Prevalence of Helicobacter Pylori Among Sudanese Patients Diagnosed with Colon Polyps and Colon Cancer using Immunohistochemistry Technique.

Aziza K. Mohamed
University of Khartoum
Nazik M. Elhassan
University of Khartoum
Zahra A. Awhag
University of Khartoum
Fadwa S. Ali
University of Khartoum
Eman T. Ali
University of Khartoum
Najwa A. Mhmoud
University of Khartoum
Emmanuel E. Siddig
University of Khartoum
Rowa Hassan
University of Khartoum
Eiman S. Ahmed
University of Khartoum
Azam Fattahi
University of Tehran
Ayman Ahmed
University of Khartoum
Mohamed S. Muneer
Mayo Clinic's Campus in Florida
Hussam A. Osman
Ahfad University for Women School of Pharmacy
Nouh Saad Mohamed (nouh_saad@outlook.com)
Ahfad University for Women School of Pharmacy https://orcid.org/0000-0001-6843-3361
Ali MM. Edris
University of Bisha

Research note

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Abstract

Objectives: infection with the bacteria *Helicobacter pylori* has been classified as class 1 carcinogen associated with increasing susceptibility of gastritis and gastric carcinoma. This study is aiming at investigating the prevalence of *H. pylori* in colon polyps and colon cancer lesions. A descriptive cross-sectional hospital-based study was conducted between February and June 2017. Sixty-nine formalin fixed paraffin blocks from colon polyps and colon cancer lesions to detect *H. pylori* using immunohistochemistry technique.

Results: Of the Sixty-nine patients included in the study, 39 (56.5%) males and 30 (43.5%) were females, their age ranged from 21 to 80 years with a mean age of 47.1 ± 19.7. Of the 69 colon polyps and colon cancer cases, 44 (63.8%) were diagnosed as adenocarcinoma, 10 (14.5%) colitis, 15 (21.7%) juvenile polyposis syndrome. The results of immunohistochemistry technique showed the presence of 16 (23.2%) positive patients for *H. pylori* infection. Of these 16, 13 (81.3%) patients were off those diagnosed with adenocarcinoma and the remaining 3 (18.7%) patients were diagnosed with juvenile polyps. the results of *H. pylori* detection among the different colon polyps and colon cancer diagnosis was showing a statistically significant association for *H. pylori* infection and adenocarcinoma, P value 0.028.

Introduction:

Colorectal cancer (CRC) account as the third most common malignancy and the third most common cause of death owed to cancer in both men and women in the US [1]. CRC mostly arises from adenomatous polyps (adenomas) and from hyperplastic polyps [2, 3]. However, early diagnosis and surgical removal of these polyps have associated with the decreased in the incidence of mortality [4–6]. Therefore, clinician start to focus in recent years on the prevention measures that decreased the incidence of developing colorectal cancer; although, researchers start to explore the role of several infectious organism and their ability to increased or induced colorectal cancer among the populations [7–10]. For instance, many epidemiological studies have linked the infection of *Helicobacter pylori* to colorectal neoplasm either through high prevalence of *H. pylori* seropositivity among CRC or colorectal polyp patients [11–14], or through the presence of bacterial products and their trophic effects on colon mucosa [15–18], Moreover, few studies have linked the presence of *H. pylori* in the stomach or colon with colon cancer and/or polyps [19–24].

It is well known that *H. pylori* predisposes to the development of gastric cancer precursor lesions, thus it has been classified as class 1 carcinogen [25]. A recent meta-analysis of the correlation between *H. pylori* and extra-gastric malignancies revealed a modest statistically significant relationship of *H. pylori* infection with both colon cancer and polyps [26]. *H. pylori* infection linked with colorectal lesions appear to be more common in African Americans compared to the Caucasian population in the US [27, 28].

Epidemiological studies have confirmed a causal relationship between *H. pylori* and gastric cancer, and colonic phenotype of *H. pylori* related intestinal metaplasia (IM) has been associated with gastric cancer

[29]. Thus, association of *H. pylori* in various gastrointestinal system organ cancers has been investigated and *Helicobacter* DNAs were positive in more than 50 percent of hepatobiliary cancer cases [30]. Helicobacter species, which may colonize the biliary tract, have been implicated as a possible cause of hepatobiliary diseases ranging from chronic cholecystitis and primary sclerosing cholangitis to gall-bladder carcinoma and primary hepatic carcinomas [31]. Therefore, the hypothesis that *H. pylori* would also be associated with colon lesions needs to be investigated. In Sudan, no reports addressing this manner were existed. Therefore, the aim of this study was to investigate the presence of *H. pylori* infections among Sudanese patients diagnosed with colon polyps and colon cancer and to correlate between its presence and the type of the lesions.

Materials And Methods:

Sample and data collection

This is a preliminary, descriptive study aimed to investigate the frequency of *H. pylori* infections among Sudanese patients diagnosed with colon lesions. Data were collected from 69 patients attended the National Laboratory and Alrahma Laboratory between February-June 2017.

Formalin fixed paraffin processed blocks collected by the pathologists were obtained for Immunohistochemistry diagnosis of *H. pylori*. Ethical approval was previously obtained by the pathologists of each hospital before colon polyps' biopsies were taken.

Preparation of the formalin fixed paraffin blocks

Four sections from each formalin fixed block were obtained with a thickness of 4 µm using Rotary microtome (LEICA RM2125RT). All sections were de-waxed with two changes of Xylene for 3 minutes and then dehydrated in descending concentrations of Methanol starting from absolute Methanol through 90% and lastly a concentration of 70% for 2 min in each concentration and then washed using distilled water.

Immunohistochemistry diagnosis

Immunohistochemistry diagnosis was performed on all the obtained sections. Known gastric sections containing *H. pylori* infection was used as positive and negative controls; for the negative control the primary antibody incubation step was omitted. All sections were pretreated to retrieve antigens at 97^{o}C for 10 minutes in citrate buffer solution and then sections were blocked by 3% Hydrogen peroxide and absolute Methanol for 20 minutes at humidified chamber. After that sections were blocked in Bovine serum Albumin (Thermo Fisher Scientific, Germany). A rabbit polyclonal antibody ULC3R (BioGenex, USA) (from tissue culture supernatant diluted in PBS, pH 7.6 containing 5% BSA and 0.09% Sodium Azaide) against *H. pylori* was applied for 40 minutes, then wash in buffer solutions for 5 minutes, then polymer solution was applied for 15 minutes, wash in buffer for 5 minutes, chromogen solution was added for 10 minutes, washed in distilled water. Finally, Mayer's Hematoxylin was added for 2 minutes and then sections were blued using running distilled water for 5 minutes. After bluing sections were dehydrated, cleared and mounted in DPX. After sections were prepared, sections were investigated microscopically by

two experts' pathologists blindly without knowing the duplication of slides sections of each patient using X40 lens. Results were recorded into categories of positive and negative results; the dot like shape denoted the coccoid form of the organism as describe previously [32–35].

Statistical analysis:

Descriptive data were analyzed using the Statistical Package for Social Science (SPSS-v20). Pearson Chi-square test was used to test the association of *H. pylori* infection with the different types of lesions.

Results:

Of the 69 patients there were 30 (43.5%) females and 39 (56.5%) males their ages ranged from 21 to 80 years with mean age 47.12 ± 19.79. Of the 69 cases, 44 (63.8%) were diagnosed with adenocarcinoma, 10 (14.5%) colitis, 15 (21.7%) juvenile polyposis syndrome. No statistically significant was observed in the association of gender with the pathological condition of each patient, P value = 0.649. Out of the 69 patients, 16 (23.2%) patients were positive for *H. pylori* infection. 13 (81.3%) patients were diagnosed with adenocarcinoma and 3 (18.7%) patients were diagnosed with juvenile polyps. the correlation between presence of *H pylori* infection and the histopathological condition of patients were positively correlated (P value 0.028) (Table 1).

Table 1
Shows the correlation between gender, immunohistochemistry detection of *H. pylori* with the histopathological diagnosis.

	Histopathological Diagnosis			**Total**	**P value**
	Adenocarcinoma	**Juvenile Polyposis Syndrome**	**Colitis**		
Gender					
Male	23 (58.9%)	10 (25.6%)	6 (15.5%)	39 (56.5%)	0.649
Female	21 (70.0%)	5 (16.7%)	4 (13.3%)	30 (43.5%)	
Total	44 (63.8%)	15 (21.7%)	10 (14.5%)	69 (100%)	
Immunohistochemistry of H. Pylori					
Negative	31 (58.5%)	12 (22.6%)	10 (18.9%)	53 (76.8%)	0.028
Positive	13 (81.3%)	3 (18.7%)	0 (0.0%)	16 (23.2%)	
Total	44 (63.8%)	15 (21.7%)	10 (14.5%)	69 (100%)	

In respect to Immunohistochemistry diagnosis, the bacteria were prominent and easier to detect in the immune-stained sections in several patterns including organisms attached to the epithelial cells or within the superficial mucus were most easily seen and in some cases the bacteria were masked by inspissated mucus or being positioned flat and closely opposed to the epithelial surface. Regarding the

morphological appearance of the organism; *H. pylori* stained brown in color and take a dot and small curved shape in different size (Fig. 1).

Discussion:

The exact role of *H. pylori* in the induction of colon cancer is still a debate between the scientific researcher communities; this is attributed to the controversial results obtained. In previous studies, *H. pylori* were linked to the development of gastric cancer [36], while others reported paradoxical results showing no association between *H. pylori* and gastric cancer susceptibility [37–39]. However, reports from Sudan regarding the possible link between *H. pylori* and colon cancer are scarce. Therefore, in the present study, we examined the presence of *H. pylori* using immunohistochemistry technique on colon polyps and colon cancer lesions of Sudanese patients underwent colonoscopy.

The results obtained from this study showed a positive correlation between the presence of *H. pylori* infection and histopathological diagnosis, as *H. pylori* was prevalent in higher frequency in patients diagnosed with adenocarcinoma compared to those diagnosed as juvenile polyposis syndrome and the result was statistically significant. This result also agrees with studies conducted by Jones et al, and Grahn et al., they investigated the presence of *H. pylori* in 59 adenocarcinomas colon biopsies using immunohistochemistry, and 77 colon and rectum cancer cases using molecular technique, correspondingly [40, 41]. Jones et al., reported that *H. pylori* were detected in 10/59 adenocarcinoma cases representing about 16.9% of the total cancer cases studied [40]. While, Grahn et al., showed that *H. pylori* were present in 27% of the cases; among the studied colon cancer, *H. pylori* were present in 11/42 (26%) cases [41].

Although, for several studies failed to demonstrate any association between *H. pylori* and colon cancer, or even if this microorganism can colonize the colon [42–46]. This could only be attributed to the ability of demonstrating the *H. pylori* bacteria in the various colon lesions of colitis, polyps and adenocarcinoma included in this study, which was achieved by the aid of the immunohistochemistry technique that allowed a better localization of *H. pylori* within the tissue sections.

Interestingly, several theories were proposed regarding the exact role by which *H. pylori* induced colon cancer, one hypothesis is that colon cancer can be induced by the produced *H. pylori* toxins; however, this theory was based only on serological data [39, 47–49]. Furthermore, some studies showed that colitis and colon cancer were also developed in experimental mice models infected with *H. hepaticus* [50]. accordingly, the development of colon cancer seems most likely due to the interaction between toxins produced by the bacteria and the immune cells of the mice [50]. Therefore, the results we obtained from our study showing that *H. pylori* were present, nevertheless, it means that *H. pylori* infection is responsible for the induction and the development of colon cancer, since the presence of *H. pylori* could be encountered as post-cancer incidence. This however, still requires more complicated experimental studies to investigate this hypothesis, yet, the role of *H. pylori* cannot be excluded due to this hypothesis.

Therefore, this preliminary report needs further advanced experimental investigations to enable the determination on the exact mechanisms by which *H. pylori* can induce colon cancer.

Conclusion:

This study was able to demonstrate the presence of *H. pylori* in colon polyps and colon cancer using immunohistochemistry marker, besides the significant association in the presence of *H. pylori* with colon adenocarcinoma, indeed further studies are required to elaborate more in-depth about the exact role of *H. pylori* in the development of colon cancer.

Limitations:

- In this study the sample size studied was relatively small, but still a significant association was observed. A bigger sample size of colon cancer lesions and benign colon lesions; non cancer lesions, should be included in future studies to determine the significant association of *H. pylori* with adenocarcinoma among the Sudanese patients diagnosed with colon cancer.

Abbreviations

DNA: Deoxyribonucleic acid; CRC: Colorectal cancer; IM: Intestinal Metaplasia.

Declarations

Ethics approval and consent to participate

The study ethical clearance was obtained from University of Khartoum, Faculty of Medical Laboratory Sciences ethical review board. Informed consent was obtained from each participant prior to enrollment using writing and verbal informed consent.

Consent to publish

Not Applicable.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not Applicable.

Authors' contributions

AKM, NME, ZAA, FSA, EES and AMME provided conceptual framework for the project. AKM, NME, ZAA, FSA, ETA, NAM, RH, HAO and ESA participated in the preparation of the samples and performed the diagnosis. NSM, AF, EES, MSM, AA, and AMME performed the data analysis and guidance for data interpretation. AA, MSM, ETA, NAM, RH, ESA, AF, and NSM drafted the manuscript. All authors read and approved the final manuscript.

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Figures

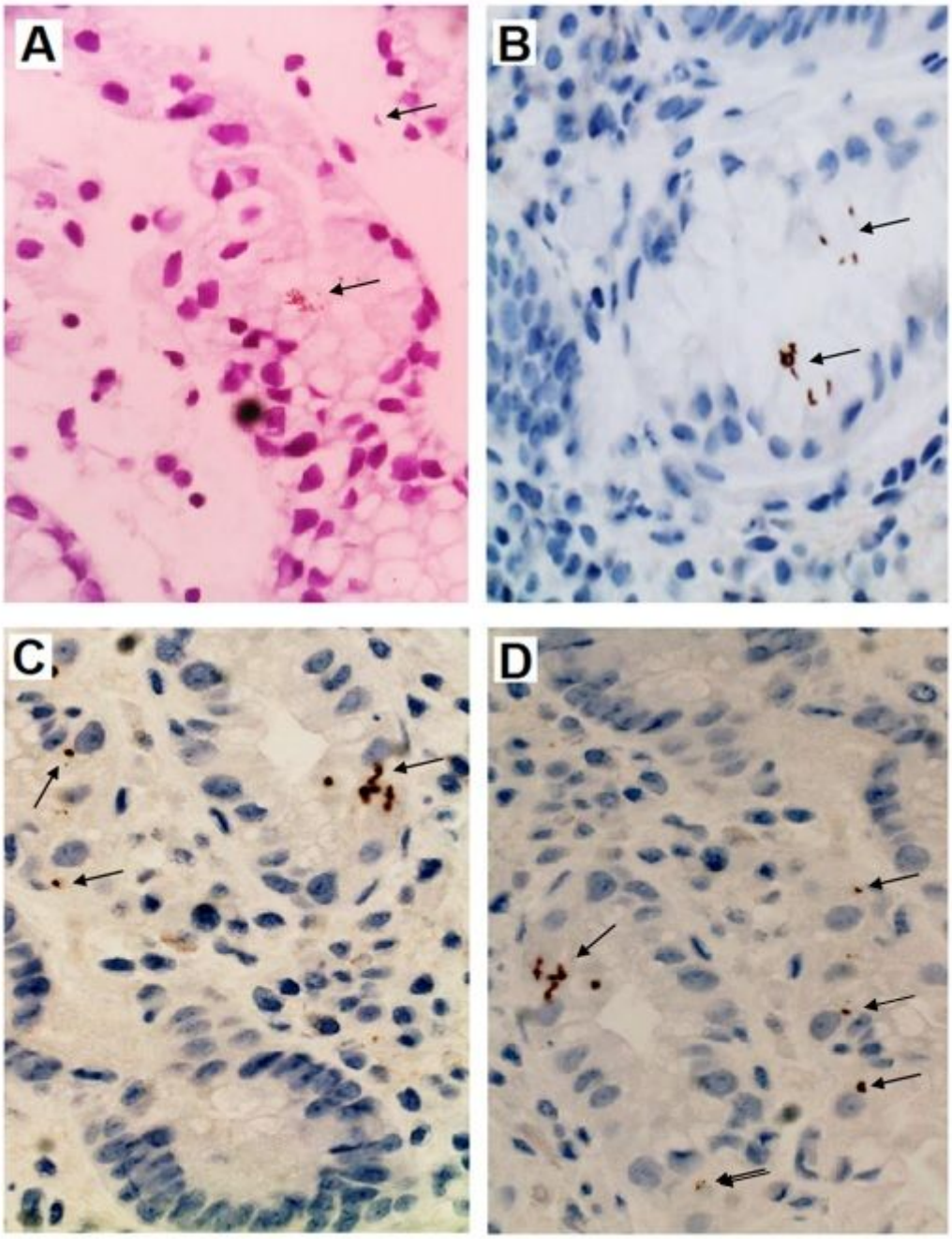

Figure 1

Detection of H. pylori using IHC staining technique. A: indicates positive control tissue section of known gastric biopsy infected with H. pylori. B, C, and D: indicates tissue sections of patients diagnosed with

colon polyps and colon cancer. Black arrows indicate H. pylori stained brown in color and take a dot and small curved shape in different size.