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Interleukin-32, interleukin-33 and acid phosphatase levels in patients 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 were taken from each patient as well as other (30) healthy control matching in age. The study included measurement the Levels of Interleukin-32, Interleukin-33, and Acid phosphatase in sera of patients and 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-32 and IL-33 and Acid phosphatase in patients sera in comparison with healthy control.

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Introduction

Helicobacter pylori is a flagellate Gram-negative spiral-shaped bacterium found on the luminal surface of the gastric epithelium with four to six polar heated flagella, which are essential for bacterial motility (1) Infection with H. pylori occurs worldwide, but the prevalence varies greatly among countries and among population groups within the same country. H. pylori colonize the stomachs of 50 % of the population in developed countries and approx. 80 % in the developing world. The infection is acquired by oral ingestion of the bacterium and is mainly transmitted within families. The main source of transmission is the mother within families (2, 3).

The overall prevalence of H. pylori is strongly correlated with socioeconomic conditions. Factors such as density of housing, overcrowding, number of siblings, birth order, sharing a bed and lack of running water have all been linked to a higher acquisition of H. pylori infection (4). Recognition of bacterial molecules by the innate immune system is mediated by TLRs (Toll-like receptors) expressed on APCs (antigen-presenting cells) such as monocytes and DCs (dendritic cells). Bacterial contact with monocytes and other APCs leads to the secretion of pro-inflammatory cytokines such as TNF-α (tumour necrosis factor-α), IL (interleukin)-1β and IL-8. H. pylori infection has been shown to be associated with increased levels of these cytokines which, in turn, act as local chemoattractants, inducing granulocytic infiltration (5). Since H. pylori rarely, if ever, invade the gastric mucosa, the host response is triggered primarily by the attachment of bacteria to epithelial cells. The organism produces a number of antigenic substances, including HSP (heat-shock protein), unease and LPS, all of which can be taken up and processed by lamina propia macrophages and activate T-cells (6, 7).

Cellular disruption, especially adjacent to epithelial tight junctions, undoubtedly enhances antigen presentation to the lamina propia and facilitates immune stimulation. The net result is increased production of inflammatory cytokines such as IL-1, IL-6, NF-α and, most notably, IL-8 (8). Chronic active gastritis is associated with an increased CD4/CD8 T-cell ratio within the gastric mucosa, dueargely to the accumulation of CD4+ T-helper lymphocytes in the lamina propia. H. pylori infection results in a Th1-predominant host immune response in the gastric mucosa, characterized by the induction of IFN-γ and IFN-γ -related genes. A Th1-pre- dominant immune response is associated with elevated levels of the pro-inflammatory cytokines IL-12, IL-18 and TNF-α. The severity of gastritis associated with H. pylori infection was correlated with mucosal expression of the TNF-α subunit CD68 and IFN-γ (9).

Materials and Methods

During a period of eight months from December 2021 to April 2022, 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 microtiter 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 thereagents 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 (green) 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 -32 (IL-32) Interleukin-33 (IL-33) estimated by ELISA according to manual procedure of Cusabio, serum Acid phosphatase(ACP) 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-32, IL-33 increased significantly ($p \le 0.05$) in patients suffering from H pylori in comparison o the healthy individual. (Table-2)

Table-2: Levels of IL-32,IL-33 (pg/ml) in patients sera and healthy control

Cytokines	IL-32	IL-33
Patients	640 ± 380	191±15
Control	201±141	37±12

Acid phosphatase Activity

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

Table: Acid phosphatase Activity in patients sera and healthy control

Groups	ACP activity (IU/ml)
Patients	0.8 ± 0.2
Control	0.3 ± 0.1

Discussion

Helicobacter pylori were identified in 115 stool samples of patient 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 Kirkuk (10), in Diyala by Hasan et al. (11), in Basrah by Al-Hamdi and Khashan (12). 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 females. The result indicated that *H. Pylori* infection in males 53.63% was higher than 51.85% in female the results in line with other results were reported in Diyala by Al-Ezzy (13).

The increasing level of IL-32 in patients sera may be due to ability of H. pylori in AGS cells to involvement of the JNK (14), p38 (15), extracellular signal-regulated kinase (ERK) (16), and NF-kB (16) pathways to induced production of IL-32 to trigger the pathogenesis of gastric diseases in other words H. pylori infection induces IL-32 through NF-kB activation in a cag-dependent manner than , IL-32 amplifies the NF-kB pathway and induces production of other inflammatory cytokines (17).

The increasing level of IL-33 in infected patients with H pylori may be due to ability of *H. pylori* via virulence factor cag A to induce maximal IL-33 expression by ERK signaling pathway. (18), in which the upregulation of IL-33 roles depending on the nature of the infection (19-21). *H. pylori*-associated virulence factor cag A induced gastric epithelial cells to express IL-33, which in turn could induce mast cell activation and pro inflammatory mediator TNF-α production. So, TNF-α plays an important role in the pathogenesis of many chronic inflammatory diseases (22,23) . Acid phoshatase increased significantly in patients with *H.pylori* (Table 3) in a general, ACP considered as virulence factor in some pathogenic microorganism or may be important for management of disease severity (24).

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-32 and IL-33 and Acid phosphatase in patients sera in comparison with healthy control

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