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ORIGINAL ARTICLE

Role of Helicobacter Bacteria in Colitis and Colorectal Neoplasms: A Histopathological and Immunohistochemically Study

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ABSTRACT

Background and Aim: Adenocarcinomas have progressed from hyper proliferative epithelium through adenomatous lesions to adenocarcinomas and adenomas. In this case, the adenomatous foci are typically neoplastic intraluminal protrusions and occasionally flat, dysplastic glandular growths, which are either filamentous or villous in nature, depending on the architectural position, etiologically, and genetic factors associated in their genesis. The present study aimed to evaluate the role of helicobacter bacteria in colitis and colorectal neoplasms.

Methodology: This retrospective study included 60 patients' samples collected of paraffin blocks at the Department of Pathology, WMC, Abbottabad and LRH Peshawar during the period from March 2020 to March 2022. Demographic details and clinical data taken from the pathology sheet included age, gender, tumor size, colorectal carcinoma site, distant metastasis presence, ulcerating, and fungating. A serial section of 5 mm thickness was cut from each block and mounted on a glass slide for histological evaluation. After staining with hematoxylin and eosin (H&E), a second section was mounted on charged slides for immune-histochemical evaluation. SPSS version 26 was used for data analysis.

Results: Of the total 60 patients, there were 32 (53.3%) men and 38 (46.7%) women. The overall mean age was 46.8±3.46 years with age ranges from 20 years to 80 years. The distribution of patients were as follows: normal colon 15 (25%), colitis 15 (25%), colonic tubular adenoma 10 (16.7%), and colorectal carcinoma 20 (33.3%). Among 60 patients stained for anti-H. pyroli, the prevalence of positive and negative antibodies was 16 (26.7%) and 44 (73.3%) respectively. The incidence of negative and positive anti-H. Pylori antibodies in normal colon, colitis, colonic tubular adenoma, and colorectal carcinoma were as follows: 4 (26.7%) and 11 (73.3%), 5 (33.3%) and 10 (66.7%), 3 (30%) and 7 (70%), and 5 (25%) and 15 (75%) respectively. Out of 20 colorectal carcinoma, the incidence of sigmoid colon, rectum, transverse colon, and right colon was 9 (45%), 6 (30%), 3 (15%), and 2 (10%) respectively. The occurrence of lesion diameter <5 cm and >5 cm was 11 (55%) and 9 (45%) respectively. The prevalence of circumferential infiltrating type, ulcerative type, and fungating type was 12 (60%), 6 (30%), and 2 (10%) respectively. Based on colorectal cancer patients histological class, the incidence of adenocarcinomas, mucoid adenocarcinoma, and adenocarcinoma with neuroendocrine differentiation was 14 (70%), 3 (15%), and 3 (15%) respectively.

Conclusion: The present study concluded that positive antibodies of H. pylori had no significant association with carcinoma, normal colon, tabular adenoma, and colitis. Still, determination of odd ratio (OR) estimating risk showed that adenoma (OR 2.0) and colitis (OR 1.39) had significant association with positivity of H. pylori. Additionally, Sigmoid colon was the most prevalent site followed by rectum, transverse colon, and right colon.

Keywords: Helicobacter bacteria, Colitis, Carcinoma, Immunohistology

INTRODUCTION

Colorectal cancer is the third most prevalent malignancy and cause for mortality in both genders [1]. Hyperplastic polyps and adenomas polyps are the reasons for colorectal carcinoma [2]. Adenocarcinomas develop from hyper proliferative epithelium to adenomatous lesions and then to adenocarcinomas and adenomas. The adenomatous foci in this case are typically neoplastic intraluminal protrusions and, on occasion, flat, dysplastic glandular growths that are filamentous or villous in nature, depending on the architectural position, etiology, and genetic factors involved in their genesis. The mortality rate could be reduced by early diagnosis and removal of colorectal carcinoma through surgical intervention [3]. Several studies revealed the role of different infectious organisms and their ability to induce colorectal cancer among the population. However, recently few researchers focused on decreasing the prevalence of colorectal cancer by taking preventive measures against the infectious organisms [4, 5]. Other epidemiological studies associated the Helicobacter pylori infection with colorectal neoplasms in colorectal cancer patients [6, 7], or colon mucosa affected by bacterial products presence and their trophic effects [8]. Additionally, few studies related the H. pylori presence in stomach with polyps or colon cancer [9, 10].

Helicobacter pylori is the global health issue affecting more than 50% of the world population and its prevalence varies from 40% to 50% and rising above 90% in the developing world [11]. H. pylori is a gram-negative bacterium that interacts with gastric epithelial cells in the human stomach. Gastric cancer, ulcers and several gastric inflammation pathologies are caused by chronic gastric H. pylori infection [12]. The gastric H. pylori infection byproduct is responsible for colorectal cancer rather than the colorectal tissue infection with H. pylori [13]. A previous study reported that hypergastrinemia caused by increased serum level of gastrin is due to gastric H. pylori infection, in turn hypothesized to have intestinal mucosa affected by proliferative effects [14]. There was a significant association between colorectal neoplasms and hypergastrinemia. H. pylori and neoplastic colorectal lesions have been associated based on indirect evidence of increasing CagA + levels or gastrin levels and direct correlation with seropositivity of H. pylori [15]. The present study aimed to evaluate the role of helicobacter bacteria in colitis and colorectal neoplasms.

METHODOLOGY

This retrospective study included 60 patients' samples collected and embedded in paraffin blocks from the Department of Pathology, WMC, Abbottabad and LRH Peshawar during the period from March 2020 to March 2022. Demographic details and clinical data taken from the pathology sheet including age, gender, tumor size, colorectal carcinoma site, distant metastasis presence, ulcerating, surgical margins (positive or negative), and fungating. A serial section of 5 mm thickness was cut from each block and mounted on a glass slide for histological evaluation. After staining with hematoxylin and eosin (H&E), a second section was mounted on charged slides for immune-histochemical evaluation. Hematoxylin and eosin stained slide histopathological examination was carried out to determine the degree of inflammation in colitis patients and dysplasia status in tubular adenoma patients. The histological grades of colorectal carcinoma were well, moderate, and poor based on extent of glandular appearance. Well-formed glands in >75% of well-differentiated tumors, 25-75% in moderately differentiated tumors, and <25% in poorly differentiated carcinomas.

Xylene was used for deparaffinization of all the sections that were then hydrated in PBS (at pH 7.5), graded alcohol (70-95%), and distilled water. Prior to Microwave treatment being done for 4 and 8 minutes, citrate buffers (pH 6) of 10 mmol/l were used for slide immersion. Evaporated fluid replenished after each step of heating. About 3% H2O2 was used for incubation for 5 minutes to perform the endogenous peroxidase activity. Tris-buffered saline was used for washing and incubating the primary antibodies for 1 hour at 25 degree Celsius. These sections are examine in high magnification camera attached with microscope and when Helicobacter organisms were detected as thin coiled brown bacilli.

Data analysis was carried out in SPSS version 26. Helicobacter positivity and colorectal neoplasm was associated suing □2 test. All the findings were statistically significant with p<0.05. The positive and negative anti-H. Pylori antibodies were compared with their relative risk using odds ratio (OR). In case OR>1 patients had greater probability of developing colitis and colorectal neoplasms.

RESULST

Of the total 60 patients, there were 32 (53.3%) men and 38 (46.7%) women. The overall mean age was 46.8±3.46 years with age ranges from 20 years to 80 years. The distribution of patients were as follows: normal colon 15 (25%), colitis 15 (25%), colonic tubular adenoma 10 (16.7%), and colorectal carcinoma 20 (33.3%). Among 60 patients stained for anti-H. pyroli, the prevalence of positive and negative antibodies was 16 (26.7%) and 44 (73.3%) respectively. The incidence of negative and positive anti-H. Pylori antibodies in normal colon, colitis, colonic tubular adenoma, and colorectal carcinoma were as follows: 4 (26.7%) and 11 (73.3%), 5 (33.3%) and 10 (66.7%), 3 (30%) and 7 (70%), and 5 (25%) and 15 (75%) respectively. Out of 20 colorectal carcinoma, the incidence of sigmoid colon, rectum, transverse colon, and right colon was 9 (45%), 6 (30%), 3 (15%), and 2 (10%) respectively.

Table-1: Gender's distribution (n=60)

Gender	Frequency N	Percentage %
Men	32	53.3
Women	28	46.7

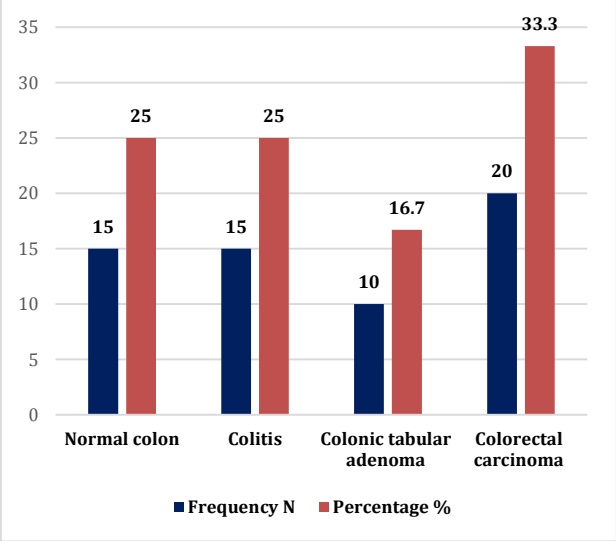

Figure-1: Patients distribution based on different colon diseases (n=60)

The occurrence of lesion diameter <5 cm and >5 cm was 11 (55%) and 9 (45%) respectively. The prevalence of circumferential infiltrating type, ulcerative type, and fungating type was 12 (60%), 6 (30%), and 2 (10%) respectively. Based on colorectal cancer patients histological class, the incidence of adenocarcinomas, mucoid adenocarcinoma, and adenocarcinoma with neuroendocrine differentiation was 14 (70%), 3 (15%), and 3 (15%) respectively. Table-I represents the gender's distribution of all the patients. Patients' distribution based on different colon diseases are illustrated in Figure-1. The staining of anti-H. Pylori antibodies are shown in Figure-2. Group-wise distribution of stained anti-H. Pylori antibodies are depicted in Figure-3. Association of H. Pylori positivity and adenoma on biopsy is shown in Table-II. Table-III shows the association of H. Pylori positivity and carcinoma.

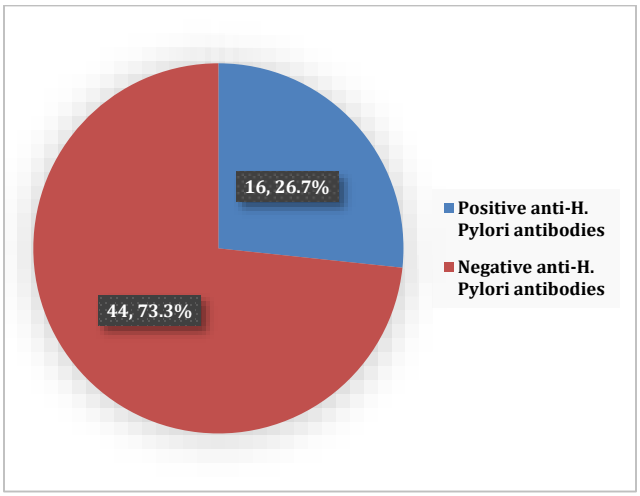

Figure-2: staining of anti-H. Pylori antibodies among the study groups (n=60)

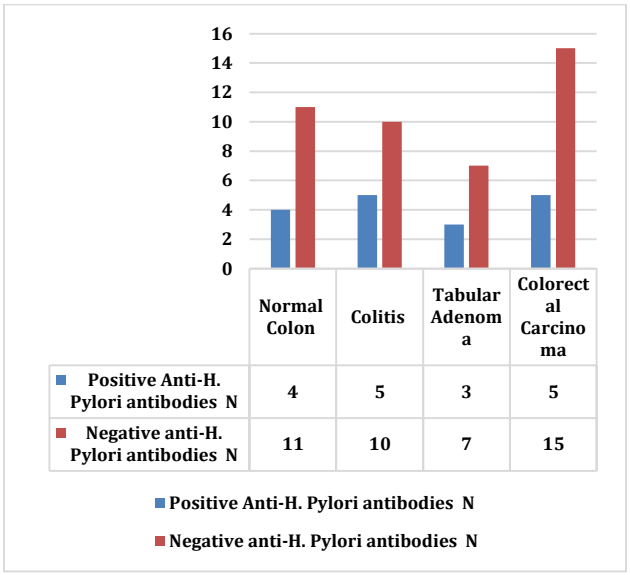

Figure-3: Group-wise distribution of stained anti-H. Pylori antibodies

Table-2: Association of H. Pylori positivity and adenoma on biopsy

Tabular Adenoma	Positive H. Pylori N (%)	Negative H. Pylori N (%)	Total N (%)
Positive	3 (30)	7 (14)	10 (16.7)
Negative	7 (70)	43 (86)	50 (83.3)
Total	10 (100)	50 (100)	60 (100)

Table-3: association of H. Pylori positivity and carcinoma

Colorectal carcinoma	Positive H. Pylori N (%)	Negative H. Pylori N (%)	Total N (%)
Positive	5 (25)	15 (37.5)	20 (33.3)
Negative	15 (75)	25 (62.5)	40 (66.7)
Total	20 (100)	40 (100)	60 (100)

DISCUSSION

The present study mainly focused on immune-histochemical methods to determine the presence of Helicobacter pylori organisms in colorectal cancer associated neoplasm. It has been found that positive antibodies of H. pylori had no significant association with carcinoma, normal colon, tubular adenoma, and colitis. Still, determination of odd ratio (OR) estimating risk showed that adenoma and colitis had significant association with positivity of H. pylori. Additionally, Sigmoid colon was the most prevalent site followed by rectum, transverse colon, and right colon. Evidently, the ambiguous results could come from serum-based methods as antibodies can be identifiable after gut colonisation and ceased due to bacteria [16]. In the current study, technique of immune-histochemical detection were used compared to routine histo-chemistry due to the former method accuracy [17] and detection of organism in non-spiral forms [18, 19].

The induction of colorectal cancer due to H. pylori is still debatable among researchers that is attributed to contentious results obtained. A previous study reported that gastric cancer is developed by H. pylori [20], whereas others found that H. pylori has no significant association with susceptibility of gastric cancer [21, 22]. There is paucity of data regarding association between H. pylori and colon cancer. Therefore, the current study investigated the H. pylori presence using immunohistochemistry technique on colon cancer lesions and colon polyps.

The present study found that H. pylori was more prevalent in colorectal cancer patients compared to those diagnosed with tubular adenoma and colitis. This result was comparable to studies done by Kawahara et al [23] and Abreu et al [24] according to them, H. pylori was present in 59 adenocarcinoma biopsy using immunohistochemistry and 77 rectum cancer cases using molecular technique, correspondingly. About 10 patients of adenocarcinoma had H. pylori detected on immunohistochemistry. Regardless of colonization by micro-organism, colon cancer had no significant association with H. pylori as demonstrated by several studies [25, 26]. The possible explanation could be H. pylori bacteria ability to be demonstrated in various lesions including adenocarcinoma, colitis, and polyps.

Several studies proposed different theories regarding H. pylori exact role in inducing colon cancer out of which one stated that H. pylori toxin introduced colon cancer among the population [27, 28]. Additionally, it has been reported that micro model infection with H. pylori could develop colon cancer and colitis. The interaction between mice immune cells and bacteria produced toxins could possibly develop colon cancer [29]. This clearly shows that H. pylori infections are responsible for the development of colon cancer.

Though H. pylori and colorectal neoplasm direct and indirect association have been identified but limited to the colorectal neoplasia and positive H. pylori present on serology were demonstrated. A previous study by Ali et al [30] investigated 83 patients of colorectal carcinoma and found that 20 patients of colorectal carcinoma had positive H. pylori on PCR analysis for specimen's biopsy with normal muscular tissues. About 36 patients had positive H. pylori IgG antibody and normal mucosa present in five patients. Yet, no statically significant association was found between colorectal carcinoma and positive H. pylori.

CONCLUSION

The present study concluded that positive antibodies of H. pylori had no significant association with carcinoma, normal colon, tubular adenoma, and colitis. Still, determination of odd ratio (OR) estimating risk showed that adenoma (OR 2.0) and colitis (OR 1.39) had significant association with positivity of H. pylori. Additionally, Sigmoid colon was the most prevalent site followed by rectum, transverse colon, and right colon.

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