Research Paper

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Comparative Evaluation Of Direct Demonstration Of H. Pylori In Gram Stained Gastric Biopsy Smear Methods And Elisa Based Igg Antibodies Detection For Diagnosis Of Helicobacter Pylori Infection In Cases Of Dyspepsia

Medical Science

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ABSTRACT

85 gastric biopsy specimens and 85 serum samples of same patients complaining of dyspepsia were collected. Biopsy specimens were processed for Gram staining. Serum samples were used for detecting H. pylori IgG antibodies using a commercially available rapid indirect solid phase ELISA kit. Gram staining was positive in 30 (35.3%). Significant IgG levels were detected in 39 (45.9%) cases. It was therefore concluded that H.pylori infection is present in a substantial number of patients with acid peptic diseases and determination of IgG levels can act as an important noninvasive screening test..

Introduction

In 1982 Marshal and Warren cultured 'campylobacter-like' organisms from the antral biopsy of 90% patients with gastritis, 87% with gastric ulcer and 87% with duodenal ulcer. [1] New name Helicobacter pylori was proposed for this organism in 1989. [2] The discovery of Helicobacter pylori revolutionized our concept of gastroduodenal pathology and diverted the world wide attention from pH to Hp.[3] Presently, its role has been established in chronic antral gastritis, duodenal ulcer, chronic gastric ulcer, dyspepsia, gastric cancer and gastric lymphoma. World Health Organization added H.pylori to its list of known carcinogens.[4] The diagnosis of H.pylori infection is currently based upon endoscopic biopsy based tests (Rapid urease, culture, Gram staining and histopathology.[5]

These procedures are invasive and hence every person complaining of symptoms suggestive of dyspepsia cannot be subjected to these tests. Non invasive, serology based tests detecting IgG antibodies (since H.pylori causes chronic infection) are commercially available. The present study was therefore planned for comparative evaluation of conventional methods (gram stained smear) and ELISA based IgG antibody detection by a commercially available kit for diagnosis of H.pylori infection in cases of dyspepsia.

Material and Method:

Eighty five patients attending the OPD of Irwin Hospital, Jamnagar, (Gujarat, India) with complain of dyspepsia were subjected to endoscopy.

Collection of specimens

Blood:

5 mL of venous blood of patients was collected, serum was separated and stored at -20°C for further processing.

Antral biopsy:

Two Antral biopsy were taken, one was crushed between two clean grease free slides and one was in oculated in 2% Christensen`s urea broth. Processing of specimens (a). Smear Biopsy specimen was crushed between two clean grease free slides, fixed by methanol and stained by Gram staining. (b)Detection of antibody titres

ELISA based Immunocomb II kit for H. pylori IgG (Orgenics, Israel) was used to detect antibodies in 85 serum samples. Manufacturer's instructions were followed and a titre equal to or above the cut off value of 20 u/mL was considered significant. Titres below the cut off value were considered insignificant.

Results:

Out of 85 cases of dyspepsia, H. pylori were detected by direct microscopy of Gram stained antral biopsy in 30 (35.3 %) cases. Significant IgG titres (> 20u/mL) were detected in 39 (45.9 %) serum samples (Table). All cases which were positive by Gram stained smear, had significant IgG levels. Nine cases were negative by Gram stained smear but had significant IgG levels. No case was detected in which Gram stained smear was positive but serology was insignificant.

	Total cases		H. pylori present in smear		H. pylori absent in smear	
	No.	%	No.	%	No.	%
Serological positive	39	45.9	30	76.9	09	23
Serological negative	46	54.1	0	0	46	100
Total	85		30		55	

Discussion:

In the present study, H.pylori could be seen in Gram stained biopsy smear only in 30 cases (35.3%). Similar findings have been reported by many workers[4], [5] in India, though some have reported higher results.[6] This low isolation rate may be due to its patchy distribution in gastric mucosa, fastidious nature, mucosal atrophy.[7],[8] The difficulties associated with invasive tests and low isolation rate of this organism has led to the development of various serological tests.[9],[10] Major antigenic determinants have been located on urease enzyme [9] as well as on 128 kDa external protein (Cag A)[10] and cytotoxin (Vac A) of pathogenic H.pylori strains.

Significant IgG titres were detected in 45.9% cases in this study which is consistent with reports of other authors. [5] In the present study, nine cases were negative by Gram stained biopsy smear, but had significant IgG levels. This may be due to past infection or patchy distribution of organism in the stomach which was responsible for negative Gram stained biopsy smear. No case was detected in which Gram stained biopsy smear was positive but serology insignificant.

Our finding can be explained by the fact that serology assays the systemic response to entire stomach, therefore serological tests may be better chronic infections where the organism may not be detected by Biopsy smear. Therefore, we conclude that H.pylori seropositivity is high among dyspeptic patients and ELISA being a non invasive technique can be used successfully for the diagnosis of H.pylori infections.

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