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RESEARCH ARTICLE

# LABORATORY DIAGNOSTICS OF HELICOBACTER PYLORI

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**ABSTRACT**

**Background**: Helicobacter Pylori(HP) diagnostics requires use of invasive and non-invasive methods. The endoscopy is considered the "gold standard", but it is expensive and difficult to accept by patients. From non-invasive methods, the most widely used is determination of HP antigens in faeces and antibodies in blood serum. We used these two tests due to low cost and ease of acceptance by the individuals.

**Methods**: The concentration of IgG in the blood serum is determined with Anti-Helycobacter pylori ELISA (IgG) (Euroimune).Antigens of HP are determined in faeces by enzyme-linked immunosorbent assay (ELISA).

**Results**: The percentage of positive results for antigens and antibodies is slightly higher in men compared to women (40% to 36% and 25% to 20%). Furthermore, the test for antigens in faeces shows higher sensitivity (40% and 25%) than that for antibodies (36% and 20%). When combining both tests the sensitivity is increased in men and women by 11%.
Conclusions: The highest is the percentage of detection of HP in combining the two tests(antigens and antibodies) and the lowest is in that for antibodies. Our results demonstrate a relatively high frequency of positive results for antigens and antibodies in our country. of HP and even higher frequency in the simultaneous use of both tests.



## INTRODUCTION

Prevalence of Helicobacter Pylori (HP) is often asymptomatic[1,2,3]. Recent studies indicate HP involvement not only in gastritis, ulcers and adenocarcinoma in the upper parts of gastrointestinal tract (GIT), but also in other diseases (MALT lymphoma, primary biliary cirrhosis, anemia, diabetes, hepatitis) [3,4,5]. We present our data for HP carriage in adults with discomfort in GIT. HP is responsible for 70-80% of gastric and 85-90% of duodenal ulcers. About 80% of patients live with asymptomatic persistent stomach inflammation without progression to manifested disease. HP diagnostics requires use of invasive and non-invasive methods [6,7,8,9]. The former has higher sensitivity, specificity and endoscopy is considered the "gold standard". However, they are laborious, expensive, require skilled personnel, and are difficult to accept by patients. From non-invasive methods, the most widely used is determination of HP antigens in faeces and antibodies, although antibodies are a test for exposure, not a test for active infection and effective treatment.

## MATERIALS AND METHODS

For 3 years we studied antigens in faeces in 2500 individuals and IgG antibodies in blood serum in 4000 individuals aged from 18 to 70, 3705 of them or 57% were men and 2795 or 42% were women. Along with HP antibodies 29.5% of subjects (1180) were also tested for HP antigens in faeces. All these individuals were referred for investigation of HP by their GP because of complaints of discomfort in GIT, suspicion of functional dyspepsia, epigastric pain, burning, belching, etc. By the time of the test the individuals did not take NSAIDs and aspirin. Blood for antibodies and faeces for antigens of HP should be taken in the morning on an empty stomach. The concentration of IgG in the blood serum is determined with Anti-Helycobacter pylori ELISA (IgG) (Euroimune). Antigens of HP are determined in faeces by enzyme-linked immunosorbent assay (ELISA), which is widespread and has a high sensitivity (94-96% ) and specificity (92-98%), speed and simplicity.

## RESULTS

The results obtained are presented in Table 1. We assume that the positive result for an antigen or antibody is indicative of

the presence of HP in research, and the negative result excludes this. The percentage of positive results for antigens and antibodies is slightly higher in men compared to women (40% to 36% and 25% to 20%). Furthermore, the test for antigens in faeces shows higher sensitivity (40% and 25%) than that for antibodies (36% and 20%). When combining both tests the sensitivity is increased in men and women by 11%.

## DISCUSSION

Our results demonstrate a relatively high frequency of positive results for antigens and antibodies of HP and even higher frequency in the simultaneous use of both tests. This is one of the most frequent and persistent bacterial infections worldwide. In some developing countries, the prevalence of infection is higher than 90%, whereas in developed countries it is about 25- 40% [10,11]. The infection is usually acquired in infancy in all countries. However, the degree of infection of children in developing countries is higher than in the industrialized countries. Approximately 50% are the infected people over 60 years of age, compared to about 10% between 18 and 30 years. Annually, the percentage increase of the infected individuals is from 0.5% to 2% and after 60 years of age it is above 50% [10,11]. HP is a gram-negative bacterium, which has microaerophilic and nitrogen metabolism, as it requires a near-neutral pH of the environment in which it evolves. On its outer membrane it contains protein families (adhesins, porins, etc.), phospholipids and lipopolysaccharide required for its colonization and biofilm formation. This provides resistance to the bacteria in the gastric mucosa and its strong mobility. Colonization takes place in a very narrow layer of gastric and / or duodenal mucosa, near the epithelium, where they are guided by the gradient of mucus pH.

Biofilm formed in the upper parts of GIT can exist without showing clinical symptoms, as it is possible in some of our individuals. In sensitive to HP hosts it is observed an infiltration of the gastrointestinal epithelium and the underlying lamina with neutrophils, B- and T-lymphocytes, macrophages and fat cells [3,6,11] (Figure 1).

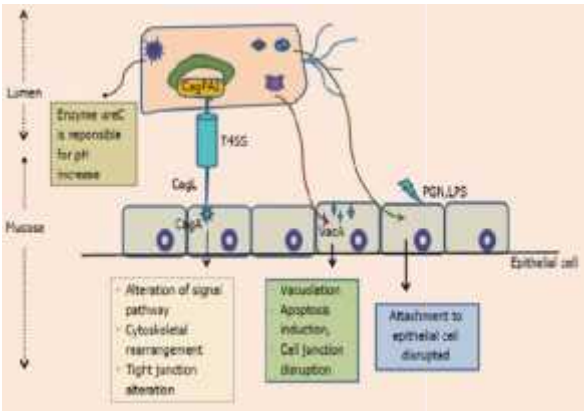


**Figure 1** Schematic representation of the pathogenes is of HP featuring virulence factors, such as CagA, CagL, lipopolysaccharides and vacuolating cytotoxingene, and subsequent damage of them ucosa (N 14, Khatoon, 2016)

Crucial bacterial factors associated with pathogenicity include type IV secretion system and the formation of a CagA effector protein, vacuolating cytotoxin (VacA), peptidoglycan, lipopolysaccharide (LPS), γ-glutamyl transpeptidase (GGT), protease HtrA and BabA and SabA adhesins [3,6,11,12,13]. In this study, our aim is to investigate what is the frequency of positive tests for antigens and antibodies of HP in individuals with mild symptoms. Apparently, despite the colonization and presence of virulence factors (SagA, vacA, babA, etc.) in patients with positive tests still this is not enough for the development of clinically manifested disease, such as gastritis, ulcers, etc. [13, 14]. The infection is transmitted by faecal-oral route and exposure of family members to HP has emerged as the most likely chance of infection transmission [15,16,17,18]. Finding carriage of HP depends on the methods used. Each of them has its advantages, disadvantages and limitations. Using a particular method is highly dependent on whether we are searching for carriage, diagnostics or effect of therapy. Invasive or direct detection methods of HP accepted by some researchers to be the "gold standard" require endoscopic biopsy for taking and testing the material by fast urease test, histology, Pap (cytology), culture, polymerase chain reaction, fluorescent in situ hybridization (FISH), etc. Despite the high specificity and sensitivity, invasive methods are labor-intensive, expensive, require appropriate qualifications and are difficult to accept by patients. Non-invasive or indirect methods prove antigens, antibodies or release of radioactive CO2 in exhaled air. Methods for determination of antigens and antibodies are more widespread, more available, faster, more acceptable by individuals, they are cheaper and with better sensitivity and specificity [9,10,19,20]. They are suitable for a large-scale analysis. That's why we used determination of IgG antibodies in blood serum and antigens in faeces. We exclude the interference of drugs in determining antigens because our individuals are without drug intake. We obtained relatively high positive results for IgG antibodies and antigens (Table 1).

**Table 1** Results from antigens and antibodies of HP in the tested individuals

| | **Helicobacter Ag** | **Totalnumberexamined 2500** | | |
|---|---|---|---|---|
| Gender | males (1425) | | females (1075) | |
| Results | positiveresults | negativeresults | positiveresults | negativeresults |
| Percentage | 40 | 60 | 36 | 64 |
| Number | 570 | 855 | 387 | 688 |
| | **HelicobacterIgG** | **Totalnumberexamined 4000** | | |
| Gender | males (2400) | | females (1600) | |
| Results | positiveresults | negativeresults | positiveresults | negativeresults |
| Percentage | 25 | 75 | 20 | 80 |
| Number | 600 | 1800 | 320 | 1280 |

The highest is the percentage of detection of HP in combining the two tests and the lowest is in that for antibodies. Failure to detect antibodies in some of our individuals may be a result of an early stage of infection because IgG antibodies are only detected only after 21 days of infection. At this stage we are not discussing the wide variety of HP strains. The evidence of antibodies cannot guarantee whether it is a long-standing or recent infection because they are stored for more than 20 years and they cannot be used for differentiation of previous from current infection (3,8,18). If the test is negative, then it is unlikely that the patient has had or has an infection with HP. In negative result there may already be colonization but at an early stage or the titre of antibodies is low to be detected. Serology has a relatively lower diagnostic accuracy (80-84%), but is useful when other tests are falsely negative, especially in patients with bleeding ulcer, atrophic gastritis, MALT lymphoma, receiving antibiotics, etc. The negative predictive value, however, is excellent and a negative result almost excludes current infection. This method is suitable for screening because it is cheap, fast, convenient and non-invasive. The other test for HP antigens is highly specific. Its key advantage is that like the UBT positive result it is an evidence for active infection. Also, similar to the UBT, the

faecal antigens test may be used as an initial diagnostic tool and assessment of treatment.

We used these two tests due to low cost and ease of acceptance by the individuals. Currently neither test can guarantee 100% detection of HP colonization, therefore a combination of two tests is recommended. A reliable non-invasive test for detecting H. Pylori may have a major impact on the treatment of patients with epigastric pain or dyspepsia or other symptoms of gastro-intestinal tract [3,20].

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