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RESEARCH TITLE

Evaluation of Serum Interleukin -6 Levels in *Helicobacter Pylori* Infected Patients

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Abstract

Interleukin -6 has various immunological effects that is mainly considered as a pro-inflammatory cytokine as well as an anti-inflammatory one. IL-6 has been shown to be upregulated during *Helicobacter plylori* infection of gastric mucosa. The serum levels of IL-6 determination during the infection with *H. pylori* were the aim of current study. During the study period a total number 75 blood samples were collected. The obtained serum was tested for anti- *H. pylori* IgG. Subjects comprised 28 males and 47 females with age ranged from 14-65 years. The other parts of serum samples were stored at -20 ˚C till use. Il-6 levels were examined by using specific human IL-6 antibody kit detected by highly sensitive enzyme -linked immunosorbent assay (ELISA).

According to the results of anti- *H. pylori* IgG subjects were divided into two groups one which comprised subjects tested positive (seropositive) which comprised (60) subjects and the other included those tested negative (seronegative) which included (15) subjects. The mean age of seropositive group was (28.97± 9.9) years while that of seronegative group was (26.92±8.9) years. Females were more than males in both groups ;(36, 11) in seropositive and seronegatve groups respectively as compared with (24, 4) males in seropositive and seronegative groups respectively. Regarding serum levels of IL-6 results showed that they were (8.5±3.46) pg/ml and (8.4±2.11) pg/ml for seropositive and seronegative groups respectively, with no significant differences. However these mean value represent higher estimate when compared with globally registered IL-6 normal values. Similarly no statistical differences were shown between genders.

1. Introduction

Helicobacter pylori is one of the most important pathogens that has the ability to colonize human stomach. This may result in chronic inflammation and consequently causes various diseases including gastritis, peptic ulcer, in some cases it may develop into autoimmunity, or allergic response and more seriously into gastric cancer.(Crowe, 2019; Bakhati *et al*., 2020) When innate immunity fails, the acquired adaptive immune response is developed against *H. pylori* infection. Studies revealed that both types of Th1- and Th2-type cells are involved although the latter comes secondly to the first. IL-6 is considered as Th2 associates with anti-inflammatory cytokines. Besides, preceding studies illustrated that IL-6 polymorphisms are associated with H. pylori infection (Bucci *et al* ., 2023). Several types of cytokines are secreted due to *H. pylori* infection such IL-6 by the inflamed gastric mucosa. Interleukin 6 is believed to play a pivotal role in inflammatory cells recruitment to the gastric mucosa which in some cases may be responsible for the persistence of local inflammation(Xu *et al* ., 2020). Large surface of stomach may cause dribbling over of locally produced immunological mediators into circulation. Studies concerning determination of circulating levels of IL-6 are rather controversial. Since the gastric inflammation of *H. pylori* is limited to gastric mucosa or results in systemic inflammation is still tentative (Bayraktaroglu˘ *et al*., 2004). An extensive study showed that the geographic region has an impact on cytokine production in infections with *H. pylori* (Yu et al., 2023). Local studies concerning the detection of IL-6 in patients with *H.pylori* still minor, accordingly this study was proposed to evaluate the serum levels of IL-6 in patients with *H. pylori* in Najaf Province.

2. Methods

2.1 Subjects

The collection of samples process was from July 2023 to February 2024, and the research is based on 75 individuals of both genders (28) male and (47) female with an age range from (14-65) years.

2.2 Detection of *H. pylori* Seropositivity

Samples were collected from the enrolled subjects as follows: (5) ml of venous blood was collected by venipuncture. After fully clotting, samples were centrifuged 1000-2000 g for 10 min. Obtained serum volumes were divided into two portions the first was submitted to anti-*H.pylori* IgG test, and the second part was frozen in -20 ˚C till use to examine the IL-6 levels by ELISA. Based on the positivity of anti-*H.pylori* IgG test, samples were registered as seropositive otherwise considered as seronegative.

2.2 Detection of serum Interleukin -6

This was done by using high sensitive kit from (Solarbio-China) for the quantitative determination of human interleukin -6 concentrations in cell culture ,supernates, serum, and plasma by Enzyme Linked Immunosorbent Assay (ELISA). Samples of this study included sera from study groups. Test was performed according to the manufacturer instructions.

2.3 Statistical Analysis

Statistical analysis was performed by the software SPSS version 22. Analysis of variance (ANOVA) of sing factor was used. Expression of results was as the mean± standard deviation. Chi Square was also used. Statistical significance was tested at $p \leq 0.05$.

3. Results and Discussion

3.1 Subjects Data

During the study period (75) blood samples were collected from patients visited different hospitals and private laboratories that were suspected of infection with *H. pylori*. Age range was (14-56) years .Based on the results of anti- *H. pylori* IgG subjects were divided into two groups. The seropositive group (positive for the two tests) contained 60 subjects, included (24) male and (36) female, and the seronegative group comprised 15 subjects which included (4) male and(11) female, those subjects. Analysis of data is shown in table -1. No significant differences were shown by comparing males and female in both groups. Similar results were shown in several studies such as that of Omar *et al* ., (2019) and Santos *et al* ., (2021) who examined the expression of Il-6 in patients with gastritis and gastric cancer. Same thing was seen in a study by Al-Karawi *et al* .,(2023) who aimed to analyze some physiological and biological parameters in H pylori patients. The mean age of seropositive group was (28.97± 9.9) while that of seronegative group was (26.92± 8.9) years as shown in table -1.

Table -1: Parameters of Study Groups

Parameters	Seropositive group N=60	Seronegative group N=15	p-value
Gender no, %			
Male	24(40)	4(27)	0.3
Female	36(60)	11(73)	
Age(years, mean±SD)	28.97±9.9	26.92±8.9	

3.2 Detection of Circulating Interleukin -6

Detection of circulating levels of interleukin six was determined by ELISA technique with the previously mentioned kit. Obtained readings of optical density were calculated according to the obtained curve. The concentration of both groups was submitted for further data analysis. Serum levels of IL-6 in seropositive group was (8.5±3.46) while that of seronegative group was (8.4±2.11) showing no significant differences p=0.9, (p>0.05) figure (3-1). Statistically no obvious differences were shown regarding the mean of IL-6 between the genders of the study groups, were p -value was 0.7

Regarding the IL-6 values a study by Stavileci *et al*., (2020) showed values similar to ours equaling 8.9 ng/ml. Similarly a study by Bayraktaroglu˘ *et al*.,(2004) elucidated that they could not show elevated circulatory levels of IL-6, IL-8 and TNF-a in *H. pylori*-infected cases.

In another study in Turkey which compared between healthy and *H.pylori* infected individuals no significant differences were shown regarding serum levels of IL-6 as well as other cytokines (Bayraktaroglu˘ *et al*., 2004).

Likewise Zumkeller and colleagues (2005) found that the concentration of serum interleukin -

6 and other two cytokines were not significantly different between subjects having *H.pylori* antibodies and those who did not have them. Also in a Japanese study by Nakagawa *et al* ., (2013) there was no significant difference in serum IL-6 levels between infected and uninfected subjects although they found out that there was significant association between levels of IL-6 and levels of IgG titers of *H. pylori* patients. Correspondingly a study in Taiwan aimed to correlate levels of interleukin 6 and hepcidin, showed that no significant relationship between seropositive and seropositive regarding interleukin 6 (Chen *et al* ., 2018). In a study by Afsharpooyan *et al* ., (2019) exhibited no prominent difference of serum IL-6 levels between seropositive and seronegative subjects.

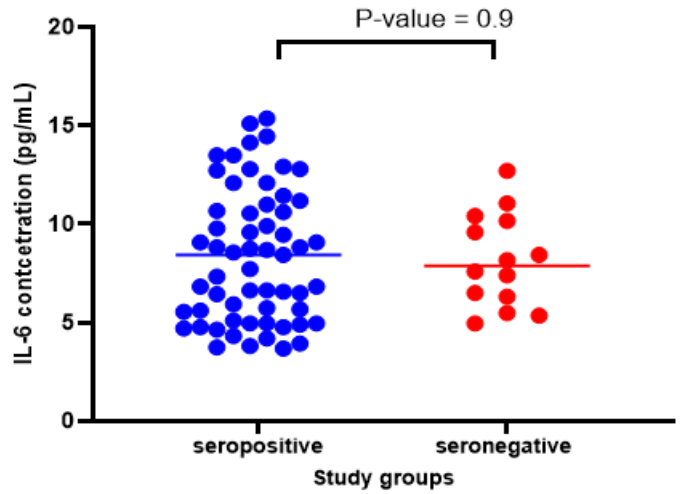

Figure (3-1) The Distribution of serum Interleukin -6 between the seropositive and seronegative groups

On the other hand, an Indonesian study revealed that only IL-8 showed no statistical differences between infected with *H. pylori* versus non infected subjects while different result was recorded regarding IL-6 of which was statistically higher in infected group.(Siregar *et al* .,2014). Similarly an Egyptian study revealed that there was significant increase in levels in seropositive as compared with seronegative subjects and is correlated with gastric ulcer (Omar *et al* ., 2019). Recent study in Iraq, researchers concluded that there was a Significant increase of IL-4 and IL-6 concentration in seropositive patients compared with seronegative controls (Rasool *et al* .,2022).

Regarding gender, means of serum IL-6 showed no significance differences between males and females as shown in figure (3-2). This comes in accordance with outcomes of Bayraktaroglu˘ *et al*., (2004) who showed no significant differences between genders regarding distribution of infection as well as levels of serum IL-6.

Interleukin-6 together with tumor necrosis factor –α and IL-1 in case of threat conditions are induced resulting in acute phase response. Whether the inflammatory response was a local or a systemic one, IL-6 play a pivotal role in modulating the immune response from proinflammatory towards an anti-inflammatory immune response (Xing *et al* ., 1998). Where several studies had correlated incidence of IL-6 high levels with generation of gastric cancers; where it seemed that high expression of IL-6 is correlated with elevated incidence of gastric cancer making it helpful tool in detection of this disease as Wang *et al*.,(2021) had concluded.

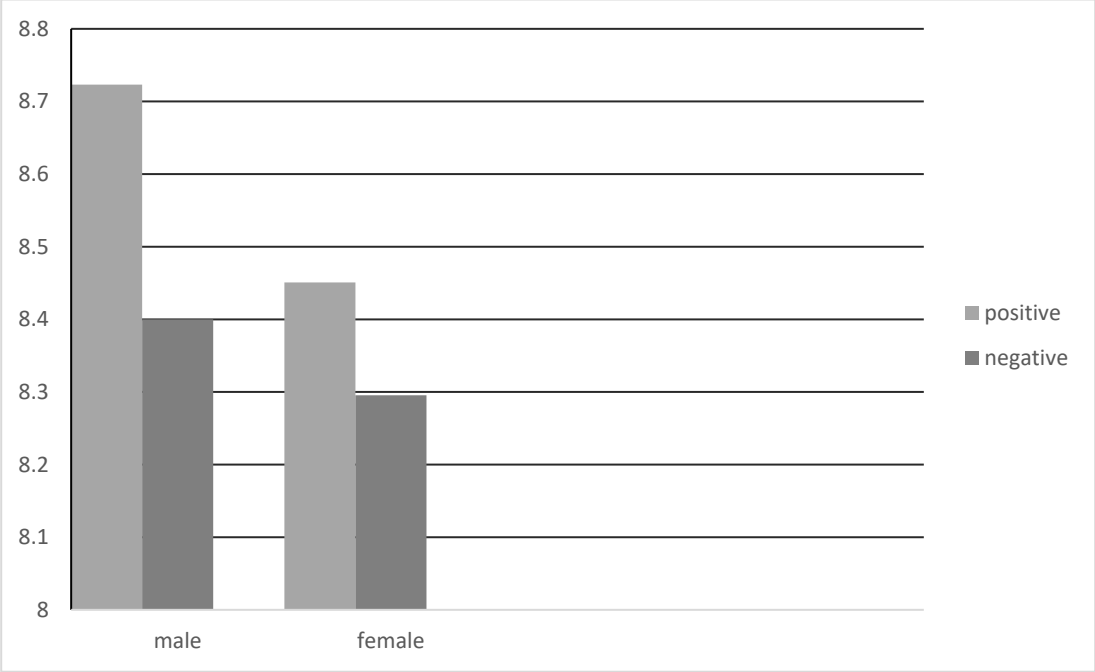

Figure (3-2) Distribution of serum Interleukin -6 Levels (ng/ml) According to Gender

As demonstrated above we noticed clear diversity regarding results extracted from studies of various world regions and upon different periods of time concerning evaluating circulatory levels of interleukin -6 in *H. pylori* patients, this controversial results may be attributed to (site of infection or infected organ) and/or the phase of infection. Since this bacterium has the ability to cause various kinds of infections ranging from gastritis to gastric cancer (Siavoshi *et al* ., 2005). Besides its infection either be acute or chronic one.

The opinion that support the idea of that circulating levels IL-6 does not affected by *H. pylori* infection tends to attribute it to that *H. pylori* inflammation is limited to and concentrated on mucosal level (Xing *et al* ., 1998; Bayraktaroglu˘ *et al*., 2004). Supporting those Yamaoka *et al* (2001) who showed that levels of mucosal IL-6 were elevated in patients with gastric cancer caused by *H. pylori* infection

Further studies are recommended to simultaneously evaluate of this cytokine at both circulating as well as mucosal levels and correlate them with site and the phase of infection

References

1 Afsharpooyan, S. and T. Mohammadian (2019). Seroepidemiology study of *Helicobacterpylori* infection and its effect on cardiovascular diseases in Karaj, Iran. Monoclonal Antibodies in Immunodiagnosis and Immunotherapy, 38(6): 277-281

2 Al-Karawi, A. S., Rasool, K. H., Atoom, A. M., & Kadhim, A. S. (2023). Correlation between H. pylori infection and serum levels of inflammatory markers: A retrospective study. Al-Salam Journal for Medical Science, 2(2), 20-4.

3 Bakhti, S. Z., Latifi-Navid, S., & Safaralizadeh, R. (2020). Helicobacter pylori-related risk predictors of gastric cancer: The latest models, challenges, and future prospects. *Cancer Medicine, 9*(13), 4808–4822. https://doi.org/10.1002/cam4.3068

4 Bayraktaroğlu, T., Aras, A. Ş., Aydemir, S., Davutoğlu, C., Üstündağ, Y., Atmaca, H., & Borazan, A. (2004). Serum levels of tumor necrosis factor-α, interleukin-6 and interleukin-8 are not increased in dyspeptic patients with Helicobacter pylori-associated gastritis. *Mediators of Inflammation, 13*(1), 25–28. https://doi.org/10.1080/09629350410001664789

5 Begolli-Stavileci, G., Begolli, G., & Begolli, L. (2020). Interleukin-6, tumor necrosis factor-α, and high-sensitivity c-reactive protein in diabetic patients with helicobacter pylori in Kosovo.

Open Access Macedonian Journal of Medical Sciences, *8*(B), 172–175. https://doi.org/10.3889/OAMJMS.2020.3199

6 Bucci, P., Barbaglia, Y., Tedeschi, F., & Zalazar, F. (2023). Helicobacter pylori infection: A balance between bacteria and host. *Revista Argentina de Microbiologia*, *55*(1), 60–67. https://doi.org/10.1016/j.ram.2022.04.003

7 Chen, S. T., Ni, Y. H., Li, C. C., & Liu, S. H. (2018). Hepcidin correlates with interleukin-1β and interleukin-6 but not iron deficiency in children with Helicobacter pylori infection. *Pediatrics and Neonatology*, *59*(6), 611–617. https://doi.org/10.1016/j.pedneo.2018.02.007

8 Crowe, S. (2019).*Helicobacter pylori* Infection. English journal of Medicine 380(12) : 1158-1165

9 Nakagawa, H., Tamura, T., Mitsuda, Y., Goto, Y., Kamiya, Y., Kondo, T., Wakai, K., & Hamajima, N. (2013). Significant association between serum interleukin-6 and helicobacter pylori antibody levels among h. pylori -positive japanese adults. *Mediators of Inflammation*, *2013*. https://doi.org/10.1155/2013/142358

10 Omar, S., Hassan, M., & Hasan, B. (2019). Impact of Serum Interleukin 6 among Helicobacter Pylori-Positive Adult Patients in Relation to upper gastrointestinal endoscopy findings. *Suez Canal University Medical Journal*, *22*(2), 117–121. https://doi.org/10.21608/scumj.2019.94803

11 Rasool, K. H., Mahmood Alubadi, A. E., & Al-Bayati, I. F. I. (2022). The role of Serum Interleukin-4 and Interleukin-6 in Helicobacter pylori-infected patients. *Microbial Pathogenesis*, *162*(August 2021), 105362. https://doi.org/10.1016/j.micpath.2021.105362

12 Santos, M. P., Pereira, J. N., Delabio, R. W., Smith, M. A. C., Payão, S. L. M., Carneiro, L. C., ... & Rasmussen, L. T. (2021). Increased expression of interleukin-6 gene in gastritis and gastric cancer. Brazilian Journal of Medical and Biological Research, 54, e10687

13 .Siregar, G. A., Salim, S., & Sitepu, R. R. (2014). Comparison of IL-6, IL-8 Concentrations in H. pylori- and non-H. pylori-associated Gastritis. *The Indonesian Biomedical Journal*, *6*(3), 163. https://doi.org/10.18585/inabj.v6i3.29

14 Siavoshi, F., Malekzadeh, R., Danesh-mand M. et al. (2005). Helicobacter pylori endemic and gastric disease. Dig Dis Sci. 50:2075-80

15 Wang, X., Li, J., Liu, W., Zhang, X., & Xue, L. (2021). The diagnostic value of interleukin 6 as a biomarker for gastric cancer A meta-analysis and systematic review. *Medicine (United States)*, *100*(47). https://doi.org/10.1097/MD.0000000000027945

16 Xing, Z., Gauldie, J., Cox, G., Baumann, H., Jordana, M., Lei, X. F., & Achong, M. K. (1998). IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *Journal of Clinical Investigation*, *101*(2), 311–320. https://doi.org/10.1172/JCI1368

17 Xu, S., Wu, X., Zhang, X., Chen, C., Chen, H.,and She, S (2020). CagA orchestrates eEF1A1 and PKCδto induce interleukin-6 expression in Helicobacter pylori-infected gastric epithelial cells. *Mediators of Inflammation*, *101*(6), 525. https://doi.org/10.1186/s13099-020-00368-3

18 Yamaoka ,Y., Kodama, T., Kita, M., Imanishi. J, Kashima K., Graham, D. (2001). Relation between cytokines and Helicobacter pylori in gastric cancer. Helicobacter .6: 116-24

19 Yu, B., Xiang, L., Peppelenbosch, M. P., & Fuhler, G. M. (2023). Overlapping cytokines in H. pylori infection and gastric cancer: A tandem meta-analysis. Frontiers in Immunology, 14, 1125658.

20 Zumkeller, N., Koenig, W., Hoffmann, M. M., Kolb, H., Brenner, H., & Rothenbacher, D. (2005). Helicobacter pylori seropositive subjects do not show a pronounced systemic inflammatory response even in the presence of the interleukin-1 receptor antagonist gene polymorphism. *Epidemiology and Infection*, *133*(3), 569–572. https://doi.org/10.1017/S095026880400319X