison to the biopsy-based tests; review of the overall performance of the commercially available serology kits that measure IgG antibodies highlighted that serology is an accurate method of diagnosing *H. pylori* infection. Comparison between serology and the combination of the rapid urease test: Campylobacter-like organism test (CLO), histology and culture showed up to

97.8% sensitivity and 100% specificity (Abasiyanik *et al.*, 2002).

In the present study, immuno-chromatographic assay was used for detection of stool Ag as it has the advantage of rapid report of results within minutes and it does not need the use of expensive laboratory equipment. The test was positive in 55/100 of the screened students (55%). All collected stool samples in the current work were well formed which could be the reason for the enhanced stool Ag test results, because the results of this test are inaccurate when stool samples are unformed or watery, as *H. pylori* specific Ags are diluted (Shimoyama, 2013).

In addition, students in the present study were also screened for *H. pylori* using the serum IgG Ab test (Immunospec Helicobacter pylori IgG ELISA).

Out of the 100 students screened for *H. pylori* in the current work, 55% (55/100) were positive for both stool Ag test and serum Ab test; 54 of whom were simultaneously positive for both tests while only 1 was positive for stool Ag test but negative for serum Ab test and another one was positive for serum Ab test but negative for stool Ag test. This can be explained by the fact that a negative result does not confirm the absence of Abs to *H. pylori*. Early stages of colonization may be present or the Ab titer may be too low for the assay to detect. Similar findings were reported by Couturier (Couturier, 2013) and Mohamed *et al.*, (Mohamed *et al.*, 2016).

Compared to the stool Ag test, a sensitivity, specificity and agreement of 98.18, 97.78 and 98%, respectively were recorded for serum IgG Ab test (Table 2)

These results were in accordance with the findings of Pandya *et al.*, (Pandya *et al.*,

2014), who reported that the IgG Ab test evaluated in their study, had a sensitivity of 100%, which permits the safe use of this test in screening surveys. Unlikely, Shah *et al.*, (Shah *et al.*, 2014), reported that serum IgG sensitivity ranged from 90 to 97%, but the specificity ranged from 50 to 96%. In addition, Iqbal *et al.*, (Iqbal *et al.*, 2013), reported a higher specificity rate of 80%.

Considering the substantial efforts that *H. pylori* is forcing us daily, both to avoid serious complications as peptic ulcer and gastric cancer and the consequent extensive financial burden, it is recommended to take accurate steps towards screening for this infection on a wide scale and to adopt a strategy to prevent and eradicate it, globally.

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