Original Research Article

Determination of seroprevalence and associated factors of Helicobacter pylori infection among Bangladeshi and Somalian students.

Abstract

Helicobacter pylori (*H. pylori*) infections are the prime health concern nowadays as this may lead to peptic ulcer diseases and several types of stomach cancer. Factors including genetic, socioeconomic, ecological, personal hygiene and sanitation, and bacterial features are accountable for the predominance and development of disease. This study aimed to determine the prevalence of *H. pylori* and their association with age, sex, food habits and source, irregular meals, and physical activities among Bangladeshi and Somalian students. Cross-sectional structured questionnaires were used to collect demographic, food habits and lifestyle data. A total 80 Bangladeshi (32) and Somalian (48) students participated in this study. Selected students were invited to the laboratory to provide blood samples for H. pylori IgG antibody detection through Enzyme Linked Immuno-sorbent Assay (ELISA) method. Data from the survey and laboratory were analyzed using SPPS v.26. Multivariate logistic regression analysis was conducted to find an association between H. pylori infection and factors. Around 55% of the participants were found seropositive. Among them Somalian students were prevalently higher than Bangladeshi students. Mostly younger males were found seropositive regardless of age and educational level. Multivariate analysis revealed gender (OR:4.524, CI:1.526-13.407), ethnic origin (OR:4.200, CI:1.622-10.872), skipping of meals (OR:8.600, CI:0.984-75.151) and breakfast (OR:11.205, CI: 3.740-33.568) and physical activity levels (OR:0.286, CI:0.113-0.721) were significantly linked with H. pylori infections. More than two-third Somalian students (68.7%) were infected with H. pylori whereas one-third of Bangladeshi students were found positive. Somalian students were found to be less active and the pattern of skipping meals and breakfast was higher compared to Bangladeshi counterparts. Somalian students probably carried H. pylori infection from their native land, and less physical activity and mistiming of taking meals enhanced the infection. Communication barriers and food preferences could be other reasons. To prevent this infection, we suggest cleanliness, a healthy life style and nutritious meals with adequate physical activities are obligatory.

Keywords
Helicobacter pylori, Serological analysis, ELISA, IgG, Food habit.

Introduction

H. pylori is a helix-formed, curved rod shaped gram negative-bacteria, estimating a length of about 3µm with about 0.5µm in diameter. It colonizes at the host stomach more than half of the individuals worldwide are infected with *H. pylori* and the infection is the significant reason for peptic ulcers disease[1-4], gastritis[4, 5], gastric lymphoma[6, 7], and gastric carcinoma[8, 9] particularly in adulthood[10-13]. However, the connection between non-ulcer indigestion and *H. pylori* has not been elucidated yet. [10]. The diseases, caused by *H. pylori,* have been proved to be a great public health concern in developing countries. Poor hygiene and sanitation, and swarmed conditions have been reported as the major factors for *H. pylori* infections[14]. Infection with *H. pylori* is common in the early days of life, which remains regardless of innate and acquired immune responses [15, 16]. Most of the *H. pylori* infections are asymptomatic, although, only 10- 20% of infections develop clinical manifestations [17-20]. *H. pylori* infection is linked with a composite relation with hereditary[21], socioeconomic [17], ecological[22], and microbial factors[23]. A pilot serological study was conducted in 1997 revealed that 90% of asymptomatic individuals contained *H. pylori* infections. However, later the prevalence considerably declined to 67%[24, 25]. Overall *H. pylori* infection prevalence in Pakistan (84%), India (79%), and Japan (41%) showed higher compared to the United States (USA) and Europe (<40%)[25]. A seroprevalence study on adult populations of Canada demonstrated that men (29.4%) were infected

more compared to women (14.9%) [26]. The prevalence of *H. pylori* infection in the USA is higher in lower educated, low-income groups and among African Americans implying that socioeconomic conditions may have roles in disease progression after exposure to bacteria [27]. In addition, lifestyle and food habits have been showing potential factors of *H. pylori* infections [23]. Frequent meal skipping over a prolonged time could result in the augmented risk of increasing *H pylori* infection and gastritis [28]. More than 90% of gastric cancers have been found linked to current or previous *H. pylori* infection and decreasing *H. pylori* infection is considered to be a key preventive measure against it [29, 30]. Surprisingly, the reduction of gastric cancers has been found related to consuming more fresh fruits and vegetables and reducing consumption of salted and stored foods[31]. Additionally, physical activity possesses several benefits for physical and psychological wellbeing, which are thought to effect on peptic ulcer disease through somephysiochemicalsystems. This event could also increase the immune system's capability to defuse the adverse effects of *H. pylori*and associated disorders [32-34].

Dhaka is capital and central hub for all socioeconomic, education, and technology of Bangladesh. In recent days, numbers of foreign students especially from Somalia, Nepal, Bhutan, India, Kashmir, and other countries, have been conferred their degrees in both private and public universities. Students, especially from African origin, face some obstacles in language, lifestyle, food habits, etc. in Bangladesh. To date, there is no available literature regarding the impact of irregular meals, lifestyle, and physical activities along with gender and age group on H. pylori infections among African, and Bangladeshi adults. This study aims to compare and conclude whether a prolonged meal skipping pattern, food habits, and physical activities are associated with increased risk of *H. pylori* infection among Somalian and Bangladeshi university students.

Materials and Methods

Study period and location

This study was initiated in the last quarter of 2020 by the Bangladesh University of Health Sciences (BUHS). Formal approval of the study was obtained from the Institutional Review Board of BUHS Memo No: BUHS/ERC/EA/20/262 dated 13.11.2020. Data and blood specimens were collected from December 2020 to May 2021 from the local and foreign students residing in Dhaka city. All the laboratory works were conducted at the Immunology laboratory of Bangladesh University of Health Sciences.

Study population

This study aimed to collect data and samples from 140 (equally divided into Bangladeshi and Somalian students) with a 95% confidence interval and ±5% margin of errors. Due to the COVID-19 pandemic, the study population was limited to 80 (32 Bangladeshi and 48 Somalian students) which finally have a 95% confidence interval and a 6.57% margin of errors. A cross-sectional method was applied to select participants. Each participant was informed about the aim and objectives of the research and written consent was taken from all participants. The consent forms were designed to explain the aims and nature of the study and the procedure they were needed to undergo. In addition, they were also clearly informed about the nature, purpose, and confidentiality in data handling. Participants had a choice to refuse to respond to any or the entire survey questions and they also have the right to withdraw from the on-going interview.

Questionnaire and data collection technique

Participants were interviewed by using a pre-structured questionnaire. Socio-demographic information (e.g. ethnic origin, age, gender, and education level), Food habits (home/self-made foods, skipping

meals pattern), and physical activity level data were also collected. Once theconsent was obtained, participants were informed to be present at the laboratory for the interview and provide blood samples.

Blood specimen collection

A total of 80 blood samples were collected by trained medical assistants strictly following standard operational procedures. Disinfection of the phlebotomy site was done by swabbing the skin in small outward circles with 70% alcohol swab or cotton wool soaked in isopropyl alcohol. Approximately 5 ml venous blood from each participant was collected in the dry clean tube. Blood samples were allowed to clot to get serum samples. Centrifugation was done at 3000rpm for 10 minutes and serum was separated and then stored at -20^{0}Ċ till analysis. Detection of Anti *H. pylori* IgG in serum by ELISA was done by commercial test kit (Monobind Inc. Lake Forest, CA 92630, USA, AccuBind, ELISA Microwells)

Data analysis

Survey data and laboratory data were analyzed using SPSS v. 26. Mean along with standard error of the mean, percentages were calculated. Odds ratio (OR) were calculated where the distribution of *H. pylori* IgG seronegative vs seropositive were dependent variables. Took P<0.05 as significant.

Results

Eighty blood samples (59 male and 21 female) were collected from 32 Bangladeshi students and 48 Somalian students (Table 1) and the average age of participant's was 24.90±0.477 years. Among 80 students, more than half (55%) were diagnosed with *H. pylori* Compare to Somalian (33 out of 48) students, Bangladeshi (11 out of 32) students found less positive in *H. pylori* IgG analysis and it has been found significant (p<0.002). The age and gender distribution pattern of their samples showed that most of the samples were obtained from age between 20–24 years old (56.3%).*H. pylori* IgG was predominating in males(38 out of 59) rather than females(6 out of 21) and it was significant (p=0.006). Within 44 seropositive most of them were from undergraduate level (29 participants). But there was no significant association with regards to age group, level of education distribution of participants, and presence of *H. pylori* IgG.

Total numbers of participants were subjected to analyze their food habits and lifestyle to know whether the bacterial infections were related to their food sources, lifestyles. Students were asked whether their foods were homemade/self-made or purchased from a restaurant. The results are summarized in Table 2. Where most of them 59 (73.3%) had restaurant-made food, however, food sources showed no statistical association to be seropositive (presence of IgG antibody). We further enquired about their food habits particularly the meal and breakfast skipping pattern and physical activity level. It is noteworthy that maximum entrant 22 (27.5%) had their meals 2 hours delay where only 7 had a meal on time. Among 80 participants 19 (23.8%) deviated their meal for more than 4 days per week. Significant associations were found in the case of breakfast (p<0.001) and physical activity (p=0.007). Around 51 (63.7%) students skipped their breakfast which is shocking. Physically inactive or less active students were prone to seropositive compared to moderately and highly active students.

Table-1: Demographic Information among participants

Parameter	Participants, N (%)	Distribution of participants on basis of *H. pylori* IgG		P-value
		Seronegative	Seropositive	

		Number	% of N	Number	% of N	
Total Participants	80 (100%)	36	45%	44	55%	
Ethnic Origin (Nationality)						
Bangladeshi Student	32 (40.0%)	21	26.3%	11	13.8%	0.002
Somalian Student	48 (60.0%)	15	18.8%	33	41.3%	
Age	Mean±SEM: 24.90±0.477					
Age Group						
20 to 24 Years Old	45 (56.3%)	22	27.5%	23	28.7%	0.790
25-29 Years Old	26 (32.5%)	11	13.8%	15	18.8%	
30-34 Years Old	4 (5.0%)	1	1.3%	3	3.8%	
34+ Years Old	5 (6.3%)	2	2.5%	3	3.8%	
Gender						
Female	21 (26.3%)	15	18.8%	6	7.5%	0.006
Male	59 (73.8%)	21	26.3%	38	47.5%	
Education						
Postgraduate	25 (31.3%)	10	12.5%	15	18.8%	0.545
Undergraduate	55 (68.8%)	26	32.5%	29	36.3%	

SEM=standard error of the mean. $p \leq 0.05$ considered statistically significant

A multivariate logistic regression analysis was conducted to find the association between microbial infections and a number of variables (food consumption timing, lifestyle, age , gender, education, and ethnic origin). In Table-3the associations are summarized. With 95% confidence interval, gender (OR: 4.524, CI: 1.526-13.407, p=0.006), ethnic origin (OR: 4.200, CI: 1.622-10.872, p=0.002), skipping of meals (OR: 8.600, CI: 0.984-75.151, p=0.023), skipping of breakfast (OR: 11.205, CI: 3.740-33.568 p<0.001) and physical activity levels (OR: 0.286, CI: 0.113-0.721, p=0.007) were found significantly associated with *H. pylori* infections. Seropositivity of *H. pylori* IgG was not linked with age, education level, and sources of food.

While analyzing the data of Table-1, we have found that foreign students possessed much more infections than local students. To know the reason within our data, we have further compared food habits and lifestyles between them (Table-4). One-third of Bangladeshi students were infected with *H. pylori*, however, for the Somalian students (68.7%) the results were just the opposite. More than 95% of Somalian students skipped at least one of the daily meals. Half of the Bangladeshi student skipped breakfast, on the other, hand the rate in Somalian students was 72.9%. More than half of the Somalian students (56.3%) were either inactive or less active, the results in Bangladeshi students (40.6%) were found contrary.

Table-2: Lifestyle and food habits of participants

Parameter	Participants, N (%)	Distribution of participants on basis of *H. pylori* IgG				P-value
		Seronegative		Seropositive		
		Number	% of N	Number	% of N	
Food Sources						
Homemade/Self-made Foods	21 (16.3%)	9	11.3%	12	15.0%	0.818
Restaurant Made Foods	59 (73.3%)	27	33.8%	32	40.0%	

Regular meals taken habits						
Meal deviation (meals have been taken within hour of regular time)	7 (8.8%)	6	7.5%	1	1.3%	
1 hour late of regular time	21 (26.3%)	13	16.3%	8	10.0%	0.022
2 hours late of regular time	22 (27.5%)	6	7.5%	16	20.0%	
3 hours late of regular time	16 (20.0%)	7	8.8%	9	11.3%	
3+ hours late of regular time	14 (17.5%)	4	5.0%	10	12.5%	
Frequency of meal deviation per week						
Not skipped	15 (18.8%)	12	15.0%	3	3.8%	
1 to 2 days per week	13 (16.3%)	7	8.8%	6	7.5%	
2 to 3 days per week	16 (20.0%)	6	7.5%	10	12.5%	0.021
3 to 4 days per week	17 (21.3%)	6	7.5%	11	13.8%	
4+ days per week	19 (23.8%)	5	6.3%	14	17.5%	
Breakfast						
Not Skipped	29 (36.3%)	23	28.7%	6	7.5%	0.000
Skipped	51 (63.7%)	13	16.3%	38	47.5%	
Physical activity level						
Not active	13 (16.3%)	2	2.5%	11	13.8%	
Less active	27 (33.8%)	10	12.5%	17	21.3%	0.007
Moderately active	22 (27.5%)	16	20.0%	6	7.5%	
Highly active	18 (22.5%)	8	10.0%	10	12.5%	

SEM=standard error of the mean. $p \leq 0.05$ considered statistically significant

Table-3: Factor Associated with *H. pylori* infection

Factors	Odd Ratio	95% Confidence Interval		P-Value
		Lower	Upper	
Age (20 to 24 years old vs 24+ years old)	1.435	.587	3.507	0.428
Gender (Female vs Male)	4.524	1.526	13.407	0.006
Education (Postgraduate vs Undergraduate)	0.744	0.285	1.941	0.545
Ethnic Origin (Bangladeshi vsSomalian Students)	4.200	1.622	10.872	0.002
Food Sources (Home/Self made foods vsRestaurant Made Foods	0.889	0.325	2.427	0.818
Skips Meals (Not skipped vs skipped	8.600	0.984	75.151	0.023
Breakfast (Not skipped vs skipped)	11.205	3.740	33.568	0.000
Physical activity (Not	0.286	0.113	0.721	0.007

active/Less active vs Moderately/Highly active)				

$p \leq 0.05$ considered statistically significant

Table-4: Comparison of Lifestyle and Food habits between Bangladeshi and Somalian Students

Parameters	Bangladeshi Students (n=32)		Somalian Students (n=48)	
	Number	% of within ethnic origin	Number	% of within ethnic origin
Distribution of participants on basis of *H. pylori* IgG				
Seronegative	21	65.6%	15	31.3%
Seropositive	11	34.4%	33	68.7%
Meals				
Not Skipped	5	15.6%	2	4.2%
Skipped	27	84.4%	46	95.8%
Breakfast				
Not skipped	16	50.0%	13	27.1%
Skipped	16	50.0%	35	72.9%
Physical Activity				
Not or Less active	13	40.6%	27	56.3%
Moderately or Highly active	19	59.4%	21	43.7%

Discussion

H. pylori infection is one of the major public health issues and approximately 4.4 billion individuals were estimated to be positive for *H. pylori* infection worldwide [35].The infection is prominent in developing countries. Particularly poor cleanliness and swarmed environment have been found as risk factors[14]. *H. pylori* is frequent and was acquired in childhood, however, it bypasses both immune response and becomes a carrier *of H. pylori* [15, 16]. Most of the people with *H. pylori* are found asymptomatic [18, 19]. *Helicobacter pylori* infection is coupled with several factors including races, environments, microbial factors, etc. H. pylori infections are predominant reasons for peptic ulcers disease, gastritis, and gastritis cancer [1-4].

The present study aimed to determine the prevalence of *H. pylori* infections and their relation with food sources, food consumption habits, and lifestyles. Among 80 students were surveyed and diagnosed. Around 55% were found seropositive which confirms the higher prevalence in developing countries [14, 24,25].The result is almost similar to the two studies[36,37]. The prevalence of *H. pylori* IgG antibody was higher in African (Somalian) students compared to Bangladeshi students. This result was similar to the study conducted in the USA [27] where it was found that *H. pylori* infections were more prevalent in African American population. Regardless of age and education, bacterial infection can be observed in most of the country [18, 37]. However, a study conducted in New Zealand found that age along with socioeconomic status and lower household income are significantly associated with infection[38]. Our study demonstrated that males are more ubiquitous than females. This observation supports some

studies[26, 38]. In 2019 Ahmed et. al. showed the prevalence of *H. pylori* infection was among 23% of hospital patients [25]. The deflection from our study was probably we are working with university students. A study conducted in 2018 concluded that the risk of *H. pylori* infection was higher in university students [36]. The USA [27] study showed that lower educated people are more susceptible. In this study, undergraduate students are found seropositive than postgraduate study and signify the USA [27] study results. The probable causes might be the youngster's indifference towards food consumption habits and lifestyle. Lack of knowledge on hygiene and sanitation also might play a significant role in this regard.

The prevalence of *H. pylori* infection was shown at 55%, which is lower than in neighboring countries e.g. India at 79%, Pakistan at 84% [25]. Probably because very few Bangladeshi people (1.15%) live without improved sanitation compared to India (4.53%) and Pakistan (6.03%)[39]. Moreover, no Bangladeshis paretic open defecation whereas Indians (14.93%) and Pakistani (7.3%) people go for it[39]. As a result, among 179 countries in the sanitation and drinking water index 2020, Bangladesh (129) remains better than that India (139) and Pakistan (144)[40]. An article published in the Bulletin of the World Health Organization reported that *H. pylori* is mostly transmitted through water[41].

Somalian students (68.7% among Somalians) were found to be more prevalent compared to Bangladeshi students (34.4% among Bangladeshis). There could be many reasons as H. pylori infections show composite factors. One of the factors could be Somalian students were infected earlier in Somalia and remain asymptomatic. Due to socioeconomic conditions, 15.82% of Somalians do not have access to improved sanitation and 23.12% practices open defecation [39]. These could be a major reason for being infected with *H. pylori*. Other factors might include the language barrier in Bangladesh and native food habits that probably held back them to get proper and on-time meals. Similarly, lack of proficiency in common language thwarts them to be friendly with local students which made them engage in their room. Thus, they became less active compared to Bangladeshi students. Race-related connections were discussed above.

Multivariate analysis of current studies demonstrated that skipping of meals (OR: 8.600, CI: 0.984-75.151, p=0.023) and breakfast (OR: 11.205, CI: 3.740-33.568 p<0.001) are significantly associated with *H. pylori* infection. A similar pattern was also reported in a 2013 study [28] and concluded that prolonged meal skipping and deviation emerge augmented the possibility of rising gastritis and *H. pylori* infection[42]. Skipping or irregular timing of meals including breakfast perhaps changes the mucosal membrane that could raise susceptibility *H. pylori* infection. The mucosa membrane can be then disturbed and facilitate bacterial infection [43]. Nevertheless, there was no association between food sources and *H. pylori* infection in this study which is in line with others[44]. A recent study [45] found that physical activity levels are linked with *H. pylori* infection as we have found so. The active human body activates several biochemical pathways as well as defense systems that might prevent bacterial infections.

Conclusion

Helicobacter pylori infections become a prime concern for human wellbeing at present. Several factors including the human race, socioeconomic conditions, environments, personal hygiene, and sanitation as well as microbial factors are responsible for the prevalence and progression of the disease. Prolonged infections are a risk factor for peptic ulcer diseases as well as stomach cancer. To prevent this infection,

we need to follow a healthy lifestyle. As this bacterial infection occurs mostly in childhood with unwariness and carefree lifestyle of youngsters might create problems in late stages of life. Cleanliness can prevent these bacteria as well as on-time meals and adequate physical activity could be major protective measures. Somalian students perhaps carry *H. pylori* from their native; however, staying in Bangladesh with some obstacles including communication and having foods of their choice enhances the risks. Universities as well and authorities responsible for foreign students need to be cautious about their health. Authority should motivate students to take their meals on time and to exercise regularly as well.

Limitations of the study

The major limitation of the study was the sample size. Due to the COVID-19 pandemic, we had to limit data and blood sample collection. Cluster-based equal number of samples could be solidified our results. As the youngsters were the subject and for the time constrain, we could not have many demographic, physiological, health, habit, and lifestyle related questions in our questionnaire. A bigger sample size and more variables could strengthen our findings. There are also limitations, in fund accusation. However, despite all limitations we are thankful to all who helped and supported us to complete the experiments.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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