

# Association of Helicobacter Pylori Infection and Stomach Cancer

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## ABSTRACT

**Introduction:** Helicobacter pylori (H. Pylori) is a primary etiological factor in carcinoma stomach. Association of H. Pylori in gastric cancer has been documented to be in more than 50% of cases. In underdeveloped countries, this association is shown to be much higher according to different studies.

**Methods:** A prospective observational study of 40 consecutive cases of carcinoma stomach was under taken in surgical department of Shree Birendra Hospital and Bir Hospital, for a period of two years 2009 to 2011. Location and pathological types of the lesion were noted and all specimens were investigated to see presence of helicobacter pylori by rapid urease test (RUT) and histological examination.

**Results:** Out of 40 patients, helicobacter pylori positivity was seen in 27 (67.5%) cases by both rapid urease test and Histopathological examination (HPE). Regarding the pathological types, out of 26 intestinal type of ca stomach, 20 (76.92%) cases were positive for H. pylori infection, whereas out of 14 diffuse type of ca stomach, 7 (50%) cases were positive for H. pylori. In 29 cases of distal ca stomach, H. pylori positivity was seen in 22 (75.86%) cases, whereas in rest of the 11 cases the lesion involved other part of the stomach, H. pylori positivity was seen in 5 (45.45%) cases.

**Conclusions:** Helicobacter pylori infection is higher in prevalence in cases of stomach cancer. Its association with intestinal histological type of stomach cancer is more common than diffuse type. There is higher prevalence of Helicobacter pylori infection in distal carcinoma.

**Keywords:** stomach carcinoma; h. pylori; incidence.

## INTRODUCTION

Gastric cancer still remains a major killer disease throughout the world. Even after advances in detection and treatment of gastric cancer, the 5 year survival rate, outside of Japan is still around 15- 20%. According to Lauren classification Ca stomach has been broadly classified into two groups, intestinal and diffused types<sup>1</sup>.

Helicobacter Pylori is a well-recognized etiological agent of gastric carcinoma. Its association was established first in 1983 by two Australian scientists

Barry Marshal and Robin Warren. H. pylori can establish a chronic, persistent infection which may lead to gastric or duodenal ulcer, gastric cancer, gastric lymphoma and other gastrointestinal disorder<sup>2</sup>.

Various studies revealed that approximately more than 50% of the world's human population is infected by H. pylori<sup>3</sup>. In Nepal, association of H. pylori has been found in significantly higher number of duodenal, gastric ulcer and gastritis cases reported in various studies and there have been various papers showing the association of H. Pylori infection and gastric cancer. However such studies are still lacking in

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underdeveloped world, where the incidence of *H. pylori* is thought to be still on the rise.

## METHODS

This was a prospective, observational, hospital based study. All patients who presented in surgery department of Shree Birendra Hospital and Bir Hospital with diagnosis of gastric carcinoma over a period of 2 years (2009-2011) were included in this study.

Thorough evaluation was done, a detailed history of all enrolled patients were taken. Demographical variables including age, sex, type and site of lesions were noted. Patients were subjected to endoscopy examination using video endoscope. In all patients, trained endoscopist at Birendra Hospital and Bir Hospital carried out an upper gastrointestinal endoscopy. Multiple biopsies were taken from the lesion, further targeted punch biopsy was taken from anterior and posterior wall of antrum, body and cardia. At least 6 mucosal biopsies were taken from the stomach in each patient for the histology and RUT.

RUT reagent was prepared as described by Thillainayagam et al<sup>4</sup>. Unbuffered solutions of urea in deionized water (10 gm of urea in 100 ml deionized water) at a pH of 6-8 and 1% phenol red solution (1gm of phenol red in 100 ml deionized water) were prepared and stored at 2 – 8 degree centigrade separately. Specimens were placed in the RUT reagent and also sent for histology. Tissues were placed immediately into a tube containing 0.5 ml of a freshly prepared solution

of 10% urea in deionized water, which was added on with two drops of 1% phenol red as a pH indicator. The original color of the solution was yellowish. In each, along with the test sample, a positive control with pink color and negative control (reagent tube without tissue) was examined. The reagent was yellowish when the pH was neutral but changed to pink when the reagent became alkaline due to urease activity. The specimens were placed fully in the RUT reagent and interpreted at 10 minute, 30 minutes and 1 hour at the endoscopy room. Positive RUT results were noted immediately. The change in color of the media from yellow to deep pink was taken as positive tests. For histological examination, piece of biopsy tissue was crushed between two sterilized glass slides. Each preparation was air dried and heat fixed, de waxed and made ready for staining. Giemsa and hematoxylin and eosin (HE) stain were done in all biopsy specimens.

Expert pathologists from Birendra Hospital and Bir Hospital did the histopathological examination of *H. pylori* by Giemsa and HE stain. A specimen was read as positive if curve bacilli organisms were seen on microscopy.

## RESULT

A total of 40 histologically proved cases of gastric cancer were taken for this study. Out of 40 cases, a total of 27 (67.5%) cases were positive for *H. pylori* infection. RUT positive seen in all 27 cases, whereas HPE showed *H. pylori* in 21 of 27 positive cases.

**Table 1: Incidence of Helicobacter infection by RUT and HPE (n=40)**

Test	<i>H. pylori</i> positive	<i>H. pylori</i> negative
Rapid urease test	27 (67.5%)	13 (32.5%)
Histopathological test	21 (52.5%)	19 (47.5%)

Histopathologically, 26 (65%) were intestinal type and 14 (35%) were diffuse type. Among 26 cases of intestinal type of ca stomach, 20 (76.92%) cases were positive for *H. pylori* infection, whereas among 14 diffuse type of ca stomach, only 7(50%) were positive for *pylori* infection.

**Table 2: *H. pylori* infection and histological types (according to Lauren's).**

Types	No (n=40)	<i>H. pylori</i> positive no. (n=27)	<i>H. pylori</i> negative no.(n=13)
Intestinal	26	20 (76.92%)	6 (23.07%)
Diffused /gastric	14	7 (50%)	7 (50%)

According to location, out of 29 cases of distal Ca stomach, H. pylori positivity was seen in 22 (75.86%) cases, whereas in rest of the 11 cases the lesion involved other part of the stomach, H. pylori positivity was seen in only 5 (45.45%) cases.

**Table 3: H. pylori infection and predominant site of lesion.**

Site	No (n=40)	H. pylori positive no.(n=27)	H. pylori negative no.(n=13)
Antrum/Pylorus	29	22(75.86%)	7 (24.1%)
Body	6	3 (50%)	3 (50%)
Cardia	2	1 (50%)	1 (50%)
Diffuse	3	1(33.33%)	2 (66.66%)

**Table 4: Age distribution in H. pylori infection.**

Ages	Total (n=40)	H. pylori positive (n=27)	H. pylori negative(n=13)
31-40	2	1 (50%)	1 (50%)
41-50	8	5 (62%)	3 (38%)
51-60	9	7(77.77%)	2 (22.33%)
61-70	16	12 (75%)	4 (25%)
71-80	4	2(50%)	2 (50%)
80 and above	1		1 (100%)

**Table 5: Sex distribution in H. pylori infection.**

Sex	Total( n =40)	H. pylori positive(n=27)	H. pylori negative (n=13)
Male	25	17 (68%)	8 (32%)
Female	15	10 (66.66%)	5 (33.33%)

## DISCUSSION

Large volume of literature on the association of H. pylori infection and gastric cancer has been published since Warren and Marshall first isolated Helicobacter pylori from human gastric mucosa in 1983<sup>5</sup>. The first compelling evidence linking H. pylori infection to gastric cancer was obtained from seroepidemiologic studies in the United States and Britain<sup>6</sup>.

Gastric carcinoma is a common malignant tumor worldwide in the 1980s and is now suppressed only by lung cancer in the incidence. The high incidence occurred in Japan, Chili, Costa Rica, China, Portugal, Iceland, Scotland and considerably lower incidence in the US, it is also common in Nepal<sup>7</sup>. Shrestha et al, found that gastric carcinoma is the most common

malignancy (16.5%). Pradhan et al, also found gastric cancer is the most common cancer in a study done at Kathmandu (15.24%)<sup>8</sup>.

Since the time of discovery of Helicobacter pylori in the year 1983, much has been studied about this gram-negative spiral bacterium and its associated disease status. In 1994, the National Institute of Health (NIH) consensus conference recognized H. pylori as a cause of gastric and duodenal ulcers. Later that year, the International Agency for Research on Cancer (IARC) declared H. pylori as group I human carcinogen for gastric adenocarcinoma. There is also evidence that H. pylori infection is a risk factor for gastric mucosa-associated lymphomas (MALT lymphomas). Infection with H. pylori leads to acute gastritis, which becomes

chronic and progresses to atrophic gastritis, intestinal metaplasia (IM), and dysplasia, which have been identified as important steps in the pathogenesis of gastric cancer<sup>9</sup>.

Biochemically the bacteria produce enzymes and toxins. The presence of the infection can be diagnosed by various invasive (like upper gastrointestinal endoscopy and biopsy, culture and rapid urease test) and non-invasive methods (like H. pylori antibody assay, polymerase chain reaction (PCR), H. pylori antigen test, ELISA and the urea breath test)<sup>10</sup>. Demonstration of bacilli on histopathology of gastric biopsy specimen after staining with appropriate stains is the most sensitive (98%) and specific (98%) test<sup>11</sup>.

Gastric cancer is the second and four most common cause of cancer-related death in the world. Male and female respectively<sup>12</sup>. Adenocarcinoma which accounts for more than 90 percent of tumors arising in the stomach and histologically classified into intestinal and diffuse types. A number of studies have demonstrated a clear association between H. pylori infection and gastric adenocarcinoma. Gastric ca is one of the common malignancies and its association of H. pylori infection is the commonest condition in Nepal and other underdeveloped country<sup>13</sup>.

In this study 40 histologically proved ca stomach cases were taken. Correlative studies of 40 cases were done including age, sex, site of lesion, and histology type with H pylori infection. Our study was centered on rapid urease test and histopathology diagnosis of H. pylori from gastric mucosa in cases of ca stomach. This study showed that 67.5% (table no.1) of 40 total patients with ca stomach were associated with H. pylori infection by RUT and HPE. The prevalence of H. pylori infection (67.5%) in our present study is similar with those of other studies (41% - 70%) done in and outside Nepal<sup>14</sup>.

Relation between H. pylori infection and gastric neoplasm has been carried out in University School of Medicine in Japan, a total 105 patients with gastric carcinoma. H. pylori seroprevalence was seen higher in patients with gastric carcinoma 98 of 105, 93%<sup>15</sup>. Khana AK et al, reported is 50 proved cases of gastric cancer, studied by rapid urease test, culture, histopathology and ELISA test for H. pylori, 68% of cases of gastric cancer were found to be positive for H. pylori infection<sup>16</sup>. A study done by Makaju et al, showed 33.9% prevalence of H. pylori infection in patient with upper GI symptoms.<sup>17</sup> A study done in Nepal by Shakya et al, in 1998 showed high prevalence

rate (80%) of H. pylori infection among symptomatic patients<sup>18</sup>.

In this study, male was predominantly affected than female in case of stomach cancer, which is 25 (62.5%) of 40 cases. The common age group in both sexes is between 61 to 70 years, which is 16 (40%) of 40 cases (Table no.4). Out of these 16 patients, 12 (75%) cases were positive for H. pylori infection. Wang RT et al, had done a study, the total subjects were 2719. There were 1399 (51.5 %) subjects male, and 1320 (48.5 %) female. 1671 (61.5 %) subjects were H. pylori positive; 1, 048 (38.5 %) were H. pylori negative<sup>19</sup>.

In present study, H. pylori infection was compared between intestinal and diffuse histological types. Out of 26 intestinal type of ca stomach, 20 (76.92%) cases were positive for H. pylori infection, whereas in case of 14 diffuse type of ca stomach, 7 (50%) cases were associated with H. pylori infection (Table no.2). A prospective controlled study enrolled 56 patients from Federal University of Rio Grande do Norte, Natal, RN, Brazil, thirty-four tumors (60.7%) were intestinal-type and 22 (39.3%) diffuse type carcinomas. When examined by Giemsa and urease test, H. pylori positive rate in tumor tissue of intestinal type carcinomas was higher than that in diffuse carcinomas<sup>20</sup>. A descriptive study was carried out at the Ziauddin Medical University, Karachi. Histological evaluations of 50 cases of carcinoma stomach, H. pylori were identified in 35 cases 70%. Out of 50 cases, intestinal types were found in 30 cases, whereas 15 cases were diffuse types<sup>21</sup>.

In this study, we compared H. pylori infection in different anatomical location. We found at distal gastric cancer is more associated with H. pylori infection, out of 29 cases of distal ca stomach, H. pylori positivity was seen in 22 (75.86%) cases, whereas in rest of the 11 cases the lesion involved other part of the stomach, H. pylori positivity was seen in only 5 (45.45%) cases (Table no.3). In a study of 39 patients with gastric carcinoma, H. pylori were 47.6% in cardia, 55.5% in the corpus and 66.6% in the antrum. The gastric ca is significantly related to H. pylori infection in the antral region<sup>22</sup>. Study done by Bhasin et al, is total 80 cases of ca stomach, 48 had intestinal type, 28 diffuse type and 4 had mixed type of gastric cancer were identified. Tumour was located in antrum were 37, in body 31, and at other sites 12 cases were recorded. Association of H. Pylori infection is more with intestinal type and distally located tumour<sup>23</sup>. Overall association of H. pylori infection in ca stomach detected by rapid urease test and positive histology in this study is high. We

speculate that in cases of ca stomach the association with H. pylori could be further high if more tests are used in large volume of patients.

## CONCLUSION

Carcinoma stomach is a common malignancy and one of the leading causes of death throughout the world. Its association with H. pylori infection is higher in developing country, early detection of infection and eradication therapy will probably reduce the incidence of ca stomach and its health related burden to the country. The present study confirmed the higher association of helicobacter pylori infection with stomach cancer. But to know the accurate incidence we need to find out and employ other possible investigation with higher sensitivity and specificity in a large volume of patients.

There is significant association of H. pylori infection with intestinal type of gastric carcinoma. Among the location, the incidence and relative risk of H. pylori infection was significantly higher in distal ca stomach patients. So in both the condition, H. pylori infection could be the etiological factor. In Public health education, the recognition of transmission routes and successful eradication therapy will be the cornerstones of future prevention of gastric carcinogenesis cause by H. Pylori.

## REFERENCES

1. Paul lochhead, El-Omar EM. Helicobacter pylori infection and gastric cancer, Best practice and Research clinical gastroenterology 2007; vol-21, no 2, pp 281-297.
2. Vandanaberry, vidyasagar, rapidurease test todiagnosehelicobacter pylori infection. JK science 2006;8(2): 86-88.
3. KniggeKL. The role of H. pylori in gastrointestinal disease: a guide to identification and eradication. Postgrad Med 2001; 110(31):71-82. PMID:11570207
4. AV Thillainayagam, AS Arvind, RS Cook, Diagnostic efficiency of an ultrarapid endoscopy room test for Helicobacter pylori, Gut (1991); 32: 467-469 <http://dx.doi.org/10.1136/gut.32.5.467> PMID:2040465
5. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; 1: 1273-1275
6. Forman D, Newell DG, Fullerton F, Yarnell JWG, Stacey AR, Wald N, Sitas F. Association between infection with Helicobacterpylori and risk of gastric cancer: evidence from a prospective investigation. British Med J 1991; 302: 1302-1305. <http://dx.doi.org/10.1136/bmj.302.6788.1302>
7. James MC, Ramzi SC, Vinay K et al. Robbins pathologic basis of disease 5th edtion 1994; 779-783.
8. Shrestha et al. Present cancer scenario and its changing pattern at TU teaching hospital, Nepal. JNMA, souvenir 1997; 35:45-48.
9. Changcheng Wang, Yuhong Yuan, Richard H; association of helicobacter pylori infection and early gastric cancer. AMJ-2007;102(8) 1789-1798.
10. Nakamura RM. Laboratory test for the evaluation of H. pylori infection. J. clin. Lab. Analysis 2001; 15: 301-305. <http://dx.doi.org/10.1002/jcla.1041> PMID:11793429
11. Basnet R.B, Shrestha B, Joshi RD, et al Prevalence of helicobacter pylori in gastric biopsy specimens, PMJN Vol 6 no. Jan-Jun 2006.
12. Parkin, DM, Bray, F, Ferlay, J, Pisani, P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55:74. <http://dx.doi.org/10.3322/canjclin.55.2.74> PMID:15761078
13. Smriti Agnihotri, Association of helicobacter pylori with gastric cancer IJMU, Jan-Jun 2008; vol 3, No 1.
14. Konturek SJ, bielanski W, Plonka M, et al. Helicobacter pylori, non-steroidal anti-inflammatory drugs and smoking in risk pattern of gastro duodenal ulcers.
15. Komoto K, Haruma k, Kamada T, et al. Helicobacter pylori infection and gastric neoplasia. Am J Gastroenterology Aug, 1998; 93(8):1271-6. <http://dx.doi.org/10.1111/j.1572-0241.1998.00408.x> PMID:9707050
16. Khanna AK, Seth P, Nath G, Correlation of Helicobacter pylori and gastric carcinoma. J Postgrad Med 2002 Mar;48(1):27-8. PMID:12082323
17. Makaju RK et al. Comparative study on a homemade rapid urease test with gastric biopsy for diagnosis of Helicobacter pylori infection. Nepal Med Coll 2006 Jun;8(2): 97-100.
18. Shakya et al. Association of Helicobacter pylori with duodenal ulcer, gastric ulcer and gastritis

in suburban patients attending NMCTH-a preliminary study of dyspeptic patients. Nepal Med College J 2001; 3:1-4.

19. Wang RT, Wang T, Chen K. Helicobacter pylori infection and gastric cancer: evidence from a retrospective cohort study and nested case-control study in China. World J Gastroenterology 2002; 8(6):1103-110. PMID:12439934
20. Araujo-Filho I, Brandao-Neto, Pinheiro LA, Arq Gastroenterol. 2006 Oct-Dec;43(4):288-92. PMID:17406757
21. Arif M, Sayed S. Association of Helicobacter pylori with carcinoma of stomach. J Pak Med association July, 2007; 57(7):337-41. PMID:17867254
22. Turkdogan M. Kursad, AliciSuleyman, Ilhan Mahmut et al. The Turkish Journal of Gastroenterology 1999; vol 10, N.
23. Bhasin DK, Kakkar N, Sharma BC, H. pylori in gastric cancer in India. Trop Gastroenterol. 1999 Apr-Jun;20(2):70-2. PMID:10484891