Study the Prevalence of *Helicobacter pylori* Infection by Different Diagnostic Methods

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Abstract

A total of 41 patients with gastro duodenal symptoms (show signs of inflammation with or without duodenal ulcer) . 21 males (51.2%) and 20 female (48.8%) with an average age 0f (20 – 80) years old under going gastrointestinal endoscopy at Baghdad teaching hospital in internal disease clinical laboratory , between (February – June) 2009 . Biopsies specimen of antrum , gastric fundus ,& duodenal bulb were examined by the following methods (rapid urease test , Giemsa stain section to detect bacteria , & Haematoxilin and Eosin stained section for pathological study which are considered the gold standard methods , sera or plasma from these patients were tested by immunochromotography (ICM),serological method for IgG antibodies to *H. pylori*. History picture are(use of certain medication , tobacco , alcohol, and current infection are taken). The results showed that the percentage of prevalence (positive results)were (83%) by histopathological method while it gave only(73%) by serological method and(66%) by rapid urease test, and the prevalence in males was more than in females (44%), (39%)respectively ,and also the prevalence increase with age (40 – 60) 14 out of 15, most patients show gastritis and duodenal ulcer, 25 (60%) by endoscopy diagnosis and 7 (17%) show malignant cancer ,while 9(22%) without any symptoms. The sensitivity of urease test (82%) and specificity (88.1%) and by ICM sensitivity (86%) and specificity (67%) comparing with gold standard methods 100% . The aim of this study is to compare the different diagnostic techniques of *Helicobacter pylori* infection by using invasive methods (histological examination of gastric & duodenal biopsies stained by Giemsa &Haematoxilin & Eosin methods , & rapid urease test which is considered the gold standard methods & non-invasive serological methods such as ICM rapid test , all these tests provide information about the incidence and prevalence of *H. pylori* in population , diagnostic value for each test also the eradication of person.

Keywords: Serology, *Helicobacter pylori* , gastric ulcer , Diagnosis

الخلاصة

يتضمن البحث دراسة انتشار بكتريا *Helicobacter pylori* المسببة لقرحة المعدة والاثنى عشري في عينة عشوائية من المرضى الذين ادخلوا إلى شعبة الناظور في مستشفى مدينة الطب التعليمي في بغداد وذلك باستخدام طرق تشخيصية مختلفة منها الفحص النسيجي باستخدام صبغة الهيماتوكسيلين وصبغة الكمزا وذلك لدراسة التغيرات النسيجية وفحص تواجد البكتريا في النسيج , كذلك فحص العينات بطريقة التحقق من إنتاج إنزيم اليورياز بالاعتماد على وسط اليوريا السائل الحاوي على دليل الفينول الأحمر وكذلك بالطريقة الغير مباشرة بالفحص السير ولوجي للأجسام المضادة IgG بالاعتماد على الطريقة السريعة ICM , وقد وجد ان نسبة الانتشار والإصابة تزداد مع تقدم العمر وخاصة بين الأعمار ٤٥-٦٠ سنة وان الإصابة بالرجال ٤٤% نسبيا أعلى من الإناث ٣٩% . ان التشخيص الأولي بالناظور يبين انه المرضى المصابين بقرحة المعدة والاثنى عشري هم حوالي ٦٠% بينما المصابين بسرطان المعدة ٢٢% والمرضى العاديين ١٧%وان اعلى نسبة للانتشار شخصت بطريقة الدراسة النسيجية ٨٣% بينما كانت ٧٣% بالطريقة السيرولوجية و ٦٦% عن طريق فحص انزيم اليورياز , اما كفاءة وحساسية هذه الطرق فتتراوح بين ١٠٠% بالدراسة النسيجية و ٨٦% , ٨٢% بالطرق السيرولوجية والانزيمية على التواليز.

Introduction

In 1983 Warren and Marshal [(1)] isolated a new curved gram negative bacillus from gastric mucosa of patients with active chronic gastritis , this bacteria was first named *Campylobacter pyloris* then *C. pylori* and finally *Helicobacter pylori* [(2)] , establishing an association between the bacteria , gastritis and peptic ulcer disease . *H. pylori* is the most important cause of chronic gastritis [(3,4,5)] , it is also the most important etiological factor responsible for duodenal ulcer [(3,4,5)] ,gastric ulcer [(3,4,5)] , and has an important role in the pathogenesis of gastric cancer [(6,8)] . *H. pylori* is also responsible for dyspeptic patients, and screening for *H. pylori* in those patients improve selectivity for gastroscopy [(5)]. The identified virulence factors of *H. pylori* include the flagella used for motility through the mucus , the urease activity used for neutralizing the acid from the stomach . The cytotoxin activity which vascuolize the epithelial cells [(10,11)] and this examined by histopathological study . Since Marshal and Warren established the association between *H. pylori* , gastritis ,& peptic ulcer, a great number of diagnostic techniques have been developed [(12)].The first rapid and simple test developed for the diagnosis of *H. pylori* infection was urease test based on the capacity of the organism to produce great quantities of this enzyme [(13,14,15,16)] .

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The urease catalyzes the degradation of urea to ammonia and bicarbonate. This reaction produces an increase in the pH of the medium that can be detected by an acid – base indicator such as phenol red , that changes color from yellow to pink [15] .The velocity of the change of color depends on the urease concentration according to the numbers of bacteria present [17] .The great advantage of the urease test in the diagnosis of *H. pylori* is that the result can be obtained before the patient leaves the endoscopy room. The result were comparable in sensitivity and specificity with the histological and culture techniques and staining section by Giemsa stain which are considered the gold standard methods (gastric biopsy is required to perform the test) [18,19] . McNulty and Wise [15] were the first ones to use this test to detect *H. pylori* infection . Serological tests are useful in *H. pylori* infection because virtually all patients colonized with this organism under a local antibody response directed against antigens covering the surface and flagella of the organism and this antibody response detected in the serum[23,24,25]. Also serological methods used to diagnose *H. pylori* in which no upper gastrointestinal endoscopy is required . Maastrich 1996 working group cited by Anon suggested that screening dyspeptic patient under 45 years of age for *H. pylori* might reduce the need of endoscopy [20,21] , but blood must be obtained to detect *H. pylori* antibodies[22] .H. pylori serology is alterative in comparison with other methods because it is simple , inexpensive , & less of a burden for the patient .Several kits for detection *H. pylori* by serology have become commercially available since the discovery of *H. pylori* by Warren in 1983 [1], most of these kits are based on various antibody preparations and different techniques , this lead to an increase in the number of studies that have evaluated kit characteristics . Different studies for comparison between kits to account for the different reference standards and designs used by various investigators [22,23,24,25] . Serological diagnosis simplest and least expensive , non – invasive method for IgG and or IgA antibodies , latex agglutination methods are quick tests , useful for screening purposes .ELISA based tests accurately quantities the amount of antibody (titer) present and are promising tool for assessing the efficacy of *H. pylori* eradication treatment [27] , also for rapid office – based serologic test , using immunochromotography (ICM) , and the immunoblott for the diagnosis of *H. pylori* [26] . C13 / C 14 , urea breath test are reliable non – invasive methods for diagnosis of on going *H. pylori* infection [30,31] .

Material and methods

Samples : 41 gastric and duodenal biopsies from patients of the endoscopy department of Baghdad teaching hospital – Baghdad / Iraq , were analyzed between (February – June) 2009 , at least two biopsies were taken from the antrum of each patient for histological study send to histopathological laboratory of the hospital stained by Giemza method (Luna 1968) [32] & Haematoxilin & Eosin method (Modified m. of Guyer ,1953) [33]by (Gram Wegurt) to study the histological change and detecting rod shaped *H. pylori*.

Phenol red rapid urease test

A solution of urea 10% and solution of phenol red 1% were prepared for the working solution , 0.1ml of phenol red solution were mixed in 1 ml of the urea solution . The reagent is stable for two weeks of 4 – 8 °C each biopsy was embedded in 0.2 ml of the reagent and incubated at room temperature (22°C) for 1 min.

Serological diagnosis by (ICM) immunochromotography method of (ACON H. pylori one step –rapid test Devise)

Serum / plasma is a sample test that utilized a combination of *H. pylori* antigen coated particles and anti – human IgG to qualitatively and selectively detect *H. pylori* antibodies in serum or plasma in10 minutes after serum or plasma specimen is placed in the specimen well., it reads with *H. pylori* antigen coated particles in the test .The mixture migrate chromatographically along the length of the test strip and interacts with the immobilized anti – human IgG , if the specimen contain *H. pylori* antibodies , a colored line appear in the test line region , indicating a positive result , if the specimen dose not contain *H. pylori* antibodies a colored line will not appear in this region , indicating a negative result comparing with positive control – test , the result should be read at 10 min.(ACON lab. Inc – 4/08 Sorrento Valley Boulevard .San Diego ,CA 9212,USA). Personal information about past infection , treated use of certain medication , alcohol and tobacco , this result were analyzed according to age , sex ,race and another characteristics .

Calculation of sensitivity and specificity

Positive and negative predictive values were made using the following formula :

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + false negative}} X\ 100$$

$$\text{Specificity} = \frac{\text{True positive}}{\text{True positive + false negative}} X\ 100$$

$$\text{Positive productive value} = \frac{\text{True positive}}{\text{True positive + false positive}} X\ 100$$

$$\text{Negative productive value} = \frac{\text{True negative}}{\text{True negative + false negative}} X\ 100$$

Results

A total of 41 patients were investigated, 21(51.2%) male and 20(48.8%) female with mean age 45 years old (range 20-80year), these patients under examination showed by endoscopy diagnosis that 14(34%) of them have gastric ulcer, 11(27%) duodenal ulcer, 7(17%) with gastric cancer and 9(22%) non ulcer dyspepsia. Tables 1&2 show that the percentage of infection with *Helicobacter pylori* or the prevalence of infection which studed by histopathological and Giemsa staining section methods increase in males 18(44%) more than in females 16(39%) and also the percentage of infections increase with age between (40-60) years old 14(34%) out of 15(36.5%) patients, the percentage of infections more than in younger and older patients. Table (3) shows the relation between the endoscopy diagnosis with positive and negative result of infection done by different diagnostic methods , in which histopathology and Giemsa staining methods gives 34(83.0%) positive, 7(17%) negative, by serodaignosis (ICM) test give 30(73%) positive, 11(27%) patients negative while by rapid urease test 27(66%) positive, 14(34%) patients negative. The positive value of serodignosis and urease test consist 88%,79% respectively from the true positive value by histopathological study (34+) patients.From these result the high prevalence of infections were obtained first by histopathological study then by serodiagnosis methods and later the lowest value by urease test. In all test used the prevalence over 75% considered high prevalence of infection in population [6]. Also if we determined the positive value of diagnosis in relation with disease or endoscopy finding, histopathological study gives 90% positive in duodenal ulcer and 80% with gastric ulcer comparing with serodiagnosis (82%),(72%) and urease test (72%),(54%) irrespectively.

Table (5) show the sensitivity& specificity for each test depending on true positive, true negative, false positive, false negative values determined in table (4), comparing with a gold standard method of diagnosis and this gives the sensitivity & specificity of histopathology & Giemsa staining methods 100% , by serodiagnosis (ICM) method (86%), (67%) and by urease broth test (82%), (87%), means that the first method gives more accuracy result than others, with many disadvantage, and the other methods give less accuracy with many advantages discussed later in discussion.

Table 1 : Show the prevalence of H. pylori infection in male & female.

Total number	Percentage	Number of positive H. pylori	Percentage of infection
Male (21)	51.3%	18	44%
Female (20)	48.7%	16	39%

Table 2 : Show the prevalence of infection in different age groups.

Age in years	Total number (41)	Percentage (%)	Number of positive	Result (%)
20 – 30	9	21.95	6	14.6
31 – 40	12	29.2	9	12.5
41 – 50	7	17	7	17.0
51 – 60	8	19.5	7	17.0
61 – 70	3	7.3	3	7.3
71 - 80	2	4.8	2	4.8

Table 3 : Show the percentage of positive and negative H. pylori infection diagnosed by urease , serological kit and endoscopy biopsies.

Total number (41)		Gastric ulcer No.(%)	Duodenal ulcer No.(%)	Gastric cancer No. (%)	Non-Ulcer Dyspepsia No.(%)	Sum.
		14 (34%)	11 (26 %)	7 (17%)	9 (22%)	41(100%)
H. pylori (+)	Histopatholog Giemsa stain	11(26.8%)	10(24.4%)	7(17%)	6(14.63)	34(83%)
	Sero.Kit	10(24.4%)	9(21.95%)	4(9.75%)	7(17%)	30(73%)
	Urease	9(21.95%)	8 (19.5%)	5 (12.19)	5 (12.19)	27(66%)
H . pylori (-)	Histopatholog Giemsa stain	3(7.3%)	1(2.43%)	0	3(7.3%)	7(17%)
	Sero. kit	4 (27%)	2 (4.87%)	3 (7.31%)	2 (4.8%)	11(27%)
	Urease	5 (12.2%)	3 (7.3%)	2 (4.87%)	4 (9.75)	14(34%)

Table 4 : Number of true positive , true negative ,false positive ,false negative by different diagnostic methods.

Test	True positive	False positive	True negative	False negative
Histological changes	34	0	7	0
Geimza stain	34	0	7	0
Urease	27	1	7	6
ICM (Kit) serology	30	2	4	5

Table 5 : values of different diagnostic methods .

Test	Sensitivity %	Specificity %	P.P.V. %	N.P.V. %
Histopath.	100	100	100	100
Giemsa stain	100	100	100	100
Rapid Urease test	82	88	96	54
Rapid serodiagnosis (ICM)	86	67	94	45

If you know the prevalence of Helicobacter pylori in your population you can make a judgment about the predictive value of a positive or negative test from the table(6).

Table 6 : Predictive value of a test with 85% sensitivity and 79% specificity

Prevalence of disease	Probability of disease with positive result(%)	Probability of disease with negative result(%)
10	**31**	**2**
50	**80**	**16**
90	**97**	**63**

Discussion

The aim of this study is to determine the prevalence of infection with *H. pylori* by different diagnostic methods in random population enter the endoscopy department for gastric and duodenal examination with or without symptoms of inflammation. From the 41 patients under investigations results in table (1) showed that prevalence of infection in males more than in females and these results agree with other studies done by Nicholas *et al* (1992), in which they found that 47% males out of 82 patients, (44%) 0f them were infected by *H. pylori*[25]. Table (2) showed that prevalence of infection increase with age between (40-60) years old agreed with Nulty (1999) which found that the more likely age of infection in patients over 50 years old (42%) than in younger patients (21%) [22], another group of Liston R, *et al* (1996) cited by Nulty(1999), found that (31.7%) of elderly patients with seropositive result had no evidence of active infection determined by endoscopy and urease test. Older patients are more likely to have developed atrophic gastritis and *H. pylori* can not readily colonize this type of gastric mucosa [22]. It was recognized that prevalence of *H. pylori* infection increase with age in a symptomatic persons in developed countries and this tend to plateau at around the age of 60 years, related to socioeconomic status and ethnicity [5,23,25].Table(3)which showed high prevalence of infection given by histopathological study which are considered invasive gold standard methods [22,25,26] , (83%) of patients gave positive result ,while by serodiagnosis (ICM) method (73%) and (65%) by urease test method which means that these methods gives lowest value of prevalence comparing with histopathological study and this because many factors were affecting on the value of the result such as for example.

- Negative values in biopsy methods histopathology & Giemsa staining section depend on patient that may be under treatment or past proven *H. pylori* infection treated with a course of antimicrobial drugs with proton pump inhibitors , that patient give negative and clearance of the disease in biopsy specimen but can give positive result with serodiagnosis, and so give false positive result and high prevalence than biopsies[23,25].
- A negative value in urease test depend on non homogeneous distribution of the microorganism in the stomach and this situation is overcomed by use of several specimen from (3-5) for the same patient [34,35,36] so we minimize the specimen error and this explain the 13 patients which give negative result by this method, which lowering the percentage of infection comparing with other methods.
- In serodiagnostic method the percentage of infection (73%) which gives positive result and 11(27%) patients comparing with histopathological study , 4 patients only were true negative and 5 patients were false negative and only 2 patients gave false positive result , this can be explained by:
  - Patients who are in acute case of infection before an IgG response has developed gave false negative serological result ,means that it may be positive result in biopsy method, also negative result may be due to patient not produce circulating antibody response which detectable only with complex type of antigen (Preez-Preez, *et al*. cited by Nicholas(1992) [25] .
  - False positive result according to cross reaction antigen (3-9%) of patient have false positive result with *H. pylori* that might produce antibodies such as Gastrospirillum hominis and this also depend on type of antigen used in test [23,25] or it may be that patient with past infection that gives false positive result with slowly return antibody, it may give positive test for over 6 months from clearance of the disease [25,26,34].

Table (5)show the sensitivity & specificity of each test depend on the true positive ,true negative , false positive ,& false negative value in table (4), each positive value in histopathological study considered true positive value and each negative value considered true negative value (gold standard methods) and so sensitivity & specificity (100%) and this also agreed with other study which find that these methods gives sensitivity & specificity between (98-100%) [34], and for serodiagnosis (86%) , (67%) while urease test gives sensitivity & specificity (82%),(87%).The sensitivity & specificity of serological test reported by many workers varies from (76-96%) and half of the patients with false positive result were over 50 years age. Other group found that elderly patient with positive serology had no evidence of active infection determined by endoscopy and urea breath test [14,22], other workers for the same methods (ICM) test find that the sensitivity is (96%) with(94%) specificity [26] which used this test to diagnose *H. pylori*

infections in thai children between(1.5-16 years old), other study compare different serological kits for *H. pylori* infection and also found that the sensitivity and specificity around (67%-86%) [22], other outhers [25] find that sensitivity of commercial ELISA kits had an accuracy between (89%- 95%).The sensitivity of urease test according to Eugenia [34] is record to be (97%) by phenol red broth test with (100%) specificity also he mention that other authors have reported that urease test with specificity between (98%-100%) and sensitivity between(64%-98%),and this agreed by Vaira[37,38], Thillainayagam [39], Mal.fertheiner[40], McNulty[15] , Arvid, Morris[42], &Westblom[14] reports specificities of (86%,98%,92%,100%) and sensitivities of (84%,92%,100%,88%) respectively, only Hernandez reports a sensitivity of (72%) and specificity of (83%) for Christensen's urea broth [43].The presence of false negative and false positive result may be explained by the patchy distribution that *H. pylori* present in gastric mucosa ,especially in the body and fundus of the stomach, so the microorganism can be present in one biopsy and absent in another from the same patient [34, 44,45]. So the false negative value by this test were caused by the patchy distribution of the bacteria.

Conclusion

It has been proposal that patient with dyspepsia could be screened for *H. pylori* status before it is recommended [25,22,23] and as *H. pylori* occurs in over (90%) of patient with chronic duodenal ulceration and up to (80%) of patient with chronic gastric ulceration [25,16,21], it has been proposal that such an approach would help to reduce the need for endoscopy as well as cost, if such a policy were adopted only seropositive patient would undergo endoscopy and over 45 years of age or those taking anti inflammatory drugs, this would avoid up to (23%) of endoscopies. However ,further large prospective clinical studies are needed before such an approach can be accepted. Also serological methods can be used for monitoring treatment and successful eradication of infection by detecting the fall in level of IgG antibodies in serum after 3 months of treatment. The great advantage of the urease test in the diagnosis of *H. pylori* is that the result can be obtained before the patient leaves the endoscopy room, making clinical management easier. The studies suggest that urease result comparable in sensitivity and specificity with histological and culture techniques, being more economic and faster [12,34], Nevertheless an endoscopy is always necessary because a gastric biopsy is required to perform the test and also culture can be required for evaluating the sensitivity to antibiotics, so urease test should be done jointly with another diagnostic test as histology or culture. Some authors and reports go to connect in a table between prevalence and sensitivity & specificity of different methods (Table6).

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