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PREVALENCE AND RISK FACTORS OF *HELICOBACTER PYLORI* INFECTION AMONG GASTRIC BIOPSIES IN TIRUCHIRAPALLI – ONE YEAR HOSPITAL BASED STUDY

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ABSTRACT

Helicobacter pylori infection is one of the common gastrointestinal infections worldwide. It is closely associated with acute to chronic gastritis followed by peptic ulcer and gastric cancer. This study is mainly aimed to analyze the prevalence of *H. pylori* infection among suspected patients who are enrolled at gastroenterology OP with specific symptoms thereby the tissue samples were determined for infection by histopathologically and microbiologically. The socio-economic details and environmental risk factors were also well analyzed in this study. This prospective study was achieved in 2016 to 2017 by recruiting total of 157 suspected OP and IP patients. Endoscopic examination was done; gastric biopsies were obtained from antrum and corpus, and *H. pylori* infection was diagnosed using histopathology and rapid urease test. Out of 157 patients included, 82 (52.2%) were positive for *H. pylori* infection. The positive observation was found higher 65.1% among males and 25.5% in females and increased in increasing age. The risk factor variables like animal contact, more family members are highly correlated with the prevalence while other factors are not having any associations. The histopathology and rapid urease test supported the existence of presence of *H. pylori* infection. The prevalence of *H. pylori* infection is moderate in the study area compared with the literature. The aged individuals, more children in the family, crowded environment, poor living conditions and history of ulcers are closely associated with *H. pylori* infections. Further formulation and designing preventive health strategies are very important to protect young generations against this infection.

KEYWORDS: *Helicobacter pylori*, infections, gastric biopsies, histopathology, microbiology.

INTRODUCTION

The *Helicobacter pylori* infection is the most common chronic gastric diseases in which found more in developing countries than the developed one. This type of organ specific infections is closely related to crowded environment and poor living conditions from childhood onwards.[1] The major clinical symptoms observed in this infectious state are abdominal pain and anorexia. Some studies suggested that the source is intrafamilial than from a community. This disease is largely found among siblings from father but not from mother.[2]

H. pylori colonize nearly 60% of the world's population,[3] that cause chronic gastritis and have been closely associated with various gastrointestinal disorders like duodenal ulcer and gastric cancer.[4] The humans are considered as a potent reservoir whose saliva, feces and gastric juice harbours this infectious agent. The possible routes of infection are oral-oral, fecal-oral, iatragenic with inadvertent usage of endoscopes, flies and domestic animals are also play a vital role in spreading the infection.[5,6]

The prevalence of infection varies worldwide and found lower in developed countries and higher percentage was observed in indigent population of developing countries.[7] Initially the infection occurs in the upper gastrointestinal tract and further progressing to acute and chronic gastro-duodenal inflammation that are muted but symptoms observed among 20% after a long latent period. Clinical manifestations of the infection include duodenal ulcer disease, gastritis, gastric atrophy, gastric ulcer disease, gastric B-cell lymphoma, gastric adenocarcinoma, iron deficiency anaemia and vitamin B_{12} deficiency. The observation of anaemia due to iron deficiency in childhood to gastric cancer in the elderly varies from region to region. There is a progressive decline in the incidence of gastric cancer whereas at the

same time, a sharp rise in the incidence of duodenal ulcer is recorded.[7]

The most significant outcome of *H. pylori* infection is gastric cancer. In many places like Africa, southern India etc, the infection is very common but gastric cancer is rare.[2,8,9,10] The most problematic clinical emergency in *H. pylori* gastritis is the susceptibility to enteric infections; thereby earlier diagnose and prompt treatment may help the patients to survive. In some cases, infection mediated hypochlorhydria and iron deficiency anaemia leads to deleterious effects on imposing endocrinology in adults, and physical and mental deterioration in children.[4,11]

The major risks of this infection are inversely related to environmental cleanliness, food and diet, water sanitation and unhygienic practices lead to the invasion of infectious pathogens into the susceptible individuals that cause severe infections.[12,13] Other factors including smoking, non-steroidal anti-inflammatory drugs and reflux of gastric juice are also implicated to cause chronic gastritis. Even though *H. pylori*, is considered as the major etiology of gastritis, it also act synergistically with other factors.[14] However more studies have already been reported, the scantiness of information about this infection in this study area is found. Thus, the current investigation was to estimate the prevalence of *H. pylori* infection among patients presenting with various gastrointestinal lesions, in a rural teaching hospital in Tiruchirapalli, India.

MATERIALS AND METHODS

The study was a cross sectional prospective study and was conducted with 157 out patients and in-patients of tertiary care teaching hospital of Tiruchirapalli. The study conducted was a cross sectional retrospective study, during the period of July 2016 to December 2017. The study was approved by institutional research board and ethical clearance was obtained from institutional ethical committee. The study involved the data analysis of routine procedure carried out in an institution and informed consent was obtained from each patient before the procedure initiated.

The patients were selected on the basis of chief clinical observation of dyspepsia and the age of patients ranged from 22 to 72. The patients who are undergoing antibiotic therapy or having antibiotic therapy within one month exclusively for *H. pylori* infection were excluded. The endoscopy was done by viewing esophago gastro duodenoscope which were taken for upper gastrointestinal endoscopy after making them fast overnight. It was considered on visualizing mucosa which is pink in color, smooth and lustrous, and two samples were obtained from the antrum.

One sample is sent for histopathology and another for microbiological investigations. Two different staining procedures were followed for staining the sections – Giemsa and H&E stain (haematoxylin and eosin stain), thereby mononuclear cell infiltration, neutrophilic infiltration, atrophy, intestinal metaplasia and *H. pylori* density were determined. Microscopic observations showing light bluish rods and dark blue rods in H&E and Giemsa stain respectively and the diagnosis was based on the standard criteria.[15,16,17]

The second sample (biopsy fragment) was sent for Microbiological analysis especially rapid urease test (RUT). The RUT was performed by the following the standard protocol,[18] with the following steps.

Step 1: Two grams of Urea was dissolved in 20ml sterile distilled water.
Step 2: Twenty drops of phenol red were added to the solution and pH was adjusted to 6.8; now the solution was faint yellow tint at this stage.
Step 3: This was transferred to sterile vial (2 ml per vial).
Step 4: Biopsy material was dispensed and incubated at 37°C.

Interpretation: If the color changed within 30 minutes, then it is considered as positive whereas week positive was reported when the color change occurred after 2 hours. Negative report was recorded when no color change occur after authorized period of time.

RESULTS

The subjects included in this study and the prevalence of *H. pylori* infection was found in both the sexes but recruitment of male (n=106; 67.5%) is more than female (n=51; 32.5%). Among the subjects total of 82 patients showed positive to *H. pylori* infections, thereby 69 males and 13 female patients were positive by all the three evaluation. The detailed description of the total number of subjects included verses number of positive cases for *H. pylori* was depicted in figure 1.

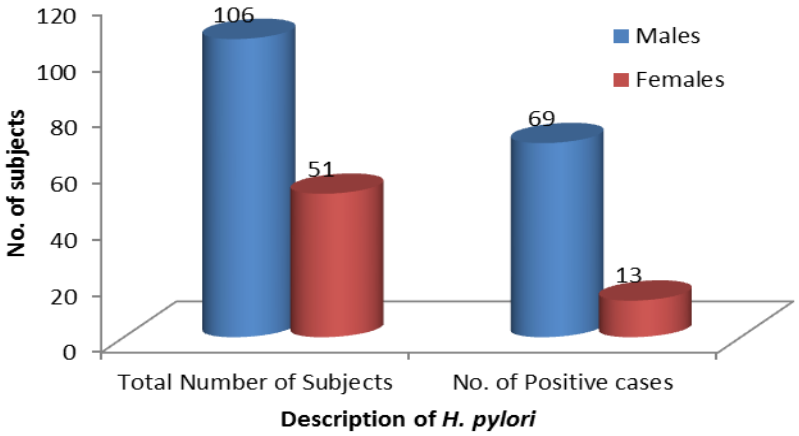

Figure 1: Total subjects verses positive cases.

Out of the subjects, 46 patients were aged 41 – 50 years, followed by 43 patients aged 31 – 40 years and 28 patients aged 21 – 30 years. The *H. pylori* infection was found positive maximum in the age group of 41 – 50 years followed by 31 – 40 years and 51 – 60 years (Table 1). The socio demographic data of the patients are well analyzed and the detailed descriptions were impregnated in table 2. Comparing with urban habitat (29.3%), rural participants (70.7%) are higher in this study. Among occupational status of the participants, the maximum was recruited from private employment; whereas 46.5% were completed their high school education. Equally the subjects were having frequent contact with the animals and 104 subjects were living in a joint family system with minimum 7 to maximum 10 members in the family.

Table 1: Age wise description of subjects and positive cases towards *H. pylori*.

Age groups (in years)	No. of subjects (n=157)	No. of positive cases (n=82)
1 - 10	1 (0.6)	0
11- 20	1 (0.6)	0
21 - 30	28 (17.8)	7 (8.5)
31 – 40	43 (27.4)	21 (25.6)
41 - 50	46 (29.3)	25 (30.5)
51 - 60	18 (11.5)	13 (15.9)
61 - 70	13 (8.3)	10 (12.2)
71 - 80	7 (4.5)	6 (7.3)

[Figure in parenthesis denoted percentages].

Table 2: Demographic data of the subjects and control.

Characteristics		Patients (n=157)
Residential status	Rural	111 (70.7)
	Urban	46 (29.3)
Occupational status	Agricultural labourers	26 (16.6)
	Domestic servant	12 (7.6)
	Semi skilled workers	14 (8.9)
	Petty business	07 (4.5)
	Government employee	29 (18.5)
	Private employee	30 (19.1)
	Student	11 (7.0)
	Drivers	04 (2.5)
	Hotel Staff	09 (5.7)
	Unemployed	08 (5.1)
	Unclassified	07 (4.5)
Education	Illiterate	23 (14.6)
	Primary level	21 (13.5)
	Middle level	17 (10.8)

	High school level	73 (46.5)
	Collegiate education	23 (14.6)
Animal contact	Yes	79 (50.3)
	No	78 (49.7)
Source of drinking water	Well water	83 (52.9)
	Bore well water	42 (26.7)
	Municipal water	32 (20.4)
No. of family members	4 – 6	34 (21.7)
	7 - 10	104 (66.2)
	More than 10	19 (12.1)
Substances abuse	Alcoholic alone	27 (17.2)
	Tobacco alone	19 (12.1)
	Smoking alone	37 (23.6)
	All	16 (10.2)
	None	58 (36.9)
Diet	Seasonal fruits	56 (35.7)
	Dried and processed fruits/ juices	101 (64.3)

[Figure in parenthesis denoted percentages].

The clinical symptoms related to gastrointestinal system are also analyzed in this study thereby, epigastric pain dominated with 92 patients followed by bloating (n=39) and vomiting (n=23). The detailed descriptions related to gastrointestinal symptoms among the positive and negative cases were depicted in table 3. Further, the related illnesses were also analyzed by which, diabetes showed in high numbers (n=59) followed by hypertension (n=51) and dyslipidemia (n=19); the complete details about the related illnesses were impregnated in figure 1.

Table 3: Details of gastrointestinal symptoms (n=157).

Clinical manifestation	No. of subjects (n=157)	Positive (n=82)	Negative (n=75)
Abdominal pain	22 (14.0)	13 (15.8)	9 (12.0)
Bloating	39 (24.8)	21 (25.6)	18 (11.5)
Epigastric pain	92 (58.6)	54 (34.4)	38 (24.2)
Haematemesis/ melena	21 (13.4)	10 (6.4)	11 (7.0)
Heart burn	19 (12.1)	11 (7.0)	8 (5.1)
Nausea	22 (14.0)	9 (5.7)	13 (8.3)
Vomiting	23 (14.6)	11 (7.0)	12 (16.0)

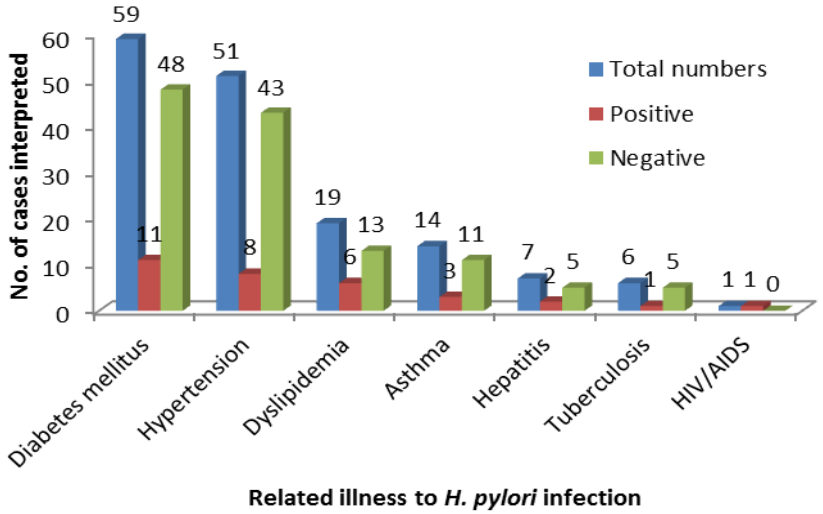

Figure 1: Descriptive analysis of related illness to *H. pylori* infection.

Among the endoscopic examinations, duodenal ulcers dominated with 59 subjects followed by gastric cancer dysphagia with 33 cases and the detailed analysis of reports is impregnated in table 4. The combinational observations among the gastrointestinal endoscopic analysis were also studied thereby gastric cancer with

gastric polyps followed by gastric cancer and gastric ulcer.

Table 4: Endoscopic examinations of the suspected subjects (n=157).

Description	Number of subjects	Percentage
Adeno-carcinoma	6	3.8
Duodenal ulcer	59	37.6
Dysphasia and ulceration	6	3.8
Excavating ulcer	5	3.2
Gastric cancer dysphagia	33	21.0
Gastric outlet obstruction	3	1.9
Gastric polyps	11	7.0
Gastric ulcer	18	11.5
Growth stomach	4	2.5
Infiltrate growth	6	3.8
Prepyloric ulcer	20	12.7
Sessie autrum	3	1.9
Umbillated ulcer	6	3.8

The histopathological analysis of the samples showed observable changes in the tissue morphology thereby monostructural changes including mononuclear cell infiltration were found among 31 samples followed by neutrophilic infiltration, atrophy and intestinal metaplasia with 16, 9 and 9 subjects respectively. All the above tissue analysis was identified along with reasonable *H. pylori* density. The combinational descriptions including mononuclear cell infiltration and neutrophilic infiltration with *H. pylori* density was found among 9 cases. The table 5 described the detailed analysis histopathology of subjects included in this study.

Table 5: Histopathological descriptions of *H. pylori* cases (n=82).

Histopathological analysis	No. of cases	Percentage
Monostructural changes with *H. pylori* density		
Mononuclear cell infiltration	31	37.8
Neutrophilic infiltration	16	19.5
Atrophy	9	11.0
Intestinal metaphasia	9	11.0
Polystructural changes with *H. pylori* density		
Mononuclear cell infiltration + Neutrophilic infiltration + Atrophy	9	11.0
Mononuclear cell infiltration + Intestinal metaphasia + Atrophy	5	6.1
Neutrophilic infiltration + Intestinal metaphasia + Atrophy	3	3.6

The results of microbiological analysis of the biopsy tissue by rapid urease test was done and showed observable reactions thereby the data supported the endoscopic and histopathological studies. The results were classified as 6 categories including color change within 30 minutes, between 31 to 45 minutes, 46 to 60 minutes, 61 – 120 minutes, 121 - 130 minutes and no color change were supported with 64, 10, 4, 3, 1 and 75 samples respectively (Figure 2).

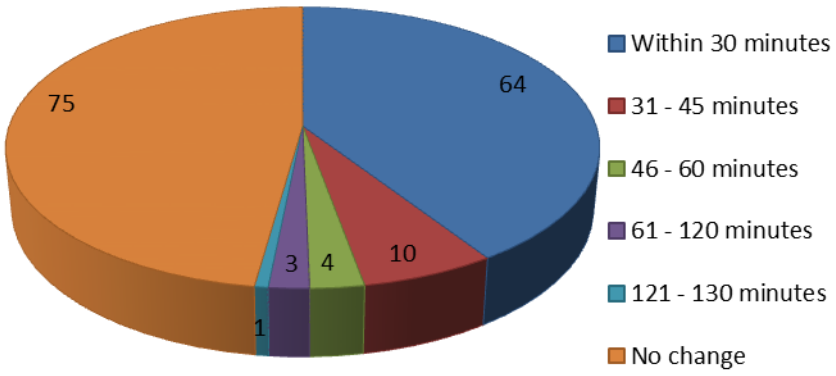

Figure 2: Period of change in color by RUT verses biopsy samples.

DISCUSSION

The prevalence of *H. pylori* infection is found higher in rural areas in India with peptic ulcer as the major clinical manifestation and found 9% lifetime prevalence.[4,7] But most of the studies suggested that the actual risk of the upshot of *H. pylori* infection is by the prototype of gastritis.[10,19,20,21] The same also found correlated in this study by which gastric cancer dominated. Studies highlighted that the gastric microbiome harbouring *H. pylori* in the presence or absence of clinical symptoms like gastritis, peptic ulcer and gastric carcinoma in animals and humans.[22,23]

The environmental and diet factors are also play a vital role in the pattern of gastritis and found more in India after the inculcation of the western type of food habits. The direct uptake of seasonal fruits and vegetables provide rich nutritive value and having high antioxidant properties. If the tropical diets with vegetables and fruits throughout the year enhance antral dominating non-atrophic gastritis and duodenal ulcer. The preservatives and salt using in drying such non-seasonal foods promote pangastritis leads to duodenal ulcer and increased age related gastric cancer.[10,24,25] Another study described the *H. pylori* infection increase the risk of diabetes by means of chronic inflammation that enhances insulin resistance.[26,27,28]

The Indian scenario highlighted that approximately 60% of the population would be infected with *H. pylori* infection; where 3% are estimated with duodenal ulcer required appropriate therapy.[7,29] Worldwide, the diagnosis for the detection of H. pylori infections is followed by standard steps including urea breath test, stool antigen test, endoscopy, histopathology, rapid urease test and microbiological culturing.[29,30,31,32]

The high rate of re-infection after treatment was observed in various countries thus requirement of appropriate eradication procedures for individuals and large groups.[33] The epidemiological analysis of *H. pylori* infection has been changed with different improvements in sanitation and methods of eradication.[34] The commonest source of infection by *H. pylori* are smoking, alcoholic, genetic factors and diet (restaurant food, meat, water, chilli peppers); further it was determined that the *H. pylori c*an grow impudently in the acidic stomach.[19,35]

The screening of all gastric and GIT based carcinoma for *H. pylori* infection may reduce the risk of mortality and also treat the patients appropriately. From this study, it was concluded that the association of *H. pylori* infection associated with various clinical manifestations are quite high. After appropriate diagnosis, it is possible to treat or eradicate *H. pylori*.[36] From this study we find that the endoscopic examination alone is not accurate; thus all necessary confirmatory tests to be done before starting empirical treatment.

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