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Serological Evidence of *Helicobacter Pylori* Exposure in Asymptomatic University Students

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ABSTRACT

Helicobacter pylori (H. pylori) infection is one of the most common chronic bacterial infections globally, closely linked to multiple gastrointestinal diseases, suffering from gastritis, peptic ulcer disease, and gastric cancer. Epidemiological data about H. pylori infection in Libya are rare, even more so among asymptomatic populations. Note: This cross-sectional study examines the seroprevalence of *H. pylori* antibodies among university students in Libya and their potential associations with gastrointestinal symptoms and selected medical history variables. The study involved 178 asymptomatic students from five faculties at Misurata University. Demographic and clinical information were obtained using a structured questionnaire, and blood samples were collected via a finger prick. Serological detection of anti-H. There was a test for anti-HP. In all, 10 participants were positive for *H. pylori* antibodies, with an overall seroprevalence of 5.6%. There was no statistically significant gender difference in seropositivity amongst females (5.5%), and males (6.3%) were found to be comparable. Also, no association was found between infection status and gastrointestinal symptoms or medical history variables, including anaemia, vitamin B12 deficiency, allergy, previous surgery to the gastrointestinal tract, and psychological stress. These findings show that asymptomatic university students had a lower sero-prevalence of *H. pylori* infection and also suggest the need for larger epidemiological studies to get more information about the extent of this infection in Libya.

1. INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is the most common chronic human infection worldwide, which can be clinically relevant for a range of gastroduodenal diseases including chronic gastritis, peptic ulcer disease, and gastric cancer. Early infection commonly occurs in childhood and persists when untreated; incidence rates and prevalence rates for this infection group vary widely between geographical spaces (PD & KJS, 2020; Polse et al., 2024).

In Libya, epidemiological data on *H. pylori* seroprevalence are limited; therefore, sound research on the population level is a major guide for a baseline understanding of infection dynamics (Almehdawi & Ali, 2016; Najia Mahdawi et al., 2024). Hence, the objective of this study was to determine the seroprevalence of *H. pylori* immunoglobulin G antibody among a nondescript Libyan cohort, which would help to better define potential epidemiological trends at population level (Almehdawi & Ali, 2016). Determining the sero-epidemiology of *H. pylori* is especially important in regions such as Libya, where public health strategies for both eradication and prevention rely on obtaining adequate prevalence data (Alkali et al., 2020). Here, an enzyme-linked immunosorbent assay was used to detect anti-H. pylori IgG in venous blood samples, which is a method that can be considered the gold standard for epidemiological surveys (Tshibangu-Kabamba et al., 2021). Although serological methods were not suitable to be used for differentiating active from past infections or in some cases of early *H. pylori* infection, the presence of IgG antibodies against *H. pylori* which is cost effective and more convenient could be utilized to screen larger populations (Omosor et al., 2017) This serological method thus serves as an important marker of potential cumulative exposure in asymptomatic populations and provides insight into the long-term trends rather than acute incidence. More specifically, this cross-sectional study outlines the prevalence of *H. pylori* infection in an asymptomatic Libyan population using serodiagnostic assays to measure exposure (AHMED & Fujeyra, 2024; FORMAN et al., 1993). This is especially relevant as most *H. pylori* infections are established in early childhood and can remain for decades, often in the absence of symptoms (Al-Brefkani et al., 2021).

2. MATERIALS AND METHOD

The researchers developed a structured questionnaire based on the objectives of the study and relevant literature to collect demographic and clinical information from participants." Detection of antibodies against *Helicobacter pylori* was performed using a commercial rapid immunochromatographic cassette test (*Helicobacter pylori* Antibody Rapid Test, ARTRUST, Catalog No. CA02-11-322), which can be used with whole blood, serum, or plasma according to the manufacturer's instructions.

Study Design and Population

Methodology: A cross-sectional study was performed among the students of Misurata University. In the study period, a total of 176 students from five faculties of the university were selected by simple randomization. The faculties included the Faculty of Economics and Political Sciences, the Faculty of Engineering, the Faculty of Environment and Natural Resources, Faculty of Education & Health. Participation was voluntary, and informed consent was obtained from all participants before participating.

Questionnaire Data Collection

At the point of sample collection, a structured questionnaire was administered to each participant. Demographic, clinical, and behavioral data Questionnaire Reliable demographic information was collected on sex, faculty affiliation, recent diarrhea (within the month before stool collection), previous diagnosis of *H. pylori* infection, use of antibiotics in the last two months before stool collection and use of antacid or anti-ulcer medications (within two weeks before sample collection); selected dietary habits were also recorded. But to avoid missing information, they filled out questionnaires under supervision.

Sample Collection

All participants were subjected to sterile finger-pricking of their finger. Following disinfection of the fingertip, a single-use sterile lancet was used to obtain a small amount of blood that was immediately used for serological testing.

Detection of Helicobacter pylori Antibodies

Serological detection for antibodies against Helicobacter pylori was performed by a commercial rapid immunochromatographic assay (*Helicobacter pylori* Antibody Rapid Test. The test is a qualitative lateral flow immunoassay for the detection of anti-H. pylori antibodies in serum, plasma or whole blood samples.

For each test, a small amount of the blood sample was added on the sample well of test cassette and followed by addition of supplied buffer as per manufacturer's instructions. Capillary action moves the mixture along the membrane. If the sample contains antibodies against *H. pylori*, they will bind to conjugated recombinant *H. pylori* antigens and form an antigen–antibody complex that is captured by immobilized antibodies at the test region to generate a colored line visible. A control line means the test has worked. Subsequent to the incubation period described above, results were interpreted as positive or negative depending on whether or not there was a test line present. To guarantee the accuracy of the results, all procedures were performed in strict accordance with manufacturer's instructions. A positive result was defined by the appearance of both control and test lines on the test cassette, while a negative result was defined by the appearance of the control line only.

3. ETHIC APPROVAL

The relevant research ethics committee at Misurata University provided ethical approval for this study and the study had started after obtaining the approval. The research in human participants was performed in accordance with the ethical standards of the institutional research committee. Informed consent was obtained from all participants prior to sample collection and questionnaire administration, and participation in the study was entirely voluntary. Data were collected in a confidential manner and used exclusively for research purposes.

4. RESULT

This cross-sectional study included 178 asymptomatic participants. The serological assay indicated that 10 individuals were positive for Helicobacter pylori antibodies, while 168 were negative, resulting in an overall seroprevalence of 5.6% in the study population.

Demographic Characteristics

Of the participants, 146 (82.0%) were female and 32 (18.0%) were male. Out of the above, seropositivity was found among 8 (5.5%) females and 2 (6.3%) males showing a similar prevalence across genders. Participants did not differ markedly in the rates of infection by gender **(Table 1)**.

Gastrointestinal Symptoms

While the study population was composed predominantly of non-symptomatic individuals, a minority of subjects noted occasional abdominal complaints. Among the most common symptoms were stomach pain or burning sensation, bloating, nausea, and excessive belching or heartburn. Some of the seropositive individuals complained of these symptoms, but similar complaints were prevalent even among seronegative participants **(Table 2)**.

For example, 7 infected individuals reported one of "stomach pain" or a "burning sensation", while the sensations of "bloating" and "nausea" were reported by 4 and 5 subjects, respectively. Other symptoms, including vomiting, anorexia (lack of appetite), weight loss for no apparent reason, early satiety, and heartburn, were reported by both infected and non-infected subjects, but without a clear pattern providing a strong association with infection status in this series.

More serious gastrointestinal signs, such as black stools, were infrequently observed in participants and not strongly linked to any infection. The distribution of gastrointestinal symptoms was similar overall among infected and non-infected participants, indicating that many of those infected were clinically asymptomatic.

Table 1 Distribution of Helicobacter pylori infection according to gender

Gender	Negative	Positive	Total	Prevalence (%)
Male	30	2	32	6.25
Female	138	8	146	5.48
Total	168	10	178	5.6

Table 2 Association between Helicobacter pylori infection and gastrointestinal symptoms

Symptom	Negative (n)	Positive (n)	Total (n)
Stomach pain / burning sensation	121	7	128
Bloating	100	4	104
Nausea	85	5	90
Vomiting	108	8	116
Loss of appetite	99	5	104
Unexplained weight loss	126	8	134
Excessive belching	132	10	142
Early satiety during meals	80	5	85
Passing black stools	153	10	163
Heartburn / reflux	125	8	133
Pain relieved after eating	137	9	146
Stomach pain worsens with hunger	115	7	122
Persistent bad breath	**143**	**8**	**151**

Medical History

Other relevant medical history variables were also tested for association with *H. pylori* infection. Among these were chronic diseases, anemia, vitamin B12 deficiency, allergies, past surgery on the gastrointestinal tract and psychological stress.

Sixty-nine participants (including four infected individuals) with a history of anemia, and 43 (including three seropositive cases) evaluated for vitamin B12 deficiency were reported. Also, 28 patients had food or drug allergies and three of these participants had a diagnosis of H. pylori infection.

Few participants (n = 11) had previous gastrointestinal surgery and tested positive for the infection. 86 participants (four of whom were seropositive) reported severe psychological empirical stress.

Fisher's exact test revealed no significant association between *H. pylori* infection and any of the medical history variables assessed ($p > 0.05$ for all comparisons).

The current study revealed a low seroprevalence (5.6%) of *Helicobacter pylori* infection in the asymptomatic subjects under investigation. The infection was identified in males and females at similar rates, and there were not significant associations between those infected or not with the gastrointestinal symptoms or medical history variables we evaluated **(Table 3)**.

Table 3 Association between medical history variables and *Helicobacter pylori* seropositivity

Variable	Category	Negative n (%)	Positive n (%)	Odds Ratio	p-value*
Chronic diseases	Yes	4 (2.4)	0 (0.0)	–	1.000
	No	162 (97.6)	10 (100)	Reference	
Anemia	Yes	65 (38.9)	4 (40.0)	0.96	1.000
	No	102 (61.1)	6 (60.0)	Reference	
Vitamin B12 deficiency	Yes	40 (24.0)	3 (30.0)	0.64	0.691
	No	125 (76.0)	6 (70.0)	Reference	
Food/medicine allergies	Yes	25 (14.9)	3 (30.0)	0.41	0.194
	No	143 (85.1)	7 (70.0)	Reference	
Previous gastrointestinal surgery	Yes	9 (5.4)	2 (20.0)	0.24	0.129
	No	151 (94.6)	8 (80.0)	Reference	
Severe psychological stress	Yes	82 (49.4)	4 (40.0)	1.46	0.747
	No	84 (50.6)	6 (60.0)	Reference	

5. DISCUSSION

The results of this study provide a unique insight into the epidemiology of *Helicobacter pylori* (*H. pylori*) in Libya, revealing a seroprevalence of 5.6% among 178 asymptomatic participants. This finding is particularly significant when contrasted with existing literature, as it suggests a much lower infection rate in this specific cohort than previously estimated for the region.

Comparison with Regional and National Data

The seroprevalence rate of 5.6% reported here is considerably lower than previous studies in Libya and surrounding North African countries. Previous studies done in Libya have generally reported much higher rates (especially from clinical settings), with reported infection rates for symptomatic patients in various regions of Libya ranging from 69.7% to 81%. (Almehdawi & Ali, 2016). Similarly, a recent study in Gharyan, Libya, focused on adult patients and found high levels of infection, particularly among those with lower vitamin D levels and high stress. (Najia Mahdawi et al., 2024).

In a wider region, high *H. pylori* prevalence rates in some areas of North Africa have been reported as being amongst the highest globally. Seropositivities of symptomatic and asymptomatic cases in Morocco were found to be 89.6% (Emmanuel et al., 2024), and 92.6%, respectively. Similar rates were also reported in Algeria and Tunisia with the prevalence reported as 71.4% and 72.8%, respectively (Emmanuel et al., 2024; Melese et al., 2019). Moreover, prevalence of around 64.6% was found among children and 66.12% in adult populations from similar reports done in Egypt (Emmanuel et al., 2024; Jayte, 2023). Such a low prevalence of 5.6% in this study is markedly different from these values and could be explained by the asymptomatic natural history in the cohort, or that local sanitation and healthcare access within the demographic of this study has improved.

H. pylori prevalence is declining globally, from 58.2% in the 1980–90 period to a rate of 43.1% between, as noted between the years of 2011 and 2022 (Li et al., 2023). But, the rates in the Eastern Mediterranean Region are consistently high and it is about 22%–87.6% (Sabah et al., 2015). The 5.6% incidence found here places this Libyan cohort below average in developing nations and even below the rich European countries at 20%, which is frequent (Nasri et al., 2022).

In this study, H. pylori seropositivity showed no significant correlation with gender; the prevalence of seropositive patients was 5.5% in females and 6.3% males. These findings agree with certain regional studies; example of which was research conducted in Republic of Benin where no significant relationship between gender and infection status was observed (Jayte, 2023). In contrast, the prevalence in this report is lower than others such as Gharyan (Libya) where prevalence was significantly higher among females (Najia Mahdawi et al., 2024).

With respect to symptoms, it is interesting that there was no substantial association between infection and GI-seeking complaints such as stomach pain or bloating in this study. Whereas some literature highlights the fact that H. pylori is a leading cause of dyspepsia and gastritis (Jayte, 2023), our results reinforce the observation that many colonised subjects are asymptomatic (Nasri et al., 2022). Moreover, despite the external studies regarding the association between *H. pylori* and anemia (Jayte, 2023), no such finding was reported in this study, indicating that other factors may be accounting for the hematological disorders seen here among Libyan subjects.

According to the Methodological Considerations IgG is an established gold standard for epidemiological mapping using ELISA. Serology is useful for quantifying cumulative exposure, but since it cannot separate active versus old infections, the actual proportion of this group with active infections may well be <5.6%. This is a widely recognized limitation of comparable studies using qualitative serological tests (Omosor et al., 2017).

6. CONCLUSION

In summary, the 5.6% seroprevalence seen in this study is an important and unexpectedly low baseline for asymptomatic Libyan individuals. These findings call into question the assumed high universal prevalence in the region and emphasize an urgent need for targeted public health screening and further nationwide epidemiological work across Libya (Almehdawi & Ali, 2016).

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