



Association of *Helicobacter pylori* Infection with Colon Polyp and Colorectal Cancer

Hossein Ajdarkosh¹, Fahimeh Safarnezhad Tameshkel¹,
Masoud Reza Sohrabi¹, Mohammad Hadi Karbalaie Niya²,
Gholamreza Hemmasi¹, Bahare Amirkalali¹, Farhad Zamani¹
and Ameneh Mehraban Sarasiab^{1*}

¹Gastrointestinal and Liver Diseases Research Center, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran.

²Department of Virology, Iran University of Medical Sciences, Tehran, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background/Objective: *Helicobacter pylori* (Hp) is an etiology of gastrointestinal problems like gastric cancer. Its role in colorectal cancer is under investigations. Therefore, in this study we proposed the association of Hp infection with colon polyp and colorectal cancer.

Methods: Patients who referred to Gastrointestinal Clinic of Firoozgar Hospital, Tehran, Iran from May 2014 to March 2015, were enrolled in a prospective case control study. Two groups of colorectal cancer (CRC) and colon polyps were compared with a group of healthy individuals. All participants underwent endoscopy, total colonoscopy, PCR test for Hp, Rapid Urease Test (RUT), stomach histological sections, anti- Hp IgG, CagA protein expression, and serum gastrin levels. SPSS v.20.0 used to analysis of variables.

Results: a total of 240 participants, 138 (57.5%) males and 102 (42.5%) females, were divided into three groups of colon polyp (66/240), CRC (58/240), and health control (116/240). Results of PCR for Hp detection in colon samples were negative in all three groups. The association of

presence of colorectal cancer and positive RUT in stomach was not significant ($p=0.09$). There was no significant relationship between positive Hp in the stomach and the site and the type of polyps in colon and anti- Hp IgG, CagA protein expression and serum gastrin levels of three groups ($p>0.05$).

Conclusion: Our findings revealed that the Hp infection does not show a significant association with CRC and colon polyps.

Keywords: *Helicobacter pylori*; colonic polyps; colorectal neoplasms.

1. INTRODUCTION

Colorectal cancer (CRC) still remains as one of the most common causes of mortality worldwide. It is the third lethal cancer in many countries such as United States [1]. Most of CRCs originate from adenomatous polyps which are benign neoplastic tumors of epithelium with variable potential for malignancy [2]. It is generally accepted that colorectal cancer arises from precancerous lesions such as colonic adenoma. Generally, 5% of adenomas are at the risk of malignancy [2]. How these precursors develop and evolve to cancer is not known, yet. In this context, the role of environmental factors like gastrointestinal infections becomes predominant. Studies investigating the etiologies and risk factors of colorectal adenomas have suggested the *Helicobacter pylori* (Hp) as a gram-negative bacteria to have a significant role in a variety of upper gastrointestinal disorders, including gastric mucosa associated lymphoid tissue (MALT) lymphoma, and gastric cancer [3-5]. Thus, it has been hypothesized that Hp may have a role in developing colorectal adenomas or CRCs. Therefore, the association between Hp and CRC is under investigation [3,6]. There are limited data in this issue, recently and a few studies revealed the association between Hp and CRCs [6,7]. Wu et al. in a meta-analysis showed a positive association between Hp infection and the risk of colorectal neoplasia [8,9]. Although this association was not confirmed in other studies, in general, there is not a conclusive result [7,8].

In this regard, we designed this study by the histopathology as a source of data, applying PCR for Hp detection, also used the location and type of adenoma and some other risk factors to investigate the association of *Helicobacter pylori* infection with colon polyp and colorectal cancer.

2. MATERIALS AND METHODS

2.1 Patient Selection

This prospective case-control study was conducted at Gastrointestinal and Liver Disease

Research Center (GILDRC), Firoozgar Hospital, by the grant of Iran University of Medical Sciences, Tehran, Iran. All patients with lower gastrointestinal symptoms who referred to gastrointestinal clinics of Firoozgar Hospital from May 2014 to march 2015, were invited to this project. The Control group is composed of healthy individuals who underwent screening colonoscopy. Patients with polyps or sporadic CRC were divided into the other two groups. The patients with gastric Hp positive were enrolled in this study. Inclusion criteria included gastric Hp positive patients who underwent screening colonoscopy, had positive Rapid Urease Test (RUT) or positive histopathological exam, or antigen detection test for Hp. Included participants were contain at least one of criteria. The exclusion criteria were familial CRCs, CRC patients who operated or received cancer chemotherapy and refused upper and lower endoscopy. Both groups were similar according to age and sex.

2.2 Laboratory Analyses

Upper and lower endoscopy exams were carried out for all the patients. Digital processor Fujinon 4400 (Tokyo, Japan) was used for endoscopy. The endoscopy was performed in a standard fashion. Anesthesia was applied by a trained nurse with Petedin and Midazolam as appropriate. Anti- Hp IgG, CagA protein expression and serum gastrin levels performed for all patients. IgG serologic test (HEL-P test, Park Co, Athens, Greece) for the diagnosis trace of Hp infection was carried out by ELISA method according to manufacturer's instructions. The IgG antibodies against Hp results of greater than 25 U/mL were assigned positive. Western-blot IgG assay for the positive specimens were examined for cytotoxin-associated gene A (CagA) protein. Then, competitive radioimmunoassay performed for the evaluation of serum Gastrin by rabbit antiserum raised against a gastrin 17 albumin conjugate (Gastrin RIA, DIAsource ImmunoAssays S.A, Belgium). The values < 100 pg/mL were assigned as normal.

During gastroscopy, antral biopsy was taken for evaluation of Hp by Rapid Urease Test (RUT). One sample was also sent for histopathology exam to detect Hp in tissue. Hematoxylin and Eosin (H&E) staining and immunohistochemistry were used to detect Hp antigen. During colonoscopy exam, the scope was advanced to cecum and all polypoid lesions were removed by biopsy or polypectomy. At the same time, the specimens of polypoid-free tissue were obtained. All of the colon samples underwent staining and pathology evaluation according to a standard fashion. Moreover, after DNA extraction of all three group colon samples, evaluation for Ure C gene of Hp by PCR in order to specify the primers and conditions described by the previous study [10] carried out.

2.3 Statistical Analysis

The results were analyzed by the SPSS Software, version 20.0 (IBM, SPSS, IL.USA) for Windows. Data was analyzed using the statistical tests, including Analysis of variance (ANOVA) and Chi square. In detail the age and serum gastrin levels analyzed by ANOVA and the sex, Anti- Hp IgG, cagA protein expression, gastric dysplasia and RUT assay analyzed by Chi square statistical tests. Descriptive analysis was carried out to report the prevalence of lesions, sex, and age distributions. A p -value of < 0.05 was considered statistically significant.

2.4 Ethics

In this study we were engaged on Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The ethical laws of Iranian Ministry of Health, Treatment, and Medical Education were applied, as well. The protocol was explained for each patient and a written informed consent was obtained. There were no financial costs for

participants. The data were conserved secure in GILDRC data bank.

3. RESULTS

A total of 240 patients, 138 (57.5%) males and 102 (42.5%) females, were enrolled in our study and were divided into three groups: a) CRC with 58 patients; b) colonic polyps with 66 patients; and c) the control group with 116 healthy individuals. Table 1 summarized the analysis of these three groups.

There is no significant difference between the three groups regarding gender and age. Anti- Hp IgG antibodies were performed in three groups, the difference has no statistical significance. The cagA protein expressed higher in the colorectal cancer group in comparison with the control group and polyp group, where all members were anti-Hp IgG positive patients but the difference was not of statistical significance. The serum gastrin levels did not significantly differ between the three groups. Hence, gastrin levels were not different in three groups, significantly. In this study, the coincidence of gastric dysplasia with colon cancer or polyp was performed. Coincidence of gastric dysplasia with CRC was not significantly different among the three investigated groups ($p=0.1$). RUT (to detect Hp infection in the stomach) were performed in three groups. There was not a significant relationship between the presence of colon cancer or polyp and positive RUT in stomach ($p=0.09$), Table 1 summarized data; but Hp was positive in 34 (32.7%) patients without gastric dysplasia. There was no significant relationship between positive Hp in the stomach with the site and grade of dysplasia or the type of the polyp in colon ($p=0.1$). There was a significant relationship between the presences of gastritis and polyp or colon cancer ($p= 0.083$). The results of PCR for Hp detection in colon samples were negative in all three groups including, CRC, polyp, and control groups.

Table 1. Characteristics and variables association details of three groups of our study population

Variables\groups	CRC N=58 (%)	Colonic polyps N=66 (%)	Control group N=116 (%)	p -value
Male	34 (58.6)	34 (51.5)	70 (60.3%)	0.62
Female	24 (41.4)	32 (48.5)	46 (39.7%)	0.7
Mean age	58.3±13.2	55.6±11.8	50.0±15.2	0.2
Anti- Hp IgG	39 (67.0)	70 (60.0)	42 (63.0)	0.91
cagA protein expression	30 (53.0)	46 (40.0)	23 (35.0)	0.18
Gastric dysplasia	8 (12.4)	10 (15.2)	4 (3.4)	0.1
RUT	26 (46.4)	26 (39.4)	38 (32.8)	0.09
Serum gastrin levels (pg/mL)	50.6±35.1	51.1±21.4	47.3 ± 16.2	0.68

4. DISCUSSION

Hp has a leading role for hypergastrinemia, and according to the previous studies hypergastrinemia might be associated with the risk of colorectal adenoma and consequently the malignancy [11]. There are controversies, however, over the role of Hp infection in colorectal adenoma and malignancies and its mechanism of action [11]. The strengths of the present study include using histopathology as a source of data, applying PCR for Hp detection, RUT assay, Gastrin level, Hp IgG antibody and cagA protein expression also performed. We also specify the location and type of adenoma.

The presence of colorectal polyps had some relationships with cancer of colon and is in line with the findings of wood et al. [12] who reported that the presence of colorectal polyps was significantly associated with the gastric adenoma. There are also some other studies which conclude that gastric adenoma and carcinoma might be indicators of colorectal adenoma or carcinoma and have a high potential for malignant transformation [13]. However, in the present study the prevalence of gastric dysplasia was not significantly different among the three investigated groups (CRC, colonic polyps and the control group). It could be due to other causes like genetic susceptibility of our region or patients in CRC and colonic polyps groups.

Gastrin is an important enzyme of gastrointestinal tract, the level of which is elevated among patients with Zollinger-Ellison syndrome with colonic proliferation or the reduction of serum gastrin level after CRC surgery [14-16]. In a study, gastrin had an influence on the growth of normal colonic epithelium [16]. It has also been hypothesized that gastrin could play its role via up-regulation of COX-2 and IL-8 expressions (as inflammatory agents) that consequently may create defects in cancer prevention system [17]. On the other hand, the association between gastrin and K-ras has been reported [15,18]. Furthermore, It is hypothesized that gastrin precursors with/without gastrin may be involved in the process of colorectal cancer [19]. In our study, serum gastrin level was also not significantly different among CRC, colonic polyps and the control groups which could be because of some confounding agents like inhibitors or some abuses of drugs indiscriminately.

RUT is the most common biopsy-based method in diagnostic tools of Hp infection due to its simplicity, rapidity and accurate procedure [20]. But it needs a high density of bacteria and the reduction of bacterial load may produce false-negative results [21]. It may be the consequence of Hp count reduction due to drug therapy [22]. Our study included patients with positive RUT in stomach also showed that the RUT results in colon were significantly different among the three groups of CRC, polyp and, control which may be due to the low density of bacteria or different locations of examination (stomach and colon).

Hp detection by PCR method was also not positive in any samples of the three groups in this study. It may be due to the use of medicines that reduce the Hp count, considering the presence of infection history among patients (positive Hp IgG antibody). Also, although gastric RUT, Ag and etc. positive patients for Hp used in this study, PCR exam in colon tissue may be due to the non-specific location for Hp was negative.

Positive Hp IgG antibody was found in 67% of the colorectal cancer group, 60% of healthy group, and 63% of polyp group that were not statistically significant. The Strofilas et al. study [15] showed similar results for positive Hp IgG antibodies with 71% of the patients in colorectal cancer group and 65% of the control group, but the difference was not significant either.

The CagA- positive Hp strains, almost always lead to atrophic gastritis and subsequently increase the probability of evolving precancerous or cancerous lesions [23]. This condition synergistically stimulate the performance of some other precursors and proteins that are involved in cancers of digestive system [24,25]. CagA-positive strains increase the risk of gastric cancer in comparison with cagA-negative strains [16]. In this study positive cagA protein expression was 53% in colorectal cancer group, 35% in the control group, and 40% in the polyp group and corresponds to the findings of Strofilas et al. [15] who reported the prevalence of 56% in colorectal cancer group and 38.4% in the control group. However, these results are in contrast with other studies which have been reported.

In this study we did not find any association between characteristics of adenoma and Hp infection. This finding is comparable to the previous reports that indicated no association between Hp and colonic neoplasm [7,24,26]. However, some studies from African or African

American populations revealed positive association [27]. In fact, we cannot offer a good explanation for this finding, but it could be possible that the subjects had used antibiotics for any reason before endoscopy procedures. It is noticeable that the abuse of antibiotic agents is common in our regions especially during the seasons that the study was conducted.

Despite the presence of a significant positive association between gastritis and occurrence of polyp or colon in the previous study [26], in this study we did not find any association between gastric dysplasia and colonic neoplasm, that is in disagreement with some previous reports [7,24,26,28,29]. In this study we did not evaluate the strain of Hp. Restricted sample size and had not any considering on antibiotic treatment were among limitations of this study.

5. CONCLUSION

In conclusion, despite usage of polyp-free, polypoid, and control tissues we did not reveal the presence of Hp particles in colonic samples, while the partial associations were found. It needs further studies in this area by other variables and under better-controlled conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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