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Direct Diagnosis of Helicobacter pylori Infection by Detection of specific IgG antibodies in saliva of Iraqi patients

التشخيص المباشر لاصابة بكتريا Helicobacter pylori من خلال التحري عن الامينوكلوبيولين IgG النوعي في لعاب المرضى العراقيين

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ABSTRACT

Detection of helicobacter pylori (H. pylori) by invasive methods is costy and unpleasant for patients and is not free form complication ,thus in our study we evaluated an enzyme - linked immunosorbent assay (ELISA) for measurement of H. pylori specific IgG in saliva in comparison with biopsy urease test and serum IgG.from 100 patients consult gastrointestinal in Najaf Hospitals during the period from February 2006 to March 2007 detailed history was taken and blood group was detected for each one .then the patients underwent endoscopy and serum and saliva was collected from each one. urease was done for samples collected by endoscopy . serum and saliva was evaluated for presence of specific IgG by ELISA kit. Our results showed that 42(82%) of urease positive patients had IgG in saliva, whereas 47(94%) urease positive patients had IgG in serum and no statistical differences (p-value >0.1).the result show highest concentration of saliva IgG in urease positive patients. There is significant difference between saliva specific IgG for H.pylori with age groups 31-40 years, drink Pepsi more than 4 cup/day, patients who are smoker and those with blood group O. we conclude that salivary IgG detected by ELISA is a rapid ,easy and accurate method for detection and screening for H.pylori among different age groups.

الخلاصة

ان التحري عن جرثومة Helicobacter pylori بالطرق الاجتياحية تعتبر مكلفة ومرهقة للمريض وقد لايسلم المريض من مضاعفاتها . حيث تم في دراستنا الحالية تقييم فحص الاليزا لقياس الامينوكلوبيولين النوعي للعاب بالمقارنة مع فحص اليوريز للخزعة وكذلك الامينوكلوبيولين للمصل. حيث تم اخد معلومات تفصيلية والتحري عن فصيلة الدم لكل مريض قاموا بزيارة قسم امراض الجهاز الهظمي في مستشفيات محافظة النجف من شباط 2006 ولغاية ادار 2007. حيث خضع المرضى الى فحص ناظور الجهاز الهضمي .وتم اجراء فحص اليوريز على الخزعة الماخودة بالناظور . تم اخد عينة من لعاب كل مريض بالاضافة الى عينات المصل وتم تحديد وجود الامينوكلوبيولين النوعيIgG لجرثومة Helicobacter pylori باستخدام عدة الاليزا.اظهرت النتائج ان 41(82%) من المرضى اظهروا فحص اليوريز الموجب كان لديهم الامينوكلوبيولين النوعيIgG لجرثومة Helicobacter pylori في اللعاب و 47(94%) من نفس المجموعة كان لديهم الامينوكلوبيولين النوعيIgG لجرثومة Helicobacter pylori في المصل . كما اظهرت النتائج ان تركيز الامينوكلوبيولين النوعيIgG لجرثومة Helicobacter pylori في اللعاب هو اكثر في المرضى الموجبين لفحص اليوريز.ايضا بينت الدراسة وجود علاقة معنوية بين وجود الامينوكلوبيولين النوعيIgG لجرثومة Helicobacter pylori في اللعاب مع المرضى الدين يتراوح اعمارهم 31-40 سنة والدين يشربون الصودا اكثر من اربعة اكواب يوميا وعند المدخنين وزمرة الدم نوع O. تبين هده الدراسة بان وجود الامينوكلوبيولين النوعيIgG لجرثومة Helicobacter pylori في اللعاب هو فحص سهل ودقيق للتحري والمسح عن وجود جرثومة Helicobacter pylori بمختلف الاعمار.

INTRODUCTION

Helicobacter pylori (H. pylori) was originaly discovered by W.jaworski (1) while the recognize of the patholegical feature of H. pylori inoculation of upper gasterointestinal tract,based on the discovery of H. pylori by Autralians clinicions, Marshal and Warren in 1982(2). Helicobacter pylori colonizes about 50% of the world

population; less than 20% of affected individuals develop gastroduodenal diseases. Gastroduodenal diseases associated with H. pylori occur mainly in adults.Nevertheless, the infection is usually acquired

during childhood (3) and it is possible that the humoral and mucosal tissue responses at that time can determine the natural course of the infection (4).

The high prevalence of H. pylori, arguably the most common cause of chronic bacterial infection in humans (infecting 40 and 80% of individuals in developed and developing countries,respectively) (5, 6), demands the development of a noninvasive, sensitive, economic, and rapid technique of screening to detect infection. The current accepted diagnostic procedures for H. pylori infection include staining of histological samples, culturing of biopsy specimens, and the [13C]urea/ [14C]urea breath test (7, 8, 9). The first two methods require invasive procedures and are therefore performed only for symptomatic subjects. The urea breath test, while not invasive,may be limited by its cost or the use of radiolabeled compounds(10).

However ,endoscopy is expensive ,is unpleasant for patients,and carriers a small but defined risk of complication (11).thus, the use of non-invasive tests to diagnose H.pylori infections is becoming more frequent.furthermore,several tests as first tal tests as first line treatment in the mangement of dyspepsia(12,13)

Several serological methods have also become available, and the presence of H. pylori IgG and IgA antibodies in serum have been found to correlate with infection. It has been recommended that salivary antibody test results can be used to screen patients prior to gastroscopy or to determine the effect of antimicrobial therapy in the eradication of H. Pylori. For such an assay to be clinically useful, it must accurately detect the presence or absence of H. pylori infection. (14) In the current study, we compared the diagnostic accuracy of the enzyme– linked immunosorbent assay (ELISA) for the detection of anti-H. pylori specific IgG in oral fluid with rapid urease test and with serum-based assay and the risk factors relation with elevated of anti-H. pylori specific IgG in saliva .

MATERIALS AND METHODS

Patients clinical specinems

A total of 100 patients undergoning upper gastrointestinal endoscopy in the endoscopic units of Najaf Hospitals were included in this study between 1/2/2006 to 1/3/2007. Detield history was taken from all patients including age ,sex,nutritienal habbt (drink pepsi and tea),smoking, blood groups and family history of upper gastrointestinal diseases,blood was aspirated from each patients to detect ABO and Rh by direct haemoaglutination test .Blood ,saliva (5 milliliters of unstimulated saliva, Saliva, were collected with sterile toothpicks and filter paper,

respectively,) and gastric biopsy specimens taken by endoscope were collected from each patiens.

Rapid Urease Testing of Biopsy Tissues

Urease testing provides indirect identification of H. pylori infection within a few hours of endoscopy (15).

The urease test (CP test: yamanouchi Pharma) was carried out according to the manufacturer's instruct instructtions, and samples were examined for the presence of positive red color at 30 minutes and 24 hours.

serum and saliva antibodies against H. pylori antigen were detected by ELISA, using a commercially available kit (Italain Radim Kit). In order to achieve optimal accuracy saliva samples were diluted to 1:2 and serum samples were diluted to 1:300. In brief, a whole H. pylori strain isolated from samples was used as a source of antigen, diluted with coating buffer, added to each well, and incubated for 2h at 37ºC, the plates were washed with washing buffer, and binding sites were blocked by addition of 2% serum albumin in washing buffer and incubation for 18h at 4ºC, diluted saliva samples were added separately to each well and incubated for 90 minutes at 37ºC. Anti-human antibody conjugated with horsradish peroxidase was added and incubated for 1h at 37ºC, and mixed with tetra methylbenzidine as a chromogen. The colorimetric reaction was then prolonged for 15min at room temperature in the

dark and terminated with the addition of 50µ lit of 4 N H2So4 per well. The absorbance value optical Density) at 450 nm was recorded with an automated plate reader

RESULTS

The study population comprised 100 patients divided into two groups ,the first group including 50 patients who are urease positive for H.pylori and other 50 patients were urease negative. The relation between saliva and serum IgG detection according to the type of disease in urease positive patients, revealed that gastritis significantly associated with Saliva IgG Positive and serum positive specific IgG to H.pyori in urease positive patients (P-value : 0.001) (Table 1).

Table 1: Urease positive patients in deifferent diseases relation with saliva and serum positive specific IgG to H.pyori

Disease	Saliva IgG Positive		Saliva IgG Negative		Total		Serum IgG Positive		Serum IgG Negative		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
Gastritis	26	89.6	3	10.4	29	58	27	93.1	2	6.2	29	58
Gastric ulcer	14	77.8	4	22.2	18	36	17	94.4	1	5.6	18	36
Gastric carcinoma	1	50	1	50	2	4	2	100	0	0	2	4
MALT carcinoma	0	0	1	100	1	2	1	100	0	0	1	2
Total	41	82	9	18	50	100	47	94	3	6	50	100

(P-value : 0.001)

Table 2:shows antibody concentration in serum and saliva in Urease positive patients.

Samples	No.	IgG (IU/ml) concentration	Mean Titers	Standard Deviation	Standard Error
Serum	50	3-110	41.3	29.1	4.8
Saliva	50	12-60	40.5	28.5	4.4

Similarly in Urease negative group, the patients underwent the same assay for IgG in serum and saliva and the concentration of antibody was detected as shown in table 3 and 4.

Table 3: Urease Negative patients in deifferent diseases relation with saliva and serum positive specific IgG to H.pyori

Disease	Saliva IgG Positive		Saliva IgG Negative		Total		Serum IgG Positive		Serum IgG Negative		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
Gastritis	2	5.7	33	94.3	35	70	3	8.6	32	91.4	35	70
Gastric ulcer	3	21.4	11	78.6	14	28	2	14.3	12	85.7	14	28
Gastric carcinoma	0	0	1	100	1	2	0	0	1	100	1	2
MALT carcinoma	0	0	0	0	0	0	0		0		0	0
Total	5	10	45	90	50	100	5	10	45	90	50	100

P-value : 0.001

Table 4. IgG (IU/ml) concentration in serum and saliva of Urease negative patients.

Samples	No.	IgG (IU/ml) concentration	Mean Titers	Standard Deviation	Standard Error
Serum	50	0.2-20	11.2	10.5	2.1
Saliva	50	0.3-15	10.9	5.9	0.93

Table 5 shows a comparison between concentration of saliva IgG of two groups Urease positive and Urease negative patients revealed that there is statistical difference in the concentration of IgG and the highest concentration was found in Urease positive patients

Table 5. Mean of oral fluid IgG (IU/ml) concentration in two groups Urease positive and Urease negative patients.

Samples	No.	Mean IgG (IU/ml) concentration	Standard Deviation	Standard Error
Positive	50	40.5	30.1	5.1
Negative	50	10.9	5.3	0.8

The correlation between studied risk factors and salivary IgG positive patients revealed that most of positive patients in age from 31-40 years, male, drink pepsi cups >4 days, smokers, not drink tea and those with ABO blood group O as shown in table 6.

Table 6: Association of risk factors with saliva positive specific IgG to H.pyori

Risk factor		Negative specific IgG to H.pyori		Positive specific IgG to H.pyori		Total
		No	%	No	%	
Age	20-30	28	84.8	5	15.1	33
	31-40	12	28.6	30	71.4	42
	41-50	10	55.6	8	44.4	18
	>51	4	57.1	3	42.9	7
Sex	Male	19	38	31	62	50
	female	35	70	15	30	50
Drink pepsi cup/day	0	40	87	6	13	46
	1-3	8	44.4	10	55.6	18
	>4	6	16.7	30	83.3	36
smoking	Yes	12	28.6	30	71.4	42
	No	42	72.4	16	27.6	58
Tea	Yes	43	62.3	26	37.7	69
	No	11	35.5	20	64.5	31
Blood group	A	12	54.5	10	45.5	22
	B	28	87.5	4	12.5	32
	AB	3	30	7	70	10
	O	11	30.6	25	69.4	36

DISCUSSION

Perfect diagnosis of H. pylori infection is essential to management of patients with gastrointestinal diseases. Usually, a combination of different methods is used, depending on the accebility of different methods in diagnosis.

Endoscopy with gastric biopsy has been the standard diagnostic procedure. This allows visualization of the gastro duodenal mucosa, confirmation of the presence of ulcers, and provision of tissue for microbiology and histological examinations. However, it is a costly and invasive procedure with potential risks and discomfort for the patients(16 and 17).currently, commercial enzyme immunoassays have been developed for the detection of serum H. pylori antibodies. Although several studies have found an excellent correlation between H. pylori serology and the presence of infection,(18) our results indicate that the present of specific IgG in the saliva seem to be significantly (P-value : 0.001)associated with diseases caused by H.pylori in urease positive patients and highest positive result was observed in patients with gastritis(P-value : 0.001)

and no significant difference between serum and saliva IgG antibodies.

In other study (19) showed Specificity and sensitivity of ELISA sera were 83.6% and 71.7% for saliva and 86.8% and 90%for sera, respectively. There was a good correlation between levels of salivary and serum IgG antibodies, and there was no significant different between them regarding specificity and sensitivity ($p > 0.05$). also the current study was in agreement with others (20 and 21). It is possible that antibody degradation by salivary protease might have contributed to False-Negative results. False-Negative results may also possibly occur in patients recently infected, before an antibody response has developed. False-Positive test results may be due to the presence of cross- reacting bacterial antibodies. Apparently False-Positive results may also, occur because of sampling error in obtaining gastric biopsy specimen: infection of the gastric mucosa may be patchy, so that examination of biopsy specimens may occasionally fail to identify truly infected patients.

the present study supports a role for the salivary IgG antibody response in screening patients. Although certain ulcers and gastritis occur independently of H. pylori infection, a negative anti-H. pylori salivary IgG status may help in reducing the number of unnecessary endoscopies, specially in lowrisk patients, such as subjects under 45 years of age.(22,23) These results indicate that the "ELISA" test in detection of salivary IgG antibody against H. pylori is moderately accurate enough and its sensitivity and specificity is the same as the "ELISA" test which is used to detect the IgG level in serum. Therefore, this test is moderately accurate method with a high sensitivity and a high negative predictive value and a high positive likelihood ratio for detecting H. pylori infection in adults.

Many risk factors associated with presence of specific IgG of H.pylori in saliva ,included There is a significant increase in specific IgG of H.pylori in saliva with age group (31-40).

This may be due to the limited number of old age and very young patients that were included in our study. Our result was not similar to a study in Brazil (2005) and in gasa(2008) (22 and 23))while in other study(24) showes Prevalence of Helicobacter pylori-specific IgG antibodies increases with age. In childhood a 5% seroprevalence is observed. In healthy young adults and persons at ages of 60 years, IgG positivity is 10 % and 70 %, respectively. Specific IgG antibody titres increase by 1% per year of life.

there is no significant difference in the specific IgG of H.pylori in saliva between males and female. And both of them appear to be equally exposed. This result is in agreement with study done in Gaza (2008)(23).Smoking is considered as a significantly risk factor for presence of specific IgG of H.pylori in saliva by several studies in the literature as a risk factor for H. pylori infection.However, the results of this study showed statistical differences between smokers and non-smokers .In the past, when idiopathic gastric hyperacidity was considered to be the chief cause of dyspeptic symptoms, smoking, was often implicated as exacerbating the condition and advice given to eliminate this habit (25) There was statistically significant difference between those who drink or don't drink tea. There is a high percentage of positive specific IgG of H.pylori in saliva was found in those who drink tea than those who didnt.

Our result is supported by other study (26) on the benefits of tea. Recent studies have presented data that show a variety of biological activities of tea catechins, compounds which constitute about 15% (dry weight) of tea against H. pylori (26).

our study regarding ABO and positive specific IgG of H.pylori in saliva indicated that individuals with O blood group tendency towards a higher rate of specific IgG of H.pylori in saliva. Studies performed by Bóren et al (27 and 28) in 1993 and 1994 have suggested that H pylori uses carbohydrate structures with terminal fucose as receptors in the gastric mucosa containing Leb and H blood group specifi cities. Later on, Ilver et al(29) in 1998 and Gerhard et al(30) in 1999 showed that there is a higher susceptibility to H pylori infection in individuals of O and Leb blood groups, because they have a higher quantity of fucosylated antigens.

Although the advances in invasive and non invasie tests, oral fluid testing in clinical and research settings is rapidly proving to be a practical and accurable means of recognizing oral signs of systemic illness and exposure to risk factors. The components of saliva act as a "mirror of the body's health," and the widespread use and growing acceptability of saliva as a diagnostic tool is helping individuals, researchers, health care professionals and community health programs to better detect and monitor disease and to improve the general health of the public.

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