Original article

Sere-prevalence of Helicobacter pylori infection in unselected adult population in Iraq

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Abstract:

IgG antibodies against H. pylori were studied by home made ELISA using whole cell antigen. The performance indices of the ELISA plates were evaluated against the "gold standard" invasive methods (culture, urease production and histology) and found to have a sensitivity of 92.5%, specificity of 70%, positive predictive value of 91.1 % and negative predictive value of 73.6%.

One thousand adults who had no Gastrointestinal or cardiovascular complaints were randomly selected and studied. Their age ranged from 15-68 years. Possible risk factors as age, sex, socioeconomic status, educational level and smoking habits were evaluated.

This study shows that 77% of the population studied was infected and that the age was a strong risk factor for infection.

Key words: Helicobacter pylori, prevalence, ELISA, Risk factors

Introduction

H. pylori is a spiral shaped microaerophilic bacterium that colonizes the gastric mucosa and causes both acute and chronic gastritis, duodenal ulcer and infection is considered an important risk factor for the development of gastric cancer[1]. Infection with H. pylori may be detected by invasive techniques as culture, urease test and histological staining of endoscopic biopsy specimen, or by less invasive techniques such as urea breath test[1,2,3]. Sero-diagnostic test is most frequently used for epidemiological studies and has been used to measure the prevalence of infection in various populations.

The seropositivity show marked differences among different parts of the world being very high in developing countries with less, though variable prevalence in developed countries. Figures of prevalence vary in different studies in different parts of the world according to the population studied and possible risk factors. These factors include age, gender, socioeconomic status, educational level, crowding and sanitation. Figures as 34%, 54%, 59%, 69%, 75%, 77%, 83% were recorded[4,5,6,7,8,9,10].

Materials And Methods

Blood was collected from 1000 consecutive adults presenting to Saddam General Hospital in Al-Anbar Governorate through the period from May-July 2000, excluding those with dyspeptic symptoms and possible myocardial ischaemic complains. The blood specimens collected were allowed to clot and the sera were separated. The sera were aliquoted, frozen and stored at -20C until

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required. Sex, age education standard smoking habits and crowding index were recorded.

Home made ELISA

The antigen used was a centrifuged sonic extract of local strains of H. pylori isolated from antral biopsy specimens and proved by biochemical tests (API Campy) (bioMerieux). H. pylori bacterial lysate was prepared as described by Blanchard et al[11]. Briefly, H. pylori organisms were harvested from liquid culture by centrifugation and resuspended in 2 mL PBS per 200 mL of bacterial culture. Bacteria were lysed by sonication, and the remaining whole organisms were removed by centrifugation and filtration through a 0.22 uM pores filter. The concentration of protein was determined by the Lowery assay[12].

The prepared antigen stored at-20° C until used. Before coating the ELISA tray, the concentrated antigen was diluted in 0.5 M carbonate buffer (pH 9.6) to give a final concentration of 2.0 ug of protein/ml. Coating was done by applying 200 uL of the diluted antigen to each well of a polyvinly flat-bottomed plates (Linbro, Flow laboratories). The plates were incubated for 24 hr at 4° C. Each well was then aspirated and refilled with 300 uL blocking buffer*. The plates were kept at 4°C until used.

Antigen-labeled plates were removed from the refrigerator and brought to room temperature before use. Wells were washed three times with washing buffer(PBS containing Tween 20 and preservative)(bioelisa). Each test serum was diluted 1:200 in diluent buffer (phosphate buffer containing protein, Tween 20 and preservative) (bioelisa). A 100 uL sample of each test serum dilution was added in duplicate to the microtiter plate and incubated at room temperature for one hour. Wells were washed five times with washing buffer. Peroxidase labeled antibody to human IgG (HRP-conjugated monoclonal anti-human IgG in stabilizing buffer) (bioelisa) was diluted in diluent buffer. One hundred uL of the diluted conjugate was applied for each well and incubated for one hour at room temperature. The washing steps were repeated and lastly a 100 uL of the substrate (TMB in buffer containing preservative) (bioelisa) was applied for each well and incubated at room temperature in the dark for 30 minutes. The reaction was stopped by 100 uL of 0,1 mol/L sulfuric acid. The absorbance was measured at 450 nm.

Optimal dilutions of all reagents were determined by checkerboard titration.

Requirements for acceptance of ELISA plates:

Each plate was examined by three control sera:

One control is a pool of positive sera with high titers of antibody (commercially available from biohit, Finland and Biokit, Spain as well as high titer sera obtained from previous studies). This was diluted from 1:200 in doubling dilutions to 1:25,600 to allow the construction of a standard curve. The highest dilution of the serum was arbitrarily designated as being equal to one enzyme unit The plate was accepted if the correlation coefficient (r) of this standard curve was >0.9.

The second control was a pool of negative sera ready for use obtained commercially and was put on each plate in a duplicate. The absorbance of these controls had to be < 0.1 (after blank subtraction).

The third control was a low positive control, which was handled as the second control.

Determination of the cut-off value

The cut-off value was determined by measuring the mean optical density of the known negative samples + 2 standard deviations[13]. The upper limit of these samples was 0.3. Weak positive samples should exceed this lower limit.

Evaluation of the ELISA performance:

To determine sensitivity, specificity, positive and negative predictive value of our ELISA system, 87 patients attending the endoscopy unit of Saddam general hospital in al Al-Anbar govornorate with different types of gastric complains were enrolled in this study.

Blood samples were collected before endoscopy. Gastric antral biopsy specimens were taken.

Patients aged less than 18 years; patients who had taken antibiotics or proton pump inhibitors or bismuth preparations in the previous four weeks were excluded from the study.

The blood specimens collected were allowed to clot and the sera were separated. The

sera were frozen and stored at -20° C until required.

Antral biopsy specimens were collected for culture of H. pylori, histology and urease production.

Culture

Biopsy specimens for culture were transported to bacteriological laboratory in sterile brain heart infusion broth and were kept in a cool bag or 4°C until cultured. The specimens were processed within a limited time of not more than four hours. Antral biopsies were crushed on sterile glass slides, homogenized with sterile needles and then cultured on brain heart infusion agar containing 7% horse blood, 0.25% yeast extract and Campylobacter selective supplement (skirrow- Oxoid SR 69) containing vancomycin, polymyxin and trimethoprim. The pH was adjusted to 6.8-6.9. Plates were incubated in microaerophilic environment generated by gas pack (Generbag Microaer, BioMerieux 45531) at 37°C for up to seven days. Suspected colonies of H. pylori were identified by Grams staining, catalase and oxidase test. Confirmation of the isolate was done by API campy system (Bio Merieux). Subculturing was done in brain heart infusion broth-filled containers, incubated for 3 days under microaerophilic conditions [14,15].

Histology

Hematoxylin and Eosin stain was used by pathologists for identification of the bacteria in the biopsy specimens [14,15].

Urease test

Presumptive evidence of the presence of H. pylori in biopsy material was obtained by placing a portion of the crushed tissue biopsy material directly into urea containing agar (Zurcal Hp test, Nycomed, gennany and Cambridge life science, UK). Ten to 12 drops of a supplied buffer was added and the kit was incubated at 37°C. A positive test manifested by color changes (yellow to pink) due to alkalinization of media within 30 minutes is considered indicative of the organism presence. The test is considered negative if no color change is seen in 24 hours.

Statistical analysis was done using Chi-square test of significance. Correlation coefficient (r) was calculated as an indicator of association between antibody titer and optical density in assessing ELISA plates performance. Moreover, this test was used as indicator to any association between H. pylori seropositivity and age in years.

Results:

Of the 87 patients participated in this study 67 were positive for H. pylori by one or more of the 'gold standard' tests (culture, histology and direct urease test).The remaining 20 were negative for H. pylori by all the three tests. The pattern of these results is shown in table 1.

The evaluation of the performance of serological test (ELISA) in comparison with the gold standard biopsy related tests is shown in table 2. Evaluation of performance indices against known sera has to show acceptable parameters.

Of the one thousand individuals enrolled in our study 41.3% were females and 58,7% were males with age ranging from 15-65 years. Age and sex distribution of the tested population is seen in figure (1).

Distribution of sera positive for anti- H. pylori by age and sex is shown in figure (2). This figure shows that a large percent of the population are seropositive for H. pylori. The percent of sera positivety increased with advancing age. Overall seropositivity was 77%.

Concerning sex, 75.7% of the females were seropositive which is almost equal to the 77.8% of seropositivity seen in the male gender. There was no significant effect of gender on the acquisition of H. pylori infection ($p>0.05$).

Table (3) shows the details of possible risk factors including age, some household living conditions, socioeconomic status, crowding and some personal habits.

Seroprevalence of H. pylori infection increased with age. The correlation coefficient (r) between percentages of seropositivity and age was found positive and very high ($r = 0.987$).

Though the percentage of positive cases was higher with reduced level of education, this difference did not reach statistical significance ($p>0.05$).

Smoking was significantly associated with the seroprevalence of H. pylori infection ($p= 0.016$).

As to the effect of socioeconomic state, seropositivity was higher in the middle and low

status were seropositive whereas 77.8% and 66.6% from medium and high socioeconomic classes were seen respectively. This difference is statistically significant (p= 0.004) Similarly, the effect of crowding was significant in the acquisition of H. pylori infection (p= 0.0001).

Table 1
Biopsy related tests for the detection of H. Pylori infection in gastric biopsies

Urease	Histology	Culture	No. of Patients
+	+	+	4
+	+	-	54
+	-	+	7
-	+	+	2
-	-	-	20
65(97%)	60(89.5%)	13(19.4%)	87

Table 2
Evaluation of the performance of home-made ELISA in comparison with the gold standard biopsy related tests

		Gold Standard		Sensitivity %	Specificity %	PPV%	NPV%	Overall accuracy %
		+	-					
ELISA	+	62	6	92.5	70	91.1	73.6	87.3
	-	5	14					

Table 3
possible risk factors associated with H. Pylori infection

Possible risk factor		N0. Of seropositive (%)	Total No. of cases	X^2	P
Sex	male	456(77.8%)	587		
	female	313(75.5%)	413	0.585	0.445
Age	15-24	69(62.7%)	110		
	25-34	182(69.5%)	262		
	35-44	219(77.1%)	284		
	45-54	213(86.6%)	246		
	55-over	87(88.7%)	98	41.488	<0.0001
Education					
	Illiterate	115(82.7%)	139		
	Primary	215(76.7%)	280		
	Secondary	390(76.4%)	510		
	Higher	50(70.4%)	71	4.403	0.221
Smoking	Never	248(76.5%)	324		
	Former	103(87.2%)	118		
	Current	419(75.1%)	558	8.240	0.016
Socioeconomic state					
	Low	265(80.3%)	330		
	Medium	405(77.8%)	520		
	High	100(66.6%)	150	11.307	0.004
Crowding (house density) person/room					
	<1	49(59%)	83		
	>1	721(78.6%)	917	16.493	<0.0001

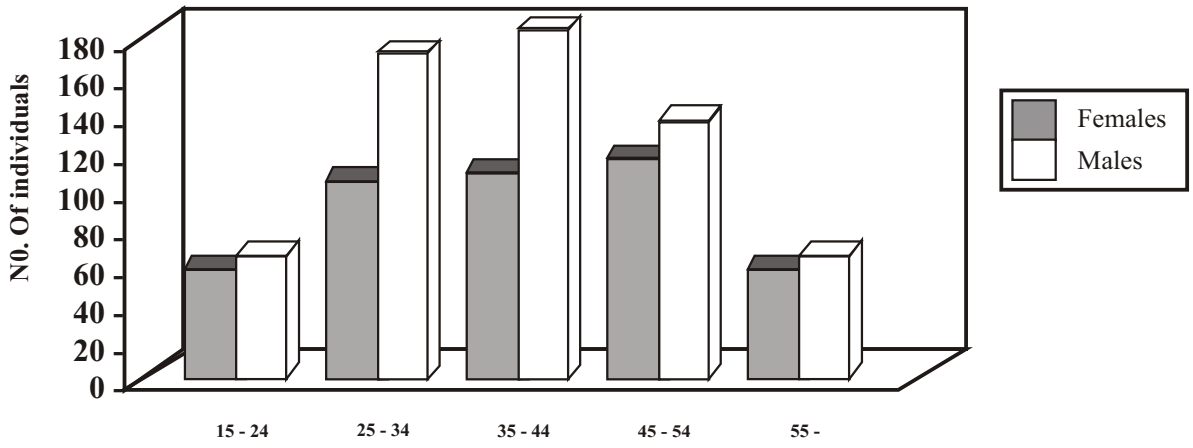

Fig -1
Distribution of individuals enrolled in the study of H. Pylori prevelance by age and sex

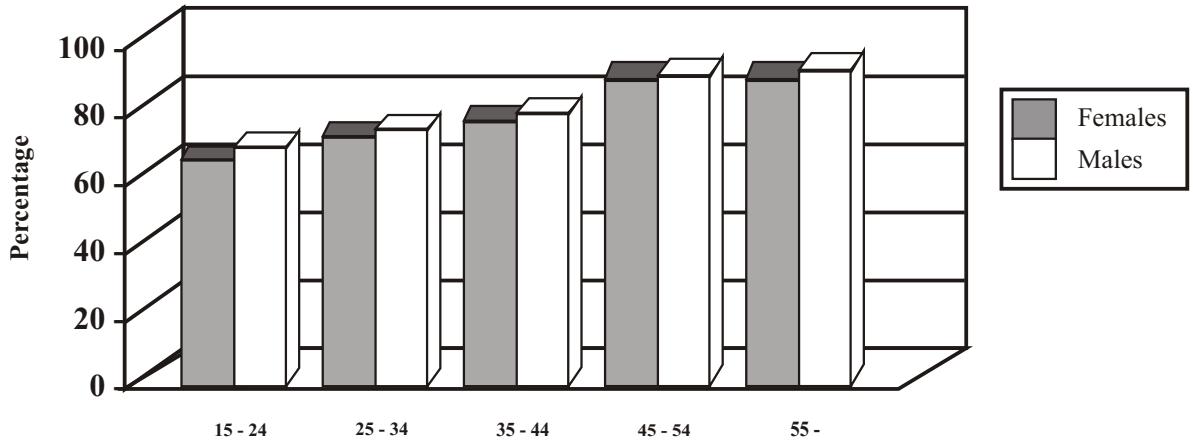

Fig -2
Distribution of percentages of positive sera for H. Pylori IgG antibodies by age and sex

Discussion:

The epidemiology of H pylori infection is a key point in developing a more comprehensive approach to the natural history of nonulcer dyspepsia, duodenal ulcer and carcinoma of the stomach. Moreover, the study of the risk factors and relations of the infection with household conditions might cast a light on source, mode of transmission and pathogenesis of H. pylori infection.

To perform such study we need to prepare an adequate material for screening a reasonable number of Iraqi people. For seroprevalence of H. pylori, we chose to perform the study by ELISA system that was made from locally isolated strains. Moreover, rigid criteria were selected for acceptance of these kits. Each ELISA plate was tested through the following steps:

1. Each plate was examined by three groups of control sera, which were negative, positive and weak positive for anti- H. pylori antibodies. Values were plotted and a correlation coefficient of standard curve of > 0.9 is required for acceptance.
2. Evaluation of performance against known sera by the gold standard biopsy related tests were done and adequate performance indices of sensitivity, specificity and predictive values is required for acceptance.
3. The cut-off value was determined by the accepted method of calculated a mean of well known negative samples + two standard deviations.

Table (2) shows the acceptable performance of our ELISA plates.

In an epidemiological study, the first question, which arises, is the representativeness of the population. To perform such a study, our sample need to represent me population by random selection of normal healthy people who are ages, sex, residence, and socioeconomic matched to the total population. Such a criteria are difficult to obtain in the time period and facilities for this study. So we were left with two possibilities. The first is to choose sample of blood from the blood banks or we randomly select patients from a general hospital. The next choice seems more representative of the population and we examined 1000 patient for anti-H. pylori antibodies. Figure (1) shows the age and sex distribution of our sample studies and figure (2) shows the percentage of seropositive cases. The overall seropositivity in our sample was 77%.

By comparing these figures with different studies from different populations it became quite clear that comparison is a difficult task due to different selection criteria of the examined population (mainly age factor) and different methods of detection. For example 34%, 54%. 59%, 69%, 75%, 77% and 83% of the healthy population is Denmark, United state of America. Ethiopia. Colombia, South Korea, San Marino, and India were seropositive [4,5,6,7,8,9,10].

The task was more difficult when we try to

compare our figures with other studies done in Iraq. The majority of work done was on patients with gastrointestinal symptoms and on a limited number of patients. Figures on individuals with nonulcer dyspepsia vary from 57%, 64%. 80%, and 83%[16,17,18,19].

Concerning possible risk factors, gender was the first to be considered. In our study, 77.8% of the males were affected compared to 75.7% in females. This difference was non significant statistically ($p>0.05$). Actually, the effect of gender on seroprevalence is variable in different studies. Many showed that the prevalence is independent on sex[20], some showed more prevalence in females[21,22] while the other showed the opposite[23,24].

Age shows a significant effect on the prevalence of H. pylori. This is may. be due to the possible mode of transmission whereby spread infection is acquired from person to person by oral-oral route or fecal-oral route. Definitely, chances of acquisition of infection increase with age.

Smoking was another factor that plays a significant role with the seroprevalence of H. pylori. This is possibly due to some of the non hygienic habits associated with smoking, which might involve the transmission of bacteria through the saliva of smokers.

Regarding socioeconomic status, we do agree with other studies done in the developing countries, which showed higher prevalence in low socioeconomic group[21,22]. This could be explained again by the increased chances of transmission in this population due to improper hygiene and health care, the same is applicable with crowding and increased number of persons per room.

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