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Levels of Interleukin -17, Interleukin-23 and Alkalinephosphatase in Patients Serum with *Helicobacter pylori*

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Abstract

The study was carried out to detection of *H.pylori* in (218) patients who attended two teaching hospitals in Baghdad. The diagnosis was done by Immunochromatography methods. Stools and blood samples was taken from each patient as well as other (30) healthy control matching in age. The study included detection the Levels of Interleukin-17, Interleukin-23, and Alkaline phosphatase in sera of patients and healthy control. The result indicated presence of H pylori antigen in 115 cases 59 cases of males and 51 of females, Also, the result indicated increasing levels of IL-17 and IL-23 and Alkalinephosphatase in patients sera in comparison with healthy control.

Keywords: *Helicobacter pylori*, Interleukin-17, Interleukin-23, Alkaline phosphatase.

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INTRODUCTION

Helicobacter pylori are a flagellate Gram-negative spiral-shaped bacterium found on the luminal surface of the gastric epithelium with four to six polar sheathed flagella, which are essential for bacterial motility [1]. Half the world's population is infected by the bacterium, making it the most widespread infection in the world. Actual infection rates vary from nation to nation; the developing world has much higher infection rates than the West, where rates are estimated to be around 25% [2], bacterium seems to influence the pathologic outcome of the infection. People infected at an early age are likely to develop more intense inflammation that may be followed by atrophic gastritis with a higher subsequent risk of gastric ulcer, gastric cancer, or both. Acquisition at an older age brings different gastric changes more likely to lead to duodenal ulcer [3]. Infections are usually acquired in early childhood in all countries but the percentage of infected people increases with age, with about 50% infected for those over the age of 60 compared with around 10% between 18 and 30 years. The higher prevalence among the elderly reflects higher infection rates in the past when the individuals were children rather than more recent infection at a later age of the individual. Most likely due

to socioeconomic factors [4, 5]. *H. pylori* transmission occurs mainly within families in developed nations orally by means of fecal matter through the ingestion of waste-tainted water [6-8]. *H. pylori* is able to sense the pH gradient in the mucus and move towards the less acidic region (chemotaxis). This also keeps the bacteria from being swept away into the lumen with the bacteria's mucus environment, which is constantly moving from its site of creation at the epithelium to its dissolution at the lumen interface [9]. *H. pylori* adherence via BabA is acid sensitive and can be fully reversed by increased pH. It has been proposed that BabA's acid responsiveness enables adherence while also allowing an effective escape from.

Unfavorable environment at pH that is harmful to the organism [10]. Following attachment of *H. pylori* to stomach epithelial cells, the type IV secretion system expressed by the cag PAI "injects" the inflammation inducing agent, peptidoglycan, from their own cell walls into the epithelial cells. The injected peptidoglycan is recognized by the cytoplasmic pattern recognition receptor (immune sensor) Nod1, which then stimulates expression of cytokines that promote inflammation [11]. *H. pylori* harms the stomach and duodenal linings by

several mechanisms. The ammonia produced to regulate pH is toxic to epithelial cells, as are biochemical products produced by *H. pylori* such as proteases, vacuolating cytotoxin A (VacA) (this damages epithelial cells, disrupts tight junctions and causes apoptosis), and certain phospholipase. Cytotoxin associated gene CagA can also cause inflammation and is potentially a carcinogen [11]. Colonization of the stomach by *H. pylori* can result in chronic gastritis, an inflammation of the stomach lining, at the site of infection. Helicobacter cysteine rich proteins (Hcp), particularly HcpA are known to trigger an immune response, causing inflammation [12].

The study was carried out to detection of *H.pylori* in (218) patients and also to detect the Levels of Interleukin-17, Interleukin-23, and Alkaline phosphatase in sera of patients and healthy control.

MATERIALS AND METHODS

During a period of eight months from December 2022 to April 2023, a study was conducted at two teaching hospitals in Baghdad on freshly collected stool samples from a total number of 218 cases of gastroenteritis among adult patients. Stool samples were collected from each patient in sterile disposable screw cap containers. These were labeled with number, date, and name of each subject. A questionnaire containing demographic, clinical, and environmental data was obtained from each case. The existence of *H. pylori* in fresh stool samples was investigated at the microbiology laboratory of the same hospital using an immunochromatographic test.

H. pylori Antigen Detection

Immunochromatographic assay (purchased from CerTest Biotech Spain) for antigenic detection of *H.pylori* and were done according to instructions of the manufacturers. Allowing the card –device, test reagents and stool samples to reach to room temperature prior to testing. A separate stool collection tube and device were used for each sample and the assay was done right after collection. To detect *H.pylori*, approximately 100mg or 100 microliter of stool sample was put and shaken in collection tube containing the diluents. Four drops or 100µl was dispensed in the circular window of the card.

The results (appearance of the colored bands) were read after 10 minutes. This CerTest-*H.pylori* KIT is qualitative Immunochromatographic assay for determination of *H.pylori* in fecal samples. The membrane on the test band region is pre – coated with mouse monoclonal antibodies against *H.pylori* antigens.

During testing, the sample is allowed to react with the colored conjugates (anti-*H.pylori* mouse monoclonal antibodies-red microspheres) which were pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the colored particles migrate. In the case of positive result, the specific antibodies present on the membrane will capture the colored particles and a red colored line becomes visible.

The mixture captures the colored particles and a red colored line becomes visible. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a green-colored band always appear. The presence of this green band serves as 1-verification that sufficient volume is added, 2-that proper flow is obtained and 3-as an internal control for the reagents. Insufficient specimen volume, incorrect procedural or deterioration of the reagents is the most likely reasons for control line failure. Negative results were indicated by only one green band (control line). For positive result, in addition to the green control band, a red band also appear on the site of result line. A total absence of the control colored band (red) regardless the appearance or not of the result line (red) was evaluated as an invalid result.

Blood Samples

Three mL of Venous blood was obtained from each patients and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in eppendorf tubes and stored in deep freeze at -20 °C until the time for using. The current study included Immunological & Clinical biochemical aspects. The level of interleukin -17 (IL-17) Interleukin-23 (IL-23) estimated by ELISA according to manual procedure of (Sunlong Biotech). Serum Alkalinephosphatase (ALP) activity determined according to manufactures instructions of Biosystem (Spain).

Statistical Analysis: The results were analyzed using statistical system SPSS version -18 (T-testing).

RESULTS

Gender

Distribution of *H. pylori* patients according to their gender, were studied, among them 59 were males out of 110 and 56 were females out of 108. In a general *H. pylori* antigen was revealed in 115 of fecal samples out 218. (Table-1)

Table 1: Distribution of H. pylori patients according to their gender

H pylori Antigen	Total	Positive		Negative	
		No.	%	No.	%
Male	110	59	53.63	51	46.37
Female	108	56	51.85	52	48.15
Total	218	115	52.75	103	47.25

Immunological Parameters

The levels of the IL-17, IL-23 increased significantly ($p \leq 0.05$) in patients suffering from *H. pylori* in comparison of the healthy individual. (Table-2)

Table 2: Levels of IL-17, IL-23 (pg/ml) in patients sera and healthy control

Groups	IL-17	IL-23
patients	264.98± 6.94	321.34±37.28
control	190.92±15.29	165.65±17.16

Alkaline Phosphatase Activity

The level of alkaline phosphatase activity increased significantly in patients serum in comparison with healthy control (Table-3).

Table 3: Alkaline phosphatase Activity (IU/ml) in patients sera and healthy control

Groups	ALP activity
patients	194.45 ± 0.21
control	112.43 ± 0.16

DISCUSSION

Helicobacter pylori was identified in 115 stool samples of patients out of 218 samples (Table - 1). The infections may be due to lack of sanitary facilities and poor living condition among the major causes of infection. The result was consistent with that reported in Diyala by Hasan *et al.*, [13], in Basrah by Al-Hamdi and Khashan [14]. But the variation in the rate of infection between different studies may be due to the type of the sample (blood, stool and tissue), size of the sample, place and period of the study and techniques used for detection of the bacteria. The rate of infection in males was higher than the females.

The result indicated that *H. Pylori* infection in males 53.63% was higher than 51.85% in female and in a line with other results were reported in Diyala by Al-Ezzy [15]. *H. pylori* infection increases IL-17 in the gastric mucosa of humans, IL-17 induces the secretion of IL-8 by activating the ERK 1/2 MAP kinase pathway and the released of IL-8 attracts neutrophils promoting inflammation. The immune response to *H. pylori* include the recruitment of T cells and the production of IL-7 and Neutrophil attracting chemokines, and the bacterial load is considerably reduced so IL-17 plays role in infection. T regulatory cells (Tregs) suppress the inflammatory reaction driven by IL-17 there by favoring bacterial persistence. Immunization produces *Helicobacter*-specific memory T-helper cells that can possibly alter the ratio between T-helper 17 and Treg responses so that the IL-17-driven inflammatory reaction can overcome the Treg response leading to bacterial clearance [16]. IL-17A secretion by Th17 cell is regulated by IL-23 through a STAT3-dependent pathway [17].

IL-23 is secreted by several immune cells including dendritic cells, macrophages, and neutrophils in the gastric mucosa. Elevated IL -23 levels in the *H. pylori* infected gastric biopsies have been reported by Koussoulas in 2008 [18]. Horvath *et al.*, reported a positive correlation between levels of IL-23 and the

infiltration levels of neutrophils and monocytes in patients with *H. pylori* infection. It is suggested that IL-23 plays a role in the activation of the immune response and induction of gastritis in response to *H. pylori* [19]. The level of ALP increased significantly. The increasing level of alkaline phosphatase in the serum may be as a result to damage of infected cells and released the enzymes to blood stream [20].

CONCLUSION

The result indicated presence the of *H. pylori* antigen in 115 cases 59 cases of males and 51 of females, Also, the result indicated increasing levels of IL-17 and IL-23 and Alkalinephosphatase in patients sera in comparison with healthy control

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