Histomorphological Patterns and Helicobacter Pylori status of Gastric tissue biopsy by Giemsa histochemical stain at Bugando Medical Centre, Mwanza Tanzania

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Research Article

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Abstract

Background information: Helicobacter pylori (H. pylori) is one of the major public health problems worldwide, affecting more than 50% of the world's population. In Tanzania, more than 50% of the population is infected by the H.pylori. Identification of its infection is essential for its eradication. There is no specific method for detection of H. Pylori in gastric tissue biopsy at Bugando Medical Centre (BMC) rather than the routine Hematoxylin and Eosin (H&E) stain which has lower detection rate of 11.2%.

Objective: To determine the histomorphological patterns and H. pylori status of gastric tissue biopsy by Giemsa stain at BMC.

Methodology: This was retrospective cross-sectional study involves 300 gastric tissue blocks from January 2020 to January 2022 archived in histopathology laboratory at BMC. Gastric tissue blocks were sectioned, stained by H&E and Giemsa for histomorphological diagnosis and H. pylori status.

Results: A 300 gastric biopsies were identified, of which 53.75% were males and 46.3% were females. Majority of participants were 40 years and above 202 (67.3%), most diagnosed with chronic gastritis 131 (43.7%), followed by gastric adenocarcinoma 115 (38.3%), those aged below 20 years were only 24 (8%). Males were predominantly diagnosed with Gastric adenocarcinoma (35.4%) and Gastric ulcers (4.9%) while majority of females had chronic gastritis (43.2%). by Giemsa stain, H. pylori was detected in only 106 (35.3%). The association between H. pylori status with chronic gastritis, gastric adenocarcinoma, gastric polyps, gastric ulcers and gastric perforation were statistically significance (p-value<0.05).

Conclusion: By the use of Giemsa stain, H. pylori was detected in about one third of gastric tissue biopsy. Most of gastric tissue biopsy with H. pylori infection were those with chronic gastritis. H pylori infection was associated with chronic gastritis, gastric adenocarcinoma, gastric ulcers, and gastric perforation. More studies focus on molecular pathogenesis of H.pylori on cause of various cancer disease like adenocarcinoma and MALT lymphoma is recommended in Mwanza, Tanzania.

Introduction

Helicobacter pylori (H.pylori) is a small, curved, highly mobile, gram negative rod that colonizes the mucus layer of the human stomach particularly in parietal cells of gastric glands and commonly identified in patients with gastric pathologies (1). H.pylori is one of the major public health problems worldwide affecting more than 50% of the world's population and is associated with gastric lesions includes chronic and atrophic gastritis, malignancies such as gastric adenocarcinoma and MALT lymphoma (2).

Infection with H.pylori seems to be a risk factor for gastric cancer (3). A study done among 17 populations from 13 countries to reflect the global range of gastric cancer incidence showed that, there is an approximately six-fold increase in the risk of gastric cancer in populations with H.pylori infection compared to populations that have no infection (3). One study done in Asia countries showed the
incidence rate of gastric adenocarcinoma was directly proportional to the sero-prevalence rate of *H. pylori* infection (4).

Identification of *H. pylori* infection is essential for its eradication (2). Detection of *H. pylori* in gastric tissue biopsy can be done by Immunohistochemical stain, special stains (Giemsa and Warthin Starry stain), or Hematoxylin and Eosin (H&E) stain (2). The gold standard is Immunohistochemistry method which is relatively expensive and requires a well-equipped laboratory (1). Modified Giemsa stain is superior to H&E stain and a cost-effective method of detecting *H. pylori* in gastric biopsies and have shown good sensitivity (85%) and specificity (89%) and can be used to improve the early management of patients infected with *H. pylori* (2).

In our setting at Bugando Medical Centre (BMC) Central Pathology Laboratory, there is no specific method for detection of *H. Pylori* in gastric tissue biopsy rather than the routine H&E stain which has lower detection rate of 11.2% (2). Thus, this study aims to determine the histomorphological patterns and *H. pylori* status of gastric tissue biopsy by Giemsa stain at BMC.

**Methods**

This was a cross-sectional study design involving all archived gastric tissue blocks from January 2020 to January 2022 from the histopathology laboratory at BMC, Mwanza Norwest, Tanzania. A total number of 300 gastric tissue blocks were retrieved from the Central Pathology Laboratory.

A consecutive sampling technique was used in which all gastric tissue blocks registered in January 2020 to January 2022 were recruited into the study. Any Blocks with no tissue section and with unclear labelling were excluded from the study. Retrieved gastric tissue blocks were sectioned and stained with H&E then examined microscopically by the researcher and verified by two registered Anatomical pathologists using a light microscope (OLYMPUS CX21) to establish the histomorphological diagnosis .Moreover, the gastric tissue blocks sectioned and stained by Modified Giemsa stain as per protocol and Standard Operating Procedures together with known case of positive control *H. pylori* tissue blocks then reviewed and verified by two registered Anatomical pathologists using a light microscope (OLYMPUS CX21) to establish the *H.Pylori* status

Data collected were entered into Microsoft excel 2016 for data cleaning and finally transferred to Statistical Package for Social Science software (SPSS) version 20 for statistical analysis. The results were presented by using tables, bar graphs, pie charts. The association between *H. pylori* and histomorphological diagnosis were assessed and p-value < 0.05 considered to be statistical significance.

Ethical approval to conduct the study was obtained from the joint Catholic University of Health and Allied Sciences (CUHAS) and BMC research ethical and review committee. The permission for data collection retrieved blocks, sectioned and reviewed all gastric tissue biopsy was obtained from administration BMC under head of Central Pathology Department in Histopathology Unit.
Results

The total number of 348 gastric tissue blocks of January 2020 to January 2022 were retrieved from Central Pathology Laboratory (CPL), Bugando Medical Centre in Mwanza, Tanzania (see Fig. 1). 24(6.9%) gastric tissue blocks were missing and 324(93.1%) recruited. Out of 324 gastric tissue blocks 16 tissue blocks were over sectioned and no tissue sample left of the block and 8 tissue blocks were inadequate for histological assessment. Therefore, only 300 gastric tissue blocks were sectioned and stained for review of histological diagnosis and presence *H. pylori* infection (see Fig. 1).

Out of 300 study participants whose gastric biopsies were studied, majority were over 40 years which accounted for 202 (67.3%) cases of which most had chronic gastritis 131 (43.7%), followed by gastric adenocarcinoma 115(38.3%). Of the 24 cases aged less than 20 years, 15 (62.5%) had inflammatory lesions. The results showed that gastric lesions were more common with advancing age (see Fig. 2)

Of the 300 gastric cases, the majority were males 161 (53.7%) while females were 139 (46.3%). Out 161 males with various gastric lesions majority had chronic gastritis 71 (44.1%) followed by Gastric adenocarcinoma 57(35.4%). The majority of female were also found to have chronic gastritis 60 (43.2%) while those with gastric adenocarcinoma were 58 (41.7%). Gastric ulcers was also found to be higher in males 8 (4.9%) than in females 2 (1.4%) (See Fig. 3).

Out of 300 gastric tissue blocks sectioned and stained with Giemsa stain for detection of *H. pylori* only 106 (35.5%) had positive results while the remaining 194 (64.7%) stain negative. Therefore the overall detection rate of *H. pylori* infection in gastric tissue biopsy by Giemsa stain was 35.3% (see Fig. 4).

Among 131 tissues biopsies with chronic gastritis, 59(45%) were positive for *H. Pylori* infection. Those with gastric adenocarcinoma only 26(22.6%) were positive for infection. In tissue biopsy with gastric ulcer 90% were positive for *H. pylori* infection and H. pylori was detected in all 4 cases (100%) with gastric perforation

The association between *H. pylori* status with chronic gastritis, gastric adenocarcinoma, gastric polyps, gastric ulcers and gastric perforation was statistically significance (p-value < 0.05) (see Table 1).
Table 1
Association of histomorphological features with H. pylori status of gastric tissue biopsy

<table>
<thead>
<tr>
<th>Histomorphological Diagnosis</th>
<th>H. pylori status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Chronic Gastritis</td>
<td>59(45%)</td>
</tr>
<tr>
<td>Gastric Adenocarcinoma</td>
<td>26(22.6%)</td>
</tr>
<tr>
<td>Gastric polyp</td>
<td>3(11.11%)</td>
</tr>
<tr>
<td>Gastric ulcers</td>
<td>9(90%)</td>
</tr>
<tr>
<td>Normal gastric mucosa</td>
<td>4(57.14%)</td>
</tr>
<tr>
<td>GIST</td>
<td>1(16.67%)</td>
</tr>
<tr>
<td>Gastric perforation</td>
<td>4(100%)</td>
</tr>
<tr>
<td>MALT</td>
<td>0(0.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
</tr>
</tbody>
</table>

**Discussion**

*H. pylori* infection is among the most common bacterial infection which is associated with various gastric lesions including benign and malignant conditions such as inflammatory conditions, ulceration, adenocarcinoma, and MALT lymphoma (2, 5, 6). It is estimated that about 50–90% of the population globally is infected by *H. pylori* (7). The pathological conditions related to *H. pylori* infection are associated with significant morbidity and mortality in both developed and developing countries. This study aims to determine the histomorphological patterns and *H. pylori* status of gastric tissue biopsy submitted at BMC.

About seventy percent of the study participants were aged 40 years and above and most had chronic gastritis as the commonest gastric lesion followed by gastric adenocarcinoma. This was also seen in other studies done at Muhimbili National hospital (1, 6). Patients aged 20 to 39 years had also diagnosed mostly with chronic gastritis followed by gastric adenocarcinoma. These findings were also similar to the study done at Muhimbili National Hospital (1, 6). The similar findings may be due to similarities in study design and probably the same pathogenic mechanism of various gastric conditions. Participants aged less than 20 were the minority and most had diagnosed with chronic gastritis followed by gastric adenocarcinoma. In this age group none had diagnosed with gastric ulcers. The probable reason of small number of young participants in this study, may be explained by the fact that children get contaminated with bacteria and remain asymptomatic through adulthood (8).
The study has pronounced male predominance among patients with chronic gastritis, gastric polyp, and gastric ulcers but females were slightly higher diagnosed with gastric adenocarcinoma, there were equal number of males and females with gastric perforation and GIST. Similar findings were also seen in other studies (2, 7, 9). The reason for difference among male and female on distribution of gastric pathology may be due to differences in late health-seeking behaviors among males as compared to females.

This study had a low frequency of \textit{H. pylori} positive gastric tissue as compared to other studies done elsewhere where the proportion was slightly higher, ranging from 40–60\% in different countries (8, 10, 11, 13). The difference can be due to low seroprevalance of Pylori infection (39.1\%) in general population at Mwanza region, (14) as compared to estimated Tanzania H.Pylori seroprevalance be 65%-79\% in general population (15, 16). Also the methodologies used in this study such as low number of gastric tissue biopsy compared to other studies may result to low frequency \textit{H. pylori} positive gastric tissue (10, 11, 13). However, the prevalence of \textit{H. pylori} in gastric tissue in this study was slight higher than in a study was done at MNH (24.9\%) (6). The reason was due to be due to the small sample size used in the study.

Chronic gastritis was the most frequent diagnosis among the gastric tissues followed by gastric adenocarcinoma. This is closely like another study done at Muhimbili National Hospital (1). The main reason was due to similarity on pathogenic mechanisms because of the same risk factors such as \textit{H. pylori} infection, environmental and dietary in Tanzania.

Inflammatory lesions, particularly chronic gastritis, accounted for most of the gastric lesions, which expressed \textit{Helicobacter pylori} infection. These findings were closely like other studies (2, 6), where \textit{H. pylori} infection was associated with chronic gastritis as one of its major causative factors. Gastric Adenocarcinoma was the next gastric lesion mostly associated with \textit{H. Pylori} infection. Gastrointestinal Stromal Tumor (GIST) was the least of all gastric lesions with \textit{H.pylori} infection like findings in other studies (2, 6, and 9). The similar findings may be due to similar pathogenic mechanism of \textit{H. pylori} infection.

In the study there was a significant association between \textit{H. pylori} infection with chronic gastritis, gastric adenocarcinoma, gastric polyps, gastric ulcers, and gastric perforation. Similar to this study, no study has showed association of \textit{H.pylori} with GIST this is because of the pathogenesis of the GIST type of tumor which arises from intestinal cajal cells which are found in the submucosa and not in the mucosa. (6, 12).

\textbf{Conclusion And Recomendation}

Majority of participants with gastric lesions were adults with 40 years and above, and most presented with chronic gastritis. Males with gastric lesions were more than females, most presented with chronic gastritis and gastric ulcers. About one third of gastric tissue biopsy detected with \textit{H. pylori} by Giemsa stain. Most of gastric tissue biopsy with \textit{H. pylori} infection were those with chronic gastritis. \textit{Helicobacter pylori} infection persistance among adult patients may associate with chronic gastritis, gastric adenocarcinoma, gastric polyps, gastric ulcers and gastric perforation diseases.
More studies focus on molecular pathogenesis of \textit{H.pylori} on cause of various cancer disease like adenocarcinoma and MALT lymphoma is recommended. Also differences on environmental or genetic factors on how it can interfere with pathogenesis of \textit{H.Pylori} on gastric cancer with comparison to other geographical area of high incidence of \textit{H.plori} and gastric cancer disease is recommended in our Lake zone region, Tanzania.

\textbf{Limitation}

The gold standard Immunohistochemical detection method for \textit{H. pylori} in tissue biopsy was not done due to shortage of fund and availability of reagents.

\textbf{Declarations}

\textbf{CONFLICT OF INTEREST}

None declared

\textbf{AUTHOR’S CONTRIBUTION}

OO, MS, JB and EE participated in the design of the work. MS and OO participated in retrieved gastric tissue blocks and patient clinical data. MS, ER and JB performed tissue section and staining with routine Hematoxylin and Eosin then followed by Giemsa stain. OO and EE reviewed gastric tissue slide for histomorphological diagnosis and Pylori status. OO, MS, EE, JB analyzed and interpreted the data. OO, EE, ER, HJ, MS, JB wrote the first draft of the manuscript. OO, EE and HJ critically revised the final draft of the manuscript. All authors read and approved the final version of the manuscript

\textbf{ACKNOWLEDGEMENT}

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\textbf{References}


Archived gastric tissue blocks.  
(January 2020 – January 2022)  
(n = 348)

Tissue blocks retrieved.  
(n = 324)

Excludes (n = 24)  
Missing blocks

Excluded (n = 24)  
- Had no tissue left 16.  
- Inadequate for diagnosis = 8

Total gastric tissue blocks sectioned and stained with Giemsa stain for distribution of histomorphological diagnosis by age, sex, and detection of *H. pylori* (n = 300)

Figure 1  
Study flow chart
Figure 2

Histomorphological features of gastric lesions by Age.
Figure 3

Shows distribution of histomorphological features of gastric lesions by sex
Figure 4

H. pylori status by Giemsa stain in gastric tissue biopsy

Figure 5

Hematoxylin and Eosin section showing different Gastric tissue biopsy with histomorphological diagnosis. A-Gastric ulcers pointed by black arrow (x10hpf). B-Chronic gastritis with numerous lymphocytes, plasma cells accumulation pointed by black arrow. C- Infiltrating Gastric adenocarcinoma of intestinal type pointed by black arrow (x 10hpf)

Figure 6

Giemsa stain showing different Gastric tissue biopsy with H. pylori infection in gastric pits pointed by black arrow (x40hpf)