Epidemiology of *Helicobacter pylori* in Bhutan: The Role of Environment and Geographical Location

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Abstract:

Background: Bhutan is small mountainous country bordering India and China and consists of four geographical regions, west, east, central, and south. The epidemiology of *Helicobacter pylori* infection and risk factors associated with in Bhutan are not previously studied. The World Health Organization reported the incidence of stomach cancer to be very high in Bhutan**. Aim**: We conducted a cross-sectional study to determine the seroepidemiologic pattern of *H. pylori* among Bhutanese from the four regions with emphasis on water source and household sanitation. **Methods:** Between June and November 2012, blood sample from patients with complaints of dyspepsia and were collected after an informed consent. Demographic information, occupation, family size living in the same household, consumption of betel nut, and aspects household environment including type of latrines, source of drinking water were collected. All serum samples were tested for *H. pylori* Immunoglobin G (IgG) by Enzyme Linked Immuno-sorbent Assay (ELISA) using MAGIWELL ELISA kit from United Biotec, USA. **Results:** Two hundred forty four patients between the ages of 17 and 75 years participated in the study; 102 males, and the mean age was 38 ($\pm$ 14.2) years. The overall prevalence of *H. pylori* among patients was 86% with no difference between males and females (90% vs. 83%, respectively, p=0.12). The prevalence was almost identical among all age groups; 81% at age 17-20, 84% (20-29), 93% (30-39), 82% (40-49), 87% (50-59) and 82% at $\geq$60 years (p=0.51). *H. pylori* prevalence was lower in the south region of Bhutan (78%) compared to the central region (97%) (OR=8.6; 95%CI=1.1-55; p=0.02), Eastern region (91%) (OR=2.7; 95%CI=1.1-7.2, p=0.004) or the western region (83%)

(OR=1.4, 95% CI=0.8-3.1, p=0.07). . The prevalence of *H. pylori* was significantly lower among household with less than 4 persons living in the same household. Source of drinking water, type of occupation, type of latrines or consumption of betel nut, showed no association with *H. pylori* prevalence. Logistic regression analysis revealed that residing region was the only significant variable. **Conclusions:** The high prevalence of antibodies to *H. pylori* among patients and in all groups could contribute to the high incident rate of gastric cancer in Bhutan. Crowded living condition and the residing region contribute to the variation of the prevalence of the infection. **The lowest prevalence in southern part of the country could be due to the difference in the ethnicity as most of its population is of Indian and Nepal origin.** Further data regarding *H. pylori* in Bhutan are critical to developing surveillance and prevention strategies for gastric cancer.

Introduction:

Helicobacter pylori infection has been associated with gastritis and the gastritis-associated diseases, peptic ulcer, and gastric cancer (1-3). The prevalence of *H. pylori* infection varies both among and within populations and is inversely related to standards of living and **hygiene and sanitation** (4-7) The increased risk of acquisition of the infection is especially high among those living in developing countries (8-11) The risk is multi-factorial that may include interaction with potentially contaminated environmental sources as local drinking water, swimming in rivers, and the ingestion of fecally contaminated vegetables have all been reported as risk factors for the acquisition of *H. pylori* infection (10, 12). In 1994, the International Agency for Research on Cancer categorized *H. pylori* infection as a definite group I carcinogen (13). Gastric cancer is the second leading cause of cancer-related death in the world and its risk varies among the countries and populations in the world (14).

Bhutan is a small country located in South Asia, at the eastern end of the Himalayas and it shares borders with south, east and west by India and to the north China. Though the prevalence of *H. pylori* in Bhutan has not been elucidated, the World Health Organization (WHO) reported the incidence of gastric cancer to be very high in Bhutan (15). Moreover, it has been reported that **mortality from** gastric cancer in Bhutan is very high (24.2 deaths/100,000 population) comparing to India, Bangladesh and Thailand (16). The reason for this high incidence of gastric cancer has not been explained. **Further data regarding *H. pylori* infection in Bhutan are critical to understand the epidemiology of the infection and *H. pylori* related diseases including gastric cancer. Therefore, we**

conducted the current study to determine the prevalence of *H. pylori* infection by age, gender, occupation, sanitation, crowding, and geographic area within Bhutan.

Materials & Methods:

Study Population:

A cross-sectional sero-epidemiologic study was carried out among unrelated Bhutanese individuals between June and September 2012. The study population consisted who attended the digestive disease out patient clinic at the National Referral Hospital, Thimphu, for upper gastrointestinal complains and dyspepsia were included in the study after informed consent. **All patients were qualified and went under upper endoscopic examination during the study period participated in the study.** Demographic information, occupation, family size living in the same household, consumption of betel nut, and aspects household environment including type of latrines, source of drinking water were collected. The study started in June and ended November 2012. Informed consent was obtained from all participants.

Geography, Population, and Climate of Bhutan

Bhutan is a remote Himalayan country between India and Tibet (China) with a population consists of only 800,000 citizens residing in 18,147 sq mi (47,000 sq km) (Fig-1). Seventy percent of country is rural and agriculture based and the literacy rate is 47% (2011 Census). More than 30% of Bhutan populations live below poverty level. The climate in Bhutan varies with elevation, from subtropical in the

south to temperate in the highlands and polar-type climate, with year-round snow in the north.

Bhutan is demographically divided into four main regions, southern, western, eastern, and central regions. The southern region shares border with India and ethnically they are of Indian and Nepal origin. The Western region is mostly on higher altitudes and socioeconomic standard is higher than Sothern region. The normal water supply is through rural water scheme that is supported by the government and most people use local streams, rivers and piped water supply. The Central region shares similarity with the western region both socioeconomically and geographically though it is little warmer. The eastern region is lower in altitude than the Western region and have similar rural water supply scheme as the western region.

Serologic Methods:

Each participant provided a blood sample. Sera were stored at −20 C until analyzed. Evaluation of IgG antibody to the high-molecular-weight cell-associated proteins of *H. pylori* was determined using a well characterized enzyme-linked immunosorbent assay (using MAGIWELL ELISA kit from United Biotec, USA). This ELISA measured IgG antibodies to *H. pylori* infection. *H. pylori*–specific IgG antibodies indicate current or recently cured infection. The results were interpreted according to the manufacturer's instructions. *H. pylori* infection was defined as a positive ELISA result.

Statistical Analysis:

The Mantel-Haenszel *X2* test was used to assess the associations between each independent factor of the study and the prevalence of *H. pylori* infection. Univariate and multivariate analyses, ORs, and 95% CI were calculated for *H. pylori* seropositivity associated with the study variables. Risk factors that were significant in the univariate analysis were used in the multiple logistic **regression models using the level of type I error=0.05.** These models help to assess the relative importance of risk factors while controlling for other factors.

Results:

Prevalence of *H. pylori* infection among the studied patients:

Two hundred forty four patients between the ages of 17 and 75 years participated in the study; 102 males, and the mean age was 38 **(SD± 14.2**) years. The overall prevalence of *H. pylori* among patients was 86% with no difference between males and females (90% vs. 83%, respectively, p=0.12). The prevalence was almost identical among all age groups; 81% at age 17-20, 84% (20-29), 93% (30-39), 82% (40-49), 87% (50-59) and 82% at ≥60 years (p=0.51).

H. pylori* sero-positivity and demographic, geographic, and environmental variables:*

Adjusted ORs were calculated for *H. pylori* seropositivity in relation to the study variables (Table-1).). **The overall prevalence of *H. pylori* infection people reside in the central region** of Bhutan was 97% and was significantly higher than the prevalence in the south region of Bhutan (78%); (OR=8.6; 95% CI=1.1-55; p=0.02), Eastern region

(91%) (OR=2.7; 95%CI=1.1-7.2, p=0.004) or the western region (83%) (OR=1.4, 95% CI=0.8-3.1, p=0.07), (Fig-2). The prevalence of *H. pylori* infection was examined in relation to the number of family members living in the same household which reflects crowding living condition. It was significantly lower among household with less than 4 persons living in the same household (Table-1). Source of drinking water, type of occupation, type of latrines or consumption of betel nut, showed no association with *H. pylori* prevalence. When logistic regression analysis was applied with all the variables in the model, the residing region was the only variable emerged to be significant.

Discussion:

This is the first study to report on the epidemiology of *H. pylori* in Bhutan. One of the marked finding of the study is the significant difference of the prevalence of the infection in different geographic regions. The Southern part had significant lower prevalence of *H. pylori* infection than the other regions of the country in spite of its lower socioeconomic leve l compared to the central and western part. The marked lower prevalence in the southern region could be due is the different of the ethnicity in the region as they are of Indian and Nepal origin and they have different food habits than the original Bhutanese. **It is known that Bhutanese are broadly from three ethnic backgrounds. The first ethnic group is from Tibetan descent that mainly from the western parts of the country while the second is the Indoburmese ethnic group where mostly from the population in the eastern parts of the country. Southern Bhutanese, the third group, is of the Nepali origin and mainly Aryan descent. Interstitially, the majority of cases of gastric cancer are reported mostly among the western Bhutanese and to some**

extent the eastern Bhutanese but it is less common among the southern Bhutanese which correlate with our results and the finding of lower prevalence of *H. pylori* infection in the southern part of Bhutan. Variation in acquisition of infection among ethnic and racial groups appeared to be primarily related to differential exposure (e.g., cultural background, social, dietary and environmental factors) (5, 8, 17, 18) and not to (or less) possible differences in genetic predisposition (19). Moreover, *H. pylori* infection has been shown to follow the routes of human migration by their geographical origin and several studies have studied the effect of immigration on the prevalence of the infection. A recent study examined *H. pylori* strains among three major ethnic populations in Malaysia, Malay, Chinese and Indian. The study reported that the majority of the Malay and Indian *H. pylori* isolates share the same origin while the Malaysian Chinese *H. pylori* is distinctive. The study concluded that the Malay population was likely to be initially *H. pylori* free, and gained the pathogen recently from cross infection from other populations (20).

It has been also established that the prevalence of *H. pylori* is inversely related to socioeconomic status (4-7). However, for populations in which the social class is more or less homogeneous, such as China and Russia, density of living has been shown to be the most significant risk factor (8, 11). Bhutan socioeconomic levels do not seem to differ markedly; therefore we used crowded living condition as a measure for socioeconomic condition. We found that the number of people living in the same household is significantly associated with increase the risk of *H. pylori* acquisition and that in consistent with several previous studies reported similar findings (21-22).

Cross-sectional studies have consistently shown a gradual increase in *H. pylori* sero-prevalence with age, which has been interpreted as a birth cohort effect reflecting a decrease in the rate of acquisition in successive generations of children as sanitation improved and standards of living increased (23-24). **Our results from Bhutan showed high prevalence of antibodies to *H. pylori* among patients in all groups.** It is likely that the socioeconomic levels in Bhutan did not differ markedly over time and the high prevalence among all ages could be a marker that contributes to the high incident rate of gastric cancer in Bhutan (15).

Although our current study is a prospective study examined several variables, it has some shortcomings. First, the studied population is a symptomatic population whom presented to a tertiary care.

In conclusion, this study demonstrates clear evidence of the high prevalence of antibodies to *H. pylori* among patients and volunteers in all groups that could contribute to the high incident rate of gastric cancer in Bhutan. Further data regarding *H. pylori* infection in Bhutan with emphasis on children are critical to understanding the epidemiology of the infection and to developing surveillance and prevention strategies for gastric cancer.

Figures Legends:

Fig-1: The geographic studied areas on the Map of Bhutan.

Fig-2- Shows the age specific prevalence of *Helicobacter pylori* infection in the studied population.

Fig-3: Shows the overall prevalence of *Helicobacter pylori* in the four regions of Bhutan

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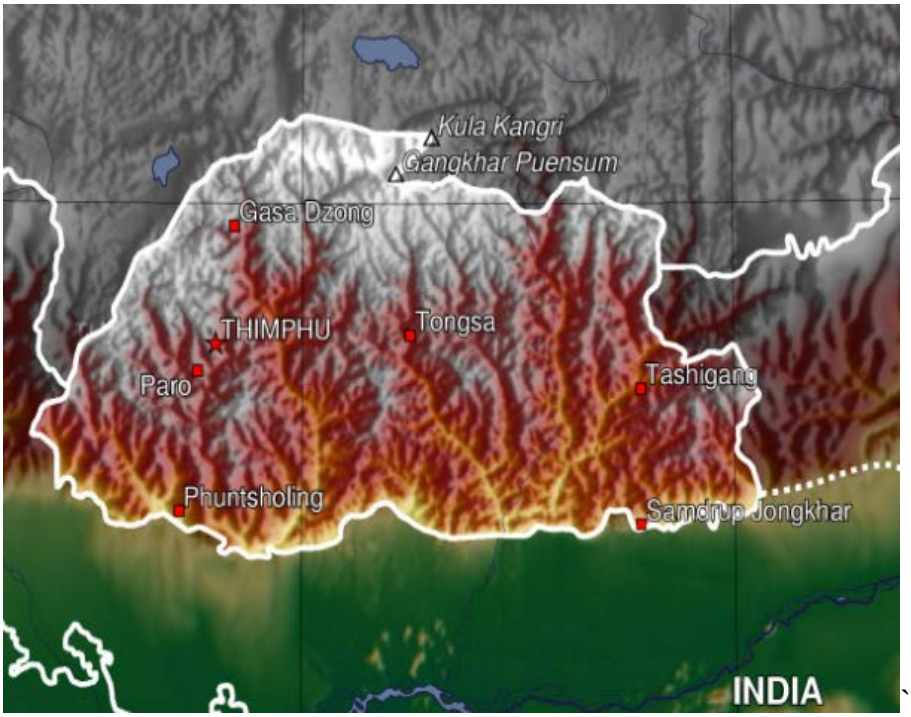
TABLE-1: Distribution of *H. pylori* (*Hp*) infection according to the study variables among 244 Bhutanese

Variable	Total (+*Hp*%)	Crude OR 95% CI	Region Adjusted OR 95% CI
Age:			
< 20	26 (81%)	Ref	Ref
… .20-29	64 (84%)	1.3 (0.6-3.6)	1.1 (0.4-4.2)
30-30	61 (93%)	2.8 (1.4-10.4)**	2.0 (1.2-8.1)**
40-49	45 (82%)	1.1 (0.7-2.1)	1.0 (0.6-2.0)
50-59	31 (87%)	1.8 (0.8-4.6)	1.4 (0.7-3.6)
≥60	17 (82%)	1.2 (0.6-2.6)	1.0 (0.5-2.1)
Gender			
Females	142 (83%)	Ref	Ref
Males	102 (90%)	1.8 (0.6-4.1)	1.4 (0.5-3.8)
Region:			
South	66 (79%)	Ref	Ref
Central	33 (97%)	8.6 (1.6-68.1)**	4.2 (1.1-50.1)*
East	67 (91%)	2.7 (1.1-7.2)**	2.0 (1.1-3.0)*
West	78 (83%)	1.4 (0.6-3.1)	1.0 (0.4-2.1)
Latrine:			
Flush	183 (86%)	Ref	Ref
Open air	7 (86%)	1.1 (0.5-8.1)	1.0 (0.5-6.1)
Pit Latrine	54 (87%)	1.1 (0.5-7.2)	1.0 (0.5-6.2)
Source of drinking water:			
Spring	43 (83%)	Ref	Ref
Pipe protected	192 (86%)	1.2 (0.5-3.1)	1.0 (0.5-2.8)
Not protected	9 (89%)	1.2 (0.2-10.2)	1.0 (0.2-8.2)
# of people in the household:			
1-3 persons	53 (77%)	Ref	Ref
4-5 persons	115 (90%)	2.5 (1.2-6.0)**	1.8 (1.0-4.0)**
≥ 6 persons	76 (87%)	2.0 (0.9-5.1)	1.6 (0.7-4.1)
Chew Betel Nut			
No	167 (86%)	Ref	Ref
Yes	77 (87%)	1.1 (0.5-2.4)	1.0 (0.4-1.6)
Occupation:			
Students	20 (75%)	Ref	Ref
Business	20 (94%)	5.2 (0.7-54.0)	4.0 (0.5-45.1)
Farmers	57 (86%)	2.1 (0.6-7.2)	1.6 (0.6-3.2)
Government job	34 (85%)	1.9 (0.5-7.7)	1.1 (0.4-5.2)
Private job	19 (905)	6.1 (0.6-57.2)	3.2 (0.5-55.2)
Unemployed	94 (85%)	1.9 (0.6-6.1)	1.5 (0.4-8.1)

Fig-1: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