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Original Research Article

Levels of Interleukin-39, C–Reactive Protein in Patient's Serum with *H. Pylori* Infections

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Abstract

The study was carried out to detection of *H.pylori* in (200) patients who attended two teaching hospitals in Baghdad. The diagnosis done by Immunochromatography methods. Stools and blood samples was taken from each patient as well as other (30) healthy control matching in their age. The study included detection the Levels of Interleukin-39 and CRP 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-39 and CRP in patients sera in comparison with healthy control.

Keywords: Helicobacter pylori, Interleukin-39, C - reactive protein.

INTRODUCTION

Helicobacter pylori is a gram-negative, flagellated, microaerophilic bacterium that selectively colonizes the gastric mucosa [1]. After entering the stomach, this spiral, Gram-negative, microaerophilic bacterium penetrates the mucus gastric layer [2], but does not traverse the epithelial barrier [3], and therefore it is considered as a non-invasive bacteria. Most of H. pylori organisms are free living in the mucus layer, but some organisms attach to the apical surface of gastric epithelial cells [3]. Upon infection, H. pylori uses urease and α-carbonic anhydrase to generate ammonia and HCO32- which mitigate the effects of low pH [4, 5]. Once established in the inner mucus layer, several outer membrane proteins, including BabA, SabA, AlpA, AlpB and HopZcan mediate bacterial adherence to gastric epithelial cells. Once attached, bacterial effector molecules, both secreted [vacuolating cytotoxin (VacA) and cytotoxin associated gene A (CagA)] or attached [components of the type IV secretion system (CagL)], modulate gastric epithelial cell behavior leading to loss of cell polarity, release of nutrients and chemokines [e.g., interleukin (IL-8], and regulation of acid secretion via control of gastrin and H +/ K+ ATPase [9, 10]. H. pylori infection can be associated with several clinical complications such as gastritis, peptic ulcer disease, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma [6-9]. Infection showed infiltration of lymphocytes and monocytes, along with significantly increased expression ofIL-1, IL-8, and IL-6 in the gastric antrum [10]. Anti-H.pylori immunoglobulin (Ig)M and IgG responses were detected in the serum of infected individuals 4 wk after infection, the numbers of gastric CD4 +and CD8+ T cells were increased compared to pre infection levels [11]. Levels of cytokines [interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-1, IL-6, IL-7, IL-8, IL-10, and IL-18], are increased in the stomach of H. pylori-infected humans compared to uninfected humans [12]. IL-4 has not been detected in the gastric mucosa of most H. pylori-infected individuals [13]. H. pylori infection leads to a T helper cell (Th)1-polarized response. H. pylori infection has also been associated with upregulation of IL-17A expression in the gastric mucosa [14]. IL-17A is the most widely studied member of the IL-17 family of cytokines (IL-17A-F), and is produced by Th17 CD4 + T cells as well as other subsets of immune cells [15]. H.pylori infection also leads to the generation of regulatory T cells (Treg) [16,17,18] associated with low Th17 and Th1 responses, high Treg response and reduced gastritis as compared with adults, suggesting that H. pylori specific T- reg play key roles in bacterial persistence. The pro-inflammatory cytokine IL-39, a member of the IL-12 family plays a key role in the

inflammatory response by modulating immune cell activity and inflammation. The purpose of this study was to investigate the Level of IL-39 and CRP in H.pylori patients.

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 200 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 (Weifang Kanghua Biotech china) 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 *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 continues to move across the membrane to the immobilized antibody placed in the control band region, a red colored band always appear. The presence of this red 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 are the most likely reasons for control line failure. Negative results were indicated by only one red band (control line). For positive result, in addition to the control band, a red band also appear on the site of result line. A total absence of the control colored band regardless the appearance or not of the result line 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 –20c°until the time for using. The current study included Immunological level of interleukin -39 (IL-39) which estimated by ELISA according to manual procedure of (Sunlong Biotech, China). Serum C-Reactive protein (CRP) level determined according to manufactures instructions of Mindray (China). The Reference range of CRP (<5 mg/L) in adults.

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 90. In a general *H. pylori* antigen was revealed in 115 of fecal samples out 200. (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	90	56	62.23	34	37.77
Total	200	115	57.75	85	42.25

Immunological Study

The level of IL-39 statistically increased significantly in patients suffering from H. pylori in comparison to healthy individual. (Table-2)

Table-2: Level of IL-39 (ng/L) in patients sera and healthy control

Groups	IL-39
Patients (HP+)	116.32± 11.90
Control (HP-)	44.62± 10.29

*** (P<0.05) significant**

The level of CRP increased significantly ($p \leq 0.05$) in patients suffering from H. pylori in comparison to healthy individual. (Table-3)

Table 3: Level of CRP (mg/L) in patients sera and healthy control

Groups	CRP
Patients (HP+)	7.23± 1.94
Control (HP-)	1.90±1.29

***(P<0.05) significant**

DISCUSSION

Helicobacter pylori was identified in 115 stool samples of patient out of 200 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*., [19], in Basrah by Al-Hamdi and Khashan [20]. 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 was higher than in female the results in line with other results were reported in Diyala by Al-Ezzy [21]. The Result indicated an increased level of IL-39 in H pylori patients (Table-2) in comparison to healthy control probably contributes to inflammatory response in H pylori patients. In a general ,The cytokine IL-39 secreted by activated B-cells has been shown to mediate its effect through the activation of STAT1/STAT3 of the signal transduction pathway [22, 23]. The results shows IL-39 linked to common immune processes and assume that IL-39 may be playing a key role in contributing to the immunopathogenic mechanisms, mediating an inflammatory response. The significant increment of CRP levels (Table-3) among H. pylori patients might be due to the inflammatory effect of H. pylori bacterium since the bacterium contain various virulent factors such as CagA and VacA. CagA, a highly virulent factor that is produced by H. pylori bacterium that has a greater role in H.pylori-induced inflammation. H. pylori infection promotes inflammation by activating nuclear factor Kappa-B (NF-κB) which mediates inflammation through activation of pro-inflammatory cytokines such as IL-6, TNF-alpha, and IL-18. The activated IL-6 stimulates acute phase reactant protein production such as CRP from the liver [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-39 and CRP in patients sera in comparison with healthy control.

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