POTENTIAL RISK FACTORS AND SOCIOECONOMIC FINDINGS OF *HELICOBACTER PYLORI* INFECTION AMONG MALE UNIVERSITY STUDENTS IN PAKISTAN; A CASE-CONTROL STUDY

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ABSTRACT

Helicobacter pylori is one of the leading risk factors for human health-related issues and has colonized more than half of the global population. However, studies on *H. pylori* infection among students are few, especially in Pakistan. Thus, this case-control study was planned from November 2017 to February 2019 on male students of Hazara University, Pakistan. A case was defined as a student having digestive complaints and tested positive for *H. pylori*, while a control was defined as having no digestive complaints and tested negative during the current study. Socioeconomic information, family history, blood and stool samples were collected from the students. The *H. pylori* was tested through three different non-invasive diagnostic techniques, e.g., one-step blood antibody test, *H. pylori* stool antigen (HpSA) test and IgG ELISA. Out of 120 selected cases, 61 (50.8%) were identified positive through ELISA, 60 (50%) through the ICT Blood antibody test, and 58 (48.3%) through the HpSA test. Overall, 43 (35.8%) were identified as positive, while 44 (36.6%) were found negative through all three testing techniques. The IgG ELISA-positive cases were compared with the control group of 122 students (two controls for each case). The leading risk factors were found to be drinking tap water (P=0.0129) followed by snuffing (P<0.0001), smoking (P<0.0001), low income (P=0.027), irregular use of toothpaste (P=0.0005) and soft drinks consumption (P=0.0016). Furthermore, the family history with gastric complications (P<0.0001), stomach or intestinal ulcer (P<0.0001) and acid reflex (P=0.0007) also showed significant association with the *H. pylori* infection.

Keywords: *Helicobacter pylori*; Students; Prevalence; Risk Factors; Case-control

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral, motile, Gram-negative and micro-aerophilic bacterium of the family *Helicobacteraceae,* which Barry and Warren first identified in 1983 at Royal Perth Hospital, Australia (Marshall and Warren, 1984). It is the leading causative agent of gastritis, peptic and duodenal ulcer, chronic gastritis, and the development of gastric cancer (Kuo *et al.,* 2014; Mobzon *et al.,* 2013; Symyk *et al.,* 2014). *H. pylori* infections are frequently reported around the globe, particularly in developing countries, affecting about half of the global population (Marshal and Warren, 1984; Tonkie *et al.,* 2012). It has been reported that *H. pylori*-positive individuals have a 10-20% increased risk of peptic ulcer development while 1-2% risk of distal gastric cancer; hence, it is included in Class 1 carcinogen by WHO (CDC, 1998). *H. pylori* infections are mainly acquired and related to dietary factors, drinking water sources, socio-demographic conditions, and if not treated, they typically remain life-long (Sjomina *et al.,* 2018; Miftahussurur *et al.,* 2017). The bacterium causes active inflammation of the gastric mucosa and genetic instability of the gastric epithelial cells, eventually leading to gastric & peptic ulcers and other gastric complications. Recent population-based publications revealed that the incidence of gastric cancer decreased by 50% with the eradication of *H. pylori* in a follow-up study of more than ten years (Chiang *et al.,* 2021; Li *et al.,* 2019).

There is no single route of *H. pylori* transmission; however, it is reported that the housefly could probably transfer bacteria unconsciously, and the food might thus get contaminated by fly excreta. This hypothesis is significant for the global regions with poor hygienic conditions (Brown, 2000). Though vertical or direct contact is the most possible route of transmission, there are three likely routes described for the spread of the bacteria from one person's stomach to another (Antoon *et al.,* 2004). Iatrogenic is the primary and most common mode in which the

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endoscope that has been in contact with one individual's gastric mucosa is used for others. The fecal-oral route is the second likely mode, while the direct-oral-contact is the third probable route of transmission, as many studies have reported the culturing of *H. pylori* from the oral cavity and saliva (Momtaz *et al.*, 2012).

It is reported that about 90% of the infected persons do not show complications or clinical indications (Bytzer *et al.*, 2011). The infection can be lifelong and lead to chronic gastritis, which may develop into ulcer and carcinoma. Thus, proper and early diagnosis and treatment are greatly important to eradicate the infection. Consequently, understanding the *H. pylori* infection's occurrence and its association with socio-demographic factors is necessary to identify the pathology and epidemiology of the disease. Diagnosis of *H. pylori* infection can be obtained by many techniques, including Gram-stained smear and gastric mucosal biopsy specimens, culturing on Skirrows medium, and non-invasive diagnostic tests like IgG antibodies detection tests, *H. pylori* stool antigen test (HpSA) and urea breathe test (Levinson and Jawetz, 2006). The immune system produces immunoglobulins (IgG) against *H. pylori*-specific antigens during the infection that can be detected in blood samples using Rapid Diagnostic Tests (RDTs). These serological diagnostic techniques are comparatively low-cost, easy and fast (Gholi *et al.*, 2013).

A significant amount of data has been published about the mode of transmission, risk factors, and prevalence; however, few studies have been conducted on Pakistan's student population, particularly in the KPK province, who live in hostels of schools, colleges, or universities. Thus, the current study was carried out to determine the prevalence, risk factors, and possible preventive measures for H. pylori infection among students residing in different hostels of Hazara University, Pakistan. The current work is the first study carried out on Hazara University's hostel students to the best of our knowledge. The study may efficiently help to disclose the baseline information and related factors of the occurrence of *H. pylori* infection in hostel students. Furthermore, the study may spread awareness among the students. Still, the results may offer productive help to the health authorities in launching *H. pylori* control and eradication programs in the future.

MATERIALS AND METHODS

Study subjects and area

We carried out this study from November 2017 to February 2019 on male hostel students of Hazara University, Pakistan. Hazara University is a public sector university situated in the KPK province of Pakistan, with about 10,000 students. More than 6000 students lived in different hostels up to 2019 data. The University is located in a semi-rural area of the district Mansehra.

Collection of students' history

Students' health history and socioeconomic factors related to gastric complications were collected by conducting face-to-face interviews and filling out a standard self-designed questionnaire (both cases and controls).

Questionnaire

The questionnaire included three sections: history of the disease factors, socioeconomic information, and family history. The disease factors included being diagnosed with gastritis, stomach or intestinal ulcer, frequent pain or burning in the upper abdominal/chest area, experiencing belching or burping after meals, nausea, feeling anxious or depressed for no apparent reason, and being medicated for gastritis. Similarly, the remaining part included monthly income in the hostel, drinking water, carbonated drink use, food self-prepared or restaurant, toothpaste use, smoking, snuffing, family history of digestive complaints, stomach/intestinal ulcer and acid reflex.

Samples Collection

In the current study, 242 students were screened for *H. pylori*. A case was defined as a patient/student having digestive complaints and tested positive on the ICT/Blood Antibody test, *H. pylori* Stool Antigen test, or ELISA during our study. Finally, 120 eligible students were selected. Blood samples (about 8 cc) were collected in disposable sterile syringes for ICT and ELISA, while stool samples (1-2g/1-2mL) were collected in a clean and dry specimen collection container. The samples were instantly transferred to the central laboratory of the Department of Microbiology, Hazara University, for further processing.

Similarly, samples were collected and processed from the control students, who were defined as having no digestive complaints, medicated, or tested positive previously. The medicated or tested positive (previously or in our study) were excluded from the control. Finally, 122 control students were selected (adjusted according to 2 controls for each case). Both the case and control students lived in Hazara University hostels.

Laboratory Diagnostic Tests

For each student (both case and control), a total of three different diagnostic tests were performed, including two serological tests, e.g., ELISA and ICT/Blood antibody tests and H. pylori stool antigen test (HpSA). The Healgen One Step Rapid Test strip kit was used for the blood antibody test and the Healgen Rapid Test Cassette (Faeces) for HpSA tests (both kits from Healgen Scientific Limited Liability Company, USA), following manufacturer's guidelines. Similarly, the BioCheck, Inc. test Kit was used to perform the IgM ELISA (IgM enzyme immunoassay).

Ethical Approval of the Study

The synopsis of the current study was submitted to the Board of Studies, Department of Microbiology. The Advanced Studies and Research Board (ASRB) and the Institutional Bioethical Committee (IBC) of the university granted the final approval (F.No.129/HU/ORIC/IBC/2019/ and F.No.73/HU/ORIC/IBC/2018/). Consent permission was acquired from each student during sampling and socioeconomic information collection, and the students were informed about the purpose of the study.

Statistical Analysis

All the data in our study were statistically analyzed by GraphPad Prism 8. The results are expressed in numbers and percentages. The variables, baseline categories, association of risk factors and significance were measured using Chi-Square and Fishers Exact test with odd ratio and 95% confidence interval. A p-value less than 0.05 was considered significant. The specificity of the RDTs was assessed by TP/(TP+FP) while the sensitivity was measured as TP/(TP+TN) where TP; Total Positive results, TN; Total Negative results and FP; False Positive with respect to ELISA results.

RESULTS AND DISCUSSION

Rapid Diagnostic Tests (RDTs) results

The overall seroprevalence among the case students was tested to be 43 (35.8%). In comparison, 44 (36.6%) case students were tested negative by all three diagnostic techniques, e.g., IgG ELISA, ICT blood antibodies test and HpSA (*H. pylori* Stool Antigen) test **(Fig. 1)**. Separately, 60 (50%) cases were tested positive by ICT blood antibodies test, while 58 (48.3%) cases were tested positive by the HpSA test out of 120 case students. We found that some cases detected positive by the ICT blood antibodies test were seen as negative on HpSA and vice versa. Thus, we also performed the *H. pylori* IgM ELISA test for further confirmation, where 61 (50.8%) cases were identified as positive among the total 120 cases. The ELISA-positive cases were greater than the HpSA and ICT blood antibodies tests **(Table 1)**. Thus, we compared the ELISA-positive cases (61) with control (122) students and adapted two controls for each case.

The IgG ELISA results also showed that some of the cases identified positive on blood antibodies or HpSA test were negative for ELISA and vice versa. Three cases detected positive on HpSA were detected negative on ELISA, 16 cases positive on ICT blood antibodies test were found to be negative on HpSA. In contrast, 15 HpSA-positive cases were detected negative on the ICT blood antibodies test **(Table 1)**. The IgG ELISA positive cases were highest (61/120), followed by blood antibodies test (60/120) and HpSA (58/160).

We compared the ICT blood antibodies test and HpSA with IgG ELISA for *H. pylori* detection. The ICT one-step blood antibodies test for *H. pylori* was found to be readily available, low cost and showed more specificity, while the HpSA, on the other hand, was found to be more accurate, sensitive and easy to perform but was unpleasant for both the students and laboratory personnel **(Table 2)**. However, no significant variance among the results of the tests was observed, suggesting that all of these rapid diagnostic procedures can be considered easy and accurate to detect *H. pylori*.

Socioeconomic characteristics and personal habits

Table 3 represents the personal habits and characteristics of the ELISA-positive cases (n=61) and controls (n=122). Tap water was found to be the highest risk factor among the cases, e.g., 60 (98.3%), followed by consumption of snuff (a local tobacco product), e.g., 21 (34.4%), smoking, e.g., 28 (45.9%), carbonated drink users, e.g., 43 (70.4%) and lower pocket money (<8000 Rs/month) group, e.g., 27 (44.2%) with the Odd ratio of 9.057, 5.298, 5.241, 2.910 and 2.00, respectively **(Table 3)**. Furthermore, there was significant variance in the type of food, e.g., self-prepared and at restaurants/hotels (P=0.0895) and no use or improper and irregular use of toothpaste and Misvaak (P=0.0005) (local traditional and religious way of cleaning the teeth using special plant roots as a tooth-brush) **(Table 3)**.

Table 1. Comparative results of *H. pylori* rapid diagnostic tests (RDTs).

RDTs	Positive Cases	Negative Cases	Positive/Negative Cases	Negative/Positive Cases
ICT Ab/HpSA	43 (35.8%) (++)	45 (37.5%) (--)	16 (13.3%) $ICT^{(+)}$, $HpSA^{(-)}$	15 (12.5%) $ICT^{(-)}$,$HpSA^{(+)}$
ICT Ab/ELISA	50 (41.6%) (++)	50 (41.6%) (--)	9 (7.5%) $ICT^{(+)}$, $ELISA^{(-)}$	10 (8.3%) $ICT^{(-)}$, $ELISA^{(+)}$
HpSA/ELISA	54 (45%) (++)	55 (45.8%) (--)	3 (2.5%) $HpSA^{(+)}$, $ELISA^{(-)}$	7 (5.8%) $HpSA^{(-)}$, $ELISA^{(+)}$
ICT Ab/HpSA/ELISA	43 (35.8%) (++)	44 (36.6%) (--)		

Table 2. *H. pylori* RDTs comparative costs, accuracy and availability.

Technique	Specificity	Sensitivity	Cost	Availability
ICT Ab	86%	50%	300 PKR (2.24 US$)	+++
HpSA	89%	48%	400 PKR (3.23 US$)	++

Table 3. Socioeconomic characteristics and personal habits of the cases and controls.

Characteristics	**Variables**	**Case (%) n=61**	**Control (%) n=122**	***p*-value**	**Odd ratio (95% Cl)**
Per Month Expenditure in Hostel (PKR)	≤ 8,000	27 (44.2%)	16 (13.1%)	0.027	2.00 (1.07-3.74)
	8,000-12,000	21 (34.4%)	59 (48.3%)	0.0001	0.590 (0.47-0.72)
	≥ 12,000	13 (21.3%)	47 (38.5%)	0.001	0.271 (0.12-0.61)
Drinking water	Tap water	60 (98.3%)	106 (86.8%)	0.0129	9.057 (1.46-96.90)
	Filtered water	1 (1.6%)	16 (13.1%)		
Carbonated Drink Users	Yes	43 (70.4%)	55 (45%)	0.0016	2.910 (1.54-5.67)
	No	18 (29.5%)	67 (54.9%)		
Food	Restaurant	23 (37.7%)	31 (25.4%)	0.0895	0.562 (0.28-1.06)
	Self-Prepared	38 (62.2%)	91 (74.5%)		
Misvaak/Toothpaste Users	Yes	49 (80.3%)	118 (96.7%)	0.0005	0.138 (0.04-0.42)
	No	12 (19.6%)	4 (3.2%)		
Smoking	Yes	28 (45.9%)	17 (13.9%)	<0.0001	5.241 (2.57-10.69)
	No	33 (54%)	105 (86%)		
Snuffing	Yes	21 (34.4%)	11 (9%)	<0.0001	5.298 (2.34-11.30)
	No	40 (65.5%)	111 (90.9%)		

Table 4. Family history of the gastric complaints of cases and controls.

Characteristics	**Variables**	**Case (%) n=61**	**Control (%) n=122**	***p*-value**	**Odd ratio (95% Cl)**
Digestive Complaints	Yes	47 (77%)	49 (40.1%)	<0.0001	5.001 (2.49-10.25)
	No	14 (22.9%)	73 (59.8%)		
Stomach/Intestinal Ulcer	Yes	3 (4.9%)	36 (29.5%)	<0.0001	0.123 (0.03-0.39)
	No	58 (95%)	86 (70.4%)		
Acid Reflex	Yes	45 (73.7%)	37 (30.3%)	0.0007	3.421 (1.70-7.05)

No	16 (26.2%)	85 (69.6%)

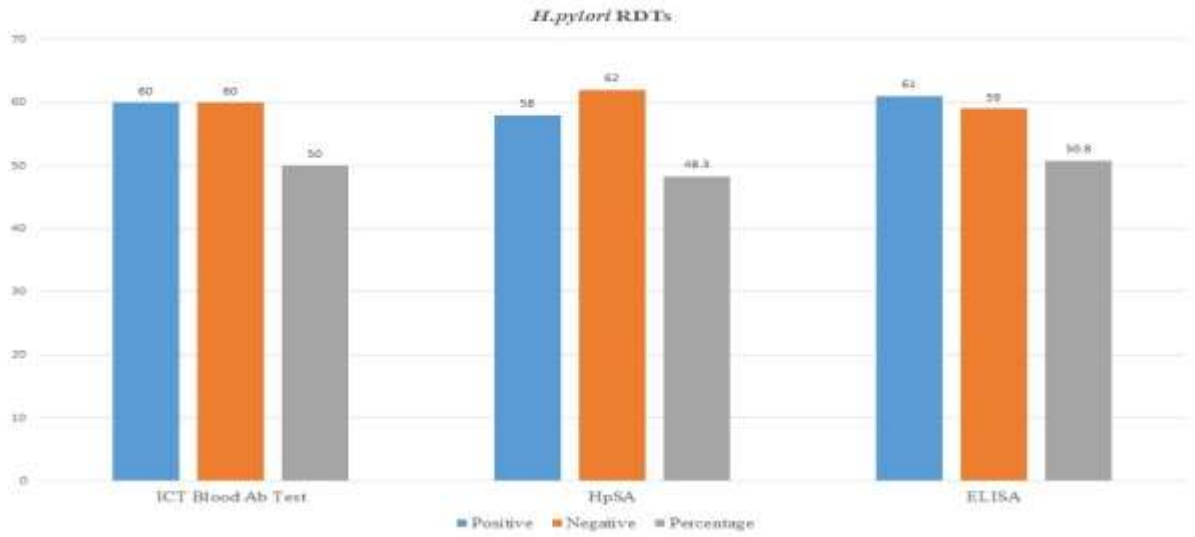

Fig. 1. Test results of cases from each RDT for *H. pylori.*

It is worth mentioning that the university has no water filtration system on campus or in the hostels. The students commonly use tap water for drinking, cooking and laundry. Similarly, many students were consuming snuff and smokers among the cases. Snuff is a local traditional tobacco product like a cigarette, but it is kept in the mouth under the lips for some time as an addiction. It is also shared among the students and is not considered a high-risk addictive drug in Pakistan and many other countries, including Afghanistan and Yemen.

Family history of gastric complaints

Table 4 shows the family history of the cases and controls related to gastric complications. It was observed that digestive complications (P<0.0001) and acid reflex (P=0.0007) history were present in most of the students' families that may present a significant risk factor for *H. pylori* infection and its spread among the family members. Similarly, a significant difference was observed for the family history of stomach and/or intestinal ulcers (P<0.0001) of both the cases and controls.

Infectious diseases are among the leading human well-being-related issues confronted by this advanced world. The lower-income countries, particularly those where a significant number of populations lives below the line of poverty, have been reported with high mortality and morbidity caused by infectious diseases. The *Helicobacter pylori* infection is the foremost public health-related issue among these infectious diseases. Previously, significant work has been done on the mode of transmission, risk factors, and prevalence of *H. pylori* globally; however, few studies have been conducted on Pakistan's student population who live in hostels of schools, colleges, or universities. Studies intended to identify the risk factors and epidemiology of diseases are necessary that are helpful in the diagnosis, treatment, and control of infectious diseases (Frerot *et al.,* 2018).

H. pylori infections are diagnosed via several approaches; however, the non-invasive and serological techniques are widely used, low-cost, and rapid methods. We conducted the current study of *H. pylori* infection in students' population of Hazara University Mansehra, targeting both the infected (cases) and healthy (students). The overall prevalence among the case population was 35.8% via all three selected diagnostic techniques, e.g., ELISA, ICT Antibody test and HpSA test. However, every second student (61/120) in the case group was detected with *H. pylori*. Although the case students were enrolled according to the gastric complaints, the prevalence is significantly high. A previous study conducted by Ahmad *et al*. (2009) reported a 72.3% prevalence of *H. pylori* infection among school-going children in Islamabad, Pakistan, which approves our study results (Ahmad *et al.,* 2009). Similarly, Jafar Khan *et al.* (2014) reported a prevalence of 34.18% among the general population of district Swat of Pakistan (Khan *et al.,* 2014) and 22.1% prevalence rate by Shah *et al.* (2021) among the general population of Timergara City of district Dir lower of Pakistan (Shah *et al.,* 2021).

We found that some of the cases were detected for *H. pylori* on one test not detected by other tests in our study. The highest cases were detected through IgG ELISA (61, 50.8%), followed by a one-step blood antibody test (60,

50%) and HpSA (58, 48.3%). Among these, 9 of the one-step blood antibodies test and 3 of the HpSA-detected cases were not positive on ELISA, suggesting the HpSA test to be more accurate. Vakil *et al*. (2000) have previously reported that the HpSA test is more accurate and time-efficient for *H. pylori* diagnosis.

Our study showed that the sanitation and hygienic condition of Hazara University is poor, having no water filtration system. The majority of the case students were found to be using tap water for drinking and multiple other purposes that may be the high-risk factor for *H. pylori*. Abebaw *et al*. (2014) have reported that individuals using unprotected surface water have a high prevalence of *H. pylori* (76.4%) as compared to individuals using piped water (65.9%). Unprotected and contaminated water provides one of the main habitats for *H. pylori*, which directly affects human hygiene, health, and water fauna. The rainfall floods contaminate the water reservoir of Hazara University and its hostels, which may cause gastric complications and other health-related issues.

We also found that snuffing, smoking, and other personal habits like toothpaste use, food type and carbonated drinks may also present a potential risk for gastric complications and *H. pylori* infections. There is a significant correlation between *H. pylori* infection and smoking and the type of drinking water (Ameri *et al.*, 2013). Smoking, in the long term, alters the normal physiology of the gastrointestinal tract. It disturbs the factors protecting or healing the lining, including mucus secretion, bicarbonate production and blood flow that contribute to susceptibility to *H. pylori*. Similarly, smoking has been associated with peptic ulcers (Masarat, 2008). The evidence of *H. pylori's* relation with the addiction to snuff, no use or irregular use of toothpaste and regular consumption of soft drinks might support the findings (Nseir *et al.*, 2012; Stenstrom *et al.*, 2007; Contractor *et al.*, 1998).

Though the current study may be affected by selecting only male students, small size and shortage of data for several variables and molecular level investigation, it may help provide meaningful information related to the students' health, hygiene, and habits. The study may also provide data for students' awareness, the planning of health programs by authorities, and further insights into the investigations of students' infectious diseases.

CONCLUSION

In the current case-control study, we investigated the *H. pylori* infection among male students of Hazara University, Pakistan. Our finding revealed that *H. pylori* infects every second of students with gastric complaints. Among the 120 suspected cases, 61 (50.8 %) were detected through the IgG ELISA test for *H. pylori*. In contrast, 43 (35.8%) cases were detected positive by all three tested techniques, e.g., ELISA, ICT one-step blood antibodies test and HpSA test. The leading risk factors included drinking tap water followed by snuffing, smoking, low income, irregular use of toothpaste and soft drinks consumption. Furthermore, the family history of acid reflex, gastric complications and stomach or intestinal ulcer showed a significant association with *H. pylori* infection.

Future Recommendations

- The female students of Hazara University should also be screened for *H. pylori*, as they were not included in the study due to cultural and ethical issues.
- The antibiotic sensitivity and molecular epidemiology of students living in different hostels of Hazara University should be further checked.
- Water filtration and hygiene food supply should be provided at Hazara University.
- Smoking, carbonated drinks consumption and snuffing should be avoided, and proper use of Misvaak/toothpaste is recommended to the students.
- The study may prove helpful in determining *H. pylori* risk factors and provide assistance to health authorities in the implementation of disease control and eradication measures.

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