Research Article

Serologic Detection of CagA Positive Helicobacter pylori in Patients with Different Gastroduodenal Diseases

Haissam Yazigi*
Department Of Laboratory Medicine, Faculty of Medicine, Tishreen University, Latakia, Syria.
***Corresponding author's E-mail:** mansouroussama@yahoo.fr

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ABSTRACT

This study aimed to know the relation between cagA positive *Helicobacter pylori* (HP) strains ad diseases such as gastric and duodenal ulcers and gastric adenocarcinoma. The study included 61 dyspeptic patients from gastrointestinal (GI) endoscopy department in Al-Assad university hospital in Lattakia. During upper (GI) endoscopy, gastric biopsy specimens were obtained for Clo-test and histological examination. Blood was obtained from Clo-test positive patients for serologic tests of HP and cagA. After upper (GI) endoscopy and histological examination, patients were divided into 4 groups: chronic gastritis (28 patients), gastric ulcer (5 patients), duodenal ulcer (17 patients) and gastric adenocarcinoma (11 patients). CagA antibodies were positive in 68.85% of patients. The difference in the proportion of cagA antibody positive between the four groups was highly significant (P = 0.006). The highest positive proportion was in gastric adenocarcinoma (90.91%), then duodenal ulcer (88.24%) then gastric ulcer (80%), while the lowest positive proportion was in chronic gastritis (46.43%). There were no difference in HP antibody levels between the four groups (P = 0.090) but these levels were significantly higher in cagA positive patients compared to cagA negative (P = 0.05). This study found a positive relation between cagA positive and diseases such as peptic ulcer and gastric adenocarcinoma in Syria.

Keywords: Helicobacter pylori, CagA, gastro duodenal diseases, gastric ulcer, duodenal ulcer, gastric adenocarcinoma, chronic gastritis.

INTRODUCTION

Helicobacter pylori (HP) infection represents the key agent in the pathogenesis of several digestive pathologies extending from asymptomatic chronic gastritis through peptic ulcer and gastric adenocarcinoma and MALT lymphoma. Disease development is conditioned by several factors belonging to bacteria, host, and environment. All are involved in type and intensity of inflammation.

The disease occurs as a result of complicated reactions between host and bacteria where host diversity genotype and gastric acid secretion determine to a great extension the microbial ability of gastric colonization. Both microbial virulence factors such as pathogenic islets (cag PAI) carrier which coding cagA protein and VACA protein existence help bacteria to colonize gastric mucosa and as a consequence to modify the host immunity.[1]

Cytotoxin- associated gene A (CagA)

Gastric colonization by HP always leads to active chronic gastritis but most of the infected patients are asymptomatic,[2] so some HP strains are more virulent than others. Consequently to studies on different strains of HP, it has been appeared that the high pathogenic ability of HP is secondary to its power in developing morphological changes, vacuolation, and progressive degeneration *in vitro*.[3] This activity was linked to a protein with molecular weight of 140 K.D called cagA.

CagA protein is a strong immunogene coded for by CagA gene[4], presents in approximately 50-70% of HP strains, and indicates CagPAI existence which codes for 27-31 proteins[4]. CagPAI carrying strains are called CagA positive strains because they are discovered in patients owing to its power in stimulating high levels anti-CagA antibodies production[1], so CagA is used in epidemical studies to indicate location of Cag PAI in total. CagA positive strains infected patients exhibit more intense inflammatory response, so they are more susceptible to develop digestive ulcer and gastric cancer. This point was confirmed in west countries[7] but not in Asian ones.[8]

CagA negative strains may involve in peptic ulcer or gastric cancer in a slight proportion. The majority of CagA positive strains contain the entire Cag PAI location[9], but about 10% of strains contain abortive one hence hypo functioning[10], the influence of these facts on digestive pathology is unclear yet.

18 proteins of those CagPAI coded for, take part in type 4 secretory system constitution which resemble an injector able both to penetrate gastric epithelial cells and to facilitate host cells inside to be traversed by microbial structures such as CagA, peptidoglycan and others [4-6]. After getting inside host cells, CagA is phosphorylated; then it reacts with signal firing molecules group such as Tyrosine Phosphatase SHP-2[11] which causes morphologic changes in epithelium[12].

CagPAI affects immunity response because type 4 secretory system-host cells reaction results in epithelial cells to produce pro-inflammatory cytokines, particularly IL-8[6] After epithelial cells CagA injection phosphorylated and non-phosphorylated CagA reacts with host cells'

proteins to release definite signal pathway leading to epithelial cell elongation, cells' connection disturbance, polarity loss, reproduction disorders and apoptosis which are tumor generating phenomena.

Research importance comes from frequent worldwide HP infection, it attains more than half of world population; more common in developing than developed countries. It causes digestive ulcers and gastric adenocarcinoma, and WHO –in 1994- considered HP as tumor generating agent 1st degree.

More than 80% of HP infected subjects are asymptomatic. Symptoms appearance depends on inflammatory response to the bacteria; which rely on pathogenic strain virulence, patient hereditary predisposition and environmental aids.

CagA is considered the most important factor in virulence. It has been noticed in west countries that digestive ulcers and gastric adenocarcinoma are more CagA positive than CagA negative.

This study aimed to detect CagA in four groups of dyspepsia patients suffers from chronic gastritis, gastric ulcer, duodenal ulcer or gastric adenocarcinoma; and to determine the relation between CagA positive HP and those diseases.

MATERIALS AND METHODS

Study sample comprised 61 patients sent to endoscopy department in Al-Assad university hospital – Latakia because of dyspepsia. This study took place between April 2011 and May 2012. Basing on endoscopy findings and histological examination, the patients were divided into four groups: chronic gastritis, gastric ulcer, duodenal ulcer and gastric adenocarcinoma. Clo-test was carried out in the course of endoscopy for HP detection, if positive a three antral biopsies were taken in view of histological HP detection and gastritis screening. Blood was collected, and serum had been conserved at freezing temperature (-20) until antibodies detection (HP IgG antibodies, CagA IgG antibodies) by ELISA method took place. Finally, all data was collected and statistically analyzed. There are different ELISA methods. Indirect one was followed in our studies.

Indirect ELISA

1. Known antigen represents the solid phase, patients serum is added to the wells. Thereafter, incubation and washing to remove unconjugated antibodies.
2. Enzyme linked anti human globulin antibodies were added to conjugate with antibodies present in patient serum previously bound to solid phase antigen.
3. Incubation and washing to remove the unbounded anti human globulin antibodies.
4. Substrate is added and leads a reaction by the enzyme causing color change. After a definite period of time, the reaction should be stopped either by milieu acidity modification or by enzyme devastation.

HP-IgG (DIA.PRO) and CagA-IgG (DIA.PRO) were used in ELISA test in order to detect HP and CagA antibodies respectively; six calibrators were used to draw the graph where CAL1=0 arbU/ml, CAL2=5 arbU/ml, CAL3=10 arbU/ml, CAL4=20 arbU/ml, CAL5=50 arbU/ml, CAL6=100 arbU/ml. The same working method was used in both HP and CagA.

Working Procedure

1. Serum dilution in the 1:101 proportion; i.e. mix 1000ml of sample diluent (DILSPE) with 10 µl of serum.
2. Two first wells were left empty as a blank. 100 µl of each calibrator were added into two wells allotted for each one.
3. 100 µl of the diluted samples to their allotted wells.
4. Wells were covered by adhesive band and incubated for 60 minutes at 37 temperature
5. Wells were washed 5 times with wash buffer
6. 100 µl of conjugated enzyme were added to all wells except the two left for blank
7. Repeating steps 4 and 5 respectively
8. 100 µl of chromogen-substrate (SUBS-TMB) to all wells including the two left for blank
9. Wells were covered by adhesive band; incubated at room temperature (18-24), in a dark place, for 20 minutes.
10. Reaction was stopped by adding 100 µl of H_2SO_4 to all wells; the color would change from blue to yellow.
11. Light absorbance was measured upon wave length 450 and 620-630 nm.
12. The graph was drawn after the calibrators values had been inserted, and the titer of patients' antibodies in the samples was determined.

Results Interpretation

CagA-Abs and HP-Abs cut-off = 5 arbU/ml, i.e. antibodies concentration higher than 5 arbU/ml means the sample is positive.

Statistical analysis

The following statistical tests were used:

1. Chi square test to study the relationship between separated variables, or Ficher's exact test Chi square conditions weren't fulfilled.
2. ANOVA contrast analysis test to compare the arithmetic means of connected variables between the three groups of patients

3. CagA and HP antibodies values distributions were highly bent; therefore they were changed into logarithmic variables so they became more suitable for statistical analysis.
4. T-Student test to compare two arithmetic means

The differences were considered statistically significant at the threshold design *P* value ≤ 0.05. The statistical analysis was made by STATA program.

RESULTS AND DISCUSSION

According to their diseases, patients were divided into four groups:

1. Chronic gastritis group (CG) 28 patients
2. Duodenal ulcer group (DU) 17 patients
3. Gastric ulcer groups (GU) 5 patients
4. Gastric adenocarcinoma group (GA) 11 patients

Table 1: Exhibits some characteristics of these groups:

Table 1: Study patients features

Number of Patients			CG	DU	GU	GA	Total	*P* value
			28	17	5	11	61	
SEX	Females	Freq.*	13	6	1	6	26	0.53
		Per.**	46.43	35.29	20	54.55	42.62	
	Males	Freq.	15	11	4	5	35	
		Per.	53.57	64.71	80	45.45	57.38	
AGE	Year	Mean	42.57	50.06	56.60	62.91		0.009
		SD***	15.37	18.47	18.47	17.34		
SMOKING	No	Freq.	14	5	3	6	28	0.41
		Per.	50	29.41	60	54.55	45.9	
	Yes	Freq.	14	12	2	5	33	
		Per.	50	70.59	40	45.45	54.1	
ALCOHOLISM	No	Freq.	26	16	4	11	57	0.51
		Per.	92.86	94.12	80	100	93.44	
	Yes	Freq.	2	1	1	0	4	
		Per.	17.4	5.88	20	0	6.56	

***freq. = frequency** ****per. = percentage** *****SD. = standard deviation**

Patients ages ranged from 16 to 87 years; there was a significant statistical difference in the mean age between groups (*P* value = 0.009). The lowest mean age was in chronic gastritis group (43 years), whereas the highest mean age was in gastric adenocarcinoma group (63 years).see table (1)

In opposition to age, there was no statistically significant difference between groups across sex, smoking and alcoholism.

Women proportion reached approximately 43%, ranged between 20% in gastric ulcer group and 55% in gastric adenocarcinoma group. Fig (1)

Smokers' proportion reached 54%, the highest proportion was across duodenal ulcer patients (70.59%) and the lowest one was in gastric ulcer patients (40%). These differences weren't statistically significant (statistical importance degree = 0.51). Fig (2)

Alcoholism was presented in nearly 7%, ranged between 0% (in gastric adenocarcinoma) and 20% (in gastric ulcer) without statistically significant differences. Fig (2)

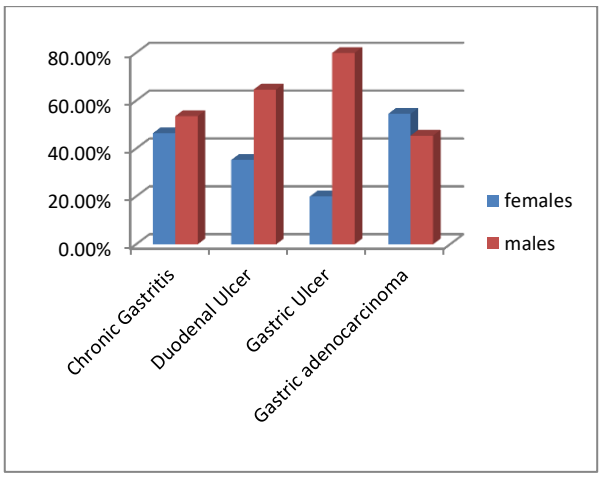

Figure 1: Sex distribution between studied groups

Positive CagA antibodies were presented in 68.85% of patients as demonstrated in Tab(2) and Fig(3). Positivity proportion differences between patients groups were statistically significant (statistical importance degree = 0.006). The highest positivity proportion recorded in gastric adenocarcinoma group (nearly 91%) followed by

duodenal ulcer (88.24%), gastric ulcer (80%) and lastly came chronic gastritis with (46.43%) proportion.

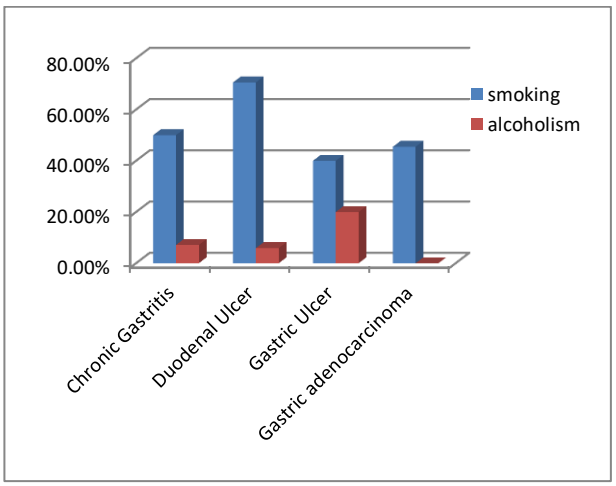

Figure 2: Smoking and alcoholism distribution between studied groups

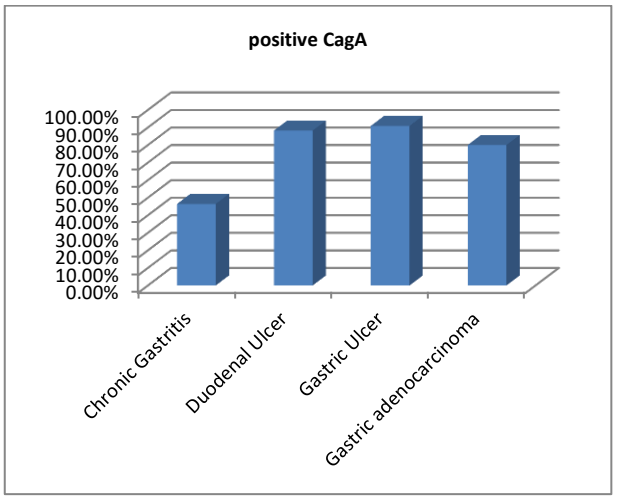

Figure 3: Positive CagA proportion in studied groups

Table 2: Relationship between studied digestive diseases and each of CagA antibodies and HP antibodies

Patients Number			CG	DU	GU	GA	Total	*P* value
			28	17	5	11	61	
CagA	Negative	Freq	15	2	1	1	19	
		Per	53.57	11.76	20	9.09	31.15	0.006
	Positive	Freq	13	15	4	10	42	
		Per	46.43	88.24	80	90.91	68.85	
CagA antibodies Titer arbU/ml		Mean	44.14	88.65	153	43.45		0.02
		S.D	67.18	93.45	134.22	38.95		
HP antibodies Titer arbU/ml		Mean	112.93	96.47	118	80.91		0.90
		S.D	98.56	80.22	97.23	74.17		

Table (2) also reveals the mean CagA antibodies titer in the four inspected groups; it's observed –in each group- a high divergence in the antibodies values (great standard deviation and equals nearly to the mean)

Table 3: Relationship between CagA and HP antibodies

HP antibodies Titer arbU/ml		CagA		*P* value
		Negative	Positive	
	Freq	19	42	0.05
	Mean	73.47	116.33	
	SD	78.58	90.11	

Table 3 reveals the relationship between HP antibodies concentration and CagA antibodies positivity, statistical significance degree = 0.05. The mean of HP antibodies level is higher when CagA is positive (the mean is 116.33) than it is negative (the mean is 73.47)

RESULTS AND DISCUSSION

Duodenal ulcers reached 17 versus 5 for gastric ulcers, i.e. duodenal ulcers = 3.4 gastric ulcer. This proportion is next to that encountered in west countries (DU=4 GU)

Mean age was 57 years in gastric ulcer versus 50 years in duodenal ulcer. This is parallel to international studies which pointed that gastric ulcers develop in the age over 50 years whereas duodenal ulcers between 30 and 60 years. [14]

Gastric adenocarcinoma mean age was 63 years old and this was similar to international studies, but female to male ratio was 1.2:1 which wasn't in agreement with internationals. Gastric adenocarcinoma occurs between 50 and 70 years old with male predominance; male to

female ratio increases with age; it's 1:1 in young and becomes 2:1 in the age of 60 to 70 years[15].

CagA antibodies were positive in 68.85% of study patients; chronic gastritis had the lowest positivity proportion (46.43%) preceded by gastric ulcer (80%), duodenal ulcer (88.24%) and the highest positivity proportion was observed in gastric adenocarcinoma (91%).

Table (4) compares our results with similar Chinese study design in 2001 [16]. The two studies were in agreement about that positive CagA antibodies proportion in gastric ulcer, duodenal ulcer and gastric adenocarcinoma were significantly higher than chronic gastritis proportion.

Table 4: CagA positivity proportion in our study in comparison to Chinese one

Digestive Disease	CG		GU		DU		GA	
CagA IgG proportion	China	Syria	China	Syria	China	Syria	China	Syria
	55.4	46.43	83.2	80	90.8	88.24	89.7	91

Table 4: CagA positivity proportion in our study in comparison to Chinese one

In Iranian study done in 2009, Positive CagA antibodies proportion was 100% in gastric cancer and 94.1% in peptic ulcers [17]. These results were superior to ours.

In Greek study done in 1999, positive CagA antibodies proportion was 94.4% in peptic ulcers and 45% in non-ulcerative patients. These results were in agreement with ours. [18]

Our study found that gastric adenocarcinoma CagA antibodies mean value was (43.45), this value was inferior to that encountered in gastric ulcer (153) and duodenal ulcer (88.65), but next to chronic gastritis value (44.14). These low values in gastric carcinoma might be due to longstanding hypochlorhydria secondary to pre-cancer atrophic gastritis; CagA transcription is acid dependent; therefore CagA hypo-expression in hypochlorhydria patients is a predictable thing. [19]

A Chinese study done in 2002 found no statistical differences in HP antibodies titers between normal mucosa, chronic gastritis and duodenal ulcer (P = 0.99) [20]. This finding is similar to one observed in our study where there was no statistical differences in HP antibodies values between the different endoscopic findings (P = 0.90)

The present study found that HP antibodies values were higher in the state of CagA positive than in CagA negative. This observation is in accordance with Holland study in 2000 where (P = 0.033)

CONCLUSION

HP antibodies-IgG fall more rapidly than CagA antibodies-IgG do. This unparallel regression gives false positive when CagA antibodies measured alone. Therefore, it's not permitted to test for CagA without HP-Ab-IgG especially when we control *Helicobacter. Pylori* treatment.

In dyspepsia patients, test for both HP and CagA antibodies at the same time because the state of CagA (negative or positive) points to inflammation intensity more than HP-Ab-IgG do and so to ulcers existence.

CagA positive dyspeptic patients especially with low titer should be endoscopied because gastric cancer-CagA positive low titer is a frequent association.

Other HP virulence factors should be researched and their relationship to digestive ulcers and gastric cancer should be detected in view to outline non-invasive methods to discover these pathologies and add these methods to other diagnostic ones.

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