Research Article

Seroepidemiological investigation of antigen and antibody for the detection of Helicobacter pylori infection in a rural area of Nepal

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Abstract:

Objective: *Helicobacter pylori* (*H. pylori*) is now recognized as a worldwide problem. The objective of the study was to investigate the seroprevalence of *H. pylori* infection and factors associated in a rural area of Nepal.

Methods: A community based cross-sectional study was carried out on 120 individuals based on cluster sampling of residential location in Province 2, Nepal. *H. pylori* infection status was determined by serology test on stool and blood samples for antigen and antibody. A questionnaire was filled out and written consent was taken. Data were analyzed using chi- square test and P-value (≤ 0.05) were considered statistically significant.

Results: Out of 120 participants 57(47.5%) and 63(52.5%) were female and male respectively. Similarly 5.8% were less than 20 years of age 49.2% were 20 to 40 age and 45% were more than 40 years age. Significant association was seen with occupation P=0.023 with antigen and P=0.042 with antibody result, consumption of water P=0.04 with antigen and P=0.005 with antibody, with onion and garlic P=0.032, consumption fried food P=0.024 and consumption and spicy food consumption P=0.050 with antigen, with onion and garlic P=0.027, consumption of fried food P=0.024 and consumption of spicy food P=0.050 with antibody positive result showed significant association.

Conclusions: The result of our research suggests the periodic screening and checkup of the patients in order to detect the infecting agent among the rural areas patient and effective treatment is required.

Keywords: Gastritis; Retrosternal- burn; Helicobacter pylori; Antigen; Antibody

Introduction

Helicobacter pylori is associated with gastro-duodenal infections in human [1] *H. pylori* has been found strongly related to chronic gastritis, duodenal ulcer, gastric carcinoma, and mucosa-associated lymphoid malignancies. Gastric cancer is the most common disease related cancer globally [2,3,4].

The infection is widespread throughout the world, and is present in about 50% of the global human population; with 80% in developing countries and 20 - 50% in industrialized countries. It is the major cause of gastritis, which plays a key role in the etiology of peptic ulcer and is a risk factor for gastric carcinoma [5].

Sero-epidemiological investigations represent the most rapid and convenient way of obtaining a picture of the prevalence of *H. pylori* infection in a population, but the assays used need to be validated in the population studied [6, 7, 8]. Serologic investigations are usually performed to detect specific antibodies against *H. pylori.* They are commercially and easily available, less expensive and easy to perform. However this diagnosis of *H. pylori* cannot differentiate between active and asymptomatic colonization, past and current infection [9]. Stool antigen Immunochromatography Rapid Detection Test (RDT) is used to detect *H. pylori* antigens in the feces. However this is a reliable and accurate diagnostic technique for *H. pylori* infection and verification of its cure after treatment. It is convenient for the patients and can be easily performed even in small laboratories [10,11]. However its accuracy in different clinical settings and controlled studies is a problem for discussion [12,13].

The present study was therefore designed for comparative evaluation of stool antigen test and blood antibody test methods detection by a commercially available kit for diagnosis of *H.pylori* infection in rural areas of Nepal.

Methodology

1. Study design, period and setting

This was a cross-sectional, community based study conducted in the Aurahi village, Mahottari district, Nepal. The study took place over a 1 year period from February 2019 till January 2020. About 120 participants were enrolled in the study.

2. Study population

All participants were from this village. The suspicious patient for *H. pylori* with symptoms such as abdominal pain, recurrent vomiting, and upper gastrointestinal bleeding were recruited. A total of 120 participants were enrolled for the study.

3. Study tools and data collection

Demographic details and clinical features

a) A pre-test structured questionnaire was used to collect information from the participants regarding possible risk factors for infection including the following: (i) socio-demographic characteristics including age, gender, residence, education, family members (number of people living in the household), and availability of potable water; and (ii) dietary practices, such as daily eating food habits. Questions used in this section were adopted from the various literature by the researchers and were revised by experts from Microbiology.

4. Ethical Consideration

An ethical permission was obtained from Institutional Review Committee, Pokhara University for ethical clearance (Ref no.97/076/077). The permission was obtained from the Aurahi, ward office, Mahottari District, Nepal. Participants were verbally informed about the study and written consents were taken from the eligible participants.

5. Laboratory tests

a. Helicobacter pylori antigen testing

H. pylori was diagnosed using a stool antigen test which is rapid, non-invasive, reliable and easy to perform and can be used to detect an existing infection. Participants were given clean, leak proof containers to provide fresh

stool samples within 2 hours. All stool specimens were transported to the laboratory maintaining at −4°C. The specimen that took more than 2 hours before processing were kept at −20°C. A lateral flow Immunochromatography assay for detection of *H. pylori* antigen in stool was used with a sensitivity of 96% and specificity of 83% by CTK diagnostic kit. Based on the intensity of the color band developed, results were reported as *H. pylori* antigen not detected or detected.

b. Helicobacter pylori antibody testing

Blood samples were collected which were labeled with unique code number and centrifuged to get serum and the samples were processed for *H. pylori* antibody by rapid kit method by CTK diagnostic kit with a sensitivity of 96% and specificity of 83%.

6. Statistical analysis

Recorded data was entered on the computer using the MS Excel and statistical analysis was done by Statistical Package of Social Science Software Program (SPSS), version 16. Data was presented using mean, median, range, and frequency and percentages for qualitative ones. Comparison between groups was performed using chi-square test for qualitative ones. P-value (≤ 0.05) was considered statistically significant.

Results

A community based cross-sectional study was carried out in a rural area, Aurahi, Mahottari district, Nepal. All together 120 participants were involved in the study. 57(47.5%) and 63(52.5%) were female and male participant respectively. 95% were Hindu and 5% were Muslim. Similarly 5.8% were less than 20 years of age 49.2% were 20 to 40 age and 45% were more than 40 years age as shown in table 1.

Table 1: *Socio-demographic characteristics of participants*

Variables	Frequency(n)	Percentages (%)
Age category (yrs.)		
Less than 20	7	5.8
20-40	59	49.2
More than 40	54	45.0
Sex		
Female	57	47.5
Male	63	52.5
Marital status		
Divorced	1	.8
Married	114	95.0
Unmarried	5	4.2
Religion		
Hindu	114	95.0
Muslim	6	5.0
Total	120	100.0

Family status of the participants show that 55% were less than 5 family numbers 42.5% were less than 10 and more than 5, 2.5% were more than 10 in their family. Similarly 15% were businessman, 21.7% were employee, 17.5 were farmer, 43.3% were housewife and 2.5% were student respectively. Similarly 44.2% were illiterate, 25% had primary education, 21.7% had secondary education and 9.2% were university graduates. 50% were those who had income less than1 lakh per year as in table 2.

Table 2: Family status of participants

Variables	Frequency(n)	Percentages (%)
Family member		
Less than 10 and more than 5	51	42.5
Less than 5	66	55.0
more than 10	3	2.5
Occupation		
Business	18	15.0
Employee	26	21.7
Farmer	21	17.5
Housewife	52	43.3
Student	3	2.5
Education		
Illiterate	53	44.2
Primary school	30	25.0
Secondary school	26	21.7
University	11	9.2
Total income		
1lakh to 2.5 lakh	40	33.3
2.5 to 5 lakh	12	10.0
Less than 1 lakh	60	50.0
More than 5 lakh	8	6.7
Total	120	100.0

Table 3: Food habits of participants

Variables	Frequency(n)	Percentages (%)
Frequency of vegetables		
Everyday	110	91.7
Never	1	.8
Some times a week	9	7.5
Fruits		
Everyday	30	25.0
Never	9	7.5

Sometimes a week	81	67.5
Milk, Meat products		
Everyday	60	50.0
Never	8	6.7
Sometimes a week	52	43.3
Onion & garlic		
Everyday	116	96.7
Never	1	.8
Sometimes a week	3	2.5
Fried foods		
Everyday	50	41.7
Never	2	1.7
Sometimes a week	68	56.7
Spicy foods		
Everyday	73	60.8
Never	8	6.7
Sometimes a week	39	32.5
Total	120	100.0

Table 4: Alcohol consumption and smoking habits of participants 25.8% were alcohol consumer while 14.2% were smokers.

Variables	Frequency(n)	Percentages (%)
Alcohol		
No	89	74.2
Yes	31	25.8
Smoking		
No	103	85.8
Yes	17	14.2
Total	120	100.0

Table 5: Hygiene practices

Variables	Frequency(n)	Percentages (%)
Using finger to eat		
Always	120	100.0
Hand washing before meal		
Always	116	96.7
Less frequent	4	3.3
Toilet facility		
No	9	7.5
Yes	111	92.5

Hand washing after toilet		
Always	117	97.5
Less frequent	3	2.5
Consumption of drinking water		
Hand pump	108	90.0
Public	8	6.7
Well	4	3.3
Total	120	100.0

Table 6: *H. pylori* antigen and antibody detection 41.7% (50) were positive for *H. pylori* antibody while 43.3% (52) were positive *H. pylori* antigen test as shown in table.

Variables	**Frequency(n)**	**Percentages (%)**
Results (Ab)		
Negative	70	58.3
Positive	50	41.7
Results (Ag)		
Negative	68	56.7
Positive	52	43.3
Total	120	100.0

Table 7: Association of socio demography with *H. pylori* antigen antibody positive test.

		H. pylori Antigen		*H. pylori* Antibody	
		(n = 120)		(n = 120)	
Variable	No.	No. (%)	p-value	No. (%)	p-value
Category		positive		positive	
Age (years)					
Less than 20	7	2(3.8%)	0.358	2(4.0%)	0.564
20-40	59	23(44.2%)		23(46.0%)	
More than 40	54	27(51.9%)		25(50.0%)	
Sex					
Female	57	28(53.8%)	0.223	26(52.0%)	0.460
Male	63	24(46.2%)		24(48.0%)	
Marital status					
Divorced	1	1(1.9%)	0.513	1(2.0%)	0.493
Married	114	49(94.2%)		47(94.0%)	
Unmarried	5	2(3.8%)		2(4.0%)	
Total	**120**	**52(100.0%)**		**50(100.0%)**	

No significant association was seen with sociodemographic study.

Table 8: Association of family status with *H. pylori* antigen antibody positive test.

		H. pylori Antigen		*H. pylori* Antibody	
		(n = 120)		(n = 120)	
Variable	No.	No. (%)	p-value	No. (%)	p-value
Category		positive		positive	
Family members					
Less than 10 and more than 5	51	25(48.1%)	0.546	23(46.0%)	0.465
Less than 5	66	26(50.0%)		26(52.0%)	
More than 10	3	1(1.9%)		1(2.0%)	
Occupation					
Business	18	7(13.5%)	0.023*	7(14.0%)	0.042*
Employee	26	5(9.6%)		5(10.0%)	
Farmer	21	13(25.0%)		13(26.0%)	
Housewife	52	25(48.1%)		23(46.0%)	
Student	3	2(3.8%)		2(4.0%)	
Education					
Illiterate	53	27(51.9%)	0.237	26(52.0%)	0.185
Primary school	30	9(17.3%)		8(16.0%)	
Secondary school	26	10(19.2%)		10(20.0%)	
University	11	6(11.5%)		6(12.0%)	
Total income					
1lakh to 2.5 lakh	40	17(32.7%)	0.498	16(32.0%)	0.498
2.5 to 5 lakh	12	3(5.8%)		3(6.0%)	
Less than 1 lakh	60	29(55.8%)		28(56.0%)	
More than 5 lakh	8	3(5.8%)		3(6.0%)	
Total	**120**	**52(100.0%)**		**50(100.0%)**	

*Significant association

Significant association was seen with occupation P-value (0.023) with antigen and P-value (0.042) with antibody positivity result.

Table 9: Association of Hygiene practice with *H. pylori* antigen antibody positive test.

		H. pylori Antigen		*H. pylori* Antibody	
		(n = 120)		(n = 120)	
Variable	No.	No. (%)	p-value	No. (%)	p-value
Category		positive		positive	
Hand washing before meal					

Always	116	48(92.3%)	0.334	47(94.0%)	0.307
Less frequent	4	4(7.7%)		3(6.0%)	
Toilet facility					
No	9	4(7.7%)	0.569	4(8.0%)	0.507
yes	111	48(92.3%)		46(92.0%)	
Hand washing after toilet					
Always	117	50(96.2%)	0.578	49(98.0%)	0.658
Less frequent	3	2(3.8%)		1(2.0%)	
Consumption of drinking water					
Hand pump	108	48(92.3%)	0.04*	46(92.0%)	0.005**
Public	8	4(7.7%)		4(8.0%)	
Well	4	0(0.0%)		0(0.0%)	
Total	**120**	**52(100.0%)**		**50(100.0%)**	

*Significant **** highly significant

Significant association was seen with consumption of water P-value (0.04) with antigen and P-value (0.005) with antibody positivity result.

Table 10: Association of eating habits with *H. pylori* antigen antibody positive test.

		H. pylori Antigen		*H. pylori* Antibody	
		(n = 120)		(n = 120)	
Variable	No.	No. (%)	p-value	No. (%)	p-value
Category		positive		positive	
Frequency of eating vegetables					
Everyday	110	46(88.5%)	0.239	44(88.0%)	0.207
Never	1	0(0.0%)		0(0.0%)	
Some times a week	9	6(11.5%)		6(12.0%)	
Consumption of fruits					
Everyday	30	16(30.8%)	0.405	15(30.0%)	0.452
Never	9	3(5.8%)		3(6.0%)	
Sometimes a week	81	33(63.4%)		32(64.0%)	
Milk and meat products					
Everyday	60	23(44.2%)	0.372	23(46.0%)	0.425
Never	8	5(9.6%)		5(10.0%)	
Sometimes a week	52	24(46.2%)		22(44.0%)	
Onion and Garlic					
Everyday	116	48(92.3%)	0.032*	46(92.0%)	0.027*
Never	1	1(1.9%)		1(2.0%)	
Sometimes a week	3	3(5.8%)		3(6.0%)	
Fried Foods					
Everyday	50	27(51.9%)		26(52.0%)	

Never	2	2(3.8%)	0.024*	2(4.0%)	0.024*
Sometimes a week	68	23(44.2%)		22(44.0%)	
Spicy foods					
Everyday	73	33(63.5%)	0.050*	32(64.0%)	0.05*
Never	8	6(11.5%)		6(12.0%)	
Sometimes a week	39	13(25.0%)		12(24.0%)	
Total	**120**	**52(100.0%)**		**50(100.0%)**	

*Significant

Significant association was seen with onion and garlic P-value (0.032), consumption of fried food P-value (0.024) and consumption of spicy food P-value (0.050) with antigen while significant association was seen with onion and garlic P-value (0.027), consumption fried food P-value (0.024) and consumption and spicy food consumption P-value (0.050) with antibody positive result.

Table 11: Association of Alcohol and smoking habits with *H. pylori* antigen antibody positive test.

		H. pylori Antigen		*H. pylori* Antibody	
		(n = 120)		(n = 120)	
Variable	No.	No. (%)	p-value	No. (%)	p-value
Category		positive		positive	
Alcohol consumption					
No	89	41(78.8%)	0.401	39(78.0%)	0.276
Yes	31	11(21.2%)		11(22.0%)	
Smoking habits					
No	103	43(82.7%)	0.412	42(84.0%)	0.409
Yes	17	9(17.3%)		8(16.0%)	
Total	**120**	**52(100.0%)**		**50(100.0%)**	

No significant association was seen with alcohol consumption and smoking habits.

Discussion

This study was a community based cross sectional study choosing individual with gastritis as a research participants. The study was carried out in a rural areas in a village called Aurahi, Mahottari district, Nepal. In our study, the total number of 120 research participants were included whose stool was collected for IRD antigen test and blood sample for serum IRD antibody test.

The prevalence of *H. pylori* was 43.3% by stool antigen test and 41.7% serum antibody by Immunochromatography Rapid Test. This prevalence was low compared to other studies done elsewhere in the developing world [14,15]. The difference in results is probably due to the variations in the study population, and the age and health conditions of the patients. In addition, differences in the geographic regions, the type of test specimens (stool and blood), the analytical methods used, and the target molecules, that is, antigen versus antibodies, are likely to have influenced the differences in the study findings.

The current study population was mainly above 14 years of age, hence a lower prevalence than that reported in other developing countries. This is supported by the findings of studies by Newton et al. [16], Jackman et al. [17], Brandi et al. [18], and Hestvik et al. [19]. A study by Triantafyllopoulou et al. [20] revealed that most

adults infected with *H. pylori* organism are asymptomatic and may therefore test negative by the antibody-detection tests and positive by the antigen- detection tests. Hence, the stool antigen tests can aid in detection of actively infected or carrier individuals.

More female patients tested positive (53.8% by stool antigen and 52.0% by serum antibody); however, this was not significantly different (p=0.460) to that of the male participants (46.2.8% by stool antigen and 48.0% by serum antibody). However, Moayyedi et al. [21] reported that males, living with a partner, and poor adult socio-economic conditions are associated with increased risk of *H. pylori* infection. Our study also examined possible predisposing factors to infection. It was found that cigarette smoking, poor sanitation, and lack of formal education were the predisposing factors to H. pylori infection.

H. pylori Seropositivity decreased linearly with cigarette consumption because Khalifa et al. [22] proved that there is no significant association between cigarette smoking with *H. pylori* infection, the most likely risk factor in the current study was poor living conditions, which is in agreement with findings from similar studies in other countries [23- 26]. Triantafyllopoulou et al. [20] reported that the acquisition of *H. pylori* could result from environmental and professional exposure issues, and poor public water supply.

The current study aimed at establishing the Seroepidemiological prevalence of *H. pylori* and the risk factors in rural parts of Nepal. The prevalence is less than what has previously been reported in other developing countries as well as in urban areas and rural areas.

Conclusion and Recommendation

Even though people were using gastritis drugs, still the prevalence of *H. pylori* in these individuals is found higher. *H. pylori* was more among female than male and was higher in age group more than 40 years. The result of our research suggests the periodic screening and checkup of the patients in order to detect the infecting agent among the rural areas patient. Only the use of gastritis drugs is not enough to complete treatment anti *H. pylori* drugs are required for treating of infective gastritis for complete treatment.

Various public awareness and health education programs should be conducted to aware people about gastritis and its consequences like having ulcers. Awareness campaigns should be focused in remote areas where education is not primary.

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