ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-4 | Issue-4 | Sept-Oct -2022 | **DOI: 10.36346/sarjbab.2022.v04i04.002**

Original Research Article

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

Khalil Ismail Abid Mohammed[1*], Saad Hasan Mohammed Ali[1], Wifaq M. Ali[1], Suha A. AL-Fakhar[1], Jinan M. Mousa[1]

[1]Communicable Disease Research Unit /College of Medicine, University of Baghdad, Baghdad, Iraq

***Corresponding Author:** Khalil Ismail Abid Mohammed
Communicable Disease Research Unit /College of Medicine, University of Baghdad, Baghdad, Iraq

Article History
Received: 18.08.2022
Accepted: 13.09.2022
Published: 17.09.2022

Abstract: The study was carried out to detection of *H.pylori* in (218) patients who attended two teaching hospitals in Baghdad. The diagnosis is 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-17, Interleukin-23, and Alkaline 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-17 and IL-23 and Alkaline phosphatase in patients sera in comparison with healthy control.

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

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 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]. 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 [3]. One of the virulence factors responsible for the progression of gastric diseases is the cag pathogenicity island (PAI) of H. pylori, a cluster of approximately 30 genes. H. pylori and its cagPAI genes are associated with severe gastric diseases, including gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [4, 5]. *H. pylori* cagPAI activates nuclear factor B (NF-kappaB) and mitogen activated protein kinase (MAPK) signaling pathways in infected epithelial cells. NF-B is a transcription factor that regulates various cellular responses, including inflammation, cell survival or death, and cell proliferation. NF-kappaB activation induces the production of inflammatory cytokines such as interleukin-1 (IL-1), IL-8, and tumor necrosis factor alpha (TNF-alpha. IL-8, which is induced by H. pylori infection via NF-B activation in gastric epithelial cells, plays a critical role in gastritis and gastric carcinogenesis [6] IL-8 cause's neutrophil infiltration into gastric tissue, which elicits additional inflammation. In Japanese populations, a single polymorphism in the IL-8 gene is associated with upregulation of IL-8 and with an increased risk of atrophic gastritis and gastric cancer [7, 8]. Similarly, polymorphisms in the IL-1and TNF-alpha genes have been associated with gastritis and gastric cancer [9, 10]. IL-32, is inflammatory cytokine and induce the production of several other cytokines, such as TNF-alpha and IL-1 [7, 11].

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 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 appears 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 -17 (IL-17)Interleukin-23 (IL-23) estimated by ELISA according to manual procedure of cusabio Biotech(Germany).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 o 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 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 [11], in Diyala by Hasan *et al*., [12], in Basrah by Al-Hamdi and Khashan [13]. 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 [14]. 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-7and 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 thereby 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 [15]. IL-17A secretion by Th17 cell is regulated by IL-23 through a STAT3-dependent pathway [16]. 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 [17]. 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 [18]. 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. 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 [19].

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

REFERENCES

1. Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A. B., Warnke, R. A., Jellum, E., ... & Friedman, G. D. (1994). Helicobacter pylori infection and gastric lymphoma. *New England Journal of Medicine*, *330*(18), 1267-1271.
2. Cave, D. R. (1996). Transmission and epidemiology of Helicobacter pylori. *The American journal of medicine*, *100*, 12S-18S.
3. Webb, P. M., Knight, T., Greaves, S., Wilson, A., Newell, D. G., Elder, J., & Forman, D. (1994). Relation between infection with Helicobacter pylori and living conditions in childhood: evidence for person to person transmission in early life. *Bmj*, *308*(6931), 750-753.
4. Maeda, S., & Mentis, A. F. (2007). Pathogenesis of Helicobacter pylori infection. *Helicobacter*, 12, 10-14.
5. Ohmae, T., Hirata, Y., Maeda, S., Shibata, W., Yanai, A., Ogura, K., ... & Omata, M. (2005). Helicobacter pylori activates NF-κB via the alternative pathway in B lymphocytes. *The Journal of Immunology*, *175*(11), 7162-7169.
6. Suzuki, M., Mori, M., Miyayama, A., Iwai, N., Tsunematsu, N., Oonuki, M., ... & Ishii, H. (1997). Enhancement of neutrophil infiltration in the corpus after failure of Helicobacter pylori eradication. *Journal of clinical gastroenterology*, *25*, S222-S228.
7. Taguchi, A., Ohmiya, N., Shirai, K., Mabuchi, N., Itoh, A., Hirooka, Y., ... & Goto, H. (2005). Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. *Cancer Epidemiology Biomarkers & Prevention*, *14*(11), 2487-2493.
8. Mohamed, K. I. A., Taha, G. I., Abu-Al-Ess, H. Q. M., Ali, S. H. M., Al-Fukhar, S. A., Al-Wattar, W. M. A., ... & Mousa, J. M. (2018). Rate Of Helicobacter pylori Antigen Detection In Relation To Laboratory Criteria Among Adult Patients With Acute Gastroenteritis. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, *9*(5), 1054-1062.

9. Sugimoto, M., Furuta, T., Shirai, N., Nakamura, A., Xiao, F., Kajimura, M., ... & Hishida, A. (2007). Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. *Journal of gastroenterology and hepatology*, *22*(1), 51-59.
10. El-Omar, E. M., Carrington, M., Chow, W. H., McColl, K. E., Bream, J. H., Young, H. A., ... & Rabkin, C. S. (2000). Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*, *404*(6776), 398-402.
11. Kim, S. H., Han, S. Y., Azam, T., Yoon, D. Y., & Dinarello, C. A. (2005). Interleukin32: a cytokine and inducer of TNF alpha. *Immunity*, 22, 131-142.
12. Hasan, A. S., Jaafer, A. M., & Athab, A. M. (2017). Rate of *Helicobacter pylori* infection among patients with irritable bowel syndrome. *Gulf Med J*, 6, 16-21.
13. I AL-Hamdi, K., & S Khashan, L. (2017). Role of Helicobacter pylori in chronic ordinary urticaria: A case-control and therapeutic study. *The Medical Journal of Basrah University*, *35*(1), 39-47.
14. Al-Ezzy, A. I. A. (2015). Evaluation of Clinicopathological and Risk Factors for Nonmalignant H. Pylori Associated Gastroduodenal Disorders in Iraqi Patients. *Open access Macedonian journal of medical sciences*, *3*(4), 645-654.
15. Kabir, S. (2011). The role of interleukin-17 in the Helicobacter pylori induced infection and immunity. *Helicobacter*, *16*(1), 1-8. doi: 10.1111/j.1523-5378.2010.00812.x.
16. Aggarwal, S., Ghilardi, N., Xie, M. H., de Sauvage, F. J., & Gurney, A. L. (2003). Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *Journal of Biological Chemistry*, *278*(3), 1910-1914.
17. Koussoulas, V., Vassiliou, S., Giamarellos-Bourboulis, E. J., Tassias, G., Kotsaki, A., Barbatzas, C., & Tzivras, M. (2009). Implications for a role of interleukin-23 in the pathogenesis of chronic gastritis and of peptic ulcer disease. *Clinical & Experimental Immunology*, *156*(1), 97-101.
18. Horvath Jr, D. J., Washington, M. K., Cope, V. A., & Algood, H. M. S. (2012). IL-23 contributes to control of chronic Helicobacter pylori infection and the development of T helper responses in a mouse model. *Frontiers in immunology*, *3*, 56.
19. Salehi, H., Minakari, M., Yaghoutkar, A., Tabesh, E., Salehi, M., & Mirbagher, L. (2014). The effect of Helicobacter pylori eradication on liver enzymes in patients referring with unexplained hypertransaminasemia. *Advanced biomedical research*, *3*(131), 1-5.