Study on Association of *Helicobactor pylori* Infections with Acid Peptic Disease (APD) Among Nepalese

Submitted by

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Content

Summary

Present study was carried out to assess the present status of *Helicobator pylori* infection among Nepalese of different age, sex and ethnic group living in both Kathmandu Valley and outside of valley. A total of 203 subjects complaining of acid peptic disease (APD) were included. A proforma was filled and informed consent was taken from each of patient included. Patients were then subjected to gastroendoscopic procedure and two samples were taken from the site of gastritis. One sample was subjected to urease test and the other one for *H. pylori* culture using specific medium. *H. pylori* thus grown were identified by studying its biochemical characters.

Overall positive rate was 29.5% (60/20. A marginally higher Helico Urease text positive rate [31.2% (48/158)] was observed in males compared with females [24.5% (12/49)] ($P>0.05$). The positive rate was higher in *Indo-Aryan* (31.9%; 31/116) ethnic group compared with *Tibeto-Burmans* (26.4%; 23/87) but without significance difference ($P>0.05$). The positive rates in three different stages of APD Mild (n=78, Moderate (n=80) and Severe (n=45) with a positive rate of 7.7%, 25.5% and 75.5%, respectively ($P<0.05$). Positive rate was highest in the age-group of 36-50 years (34.4%) followed by over 50 years (29.0%) and the age group of 21-35 years.

All Helico urease and/or culture positive patients were given a course of **triple therapy** (Omeprazole, Clarythromycin and Amoxycillin) for two weeks followed by Omeprazole for up to six weeks. Patients receiving full course of therapy and were available for follow-up were found to be recovering well from their APD problem. Of the total, 76 patients were not available follow-up. None of the patient initially negative (both Helico urease and/or culture) was positive on follow up.

Introduction

Helicobactor pylori is a slightly twisted bacilli first discovered by Warren and Marshal in 1983. Since then, it has been it has overwhelmingly been found to be associated with the pathogenesis if gastroduodenal diseases even leading to cancer. Since its discovery, *H. pylori* has been implicated as an important cause of gastro-duodenal disease. It is reportedly associated with 95.0 to 100.0% of duodenal ulcers, 96.0% of gastric ulcers, 92.0% of mucosa-associated lymphoid tissue lymphoma, 70.0% of gastric adenocarcinoma, 86.0% in atrophic gastritis and 30% of nun-ulcer dyspepsia (Dixon, 1995).

Gastric colonization of *H. pylori* can increase the risk of gastric cancer as high as 18 folds compared with those of general population. Early detection of this organism and timely treatment can prevent the occurrence of gastric cancer. However, *H. pylori* have not been detected to any extent in the environment (Fukuda *et al*, 2001).

Re-infections are very rare in adults, but uncommon in childhood (Fukuda *et al*, 2001). However, no pediatric cases with *H. pylori* associated gastric cancer have been reported, however, precancerous lesions including mucosal atrophy can develop in infected children (Kato, 2001).

Thus, the importance of *H. pylori* in the medical science particularly in the gastroduodenal diseases has been increased.

Infection with *H. pylori* is wide spread. In developing countries, 8 in 10 children by the age of 5 years, and more than 90.0% of adults are infected. It is

transmitted through person-to-person contact, and probably also from contaminated water and foods. However, *H. pylori* have not been detected to any extent in the environment (Fukuda *et al*, 2001). Eradication of *H. pylori* reportedly results in cure and reduce ulcer occurrence in 90.0% of peptic ulcer patients. Re-infections are very rare in adults, but uncommon in childhood (Fukuda *et al*, 2001). However, in most of the subjects infections are asymptomatic.

It is known to be associated with socioeconomic status (Mendal *et al*, 1992) and lower educational attainment, ethnic minorities and immigrants (Perez-Perez *et al*, 1990) and people in developing countries are infected earlier than those in developed countries (Mitchel *et al*, 1992). In Japan, it is thought to be transmitted to children from their infected father (Fukuda *et al*, 2001).

H. pylori lives closely attached to the gastric epithelial cells beneath a protective layer of mucus. Gastritis due to *H. pylori* infection begins as an inflammatory reaction directed against *H. pylori*. The presence of *H. pylori* brings about the cellular changes and reduction of mucus layer. However, in many patients with chronic *H. pylori* colonization, hardly any readily identifiable endoscopic changes are seen. Chronic gastritis may gradually progress to atrophy and interfere in the function of gastric mucosa. It may subsequently increase the risk of gastric cancer as high as 18 folds than those among general population. Therefore, gastric cancer can also be considered as a late complication of *H. pylori* infections.

In neighboring country India, the infection rate has been reported to be as high as 85-90% in gastroduodenal diseases (Nanivadekar *et al*, 1990) and the incidence increase with age (Fukuda *et al*, 2001).

Acid Peptic Disease (APD) is a common health problem in Nepal. As much as 84.4% of endoscpies were abnormal, out of them 75.0% were under 45 years of age. Over 70.0% had either gastritis or ulcer (Acharya *et al*, 1989). It is more

common among females than in males and is attributed to their "sacred fast" keeping. The reported prevalence of *H. pylori* associated APD in different series range from 39.0% to 86.6%. In Nepal, prevalence of *H. pylori* in various gastroduodenal diseases have been reported to be ranged from 39.0% to 86.6% (Larson *et al*, 1992; Shah *et al*, 1990; Shakya *et al*, 2001; Thapa *et al*, 2001). In our previous study we found a prevalence of 6.6% (Shah *et al*, 1990).

Keeping in view of this, present study was carried out to investigate the prevalence of *H. pylori* associated APD in patients of different sex, age and ethnic, and to compare the direct Helico urease test and culture of the organism as the eradication of infection is most useful in preventing gastric carcinogenesis (Dixon, 1995; Nozaki *et al*, 2001).

Objectives

General Objective

To study an association of *H. pylori* infection acid peptic disease (APD) (gastritis, gastric and duodenal ulcer, gastric cancer) in Nepal.

Specific Objectives

1. To find out the present status of *H. pylori* associated APD (gastritis, gastric and duodenal ulcer, gastric cancer) in Nepal.
2. To see the status of its association in different age sex and ethnic group.
3. To observe the status in people living in Kathmandu Valley and outside of Valley.
4. To see the effect of drug therapy.

Materials & Methods

This study was carried out in Kathmandu Valley but with the inclusion of all patients with complain of APD (gastritis, gastric and duodenal ulcer, gastric cancer) coming from both inside the valley and outside of it and undergoing endoscopic examination. A total of 200 to 300 patients were expected to be enrolled in this study. As targeted, 203 subjects were included.

(1) Filling of Proforma and Recording of Clinical Findings

After taking an informed consent, a proforma (as shown in the Annex I) was filled for each patient. This included Name of patients (in full), age, sex, address, clinical history. A written consent was planned to be taken, but it was not practicable as most of the patients were hesitant to give their signature. Therefore, an informed consent was taken either by PI and/or gastroenterologist.

Each of the patients with the symptoms of APD was examined by a gastroenterologist. They were then subjected to endoscopic procedure. For this, patients were given xylocaine viscous solution. Patients were asked lay down on the bed in a relaxed manner and endoscopy was performed. Grade of APD was recorded as (1) Mild, (2) Moderate and (3) Severe. Using the endoscope, two biopsy samples were taken from the suspected area (inflamed and/or ulcerated).

(2) Laboratory Analysis of Samples

One of the two samples was put into Helico urease test medium and incubated at 37C for overnight. Positive result was noted. In case of negative result,

the medium was further incubated till 48 hours. All medium showing negative result after 48 hours were considered as negative test.

Another sample was subjected for culture. For this, the biopsy material was placed into a vial containing sterile saline and was crushed into small pieces using sterile scalpel blades. Then, it was inoculated on *Campylobactor* medium with Screw's supplement. All samples were processed without delay. The plates were then incubated in microaerophilic condition with 10.0% CO_2 gas and adequate moisture and were incubated at 37C for up to 120 hours.

Grey translucent colonies observed after 72 hours were considered as the colonies of *H. pylori*. From colonies, Gram staining was done to look for spiral (so called "S" type) Gram negative bacilli. For confirmation, catalase, oxidase and urease tests were performed. Culture showing spiral Gram negative bacilli with catalase, oxidase positive and strong urease test positive were considered as *H. pylori*.

None of the patients included in this study were charged these investigative procedures. This was a good opportunity for the patients to get investigations done and to receive proper treatment. All microbiological investigations were carried out at National Institute of Tropical Medicine and Public Health Research (NITMPHR), Putalisadak, Kathmandu, Nepal.

(3) Treatment of Patients

Each of the patients with Helico urease and/or culture positive results was given a course of **triple therapy** (Omeprazole, Clarythromycin and Amoxycillin) for two weeks followed by Omeprazole for up to six weeks.

Each of the patients was advised to visit once after weeks for follow-up.

(4) Follow-up of patients

Of the total 203 patients initially enrolled in the study, 76 patients did not come for follow-up.

(5) Data Management

All the data obtained from the study were managed by principal investigator (stored on hard papers as well as on computer floppy discs in duplicates).

Results & Discussion

Of the total 203 subjects included, 154 were males and 49 females. This reflected the male preference and priority in the Nepalese society. Overall positive rate was 29.5% (60/203). This was lower than that reported (39.0%) by Thapa *et al* (2001). Ahough, APD problems are common in females, in this study, marginally higher Helico Urease text positive rate [31.2% (48/158)] was observed in males compared with females [24.5% (12/49)]. However, this difference was not significant ($p<0.05$) (Fig. 1). Earlier in Western Nepal, Acharya *et al* (1989) also observed a higher positive rate in males (45.0%) than in females (30.0%). However, Thapa *et al* (2001) found same positive rate in both the sexes. This could be due to the different study population and smaller samples size (n=42) in their study.

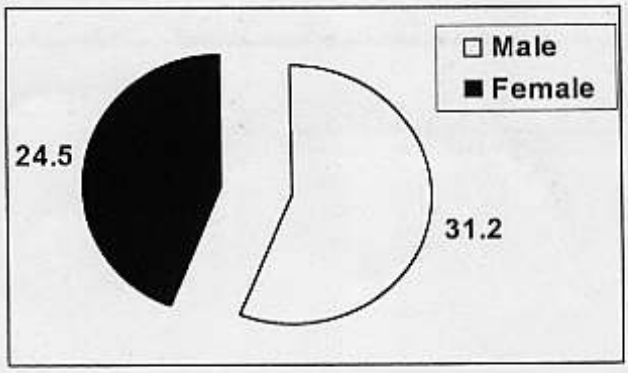

Fig. 1. Helico Urease test positive rate in male and female Nepalese patients with APD (overall positive rate was 29.5%).

The present finding, however, was much lower than that of serological findings (56.8%) reported by Kawasaki *et al* (1998) among apparently healthy Nepalese. They found a higher prevalence (67.2%) in a suburban village in

Kathmandu Valley than in a rural village in eastern Nepal (41.5%). On the other hand, Broutet *et al* (1999) reported a surprisingly very low prevalence rate of *H. pylori* infections in saliva samples obtained in a very remote hilly area in western Nepal. Such a high difference in the positive rate appeared to be due to the difference in the study method employed.

The Helico Urease test positive rate was higher in *Indo-Aryan* (31.9%; 31/116) ethnic group compared with *Tibeto-Burmans* (26.4%; 23/87) but without significance difference ($p>0.05$) (Fig. 2). This could be due to the socio-cultural difference in these two ethnic groups. *Indo-Aryans*, for instance, go on fast more frequently compared with *Tibeto-Burman* counterparts. It might be due to the genetic difference of two ethnic populations (Genta *et al*, 1995; Straus *et al*, 2002), but remains to be elucidated. In USA, *African-Americans* were more likely to be infected with *H. pylori* and suffered from more serious gastroduodenal disorders than other ethnic groups (Straus *et al*, 2002). In addition, there are reports showing strong association in *H. pylori* infections in familial aggregation (relatives) of gastric cancer (Chang *et al*, 2002).

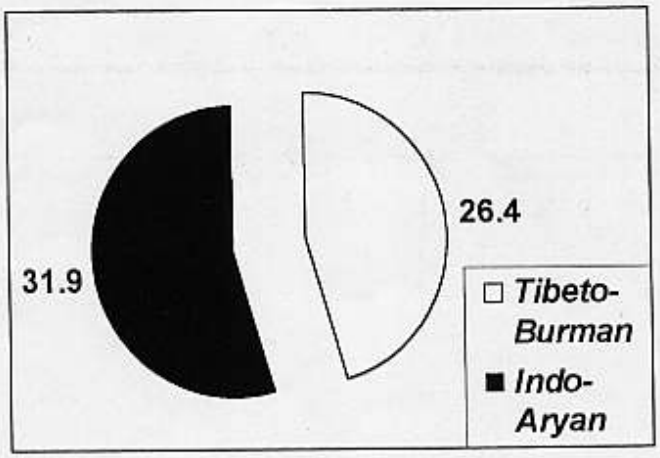

Fig. 2: Helico Urease test positive rate in two ethnic groups of patients with APD (overall positive rate was 29.5%).

As shown in Fig. 3, only one-fifth of total patients included had severe stage of APD. Cases of mild and moderate stages were nearly equal.

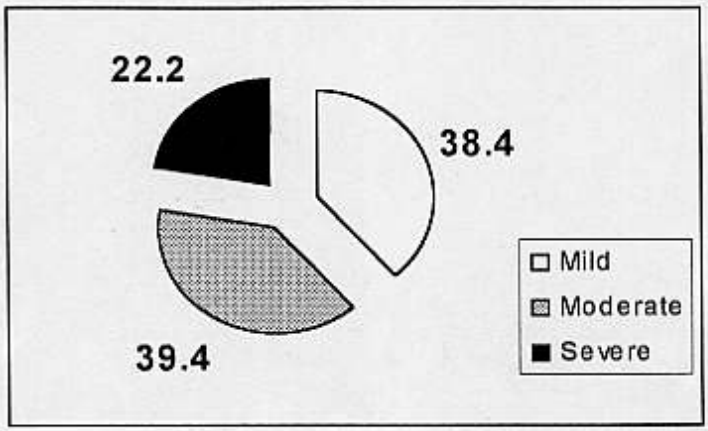

Fig. 3: Distribution pattern of severity of APD cases (n=203) included in this study.

As shown in Table-3, the Helico urease test positive rate increased significantly with the increase of severity of APD. Present finding indicated that *H. pylori* is associated with over 75.0% of severe cases of APD in Nepal.

Table-1: Helico Urease test positive rates in different stages of APD patients included in the present study.

Stage of APD	Total n (%)	Helico Urease Positive	Percentage
Mild	78	6	7.7 %*
Moderate	80	20	25.5 %*#
Severe	45	34	75.5 %#
Total	203	60	29.5 %

*P value = <0.05; #P value = <0.05.

The incidence of *H. pylori* associated gastric diseases in different age-groups of Nepalese has been shown in Table-1. Highest positive rate was in the age-group

Table-3: Stages of APD in Nepalese and Helico Urease and *H. pylori* culture positive rates in different stages of APD.

Stage of APD	Total n (%)	Helico Urease Positive (%)	*H. pylori* Culture Positive (%)
Mild	78	7 (8.9%)	6 (7.7%)
Moderate	80	20 (25.0%)	12 (15.0%)
Severe	45	34 (75.5%)	25 (55.5%)
Total	203	60 (29.5%)	44 (21.7%)

Both Helico urease and culture positive rate was highest in severe cases of APD indicating the pathogenic role of *H. pylori*. In 1994, WHO/IARC concluded that *H. pylori* as "a definitive carcinogen". However, we did not examine the biopsied material histopalogically as to rule out the cancerous state. Nozaki *et al* (2001) in Mongolian gerbil (MGs) demonstrated an important role of *H. pylori* in enhancing chemical carcinogen in the causation of gastric cancer.

All Helico urease and/or culture positive patients were given a course of **triple therapy** (Omeprazole, Clarythromycin and Amoxycillin) for two weeks followed by Omeprazole for up to six weeks. Patients receiving full course of therapy and were available for follow-up were found to be recovering well from their APD problem.

Of the total 203 patients initially enrolled in the study, 76 patients did not come for follow-up (after six weeks). One possible reason for this could be due to the effective treatment of their problem. This was predicted on the basis of the fact that none of the patients initially found to be positive (both Helico urease and/or culture) were found to be positive on follow up. None of the patient initially negative (both Helico urease and/or culture) was positive on follow up.

Present finding revealed that about one third of Nepalese patients with APD problems are infection with *H. pylori* and needs specific treatment for the same. Keeping in view of modes of infections of this organism, public health education, early detection of cases and specific treatment should be encouraged. Furthermore, study on its relationship with gastric cancer in Nepalese patients with APD should also be conducted in larger scale in future.

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Annex - I

Proforma (*Helicobactor pylori* study, 2003)

Date:

Name of Patient (Full) ..... Age/Sex.....

Address: .....

Clinical history: .....

Endoscopic finding: (1) Mild (2) Moderate (3) Severe

Further comments, if any: .....

Laboratory findings:

(1) Helico urease test: .....

(2) Culture & Sensitivity finding: .....

.....

Treatment given: .....

Follow-up Date: .....

Clinical history: .....

Endoscopic finding: (1) Normal (2) Mild (3) Moderate (4) Severe.

Laboratory findings:

(1) Helico urease test: .....

(2) Culture & Sensitivity finding: .....

.....

Treatment given: .....