ROLE OF H. PYLORI IN PATIENTS OF GASTRIC CANCER IN SOUTHERN ODISHA

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HOW TO CITE THIS ARTICLE:

Kalandi Barik, I.C. Muduli, S.N. Mallick, Lachhman Bag, Anower Hossen Halder, Rupesh Sondawle, Sarada Prasanna Sahoo, Sanjit Kumar Nayak. "Role of H. pylori in patients of gastric cancer in Southern Odisha". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 01, January 06; Page: 60-67.

ABSTRACT: Since the discovery of *Helicobacter pylori* in 1983 by Robin Warren and Barry Marshall, the diagnosis and treatment of upper gastrointestinal disease have changed greatly. As infection with *H. pylori* is the strongest known risk factor for gastric cancer, that is why our study was undertaken in our surgery department to establish the role of *H. pylori* in patients of gastric cancer in Southern Odisha. Total 70 patients of gastric cancer as cases and 32 controls were studied. *H. pylori* infection status in all cases and controls was assessed by serological test, rapid urea broth test and histopathological examination of the gastric mucosal biopsy specimens. *H. pylori* infection was positive in 64 cases of cancer stomach (91.43%) and 24 patients were positive in the control group (75%). The odds ratio was estimated to be 3.56 and p. value was found to be < 0.02.

KEY WORDS: *H. pylori*, gastric cancer, rapid urea broth test

INTRODUCTION: Cancer stomach is the fourth most common cancer and the second leading cause of cancer-related death worldwide. It is especially prevalent in East Asia and South America and has been increasing in developing countries¹.

Gastric cancer is a multi-factorial disease. The major risk factors for gastric cancer include environmental and genetic factors. *H. pylori* is the strongest known risk factor for gastritis and gastric cancer and now it is considered as the most common aetiological agent of infection-related cancers. In 1994, the International Agency for Research on Cancer labeled *H. pylori* a definite carcinogen (class-I carcinogen). A number of prospective studies have demonstrated an association with the development of gastric cancer¹.

H. pylori, a spiral-shaped gram-negative rod, has evolved the ability to colonize the highly acidic harsh environment found within the stomach by converting urea to ammonia through the production of urease enzyme, which generates a neutral environment around it². *H. pylori* infection is very common and has coexisted with humans for many thousand years. Most of the infections are acquired in childhood via the fecal-oral or oral-oral mode of transmission^{3,4}. The variable outcomes of *H. pylori* infection likely depend on various factors such as strain-specific bacterial constituents, inflammatory responses governed by host genetic diversity, or environmental influences, which ultimately influence the interactions between pathogen and host⁵. The primary mechanism is thought to be the presence of chronic gastric inflammation. Long-term infection with the bacteria *H. pylori* leads to chronic atrophic gastritis which then progresses to intestinal metaplasia, dysplasia and ultimately intestinal-type adenocarcinoma. A wide range of molecular alterations in intestinal metaplasia have been described and may affect the transformation into gastric cancer¹.

With this background the present case control study has been undertaken to find out the prevalence of *H. pylori* infection and to determine the degree of association of *H. pylori* infection in patients of gastric cancer in Southern Odisha.

MATERIALS AND METHODS: This case control study was conducted in the Department of General Surgery, M.K.C.G. Medical College, Berhampur, taking into account both indoor and outdoor patients with the symptoms of gastric cancer as the study group during the period between September 2011 and September 2013.

The patients were selected on the basis of their clinical signs and symptoms supplemented by routine and special investigations to arrive at the diagnosis of gastric cancer. Then relevant tests like serology, rapid urea broth test and histopathological examination of gastric mucosal biopsy specimens were performed for detection of *H. pylori*. Mucosal biopsy specimens were obtained either by upper GI endoscopy or from resected stomach.

Endoscopy was carried out by a flexible upper GI video endoscope by an expert endoscopist. Four biopsy specimens were taken; two from the greater curvature of the antrum and two from the upper body of the stomach (when lesions suspected to be cancerous were noted, additional biopsies were performed). Of these four specimens, two were fixed in formalin and assessed for presence of *H. pylori* (by Giemsa staining) and the degree of neutrophil infiltration and intestinal metaplasia (by staining with Hematoxylin and Eosin). The remaining two were used for a rapid urea broth test.

The degree of neutrophil infiltration was classified into four grades (0- no infiltration, 1- mild, 2- moderate, and 3- marked) and expressed as a score by two pathologists according to the updated Sydney System [6]. Consensus was reached through joint review of all the slides.

Active gastritis was classified into four categories: (no gastritis, antrum-predominant gastritis, pan-gastritis, and corpus-predominant gastritis). Intestinal metaplasia was classified in two grades (absent or present).

Gastric mucosal atrophy was evaluated according to the endoscopic-atrophic-border scale described by Kimura and Takemoto⁷ which correlates with the results of histological evaluation⁸. There were three classifications (1- mild atrophy or none, 2- moderate, and 3- severe). The pathologists were not aware of the clinical or endoscopic data. The results were scored blindly with the use of patient codes.

The gastric mucosal biopsy specimens for the detection of *H. pylori* by rapid urea broth test were immediately suspended into normal saline in aseptic condition and then put into the urea broth which was then monitored for 24 hours for detection of color change from yellow to pink.

Blood sample was collected immediately before endoscopy, serum was immediately separated and cryopreserved at -20°C until it was assayed for antibodies against *H. pylori* by ELISA test. A positive serologic test for *H. pylori* was defined as one with a titer of 1.8 or more.

H. pylori infection was identified by histological examination, the rapid urea broth test, and serologic evaluation. Patients in whom any of these assays were positive were classified as *H. pylori*-positive. Those in whom all three tests were negative were considered *H. pylori*-negative.

OBSERVATION: A total of 70 cases of cancer stomach and 32 non-cancer patients as controls were studied to determine the association of *H. pylori* with gastric cancer in patients of Southern Odisha and following observations were noted. All the gastric cancer patients were taken as cases after

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confirmation of diagnosis on the basis of presenting symptoms, signs and investigations. The observations were recorded in tabular forms and depicted in various diagrams and simultaneously the facts and figures were compared with those of other workers.

Sl. No.	Age group in years	No. of males	No. of females	Total no. of cases	Percentage
1	11 – 20	0	1	1	1.43
2	21 – 30	0	0	0	0
3	31 – 40	2	6	8	11.43
4	41 – 50	12	7	19	27.14
5	51 – 60	13	8	21	30
6	61 – 70	16	1	17	24.29
7	71 – 80	3	1	4	5.71
Total		46(65.71%)	24(34.29%)	70(100%)	100

Table – 1: AGE AND SEX DISTRIBUTION AMONG GASTRIC CANCER CASES

In our study, majority of the cases occurred after 40 years of age. The youngest patient in this series was 18 year old female and oldest patient was 80 year male. The average age was 55 year. The highest incidence was found in age group 51-60 years (30%). More than half of the total cases included in this study were found in between 40-60 years of age (57.14%). In this study 46 cases (65.71%) were male and 24 cases (34.29%) were female. The ratio between male and female was 1.9:1. Maximum no. of male patients was in age group 61-70 years and maximum no. of female patients was in age group 51-60 years.

Sl. no.	Agegroup in years	No. of males	No. of females	No. of controls	percentage
1	21 – 30	6	0	6	18.75
2	31 – 40	4	2	6	18.75
3	41 – 50	3	3	6	18.75
4	51 – 60	5	1	6	18.75
5	61 – 70	5	2	7	21.87
6	71 - 80	1	0	1	3.13
		Total = 24 (75%)	Total = 8 (25%)	Total = 32	100

Table – 2: AGE AND SEX DISTRIBUTION AMONG CONTROL GROUP

In this study, 32 patients were taken as control group among which 24 patients were male and 8 patients were female. The youngest control was 21 year old male and oldest control was 75 year old male. Maximum number of the controls was in the age group 61-70 year (n=7, 21.87%). In our study, 52 cases (74.28%) were from rural areas and 18 cases (25.72%) from urban areas. In the control group, 23 patients were from rural and 9 patients were from urban areas.

Out of 70 cases of gastric cancer, 40 cases (57.14%) were from low, 18 cases (25.71%) were from middle and 12 cases (17.15%) were from high socio-economic status group. Among 32 controls, 18 patients (56.25%) were from low, 8 patients (25%) were from middle and 6 patients (18.75%) were from high socioeconomic group. Hence cases and controls were comparable with respect to socioeconomic status.

Type ofgastro-duodenal lesion	No. of H. pylori +ve patients	No. of H. pylori -ve patients	Total no. ofpts.
Ultero-proliferative gastric cancer	28	2	30
Ulcerativecancer	24	2	26
Fungating cancer	9	2	11
Linitisplastica	3	0	3
Benign gastric ulcer	6	1	7
Chronic duodenal ulcer	7	0	7
Acute Gastritis	7	1	8
Normal(non-ulcer dyspepsia)	4	6	10
Total	88	14	102

Table - 3: Showing association of H. pylori in various gastro-duodenal lesions

On endoscopy, out of 70 cases, maximum no. of cases was found to contain ultero-proliferative growth (n=30; 42.85%). Rest of the cases were found to contain ulcerative lesion (n=26; 37.14%), fungating growth (n=11; 15.71%) and linitisplastica (n=3; 4.28%). Total no. of gastric cancers found in pre-pyloric region was 33 (47.14%), in body of stomach was 15 (21.43%), in pyloric antrum was 13 (18.57%) and in cardiac end of stomach was 6 (8.57%). Endoscopic findings in control group included that 10 patients had normal finding (non-ulcer dyspepsia), 8 patients had acute gastritis, 7 patients had chronic duodenal ulcers and rest 7 patients had benign-looking gastric ulcers.

On histopathological examination, adenocarcinoma was found in all cases. Out of 70 cases, intestinal type of adenocarcinoma was found in 44 cases (62.86%) and diffuse type was found in 26 cases (37.14%). Intestinal type was more common in males and diffuse type was more common in females.

In our study, it was found that sensitivity of the three tests used for detection of H. pylori are 78.13%, 95.31% and 75% for serology, rapid urea broth test and histopathology of direct smear respectively. The ELISA test used for detection of anti-H.pylori antibody detected only IgG antibody.

	No. of gastric cancer +ve patients (cases)	No. of gastric cancer -ve patients (controls)	Total
No. of H. pylori +ve patients	64 (a)	24 (b)	88 (a+b)
No. of H. pylori - ve patients	6 (c)	8 (d)	14 (c+d)
Total	70 (a+c)	32 (b+d)	102 (a+b+c+d)

Table – 4: TABLE FOR DETERMINATION OF DEGREE OF ASSOCIATION OF H. PYLORI WITH GASTRIC CANCER

In our study, the total no. of *H. pylori* positive cases was 64 (91.43% of cases), total no. of *H. pylori* negative cases was 6, total no. of *H. pylori* positive controls was 24 (75% of controls) and total no. of *H. pylori* negative controls was 8. Thus, *H. pylori* was found in 91.43% of gastric cancer patients and in 75% of controls. In this study of 102 patients, total no. of *H. pylori* positive patients was 88 (86.27%) and total no. of *H. pylori* negative patients was 14 (13.73%).

The odds ratio (OR) is a measure of the odds of disease in the exposed compared to the odds of disease in the unexposed (controls) and is calculated as:-

$$\text{Odds Ratio (OR)} = \text{ad/bc} \Rightarrow \text{OR} = 3.56$$

$$\text{Relative Risk (RR)} = \text{a(c+d)/c(a+b)} \Rightarrow \text{RR} = 1.69$$

Thus Odds Ratio was found to be 3.56 which estimated that *H. pylori* positive patients were 3.56 times more likely to develop gastric cancer in comparison to *H. pylori* negative patients. P-value calculated by Chi-square test was found to be <0.02.

DISCUSSION: The case-control study to establish the bacterial infective cause of gastric cancer carried out in the Department of General Surgery, M.K.C.G. Medical College, Berhampur correlates well with the results published earlier and made us think that a real association is indeed not deniable.

It was found that the prevalence of infection with *H. pylori* in male patients with gastric cancer was higher than in the female patients which correlated with the findings by Anderson et al (1994) ⁹. The infection occurs early in childhood and prevalence of *H. pylori* infection increases with age. This same trend was seen in our study. The maximum no. of *H. pylori* infected patients were in the age group 51-70 years. There was a bimodal distribution of prevalence of *H. pylori* infection with first peak between 55-60 years and second peak between 65-70 years.

H. pylori infection is more prevalent in developing countries than developed countries. In our study of total 102 patients, the maximum no. of patients were from rural areas (n=75, 73.53%) and 58 patients (56.86%) were of low socioeconomic status. *H. pylori* infection rate was highest among low socioeconomic status patients. In our study of 70 cases of gastric cancer, 40 cases occurred in persons of low socio-economic status constituting 57.14% of total cases. In our study, the prevalence of *H. pylori* infection among 70 cases of gastric cancer patients was 91.43% (n=64 cases) and prevalence of *H. pylori* infection among 32 controls was 75%.

In a study conducted by M. Kato et al., and the members of The Multi-centre Study Group ¹⁰, in the Division of Endoscopy of Hokkaido University Hospital and in the Department of Gastroenterology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; it was found that the overall prevalence of *H. pylori* infection was 50.2% in control subjects but was 82.8% in those with gastric cancer. In our study, the high prevalence of *H. pylori* infection among cases and controls might be due to the fact that maximum number of patients was from low socioeconomic status group and from rural areas.

The prevalence of *H. pylori* antibody (IgG) among gastric cancer patients was 82.85%. In our series, ELISA technique was used to detect antibody. The sensitivity of the three tests used for detection of *H. pylori* was 78.13%, 95.31% and 75% for serology, rapid urea broth test and histopathology of direct smear respectively. The increased absorbance level which is related to increased density of colonization of *H. pylori* was seen in 76.51% of 64 positive cases tested positive for IgG *H. pylori* specific antibodies. A similar finding has been reported by Lamers CBHW et al in

1995¹¹ who concluded that the serological absorbance index of IgG antibodies against *H. pylori* is related to the severity of antral gastritis and density of bacterial colonization.

All our gastric cancer cases showed both serologically as well as by histological study of gastric mucosal biopsy, of increased colonization of antrum with *H. pylori* that proves the high prevalence of infection in this population and definite association of *H. pylori* with gastric cancer as the major causative factor in its pathogenesis. IgM antibody has been found to have little diagnostic utility for *H. pylori* infections and is elevated only after infection, whereas *H. pylori* infections are generally chronic. IgM has extremely low sensitivity, confirming its lack of clinical utility in either children or adults.

Disease	Chen CJ et al	Taiwan study	Delpiano	Our study
Gastric cancer	76.2%	72.3%	87.9%	82.85%

Table-5: Comparison of various studies using sero-positivity for *H. pylori* (IgG antibodies) detection

In our case-control study, Odds Ratio was found to be 3.56 which estimated that *H. pylori* positive patients were 3.56 times more likely to develop gastric cancer in comparison to *H. pylori* negative patients. This is in accordance with existing literature that the risk of gastric cancer in patients with chronic *H. pylori* infection is increased about three fold¹². Our study showed a statistically significant association of *H. pylori* infection with gastric cancer in patients of Southern Odisha ($P < 0.02$).

Asaka et al.¹³ reported a significantly higher prevalence of *H. pylori* infection in gastric cancer patients than in asymptomatic control subjects. This association was strongest for early gastric cancer. Kikuchi et al.¹⁴ compared cancer subjects who were younger than 40 years with well-matched controls and found an OR of 13.3. In comparison to above study, the odds ratio of our study was very low due to maximum no. of patients were in advanced stage and prevalence of *H. pylori* infection decreases with advanced stage of disease.

CONCLUSION: The gastric cancer is a highly lethal and very common disease of gastro-intestinal tract. Gastric cancer is highly prevalent in this part of Odisha. The prevalence of *H. pylori* infection among gastric cancer cases of our study was 91.43%. This sort of high prevalence of infection among gastric cancer patients, the increase in absorbance level as the density of the bacterial colonization increases shown in serological study correlating well with the histological study and the odds ratio of 3.56 and p-value of < 0.02 support the significant causal association between gastric cancer and *H. pylori* infection. However further detailed study is required to firmly establish the role of *H. pylori* as a causative agent of gastric cancer.

REFERENCES:

1. Mahvi DM, Krantz SB. Stomach. In: Townsend CM, Beauchamp RD, Evers BM, Mattox KL, editors. Sabiston Textbook of Surgery. 19th ed. Philadelphia: Elsevier Saunders; 2012. p.1204 - 1205.
2. Weeks, D. L., S. Eskandari, D. R. Scott, and G. Sachs. 2000. A H⁺-gated urea channel: the link between *Helicobacter pylori* urease and gastric colonization. Science 287:482-485.

3. Everhart, J. E. 2000. Recent developments in the epidemiology of *Helicobacter pylori*. 29:559-578.
4. Ernst, P. B., and B. D. Gold. 2000. The disease spectrum of *Helicobacter pylori*: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. 54:615-640.
5. Blaser, M. J., and D. E. Berg. 2001. *Helicobacter pylori* genetic diversity and risk of human disease. 107:767-773.
6. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis: the updated Sydney System. *Am J SurgPathol* 1996; 20:1161-1181
7. Kimura K, Takemoto T. Endoscopic atrophy border. *Endoscopy* 1969; 1:1-3
8. Satoh K, Kimura K, Taniguchi Y, et al. Distribution of inflammation and atrophy in the stomach of *Helicobacter pylori*-positive and -negative patients with chronic gastritis. *Am J Gastroenterol* 1996; 91:963-969
9. Anderson SI, Haganders B, Wadstrom I, et al. *Scand J. Gastroenterol*, 1994 June29 (6) 306-10.
10. Kato M, Asaka M, Shimizu Y, Nobuta A, Takeda H, Sugiyama T et al. Relationship between *Helicobacter pylori* infection and the prevalence, site and histological type of gastric cancer. *Aliment PharmacolTher* 2004 Aug 4; 20 (Suppl. 1): 85–89.
11. Lamers CBHW et al. *J Clin Path* 1995 47, 227-231.
12. Dempsy DT. Stomach. In: Brunnicardi FC, Andersen DK, Billiar TR, Dunn DL, Hunter JG, Matthew JB, Pollock RE, editors. *Schwartz's Principles Of Surgery*. 9thed. The McGraw-Hill Companies, Inc; 2010. P.927.
13. Asaka M, Kato M, Takahashi S, Fukuda Y, Sugiyama T, Ota H, et al. Guidelines for the management of *Helicobacter pylori* infection in Japan: 2009 revised edition. *Helicobacter*. 2010; 15:1–20.
14. Kikuchi S, Wada O, Nakajima T, et al. Serum anti-*Helicobacter pylori* antibody and gastric carcinoma among young adults. *Cancer* 1995; 75:2789-93.

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Date of Submission: 05/12/2013.
Date of Peer Review: 06/12/2013.
Date of Acceptance: 18/12/2013.
Date of Publishing: 01/01/2014