

## Frequency of *Helicobacter Pylori* Antibodies among Asymptomatic Students of Shalamar School of Allied Health Sciences.

Arooma Nadeem<sup>1</sup>, Kinza Mushtaq<sup>1</sup>, Khizra Asghar<sup>1</sup>, Talha Mannan<sup>\*1</sup>, Hina Awais<sup>2</sup>, Madiha Mumtaz<sup>1</sup> and Arslan Saleem Chughtai<sup>1</sup>.

1. Department of Medical Lab Technology, Shalamar School of Allied Health Sciences, Lahore, Pakistan.
2. University Institute of Medical Lab Technology, The University of Lahore, Lahore, Pakistan.

**\*Corresponding author:** Talha Mannan (ORCID: 0000-0001-5408-8632).

### Abstract

**Background:** *Helicobacter pylori* (*H. pylori*) infection remains a major public health challenge. It is the main bacterial cause of several gastrointestinal disorders but most of the infections remain asymptomatic. More than 50% of the people are infected worldwide with *H. pylori* which causes significant public health morbidity and mortality. **Objective:** To determine frequency of *H. pylori* and associated risk factors among asymptomatic students of Shalamar School of Allied Health Sciences. **Material and Methods:** In this cross-sectional study, we collected the venous blood samples from 99 asymptomatic students from Shalamar school of allied health sciences, Lahore. The history was taken before the collection of the sample through the completion of the questionnaire and *H. pylori* antibodies (IgG, IgM and IgA) were detected in serum/plasma using a lateral flow chromatographic immunoassay. Chi square test was used to determine the association between *H. pylori* status and study variables (socio-demographic data, drinking water, lifestyle characteristics etc). **Results:** A total of 99 asymptomatic students were included in this study, in which 26 (26.3%) were males and 73 (73.7%) were females. Out of 99 students, 23(23.2%) were positive but the proportion rate of *H. pylori* antibodies was not found to be associated with different degree programs, socio-demographic data and lifestyle characteristics. Among the studied variables, the drinking water source was found to be associated with the presence of *H. pylori* antibodies (p-value 0.022). **Conclusions:** The frequency of *H. pylori* infection was significant and it was related with drinking water source. These findings emphasize the importance of implementing preventive measures and improving the quality of drinking water sources to mitigate the risk of *H. pylori* infection.

**Key words:** *Helicobacter pylori*, Students, Asymptomatic, Drinking Water, Gastrointestinal disorders.

## Introduction

*Helicobacter pylori* infection up to this time is a prevalent public health issue around the globe. [1] This infection is closely linked to peptic and gastric ulcer disease and poses a major risk factor for conditions such as atrophic gastritis, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma. [2] Among patients infected with *H. pylori*, the risk of developing peptic ulcers is elevated to 20%, while the risk of gastric cancer ranges from 1% to 2%. The prevalence of *H. pylori* infection varies, with developed countries showing a lower rate (around 14%) compared to underdeveloped countries, where it can be as high as 92%. [3]

*H. pylori* is a type of gram-negative bacteria with a distinctive spiral or comma-shaped appearance. It predominantly resides in the gastric mucosa of humans. In countries like India, the infection is highly prevalent, affecting up to 80% of the population. [4] Similarly, the infection rate among junior high school students in Poland, Grudziadz, was found to be 23.6%, [5] while Lithuanian medical students exhibited a positive seroprevalence of 14.2% for *H. pylori*. [6] In Pakistan, the prevalence of *H. pylori* is exceptionally high, ranging from 50% to 90%, surpassing the global average. [7]

Transmission of *H. pylori* occurs mainly through person-to-person contact, often within families. The primary routes of transmission include fecal-oral, gastric-oral, and oral-oral pathways. The bacterium can spread through food or water contaminated with feces. [8]

*H. pylori* attaches itself to the mucus-secreting cells in the gastric mucosa. It produces a substantial amount of ammonia by utilizing urea through its urease enzyme, which, combined with an inflammatory response, leads to mucosal damage. The loss of the protective mucus coating makes individuals susceptible to gastritis and peptic ulcers. Ammonia production by *H. pylori* also neutralizes stomach acid, enabling the bacterium to survive. Additionally, its lipopolysaccharide (LPS) surface structures play a significant role in interacting with the host and evading the immune system. [9,10]

In many cases, *H. pylori*-infected individuals do not exhibit specific symptoms, resulting in the infection going unnoticed initially. It often remains asymptomatic in the gut for an extended period. Symptoms typically arise after damage to the lining of the stomach or duodenum occurs. [11]

The objective of this study is to determine the frequency of *Helicobacter pylori* infection and identify associated risk factors among asymptomatic undergraduate students at Shalamar School of Allied Health Sciences using serum antibody detection methods.

## Material and Method

This was a cross-sectional study that was conducted at Shalamar School of Allied Health Sciences in Lahore, spanning from August 2022 to February 2023. The study involved the collection of blood samples from 99 asymptomatic students, following collection of information by using questionnaire that covers various aspects such as demographic information, family history, food consumption, sanitation methods, and drink consumption. Both male and female participants were included in the study, with a mean age of  $21.2 \pm 1.66$  and  $21.7 \pm 5.98$ , respectively. The study received approval from the Institutional Ethics Committee (SSAHS-IRB/AL/41/2022) and adhered to the guidelines set forth by the World Medical Association (WMA) in Helsinki. Upon collection, the blood samples underwent centrifugation at a gravitational force of 3000rpm to separate the serum. The obtained serum samples were then used for *H. pylori* detection. The Acu-check *H. pylori* one-step test device, which operates on the principle of sandwich lateral flow chromatographic immunoassay, was employed for qualitative detection of anti-*Helicobacter pylori* (*H. pylori*) antibodies (IgG, IgM, and IgA). The device exhibits a sensitivity of 97.1% and specificity of 99.0%. The collected data was analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0. Quantitative variables such as age were summarized using mean  $\pm$  SD, while categorical variables like gender and history of symptoms were presented as frequency and percentages. The comparison of categorical variables was assessed using either the Chi-square test or the Fisher exact test.

## Results

A total of 99 asymptomatic students were included in this study in which 26.3% (N=26) were males and 73.7% (N=73) were females. Mean age of male was  $21.2 \pm 1.66$  and mean age of female was  $21.2 \pm 1.39$  with the BMI of  $21.7 \pm 5.98$  and  $19.4 \pm 6.71$  respectively.

Out of 99 students 23(23.2%) were positive and 76(76.8%) were negative for *H. pylori* infection and these results showed no association between gender and *H. pylori* antibody (p-value 0.574). In this study, 2 (7.7%) males and 19 (26.0%) females from Doctor of Physiotherapy (DPT), 4 (15.4%) males and 13 (17.8%) females from Medical Imaging Technology (MIT), 14 (53.8%) males and 20 (27.4%) females from Medical Laboratory Technology (MLT), 1 (3.8%) male and 13 (17.8%) females from Optometry (Opto) and 5 (19.2%) males and 8 (11.0%) females from Operation Theater Technology (OTT) was included. The frequency of *H. pylori* was recorded with different degree programs. In this study, probability of *H. pylori* in DPT students was 23.8%, in MIT students was 23.5%, in MLT students was 20.6%, in Opto students was 28.6% and in OTT

students was 23. The analyzed data predicts that no association was found between *H. pylori* and other factors such as the residence of students, number of household members, family history of *H. pylori*, intake of spicy foods, increased salt intake, frequency of consumption of outside food, washing hands before meal, sharing bed with others, cola consumption, coffee consumption, tea consumption and drinking of filtered & boiled water as shown in table 1.

**Table No. 1: Frequency of *H. pylori* antibody according to categorical variables.**

Characteristics	Category	Number of positive students N (%)	Number of negative students N (%)	P-value
Residence of Students	Day scholar	18(24.3%)	56(75.7%)	0.658
	Hostelite	5(20.0%)	20(80.0%)	
Number of Household members	Less than 5	7(31.8%)	15(68.2%)	0.280
	5 or more	16(20.8%)	61(79.2%)	
Family History of <i>H. pylori</i>	Yes	3(50.0%)	3(50.0%)	0.136
	No	20(21.5%)	73(78.5%)	
Intake of Spicy Food	Yes	18(22.8%)	61(77.2%)	0.776
	No	5(25.0%)	15(75.0%)	
Consumption of Meat	Yes	19(22.9%)	64(77.1%)	1.000
	No	4(25.0%)	12(75.0%)	
Increased Salt intake	Yes	5(15.2%)	28(84.8%)	0.178
	No	18(27.3%)	48(72.7%)	
Frequency of Consumption of Outside Food	Almost Never	3(42.9%)	4(57.1%)	0.791
	Once a Week	8(21.6%)	29(78.4%)	
	Twice a Week	6(23.1%)	20(76.9%)	
	Thrice a Week	6(21.4%)	22(78.6%)	

	<b>Daily</b>	0(0.0%)	1(100.0%)	
<b>Washing Hands Before Meal</b>	<b>Seldom</b>	1(33.3%)	2(66.7%)	0.829
	<b>Often</b>	7(26.9%)	19(73.1%)	
	<b>Always</b>	15(21.4%)	55(78.6%)	
<b>Sharing Bed with Others</b>	<b>Yes</b>	8(18.6%)	35(81.4%)	0.339
	<b>No</b>	15(26.8%)	41(73.2%)	
<b>Cola Consumption</b>	<b>Yes</b>	17(22.4%)	59(77.6%)	0.711
	<b>No</b>	6(26.1%)	17(73.9%)	
<b>Coffee Consumption</b>	<b>Yes</b>	4(13.8%)	25(86.2%)	0.152
	<b>No</b>	19(27.1%)	51(72.9%)	
<b>Tea Consumption</b>	<b>Yes</b>	17(23.6%)	55(76.4%)	0.884
	<b>No</b>	6(22.2%)	21(77.8%)	
<b>Drinking of Filtered or Boiled Water</b>	<b>Yes</b>	23(25.3%)	68(74.7%)	0.192
	<b>No</b>		8(100.0%)	

6.3% students who used to drink natural water, 30.6% who used to drink mineral water and 32.3% was used to drink both natural and mineral water were positive for *H.pylori* infection. Students who used to drink mineral water were more positive than those who used to drink natural water showed in table 2. So, there was an association between Drinking water source and *H. pylori* antibody status (p-value 0.022).

**Table 2. Probability of *H. pylori* regarding Drinking water source**

<i>H. pylori</i> Antibody Test				
<b>Drinking Water Source</b>		<b>Positive</b>	<b>Negative</b>	<b>Total</b>
	<b>Natural</b>		2(6.3%)	30(93.8%)

	<b>Mineral</b>	11(30.6%)	25(69.4%)	36(36.3%)
	<b>Both</b>	10(32.3%)	21(67.7%)	31(31.3%)
	<b>Total</b>	23(23.2%)	76(76.8%)	99(100.0%)

**Pearson Chi-Square 7.673a**

## Discussion

*H. pylori* is a highly prevalent human infection worldwide, with approximately 50% of the global population being colonized by this bacterium. It represents a significant risk factor for the development of various gastrointestinal diseases. Notably, *H. pylori* plays a crucial role in the pathogenesis of stomach cancer, which ranks as the fifth most common incident cancer and the third leading cause of death worldwide. [12] Therefore, conducting epidemiological studies on *H. pylori* is essential as they provide valuable information about its prevalence rate. Furthermore, such studies aid in establishing public health interventions to prevent transmission and acquisition of the infection, as well as supporting therapeutic programs aimed at eradicating the bacterium.

The results of this study conducted among 99 asymptomatic students at Shalamar School of Allied Health Sciences showed a seroprevalence of *H. pylori* infection of 23.2%. This finding was similar to a study conducted among undergraduate students at Hawler Medical University/College of Health Sciences, which reported a seroprevalence of 23.4% for *H. pylori*. [13] However, it was lower than the rates observed in previous studies among Bangladeshi and Somalian students, where *H. pylori* infection was found in 55% of the students. [14] On the other hand, the prevalence in students of Bingham University in Karu, North-Central Nigeria, was lower at 5.5%. [15]

In a study conducted by Mekalo Nya-Nweme, an increasing trend of *H. pylori* infection was found with decreasing age [16] However, in our study, only students aged 20-24 years were included, so no significant difference was observed based on age.

It was assumed that *H. pylori* would be high among Medical Lab Technology (MLT) and Operation Theater (OTT) students because they were more exposed to infected specimens/subjects but in contrast no difference in frequency of *H. pylori* was documented. Whereas, frequency was comparatively lower to other discipline that may be because of preventive measures taken in labs. In Optometry students, frequency was higher but not statistically significant. It might be because of low participation from this group. Similarly, no significant difference was found based on gender, which is consistent with other studies. [17]

Regarding living conditions, no association was found between *H. pylori* infection and living in crowded conditions (i.e., households with five or more members) in this study. This finding aligns with a study conducted by Mekalo Nya-Nweme, which reported no significant difference between crowded and non-crowded living conditions. [16]. Additionally, a study by Mana similarly found no association between crowded families and *H. pylori* infection. [18]

In this study, it was observed that *H. pylori* infection was common among students who had family history of *H. pylori*, although the difference was not statistically significant. These findings differ from a study conducted by Hasosah, which reported a significant association between *H. pylori* infection and a family history of *H. pylori* infection. [19] In this study, no student with a family history of gastric cancer was present.

The results indicated that *H. pylori* infection was not associated with increased salt intake or consumption of spicy foods. These findings are consistent with studies conducted by Habbash and Ghalia Khoder et al. that reported no significant correlation between *H. pylori* infection and spicy or salty foods. [20]

In terms of nutritional resources, no association was found between *H. pylori* infection and consumption of meat; this is in agreement with the findings of a recent study published by Ghalia Khoder et al. who reported no correlation was found between *H. pylori* infection and dietary habits (including consumption of meat). [21]

Considering dietary habits of students, no association was found with frequency of consuming outside food. This may be because population taken in this study was students who were aware of poor sanitary measures taken by street vendors and majority of the students (42.9%) were those who almost never consumed outside foods and some students consumed it rarely and no one consume it daily. These results align with a study conducted by Hasosah, which also found no association between *H. pylori* infection and eating food from street vendors. [19]. However, a study conducted by Galal in 2019 reported a significant association between eating from street vendors and *H. pylori* infection which contrasts with our findings. [17]

Regarding hygienic conditions, it was found that *H. pylori* infection was more common in students who did not practice hand washing, although the difference was not statistically significant. This finding is in line with a study conducted by Mekalo Nya-Nweme. [16]

According to this study, no association was found between *H. pylori* infection and sharing a bed with others. But a study conducted by Shi and his colleagues in 2008, who reported similar results. [22] *H. pylori* positivity was compared with cola, tea, coffee consumption but but found no



significant difference between these factors and *H. pylori* infection. These findings are consistent with a study conducted by Mekalo Nya-Nweme et al., which also reported no association between *H. pylori* infection and the consumption of cola, tea, or coffee. [16]

An important environmental risk factor associated with the spread of *H. pylori* is poor drinking water sources. The fact that *H. pylori* infection can be transmitted through drinking of unclean water explains why it is more prevalent in developing countries where drinking water sources are not reliable. This was supported by our results in which high frequency of *H. pylori* was recorded in people whose source of drinking water was mineral (p-value 0.022). This could be because contaminated bottles of mineral water serve as source of resistant and strains virulent of *H. pylori*. Therefore, careful monitoring of bottled mineral water production is essential to reduce the risk of *H. pylori* transmission to the human population. It is important to improve the management of mineral water resources to prevent an increase in the incidence rate of *H. pylori* infection. In contrast other studies reported no association between *H. pylori* infection and drinking water sources. [23,24,25]

According to the researcher's knowledge, this study conducted at the Shalamar School of Allied Health Sciences is the first of its kind to investigate the frequency and risk factors associated with *H. pylori* infection among asymptomatic students. This study was single centered so, cannot be generalized over the entire population.

### **Conclusion:**

Among 99 students, 23 individuals (23.2%) tested positive for *H. pylori* antibodies. The frequency of *H. pylori* infection was significant and it was related with drinking water source. These findings emphasize the importance of implementing preventive measures and improving the quality of drinking water sources to mitigate the risk of *H. pylori* infection.

**Acknowledgement:** We are highly acknowledged to Shalamar school of Allied Health Sciences where all the wet work of this project was completed.

### **Conflict of interest**

The author declares that this article's content has no conflict of interest

### **References**

1. Mezmale, L., Polaka, I., Rudzite, D., Vangravs, R., Kikuste, I., Parshutin, S., Daugule, I., Tazhedinov, A., Belikhina, T. & Igissinov, N. 2021a. Prevalence and potential risk factors



- of *Helicobacter pylori* infection among asymptomatic individuals in Kazakhstan. *Asian Pacific Journal of Cancer Prevention: APJCP*, 22, 597.
2. Fang, Y.-J., Chen, M.-J., Chen, C.-C., Lee, J.-Y., Yang, T.-H., Yu, C.-C., Chiu, M.-C., Kuo, C.-C., Weng, Y.-J. & Bair, M.-J. 2020. Accuracy of rapid *Helicobacter pylori* antigen tests for the surveillance of the updated prevalence of *H. pylori* in Taiwan. *Journal of the Formosan Medical Association*, 119, 1626-1633.
  3. Mungazi, S. G., Chihaka, O. B. & Muguti, G. I. 2018a. Prevalence of *Helicobacter pylori* in asymptomatic patients at surgical outpatient department: Harare hospitals. *Annals of medicine and surgery (2012)*, 35, 153-157.
  4. Dhakal, O. & Dhakal, M. J. T. I. J. O. M. R. 2018. Prevalence of *Helicobacter pylori* infection & pattern of gastrointestinal involvement in patients undergoing upper gastrointestinal endoscopy in Sikkim. 147, 517.
  5. Szaflarska-Popławska, A. & Soroczyńska-Wrzeszcz, A. J. H. 2019b. Prevalence of *Helicobacter pylori* infection among junior high school students in Grudziadz, Poland. 24, e12552.
  6. Jonaityte, I. R., Ciupkeviciene, E., Jonaitis, P., Kupcinskas, J., Petkeviciene, J. & Jonaitis, L. J. M. 2021. Changes in the seroprevalence of *Helicobacter pylori* among the Lithuanian medical students over the last 25 years and its relation to dyspeptic symptoms. 57, 254.
  7. Rishma, M. N., Shams, S., Khan, A., Hassan, H., Shah, M. & Afridi, S. G. 2018. Frequency distribution and risk factors of *Helicobacter pylori* infection in patients with gastric problems in Mardan Pakistan. *Biomedical Journal*, 2, 5.
  8. Quaglia, N. C. & Dambrosio, A. 2018. *Helicobacter pylori*: A foodborne pathogen? *World journal of gastroenterology*, 24, 3472.
  9. Baj, J., Forma, A., Sitarz, M., Portincasa, P., Garruti, G., Krasowska, D. & Maciejewski, R. 2020. *Helicobacter pylori* Virulence Factors-Mechanisms of Bacterial Pathogenicity in the Gastric Microenvironment. *Cells*, 10, 27.
  10. Denic, M., Touati, E. & De Reuse, H. 2020a. Pathogenesis of *Helicobacter pylori* infection. *Helicobacter*, 25, e12736.
  11. Haq, I., Muhammad, A., Fazli Zahir, M. K., Anwar, F., Akhtar, M. S. & Ullah, F. 2020a. Serological and Epidemiology study of *Helicobacter pylori* infection among Dyspeptic patients in District Peshawar Pakistan. *Adv. Biores*, 11, 81-85.

12. Al-Ardawi, E. J. R., Al-Hussaini, R. M., Al-Asady, H. M. K., Sahib, A. A. & Tizkam, H. H. 2019. Prevalence and association of Helicobacter pylori infection with gastritis and its age and sex distribution in a population of Karbala. *Drug Intervention Today*, 12, 2571-2574.
13. Khaleel, A. A., Zaki Abdullah, S. M., Mohammed, A. B. & Anwer, S. S. 2020. Screening for Helicobacter Pylori IgG among Undergraduate Student of Hawler Medical University/College of Health Sciences. *Kirkuk Journal of Medical Sciences*, 8, 123-129.
14. Moniruzzaman, M., Abubakar Mohamud, B. & Nahar Ferdous, R. 2022. Determination of Seroprevalence and Associated Factors of Helicobacter Pylori Infection among Bangladeshi and Somalian Students. *Asian Journal of Research in Infectious Diseases*, 32-40.
15. Abioye, J. O. K., Anarado, K. S. & Babatunde, S. 2021. Seroprevalence of Helicobacter pylori Infection among Students of Bingham University, Karu in North-Central Nigeria. *International Journal of Pathogen Research*, 7, 38-47.
16. Mekalo Nya-Nweme, A., Eteneneng Enoh, J., Thumamo Pokam, B., Tangi Fominyam, B. & Clement Assob, J. 2021. Association of H. pylori with Serum Iron Levels and Some Risk Factors in Children Aged 1-12 Years Attending the Buea Regional Hospital.
17. Galal, Y. S., Ghobrial, C. M., Labib, J. R. & Abou-Zekri, M. E. 2019. Helicobacter pylori among symptomatic Egyptian children: prevalence, risk factors, and effect on growth. *Journal of the Egyptian Public Health Association*, 94, 1-8.
18. Mana, F., Vandebosch, S., Deyi, V. M., Haentjens, P. & Urbain, D. J. A. G.-E. B. 2013. Prevalence of and risk factors for H. pylori infection in healthy children and young adults in Belgium anno 2010/2011. 76, 381-385.
19. Hasosah, M., Satti, M., Shehzad, A., Alsahafi, A., Sukkar, G., Alzaben, A., Sunaid, A., Ahmed, A., Alhubiti, S. & Mufti, A. J. H. 2015. Prevalence and Risk Factors of Helicobacter pylori Infection in Saudi Children: A Three-Year Prospective Controlled Study. 20, 56-63.
20. Habbash, F., Alalwan, T. A., Perna, S., Ahmed, N., Sharif, O., Al Sayyad, A., Gasparri, C., Ferraris, C. & Rondanelli, M. 2022a. Association between Dietary Habits and Helicobacter pylori Infection among Bahraini Adults. *Nutrients*, 14, 4215.

21. Khoder, G., Mina, S., Mahmoud, I., Muhammad, J. S., Harati, R. & Burucoa, C. 2021a. Helicobacter pylori Infection in Tripoli, North Lebanon: assessment and risk factors. *Biology*, 10, 599.
22. Shi, R., Xu, S., Zhang, H., Ding, Y., Sun, G., Huang, X., Chen, X., Li, X., Yan, Z. & Zhang, G. J. H. 2008. Prevalence and risk factors for Helicobacter pylori infection in Chinese populations. 13, 157-165.
23. Habbash, F., Alalwan, T. A., Perna, S., Ahmed, N., Sharif, O., Al Sayyad, A., Gasparri, C., Ferraris, C. & Rondanelli, M. J. N. 2022b. Association between Dietary Habits and Helicobacter pylori Infection among Bahraini Adults. 14, 4215.
24. Khoder, G., Mina, S., Mahmoud, I., Muhammad, J. S., Harati, R. & Burucoa, C. J. B. 2021b. Helicobacter pylori Infection in Tripoli, North Lebanon: assessment and risk factors. 10, 599.
25. Hussen, B. M., Qader, S. S., Ahmed, H. F. & Ahmed, S. H. 2013. The prevalence of Helicobacter pylori among University Students in Iraq. *Indian Journal of Science and Technology*, 6, 5019-5023.