RESEARCH ARTICLE

**Editorial Process:** Submission:07/25/2024 Acceptance:01/21/2025

# Detection of *H-Pylori* in the Explanted Liver Tissue and the Enlarged Perihepatic Lymph Nodes of Cirrhotic Patients with Decompensated End-Stage Liver Disease Recruited for Liver Transplantation

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## Abstract

**Background:** Helicobacter pylori bacteria colonize the gastric mucosa and contribute to the occurrence and development of gastrointestinal diseases. According to the WHO, *H. pylori* bacteria are considered class I carcinogen. **Objectives:** To detect Helicobacter pylori organisms by IHC expression of anti-H. Pylori antibodies in the explanted liver tissue; and enlarged perihepatic lymph nodes of cirrhotic liver; to detect any relation between the presence of the organism and histopathological findings in the liver tissue. **Materials and Methods:** This retrospective cross-sectional study included forty cases of cirrhotic patients with decompensated end-stage liver, recruited for liver transplantation based on combined clinical, radiological, and histological data. Samples were immunohistochemically analyzed for anti-H-Pylori antibodies to detect Helicobacter pylori organisms in the explanted liver tissue and enlarged perihepatic lymph nodes. The presence of the organism was correlated with clinic-pathologic variables. **Results:** Eighty-five percent (34 cases) and seventy percent (28 cases) of cases were positive for anti-H-Pylori antibodies in the liver and lymph nodal tissues, respectively. More than eighty percent (14 cases) and half of the studied cases (8 cases) showed dysplasia in liver tissue expressing anti-H-Pylori-antibody in the liver tissue and the lymph nodes, respectively. All HCC cases expressed anti-H-Pylori antibody in the liver tissue and the lymph nodes. The relation between anti-H-Pylori antibody expression in lymph nodes and the presence of dysplasia or HCC in liver tissue was statistically significant (p-value = 0.037 and p-value = 0.041 respectively). **Conclusion:** Our results conclude that there is a pathogenic role of extra-gastric H-Pylori colonization in lymph nodal tissue and in liver tissue, and it may be preventable by treating *H. pylori*, especially if treatment can be started very early.

**Keywords:** Helicobacter pylori- hepatocellular carcinoma- anti-*H-Pylori* polyclonal antibody- immunohistochemistry

*Asian Pac J Cancer Prev,* **26 (1)**, 181-187

## Introduction

Gastritis and peptic ulcer are strongly related to Helicobacter pylori (*H. pylori*) infection, which is also considered a main factor in the development of gastric carcinoma and gastric lymphoma [1, 2]. H-Pylori is a gram-negative bacterium that infects more than half of the people worldwide, with incidence rates between 20 and 40% in advanced countries and up to 90% in underdeveloped ones [3, 4]. Matrix metalloproteinases (MMPs) contribute to the pathogenesis of *H. pylori* infection by causing degradation of the extracellular matrix (ECM), which is involved in the progression of different inflammatory diseases. Additionally, *H. pylori* infection has a systemic effect through its role in increasing the activity of proinflammatory cytokines [5, 6]. Finding *H-Pylori* in the liver tissue and the corresponding rise in vascular mediators and inflammatory markers plays a role in the progression of hepatitis C infection, autoimmune liver disorders, cirrhosis, and hepatocellular carcinoma, which is the fourth most prevalent cause of cancer-related death worldwide and constitutes the most common cause of mortality-related cancer in Egypt [7-11]. Portal hypertension-related gastropathy is more prominent in *H. pylori*-infected patients. Therefore, treatment of *H. pylori* infection is recommended in cirrhotic patients to inhibit

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the severity of portal hypertension gastropathy [12]. The WHO classified *H. pylori* bacteria as a class I carcinogen, colonizing the stomach, passing through the mucosa, and transferring to the lymph nodes, thereby chronically triggering the immune system [13].

*Objectives*

The rationale of this work was to: 1- Detect the presence of *H. pylori* organisms in liver tissue and enlarged perihepatic lymph nodes of cirrhotic patients with decompensated end-stage liver disease by immunohistochemistry. 2- Statistically correlate its presence with clinicopathological parameters. 3- Determine its pathological role in liver tissue, which may be preventable through early treatment of *H. pylori* infection.

## Materials and Methods

After approval by The Kasralainey Research Ethics Committee (REC) (code: MS-73-2021) on 20 -5- 2021, Forty archival blocks of formalin-fixed, paraffin-embedded explanted liver tissue and enlarged perihepatic lymph nodes were taken from cirrhotic patients with decompensated end-stage liver disease recruited for liver transplantation based on combined clinical/radiological and histological data were collected for this retrospective study from the Anatomic Pathology Department at National Hepatology and Tropical Medicine Research Institute (NHTMRI) during the period from January 2016 to December 2020.

Exclusion criteria: Poorly fixed, inadequately depicted tumors; and tumors with significant necrosis or cautery artifacts were excluded from this study. Data was collected from the pathology reports regarding personal data (age and sex), clinical & laboratory data [blood group, presence of hepatitis, viral markers, tumor markers (CA125, CA19.9, CEA and AFP), bilharzial Ag/Ab and autoimmune profile (ASMA, ANA and AMA)] and histopathological findings in the liver (Cholestasis, Dysplasia and HCC) that have been revised to confirm the diagnosis as well as the etiology of cirrhosis. Four micron-thick sections were re-cut from all chosen blocks and stained with Hematoxylin and eosin (H&E) for histopathological assessment and Masson's trichrome stain of liver tissue to confirm the diagnosis of liver cirrhosis as well as Immunohistochemical staining using the Dako immune auto-stainer by anti-Helicobacter Pylori rabbit polyclonal antibody (catalog no. E3011, rabbit source, polyclonal) used for detection of H-Pylori antigen in the liver and lymph node tissues. The slides were examined under a light microscope (Zeiss, Germany) by three pathologists. The histopathological and immunohistochemical findings were recorded. The *H. Pylori* polyclonal antibody positivity was interpreted as brown-colored particles within the liver tissue; in the Kupffer cells existing along the sinusoids and in the hepatocytes inside the hepatic cytoplasm [14, 15] as well as within the lymph node tissue; in the paracortical areas of the lymph nodes which suggest that H-Pylori invades the gastric mucosa to translocate to the draining lymph nodes [16]. The cases were classified according to the presence or absence of anti-H-Pylori antibody staining as negative or positive [17]. The associations of anti-Helicobacter Pylori antibody expression in the liver and lymph nodal tissues with the constitutional data (age and sex), clinical & laboratory data [blood group, hepatitis viral markers, tumor markers (CA125, CA19.9, CEA and AFP), bilharzial Ag/Ab and autoimmune profile (ASMA, ANA and AMA)] and histopathological findings in the liver (Cholestasis, Dysplasia and HCC) as well as the etiology of cirrhosis were evaluated and assembled on a Master chart and were analyzed by using SPSS version 25. Simple descriptive statistics were used (arithmetic mean and standard deviation) to summarize quantitative data and frequencies were used for qualitative data. The bivariate relationship was displayed in cross-tabulations and a comparison of proportions was performed using the chi-square test. The t-independent test was used to compare normally distributed quantitative data. All p-values were two-sided and those $\leq 0.05$ were used to denote statistical significance. Microscopic photos were captured using a digital camera attached to an Olympus microscope model BX 53.

## Results

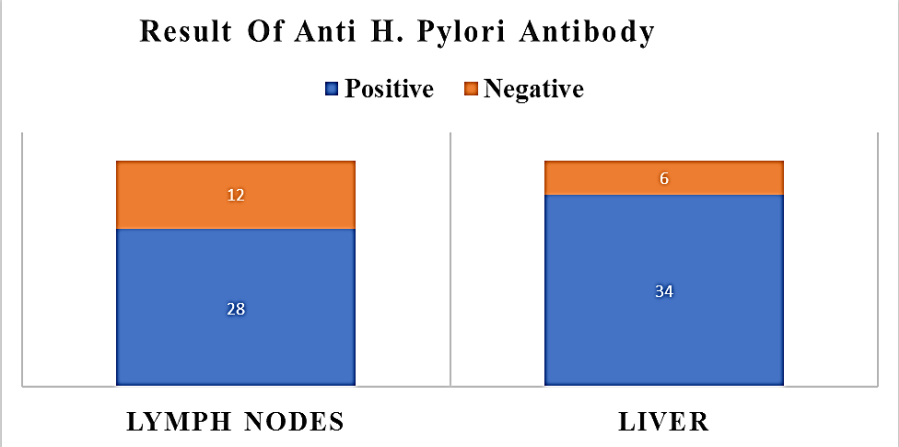
Forty archival blocks of formalin-fixed, paraffin-embedded explanted liver tissue and enlarged perihepatic lymph nodes were taken from cirrhotic patients with decompensated end-stage liver and recruited for liver transplantation over 24 months. About 90% (36 cases) were males and 4 were females. Ages ranged from 20 to 60 years, with a mean age of approximately 44 years; 70% of patients were older than forty years. Forty percent of cases were blood group B. More than half of the cases were HCV-positive, and all patients were positive for Epstein-Barr virus (EBV) IgG and Cytomegalovirus (CMV) IgG. More than 70% of cases were negative for bilharzial Ag/Ab (antigen-antibody). AFP, CEA, CA19.9, and CA125 levels were within normal in more than 50% of cases; with more than half of them were positive for anti-smooth muscle antibody (ASMA), over 72% were negative for antinuclear antibody (ANA), and all were AMA-negative. Approximately 60% of cases showed less than 5% steatosis, while 15% showed more than 66% steatosis. About 55% of cases showed cholestasis, 40% displayed liver cell dysplasia, and 25% exhibited hepatocellular carcinoma (HCC). More than 50% of cases were cirrhotic due to HCV (Figure 1).

Eighty-five percent of cases were positive for anti-H pylori antibodies in the liver tissue; while seventy percent were positive in lymph node tissues (Figures 2 & 3). The correlation between anti-*H. Pylori* antibody expression in liver and lymph nodal tissue; and histopathological findings in liver tissue was analyzed using the Chi-square test (Tables 1 & 2).

Approximately 80% of cases expressed anti-*H. Pylori* antibodies in the liver were over the age of 40 (p-value = 0.006). All cases with blood groups A and O expressed anti-*H. Pylori* antibody in liver tissue, while less than forty percent of blood group B cases did not express anti-*H.*

Table 1. The Correlation between both Anti-*H. Pylori* Antibody Expression and Histopathological Findings in the Liver Tissue

| Histopathological findings in the liver tissue | Anti-H. Pylori Antibody -ve (n=6, 15%) | Anti-H. Pylori Antibody +ve (n=34, 85%) | Total (n=40, 100%) | p-value |
|---|---|---|---|---|
| Cholestasis | | | | |
| Absent | 2 (11.1) | 16 (88.9) | 18 | 0.673 |
| Present | 4 (18.2) | 18 (81.8) | 22 | |
| Steatosis | | | | |
| < 5% | 6 (27.3) | 16 (72.7) | 22 | Not Applicable |
| 5-33% | 0 (0) | 7 (100) | 7 | |
| 34 - 66% | 0 (0) | 5 (100) | 5 | |
| > 66% | 0 (0) | 6 (100) | 6 | |
| Dysplasia | | | | |
| Absent | 4 (16.7) | 20 (83.3) | 24 | 0.715 |
| Present | 2 (12.5) | 14 (87.5) | 16 | |
| HCC | | | | |
| Absent | 6 (20) | 24 (80) | 30 | 0.307 |
| Present | 0 (0) | 10 (100) | 10 | |




Graph 1. Distribution of Cases According to Anti-*H. Pylori* Antibody Expression in Liver and Lymph Node Tissues.

*Pylori* antibody in the liver tissue (p-value = 0.006). All cases with 5-33%, 34-66%, and more than 66% steatosis in liver tissue expressed anti-H-Pylori antibody in liver tissue. More than 80% of the studied cases with cholestasis or dysplasia in liver tissue expressed anti-H-Pylori antibody in the liver tissue (p-values of 0.673 and 0.718, respectively). All cases with HCC expressed anti-*H. Pylori* antibody in liver tissue (p-value = 0.307).

All cases with 34-66% steatosis in liver tissue expressed anti-H-Pylori antibody in lymph nodes. More than 80% of the studied cases with cholestasis in liver tissue expressed anti-H-Pylori antibody in lymph nodes (p-value = 0.093). Half of the studied cases with dysplasia in liver tissue expressed anti-*H. Pylori* antibody in lymph nodes (p-value = 0.037). All cases with HCC expressed anti-*H. Pylori* antibody in the lymph nodes; while 40% of cases without HCC did not express anti-*H. Pylori* antibody in lymph nodes (p-value = 0.041).

## Discussion

One of the main causes of cirrhosis is infection with the hepatitis C virus (HCV) [11], which aligns with our study results, where over fifty percent of cases were due to HCV-related cirrhosis. The relationship between age and HCV-positive patients was statistically significant ($p < 0.001$); more than 90% of HCV-positive cases were over the age of forty, consistent with Reid et al.'s findings that HCV infection is of particular concern in older adults, likely due to an impaired immune system [18]. In our study, a significant correlation was found between sex and HCV-positive patients ($p < 0.001$), with more than 50% of HCV cases were males. Similarly, El-Ghitany and Farghaly reported a higher HCV prevalence in males (16.1%) than in females (13.4%) ($p = 0.000038$) [19].

In this work, 85% and 70% of cases expressed anti-H-Pylori antibodies in liver and lymph nodal tissues, respectively. This aligns with findings by Sakr et al., who reported that 62% of cases showed *H. pylori* immunopositivity in liver tissue [20], and by Ito et al.,

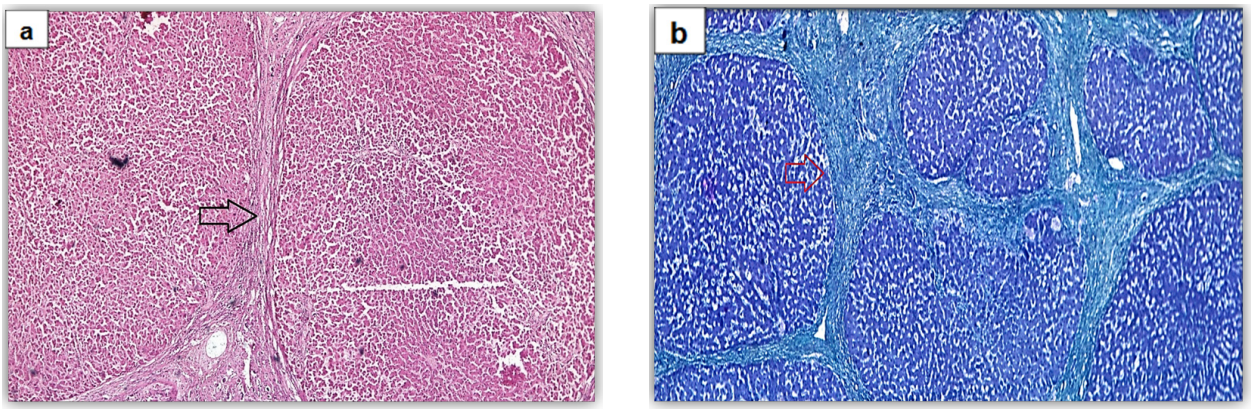



Figure 1. a- Case of liver cirrhosis showed multiple cirrhotic nodules surrounded by fibrous septate (arrow); (H&E X400 original magnification). b- Case of liver cirrhosis showed positive Masson's trichrome stain of the fibrous septate (arrow); (X400 original magnification).

who noted that *H. pylori* was captured by macrophages in the gastric mucosal layer in 87% of patients and the paracortical areas of gastric lymph nodes in 63% of patients [16]. In this study, all cases with 5-33%, 34-66%, and more than 66% steatosis in liver tissue expressed anti-*H. Pylori* antibody, consistent with Dogan et al., who found that *H. pylori*-positive patients were more frequently diagnosed with fatty liver (p = 0.02) [21], and with Sumida et al., who reported a higher prevalence of NASH in *H. pylori*-positive patients (80.8%) compared to *H. pylori*-negative individuals (50.7%, p = 0.008) [7]. However, Okushin et al. and Baeg et al. found no association between *H. pylori* infection and NAFLD [22, 23].

There was no significant correlation between anti-*H. Pylori* antibody expression in liver tissue and the presence of cholestasis (p-value = 0.673), despite over 80% of cases with cholestasis expressed anti-*H. pylori* antibody (Graph 1). This contrasts with Popescu et al.'s report of a significant association between *H. pylori* infection and hepatobiliary diseases [24]. In our study, half of the cases with dysplasia in liver tissue expressed anti-*H. Pylori* antibody in lymph nodes (p-value = 0.037), a correlation not reported in other comparative studies.

We found a significant correlation between anti-*H. Pylori* antibodies in lymph nodes and HCC (p-value = 0.041). All cases of HCC showed anti-*H. Pylori* antibodies in lymph nodes; while 40% of cases without HCC did not express anti-*H. Pylori* antibodies in lymph nodes. Similarly, Mekonnen et al. identified *H. pylori* infection as a risk factor for HCC, finding *H. pylori* in 61.7% of HCC patients (p-value < 0.01), considering *H. pylori* an additional factor contributing to HCC [25].

Abdel Razik et al. also found that *H. pylori*-infected patients had a higher incidence of HCC (p < 0.05) and suggested that *H. pylori* infection triggers a transforming growth factor B1-dependent oncogenic pathway, altering the balance between hepatocyte proliferation and apoptosis [8]. Similarly, Madala et al. concluded that HCC development is significantly related to *H. pylori* infection and reported that co-infection with HCV and *H. pylori* increases the risk of developing HCC [26]. In this study, all cases with blood groups A and O expressed anti-*H. Pylori*

Table 2. The Correlation between Anti-*H. Pylori* Antibody Expression in Lymph Node and Histopathological Findings in the Lymph Nodes

| Histopathological findings in the liver tissue | Anti-H. Pylori Antibody -ve (n=12, 30%) | Anti-H. Pylori Antibody +ve (n=28, 70%) | Total (n=40, 100%) | p-value |
|---|---|---|---|---|
| Cholestasis | | | | |
| Absent | 8 (44.4) | 10 (55.6) | 18 | 0.093 |
| Present | 4 (18.2) | 18 (81.8) | 22 | |
| Steatosis | | | | |
| < 5% | 8 (36.4) | 14 (63.6) | 22 | Not Applicable |
| 5-33% | 2 (28.6) | 5 (71.4) | 7 | |
| 34 - 66% | 0 (0) | 5 (100) | 5 | |
| > 66% | 2 (33.3) | 4 (66.7) | 6 | |
| Dysplasia | | | | |
| Absent | 4 (16.7) | 20 (83.3) | 24 | 0.037 |
| Present | 8 (50) | 8 (50) | 16 | |
| HCC | | | | |
| Absent | 12 (40) | 18 (60) | 30 | 0.041 |
| Present | 0 (0) | 10 (100) | 10 | |

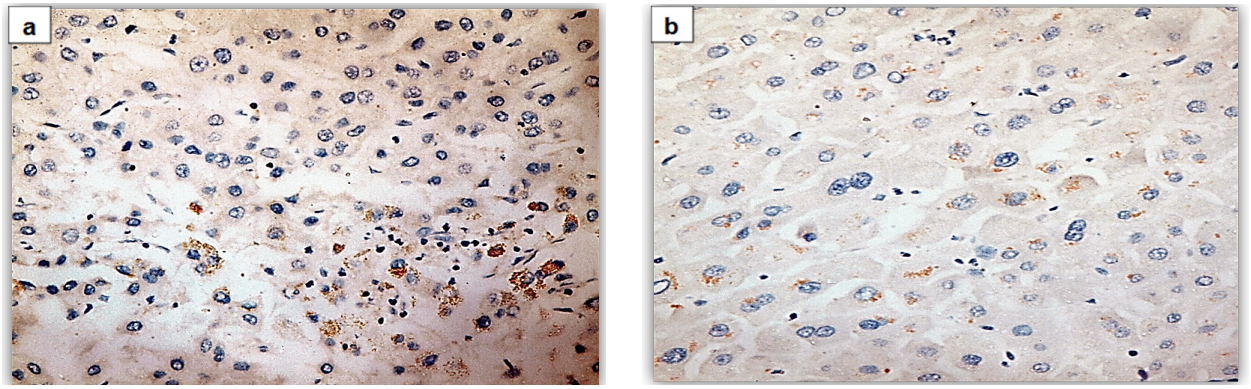



Figure 2. a- Hepatic tissue; anti-*Helicobacter Pylori* antibody positive, granular cytoplasmic brown-staining staining (IHC X400 original magnification). b- Hepatic tissue; anti-*Helicobacter Pylori* antibody positive, granular cytoplasmic brown-staining staining (IHC X400 original magnification).

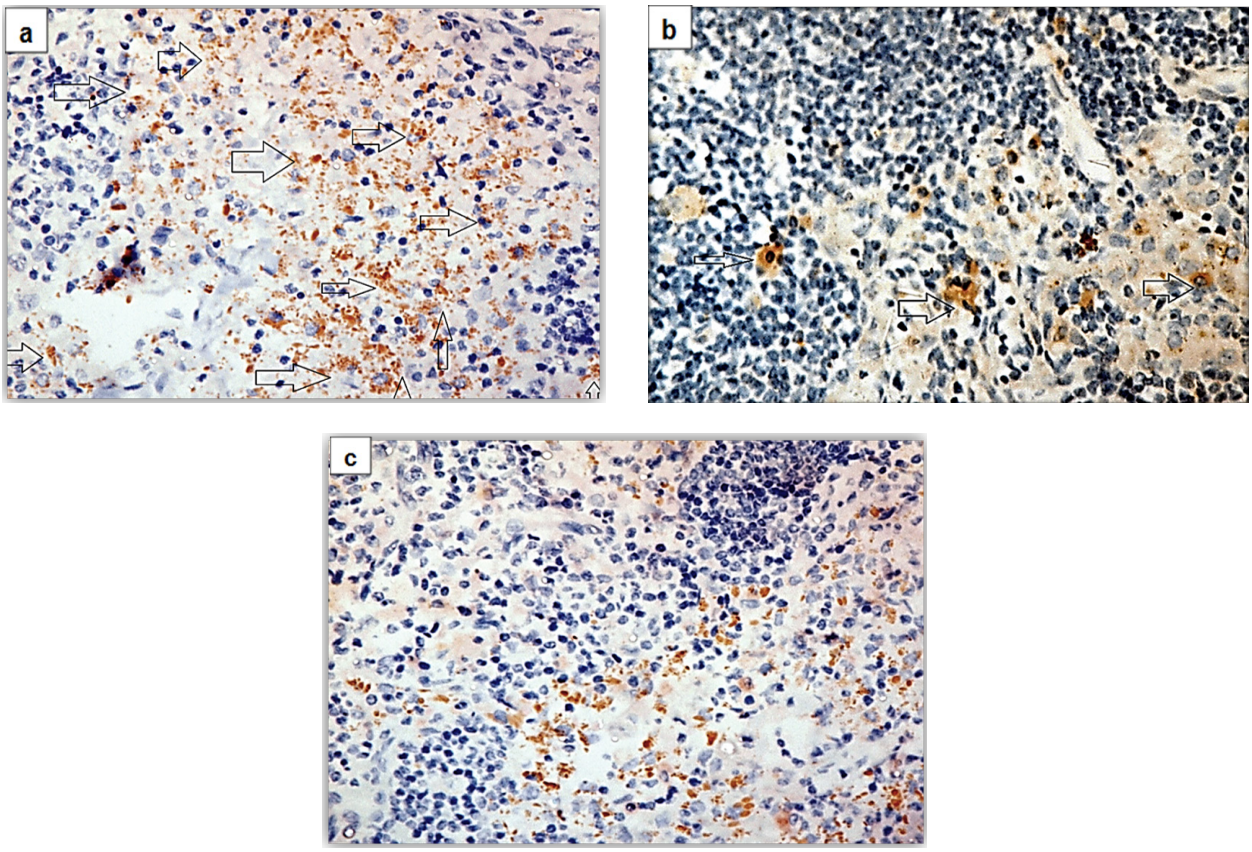



Figure 3. a- Lymph node; anti-Helicobacter Pylori antibody positive, granular cytoplasmic brown-staining staining in paracortical area (IHC X400 original magnification).b- Lymph node; anti-Helicobacter Pylori antibody positive, granular cytoplasmic brown-staining staining in paracortical area (IHC X400 original magnification). c- Lymph node; anti-Helicobacter Pylori antibody positive, granular cytoplasmic brown-staining staining in paracortical area (IHC X400 original magnification).

antibody in liver tissue, while less than forty percent of blood group B cases did not, showing a statistically significant relationship between anti-*H. Pylori* antibody in liver tissue and blood groups ($p = 0.006$). This finding is consistent with Chakrani et al.'s meta-analysis, which concluded that the O blood group has a higher incidence of *H. pylori* infection compared to non-O blood groups ($p < 0.001$) [27].

In conclusion, extra-gastric translocation of *H. pylori* was observed in liver and lymph nodal tissues. Males over forty were the most affected. HCV was the primary cause of cirrhosis among studied cases. All cases free of hepatocellular carcinoma (HCC) did not express anti-H-Pylori antibodies in the liver. There was a significant association between anti-H-Pylori antibody expression in lymph nodes and the presence of dysplasia or HCC in liver tissue. No significant association was observed between the degree of steatosis and the presence of dysplasia or HCC with *H. pylori* detection in liver tissue. There is a potential pathological role of extra-gastric *H. pylori*

colonization in lymph nodal tissue impacting liver tissue, which may be preventable by early treatment of *H. pylori*. We recommend monitoring cirrhotic patients, especially those with concurrent gastric *H. pylori* infection.

## Author Contribution Statement

All authors contributed significantly to the study: Abeer Mohammed Amal participated in reading the slides, interpreting results, analyzing data, and writing the manuscript. Ahmed El-Hennawy assisted in revising the manuscript. Ehsan H. Hassan contributed to the study design, data collection, result interpretation, data analysis, and reading of the slides. Amr Abdelraouf and Ahmed Abdelhaleem contributed to the research concept, data collection, and result interpretation. Basma Mohamed and Mariam Georgey assisted with data collection, slide reading, and result interpretation. Maha Emad participated in writing and revising the manuscript.

## Acknowledgements

We thank the staff of the Anatomic Pathology Department at Kasr Al-Ainy and the National Hepatology and Tropical Medicine Research Institute (NHTMRI), as well as the Surgical and Tropical Department staff at NHTMRI, for their continuous help and support.

*Ethical Declaration*

This study protocol was approved by the Kasr Al-Ainy Research Ethics Committee (REC) of the Faculty of Medicine, Cairo University, and conducted following ICH GCP standards, as well as relevant local and institutional regulations and guiding principles governing REC operations (Code: MS-73-2021, approved on 20-5-2021).

*Data Availability*

Data are available upon request, following institutional regulations, and with official permission.

*Conflict of Interest*

The authors declare that they have no conflict of interest.

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