Detection of *Helicobacter Pylori* IgG and IgA , Serum Biomarkers CA19-9 and CEA in Patients with Gastrointestinal Diseases

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Abstract:

Gastrointestinal diseases and especially chronic gastritis are mainly induced by *Helicobacter pylori* infection, and provides the basis for gastric carcinogenesis and colorectal cancer. The study involved the detection of serum anti-*H. pylori* IgG and IgA antibody of and some serum biomarkers ;CEA and CA19-9 in patients with gastrointestinal diseases. Fifty eight serum samples were collected from 25 males and 33 females .Peripheral venous blood was collected from each patient and sera obtained by centrifugation. Serum anti-*H. pylori* IgG and IgA ,serum CEA and CA19-9 were evaluated by enzyme-linked immunoadsorbent assays (ELISA).Forty eight serum samples were positive for IgG (82.7%) divided into 27 positive samples for females and 21 positive samples for males while ten samples were positive for IgA (17.2%) divided into eight positive samples for females and two positive samples for males .Only three samples were positive for anti IgG and serum biomarker CA19-9 .In this study most of positive results of *H.Pylori* IgA and IgG are between the age 30-50 years and low within 1-10 years ,we conclude because most of them are working and eat fast food from different sources. This study concluded that detection of serum IgG and IgA is effective in monitoring the *H. pylori* infection in gastrointestinal disorders and chronic infections .

Keywords: Gastrointestinal diseases, Gastritis , *H.Pylori*, IgG ,IgA ,CEA,CA19-9.

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التحري عن اضداد IgG و IgA لبكتريا *Helicobacter Pylori* و المعلمات البيولوجية CA19-9 و CEA لدى مرضى الجهاز الهضمي

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الخلاصة

تعد بكتريا *Helicobacter pylori* المسبب الرئيسي لأمراض الجهاز الهضمي والتهاب المعدة الزمن والمؤدي الى الأصابة بسرطان المعدة والقولون.تضمنت هذه الدراسة التحري عن الكلوبيولينات المناعية IgG و IgA بكتريا *H. pylori* والمعلمات البيولوجية CA19-9 و CEA في مصول المرضى . جمعت ٥٨ عينة مصل من مرضى الجهاز الهضمي والتهاب المعدة الزمن وتوزعت الى ٢٥ عينة من الذكور و٣٣ عينة من الأناث وقيست اضداد ال IgG و IgA والمعلمات البيولوجية CA19-9 و CEA في مصول المرضى بطريقة الأليزا . اظهرت ٤٨ عينة ايجابيتها لأضداد ال IgG (٨٢.٨ %.) توزعت ال٢٧ عينة للذكور و ٢١ عينة للأناث . بينما كانت العينات الموجبة لأضداد IgA (%١٧.٢) توزعت بين ثمانية عينات للأناث و اثنان للذكور .أوضحت النتائج ظهور ثلاث عينات موجبة لأضداد ال IgG والمعلم البيولوجي CA19-9. كانت نتائج هذه الدراسة ايجابية لأضداد IgG وIgA للأعمار ٣٠-٥٠ سنة لأنه هذه الفئة هي الأكثر نشاطا وارتباطا بالعمل والأكثر تعرضا لهذه البكتريا لأن معظمهم يتناولون الوجبات السريعة ومن مصادر مختلفة.بينما كانت الفئة العمرية ١-١٠ سنوات هي الأقل اصابة . يعد التحري عن الأجسام المضادة IgG وIgA لبكتريا *H. pylori* باستخدام الفحص السيرولوجي و الذي هو من الفحوصات الروتينية والمتوفرة فعالا في التحري عن الأصابة بهذه البكتريا خصوصا في الأصابات المزمنة والأضطرابات المعوية.

الكلمات المفتاحية: H. pylori ، IgG ، IgA ، CA19-9 ، CEA ، أمراض الجهاز الهضمي والتهاب المعدة الزمن .

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Introduction

Helicobacter pylori , a spiral - shaped pathogenic bacterium found on the human gastric mucosa , was first isolated by Warren and Marshall [1] in 1982 and soon after was Linked with chronic gastritis and peptic ulceration [2].

The world wide prevalence of *H. pylori* is more than 50% [3,4]. It is more prevalent in developing countries as compared to developed countries [5]. Its prevalence in South Asia is ranging between 55 to 90% [6]. Primary care physicians were reported that 78% of the physicians thought that contaminated water was the source of spread of infection [7] Chronic gastritis is mainly induced by *Helicobacter pylori* infection, and provides the basis for gastric carcinogenesis[8]. Several prospective studies reported a strong association between *H. pylori* infection and gastric cancer; hence , *H. pylori* is now recognized as a Group 1 carcinogen for humans [9,10] . However , only a few individuals

infected by *H. pylori* may eventually develop gastric cancer. The clinical outcome of *H. pylori* infection may depend on several factors, such as age at the time of first infection, environmental factors, immune response, and genetic characteristics [11].Among the serum tumor markers, CEA and CA19-9 are most widely used for the screening and monitoring gastric cancer, because they have been reported to be elevated in some patients with gastric cancer [12,13]. Carcinoembryonic antigen (CEA) is the most commonly used tumor marker in patients with colorectal cancer, and the tumor marker carbohydrate antigen CA19-9 is often used in combination with CEA to manage patients with colorectal cancer [14,15,16]. The aim of the study is to determine the prevalence of the serum anti-*H. pylori* immunoglobulin IgG and IgA antibodies and serum tumor markers, CEA and CA19-9 in patients with gastrointestinal diseases .

Patients and Methodes

Patients and samples:

A total of fifty eight patients with abdominal pains, cramps, diarrhea and chronic gastritis whom referred to Al-Karamah hospital in Baghdad from April 2010 to July 2011, were included in the present study . Among them 25 were males and 33 were females with gastrointestinal disorders (8 persons were considered as control) , aged from 6 to 72 years with a mean age of 55($\bar{}_+$)years old.

Serological tests:

Blood was sampled twice from patients. Enzyme-linked

immunoadsorbent assays (ELISA) were used to measure the levels of serum anti-*H. pylori* IgG and IgA antibodies. The test kits for the detection of anti-*H. pylori* -IgG and anti-*H.pylori* IgA were purchased by Biohit Oyi company (Finland) . Detection of serum markers CEA and CA19-9 were also done by ELISA technique (Biohit Oyi company) .

Results and Discussion

The results were classed as positive if anti–*H pylori* immunoglobulin IgG titers were >30 U/mL and IgA titers positive if they were >20 U/mL.
Table 1 shows the detective positive rates of serum anti *H. pylori* IgG and IgA antibodies of patients in relation to gender and age. The incidence of infection was (43.18 %, 25/58) between the males and (56.8 %, 33/58) the females patients .The *H. pylori* infection rate was the highest in the ages from 42 to 50 years.Also the detected positive rate of *H. pylori* was not remarkably associated with the age of patients .

Table (1) Age ,gender and number of anti *H.pylori* IgA and IgG positive serum samples in females and males.

Gender	Age (year)	Number and percentage of patients with positive anti -IgA	Numbers and percentage of patients with positive anti -IgG
F	7-60	8(13.7%)	27(46.5%)
M	1-58	2(3.4%)	21(36.2%)
		10(17.1%)	48(82.7%)

The results showed that anti *H.pylori* IgG positive serum samples in both females and males were elevated in ages 21-50 years as in table 2 and 3.

Table (2) Age distribution of anti *H.pylori* IgG positive serum samples in females

Age (year)	Gender	Numbers of patients
1-10	F	2
11-20	=	3
21-30	=	5
31-40	=	8
41-50	=	7
51-60	=	2
Total	=	27

Table (3)Age distribution of anti *H.pylori* IgG positive serum samples in males

Age (year)	Gender	Numbers of patients
1-10	M	1
11-20	=	1
21-30	=	4
31-40	=	5
41-50	=	7
51-60	=	3
Total	=	21

Anti *H.pylori* IgA positive serum samples in males were only 2 between ages 1-30 years as in table 4,but anti *H.pylori* IgA positive serum samples in females were elevated in ages 41-50 years as in table 5.

Table (4) Age distribution of anti *H.pylori* IgA positive serum samples in males

Age (year)	Gender	Numbers of patients
1-10	M	1
11-20	=	-
21-30	=	1
31-40	=	-
41-50	=	-
51-60	=	-
Total		2

Table (5) Age distribution of anti *H.pylori* IgA positive serum samples in females

Age (year)	Gender	Numbers of patients
1-10	F	-
11-20	=	-
21-30	=	1
31-40	=	1
41-50	=	6
51-60	=	-
Total		8

Table (6) Age distribution of anti *H.pylori* IgG and IgA positive serum samples in females .

Age (year)	Gender	Numbers of positive IgG and IgA samples
1-10	F	-
11-20	=	-
21-30	=	-
31-40	=	1
41-50	=	3
51-60	=	1
Total		5

Table (7)Age distribution of anti *H.pylori* IgG and IgA positive serum samples in males .

Age (year)	Gender	Numbers of positive IgG and IgA samples
1-10	M	1
11-20	=	-
21-30	=	-
31-40	=	1
41-50	=	-
51-60	=	-
Total		2

Table (8) Age distribution of anti *H.pylori* IgG and IgA positive and serum markers CEA and CA19-9 in males patients .

Age year	Gender	positive IgA samples	positive IgG samples	positive CEA samples	positive CA19-9 samples
1-10	M	-	-	-	-
11-20	=	-	1	-	1
21-30	=	-	1	-	1
31-40	=	-	-	-	-
41-50	=	-	1	-	1
51-60	=	-	-	-	-
Total	=	-	3	-	3

Three specimens of males gave positive result for CA19-9 test but all females specimens gave negative results for CA19-9 test. All females and males specimens gave negative results for CEA test.

It is now recognized that the clinical detection of serum antibody is effective in monitoring the *H. pylori* infection and the serology test has been used widely in epidemiologic studies and routine diagnosis of *H pylori* infection [5,6,7,8] .In this study most of positive results of IgA and IgG are between the age 30-50 years and low within 1-10 years ,we conclude because most of them are working and eat fast food from different sources. Serological testing to diagnose *H. pylori* infection in children is still controversial, although commonly used in clinical practice. A recent study speculate that young children may have a different immune response to *H. pylori*, with preferable responses to certain antigens, as well as lower titers than adults. The Pyloriset test may fail to recognize these specific antibodies [17].However, most individuals infected with *H. pylori* do not experience symptoms or have signs of recognizable disease. In most children, the presence of *H. pylori* infection does not lead to clinically apparent disease, even when the organism colonizing the gastric mucosa causes chronic active gastritis [18]. A recent study was carried in Iran and this study showed that IgG titers were high in individuals between 40 to 50 years old , Anti *H. pylori* IgA was also correlated with increasing age [19]. Serological tests are widely available and cheap, and may be helpful-in screening populations or in confirming the presence *H. pylori* infection in case of equivocal results of the other diagnostic methods due to bleeding ulcers, antibiotic and/or antisecretory treatments. A major advantage of this serologic test is that it

enables large numbers of subjects to be screened quickly and relatively inexpensively [20].In table (8) the results showed only three males infected with *H.pylori* and elevated values of CA19-9 in age between 11-50 years. According to these results our study conclude that these three males may suffered from gastric or other kind of gastrointestinal tumors . A low number of positive results were recorded on elevated values of CA19-9 by Kim et al. when they identified only four patients among 1,063 patients with CA 19-9 serum levels >37 U/ml (mean values 50.5±16.8 U/ml) [21] .Some times CA19-9 is not used for early screening because it is not present in patients with certain blood types and is often elevated in benign disease. Certain changes that occur in the sera of pancreatic cancer patients reflect the high level of inflammation associated with the disease [22]. A recent study on serum CA19-9 mention that it is the most extensively studied and clinically useful biomarker for pancreatic cancer. Unfortunately, CA 19-9 serum level evaluation in pancreatic cancer patients is limited by poor sensitivity, false negative and increased false positivity in the presence of obstructive jaundice (10–60%) [23].

We conclude that serum analysis should include CA19-9 and CEA for the diagnosis the chronic infections with *H. pylori*.

Recommendations: test the specimens of patients with chronic infections with *H.pylori*. for CA19-9 and CEA diagnosis because several studies reported a strong association between *H. pylori* infection and gastric cancer and colorectal cancer; as such, *H. pylori* is now recognized as a Group 1 carcinogen for humans .

References

1. Warren, J.R.,and Marshall,B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983;1:1273-5.
2. Marshall, B.J.and Warren,J.R. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration.Lancet 1984;1:1311-15.
3. Taylor, D.N.and Blaser, M.J. The epidemiology of *Helicobacter pylori* infection. Epidemiol. Rev. 1991; 13:42-59.
4. Parsonnet, J. *Helicobacter pylori*. Infect Dis. Clin. North Am. 1998;12(1):185-197.
5. Frenck,R.W.and Clemens,J. *Helicobacter* in developing world.Microbes Infect 2003; 5:705-713.
6. Hunt, R.H; Xiao,SD.;Megraud,F. and Bazzoli,F., *Helicobacter pylori* in developing countries. WGO Practice Guideline 2006 .[http://www. Omge.org /assets / downloads /en /pdf/ guidelines /11_ *Helicobacter _pylori* _developing _countries _en.pdf].
7. Shahid,A.;Mohammad,S.;Wasim,J.i; Hasnain, A.et al., *Helicobacter pylori* infection: approach of primary care physicians in a developing country, BMC Gastroenterol. 2009; 9:23.
8. Kawaguchi,H.;Haruma,K.;Komoto,K. and Yoshihara,M.,*Helicobacter pylori* infection is the major risk factor for atrophic gastritis.Am.J.Gastroenterol. 1996; 91:959–62.
9. Forman D, Newell DG, Fullerton F, et al. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. BMJ.1991;302:1302-1305.
10. *Helicobacter* and Cancer Collaborative Group. Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case control studies nested within prospective cohorts. Gut.2001;49:347-353.
11. Plummer M, van Doorn LJ, Franceschi S, et al. *Helicobacter pylori* cytotoxin-associated genotype and gastric precancerous lesions. J Natl. Cancer Inst. 2007;99:1328-1334.
12. Marta, Ł.; Barbara, M.;Mariusz, G. ; Bogusław, Ke. Comparison between clinical significance of serum proinflammatory proteins (IL-6 and CRP) and classic tumor Clin Exp Med.2011; 11:89–96.
13. Gao ZL, Zhang C, Du GY, Lu ZJ: Clinical significance of changes in tumor markers, extracellular matrix, MMP-9 and VEGF in patients with gastric carcinoma. Hepatogastroenter. 2007; 54:1591–1595.
14. .Reiter W, Stieber P, Reuter C, Nagel D, Lau-Werner U and Lamerz R: Multivariate analysis of the prognostic value of CEA and CA19-9 serum levels in colorectal cancer. Anticancer Res.2000;20: 5195-5198.
15. Gasser M, Gerslauer C, Grimm M, Bueter M, Lebedeva T, Lutz J,Maeder U, Ribas C, Ribas C, Nichiporuk E, Thalheimer A,Heemann U, Thiede A, Meyer D and Waaga-Grasser AM:Comparative analysis of predictive biomarkers for thera peutical strategies in colorectal cancer. Ann Surg Oncol.2007; 14: 1272-1284.

16. Gobbi PG, Valentino F, Berardi E, Tronconi C, Brugnatelli S,Luinetti O, Moratti R and Corazza GR: New insights into the role of age and carcinoembryonic antigen in the prognosis of colorectal cancer. Br J Cancer 2008, 29: 328-334.

17. Ageleka,T.Enzyme Immunoassays, Testing Immunoglobulin G (IgG) and IgA Responses, for Diagnosis of *Helicobacter pylori* Infection in Children, J. OF CLIN. MICROB. 2001; 3591–3596.

18. Drumm, B. *Helicobacter pylori* in the pediatric patient. Gast. Clin North Am. 1993;22:169–82.

19. Soheila,M.;Safar,F.; Nazli, S.,and Rana,Y.,Seroprevalence of *Helicobacter pylori* infection in patients suffering from gastric symptoms in the Northwest of Iran. African J. of Microb.Research. 2011;5(22) : 3616-3619.
.

20. Linda, M. *Helicobacter pylori:* Epidemiology and Routes of Transmission, Epidemiol. Rev.2002; (22): 1-2.

21. Kim JE, Lee KT, Lee JK et al .Clinical usefulness of carbohydrate antigen 19-9 as a screening test for pancreatic cancer in an asymptomatic population. J Gastroenterol Hepatol.2004. 19 (2):182–186

22. Fearon KC, Barber MD, Falconer JS, McMillan DC,Ross JA, Preston T. Pancreatic cancer as a model:inflammatory mediators, acute-phase response, and cancer cachexia. World J Surg.1999;23:584–8.

23. Serum CA (19-9 as a Biomarker for Pancreatic Cancer—A Comprehensive Review Umashankar K. Ballehaninna & Ronald S. Chamberlain . Indian J Surg. Oncol.2011. 2(2):88–100