1. Abstract

1.1. Purpose: H. pylori has been classified as class 1 carcinogen, this pathogen was reported to be associated with the gastritis and gastric carcinoma, also in recent years the researchers start to study the role of H. pylori in colorectal cancer. Therefore, the aim of the current study is to evaluate the presence of H. pylori in different lesions including colon polyps and colon cancer.

1.2. Methods: between February-May 2017; sixty-nine Formalin fixed paraffin blocks from different colon lesions were collected, and each one was stained using Immunohistochemistry marker for the detection of H pylori.

1.3. 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. Out of the 69 patients, 16 (23.18%) 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 and the results were statistically significant (0.028).

1.4. Conclusion: This study has demonstrated the presence of H. pylori in colon polyps and colon cancer by IHC methods, albeit with a statistical significance results. Our findings suggest a positive correlation between colon polyps and cancer and H. pylori.

2. Introduction

Colorectal cancer (CRC) is the third most common cancer and the third most common cause of death owed to cancer in both men and women in the US [1]. In its sporadic form, CRC mostly arises from adenomatous polyps (adenomas) and from hyperplastic polyps [2, 3]. However, early detection and removal of colorectal polyps have decreased the incidence of mortality as a result of CRC [4-6]. Recent interests have been directed toward CRC prevention and the possible role of infectious agents in the polyp that leads to cancer [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 [1, 27]. Epidemiological studies have confirmed a causal relationship between H. pylori and gastric cancer [28], and colonic phenotype of H. pylori related Intestinal Metaplasia (IM) has been associated with gastric cancer [28]. 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 [29]. 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 [30]. 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.

3. Materials and Methods

3.1. Sample and Data Collection

A descriptive cross-sectional hospital based study aimed to investigate the frequency of H. pylori infections among Sudanese patients diagnosed with colon lesions. Data were collected from 69 patients attending the National Laboratory and Alrahma Laboratory between February-May 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 biopsy were taken.

3.2. Preparation of The Formalin Fixed Paraffin Blocks

Four sections from each formalin fixed block were obtained with a thickness of 4µm using Rotary microtome (LEICA RM2125RT). All sections were de-waxed in 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.

3.3. Immunohistochemistry Diagnosis

Immunohistochemistry diagnosis was performed on all the obtained sections. A known section containing H. pylori infection was used as a positive control and another section not containing H. pylori infection was used as a negative control. All sections were pretreated to retrieve antigens at 97°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 (power block) (HK 083-5K). A rabbit polyclonal antibody (from tissue culture supernatant diluted in PBS, pH 7.6 containing 5% BSA and 0.09% Sodium azide) 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 Haematoxylin 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 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.

3.4. Data 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 different lesions types.

4. 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 statistical significant was observed in the association of gender with the pathological condition of each patient, p value = 0.649. (Table 1).

Table 1: Shows Correlation between the Gender and Histopathological diagnosis of our study population.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Histopathological diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>juvenile polyposis syndrome</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>15</td>
</tr>
</tbody>
</table>

(p value = 0.649)
In respect to Immunohistochemistry diagnosis, slides sections obtained were diagnosed as 276 sections, and then results of each 4 sections were used to confirm the final diagnosis of H. pylori infection. Although, the bacteria were prominent and easier to detect in the immunostained 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 insspissated mucus or being positioned flat and closely opposed to the epithelial surface, no miss diagnosis was reported for any of the sections duplications.

Out of the 69 patients, 16 (23.18%) 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 patient were positively correlated (p value 0.028).

5. 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. Furthermore, evolving body of evidence showed that H. pylori was linked to the development of the gastric cancer, however the data regarding the possible link between H. pylori and colon cancer is scarce and controversial. Therefore, in the present study, we examined the histopathological blocks of a 69 patients underwent colonoscopy for the presence of H. pylori using immunohistochemistry.

The results obtained from this report showed a positive correlation between presence of H. pylori infection and histopathological conditions, as H. pylori was successfully identified in 13 out of 44 (29.5%) patients diagnosed with adenocarcinoma; while the organism was being present in 3 out of 15 (20%) cases diagnosed as juvenile polyposis syndrome and the results was shown to be statistically significant (p value 0.028). Our results in agreement with a study conducted by Jones and her associates; they investigated the presence of H. pylori in 60 normal colon samples and colorectal neoplasia notably 59 adenocarcinoma using immunohistochemistry; their results showed that H. pylori was detected in 10 out 59 adenocarcinoma cases represent about 16.9% of total cancer cases [31]. Furthermore, Grahn and his colleagues; they investigate the presence of H. pylori in 77 cancer samples consist from 42 colon and 35 rectum cancer cases using molecular technique [21]. Their results showed that H. pylori was present in 27% of their total samples; and in colon cancer the H/ pylori was present in 11 out of 42 (26%) [21]. Additionally, Shmuely and his associates, they investigated whether there is a relationship between CagA seroprevalence and colorectal cancer [32], their results showed a positive correlation between the CagA seropositive patients and increased the risk of both gastric and colon cancer [32]. Although, several studies were able to demonstrated a positive correlation between H. pylori seropositive and increase the incidence of development of colon cancer [33, 34].

On other hand; various studies failed to demonstrate any association between H. pylori and colon cancer, or even more, if these microorganisms can have colonized the colon [35-39]. However, in our report we are able to demonstrate the presence of H. pylori in various colon lesions including colitis, Polyps and adenocarcinoma and this is achieved by the aid of immunohistochemistry which allowed us for 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 the colon cancer can be induced by toxins production and in the case toxins produced by H. pylori; however, this theory based on serological data only [32-34]. Furthermore, some researchers showed that colitis and colon cancer were developed in an experimental mice models in the presence of H. hepaticus [40], however the development of the cancer seems most likely due to the interaction between the bacteria and the immune cells of the mice [40]. Therefore the results we obtained from our study showed that H. pylori was present however, its presences not essentially mean that it is responsible for the induction and development of colon cancer and also we cannot exclude this observation too, therefore this preliminary report needs further investigation using advance molecular techniques to investigate the exact mechanisms by which H. pylori can induced colon cancer.

6. Conclusion
This study was able to demonstrate the presence of H. pylori in colon polyps and colon cancer using immunohistochemistry marker, and there is a positive association in their presence with colon adenocarcinoma. Indeed further studies are required to elaborate more in-depth about the exact role of H. pylori in development of colon cancer.

### Table 2: Shows Correlation between immunohistochemistry results and Histopathological diagnosis of our study population.

<table>
<thead>
<tr>
<th>Immunohistochemistry</th>
<th>Histopathological diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. Pylori</td>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>15</td>
</tr>
</tbody>
</table>

(p value 0.028)
References

30. Leong RW, Sung JJ. Review article: Helicobacter species and hepatobiliary.