

## ORIGINAL ARTICLE

# Association between cytotoxin producing H. pylori and gastric carcinoma

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### Abstract

**Background:** Enormous studies have been conducted worldwide regarding CagA+ status of H. pylori in gastric carcinoma.

**Objective:** No study relating CagA+ status and gastric carcinoma has been carried out in our country yet. This study has been designed to see the association between CagA+ H. pylori strain and gastric carcinoma

**Methods:** 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 which was regarded as gold standard, CagA IgG was detected by ELISA method.

**Results:** In this study, 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. In this study, no significant difference between case and control on the point of CagA-IgG status was found.

**Conclusion:** H.pylori may be a simple initiator and not the actual cause of gastric carcinoma.

**Key words:** H.pylori, Gastric carcinoma, Cytotoxin associated gene.

### Introduction

Stomach cancer has been recognized for several millennia and worldwide, however, it is the second leading cause of cancer related death.<sup>1,2</sup> Our understanding of gastric cancer underwent a marked shift with the re discovery of Helicobacter pylori.<sup>1</sup> 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 disease.<sup>3</sup> 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."<sup>4</sup>

A causal relationship between Helicobacter pylori and gastric cancer (GC) was first postulated by Marshall and Warren in 1983.<sup>4</sup> H. pylori is the first bacterium identified as being carcinogenic in humans.<sup>5</sup> 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 carcinogen. Helicobacter infection is the leading cause of gastric cancer worldwide. There is increasing evidence that persistent infection with Helicobacter pylori is a risk factor for gastric adenocarcinoma especially of the distal stomach.<sup>6</sup> The evidence comes mainly from epidemiological investigations including nested case control studies and molecular and pathological studies support its biological plausibility.<sup>7</sup> However, although H. pylori infection is highly prevalent in patients with gastric cancer, most H. pylori infected persons 'never develop these neoplasms.'<sup>8</sup>

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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 strain.<sup>9</sup>

*Helicobacter pylori* is not a clonal organism and exhibits great genetic diversity.<sup>10</sup> 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 not.<sup>11</sup> However, approximately 60% of isolates possess a gene, *cagA*, which encodes a high molecular weight protein (CagA) of variable size (MW: 1,20,000-1,40,000).<sup>12</sup> 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 not been isolated.<sup>13</sup> Persons infected with CagA+ *H. pylori* strains have enhanced expression of IL-1a, IL-1b & IL-8 in gastric biopsies compared to uninfected persons or patients not infected with CagA+ strains.<sup>14</sup> 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 colleagues stated that subjects infected with *H. pylori* who had CagA antibodies were 5.8 fold more likely than uninfected subjects to develop gastric cancer.<sup>14</sup> In our country study had been carried out showing relation of *H. pylori* with gastric malignancy but no study relating CagA+ status with gastric carcinoma has been carried out yet. Therefore, this study has been designed to see the association between CagA+ *H. pylori* strain and gastric carcinoma

## 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

**Study Population:** Patients and control subjects were selected consecutively from endoscopic unit of Dept. of Gastroenterology of Sylhet M A G Osmani medical College and private practice in Sylhet city. The clinical history of the patients were noted. The patients were examined thoroughly. History; physical finding and reports of investigations were recorded in a form prepared for this purpose. Patients having clinical features suggestive of carcinoma stomach were selected for upper GI endoscopy. The endoscopic examinations were performed by experienced endoscopists 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 in the same sitting. 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. 3 to 4 cc 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.

**Inclusion Criteria:** All the patients with age 15 years and above clinically suspected of gastric malignancy and confirmed by histopathology.

**Exclusion Criteria:** 1) Patients refuse endoscopy 2) Failed endoscopy. 3) Diagnosis of carcinoma not proved histopathologically. 4) Taking *H. pylori* eradication therapy within last four weeks of endoscopy 5) Major organ failure. 6) Pregnant women.

The following outcome variables were studied: Primarily CagA+ status in gastric carcinoma patients with histopathological report.

## Results

The age range of total 80 patients was between 21 and 90 years with the mean age of 51.89 with SD  $\pm 16.93$ . The highest number of subjects (21 in number) were seen in 6th decade (Table 1).

**Table 1**

Distribution of study subjects on the basis of age

Age group of Patients (Years)	Sex of the Patient			
	Male		Female	
	Number	%	Number	%
21-30	9	18.4	5	16.1
31-40	5	10.2	3	9.7
41-50	8	16.3	10	32.3
51-60	12	24.5	9	29.0
61-70	8	16.3	2	6.5
71-80	6	12.2	0	00
81-90	1	2.0	2	6.5

Total number of male and female in the case is 24 and 16 respectively with mean age 57.73 and SD-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)

**Table II**

Distribution of study subjects on the basis of sex.

Age group of Patients (Years)	Sex of the Patient (Case)		Sex of the Patient (Control)	
	Male (%)	Female (%)	Male (%)	Female (%)
21-30	0	0	6.3	9
31-40	1	4.2	6.3	4
41-50	3	12.5	43.8	5
51-60	10	41.7	37.5	2
61-70	5	20.8	6.3	3
71-80	4	16.7	0	0
81-90	1	4.2	0	0

Socio economic status has been considered as per income status. Per capita income per annum (Year 2000) has been considered as a reference (Table III).

**Table III**

Distribution of subjects on socio economic status.

Socio-economic status	Patients group	
	Case (%)	Control (%)
Lower class	17	42.50
Middle class	20	50.00
Higher class	3	7.50

The study subjects were divided on the basis of their income status. Majority of the subjects fall in the lower class and middle class. Our study shows all the study subjects are H. pylori IgG positive (Table IV)

## 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 1982 and claiming the organism as the causative agent of gastric carcinogenesis in 1983 had cast dim light on it for the first time<sup>2,3</sup>. 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. Another citation by Talukder and colleagues in Bangladesh is that there is significant association found between H. pylori and both intestinal and diffuse types of gastric cancer.<sup>4</sup>

Helicobacter is the leading cause of gastric cancer worldwide.<sup>5</sup> However, although H. pylori infection is highly prevalent in patients with gastric cancer, but many of the H. pylori infected persons never develop these neoplasms.<sup>6</sup> 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 not.<sup>7</sup>

GC is a multifactorial disease. The main determinant, H. pylori infection, can be considered a sine qua non for GC development; however, despite almost all individuals who get GC are currently, or have been infected, it is neither a necessary nor a sufficient condition.<sup>8</sup> The aetiology of GC is complex and multifactorial, involving environmental and host related factors as well as genetic and epigenetic alterations. H. pylori

**Table IV**

Findings of various diagnostic procedures.

Patients group	Rapid Urease test for H. pylori				Findings of Modified Giemsa Stain				H. pylori IgG status	CagA IgG status	
	Positive in antrum	Positive in fundus	Positive in antrum & fundus	Negative	H. pylori positive in fundus	H. pylori positive in antrum	H. pylori positive in antrum & fundus	H. pylori 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

infection is a necessary but not a sufficient cause for gastric cancer.<sup>9</sup>

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. The highest number of subjects (21) 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. This study shows that majority of the people belong to lower and middle class. This observation is consistent with other studies.<sup>10</sup> Here, we see positivity of H. pylori IgG antibody is 100% among study subjects. This finding is consistent with the findings of others.<sup>4,6,10</sup> A study carried out in Sylhet MAG Osmani Medical College, with the undergraduate students shows that it was 92%.

There are many studies, which reveal that there is association between gastric carcinoma and CagA positivity. Infection by the cagA positive H. pylori genotype may determine an increased inflammatory response and a consequent enhancement of mutagenesis rate, oxidative stress, reactive oxygen species generation, dysfunction of DNA repair mechanisms, genetic instability and resultant high risk of GC development. Currently, although substantial evidence supports the role of H pylori infection in GC development, the magnitude of the risk of GC associated with infection remains unclear.<sup>11</sup> Overall, it is difficult to attribute the increased risk of development of gastric cancer to a specific polymorphism, as infection with H. pylori is an essential component in the equation. Rather, it is the interaction between the different pro and anti inflammatory polymorphisms, the immune status of the host, and the characteristics of the colonizing H. pylori strain that jointly determine disease outcome.

There are controversial opinions which 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 population.<sup>12</sup> Ghoshal and colleagues 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.<sup>13</sup> Singh and Ghoshal stated that H. pylori alone is not the only independent factor in gastric carcinogenesis in India.<sup>14</sup>

Studies from India failed to show an association between H. pylori infection and gastric cancer. However, these controversies merge with the ultimate finding of this study.

## Conclusion

No significant difference between case and control on the point of CagA-IgG status was found. 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. However, it is being left open for the future researchers to prove or to disprove it.

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