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Prevalence of *Helicobacter pylori* in patients with colorectal cancer

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ABSTRACT

Colorectal cancer (CRC) has defined as the uncontrolled growth of cells in the colon or rectum. The human colon contains the greatest number of microbes Therefore; microbes may play an important role in the development of colorectal cancer. Recent studies have shown an association between the microbial infection and the risk of colorectal cancer. However, the results of these studies are controversial. The nobility of the present study was to assess the prevalence of *Helicobacter pylori* infection in the colorectal cancer in Iran. The biopsy were done and the samples were obtained from patients suspected with colorectal cancer, from 2015 to 2017.Secondary, DNA of *Helicobacter pylori* was extracted by Commercial kit. PCR was performed for *Helicobacter pylori glmM* gene eventually, the data was analysed. A total of 86 patients in which 68 of them confirmed with CRC and 17 patients considered as controls. Of 68 biopsy specimens and control group, all of them were negative for *Helicobacter pylori.* It was ultimately attained that in colorectal cancer, there was no presence of *Helicobacter pylori.*

1. Introduction

Colorectal cancer (CRC) is the over growth of cancer cells in the colon or rectum (Lynch et al., 2003). CRC has been recognized as the third most common cause of cancer which elicits mortality globally (Jemal et al., 2011; Rezasoltani et al., 2018). This condition occurs due to the growth of abnormal cell that can spread through the other tissues of the human body invasively. Colorectal cancer is the second most common cancer in females after breast cancer; in addition, it is the third common cancer in males after prostate and lung cancer (Jemal et al., 2011; Center et al., 2009). The incidence of colorectal cancer in Iran is lower than the western countries; and this cancer has been categorized in the 5th place for males and in the 3rd place for females (Akhoond et al., 2011).

Apparently, more than 80% of cancers are affected by the environmental factors, such as diet, exposure to radiation, and other factors; however, a number of cancers are caused by infectious agents. Currently, more than 20% of cancers are affected by the prevalence of

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infectious agents (Jemal et al., 2011; Mojarad). According to the high volume of bacteria in the gastrointestinal tract and available evidence points to an essential role of the intestinal microbiota in CRC pathogenesis, there is a possibility that *Helicobacter pylori* play a role in colorectal cancer development (Sears et al., 2014; Siegel et al., 2017).

Helicobacter Pylori is one of the most common gastrointestinal infections world-wide and it is the main cause of peptic ulceration, distal gastric adenocarcinoma, and gastric lymphoma (Moore et al., 2005). The bacterium colonizes the stomach and induces severe mucosal inflammation, as a result, causes a local and systemic immune response (Atherton et al., 2006; kusters et al., 2006). However, a small number of infected individuals with this bacterium are suffering from severe consequences, such as include peptic ulcer (bleeding) and stomach cancer (Dooley et al., 1989). According to the previous data, it has been shown that *Helicobacter pylori* elicits gastric cancer; therefore, further studies on oncogenicity have been conducted to examine this pathogen role in the development of other gastrointestinal malignancies (Replogle et al., 1995).

Some studies have shown the prevalence of *Helicobacter pylori* infection in colorectal cancer; nonetheless, the results of those study are controversial. The aim of the present study was to assess the prevalence of *Helicobacter pylori* infection with colorectal cancer in Iran (Brim et al., 2014).

2. Materials and Methods

2.1. Patients and specimens

This cross-sectional descriptive study was conducted on tissue samples collected from the patients suspected to CRC referring to the Research Center of Gastroenterology and Liver Diseases, Taleghani Hospital, Tehran, Iran from 2015-2017. The entire patients who were suspected of colorectal cancer were introduced for large intestine biopsy, performed by gastroenterologist under colonoscopy procedure in sterile condition. The biopsies specimens were placed in the sterile screwed test tubes and transferred to the Institute's lab. A questionnaire included demographic and past medical history information, such as age, sex, height, and education was collected for all the include patients. The informed consent was obtained from all patients, and the protocol was approved by the ethical committee of the School of Medicine in Shahid Beheshti University of Medical Science.

2.2. DNA extraction and performing Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from each tissue sample by using the commercial available DNA Extraction Kit (US, QIAamp DNA Mini) according to manufacture guideline. The quality of extracted DNA in samples was measured by using Nanodrop (Thermo Fisher Scientific, Waltham, MA, USA) absorbance at 260 nm and 280 nm by agarose gel electrophoresis. All DNA samples were stored at -20 ◦C until further processing. The presence of *H*elicobacter *pylori* was determined by polymerase chain reaction (PCR) using specific primers targeting *glmM*-F (GCT TAC TTT CTA ACA CTA ACG CGC) and *glmM*-R (GGA TAA GCT TTT AGG GGT GTT AGG GG) with a 296-bp size product (Faïs et al., 2016). All PCR reactions were performed in 0.2 mL tubes using Eppendorf thermal cycler. PCR for *glmM* genes was performed in a 25 µL solution, containing 300 ng DNA template, 1.0 µM each of the primer *glmM*-F and *glmM*-R, 0.25 U of Taq DNA polymerase, 0.5 Mm dnTp and 1 Mm MgCl2. The PCR conditions for *Helicobacter pylori* was done, one cycle for 5 min at 95°C, 34 cycles for 45 s at 95°C, 40 s at 64°C, 1 min at 72°C. Eventually, the final extension step of 5 min at 72°C was performed.

2.3 Data Analysis

Chi-squared, and Fisher's exact tests were used for the analysis of categorical data and T-test for numerical data. The analyses were done using Sigma Stat for Windows V22 (SPSS, Chicago, IL, USA). A P value less than 0.05 was accepted as statistically significant.

3. RESULTS

A total of 86 patients; 69 with CRC; comprised of 46 (62.8%) male and 23 (32.6%) female with mean age of 56±16 and 17 without CRC, 8 (44.4%) male and 5(27.8%) female with mean age of 56±16, as a control group were enrolled in the current study. In term of

exercise, diabetes, familial history, alcohol consumption, and allegiance, there was no significant differences between CRC and control groups.

The largest site of colon involvement was respectively rectum (30.2%), sigmoid colon (15.1%), transverse colon (11.6%), descending colon (11.6%), cecum (7.0%), recto sigmoid (3.5%), and other areas related to ascending colon; for example, rectal, duodenal, and stomach. Considering tumor cell differentiation degree for patients, 18.4% had a desirable degree of cell differentiation (grade I) and 34.7%-degree moderate cell differentiation (grade II) and the frequency for other degree including IIA, IIB, III, IIIA, and IIIB were 40.8, 4.1, 6.1, 2.0, and 12.2, respectively.

According to our results, all the analyzed biopsy specimens were negative for *Helicobacter pylori* by using the molecular technique.

4. Discussion

Colorectal cancer is the second most common cancer in the world after lung cancer; moreover, it is the third most common cause of death from cancer. This issue has been distinctly recognized as a major health problem globally. Various factors, such as genetics background, environment, and life style contribute to the development of this disease. However, this risk factors are not fully explaining all cases. Recently, the role of microorganisms has been proposed in pathogenesis of CRC but is not still fully understood and is controversial (Parkin et al., 2001).

This study was performed on 69 patients with colorectal cancer who referring to the Research Center of Gastroenterology and Liver Diseases, Taleghani Hospital, Tehran, Iran. The patients were mostly elderly men, who referred to the physician for abdominal pain and alterations in bowel movements; in addition, they were referred for endoscopic evaluation and surgical treatment of colorectal cancer. Santhat Nivatvongs reported the highest frequency of colorectal cancers at the age of 60 years and its proportion in men more has reported more than women, in which we attained the same data (Nivatvongs et al., 2000). In a Cross-Sectional study by Sung Noh Hong in Korea in 2010, among 2,195 eligible candidates, 1,253 subjects were *Helicobacter pylori* seropositive [*Helicobacter pylori* (+) group], and 942 subjects were *Helicobacter pylori* seronegative [*Helicobacter pylori* (-) group]. In the *Helicobacter pylori* (+) group, the prevalence of colorectal adenoma was 25.3 % (317/1,253), which was significantly higher than in the *Helicobacter pylori* (-) group at 20.1 % (189/942, p = 0.004) (Hong et al., 2012). Contrary to their results, our finding was supportive for those studies in which *Helicobacter pylori* had no significant prevalence in colorectal cancer. For example, in a study conducted in 2005 by Nicolas Grahn and the colleagues at Tucson, 77 samples of colorectal cancer biopsy were studied, with 42 cases of colon cancer and 35 of them isolated from rectal cancer; in fact, 11 of the 42 samples were obtained from the colon and 10 of 35 were obtained from rectal cancer patients. There was a sequence of DNA related to *Helicobacter pylori*. Indeed, no significant correlation was found between the incidence of colon and rectal colitis in *Helicobacter pylori* (Grahn et al., 2005). In the study of Milutin Bulajic, they applied nested-PCR amplification in which indicated the presence of urease A gene of *Helicobacter pylori* in 83 patients' colorectal cancer biopsy specimens from February 2002 to April 2003. They found *Helicobacter pylori* DNA in one case (1.2%) of colorectal cancer in the tumor tissue and in five samples (6.0%) of a normal colonic mucosa in patients with cancer (Bulajic et al., 2007). In a similar study which was done by Shahab Mahmoudvand and his colleagues at Shiraz in 2017 on colorectal cancer, they could not detect *Helicobacter pylori* DNA (Mahmoudvand et al., 2017). Seroepidemiological studies have indicated that antibodies against *Helicobacter pylori* are present in patients with colorectal cancer and adenocarcinomas. Nevertheless, the evaluation based on the serologic testing did not distinguish between the current and past infections (Grahn et al., 2005). By using molecular methods (PCR) we could not find *Helicobacter pylori* DNA in biopsy samples. Apparently, results come from using molecular methods for detection of *Helicobacter pylori* DNA in colorectal cancer is not as same as using serological techniques. A comparison demonstrates that the prevalence of *Helicobacter pylori* antibodies among patients with CRC was almost two to three times higher

compared to the outbreak of *Helicobacter pylori* DNA detected by using molecular techniques (Grahn et al., 2005).

Conclusively, our results supported the studies in which indicated that there was no association between *Helicobacter pylori* and colorectal cancer.

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Refereces

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