

PREVALENCE OF HELICOBACTER PYLORI INFECTION IN PATIENTS WITH ATROPHIC GASTRITIS AND ROLE OF DARK FIELD MICROSCOPY IN DIAGNOSIS

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ABSTRACT: BACKGROUND: Helicobacter pylori is the most common human pathogen which causes chronic gastritis, leading to serious complications. The aim was to study the incidence of H. pylori infection and the risk factors associated with chronic atrophic gastritis using simple diagnostic procedures.

MATERIALS AND METHODS: Biopsy samples were collected from 146 chronic atrophic gastritis patients. Rapid Urease Test (RUT) and dark field microscopy were used to diagnose the presence of H. pylori infection. The prevalence rate of H. pylori infection in relation to age, sex, smoking and alcohol consumption was studied.

RESULTS: 88 (60.27%) cases were positive for RUT and also for the characteristic cork screw motility under dark field microscopy. Prevalence rate was high in younger age group and among female. We found no correlation of H. pylori infection with smoking and alcohol consumption.

CONCLUSION: It has been found that H. pylori is a major causative agent for atrophic gastritis and RUT is a simple cost efficient diagnostic test for H. pylori. The association of H. pylori infection with age, sex, smoking and alcohol consumption is variable and requires further studies to establish any relationship.

KEYWORDS: Gastritis, Helicobacter pylori, RUT, Dark field microscopy.

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INTRODUCTION: Helicobacter pylori is a gram negative, microaerophile, colonizing the human gastric mucosa. It causes chronic inflammation of the mucosa which leads to various upper gastrointestinal (GI) disorders.^(1,2,3)

Helicobacter pylori is found to be a major causative agent for gastritis.^(4,5) There are evidences that support the association of H. pylori induced chronic atrophic gastritis with serious complications, like gastrointestinal bleeding, adenocarcinoma of stomach, etc.^(6,7) The bacterial colonization and the mechanism of inflammation include various factors. Adherence of the bacteria on the mucosal epithelium plays an important role in colonization, pathogenesis and inflammation.⁽⁸⁾

Adherence facilitates the absorption of nutrients and also helps the bacterium to evade immune attack.⁽⁹⁾ The cytotoxins (e.g. vacuolating cytotoxin) produced by H. pylori directly causes cell damage. Protease and lipase produced by H. pylori and ammonia produced through urease activity also contribute to cell injury and inflammation.⁽¹⁰⁾

The prevalence of H. pylori infection increases with respect to age and the risk factors associated with it varies among different parts of the world.⁽¹¹⁾

The rate of H. pylori infection is high in developing countries when compared to the developed countries.⁽¹²⁾ Studies reveal that the socioeconomic status plays a vital role in the prevalence of H. pylori. People with low socioeconomic status and those living in larger families, crowded populations, are more prone to H. pylori infection.⁽¹³⁾ H. pylori infection spreads from person to person through vomitus, saliva, through the use of surface water resources like pond, river, etc.⁽¹⁴⁾

Lifestyle factors like cigarette smoking, tobacco chewing, alcohol consumption, food habits, etc., are also found to have an association with the prevalence of H. pylori infection.^(15,16,17) We aim to study the incidence of H. pylori infection in patients with atrophic gastritis using rapid urease test (RUT) and dark field microscopic observations.

MATERIALS AND METHODS: A total of 146 patients with symptoms of Acid Peptic Disease (APD) comprising of 89 males and 57 females were diagnosed as suffering from chronic atrophic gastritis, by upper GI endoscopy. Written and informed consent was obtained from the patients. The study was conducted over a period of two months with the approval of institutional ethical committee. The age groups include <30, 31-40, 41-50, 51-60, 61-70 and >70. The habit of smoking and alcohol consumption of more than five years was found in 24 and 35 patients respectively.

Gastric antral biopsy samples taken from these patients were subjected to Rapid Urease Test (RUT) to detect the presence of urease producing Helicobacter pylori. The biopsy tissues were inoculated in 5ml of Christensen's urea broth (2% urea) and incubated for 24 hours at 37°C.

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The urease positive samples were further observed under dark field microscopy for the presence of spiral-shaped, flagellated bacteria with its characteristic cork screw motility within 24 hours of sample collection from urease broth; 10% of urease negative samples were also examined by dark field microscopy for motile bacteria as described above as control for comparison.

RESULTS: Out of the 146 cases, 88 (60.27%) were positive in the RUT. All the 88 samples which were found to be positive in urease test also exhibited the presence of spiral-shaped, flagellated, bacteria with characteristic cork screw motility by dark field microscopy. *H. pylori* positivity is found to be more (72.72%) in the <30 age group (Table. 1). There is no vast difference in the incidence rate of *H. pylori* with respect to sex, smoking and alcohol consumption (Table No. 2 and 3). The prevalence of *H. pylori* among smokers and non-smokers are 54.16% and 61.47% respectively. Among alcoholic and non-alcoholic, the prevalence rates are 54.28% and 62.16% respectively (Table No. 3).

DISCUSSION: Efficient diagnostic procedure is essential to study the prevalence of a particular pathogen. Two or more tests are said to be a gold standard for the diagnosis of *H. pylori*.⁽¹⁸⁾ Diagnosis of *H. pylori* through RUT is an invasive technique. A single RUT is accepted as a confirmatory test for *H. pylori* by taking the pretest probabilities of an infection into consideration.⁽¹⁹⁾

In our study, persistent clinical symptoms of more than six months, not responding to antacids was a measure of pretest probability for *H. pylori* infection. Thereby RUT positivity alone can be considered as a confirmatory test. However, we examined for the presence of characteristic motile bacteria in the urease positive samples by dark field microscopy.

From the study we have found that 60.24% of patients were positive for *H. pylori* infection. This shows *H. pylori* as an important causative agent of the atrophic gastritis.^(1,2,3) *H. pylori*, upon colonization, releases chemotactic substances which causes accumulation of leucocytes, neutrophils and other inflammatory cells, thereby resulting in tissue injury and inflammation.^(8,20)

The effect of age on the prevalence of *H. pylori* has been revealed through various studies. Most of the studies have shown that the prevalence of *H. pylori* increases with increasing age.^(15,21,22) But there are studies that supports decreasing prevalence of *H. pylori* infection with increasing age.⁽²³⁾ The results of our study shows that *H. pylori* infection is more prevalent in the younger age group i.e., <30 years. *H. pylori* infection acquired during early age, persists for a lifetime and if left untreated may result in *H. pylori* induced chronic gastritis and carcinoma.

H. pylori infection in developing countries may be around 70%, which is 40% in case of developed countries.⁽¹⁴⁾ The vast difference between developed and developing countries may be due to the high probability of transmission in developing countries with a large population in the low socioeconomic group and poor hygienic practices, high population which facilitates person to person transmission, sharing pond and river as sources of drinking water, etc., which are the risk factors for high *H. pylori* prevalence.^(12,13,24)

We have studied the association of smoking and alcohol consumption with *H. pylori*. Smoking suppresses the immune system which may facilitate the acquisition of *H. pylori*. There

are studies which support the fact that smokers are more susceptible to *H. pylori* infection.^(4,17) Some studies have stated no association of smoking with *H. pylori* prevalence.^(23,25) The association of smoking with *H. pylori* is contradictory. Our results show that *H. pylori* is more prevalent in non-smokers than smokers. The results of our study agree with the work of Kikuchi, which supports a negative association of smoking with *H. pylori* infection. The results supporting positive association of smoking with *H. pylori* may have some other latent risk factors co-existing along with smoking.^(14,26) Alcohol consumption was found to have no impact on the prevalence of *H. pylori* infection.⁽²⁵⁾

The incidence of *H. pylori* in our study is less in alcoholics than non-alcoholics. This may be due to the antimicrobial effect of alcohol, which suppresses the survival of *H. pylori* in the gastric mucosa. Moderate alcohol consumption may reduce the risk of *H. pylori* infection.^(27,28)

CONCLUSION: The prevalence of *H. pylori* infection is independent of smoking and alcohol consumption. The prevalence rate is high among younger population and female. Dark field microscopic examination of urease positive samples is a simple inexpensive and useful supplementary test for diagnosis of *H. pylori* infection.

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Age	Total	H. pylori Positive	Total Positive %
<30	22	16	72.72%
31-40	38	26	68.42%
41-50	32	18	56.25%
51-60	26	15	57.69%
61-70	18	8	44.44%
>70	10	5	50.00%

Table 1: Age wise Distribution of H. pylori Infected Cases

Sex	Total	H. pylori Positive	Total Positive %
Male	89	50	56.17%
Female	57	38	66.66%

Table 2: Sex wise Distribution of H. pylori Infected Cases

Total		Positive	Total Positive %
Smokers	24	13	54.16%
Non-smokers	122	75	61.47%
Alcoholic	35	19	54.28%
Non-alcoholic	111	69	62.16%

Table 3: H. pylori Infection in Relation to Smoking and Alcohol Consumption