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Full Length Research Paper

The Relationship between Helicobacter pylori anti-cagAIgG and Anti-vacAIgG and the Treatment Response

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Abstract
Background & Aims: One of the commonest pathogenic microorganisms worldwide is Helicobacter pylori. It is concluded from the previous studies, that virulence genes like cagA and vacA are not limited to the pathogenicity but extending to affect the eradication of H. pylori. Though, there are discrepancies in these results. So, the present study aimed to evaluate the relationship between Helicobacter pylori cagA and vacA virulence factors and the treatment response. Methods: From July 2017 to September 2017, 144 patients who suffered from epigastric pain, vomiting and belching and tested positive for H. Plori were recruited from KafrElsheikh University Hospitals were enrolled in this study. All the patients received triple treatment consisting of PPI (40 mg once dialy), Amoxicillin (1000 mg twice daily) and Clarithromycin (500 mg twice daily)for 2 weeks. All the patients subjected to H.Plori Ag in stool after 1 month of the end of treatment. Serum samples were screened for H.Pylori antibodies (anti-CagAIgG and anti-VacAIgG) by using the ELISA Kits. Results: In the present study, anti-Cag A IgGwas positive in 74(51.4%), while it was negative in 70(48.6%). The present study, showed that anti-Vac AIgG was positive in 58(40.3%), while 86(59.7%) were negative. H.pylori eradicated in 96 (66.7%) of the studied subjects. There were no significant correlations between anti-cagAIgG, anti-vacAIgG, sex and age as regards h.pylori treatment response. Conclusion: There is no evident relationship between Helicobacter pylorianti-cagAIgG and anti-vacAIgGand the treatment response.

Keywords: *Helicobacter pylori; eradication; treatment response; resistance; Egypt.*

Introduction

One of thecommonest pathogenic microorganism's worldwide is Helicobacter pylori (H. pylori) which isconcerned in the pathogenesis of many diseases like gastric cancer, gastroduodenal ulcers and gastritis (Hajimahmoodi et al., 2011). Treatment of H. pylori infected patients isbeneficialin order to prevent the gastric diseases progression (You et al.,2006 and Take et al.,2015). Host factors and H. pylorirelated factors like mutation, efflux pumps, biofilm formation, as well as other factors play a crucial role in H.pylori eradication. Also, H. pylori eradication affected by some virulence factors which are released from H. pylori, and play an important role in initiation of inflammatory reactions , bacterial colonization, cancer enhancement and immune evasion (He et al.,2013).The two important H. pylori virulence factors are Vacuolatingcytotoxin A (VacA) and Cytotoxin associated gene A (CagA)(Kim and Blanke, 2012).

VacA gene-encodedvacuolating toxins cabable of inducing apoptosis, inhibit T-cell activity and avoid clearance by hostimmunity (Kim and Blanke, 2012). A further virulence issue is the CagA, which is connected closely with the pathogenicity of H. pylori. Many discrepancies were found in the results of the different researches studying the cagA and H. pylori eradication relationship. Therate of recovery of patients from H.pylori infection is varied greatly according to CagA positive or negative strains, where the rate of recovery rate of patients was significantly higher in CagA positive strains than in cagA-negative strains (Van Doorn et al., 2000), whereas opposite results reported by(Huang et al.,2012).On the other hand, (Magalhaes et al., 2005) and (Baryshnikova et al., 2012) showed that eradication rates did not affected by cagA-positive or cagA-negative strains. It is concluded from the previous studies, that virulence strains like cagA and vacA are not only strictlyconnected to pathogenicity but alsoaffecting theH. pylori eradication. However, there are discrepancies in these results. So, the present study aimed to evaluate the relationship between Helicobacter pylori cagA and vacA virulence factors and the treatment response.

Patients and Methods

The study protocol was performed according to the ethical guidelines of the Helsinki Declaration and was agreed by the Clinical Research and Ethics Committee. A written informed consent was signed by all subjects participating in the investigation. From July 2017 to September 2017, 144 patients who suffered from epigastric pain, vomiting and belching and tested positive for H.Pyloriwere recruited from KafrElsheikh Univerity Hospitals were enrolled in this study.

The exclusion criteria were as follows: (1) patients who had taken any PPI, bismuth salts, antibiotics or H2 receptor antagonists for at least 4 weeks before the time of their enrolment in the study; (2) patients whose condition was complicated by severe lung, blood, heart, kidney or liver failure (3) H.Pylori negative; and (4) patients who would not comply to the study.

The patients had been subjected to testing for anti-Vac AIgG and anti-Cag AIgG before treatment. All the patients received triple treatment consisting of PPI (40 mg once dose/day), Clarithromycin (500 mg /2 doses /day) and Amoxicillin (1000 mg /2 doses per day) for 2 weeks. All the patients subjected to H.Plori Ag in stool after 1 month of the end of treatment, after stopping PPI for 2 weeks and antibiotic stoppage for 1 month. Helicobacter pylori Infection Test: The diagnosis of H. pylori infection was done by ELISA technique using IBL International Kit (Flughafenstrasse, Hamburg, Germany), and Tecan Spectra ELISA reader (supplied by Tecan Group Ltd. Switzerland) with 3 ng/ml as a cut-off value (Gulcan et al., 2005).

Serum samples were screened for H.Pylori antibodies (anti-CagAIgG and anti-VacAIgG) by using the ELISA Kits (Kits to identify IgG against H.PyloriCagA and VacA, Xingkang Company, Shenzhen) according to the method described by Han et al.,(2006) (%). The cutoff values of anti-VacAIgG and anti-CagAIgG were estimated .

The details of procedures including addition of 4drops of the washing solution to the bottom of the nitrocellulose membrane then 100 L of diluted serum was putted onto the membrane, and 5minutes later, washing solution of only 6 drops were dribbled on it to wash the residual serum. Thereafter, immunogold (100 L) was putted on the membrane, then addition of washing solution (6 drops) for 5 min. lastly, the protein array was placed into the scanner for measurement according to the instructions of the manufacture`s .The data were prearrangedbasing on the sites of the matrixes and the mean grey levels of the spots in the matrixes (Han et al., 2006).

Statistical Analysis: The obtained data were statistically analyzed by applying SPSS 25. Continuous variables were presented as means ± SD, and categorical variables were displayed as percentages (%). Differences between two groups were tested by X^2 test for categorical variables and t test for continuous parameters. Binary logistic regression was also used to evaluate the association the treatment response and other variables. $P < 0.05$ level was considered statistically significant.

Results

The investigation conducted on 144 subjects, of them 96(66.7%) were males and females were 48(33.3%) (Table 1) with the mean age was (36.4+8.2) (Table 1). In the present study, anti-Cag AIgG was positive in 74(51.4%), while it was negative in 70(48.6%) (Table 1). The present study, showed that anti-Vac AIgG was positive in 58(40.3%), while 86(59.7%) were negative (Table 1). H.Pylori eradicated in 96 (66.7%) of the studied subjects (Table 1).

There were no significant response between eradicated and non-eradicated groups regardingsex, anti-Cag A IgG, anti-Vac A IgG and age (Table 2).

There were no significant correlations between anti-Cag A IgG, anti-Vac A IgG, sex and age as regards h.pylori treatment response (Table 3).

Table 1: Descriptive data of the study participants

	Number 144(100%)
Sex	
• **Male**	96(66.7%)
• **Female**	48(33.3%)
Anti-Cag A IgG	
• **Positive**	74(51.4%)
• **Negative**	70(48.6%)
Anti-Vac A IgG	
• **Positive**	58(40.3%)
• **Negative**	86(59.7%)
H. pylori infection	
• **Eradicated**	96(66.7%)
• **Not eradicated**	48(33.3%)
Age	Mean + SD (36.4±8.2)

Table 2: Relation between treatment response and sex, anti-Cag A IgG, anti-Vac A IgGand age

		TTT response		X^2 test
		Not eradicated N=48 (33.3%)	**Eradicated N=96 (66.7%)**	P- value
Sex	Male	32 (66.7%)	64 (66.7%)	1.000
	Female	16 (33.3%)	32 (33.3%)	
Anti-Cag A	Negative (70)	28 (40%)	42 (60%)	0.099
IgG	Positive (74)	20 (27%)	54(73%)	
Anti-Vac A	Negative (86)	26 (30.2%)	60 (69.8%)	0.336
IgG	Positive (58)	22 (37.9%)	36 (62.1%)	
Age	N (%)	48 (33.3%)	96 (66.7%)	1.000
	Mean±SD	36.38±8.29	36.38±8.25	

Table 3: Binary logistic regressioncorrelation between treatment response and sex, anti-Cag A IgG, anti-Vac A IgGand age.

	B	**S.E.**	**Wald**	**Df**	**Sig.**	**Exp(B)**
Sex	-0.192	0.391	0.240	1	0.624	0.826
Age	0.002	0.022	0.013	1	0.910	1.002
Anti-Cag A IgG	0.943	0.419	5.070	1	0.024	2.569
Ant-Vac A IgG	-0.779	0.422	3.410	1	0.065	0.459
Constant	0.533	0.845	0.397	1	0.529	1.704

Discussion

Warren and Marshall discovered Helicobacter pylori (H. pylori) in 1983 and reported it in 1984 and in 2005, awarded the Noble prize (Fock et al.,2013). The incidence of H. pylori infection differs greatly among countries; where the frequency reached about 80% in developing and 30% in developed countries (Khoshbaten et al., 2013). Many Helicobacter pylori virulence factors have been detected to affect the course of infection. Of them, theretwo important factors (CagA and VacA) which will determine both Helicobacter pylori virulence and its antibiotic resistance (Atherton, 1996) and the cure rates (Sugimoto and Yamaoka,2009). The study conducted on 144 patients, males were 96(66.7%) and females were 48(33.3%) (Table 1) with the mean age was (36.4±8.2) (Table 1). Remarkably, the frequency of the cag A-positive strain are varies betweendiverse countries, where in East Asian countriesmore than 90% of H. pylori strains are cagA positive strains, regardless of clinical symptoms (Yamaoka et al. 2002). A total of 127(69.1%) of patients were established to be CagA positive, whereas, only 57 (30.9%) of patients were recorded as CagA negative strain (Saruç et al.,2001). More than 60% of the strains possessing cagA gene, and it is incidence was differ significantly (p<005) amide strains isolated from PUD patients (Biernat et al., 2014).

On the other hand, the present study anti-Cag AIgG was positive in 74(51.4%), while it was negative in 70(48.6%) (Table 1). The present study, showed that anti-Vac AIgG was positive in 58(40.3%), while 86(59.7%) were negative (Table 1). This is in agreement with (Rhead et al.,2007), who found that,H. pylorivacA gene expression causing the release of vacuolating cytotoxic protein VacA (found merely in about 40% of isolates). H.Pylori eradicated in 96(66.7%) of the studied subjects (Table 1), which is lower than detected by (Saruç et al.,2001), which was 82.6%. The present study showed that,the eradication rate in the anti-CagAIgG-positive group (73%)was more than that in anti-CagAIgG-negative group (60%), but doesnot reach the statistical significance(P = 0.099).

This is in agreement with (Saruç et al.,2001), who showed that,the incidence in the CagA-positive group was 87.4%, while it averaged 71.9% in the CagA-negative group (P = 0.019). Also, patients infected with cagA-negative strains and vacA s2 genotypes exposed highly to rise the risk of failure in extinction of H. pylori infection(Sugimoto and Yamaoka,2009) and (van der Hulst et al. 1997) also, H. pylori eradication ratein patients infected with cagA positive (73%, 89/122) was higher (p=0.017) than those which carrying cagA-negative strains (52%, 17/33).

An explanations for these results, may be attributed to the fact that H. pylori virulence factors prompttumor necrosis factor (TNF)-α and proinflammatory cytokines, such as interleukin IL-8 ,IL-1and, which effectinflammation in the mucous membranes and/or gastric acid release (Sugimoto and Yamaoka,2009).The CagA pathogenicity island has a developmentbenefit to H. pylori strains and is linked with augmentation in the inflammatory reaction at level of the gastric mucosa. These characteristic feature could permit of CagA-positive H. pylori strains to be more susceptible to antibiotics (Saruç et al.,2001). On the other hand, the overall recovery rate not differ significantly between CagA positive and negative strains between different researches (Lopez-Brea et al.,1999, Chaudhuri et al.,2003, De Francesco et al.,2002). On the other hand, the cure rate was higher in Cag A-negative than in Cag-A positive group (Greenberg and Cello, 1999). The present study showed thatthe eradication rate in the anti-Vac A IgG-positive group (62.1%) was more than that in the anti-Vac A IgG-negative group (37.9%%), but does not reach the statistical significance (P = 0.336). These results comes in agreement with that reported by Van Doorn et al., 2000, Russo et al.,2003, Zhao et al.,2007. On the other hand, Lopez-Brea et al., 1999, found that eradication rate was higher in Vac A-negative group than Vac A-positive group. However, Rudi et

al., 2002, De Francesco et al., 2004, found no significant difference between both groups regarding the eradication rate. The discrepancy in these results may be due to the difference in the ethnicity and may be due to the difference in H.Pylori treatment regimens. In conclusion, there is no evident relationship between Helicobacter pylori anti-cagAIgGand anti-vacAIgG and the treatment response.

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