Original Article

Helicobacter pylori in colorectal neoplasms of Kashmiri patients: What is the prevalence?

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ABSTRACT

Background: The purpose of the present study was to detect the presence of *Helicobacter pylori* in colorectal cancer (CRC) patients in Kashmiri population. **Materials and Methods:** A total of 86 CRC samples were studied. Samples were taken from tissues suitable for DNA amplification by polymerase chain reaction (PCR). DNA was extracted and *H. pylori* detection was carried out by PCR amplification of *glm*M gene. **Results:** Out of 86 samples, only eight were *H. pylori*-positive for amplification *glm*M gene of a 294 bp DNA fragment. **Conclusion:** *H. pylori* is not the prominent risk factor for the development of colorectal cancer in Kashmiri population.

Keywords: Colorectal cancer, *Helicobacter pylori*, Kashmir

INTRODUCTION

Helicobacter pylori is one of the most genetically diverse bacterial species.[1] Some strains may be substantially more virulent than others. The virulent strains have a unique CagA pathogenicity island, a 40 kilobase-pair segment of DNA comprising a collection of approximately 30 genes. CagA gene codes for the cytotoxic protein, which is one of the important virulence factors of *H. pylori*.[2]

A number of studies have been carried out on colorectal cancer to assess the role of *H. pylori* in carcinogenesis. In many of these studies, high prevalence of infection was found in these tissues,[3-5] while other study reported the non-association.[6]

Kashmir valley, located in the northern division of India, surrounded by Himalayas, has an unique ethnic population living in temperate environmental conditions, having distinctive food habits, which play an overwhelming role in the development of gastro intestinal tract (GIT) cancers over the genetic factors.[7,8] Colorectal cancer (CRC) is the fourth most common cancer in men and the third most common cancer in women worldwide.[9] In Kashmir valley, CRC represents the third most common GIT cancer after esophageal and gastric.[7,8,10]

In the present study, we assessed the prevalence of *H. pylori* infection in colorectal cancer patients using polymerase chain reaction (PCR) detection method.

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MATERIALS AND METHODS

Study population

This study included 86 consecutive primary CRC patients. All CRC patients were recruited from Department of Surgery, Sher-I-Kashmir Institute of Medical Science, from March 2008 to August 2009. Tumor types and stages were determined by two experienced pathologists. The mean age was 52 years old, and 56 of the patients were >50 years old; see Table 1 for details.

Data on all CRC patients were obtained from personal interviews with patients and or guardians, medical records and pathology reports. The data collected included sex, age, dwelling, tumor location, Dukes stage, lymph node status, pesticide exposure and bleeding per rectum (PR).

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All patients and/or guardians were informed about the study, and their will to participate in this study was taken on predesigned questionnaire (available on request). The collection and use of tumor and blood samples for this study was previously approved by the appropriate Institutional Ethics Committee.

DNA extraction and polymerase chain reaction-restriction fragment length polymorphism

DNA extraction was performed using ammonium acetate method. Each sample was examined by two different researchers in separate PCRs. Primers (F: 5′-AAGCTTTTAGGGGTGTTAGGGGTTT-3′ R: 5′-AAGCTTACTTTCTAACACTAACGC-3′) used in this study were designed for *glm*M gene (294 bp) as described previously to be most sensitive to detect presence of *H. pylori* infection in biopsy samples.[11]

PCR was carried out in a final volume of 25 μL containing 50 ng genomic DNA template, 1X PCR buffer (Biotools) with 2 mM $MgCl_2$, 0.8 μM of each primer (Genescript), 50 μM dNTPs (Biotools), and 0.5 U DNA polymerase (Biotools).

Table 1: Frequency distribution analysis of selected demographics and risk factors in colorectal cancer cases and controls

Variable	Cases (n=86)
Age group	
≤50	30 (34.9%)
>50	56 (65.1%)
Gender	
Female	37 (43.0%)
Male	49 (67.0%)
Dwelling	
Rural	59 (68.6%)
Urban	27 (31.4%)
Location	
Colon	36 (41.8%)
Rectum	50 (58.2%)
Smoking status	
Never	31 (36.0%)
Ever	55 (64.0%)

For PCR amplification, the standard program was used as follows: one initial denaturation step at 94°C for 7 min, followed by 35 denaturation cycles of 1 min at 94°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C, followed by a final elongation cycle at 72°C for 10 min.

PCR mixture was electrophoresed through a 2–3% agarose gel for resolution. A positive control for each PCR was used as an internal control for amplification.

RESULTS AND DISCUSSION

A total of 86 colorectal cancer patients were included in this study. The patients comprised 49 males and 37 females (M/F ratio = 1.32). Mean age in patients was 52 years. Furthermore, out of 86 confirmed cases of CRC, 59 were rural and 27 urban; 36 cases had carcinoma in colon and 50 in rectum; and 55 were smokers and 31 non smokers [Table 1].

The PCR method of detection of *H. pylori* infection in the tumor samples revealed very low prevalence of *H. pylori* in CRC in our population. Out of 86 samples that were screened, only eight tumors (9.3%) had *H. pylori* infection. Seven of the eight positive tumor samples were of higher grade (Duke's C+D Stage) [Table 2]. Another important finding of this study was that one of the *H. pylori* infected tumor was of a familial adenomatous polyposis patient.

Although *H. pylori* infection is one of the most common bacterial infections in man, known to cause gastritis and increase the risk of gastric cancer,[4] there are conflicting reports about the presence of *H. pylori* infection and colorectal cancer.[6,12-14]

In this study, we found very low prevalence of the *H. pylori* infection in CRC patients in our ethnic population, which was in tune with other studies.[6,15] These results suggest that *H. pylori* has a very small role in causing the CRC malignancy in our population.

Table 2: The clinico-pathological features of colorectal cancer patients having *H. pylori* infection

Patient id	Age / sex[a]	Rural / urban[b]	Smoking status[c]	Duke's stage[d]	Grade[e]	Site[f]	Type[g]	Nature[h]	Lymph node status[i]	Exposure to pesticides[j]
X04	47/F	R	Sk	D	III	R	MAC	S	Y	N
X07	55/M	R	Sk	D	IV	R	AC	FAP	Y	Y
X12	65/F	R	Sk	C	IIIC	C	AC	S	Y	Y
X18	30/F	R	NSk	B	IIA	R	AC	S	N	Y
X28	42/M	U	Sk	C	IIA	R	MAC	S	Y	N
X39	75/M	R	Sk	D	III	R	AC	S	Y	Y
X57	40/M	R	Sk	D	IIIB	C	AC	FAP	Y	N
X59	27/M	R	NSk	D	IV	R	AC	S	Y	Y

[a]Age/Sex: M = Male, F = Female; [b]Rural/Urban : R = Rural, U = Urban; [c]Smoking Status: Sk: Smokers; NSk: Non Smokers; [d]Duke's stage: A - Tumour confined to the intestinal wall; B - Tumour invading through the intestinal wall; C - With lymph node(s) involvement; D - With distant metastasis; [e]Grade Stage: Stage I: T1/T2,N0,M0; Stage II: T3/T4,N0,M0; Stage III: Any,N1/N2,M0; Stage IV: AnyT,AnyN,M1; [f]Site of tumor: C = Colon, R = Rectum; [g]Tumor Type: AC = Adenocarcinoma, MAC = Mucoid Adenocarcinoma; [h]Nature of tumor: S: Sporadic; FAP: Familial Adenomatous Polyposis; HNPCC: Hereditary Non Polyposis Colorectal Cancer; [i]Lymph Node Status: Y = Yes, N = No; [j]Exposure to Pesticides: Y = Yes, N = No;

CONCLUSION

In our study, we detected the presence of *H. pylori* genomic material by PCR reaction in colorectal cancer. However, we conclude that there is very less frequency of *H. pylori* infection in our CRC patients.

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