



Frequency of *Helicobacter pylori* in Stool Specimens of Patients Suspected of Upper Gastrointestinal Symptoms in District Bunir

Noor Muhammad¹, Jehanzeb Afridi¹, Nourin Mahmood² and Sajid Ali^{2,*}

¹Peshawar Medical College, Peshawar, Khyber Pakhtunkhwa, Pakistan

²Department of Biotechnology, Abdul Wali Kahn University Mardan, Mardan, Pakistan

*Corresponding author: Department of Biotechnology, Abdul Wali Kahn University Mardan, Mardan, Pakistan. Email: sajid@awkum.edu.pk

Received 2020 May 10; Revised 2020 July 26; Accepted 2020 July 27.

Abstract

Background: There are studies on *Helicobacter pylori* infection in Khyber Pakhtunkhwa. However, district Bunir is far away, and most people have the least access to more developed techniques for the diagnosis of different diseases, and no study has been conducted in this region. Therefore, this study was conducted to determine the frequency of *H. pylori* infection in patients presenting with upper gastrointestinal symptoms.

Objectives: This study aimed to determine the frequency of *H. pylori* antigen in the stool of patients with upper gastrointestinal symptoms.

Methods: A cross-sectional study was conducted at Bilal Medical Trust Hospital from February 2018 to November 30, 2019. A total of 111 patients presenting with upper gastrointestinal symptoms were included in this study. A purposive (non-probability) sampling technique was used. All of the patients were screened for *H. Pylori* in stool specimens using a specific stool *H. pylori* device.

Results: Out of 111 patients, 74 (66.66%) were reported positive for *H. pylori* infection, among whom, females had a higher ratio (54.05%) than had males (45.94%). The infection was more prevalent in patients aged 20 - 30 years (43.67%). Patients who consumed fresh milk had higher rates of infection than those who used powdered milk/packed milk (52.54% vs. 15.25%). Patients who consumed water from water wells had a higher infection rate (72.87%) than those who used water from tube wells (27.11%).

Conclusions: The present study revealed a higher prevalence of *H. pylori* in females than in males. The maximum prevalence was noticed in the age group of 20 - 30 years. The study indicated a significantly higher rate of *H. pylori* infection in patients who used uncooked milk and water from contaminated sources.

Keywords: *Helicobacter pylori*, Dyspepsia, Gastritis, Milk, Age, Gender

1. Background

Helicobacter pylori is a Gram-negative, microaerophilic bacterium that is commonly found in the stomach. It is found in approximately half of the world's population (1). The infection is mostly acquired earlier in life and is common in less developed countries. There is a positive correlation between *H. pylori* and peptic ulcers. In some patients, the disease progresses to intestinal metaplasia, dysplasia, and carcinoma (2). *Helicobacter pylori* infection can be diagnosed by different techniques, including invasive techniques, requiring endoscopy and biopsy (e.g., histological examination) and non-invasive techniques, such as serology, urea breath test, urine/blood test, or the detection of *H. pylori* antigen in stool specimens (3).

Stool antigen tests were introduced after the urea breath test. Early stool antigen tests used an Enzyme Immunoassay (EIA) based on polyclonal antibodies. Although

this test provides reliable results in the diagnosis of *H. pylori* infection, there are some controversial results in the post-eradication assessment because of false-positive results. Stool antigen tests based on monoclonal antibodies have been developed and showed to be more accurate and reliable than those using polyclonal antibodies. A meta-analysis also showed that the specificity of stool antigen tests based on monoclonal antibodies was 97%. European and Japanese guidelines have indicated stool antigen tests using monoclonal antibodies to be useful for primary diagnosis and assessment of eradication therapy (4).

The worldwide prevalence of *H. pylori* infection varies according to socioeconomic factors and levels of hygiene. According to a meta-analysis of 184 studies conducted from 1970 to 2016 to find out *H. pylori* infection in 62 countries at different time frames, it was concluded that more than half of the world's population were infected with *H. pylori*. Whereas the U.S. and Australia have a low prevalence of

H. pylori, their indigenous populations have a high prevalence. The regions with the highest prevalence were Africa, Nigeria, Portugal, Estonia, South America, and Western Asia, while those with the lowest prevalence were Switzerland, Denmark, New Zealand, Oceania, Western Europe, and North America. *Helicobacter pylori* was estimated to infect 4.4 billion people in 2015 (5). The *H. pylori* prevalence in Pakistan is at an alarming level. According to a study, 85% of chronic gastritis patients and about 100% of duodenal ulcer and duodenitis patients show evidence of infection with *H. pylori*. Most of the patients were male and relatively young (6). Another study showed a prevalence of 74.4%, with 73.5% in males and 75.4% in females. The infection increased with age, and adults were mostly affected (7).

2. Objectives

There are studies regarding *H. pylori* infection in Khyber Pakhtunkhwa. However, district Buner is far away, and most people have the least access to more developed techniques for the diagnosis of different diseases; in the same way, people have no concept of research due to the lack of education. Therefore, data are needed to highlight the prevalence of *H. pylori* infection in patients in this district.

3. Methods

A cross-sectional study was conducted from February 2018 to November 30, 2019, at Bilal Medical Trust Hospital, Buner. The consent forms were signed by all patients. The method for identifying patients depended on the consultant in the OPD. The inclusion/exclusion criteria included patients who presented to Medical OPD with upper gastrointestinal symptoms, i.e., epigastric pain, dyspepsia, heartburn, burping, blotting, nausea, and vomiting while those patients were excluded who were already diagnosed with *H. pylori* and were on eradication therapy but still presented with upper gastrointestinal symptoms.

3.1. Test and Procedure

We used a non-invasive method that did not involve radioactive isotopes. The assay was easy and did not require professional training. It provided a rapid result with high sensitivity and specificity. The test used for the detection of *H. pylori* was the "One Step Rapid HP Ag Test" (Healgen, USA), which detects antigens specific to *H. pylori* infection in stool samples. Simply, one drop of the stool sample was diluted in buffer and put on strip. After incubation for 10 minutes, the bands on the device were noted. For a valid test, both control and T lines were noted. In the case of positive tests, both lines were prominent, and in the case of negative tests, only the control line was prominent.

4. Results and Discussion

There were 52 (46.84%) males and 59 (53.15%) females among 111 patients. The total number of patients with positive stool antigen tests was 74 (66.66%), of whom females had a higher ratio (54.05%) than males (45.94%) (Table 1). The mean age of *H. pylori*-infected patients was found to be 35 years, and the prevalence was higher in the age group of 20 - 30 years (43.67%). It was found to be lower in the age group of lower than 20 years (8.55%) and more than 40 years (17.81%). We also found that people who used uncooked milk had a higher positivity rate (52.54%) than those who used powdered milk/packed milk (15.25%). The infection with *H. pylori* was found related to the source of water in the patient group. It was found more in patients using water from water wells (72.87%) than those who used water from tube wells (27.11%) (Table 1).

Table 1. Subjects and Their Positivity Rate

| Subjects/Age Groups/Sources | Percent of Positivity |
|-----------------------------|-----------------------|
| Gender | |
| Male | 45.94 |
| Female | 54.05 |
| Age groups | |
| Below 20 years | 8.55 |
| 20-30 years | 43.67 |
| Above 40 years | 17.81 |
| Milk source | |
| Fresh milk/uncooked | 52.54 |
| Packed Milk/Powdered Milk | 15.25 |
| Water source | |
| Water wells | 72.87 |
| Tube wells | 27.11 |

The various prevalence rates among the residents of different countries (8) advocate that different parameters play important roles in the prevalence of *H. pylori*. These parameters include socioeconomic status, sanitation conditions, demographics, and environmental conditions. There is a high prevalence rate of *H. pylori* infection in different regions in Pakistan. A study was conducted in the rural areas of Islamabad in asymptomatic patients, which showed a prevalence rate of 74.4% (9). Similarly, a study in the southern region of Pakistan (Karachi) showed a prevalence of 45% (10). These studies are in line with our study, which showed a high prevalence rate of 66.66%. Around 36.44% of symptomatic patients were negative on the stool antigen test. The high proportion of symptomatic patients reporting negative for *H. pylori* suggests other reasons for

the symptoms. The other relevant factors, as suggested by Levenstein et al. (11), can be stress, socioeconomic status, smoking, and over-the-counter use of Non-steroidal Anti-inflammatory Drugs (NSAIDs) by patients with upper gastrointestinal symptoms in our community. These factors might be the roots of dyspeptic symptoms among more than half of these patients, and this might not be just due to the *H. pylori* infection. The male-to-female ratio in our study showed that females had a higher prevalence rate than males (53.15% vs. 46.84%) (Table 1).

A meta-analysis conducted in 2006 contradicts our study, which showed that men predominated in *H. pylori* infection globally (12). Similarly, another study obtained the same results as ours and showed the prevalence was more in women than in men (13). The mean age of prevalence was found in patients in the age group of 20-30 years (Table 1). According to a study conducted in 2000 and 2005 (13, 14), the prevalence of *H. pylori* increased with increasing age. The results of these studies are in line with ours and strengthen the results of our research. There seemed to be an increased incidence of *H. pylori* in people consuming fresh milk (52.54%) than in people consuming powdered/packed milk (15.25%) (Table 1). There have been several studies conducted around the world that prove the transmission of *H. pylori* from the milk of livestock as one of the sources of disease transmission (15).

Patients who consumed water from water wells had a higher infection rate than those who used water from tube wells (72.87% vs. 27.11%) (Table 1). The infection rate of *H. pylori* in great part is related to the water source used by the populous for drinking and other daily activities, e.g., washing fruits, etc. According to the conclusion of a study conducted by Santiago et al., *H. pylori* can survive in water and can be very infective in drinking water; thus, it proves that water distribution systems can be a source of spread of *H. pylori* (16). Some studies conducted in Pakistan showed that samples collected from different water sources around major cities of Pakistan were infected with *H. pylori* (17, 18).

Like other studies, the present study had some limitations. Our main limitation was the study setting, as there were limited patients in our setting compared to other tertiary care hospitals. It also did not properly harbor the socioeconomic status of patients and the residence of individual patients. Thus, this study lacks to determine the prevalence of the whole region. For future studies, *H. pylori* can be correlated to the socioeconomic status, as well as the locality of patients, to determine the high-prevalence areas of the region. Further research can be done on the water supply of many sectors of this region to determine the infected sources of drinking water.

4.1. Conclusions

The present study revealed a higher prevalence of *H. pylori* in females than in males. The maximum prevalence was in the age group of 20-30-years-old. This study also showed that *H. pylori* is transmitted from consuming fresh milk and water from contaminated sources.

Acknowledgments

We are thankful to Dr. Noor Muhammad for complete support for the current study.

Footnotes

Conflict of Interests: The authors declare that they have no conflict of interest.

Funding/Support: This was a self-support study.

Informed Consent: The consent forms were signed by all patients.

References

1. Sheila EC. *Helicobacter pylori* infection and treatment (Beyond the Basics). 2020. Available from: <https://www.uptodate.com/contents/helicobacter-pylori-infection-and-treatment-beyond-the-basics>.
2. Lwai-Lume L, Ogutu EO, Amayo EO, Kariuki S. Drug susceptibility pattern of *Helicobacter pylori* in patients with dyspepsia at the Kenyatta National Hospital, Nairobi. *East Afr Med J*. 2005;82(12):603-8. doi: 10.4314/eamj.v82i12.9364. [PubMed: 16619703].
3. Ricci C, Holton J, Vaira D. Diagnosis of *Helicobacter pylori*: invasive and non-invasive tests. *Best Pract Res Clin Gastroenterol*. 2007;21(2):299-313. doi: 10.1016/j.bpg.2006.11.002.
4. Shimoyama T. Stool antigen tests for the management of *Helicobacter pylori* infection. *World J Gastroenterol*. 2013;19(45):8188-91. doi: 10.3748/wjg.v19.i45.8188. [PubMed: 24363508]. [PubMed Central: PMC3857440].
5. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of *Helicobacter pylori* Infection: Systematic Review and Meta-Analysis. *Gastroenterology*. 2017;153(2):420-9. doi: 10.1053/j.gastro.2017.04.022. [PubMed: 28456631].
6. Shah Sattar Khan AZ, Danish KF, Sauwal M, Shahid Bashir SU. Prevalence of *H. Pylori* infection in patients with Gastroduodenal disease in Pakistan. *Rawal Med J*. 2008;33(1):89-91.
7. Rasheed F, Ahmad T; Bilal R. Prevalence and risk factors of *Helicobacter pylori* infection among Pakistani population. *Pak J Med Sci*. 2012;28(4):661-5.
8. Peleteiro B, Bastos A, Ferro A, Lunet N. Erratum to: Prevalence of *Helicobacter pylori* Infection Worldwide: A systematic review of studies with national coverage. *Dig Dis Sci*. 2015;60(9):2849. doi: 10.1007/s10620-015-3779-5. [PubMed: 26160435].
9. Rasheed F, Ahmad T; Bilal R. Frequency of *Helicobacter pylori* infection using 13C-UBT in asymptomatic individuals of Barakah, Islamabad, Pakistan. *J Coll Physicians Surg Pak*. 2011;21(6):379-81.
10. Khan A, Farooqui A, Raza Y, Rasheed F, Manzoor H, Akhtar SS, et al. Prevalence, diversity and disease association of *Helicobacter pylori* in dyspeptic patients from Pakistan. *J Infect Dev Ctries*. 2013;7(3):220-8. doi: 10.3855/jidc.2942. [PubMed: 23493000].

11. Levenstein S, Rosenstock S, Jacobsen RK, Jorgensen T. Psychological stress increases risk for peptic ulcer, regardless of Helicobacter pylori infection or use of nonsteroidal anti-inflammatory drugs. *Clin Gastroenterol Hepatol.* 2015;**13**(3):498-506 e1. doi: [10.1016/j.cgh.2014.07.052](https://doi.org/10.1016/j.cgh.2014.07.052). [PubMed: [25111233](https://pubmed.ncbi.nlm.nih.gov/25111233/)].
12. de Martel C, Parsonnet J. Helicobacter pylori infection and gender: a meta-analysis of population-based prevalence surveys. *Dig Dis Sci.* 2006;**51**(12):2292-301. doi: [10.1007/s10620-006-9210-5](https://doi.org/10.1007/s10620-006-9210-5). [PubMed: [17089189](https://pubmed.ncbi.nlm.nih.gov/17089189/)].
13. Kanbay M, Gur G, Arslan H, Yilmaz U, Boyacioglu S. The relationship of ABO blood group, age, gender, smoking, and Helicobacter pylori infection. *Dig Dis Sci.* 2005;**50**(7):1214-7. doi: [10.1007/s10620-005-2762-y](https://doi.org/10.1007/s10620-005-2762-y). [PubMed: [16047462](https://pubmed.ncbi.nlm.nih.gov/16047462/)].
14. Rosenstock SJ, Jorgensen T, Andersen LP, Bonnevie O. Association of Helicobacter pylori infection with lifestyle, chronic disease, body-indices, and age at menarche in Danish adults. *Scand J Public Health.* 2000;**28**(1):32-40. doi: [10.1177/140349480002800107](https://doi.org/10.1177/140349480002800107). [PubMed: [10817312](https://pubmed.ncbi.nlm.nih.gov/10817312/)].
15. Talaei R, Souod N, Momtaz H, Dabiri H. Milk of livestock as a possible transmission route of Helicobacter pylori infection. *Gastroenterol Hepatol Bed Bench.* 2015;**8**(Suppl 1):S30-6. [PubMed: [26171135](https://pubmed.ncbi.nlm.nih.gov/26171135/)]. [PubMed Central: [PMC4495422](https://pubmed.ncbi.nlm.nih.gov/PMC4495422/)].
16. Santiago P, Moreno Y, Ferrus MA. Identification of Viable Helicobacter pylori in Drinking Water Supplies by Cultural and Molecular Techniques. *Helicobacter.* 2015;**20**(4):252-9. doi: [10.1111/hel.12205](https://doi.org/10.1111/hel.12205). [PubMed: [25655472](https://pubmed.ncbi.nlm.nih.gov/25655472/)].
17. Khan A, Farooqui A, Kazmi SU. Presence of Helicobacter pylori in drinking water of Karachi, Pakistan. *J Infect Dev Ctries.* 2012;**6**(3):251-5. doi: [10.3855/jidc.2312](https://doi.org/10.3855/jidc.2312). [PubMed: [22421606](https://pubmed.ncbi.nlm.nih.gov/22421606/)].
18. Samra ZQ, Javaid U, Ghafoor S, Batoool A, Dar N, Athar MA. PCR assay targeting virulence genes of Helicobacter pylori isolated from drinking water and clinical samples in Lahore metropolitan, Pakistan. *J Water Health.* 2011;**9**(1):208-16. doi: [10.2166/wh.2010.169](https://doi.org/10.2166/wh.2010.169). [PubMed: [21301128](https://pubmed.ncbi.nlm.nih.gov/21301128/)].