

Detection of CagA in H. Pylori Gastric Illness Using in Situ Hybridization

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Abstract

Background: H. pylori had been recognized as the causative agent of several gastric diseases and possessed wide variety of virulence factors, one of most important factors is CagA.

Objective: This study investigated the expression of CagA cytotoxin on the gastric epithelial cells in patients with Helicobacter pylori gastric illness by using In Situ Hybridization technique.

Methods: Paraffin embedded tissue had been made form biopsies taken from 30 patients undergo O.G.D. and selected according to exclusion criteria. In addition 10 apparently healthy volunteers were included as a control group.

Results: The CagA cytotoxin was detected in 21(70.0%) patients out of 30 and 9 (30.0%) gave negative results, on other hand control group showed no positive results.

Conclusions: The CagA positive H. pylori strains cause the majority of H. pylori related gastric illness.

Key words: Helicobacter pylori; Gastritis; CagA; In situ hybridization.

Introduction

Helicobacter pylori are Gram-negative spiral-shaped bacterium, specialized in colonization of the human Stomach [1]. The bacteria are 2.5-5.0 μm long and 0.5-1.0 μm in diameter [2]. H. pylori had been recognized as the etiological agent of different gastric diseases such as gastritis, chronic atrophic gastritis, peptic ulcers and gastric cancer [3]. H. pylori has been ranked as a class I carcinogen by the International Research Agency on Cancer [4].

One of the best-acknowledged virulence factors in H. pylori is the cytotoxin-associated pathogenicity island, Cag-PAI. The Cag-PAI is 40 kb in size and composed of 27 open

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reading frames [5]. CagA expression has been considered as a main marker for the presence of Cag-PAI and is one of the most immunogenic proteins of H. pylori [5]. CagA status is highly associated with the pathogenicity of H. pylori strains [6].

Materials and Methods

A total of thirty patients had been included in the present study according to the exclusion criteria which are:-(a) Receiving H. pylori eradication therapy.(b) Receiving proton pump inhibitors.(c) Receiving H2blockers.(d) In the past six weeks had received bismuth compounds or Antibiotics.(e) Receiving any of the non-steroidal anti-inflammatory drugs.(f) Rapid urease test result was negative.

According on these criteria 19 male and 11 female with a mean of age 39.47 years (range between 16 and 70 years) were chosen. Twenty one patients were presented with Antral Gastropathy and/or Gastritis, while the rest patients were suffering from chronic atrophic gastritis. Ten apparently healthy volunteers (7 male and 3 female) with the mean age 42.3 years and age range (17-63) years were enrolled as control.

Paraffin embedded sections of gastric tissue were cut into 4-5 μm thickness, mounted onto positively charged slides (Fisher brand, superfrost /plus; U.S. Pat. 4481246) and drained the slides by fluffless blotting papers and left overnight to dry at room temperature [7].

Procedure of In Situ Hybridization was carried out according to the manufacturer's instructions. Using DAKO GenoPoint Hybridization /Detection System (Code No. K0620 U.S.patent#5,196,306) with Biotinylated long DNA probe for H. pylori / CagA Gene (Maxim's product Cat. No.: IH-60061). In each In Situ Hybridization run, negative control slide were included, which omitting the probe and using hybridization solution only.

Proper use of this hybridization /Detection system was judged when an intense brown-black color at the specific site of hybridized probe in both positive test tissue and positive control was developed. The specificity of In Situ Hybridization signal was reflected by the absence of positive In Situ Hybridization signal in the negative control slides. Housekeeping gene was used as positive control probe to evaluate the staining efficiency [7], for calibration of the staining intensity, and to verify the tissue quality.

Results

Nidhal A. Mohammed, "Detection of CagA in *H. Pylori* Gastric Illness Using in Situ Hybridization"

According to clinic-pathological examination (O.G.D. and Histopath.) the patients distributed into five groups of clinical presentation (see figure 1)

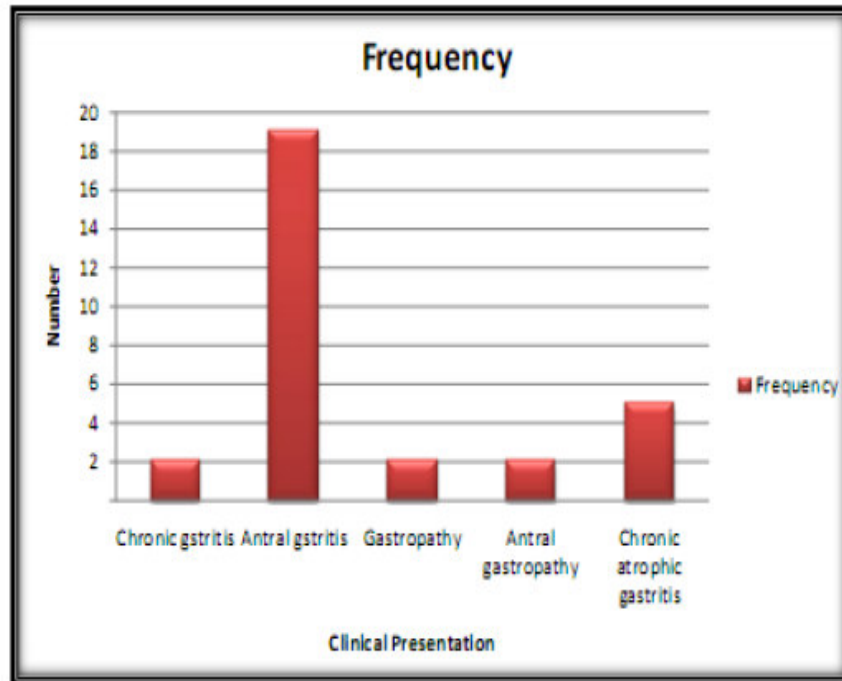


Figure (1):-Clinical presentation frequency of patients groups.

The CagA cytotoxin was detected by I.S.H. in 21(70.0%) patients out of 30 and 9 (30.0%) gave negative results (see figure 2). The CagA cytotoxin frequency among clinical presentation groups distributed unequally (see figure 3).

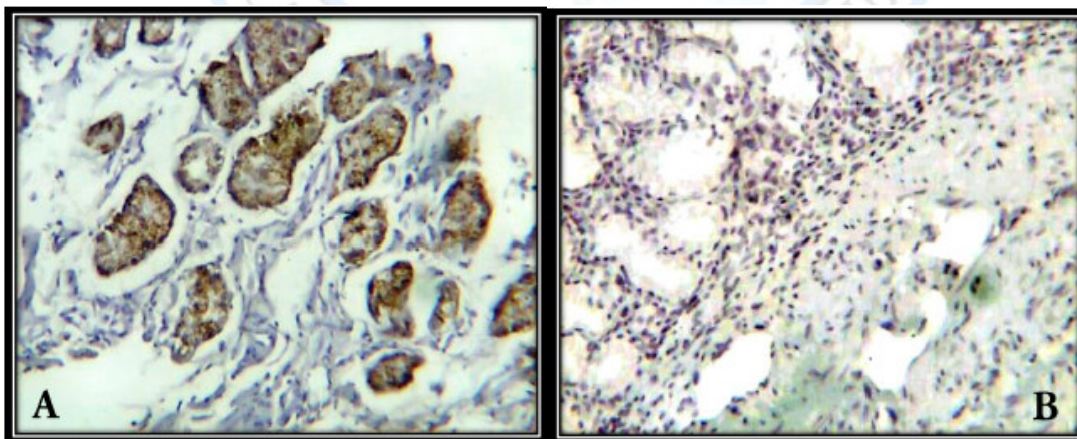


Figure (2):In situ hybridization for *CagA* positive *H. pylori* in gastric tissue section (x10) .staining by DAB and counter stained by hematoxylin.(A) positive (B) negative.

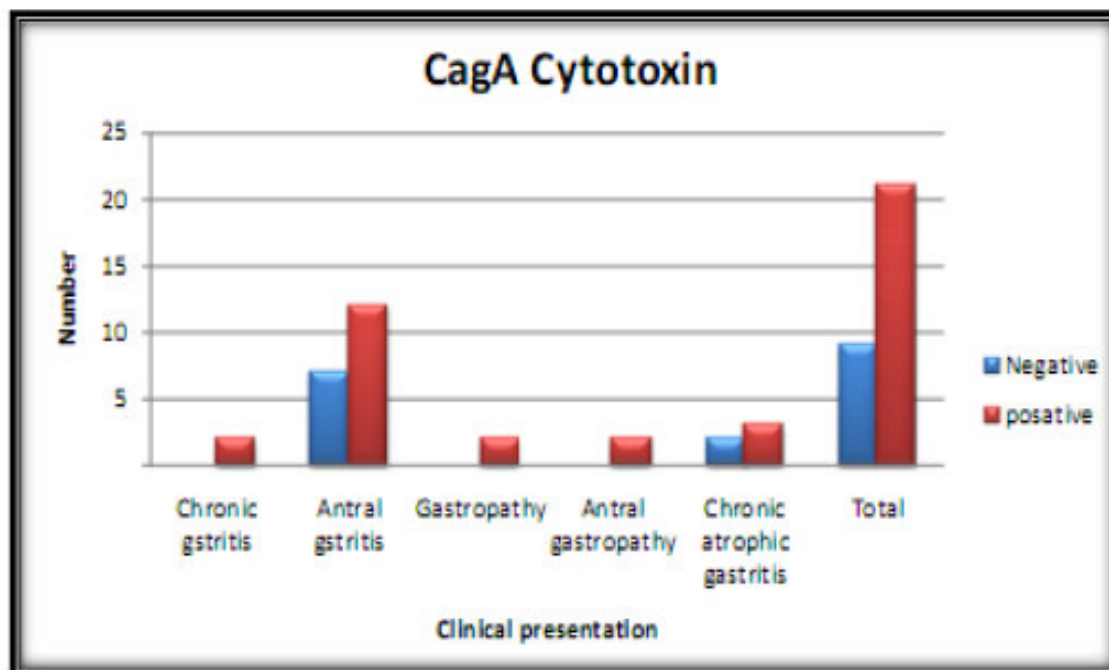


Figure (3): CagA cytotoxin frequency among clinical presentation groups

Discussion

One of most known and investigated virulence factor of H. pylori is the immunodominant CagA, although its functions and effects on cells had not been fully understood. In Iraq there were few studies that investigate the relationship between H. pylori CagA and clinical presentations of G.I.T. illness; in this study we used In Situ Hybridization technique to detect the presence of CagA. Which is the best indicator for Cag-PAI that encode also many other virulence factors.

Our findings revealed that 70.0% of patients (21 out of 30) had positive I.S.H. results for CagA, which considered high percentage if we assumed that the clinical presentation of patients are not severe (severe gastric illness include ulcers, intestinal metaplasia and cancer) and can be described as non aggressive lesions. Obtainment of such percentage may be due to that the majority of patients group were suffering from antral gastritis. There is a model proposing that infection with H. pylori begins in the antrum and then spreads throughout the fundus [8].

Our results come in concordance with recent studies in which Polymerase Chain Reaction (PCR) and or Real-time PCR had been used. Moreno-Hernandez et al., [9] detected CagA in greater percentage (83%) in the most aggressive lesions and 56% in cases with less



severity. Zali et al., detected CagA in 73.8% of the patients with gastro-duodenal diseases by using PCR [10].

The differences in ratio between the present study and the studies that mentioned above most likely related to the sensitivity of PCR technique that used for detection of the infective strain even if was present in a very limited numbers inside the infected tissue and the ability of amplification of target CagA gene for hundred times. Such feature was not available in I.S.H. technique. In addition other possible factors such as the sample size of population under investigation as well as genetic and environmental factors, economical and social habits which may have a role in high percentage of infection .

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Nidhal A. Mohammed, "Detection of CagA in H. Pylori Gastric Illness Using in Situ Hybridization"

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