Original Article

Association of helicobacter pylori with carcinoma of stomach

Muhammad Arif, Serajuddaula Syed
Department of Pathology, Sindh Medical College, Karachi

Abstract

Objective: To note the association of Helicobacter pylori in patients having carcinoma of stomach.

Methods: A descriptive study was carried out at the Department of Histopathology, Ziauddin Medical University, Karachi from April 1992 to May 1998. Histological evaluation of 50 cases of carcinoma of stomach was compared with 50 cases each of chronic gastritis and histological normal gastric mucosa. Only those cases of carcinoma of stomach were included that contained sufficient non-neoplastic mucosa in addition to tumour tissue. Three glass slides with serial sections of each case of carcinoma of stomach, chronic gastritis and normal gastric mucosa were freshly cut and stained with H&E, PAS and Giemsa stains. All slides were examined by light microscopy.

Results: Helicobacter pylori were identified in 35 cases (70%) of carcinoma of stomach, in 42 cases (84%) of chronic gastritis, and in 12 cases (24%) of normal gastric mucosa. The presence of H. pylori in cases of carcinoma of stomach and chronic gastritis was highly significant ($P<0.001$) as compared to normal gastric mucosa. Chronic gastritis was observed in the non-neoplastic mucosa in 48 cases (96%) with carcinoma of stomach. Of 50 cases with carcinoma of the stomach, intestinal type of carcinoma was found in 30 cases (70%), and diffuse type in 15 cases (30%). No significant difference was noted in the prevalence of H. pylori between intestinal type (69%) and diffuse type (71%) gastric carcinoma. Significant Helicobacter pylori associated chronic gastritis was observed in intestinal type (94%) and diffuse type (100%) of gastric carcinoma. The prevalence of H. pylori was insignificant in the presence or absence of mucosal atrophy and intestinal metaplasia in both types of gastric carcinoma.

Conclusion: A significant number of H. pylori were found in patients of carcinoma of stomach. Both intestinal and diffuse types of gastric carcinoma showed strong association with H. pylori. Chronic gastritis appears to be the background lesion while atrophy and intestinal metaplasia indicate long term infection (JPMA 57:337:2007).

Introduction

Helicobacter pylori is a gastric pathogen.[1] This bacterium is the commonest causative agent of chronic gastritis and peptic ulcer. Long term infection with this organism is considered a risk factor in the development of carcinoma of stomach.[2,3]

Carcinoma of stomach is the second commonest cancer in the world and carries a bad prognosis.[4] Various environmental and dietary factors have been investigated as agents in the pathogenesis of carcinoma of stomach. But with the discovery of H. pylori in human stomach, an inflammation-related carcinogenesis has emerged in which this bacterium is implicated in the causation of gastric carcinoma.[5] The epidemiological features of H. pylori and carcinoma of stomach are parallel in different populations of the world. Several studies have suggested that H. pylori is a risk factor in the development of carcinoma stomach.[2-5] This study was conducted to investigate the association of H. pylori with carcinoma of stomach.

Patients and Methods

The study included 50 consecutive cases of carcinoma of stomach diagnosed at the department of histopathology, Ziauddin Medical University Karachi from April 1992 to May 1998. For comparison 50 cases of chronic gastritis and 50 cases of histological normal gastric mucosa were taken as controls. The controls were selected from the same period as the cases.

The inclusion criterion of carcinoma of stomach cases was the biopsies which in addition to tumour contained area of non-neoplastic tissue, while biopsies or tissues of gastric cardiac region were excluded. Similarly those biopsies of control cases of chronic gastritis and histologically gastric mucosa were included that contained well oriented sufficient amount of lamina propria. Fresh sections were cut from each paraffin tissue block of the cases included in the study. For each case of carcinoma of stomach, chronic gastritis and histologically normal gastric mucosa, three glass slides with serial tissue sections were prepared for 03 different stains. The stains used were Haematoxylin & Eosin, Periodic Acid-Schiff and Giemsa.

All the stained tissue slides of carcinoma of stomach, chronic gastritis and histological normal gastric mucosa were examined by light microscopy. Carcinoma of stomach was typed into intestinal and diffuse varieties according to Lauren's classification[6]; in intestinal carcinoma cohesive malignant cell clusters form glands with distinct lumina, whereas diffuse type carcinoma shows dissociate neoplastic cells which lack glandular lumina. In chronic gastritis, grading of density of chronic inflammation, active inflammation (neutrophils), atrophy, intestinal metaplasia and H. pylori was done into normal, mild, moderate, and marked in the guidelines of updated Sydney System aided by the provision of visual analogue scale.[7] The histological parameters were assessed at a time in a particular or combination of stains. The histological finding of cases of carcinoma of stomach, chronic gastritis and histological normal gastric mucosa were entered in performa I, II and III respectively.

Frequency and percentage were computed for qualitative and categorical variables (gender, densities of H. pylori, morphological types of carcinoma of stomach), and mean and standard deviation for quantitative variable (age). Test of proportion was used for comparison of qualitative variable in three groups (Carcinoma of stomach, chronic gastritis and normal gastric mucosa). Analysis of variance (ANOVA) was applied for comparison of age (Mean ± S.D.) in three groups (Carcinoma of stomach, chronic gastritis and normal gastric mucosa). In all statistical analysis only P value <0.05 was considered significant.

Results

The age of patients ranged from 20 to 75 years with a mean age of 49 ± 15.5 years which was similar to both types of controls (chronic gastritis and normal gastric mucosa). However, the patients of gastric carcinoma were older than chronic gastritis and normal gastric mucosa. The age and gender distribution of all 3 groups is shown in Table 1. In all three groups gastric antrum was the predominant anatomical site.

H. pylori was identified in 35 cases (70%) of carcinoma of stomach, in 42 cases (84%) of chronic gastritis, and in 12 cases (24%) of normal gastric mucosa. The prevalence of H. pylori in carcinoma of stomach and chronic gastritis was highly significant ($p<0.001$) as compared to normal gastric mucosa. Chronic gastritis was seen in the non-neoplastic mucosa in 48 cases (96%) of carcinoma; and thirty (69%) of these had moderate chronic inflammation. Mucosal atrophic changes were seen in 41 cases (82%) of carcinoma with predominance of mild grade. Of 19 cases (38%) with intestinal metaplasia in carcinoma

Table 1. Age and sex distribution in Carcinoma of stomach, Chronic Gastritis and Normal Gastric Mucosa cases.

	Carcinoma of stomach (n=50)	Chronic Gastritis (n=50)	Normal Gastric Mucosa (n=50)
AGE (Years): Range	20 75	10 70 **	19 75
Mean ± S.D.	49 ± 15.5	39 ± 15.3	44 ± 15.0
SEX : Males	30 (60%)	27 (54%)	25 (50%)
Females	20 (40%)	23 (46%)	25 (50%)

**Significant (P<0.01) as compared to Chronic Gastritis.

Table 2. Densities of H. pylori in Carcinoma of stomach, Chronic Gastritis and Normal Gastric Mucosa cases.

Densities of H. pylori	Carcinoma of stomach (n=50)	Chronic Gastritis (n=50)	Normal Gastric Mucosa (n=50)
Normal	15 (30%)	8 (16%)	38 (76%)
Mild	20 (40%)	14 (28%)	12 (24%)
Moderat	12 (24%)	13 (26%)	000 (%)
Marked	3 (6%)	15 (30%)	000 (%)

Difference between Carcinoma of stomach, chronic Gastritis and Normal Gastric Mucosa were significant (P<0.01).

Table 3. Prevalence of H. pylori according to morphological variables of Gastritis in Carcinoma of stomach cases according to type of cancer.

Morphological varibales	**** TYPE OF CANCER ****	
	Intestinal (n=35) H.pylori	Diffuse (n=15) H.pylori
	Number +ve (%)	Number +ve (%)
Chronic gastritis	33 22 (67)	15 11 (73)
Active inflammation	12 9 (75)	4 4 (100)
Atrophy	26 17 (65)	15 11 (73)
Intestinal Metaplasia	13 8 (62)	6 5 (83)

Table 4. Topographical distribution of atrophy and intestinal metaplasia with prevalence of H. pylori in Carcinoma of stomach cases according to type of Cancer.

Morphological variables site of biopsies	**** TYPE OF CANCER ****					
	Intestinal (n=35) H.pylori			Diffuse (n=15) H.pylori		
	Number	+ve	(%)	Number	+ve	(%)
Atrophy:						
Body / Fundus	4	3	(75)	7	5	(71)
Antrum	22	14	(64)	8	6	(75)
Intestinal Metaplasia						
Body / Fundus	1	1	(100)	2	2	(100)
Antrum	12	7	(58)	4	3	(75)

No significant difference.

group, 16 cases (84%) had mild to moderate metaplasia. The density of H. pylori showed mild grade in most cases (57%) of gastric carcinoma which was significantly different (P <0.01) from grades of densities in control subjects (Table 2). The biopsy site (antrum, body,fundus) had no influence in the occurrence of bacteria in all the three groups.

In fifty cases of carcinoma of stomach, intestinal type was found in 35 cases (70%), and diffuse type in 15 cases (30%). Helicobacter pylori were seen in 24 of 34 cases (69%) of intestinal type, and 11 of 15 cases (71%) of diffuse type carcinoma. No significant difference was noted in the prevalence of H. pylori between intestinal and diffuse type of carcinoma. The mean age in intestinal type was 50 years, and 44 years in diffuse type. Young age of diffuse type carcinoma was significant (P<0.01). Males were predominant in both types of gastric carcinoma. Chronic inflammation was noted in the non-neoplastic mucosa in 33 of 35 cases (94%) of intestinal type and 100% of diffuse type carcinoma. Atrophic mucosal changes were present in 26 of 35 cases (74%) of intestinal and all 15 cases of diffuse type. Mild to moderate grade of intestinal metaplasia was seen in 11 of 13 cases (85%) with intestinal type and 05 of 06 cases (83%) with diffuse type carcinoma. There was no significant difference in the prevalence of H. pylori in the morphologic variables of gastritis (Table 3). Mucosal atrophy and intestinal metaplasia in intestinal type was mostly observed in the antrum. Same morphology showed almost equal distribution in diffuse type carcinoma. Anatomical site had no influence of H. pylori in atrophy and metaplasia in both type of gastric carcinoma (Table 4).

Discussion

In this study the association of H. pylori with carcinoma of stomach was investigated. Gastric carcinogenesis is a multi step and multi factorial process.[8] Helicobacter pylori is classified as a group I carcinogen and is linked to gastric carcinoma in humans.[9] In the present study, the prevalence of H. pylori in carcinoma of stomach was 70% with no significance difference in the prevalence of organisms between intestinal (69%) and diffuse (71%) type carcinoma. Our results concur with the histological studies from Europe[10] and Saudi Arabia[11] which reported the bacterial prevalence of 59% and 79.8% respectively; and in both studies no significant difference in the occurrence of H. pylori was found in both types of gastric carcinoma. Whereas in a similar retrospective study from United States, the frequency of H. pylori in intestinal type gastric carcinoma was 89.2% compared with 31.8% in diffuse type gastric carcinoma.[12] In the present study, the H. pylori prevalence in intestinal and diffuse type carcinoma was significantly higher as compared to 24% in controls with normal gastric mucosa (P<0.001). This high yield was due to high prevalence of bacteria in patients with chronic gastritis and peptic ulcer disease in our population.[13,14] Various other important studies have used specific serum antibodies against H. pylori, and two prospective case-control serological studies from Britain[15] and Hawaii[4] showed significant bacterial prevalence of 69% and 94% respectively in patients of gastric carcinoma. The overall prevalence of 70% in patients with gastric carcinoma in this

study is comparable with the serological-based studies of H. pylori in patients with gastric carcinoma.[4,15] Intestinal type of gastric carcinoma is the predominant type in different studies[10]; in our study the intestinal type was 2.3 times more common than diffuse type (35:15). The advantage of selecting histological examination of gastric mucosa for the detection of H. pylori was to correlate its presence or absence with the morphological changes of gastritis. The "gold standard" for H. pylori status is examination of at least two biopsies.[16] In the present study 5.6 and 1.4 good-sized tissue blocks were taken from gastrectomy specimens of carcinoma cases. The number of biopsies was 2.4 and 2.2 in control cases of chronic gastritis and normal gastric mucosa respectively.

The mean age in this study was 49 years for patients with gastric carcinoma, which was a decade older than patients of chronic gastritis. In a study from Britain, the mean age at the time of diagnosis was 60 years (range 47-76 years).[15] The prevalence of H. pylori in the present study was 83 % (05 of 06 cases) in gastric patients under 30 years of age, compared to 73% (11 of 15 cases) in controls with chronic gastritis. This suggests that the acquisition of H. pylori infection occurred earlier in life in gastric carcinoma patients. High rates of gastric cancer have been reported in areas in which H. pylori infection is common in early childhood.[17]

Gastric cancer occurs more frequently in males[18]. Male predominance was found in this study with a frequency of 60% in both types of carcinoma of stomach and there was no significant difference in the prevalence of H. pylori in either sex. Majority of intestinal type gastric carcinoma were located in the gastric antrum, which is in general agreement with western reports.[19] In diffuse type carcinoma the anatomical site showed no influence in the occurrence of neoplasia. Similar result was observed by others.[20] The location of tumors does not affect the frequency of H. pylori infection.[12,13]

The association between H. pylori and chronic gastritis is well known.[14-21] Helicobacter pylori associated chronic gastritis was the background lesion in majority of the intestinal and diffuse type of gastric carcinoma in this study. The bacteria act directly via the release of enzymes or toxins, or by the inflammatory response, which it provokes, and is thought to cause epithelial damage. Several studies have suggested the role of H. pylori in gastric carcinogenesis through stages of chronic gastritis to mucosal atrophy to intestinal metaplasia more so with intestinal type carcinoma.[5,20] However, a recent research indicates that H. pylori infection is associated with DNA damage in gastric epithelial cells, which could be a risk for gastric cancer in humans.[22] Although mucosal atrophic changes were present in significant number of intestinal and diffuse type of gastric cancer but there was no obvious correlation of mucosal atrophy and intestinal metaplasia with H. pylori in gastric carcinoma cases in the present study. In a report from Japan, no precursor lesions were found in the margins of microcarcinoma.[23] Hence, it was postulated that gastric atrophy and intestinal metaplasia appear to be an indicator of long-standing proliferation, but are not necessarily specific precancerous lesions.[24] Strain variation has impact on gastroduodenal diseases and it is shown that patients infected with H. pylori CagA strains had increased rate of gastric epithelial cell proliferation.[25]

References

1. Dixon MF, Sobala GM. Gastritis and duodenitis. The histopathological spectrum. Eur J Gastroentrol Hepatol 1992; 4 (suppl): 517-23.
2. Enroth H, Kraaz W, Engstrand L, Nyren O, Rohan T. Helicobacter pylori strain types and risk of gastric cancer: a case- control study. Cancer Epidemiol Biomarkers Prev 2000;9:981-5.
3. McColl KE. Helicobacter pylori: Clinical Aspect. J Infect 1997; 34:7-13.
4. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI,Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991; 325: 1132-6.
5. Welin M, Holmgren NMA, Nillson P, Enroth H. Statistical model of the interactions between Helicobacter pylori infection and gastric cancer development. Helicobacter 2003; 8: 72-8.
6. Lauren P. The two histological main types of gastric carcinoma: diffuse and so called intestinal type carcinoma. Attempt at a Histo-Clinical Classification. Acta Pathol Microbiol Scand 1965; 64: 31-49.
7. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and Grading of Gastritis: The Updated Sydney System. Am J Surg Path 1996; 20:1161-81.
8. Stemmermann GN. Intestinal metaplasia of the stomach: A status report. Cancer 1994; 74: 556-64.
9. Kuipers EJ, Meuwissen SG. Helicobacter pylori and gastric carcinogenesis. Scand J Gastroenterol (suppl)1996; 218: 103-5.
10. Loffeld RJ, Willems I, Flendrig JA, Arends JW. Helicobacter pylori and gastric carcinoma. Histopathology 1990; 17: 537-41.
11. Jamal H, Nader M. Helicobacter pylori infection in gastric carcinoma: A study of 84 cases from Asir region. Annals of Saudi Medicine 1994; 14: 286-9.
12. Parsonnet J, Vandersteen D, Goates J, Sibley RK, Pritikin J, Chang Y. Helicobacter pylori infection in intestinal and diffuse-type gastric carcinoma. J Natl Cancer Inst 1991; 83: 640- 43.
13. Kazi JI, Jafarey NA, Alam SM, Zuberi SJ, Kazi AM, Qureshi H, et al. Association of Helicobacter pylori with Acid-peptic disease in Karachi. JPMA 1990; 10: 240-1.
14. Shaikh AH, Akhund AA, Jafrey HA. Prevalence of Helicobacter pylori in 156 consecutive patients with dyspepsia in the interior Sind, Pakistan. Specialist 1997; 13: 363-6.
15. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, et al. Association b/w infection. Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. BMJ 1991; 302: 1302-5.
16. Williams CL. Helicobacter pylori: Bacteriology and Laboratory Diagnosis. J Infect 1997; 34: 1-5.
17. Brown LM. Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev 2000; 22: 283-97.
18. Branum GD, Fink AS. Adenocarcinoma of Stomach. In: DC Sabiston and Lyerly HK eds. Textbook of Surgery. WB Saunders Philadelphia, 1996: pp 893-907.
19. Parsonnet J. Helicobacter pylori and gastric carcinoma. Gastroenterol Clin N Am. 1993; 22:89-104.
20. Wee A, Kang JY, Teh M. Helicobacter pylori and gastric cancer: Correlation with gastritis, intestinal metaplasia, and tumour histology. Gut 1992; 33: 1029-32.
21. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in

active chronic gastritis. Lancet 1983; 1: 1273-5.

22. Ladeira MS, Rodrigves MA, Salvadori DM, Queiroz Dm, Freire-Maia DV. DNA Damage in Patients Infected by Helicobacter pylori. Cancer Epidemiology Biomarkers & Prevention 2004; 13: 631-7.

23. Hattori T. Development of adenocarcinoma in the stomach. Cancer 1986; 57: 1528-34.

24. Ekstrom AM, Held M, Hansson LE, Engstrand L, Nyren O. Helicobacter pylori in gastric cancer established by CagA immunoblot as a marker of past infection. Gastroenterology 2001; 121: 784-91.

25. Rokkas T, Ladas S, Liatsos C, Petridou E, PapatheodorouG, Theocharis S, et al. Relationship of Helicobacter pylori CagA status to gastric cell proliferation and apoptosis. Dig Dis Sci 1999; 44: 487-93.