Full Length Research Paper

Diagnosis of *Helicobacter pylori* infection in children and their mothers using some non-invasive techniques

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Helicobacter pylori (*H. pylori*) is an important global public health concern. It infects approximately one half of the world's population. Considering the broad spectrum of available *H. pylori* diagnostic methods and since a reliable diagnosis is mandatory both before and after eradication therapy; thus it is crucial to study the different methods to be able to select the highly accurate laboratory tests that could be used to efficiently diagnose *H. pylori* infection in clinical practice. In this descriptive-cross sectional study we aimed to evaluate some non-invasive techniques for the diagnosis of *H. pylori* infection in children and their mothers. Forty children and their 21 mothers who had gastrointestinal symptoms were included. Stool, serum and saliva samples were collected from each enrolled child and mother to detect *H. pylori* antigens (Ags) in stool using chromatographic immunoassay and IgG antibodies (Abs) in serum and saliva by enzyme linked immune sorbent assay (ELISA). Collected data were analyzed using IBM SPSS software package version. Thirty (75%) children were positive for stool Ags, 10 (25%) were positive for serum Abs and only 5.4% were positive for saliva Abs. *H. pylori* stool Ag test was the most reliable test in all studied individuals and testing *H. pylori* IgG Abs in serum was more useful in adults than in children.

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

INTRODUCTION

Helicobacter pylori (*H. pylori*), one of the most common bacterial pathogens in the world, is an important global public health concern (Cheng et al., 2009). This organism infects approximately one half of the world's population and represents a key factor in the etiology of various gastrointestinal diseases, ranging from chronic active gastritis without clinical symptoms to peptic ulceration, gastric adeno-carcinoma, and gastric mucosa-associated lymphoid tissue lymphoma (Estakhri et al., 2008). The prevalence varies greatly between countries and between

population groups within the same country and is inversely associated with socioeconomic status or household hygiene and sanitation (Hanafi and Mohamed, 2013). In developing countries, more than 80% of the population is *H. pylori* positive, even at young ages, while in developed countries the prevalence generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people (Yan et al., 2013).

H. pylori infection is commonly acquired in childhood (Luzza et al., 1997). The prevalence of infection among children ranges from 2.4 to 13.6% in developed countries and from 22.6 to 80% in developing countries. This difference may be attributed to poor socioeconomic status and overcrowded conditions during childhood (Ravelomanana et al., 2013). In Egypt, infection is alarmingly high (Mohammad et al., 2008). A prevalence rate of 33% has been reported among children less than 6 years and 72.38% among school children (Frenck et al., 2006).

The diagnosis of *H. pylori* can be done using invasive and non-invasive methods. Invasive methods need endoscopy and biopsy [histological examination, rapid urease test (RUT), culture, polymerase chain reaction (PCR), and fluorescent in situ hybridization (FISH)]. Non-invasive methods include ^{13}C, ^{14}C urea breath test (UBT), detection of *H. pylori* antigen (Ag) in stool, and detection of antibodies (Abs) against *H. pylori* in serum, urine, and saliva (Koletzko et al., 2011; Leal et al., 2011).

Non-invasive tests for *H. pylori* infection have been helpful in epidemiological studies of prevalence, mode of transmission and spontaneous clearance of the infection. Considering the broad spectrum of available *H. pylori* diagnostic methods and since a reliable diagnosis is mandatory both before and after eradication therapy; thus it is crucial to study the different methods to be able to select the highly accurate laboratory tests that could be used to efficiently diagnose *H. pylori* infection in clinical practice (Leal et al., 2008). In this study we aimed to evaluate some non-invasive techniques (Detection of *H. pylori* stool Ags using rapid chromatographic Immunoassay and *H. pylori* IgG Abs in serum and saliva by ELISA technique) for the diagnosis of *H. pylori* infection in children and their mothers.

MATERIALS AND METHODS

This descriptive-cross sectional study was conducted at El-Shatby University Hospital and the Microbiology Department of the High Institute of Public Health (HIPH), in Alexandria, Egypt during a five-month period from May to December 2014.

Forty children and their twenty one mothers who had gastrointestinal symptoms suggestive of *H. pylori* infection (Heart burn, epigastric pain, nausea, vomiting, anorexia, vague abdominal pain, diarrhea, indigestion, bloating, weight loss, bleeding) were included in the present study. Any child or mother who had received antimicrobial agents (including metronidazole) and /or proton pump inhibitors (PPI) within 2 weeks prior to samples collection was excluded from the study.

Sample size

Using a power of 80% to detect the sensitivity of salivary IgG at predicting the *H. pylori* in children = 87% and specificity = 73% (Roxo-Rosa et al., 2013), assuming the prevalence of *H. pylori* in children =50% with precision = 5 and at alpha = 0.05 (Mohammad et al., 2008); a minimum sample size required was calculated using STATA to be 30 children and their mothers for whom all methods were applied.

Data collection method

A questionnaire sheet was completed for every individual enrolled in the study with all the relevant information including gastrointestinal symptoms and risk factors related to *H. pylori* infection. This study was approved by the HIPH Ethics Committee, and written informed consents were obtained from all the mothers of examined children.

Samples collection and processing

Stool, blood and saliva* samples were collected from each of the 40 children and their 21 mothers enrolled in the present study. *Saliva samples were collected from 37 children as it was not feasible to collect saliva from three babies.

At the laboratory

Stool samples were tested for *H. pylori* Ag using rapid chromatographic Immunoassay (Abon one step test code no: 1155976703) (ABON Biopharm Co 2012).

Small portions of stool samples collected from three different parts (to collect approximately 50 mg) were transferred to the sample collection tube containing extraction buffer. It was then vigorously agitated and after two minutes of resting the tube, two full drops were dropped into the round window of the test cassette. Reading was made after 10 minutes of incubation at room temperature.

Interpretation of the results

Based on the appearance of colored lines across the central window of the cassette, two lines, C (control) and T (test), indicated positive test. Only one line in C indicated negative result. A pale colored line in T was also considered positive. Invalid test was considered if no line appeared in C line region.

The obtained sera were stored at -20°C until used for detection of serum *H. pylori* IgG using the Immunospec Helicobacter pylori IgG ELISA kit (Catalog No.E30-145) (Immunospec Co 2006).

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 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.

Table 1. Kappa coefficiency test analysis.

Kappa	Interpretation
< 0	Poor agreement
0.0 – 0.20	Slight agreement
0.21 – 0.40	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 1.00	Almost perfect agreement

The sample was considered:

- Positive: if the ratio was > 1.1.
- Doubtful: if+/- 10% of the cut-off.
- Negative: if the ratio was < 0.9.

Quantitative results

Units (U): Positive results were expressed in U, the OD values of the 5 calibrators were interplotted and the value of the sample was compared to this curve. Containers with collected saliva samples were stored at -80^0 C till ELISA test for salivary *H. pylori* IgG was performed using the WKEA Ab H. pylori IgG Ab ELISA Kit (WH-354) (Wkea Med Supplies Co 2009).

Preparatory work

Preparation of saliva samples: A 0.5 ml of saliva was placed in a sterile centrifugal tube, and then one ml of phosphate buffer solution (PBS) with pH value of 7.4 was added by an automatic pipette. The tube was then centrifuged for 20 min (2000-3000 rpm) and the supernatant was collected carefully in a clean Eppendorf tube.

Interpretation of results

Critical cut off was calculated:

Critical cut off = The average of negative control well + 0.15.

- If sample OD < calculated critical cut off, it is negative.
- If sample OD ≥ calculated critical cut off, it is positive.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (Kirkpatrick and Feeney, 2013). Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's exact test or Monte Carlo correction. Significance of the obtained results was judged at the 5% level. Calculation of diagnostic sensitivity and specificity were also chosen at the optimal cut off which has a highest positive likelihood ratio (+LR)

$$^+LR = \frac{\text{Sensitivity}}{1-\text{specificity}} = \frac{\text{TPrate}}{\text{FPrate}}$$

Where

N.B. +LR > 1 denotes good performance of a test.

The agreement between different tests was analyzed using Kappa coefficiency test as shown in Table 1:

RESULTS

A total of forty children and their 21 mothers who attended the outpatient clinic at El-Shatby University Hospital and had symptoms suggestive of *H. pylori* infection were included in this study. The 40 studied children included 22 (52.5%) males and 18 (47.5%) females. Their ages ranged from 7 months to 15 years, with a mean age of 6.3. The mean age of enrolled mothers was 32.6 and their ages ranged from 23 to 52 years.

Eleven (52.38%) of the 21 examined mothers and their children (20 children [50%]) were classified as of high socioeconomic class, 9 (42.85%) and their children (19 children [47.5%]) were of average socioeconomic class and only one (4.76%) and her child (one child [2.5%]) belonged to low socioeconomic class according to modified score for social leveling of families (Fahmy and El Sherbini, 1983).

Of the 21 mothers, 8 (38%) and their children (13 [32.5%]) were from urban areas, while 13 (61.9%) and their children (27 [67.5%]) were from rural areas. In addition, seven of these 21 mothers (33.33%) and 14/40 children (35%) had previous *H. pylori* infection.

Of the 40 children, 30 (75.0%) were positive for stool Ags, 10 (25.0%) were positive for serum Abs, while two (5.4%) out of 37 children were saliva Ab positive, as it was not feasible to collect saliva from 3 babies. These results were found to be statistically significant. (p1<0.001, p2<0.001, p3= 0.026) (Table 2)

As regards the 21 studied mothers, 19 (90.4%) were positive for each of stool Ags and serum Abs and only 5 (23.8%) were positive for saliva Abs. These results were statistically significant (p_2<0.001, p_3<0.001) (Table 2).

Twenty seven (27) children together with their mothers (18) were concordantly positive for *H. pylori* stool Ags, while 9 and their mothers (7) were positive for serum Abs and 2 children and their mothers were positive for saliva Abs. These results were statistically significant (p_1<0.001[*], p_2<0.001[*], p_3= 0.032[*]) (Table 3).

As regards children, the sensitivity of the serum Ab test in relation to stool Ag test was 30 % with a specificity of 90%; positive predictive value (PPV) equals 90% and negative predictive value (NPV) 30%. The agreement between the two tests in the diagnosis of *H. pylori* infection was 45% (slight agreement) (Table 4). While for mothers, the sensitivity of the *H. pylori* serum Ab test in relation to stool Ag test was 94% with a specificity of 50%. PPV was 94.74% and NPV was 50%. The agreement between the two tests in the diagnosis of *H. pylori* infection was 90.48% (moderate agreement)

Table 2. Results of *H. pylori* stool Ag, serum and saliva IgG Abs among studied children and their mothers.

Results of *H. pylori* tests / Studied individuals	Stool Ag				Serum Ab				Saliva Ab			
	Positive		Negative		Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Children (40)	30	75.0	10	25.0	10	25.0	30	75.0	2*	5.4	35*	94.6
Significance between groups	$p_1<0.001^*$, $p_2<0.001^*$, $p_3= 0.026^*$											
Mothers (21)	19	90.4	2	9.6	19	90.4	2	9.6	5	23.8	16	76.2
Significance between groups	$p_1= 1.000$, $p_2<0.001^*$, $p_3<0.001^*$											

*A total of 37 saliva samples were collected from children as we could not obtain saliva from 3 of them. (Their ages were 7months, 2 years and 5 years). p_1: p value for Chi square test or Fisher Exact test for comparing between stool and serum results. p_2: p value for Chi square test for comparing between stool and saliva results. p_3: p value for Chi square test for comparing between serum and saliva results. *: Statistically significant at $p \leq 0.05$.

Table 3. Relation between results of *H. pylori* stool Ag, serum and saliva IgG Abs among studied children and their mothers.

Studied tests for *H. pylori* / Results of studied individuals	Stool Ag	Serum Ab	Saliva Ab
Positive children and their mothers	27children /18 mothers	9 children /7 mothers	2 children /2 mothers
Significance between groups		$p_1<0.001^*$, $p_2<0.001^*$, $p_3= 0.032^*$	
Negative children and their mothers	0 children /0 mothers	2 children /1 mother	25 children /16 mothers
Significance between groups		$^{FE}p_1= 0.494$, $p_2<0.001^*$, $p_3<0.001^*$	
Negative children and positive mothers	10 children /9 mothers	28 children /17 mothers	10 children /4 mothers
Significance between groups		$p_1<0.001^*$, $p_2= 0.204$, $p_3<0.001^*$	
Positive children and negative mothers	3 children /2 mothers	1 child /1 mother	0 children /0 mothers
Significance between groups		$^{FE}p_1= 0.615$, $^{FE}p_2= 0.241$, $p_3= 1.000$	

p_1: p value for Chi square test or Fisher Exact test for comparing between stool and serum results. p_2: p value for Chi square test or Fisher Exact test for comparing between stool and saliva results. p_3: p value for Chi square test for comparing between serum and saliva results. *: Statistically significant at $p \leq 0.05$.

Table 4. Relation between results of *H. pylori* serum IgG Abs and stool Ag in 40 studied children.

Stool Ag results / Serum Abs results		Stool Ag		Sensitivity	Specificity	PPV	NPV	Agreement
		Positive	Negative					
Serum Abs	**Positive**	9	1	30.0	90.0	90.0	30.0	45.0
	Negative	21	9					
	Total (40)	30	10					
	Kappa (p)							0.120(0.206) Slight agreement

(Table 6).

Regarding saliva test, the sensitivity of the saliva Ab test in children in relation to stool Ag test was 6.67 %, with specificity and PPV of 100% each, and NPV of 26.32%. The agreement between the two tests in the diagnosis of *H. pylori* infection was 30% (slight agreement) (Table 5). While for mothers, the sensitivity of the saliva Ab test in relation to stool Ag test was 15.79%, with specificity and PPV of 100% each, while NPV was 11.11%. The agreement between the two tests in the

Table 5. Relation between results of *H. pylori* saliva IgG Ab and stool Ag among 40 studied children.

Stool Ag results / Saliva Ab results		Stool Ag Positive	Stool Ag Negative	Sensitivity	Specificity	PPV	NPV	Agreement
Saliva Ab	Positive	2	0	6.6	100.0	100.0	26.3	30.0
	Negative	28	7					
	Total (37)	30	7					
	Kappa (p)							0.026(0.482) Slight agreement

*Total collected saliva samples were 37.

Table 6. Relation between results of *H. pylori* serum IgG Ab and stool Ag among 21 studied mothers.

Stool Ag results / Serum Ab results		Stool Ag Positive	Stool Ag Negative	Sensitivity	Specificity	PPV	NPV	Agreement
Serum Ab	Positive	18	1	94.0	50.0	94.7	50.0	90.4
	Negative	1	1					
	Total (21)	19	2					
	Kappa (p)							0.447*(0.040*) Moderate agreement

Table 7. Relation between results of *H. pylori* saliva IgG Ab and stool Ag among 21 studied mothers.

Stool Ag results / Saliva Ab results		Stool Ag Positive	Stool Ag Negative	Sensitivity	Specificity	PPV	NPV	Agreement
Saliva Ab	Positive	5	0	15.7	100.0	100.0	11.1	23.8
	Negative	14	2					
	Total (21)	19	2					
	Kappa (p)							0.064(0.406) Slight agreement

* Total collected saliva samples were 37.

diagnosis of *H. pylori* infection was 23.81% (slight agreement) (Table 7).

Of the 30 children with positive stool Ags, 10 (34%) were living in urban areas and 20 (66%) were from rural areas. Of the 10 children with positive serum Abs, 6(60%) were from urban areas and 4 (40%) were from rural areas. Regarding the 2 children with positive saliva Abs, one (50%) was from an urban area and the other (50%) was from a rural area. As regards mothers, of the 19 mothers with positive stool Ags and serum Abs, 7 (37%) were from urban areas and 12 (63%) were from rural areas. Of the 5 mothers with positive saliva Abs, 3 (60%) were from urban areas and 2 (40%) were living in rural areas. There was no statistical significant difference between these results.

As regards family size, 27/30 children (90%) with positive stool Ags, had families of 4-6 members and 3(10 %) belonged to families with more than 6 members. For the mothers, 18 /19 (95%) who were positive for each of stool Ags and serum Abs had a 4-6 family size.

Regarding Unhealthy eating habits, of the 30 children who were positive for stool Ags, 25 (83%) shared spoons, glasses and plates with their family members and 8 (62%) of them, their mothers chewed food before giving it to them. While concerning the 19 mothers who were positive for each of stool Ags and serum Abs, 15 (78%) shared spoons, glasses and plates with their family members and 7 (36%) chewed food before giving it to their children.

As regards serum Abs, of 13 mothers who had symptoms suggestive of *H. pylori* infection, 12 (92.3%) mothers were positive for serum Abs and had 6 (25%) of their children positive for serum Abs. These results were found to be statistically significant (p<0.001*).

DISCUSSION

H. pylori, one of the most common bacterial pathogens of humans, is the main cause of chronic gastritis all around

the world. It colonizes the gastric mucosa and appears to persist throughout the host's life unless the patient is treated. It is the only bacterium to be classified as a carcinogen by the world health organization (WHO) because of its role in gastric cancer development. Gastric cancer is the third leading cause of cancer deaths worldwide, and 89% of gastric cancer cases are attributable to *H. pylori* infection. Thus an accurate and reliable method for diagnosis is crucial (Ahmed and Shammari, 2015; Plummer et al., 2015; Moon et al., 2013). The invasive techniques for diagnosis of *H. pylori* are difficult, very expensive and not accepted by the patients (Ni et al., 2000). So in clinical settings, a rapid and cost-effective detection method for diagnosis of *H. pylori* infection is desirable especially in children. Non-invasive testing for *H. pylori* has been strongly recommended as it is less expensive, more patient friendly than invasive methods and does not need a very complicated laboratory facility (Osman et al., 2014).

Stool Ag test is one of the non-invasive methods that is widely used in the diagnosis of *H. pylori* infection and had been known with its accuracy and comparability to invasive methods (Ni et al., 2000). In the present study *H. pylori* stool Ag test was considered the gold standard method for diagnosis of *H. pylori* infection. This is due to its high sensitivity and specificity in children and adult patients and excellent positive and negative predictive values regardless of *H. pylori* prevalence as reported by Bakri (2012). In addition, Pourakbari et al. (2013) has recorded that the performance of stool Ag test in adult patients was excellent, with a sensitivity, specificity, and accuracy of 91, 79 and 85%, respectively. Furthermore in 2014, a review done by Garza-González et al. documented that *H. pylori* stool Ag test seemed to perform well in children with sensitivity of 97%, specificity of 97%, PPV of 88%, and NPV of 99% (Garza-González et al., 2014).

Also in comparison with UBT, stool Ag proved to be equivalent in its sensitivity specificity as reported by Frenck et al. at Cairo University, who concluded that UBT and stool Ag test had the highest sensitivity (98, 94%, respectively) and specificity (89%, 81%, respectively) and that the use of the stool Ag test has been evaluated as equivalent to the UBT (Frenck et al., 2006).

In the present study, stool Ag test was positive in 30/40 examined children (75%) (Table 2). A higher percentage was recorded by Douraghi et al. (2013), where 93.1% of the children were positive for *H. pylori* stool Ags (Douraghi et al., 2013). Jafar et al. (2013) found that stool samples were positive for *H. pylori* Ags in 294 (64.2%) children (Jafar et al., 2013). A lower percentage was reported by Issa et al. (2014) where stool Ags were positive in only 45.7% of tested children. Even a much lower percentage (30%) was recorded in an early study that was conducted by Kato et al. (2003).

As regards the studied mothers in this work, 19 (90%) were positive for stool Ags (Table 2). This was in line with Karim et al. (2012) who found that *H. pylori* stool Ag was positive in 92% of the patients. Lower percentages were reported by Naji et al. (2014) and Segamwenge et al. (2014) where stool Ags were positive in 49 and 33.5%, respectively (Naji et al., 2014; Segamwenge et al., 2014). Korkmaz et al. (2015) reported a much lower percentage where *H. pylori* stool Ag test was positive in only 19.7% of patients. In this study, immuno-chromatographic assay was used for stool Ag detection as it has the advantage over other immune assay methods that the test results are available within minutes and it does not require the use of expensive laboratory equipment. In a study done in 2013 by Jekarl et al., *H. pylori* stool Ag had high sensitivity and specificity (84.5 and 96.2%, respectively) (Jekarl et al., 2013). In the current work, all collected stool samples were well formed. This fact could be the reason for the enhanced stool Ag test results, as the accuracy of the stool Ag test is lowered when stool samples are unform or watery, because *H. pylori* specific Ags in these stool samples are diluted. Therefore, watery stools should not be used, particularly in the determination of the results of eradication therapy. The sensitivity of this test is also lowered in patients with upper gastrointestinal bleeding (Shimoyama, 2013). All our studied individuals didn't have gastrointestinal bleeding.

In addition to stool Ag test, serological testings are also useful non-invasive methods for the diagnosis of *H. pylori* infection. It is easy for patients to accept the test because of its non-invasiveness; the results can be quickly obtained, less likely to be affected by colloidal bismuth, PPIs, or antibiotics (Shah et al., 2014).

In the current study, serum and the saliva IgG Ab tests were done and they were compared to the stool Ag test that was considered as the gold standard method for diagnosing *H. pylori* infection. It was found that serum IgG Ab was positive in 19 of the 21 enrolled mothers (90%) with sensitivity, specificity and agreement of 94, 50 and 90%, respectively) (Table 6). These results were in concordance with the findings of Pandya et al. (2014) who reported that the IgG Abs evaluated in their study, had a sensitivity of 100% in adults, which permits the safe use of the test in epidemiologic surveys. Also, they recorded a very low specificity, indicating that the ELISA test must be validated for different populations (Pandya et al., 2014). In addition, Shah et al. (2014) reported that serum IgG sensitivity ranged between 90 and 97%, but the specificity ranged between 50 and 96%. In contrast to the results of the current study, Iqbal et al. (2013) reported a higher specificity rate of 80%. As regards children in the present study, 10 out of 40 children (25%) were positive for IgG Abs in serum with sensitivity, specificity and agreement of the test were 30, 90 and 45%, respectively (Table 4). Sensitivity was found to be lower than that of the mothers and this could be attributed

to the immature immune response or tolerance to *H. pylori* that exists in childhood and thus serodiagnosis of *H. pylori* infection is less useful in children. Similar findings were reported by Okuda et al. (2002) who compared *H. pylori* IgG and IgA Abs with *H. pylori* stool Ags (Okuda et al., 2002). Our results were in concordance with that of Pourakbari et al. (2013) who found that ELISA-IgG serum tests in children showed high specificity, but low sensitivity. These findings have significant clinical implications, since a negative test would not be reliable for ensuring the absence of *H. pylori* infection. Low sensitivity may be attributed to the weak or immature immune response observed in young children (Pourakbari et al., 2013). IgG was also much more specific in children than adults, corroborating the fact that adults are more likely to have been frequently exposed to *H. pylori* in the past so if it is present in a child, it is so specific to the infection (She et al., 2009). This may explain the difference in specificity.

In this study, it was found that 18 out of the 19 positive mothers for stool Ags (94%) were positive for both stool Ags and serum Abs (Table 6) while in children only 9/30 positive children for stool Ags (30%), were positive for both stool Ags and serum Abs and 21 children were positive for stool Ags but negative for serum Abs. This may be explained by the fact that negative results do not preclude the absence of Abs to *H. pylori*. Colonization may be present, however it may be in its very early stages or the Ab titer may be too low for the assay to detect. Similar findings were reported by Couturier (2013).

Saliva samples have advantages over serum samples, since their collection is easy, non-invasive, less hazardous and there is a greatly reduced risk of blood-borne infections (Krishnaswamy et al., 2012). In this study we have collected saliva samples from 37 children, as we couldn't collect saliva from 3 children (their ages were 7 months, 2 years and 5 years) due to their physical resistance and continuous crying.

In the present study, the results of the salivary Abs were inferior to that of stool Ags and serum Abs. Only 5 mothers out of 21(23%) were positive for salivary Abs (Table 7) and only 2 out of 37 children (5%) were positive for salivary Abs. (Table 2) This may be due to the fact that IgG which appears in saliva by trans-capillary leakage is present in low concentration as compared to that present in serum. Factors such as low concentration and variable trans-capillary leakage may be the reasons why salivary IgG assay is less reliable than the serum IgG assay as recorded by Kabir (2003), who in his research concluded that salivary IgG assay has provided inconsistent results with less than optimum sensitivity and specificity.

The calculated sensitivity, specificity and agreement for mothers' salivary Ab tests in the current study were 15.79, 100 and 23.81%, respectively (Table 7) and the corresponding figures for children were 6.67, 100 and 30%, respectively (Table 5). A higher sensitivity and lower specificity percentages were recorded by El-Fakhfakh et al. (2014) who evaluated the frequency of anti-*H. pylori* serum and salivary Abs positivity among Egyptian patients with gastric disorders and the validity of salivary and serum serological tests for diagnosis of *H. pylori*, comparing them with tests performed on endoscopy biopsy; where salivary IgG succeeded to diagnose 19 cases from the 31 positive *H. pylori* patients with a sensitivity of 63.33% and specificity of 92.86% (El-Fakhfakh et al., 2014).

The prevalence of *H. pylori* infection seems to depend mostly not only on the rate of acquisition, but also on the rate of loss of infection and the length of the persistence period between acquisition and loss. Based on these factors, *H. pylori* prevalence differs from one country to another and may differ between different ethnic, social, or age groups within the same country. Among Egyptian children, *H. pylori* prevalence was highest in children attending school in socially-deprived areas. Inhabitants of Cairo residing in an overcrowded home had the highest prevalence among the locations studied (Muhammad et al., 2012).

In our study, *H. pylori* infection was recorded in 37% of mothers who lived in urban areas and 63% of those who lived in rural areas. It represented 34% in children who lived in urban areas and 66% among those who were from rural areas. This was comparable to what was documented by Laszewicz et al. (2014) where they demonstrated statistically significant higher prevalence of *H. pylori* infection both in adults and children in rural areas. This could be attributed to inadequate sanitary conditions. Absence or poor personal hygiene may also play a role in increasing *H. pylori* infection. This may be observed in developing countries, which may reflect the combined effects of poor living conditions, poor hygiene and overcrowded cities (Laszewicz et al., 2014). This work showed higher levels of positivity in children (83%) and mothers (78%) who shared spoons, plates and glasses. This was in accordance with the study of Kaya et al. (2014) who reported that some practices that are characterized by lack of hygiene such as sucking the teat before giving it to the baby and sharing spoons, forks and cups were associated with higher prevalence of infection.

Infection by *H. pylori* remains the most frequent and persistent bacterial infection worldwide; therefore, accurate diagnosis of infection is imperative. Determining the diagnostic method and therapies to use for each patient depends on several factors, such as the patient's clinical condition and the prevalence of infection (Garza-González et al., 2014). The ideal test for diagnosis of *H. pylori* infection should be noninvasive, highly accurate, widely available and inexpensive. Furthermore, it should be able to discriminate the colonization from *H. pylori* associated disease. That is why non-invasive tests are

useful for primary diagnosis, when a treatment indication already exists, or to monitor treatment success or failure (Jafar et al., 2013).

Limitations of the study

One of the limitations of the present study was that we could not compare our 3 tested non invasive techniques to one of the invasive techniques that need endoscopy/biopsy, as this wasn't feasible during the study period. Another limitation was the relatively small sample size, though we have abided by the statistically calculated sample size based on the prevalence rate of *H. pylori* in children. Increasing the number of patients would have yielded more profound data.

Conclusions

1) *H. pylori* stool Ag test was the most reliable, rapid, simple and easy to perform non invasive test in all studied individuals.
2) Testing *H. pylori* IgG Ab in serum was more useful in adults than in children in the diagnosis of *H. pylori* infection.
3) Although saliva IgG Ab test was easy especially among adults, yet it wasn't reliable as its results were inferior to those of stool Ag and serum IgG for the detection of *H. pylori* infection in both children and adults.

Conflict of Interests

The authors have not declared any conflict of interest.

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