

Original Article

Is There Any Association between *Helicobacter pylori* CagA Status and Patient's Habits with Gastric Carcinoma

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Abstract:

Enormous studies have been conducted worldwide regarding CagA⁺ status of *H. pylori* and patients habits in gastric carcinoma. But no study has been carried out in our country yet. Thus, this study has been designed to see the association between CagA⁺ *H. pylori* strain and patients habits with gastric carcinoma. For this purpose, a total number of 80 (eighty) patients were selected. Of the 80 (eighty) patients 40 (forty) were selected as cases (malignant) and the remainder 40 (forty) were selected as controls (non-malignant). *H. pylori* was detected by applying non-invasive (*H. pylori* IgG serology and CagA IgG serology) and invasive (Histology and rapid urease test) technique. Of them Histology was done by Modified Giemsa stain, CagA IgG was detected by ELISA method. In this study, we see that among the 40 cases, 35 (thirty five) possess the CagA⁺ *H. pylori* strain. And among the 40 controls, 33 (thirty three) bear the CagA⁺ *H. pylori* strain. This study also discloses that among cases, who were habituated with betel leaf and betel-nut ranking the highest number of gastric carcinoma. There are sufficient papers in favor of it which argues that CagA positivity as well as *H. pylori* positivity is not the sole causative agent of gastric carcinoma. If so, it merely acts as an initiator.

Key words: *Helicobacter pylori*, *H. pylori* IgG, CagA IgG, CagA⁺ *H. pylori*, Gastric carcinoma.

Introduction:

Stomach cancer has been recognized for several millennia¹ and worldwide, however, it is the second leading cause of cancer related death². Our understanding of gastric cancer underwent a marked shift with the re-discovery of *Helicobacter pylori*¹. In the late 1970s Warren also noted the bacteria, and in 1982 Barry Marshall and Robin Warren were able to culture the organism and proved the association with gastritis and peptic ulcer disease³. Further studies

suggested that gastric colonization with *H. pylori* can lead to a variety of upper gastrointestinal disorders, such as chronic gastritis, peptic ulcer disease, gastric mucosa associated lymphoid tissue lymphoma (MALT lymphoma), and gastric cancer. Robin Warren and Barry Marshall were awarded Nobel Prize 2005 in Physiology or Medicine for their "discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease"⁴.

A causal relationship between *Helicobacter pylori* and gastric cancer was first postulated by Marshall and Warren in 1983⁴. *H. pylori* is the first bacterium identified as being carcinogenic in humans⁵. The association between gastric adenocarcinoma and *H. pylori* was confirmed by many subsequent investigations, leading to the consensus that the bacterium is a class I carcinogen⁵. *H. pylori* infection is the leading cause of gastric cancer worldwide. There is increasing evidence that persistent infection with *H. pylori* is a risk factor for gastric adenocarcinoma⁶ especially of the distal stomach. The evidence comes mainly from epidemiological investigations including nested case control studies and molecular and pathological studies support its biological plausibility⁷. However, although *H. pylori* infection is highly prevalent in patients with gastric cancer, most *H. pylori*

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infected persons never develop these neoplasms⁸. A logical next step is to identify other factors that more precisely determine risk among *H. pylori* infected persons. *H. pylori* strains are highly diverse, and individuals may harbour more than one strain⁹.

H. pylori is not a clonal organism and exhibits great genetic diversity¹⁰. At the phenotypic level, strains can be characterized into two types: those that contain a gene associated with cytotoxin expression, the so called CagA gene, and those that do not¹¹. However, approximately 60% of isolates possess a gene, *cagA*, which encodes a high molecular weight protein (CagA) of variable size (M 1,20,000-1,40,000)¹². Studies suggest that persons infected with CagA⁺ strains have higher degrees of gastric inflammation and epithelial cell damage than do persons from whom CagA⁻ strains have been isolated¹³. Persons infected with CagA⁺ *H. pylori* strains have enhanced expression of IL-1 α , IL- β & IL-8 in gastric biopsies compared to uninfected persons or patients infected with CagA⁻ strains¹⁴. Since both intensity of inflammation and epithelial damage may be involved in pathogenesis of gastric cancer it is reasonable to examine the importance of CagA in this context. In one study Parsonnet and colleagues¹⁴ stated that subjects infected with *H. pylori* who had CagA antibodies were 5.8 fold more likely than uninfected subjects to develop gastric cancer. In our country study had been carried out showing relation of *H. pylori* with gastric malignancy but to the best of my knowledge no study relating CagA⁺ status with gastric carcinoma has been carried out yet. Therefore, this study has been designed to see the association between patient's habits and gastric carcinoma in CagA⁺ *H. pylori* strains.

Materials and Methods:

This prospective randomized case control study was carried out in the Department of Pathology, Sylhet M A G Osmani Medical College, during the period of July 2010 to June 2011. Patients and control subjects were selected consecutively from endoscopic unit of Department of Gastroenterology of Sylhet M A G Osmani Medical College and Popular Medical Centre in Sylhet City. Clinical histories of the patients were noted. Patients were examined thoroughly. History, physical finding and reports of investigations were recorded in a form prepared for this purpose. Patients aged > 15 years and having clinical features suggestive of carcinoma stomach were selected for upper GI endoscopy. Patients taken *H. pylori* eradication therapy within last four weeks of endoscopy, pregnant lady & patient with major organ failure were excluded from the study. The endoscopic examinations were performed by experienced endoscopist using video endoscope and biopsies were taken from the lesions suspicious of malignancy for histopathology. When

histopathology was found compatible with gastric carcinoma, the subject was selected as case. In order to detect *H. pylori*, tissue were taken from non-involved area of antrum and fundus of stomach for histopathological examination and rapid urease test. Subjects with normal upper GI endoscopy and histopathologically proved non-malignant were taken as controls and 40 (forty) persons were taken consecutively. Tissue biopsies were taken from the antrum and fundus for rapid urease test and histologic diagnosis of *H. pylori* from the control. Three to four cc venous blood was aspirated from each case as well as control for serology. Rapid urease test was done by inoculating endoscopic biopsy material in Christensen's urea agar and urea solution. Serology was done to detect *H. pylori* IgG antibody and CagA IgG antibody by ELISA method.

Result:

Table-I: Age and Sex distribution of the patients

Age years	Sex of the Patient (Case)		Sex of the Patient (Control)		M:F
	Male	Female	Male	Female	
21-30	0	0	1 6.3%	9 36.0%	4 26.7%
31-40	1 4.2%	1 6.3%	4 16.0%	2 13.3%	
41-50	3 12.5%	7 43.8%	5 20.0%	3 20.0%	
51-60	10 41.7%	6 37.5%	2 8.0%	3 20.0%	1.58:1
61-70	5 20.8%	1 6.3%	3 12.0%	1 6.7%	
71-80	4 16.7%	0 0	2 8.0%	0 0	
81-90	1 4.2%	0 0	0 0	2 13.3%	

The age range of total 80 patients was between 21 and 90 years with the mean age of 51.89 with std. deviation ± 16.93 . The highest number of subjects (21 in number) was seen in 6th decade. Total number of male and female in the case is 24 and 16 respectively with mean age 57.73 and std. deviation ± 12.21 . Total number of male and female in control is 25 and 15 respectively. The number of males was 49 and the number of females was 31. The overall Male: Female ratio was 1.58:1.

Table-II: Distribution of study subjects as per socio-economic status.

Socio-economic status	Patients group		Control	
	Case			
Lower class	17	42.50%	17	42.50%
Middle class	20	50.00%	21	52.50%
Higher class	3	7.50%	2	5.00%

Note: Socio-economic status has been considered as per income status. Per capita income per annum (Year-2000) has been considered as a reference. The study subjects were divided on the basis of their income status. Majority of the subjects fall in the lower class and middle class (17 and 20 in number in cases and 17 and 21 in number in controls respectively).

Table-III: Findings of various diagnostic procedures.

Patients group	Rapid Urease test for <i>H. pylori</i>				Findings of Modified Giemsa Stain				<i>H. pylori</i> CagA IgG status		
	Positive in antrum	Positive in fundus	Positive in antrum & fundus	Negative	<i>H. pylori</i> Positive in fundus	<i>H. pylori</i> positive in antrum	<i>H. pylori</i> positive in antrum & fundus	<i>H. pylori</i> negative	Positive	Positive	Negative
Case	7	7	10	16	6	9	11	14	40	35	5
Control	10	4	6	20	9	6	2	23	40	33	7

Table shows all the study subjects are *H. pylori* IgG positive & there is no difference in CagA IgG status among two groups.

Table-IV: Frequency table of the habit of patients (case)

Habit	Frequency	Percent
None	3	7.5
Smoker	10	25.0
Betel leaf and betel nut	16	40.0
Cigarette, betel leaf & betel nut	11	27.5
Total	40	100.0

Those who are habituated with betel leaf & betel nut ranking the highest (16 in number).

Table-V: Frequency distribution of food habit in case and control

Food Habit	Case		Control		Gastritis
	Adenocarcinoma	Normal gastric tissue	Adenocarcinoma	Normal gastric tissue	
Spice and Pepper	16	40.0%	7	41.2%	11 47.8%
Hot Tea	11	27.5%	3	17.6%	4 17.4%
Very hot tea	1	2.5%	0	00	0 00
None	12	30.0%	3	17.6%	3 13.0%
Tea and spice	00	00	4	23.5%	5 21.7%

Here, consumers of spice and peppers ranking the highest among both groups. Tea and spice consumption are common in control groups.

Discussion:

Gastric cancer has been recognized for several millennia. But people were not aware of the exact causative agent of this deadly disease. Discovery of the organism *H. pylori* by Warren and Marshall in 1982³ and claiming the organism as the causative agent of gastric carcinogenesis in 1983⁴ had cast dim light on it for the first time. The historical award winning lecture, delivered by Correa has focused great beam of light about the causation of this fatality¹⁵. Correa postulated that Gastric cancer is the end result of a sequential multistep and multifactorial process (Chronic gastritis, Atrophy, Intestinal Metaplasia, and Dysplasia); where *H. pylori* and excessive salt intake was detected as the prime initiator. He orchestrated with the findings of Warren and Marshall. Correa's postulates were supported by many investigators later on. Prospective serologic studies have reported that persons with *H. pylori* infection have a three to six fold higher risk of gastric cancer^{14,16,17}. Another citation by Talukder and colleagues¹⁸ in Bangladesh is that there is significant association found between *H. pylori* and both intestinal and diffuse types of gastric cancer. In the article "Pathogenesis of *Helicobacter pylori* Infection", Kusters and associates¹⁹ depicted that *H. pylori* colonization increases the risk of gastric cancer approximately 10 fold and *H. pylori* was designated a class I carcinogen by the WHO⁵.

Helicobacter is the leading cause of gastric cancer worldwide¹⁶. However, although *H. pylori* infection is highly prevalent in patients with gastric cancer, but many of the *H. pylori* infected persons never develop these neoplasms⁸. Certainly there is some fallacy. To answer to this fallacy, it is to be said that there are other

factors of which the most important one is the diversity of strains of *H. pylori*. At the phenotypic level, *H. pylori* strains can be characterized into two types: those that contain a gene associated with cytotoxin expression, the so called CagA gene, and those that do not²⁰. Studies of gastric carcinoma relating *H. pylori* infection had been carried out in our country. But no study relating gastric carcinoma with CagA⁺ status has been carried out yet. So, this study had been planned to see the association between CagA⁺ *H. pylori* strain and gastric carcinoma.

In this study, age range of total 80 patients (case and control) was between 21 and 90 years with the mean age of 51.89 with standard deviation 16.93 (SD±16.93). The highest number of subjects (21 in number) fall in the age group 51- 60 years. The number of males was 49 and the number of females was 31. The overall male: female ratio was 1.58: 1 (Table-1). This study shows that majority of the people belong to lower and middle class (17 and 20 in number in cases and 17 and 21 in number in controls) (Table-2). This observation is consistent with other studies (Ahmed et al. 2007)²¹. Here, we see positivity of *H. pylori* IgG antibody is 100% among study subjects (Both case & control groups) (Table-3). This finding is consistent with the findings of others^{8,18, 21}. A study carried out in Sylhet MAG Osmani Medical College, with the undergraduate students shows that it was 92%. Study also discloses that among cases, who were habituated with betel leaf and betel-nut ranking the highest (16 in number) (Table-4). Study also reveals that consumers of spice and peppers rank the highest among case & control groups (Table- 5).

There are many studies, which reveal that there is association between gastric carcinoma and CagA positivity. Such as, findings observed by Atherton²². Study carried out by Blaser and colleagues²³ also stated that patients with peptic ulceration, pre-neoplastic and neoplastic gastric epithelial lesions are more likely to be infected by CagA⁺ strains. But there are controversial opinions also. Maeda and colleagues²⁴ and Yamaoka and associates²⁵ stated that since, the majority of *H. pylori* infected individuals in Asian countries harbour CagA-positive strains, associations of CagA status and diseases are not observed in Asia. Study carried out in India by Kumar and colleagues²⁶ showed that antibodies to CagA protein are not predictive of serious gastroduodenal disease. This is contradictory to the studies from developed countries. Genotype analysis of *H. pylori* strains from India showed pathogenic strains to be present in more than 80% of adults and children with gastroduodenal diseases as well as control population²⁷. Ghoshal and colleagues²⁸ stated that a large study carried out in their centre showed that frequency of CagA IgG antibody

was similar among the patients with gastric carcinoma and the controls, suggesting that difference in virulence factors of *H. pylori*, at least CagA is unlikely to explain the variation in outcome of *H. pylori* infection. Singh and Ghoshal²⁹ stated that *H. pylori* alone is not the only independent factor in gastric carcinogenesis in India. Several studies carried out by Satarkar and colleagues³⁰ and Prabhu and associates³¹ come to conclusion that intestinal metaplasia is rare following *H. pylori* infection, and the organism may not be important in the development of gastric cancer in India. Studies from India failed to show an association between *H. pylori* infection and gastric cancer³². However, these controversies merge with the ultimate finding of this study.

Conclusion:

Different host factors like patient's habit and *H. pylori* CagA status are suspected to be associated with development of gastric carcinoma. Many studies show positive correlation and many shows no such association. In our study *H. pylori* CagA status is similar among carcinomatous & non carcinomatous groups. Patient's habits are also shows almost similar distribution among them, besides; those who are habituated with betel leaf & betel nut ranking the highest among gastric carcinoma group. Future study may be needed to prove or disprove it.

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