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Polymorphism of -765G > C *COX-2* is a risk factor for gastric adenocarcinoma and peptic ulcer disease in addition to *H pylori* infection: A study from northern India

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Abstract

AIM: To investigate -765G > C *COX-2* polymorphism and *H pylori* infection in patients with gastric adenocarcinoma, peptic ulcer disease (PUD) and non-ulcer dyspepsia (NUD).

METHODS: We enrolled 348 adult patients (62 gastric adenocarcinoma, 45 PUD and 241 NUD) undergoing upper gastrointestinal endoscopy at two referral centers between September, 2002 and May, 2007. *H pylori* infection was diagnosed when any of the four tests (RUT, culture, histopathology and PCR) were positive. Genotyping for -765G > C polymorphism of *COX-2* was performed by PCR-RFLP analysis.

RESULTS: Frequency of C carrier had significant association with gastric adenocarcinoma as compared to NUD [77.4% *vs* 29%, $P < 0.001$, odds ratio (OR) 8.20; 95% confidence interval (95% CI), 4.08-16.47] and PUD (77.4% *vs* 31.1%, $P < 0.001$; OR 8.04; 95% CI, 3.25-19.90). Risk of gastric adenocarcinoma was significantly higher in patients having C carrier with (OR 7.83; 95% CI 3.09-19.85) and without *H pylori* infection (OR 7.06; 95% CI, 2.61-19.09). Patients with C carrier and *H pylori* infection had significant risk for the development of PUD ($P < 0.001$; OR 5.65; 95% CI, 2.07-15.34).

CONCLUSION: -765G > C *COX-2* polymorphism with or without *H pylori* could be a marker for genetic susceptibility to gastric adenocarcinoma. *COX-2* polymorphism in presence of *H pylori* infection might be useful in predicting the risk of PUD.

Key words: *COX-2* polymorphism; Gastric adenocarcinoma; Peptic ulcer disease; *Helicobacter pylori* infection

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INTRODUCTION

H pylori has been classified as a major cause of chronic gastritis and peptic ulcer disease (PUD), as well as a risk factor for gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma[1-3]. Although there is a considerable high rate of *H pylori* infection in Asian countries such as Japan, China, Thailand, Indonesia and India, there is a remarkable difference in incidence of gastric cancer within these countries (Asian enigma)[4,5]. The annual incidence of gastric cancer is disproportionately high in Japan and China in spite of a lower *H pylori* seropositivity[5]. In contrast, in India

seropositivity of *H pylori* is very high, but the annual incidence of gastric cancer is low (Indian enigma)[5]. Even studies based on differences in *H pylori* virulence factors couldn't explain such contradictory findings[6]. This shows that *H pylori* may not be the sole factor, but may play a synergistic role with factors like diet and host genetic make up in gastric carcinogenesis[5].

The role of host-related factors in the pathogenesis of diseases caused by *H pylori* infection has largely been ignored[7,8]. Overexpression of cyclooxygenase-2 (*COX-2*) has been observed in several forms of cancer[9-16], including gastric cancer and precancerous lesions[17-19]. COX, (also known as prostaglandin endoperoxide synthase) is a rate-limiting enzyme for the synthesis of prostaglandins (PGs) from free arachidonic acid. Two isoforms of COX have been identified; *COX-1* is constitutively expressed in most normal tissues and considered to be a housekeeping enzyme responsible for the maintenance of vascular homeostasis and gastroprotection. In contrast, *COX-2* is the inducible isoform of the enzyme and is rarely expressed in normal tissues, but it is rapidly induced by bacterial lipopolysaccharide (LPS), cytokines, growth factors, mitogens and tumor promoters[9,10]. Up-regulation of *COX-2* plays an important role in the inhibition of apoptosis, tumor growth, angiogenesis, invasion and metastasis, which are considered to be important steps in cancer development[9,17,20,21]. Several polymorphisms in *COX-2* have been identified so far. However, only a few seemed to have a functional effect on the transcription. Recently, Papafili *et al*[22] described a new polymorphism in the promoter region of *COX-2*, characterized by a guanine (G) to cytosine (C) transition at position -765 (-765G > C). This polymorphism appears to disrupt a stimulatory protein 1 (Sp1) binding site, which is considered to be a positive activator of transcription and leads to a 30% reduction of the *COX-2* promoter activity *in vitro*[22]. Only a few studies have been published on *COX-2* polymorphisms either in cancer or related diseases[23-29] or non-malignant diseases[30-32]. The aim of the present study was to evaluate the role of -765G > C *COX-2* polymorphism and *H pylori* infection in patients with gastric adenocarcinoma and PUD.

MATERIALS AND METHODS

Patients

We studied 348 adult patients, including 62 with gastric adenocarcinoma, 45 PUD and 241 non ulcer dyspepsia (NUD), undergoing upper gastrointestinal endoscopy at two referral centers in northern India between September, 2002 and May, 2007. The diagnosis of gastroduodenal diseases was based on clinical, endoscopic and histopathological parameters. Patients with NUD were considered as disease control in our study. The Ethics Committee of the institute granted approval for the study and consents were obtained from all the patients. Subjects who had received anti-microbial therapy, H_2 receptor blockers, proton pump inhibitors and non-steroidal anti-inflammatory drugs in the last 4 weeks before endoscopy or anti-*H pylori* treatment in the past were excluded from the study.

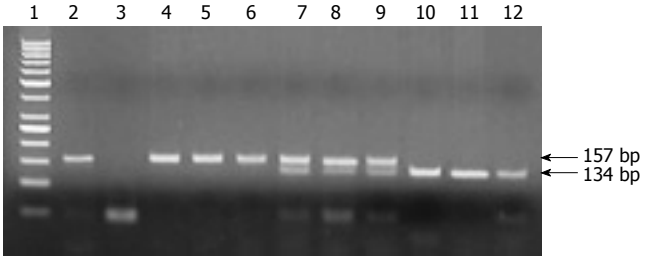

Figure 1 PCR-RFLP analysis of -765 G > C *COX-2* polymorphism. Lane 1: 50 bp DNA ladder; lane 2: PCR product (undigested); lane 3: Negative control; lanes 4 to 6: Homozygous -765 CC genotype; lanes 7 to 9: Heterozygous-765 GC genotype; lanes 10 to 12: Homozygous -765 GG genotype.

DNA extraction

Genomic DNA was isolated from gastric tissues using the QIAamp DNA mini kit (QIAGEN, Hilden, Germany) as per the manufacturer's instruction.

Diagnosis of H pylori infection

During each endoscopy, antral biopsies were obtained and subjected to the following tests: rapid urease test (RUT), culture, histopathology and *H pylori* specific *ureA* PCR following the standard protocol as described earlier[33]. *H pylori* infection was diagnosed if any of the above tests was positive.

-765G > C COX-2 polymorphism

Analysis of -765G > C *COX-2* polymorphism was performed by PCR-based restriction fragment length polymorphism (PCR-RFLP) as previously described[30]. The sequences of PCR primers were: forward 5'-ATTCTGGCCATCGCCGCTTC-3' and reverse 5'-CTCCT TGTTTCTTGGAAAGAGACG-3' (Metabion, Martinsried, Deutschland). PCR conditions were as follows: an initial denaturation at 94℃ for 10 min, followed by 35 cycles of denaturing at 94℃ for 1 min, annealing at 59℃ for 1 min, and extension at 72℃ for 1 min. The final extension was continued at 72℃ for 10 min and cooling to 4℃. Template free water was used as negative control. After PCR amplification, PCR products were subjected to restriction digestion by *Bsh* 1236I restriction endonuclease (Fermentas, Vilnius, Lithuania) for 8 h at 37℃. The DNA fragments were then separated on 3% agarose gel electrophoresis. Fragments size of 134 and 23 bp indicated the presence of a wild type homozygous -765GG genotype, a single 157 bp fragment indicated the presence of homozygous -765CC genotype and three fragments of 157, 134 and 23 bp indicated the presence of heterozygous -765GC genotype (Figure 1). The 23 bp fragment cannot be distinguished from the primer-dimer band in the agarose gel. All the experiments were repeated twice for the confirmation of RFLP results.

Statistical analysis

The data analysis was performed by SPSS software (Version 12.0, SPSS, Chicago, IL, USA). *H pylori* status in relation to gastroduodenal diseases was analyzed using Chi-square test. Multivariate logistic regression analyses were

Table 1 Demography of the study populations and *H pylori* infection

Parameter	Gastric adenocarcinoma (*n* = 62)	Peptic ulcer disease (*n* = 45)	Non-ulcer dyspepsia (*n* = 241)	Overall (348)
Age (yr)	56.60 ± 15.42	49.47 ± 17.22	43.75 ± 14.76	46.78 ± 15.96
Male:Female	47:15	31:14	138:103	216:132
H pylori infection[1] (%)	35 (56.5)	36 (80)	133 (55.2)	204 (58.6)

[1]Peptic ulcer disease vs non-ulcer dyspepsia: 80% *vs* 55.2%, $P = 0.002$; Gastric adenocarcinoma *vs* non-ulcer dyspepsia: 56.5% *vs* 55.2%, $P = 0.858$; Peptic ulcer disease *vs* gastric adenocarcinoma: 80% *vs* 56.5%, $P = 0.01$.

Table 2 Allelic distribution of *COX-2* polymorphism in gastric adenocarcinoma, peptic ulcer disease and non-ulcer dyspepsia

COX-2 genotype	Gastric adenocarcinoma (*n* = 62)	Peptic ulcer disease (*n* = 45)	Non-ulcer dyspepsia (*n* = 241)
GG (%)	14 (22.6)	31 (68.9)	171 (71)
GC (%)	29 (46.8)	12 (26.7)	62 (25.7)
CC (%)	19 (30.6)	2 (4.4)	8 (3.3)
C carrier (%)[1]	48 (77.4)	14 (31.1)	70 (29)

[1]Gastric adenocarcinoma *vs* non-ulcer dyspepsia: $P < 0.001$; Gastric adenocarcinoma *vs* peptic ulcer disease: $P < 0.001$; Peptic ulcer disease *vs* non-ulcer dyspepsia: $P = 0.74$.

used to identify the independent risk factors for gastric adenocarcinoma and PUD. Gender and age were included in regression analysis, and assessment for interaction was considered in the model. A two-sided P value of less than 0.05 was considered significant.

RESULTS

Patient characteristics

A total of 348 patients (mean age: 46.78 ± 15.96; 216 male) were enrolled in the study and their distributions were gastric adenocarcinoma 62 (mean age: 56.60 ± 15.42; 47 male), PUD 45 (mean age: 49.47 ± 17.22; 31 male) and NUD 241 (mean age: 43.75 ± 14.76; 138 male, Table 1).

Diagnosis of H pylori infection

Prevalence of *H pylori* infection in our study population was 58.6%. *H pylori* infection was significantly higher in patients with PUD than with gastric adenocarcinoma (80% *vs* 56.5%, $P = 0.01$) and NUD (80% *vs* 55.2%, $P = 0.002$, Table 1).

-765G > C COX-2 polymorphism

All genotypic distributions were in Hardy-Weinberg equilibrium. The potential association of -765G > C *COX-2* polymorphism in patients with gastroduodenal diseases is shown in Table 2. The frequency of the -765 GG, GC and CC genotypes were 71%, 25.7% and 3.3%, in patients with NUD, 68.9%, 26.7% and 4.4% in patients with PUD and 22.6%, 46.8% and 30.6% in patients with gastric adenocarcinoma, respectively. The frequency of C carrier was more common in patients with gastric adenocarcinoma as compared with NUD [77.4% *vs* 29%, $P < 0.001$, odds ratio (OR) 8.20; 95% confidence interval (95% CI), 4.08-16.47] and PUD (77.4% *vs* 31.1%, $P < 0.001$, OR 8.04; 95% CI, 3.25-19.90). However, the frequency of C carrier in patients with PUD and NUD was similar (PUD *vs* NUD 31.1% *vs* 29%, $P = 0.74$; OR 1.13; 95% CI, 0.56-2.27).

Table 3 *H pylori* infection and *COX-2* polymorphism as risk for gastric adenocarcinoma

H pylori status	*COX-2* genotype	Gastric adenocarcinoma (*n* = 62)	Non-ulcer dyspepsia (Controls, *n* = 241)	OR (95% CI)	*P*-value
HP-	GG	8	79	Referent	
HP-	C carriers	19	29	7.06 (2.61-19.09)	< 0.001
HP+	GG	6	92	0.83 (0.26-2.59)	0.747
HP+	C carriers	29	41	7.83 (3.09-19.85)	< 0.001

HP-: *H pylori* negative; HP+: *H pylori* positive.

Table 4 *H pylori* infection and *COX-2* polymorphism as risk for peptic ulcer disease

H pylori status	*COX-2* genotype	Peptic ulcer disease (*n* = 45)	Non-ulcer dyspepsia (Controls, *n* = 241)	OR (95% CI)	*P*-value
HP-	GG	8	79	Referent	
HP-	C carriers	1	29	0.86 (0.09-7.48)	0.89
HP+	GG	23	92	2.83 (1.18-6.73)	0.019
HP+	C carriers	13	41	5.65 (2.07-15.34)	< 0.001

HP-: *H pylori* negative; HP+: *H pylori* positive.

Interaction between H pylori infection and -765G > C COX-2 polymorphism

We also examined the potential interaction between *H pylori* infection and -765G > C *COX-2* polymorphism in the development of gastric adenocarcinoma and PUD in our population. Presence of C carrier with ($P < 0.001$, OR 7.83, 95% CI, 3.09-19.85) and without *H pylori* infection ($P < 0.001$, OR 7.06, 95% CI, 2.61-19.09) was significantly associated with gastric adenocarcinoma (Table 3). We also found that patients with C carrier and *H pylori* infection had significant risk for the development of PUD ($P < 0.001$, OR 5.65, 95% CI, 2.07-15.34; Table 4).

DISCUSSION

We investigated the potential association of -765G > C *COX-2* polymorphism and *H pylori* infection with gastric adenocarcinoma and PUD. We report for the first time that -765G > C *COX-2* polymorphism with or without *H pylori* infection could be a marker for genetic susceptibility to gastric adenocarcinoma. To the best of our knowledge, this is the first study to show that patients with C carriers of *COX-2* gene are susceptible to develop PUD in the presence of *H pylori* infection.

Table 5 C allele frequency and C carrier distribution in control populations from various countries

Country	Control population (*n*)	C allele frequency (%)	C carrier distribution (%)	Reference
America				
USA	228	21	37	27
USA (African American)	100	32	52	27
Europe				
Portugal	210	22	38	34
Italy	864	28	50	31
UK	454	14	25	22
Poland	547	17	31	30
Australia				
Australia	168	17	31	32
Asia				
India	241	16	29	Present study
Singapore	1177	5	9	23
Japan	241	2	5	24
China	1270	2	4	19

Genetic polymorphism is considered an important determinant for the development of cancer. An association between increased C*OX-2* gene expression and cancer including gastric adenocarcinoma has been reported[17-19]. A new polymorphism in the promoter region of *COX-2*, characterized by a guanine (G) to cytosine (C) transition at position -765 (-765G > C) appears to disrupt Sp1 binding site, which reduces the *COX-2* promoter activity to the extent of 30% *in vitro*[22]. An altered susceptibility to develop cancer due to the disruption in Sp1 binding site is attributed to this polymorphism (-765G > C) of *COX-2* gene[22,23]. The frequency of this polymorphism seems to vary, especially among different ethnic populations (Table 5). C allele and C carriers have been frequently reported in the western populations than the Asians. Both C allele (16.2%) and C carriers (29%) in our control populations are more close to the West, but much higher than the other Asian countries[19,22- 24,27,30-32].

In our study, -765 C carriers were frequently present in gastric adenocarcinoma as compared to disease controls (Table 3). Patients with C carriers had 8.2-fold increased risk of progression to gastric adenocarcinoma. Pereira *et al* reported nearly 3-fold increased risk of progression to gastric adenocarcinoma in patients with atrophy or intestinal metaplasia carrying C allele[34]. Zhang *et al* also reported that patients with C carriers had 2.66-fold increased risk of gastric adenocarcinoma[35]. Recently, Guo *et al* described a 2-fold increased risk of esophageal squamous cell carcinoma due to the polymorphism in *COX-2* gene[36]. It appears that the risk for the development of gastric adenocarcinoma related to *COX-2* polymorphism in our population is much higher than other published studies. Although the exact molecular mechanism by which *COX-2* polymorphism may increase the risk of gastric adenocarcinoma development is still unclear, studies in the *COX-2* promoter revealed that *COX-2* transcription is activated by E2 promoter binding factor 1 (E2F1)[37,38]. Hence, the ability of this polymorphism to create an E2F binding site, essential for the expression of several genes may be the reason for the increased risk[30]. The contribution of genetic polymorphism to the risk of gastric adenocarcinoma may depend on the study population as well as on several environmental and dietary factors. Therefore, each population has to be evaluated for its own genetic profile for cancer risk that may help to understand the geographic and racial differences for development of gastric adenocarcinoma[39]. When we analyzed the combination of C carrier and *H pylori* infection, the risk was nearly 8-fold (OR 7.83; 95% CI, 3.09-19.85) in *H pylori* positive individuals and 7-fold (OR 7.06, 95% CI, 2.61-19.09) in *H pylori* negative individuals. So far, association between *H pylori* infection and *COX-2* gene polymorphism in the development of gastric adenocarcinoma has not been studied. The present study clearly shows that C carriers either in presence or absence of *H pylori* infection are susceptible to develop gastric adenocarcinoma. The frequency of C carriers was almost equal in our patients with PUD (31.1%) and disease controls (29%). But the combination of C carrier and *H pylori* infection had nearly 6-fold increased risk to develop PUD (OR 5.65, 95% CI 2.07-15.34). Interestingly, the risk was not increased in *H pylori* negative individuals, implicating a potential interplay between *H pylori* infection and *COX-2* polymorphism in the development of PUD (Table 4). There are no data available in literature to compare our observations.

In conclusion, this study suggests that -765G > C *COX-2* polymorphism could be a marker for genetic susceptibility to gastric adenocarcinoma. This polymorphism in gastric adenocarcinoma was independent to *H pylori* infection. However, patients with *H pylori* infection and *COX-2* polymorphism had higher risk to develop PUD. Thus, *COX-2* polymorphism might be useful in predicting the risk of PUD in presence of *H pylori* infection. Further studies on different ethnic groups are warranted to confirm the association of this polymorphism with the risk of gastric adenocarcinoma and PUD.

COMMENTS

Background

It remains unclear why only a subpopulation of *H pylori* infected individuals develop peptic ulcer disease (PUD) and gastric adenocarcinoma. This raises the possibility that host genetic factors play an important role in the pathogenesis of *H pylori* infection. Differential expression of *COX-2* enzyme might confer inter-individual susceptibility to gastric cancer and PUD. Hence we investigated -765G > C *COX-2* polymorphism and *H pylori* infection in patients with gastric adenocarcinoma, PUD and non ulcer dyspepsia (NUD).

Research frontiers

The role of host-related factors in the pathogenesis of diseases caused by *H pylori* infection has largely been ignored. Role of *COX-2* polymorphism in patients with gastric adenocarcinoma and PUD in presence or absence of *H pylori* infection has not been studied. The study showed that *COX-2* polymorphism was associated with gastric adenocarcinoma independent of *H pylori* infection. However, patients with combined *H pylori* infection and *COX-2* polymorphism had higher risk to develop PUD. Further studies on different ethnic groups are warranted to confirm the association of this polymorphism with the risk of gastric adenocarcinoma and PUD.

Innovations and breakthroughs

We report for the first time that -765G > C *COX-2* polymorphism with or without *H pylori* infection could be a potential marker for genetic susceptibility to gastric adenocarcinoma. To the best of our knowledge, this is the first study to show that patients with C carriers of *COX-2* gene are susceptible to develop PUD in the presence of *H pylori* infection.

Applications

Detection of *COX-2* polymorphism might help to identify the subgroup of patients having greater susceptibility to develop gastric adenocarcinoma, and peptic ulcer disease in presence of *H pyl*ori infection.

Peer review

COX-2 polymorphism and its role in carcinogenesis is an extremely exciting field. Most of the work in this area has been done in the investigation of colorectal cancer, more because of the relatively higher incidence of colorectal cancer and its ease of detection. This is probably the first study to document the role of *COX-2* polymorphism in gastric cancer and PUD in addition to *H pylori* infection.

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