Iraqi Journal of Biotechnology, 2023, Vol. 22, No. 1, 25-30

Impact of *Helicobacter pylori* in some Blood Parameters Change of Iraqi Patients with Gastritis Disease

[1]Hussein S. Qasim, [2] Rana S. Aboud

[1]Department of Biology, College of Sciences, University of Baghdad
[2]Department of Medical Laboratory Techniques, Al-Esraa University College

Received: June 15, 2022/ Accepted: August 17, 2022 / Published: June 4, 2023

Abstract: In order to find the relationship between *Helicobacter pylori* infection and hematological disease are disorders which primarily affect the blood and blood-forming organs. One hundred and three blood samples were taken for people aged (20-68) years for the period from 10/1/2021 to 1/3/2022, divided into three groups. The first group included 44-person *H. pylori*-infected with symptoms of infection, the second group had 19-person *H. pylori*-infected but without symptoms, and the third group included 40 people without *H. pylori* infection. All studied groups were carried out to measure anti-IgG Ab, Vac A and Ferritin by Enzyme Linked Immunosorbent Assay (ELISA) technique. The statistical analysis indicates a non-significant difference in Vac A ($p>0.05$) but a significant difference in IgG and ferritin ($p < 0.05$) in the positive group. It was concluded *H .pylori* infection may contribute to hematological disease.

Keywords: IgG;Ferritin ;Vac A.**; *Helicobacter pylori***

Corresponding author: (Email: hussein.salmaan449@gmail.com).

Introduction

The bacterial infection *H. pylori* is the most common in humans, infecting around half of the world's population. Despite the fact that the majority of people infected with *H. pylori* remain asymptomatic for the rest of their lives, they all develop chronic inflammation (1). Peptic ulcer disease affects about 10% of infected persons, stomach adenocarcinoma affects 1–3% of infected people, and mucosa-associated lymphoid tissue (MALT) affects less than 0.1 percent of infected people (2). The presence, severity, and types of cytotoxin-associated gene A (CagA) type IV secretion system (CagL polymorphism) responsible for its hybridization into host cells, genetic markers of vacuolating cytotoxin A (vacA, s1/i1/m1 type), and expression intensity of blood type antigen binding adhesion (BabA) are all linked to the development of cytotoxin-associated gene A (Cag (BabA, low-producer or chimeric with BabB) (3). CagA, VacA, UreaB, OipA, Heat shock protein B, Flagellar proteins, and others are possible components of vaccines or ELISA systems. During *H. pylori* colonization of the human stomach, these antigens trigger humoral and cell-mediated immune responses, resulting in elevated serum immunoglobulin titers, particularly of the IgG class (4).

Vacuolating cytotoxin A is one of the most well-studied toxins generated by *H. pylori* (VacA).

Infection with *H. pylori* strains that carry the toxigenic allelic s1 type of

VacA has been linked to a higher incidence of peptic ulcers and stomach cancer. VacA is also required for *H. pylori* colonization in humans (5). The bacteria have been classified as a class I carcinogen based on epidemiological evidence since it is the strongest known risk factor for the development of severe gastrointestinal disorders (6). A complicated interplay between bacterial virulence factors, host, and environmental variables is mediated by *H. pylori* pathogenesis and illness consequences (7).

The *H. pylori* infection is usually asymptomatic, although it can cause gastritis (stomach inflammation) or ulcers in the stomach or the first section of the small intestine. In less than 20% of cases, the infection has also been linked to the development of some malignancies (8).

As a result of chronic gastritis, which produces stomach hypochlorhydria, *H. pylori* infection causes anemia through lowering iron absorption, preventing the conversion of ferric to ferrous iron in the food (9). Patients were not followed up with after finishing oral iron therapy, and IDA relapse after *H. pylori* eradication was not investigated, so it was impossible to ascertain whether *H. pylori* infection was the cause of IDA. The majority of the intervention trials took performed in areas where both IDA and *H. pylori* infection are frequent, and the etiology of IDA is unknown (malnutrition, vitamin deficiencies, chronic parasitic infections, malaria). Only a few uncontrolled intervention studies in western nations have indicated anemia recovery following H. pylori removal (10). The goal of this study was to see what influence Helicobacter pylori infection had in hematological illness.

Materials and methods

Studied subject samples

During the period from October 2021 to March 2022, One hundred and three patients with gastritis disease from AL-Kadhimya Teaching Hospital in Iraq were enrolled in the study. The patients' ages ranged from 20 to 68 years old from both gender. The total number of patients was divided into three groups: The first group included of 44 patients who tested positive for *H. pylori*. Group two: As a control group, 40 apparently healthy people were included. As an asymptomatic group, 19 subjects with *H. pylori* without symptoms were included.

Blood samples collection

Blood samples (5 mL) were taken and placed in a vacuum gel plain tube, which was left at room temperature until the coagulant formed. The materials were then centrifuged for 5 minutes at 3000 rpm. Eppendorf tubes were used to separate the serum samples. The serum samples were kept at -20 C until the immunological assays were completed.

Estimation of anti-*Helicobacter pylori IgG,* VacA Ab and ferritin level

All the studied patient group, asymptomatic group and healthy individuals were submitted to estimate the level of anti-*H.pylori* Ab IgG (Demeditec / Germany), ferritin level (Demeditec / Germany) , anti Vac A (Shanghai YL Biotec / China), by using enzyme-linked immunosorbent-assay(ELISA) technique according to the protocol of the kit as per method (11,12). All tests were carried out on the basis of kit.

Statistical analysis

To detect the effect of differential factors on study parameters, the statistical analysis system-SAS (2012) software was used. The Chi-square test was used to

compare percentages in this investigation (0.05 and 0.01 likelihood).

To compare the means statistically, the LSD test was used. In this study, the Chi-square test was utilized to compare percentages (0.05 and 0.01 likelihood). The students' test was employed, as well as statistical comparisons between groups (Analysis of variation-ANOVA) (13).

Results and discussion

According to the results in table (1). Level of IgG Ab revealed high significant difference in positive group (172.94 ±13.14 U/ml) compared with negative group (56.67 ±12.26) and asymptomatic group (114.05 ±20.95 U/ml). While, high significant (P<0.01) decrease was found in negative group as compared with asymptomatic group.

Table (1): Mean levels of anti IgG Antibody in sera of studied group.

Group	Mean ± SE
	IgG (U/ml)
Positive	172.94 ±13.14 a
Negative	56.67 ±12.26 c
Asymptomatic	114.05 ±20.95 b
LSD value	44.773 **
P-value	0.0001

• Non-significant differences (P>0.05) are shown by means with similar letters in the same column.

• Significant differences (P<0.01) are shown by means with different letters in the same column.

The result of the present study coincide with (14) who demonstrated against the control group (4.48_+0.61 U/ml), there was a substantial increase (p>0.05) in *H. pylori* IgG concentration (6.30 +0.99 U/ml) and the prevalence of anti *H. pylori* antibodies IgG was 20% (4/20). Between the test and control groups studied, there was a significant difference (p>0.01).

The result of the present study showed coincide with syudy done by (15) who showed in these groupings, there was a significant difference in *H. pylori* exposure between cases and controls based on IgG tests: P=0.004 for age under 30 years old, females (P=0.047 for men and P=0.014 for women), urban residency (P=0.002), and academic degree (P=0.013). Antibodies with a high percentage indicate the presence of an infection inside the body, so their percentage increases in the blood of infected people and decreases in the blood of those who are not infected.

The most common test for *H. pylori* infection is a serological blood test to determine IgG levels. The geographical variety of *H. pylori* bacteria has a big impact on this test. In patients with a low frequency of *H. pylori* infection, this test has a high false positive rate, hence it must be verified using other methods (16). Antigen testing for *H. pylori* were able to distinguish between individuals who were actively infected and those who had been treated, as evidenced by the findings of numerous additional investigations (17).Figure (1) show the levels of and VacA in different groups of the study. There were non-significant

(P>0.05) differences in levels of VacA among the different groups.

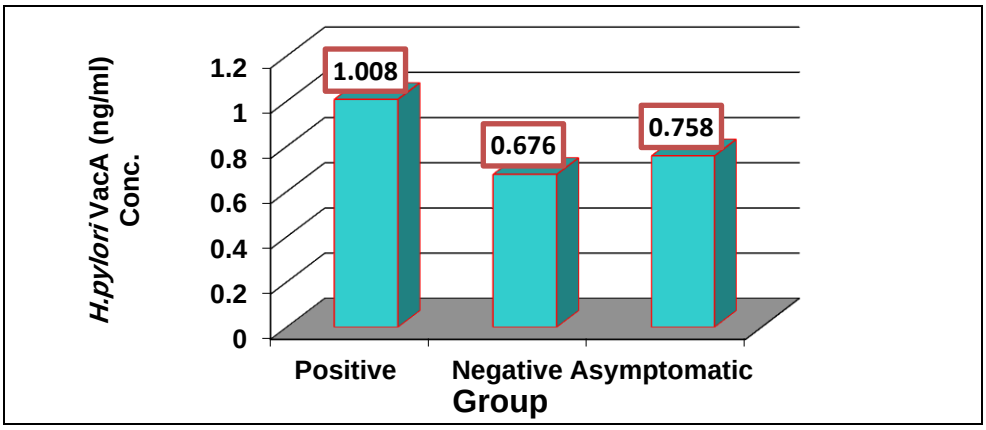

Figure (1): Mean level of VacA Ab sera of studied group.

The result of the present study showed not coincide with other study by (18) revealed that vacA were significantly associated with gastric erosion (P = 0.013) and ulceration (P = 0.006).

VacA, a vacuolating cytotoxin, has been associated to the progression of gastroduodenal disease. An auto transporter secretes a pore-forming toxin produced by the gene. Binding to the lipid sphingomyelin receptor in eukaryotic cells causes it to be toxic. The gene specifically targets mitochondria, causing death and the creation of massive intracellular vacuoles. Signal, intermediate, middle, and deletion areas exist in VacA polymorphisms. The vacA s1/m1 alleles are the most harmful of all the allelic combinations, whereas the s1/m2, s2/m1, and s2/m2 genotypes are quite harmless (19).Cytotoxin-associated gene A (Caga) and vacuolating cytotoxin A (VacA) (CagA)were discovered to have a two- to four-fold greater risk of gastric cancer (20). Levels of ferritin in different studied groups are shown in table (2) Significant (P<0.05) differences were found in level of ferritin in positive group (133.25 ±14.32 ng/ml) as compared with negative group (147.64 ±16.67 ng/ml) and asymptomatic group (108.19 ±24.24 ng/ml).

Table (2): Levels of ferritin in different studied groups

Group	Mean ± SE
	Ferritin (ng/ml)
Positive	133.25 ±14.32 ab
Negative	147.64 ±16.67 a
Asymptomatic	108.19 ±24.24 b
LSD value	33.750 *
P-value	0.04088
Means in the same column with different letters differed considerably. NS: Non-Significant (P<0.05).	

Another study showed there was a substantial difference in serum iron and serum ferritin between *H. pylori* infected participants and healthy ones (p-value less than 0.05). Serum iron and ferritin concentrations in cases were lower than in control (6218.1, 91.316.7) and (36.816.5, 6416.4), respectively. Total iron binding capacity was unaltered in all instances and controls (21). Another study showed there was no link discovered between *H. pylori* infection and serum iron or ferritin levels. The mean corpuscular volumes of the *H. pylori* positive and negative groups were 91.17, 3.94 fl and 91.17, 4.09 fl, respectively (P =.986) (22,23,24).

Ferritin is yet another crucial protein in the homeostasis of iron. It has 88 molecules and 88 different functions, including as storing iron and acting as an antioxidant. 89 This gene's overexpression has been observed in a number of cancers, and in a few of them, a tumor marker has been developed. The 70-year-old protein ferritin has undergone intensive study, and daily 310-point discoveries of its numerous bioactivity properties have been made. The constituents of this protein play additional, unrelated functions (25,26).

Conclusion

The present study shows that infection with *Helicobacter pylori* may pay in hematological disorders. Anti-*H. pylori* IgG concentration was higher in positive group with *H. pylori* infection compared to negative group with significance difference. The level of ferritin was significantly greater in the positive group with *H. pylori* infection compared to the negative group.

References

1. Peek, Jr.; R. M. (2002). New insights into microbially initiated gastric malignancies: beyond the usualsuspects. Gastroenterology, 123(5), 1739-1740.
2. Peek, Jr.; R. M. and Crabtree, J. E. (2006). *Helicobacter* infection and gastric neoplasia. The Journal of Pathology*: A* Journal of the Pathological Society of Great Britain and Ireland, 208(2), 233-248.
3. Chang, W. L.; Yeh, Y. C. and Sheu, B. S. (2018). The impacts of *H. pylori* virulence factors on the development of gastroduodenal diseases. Journal of Biomedical Science, 25(1), 1-9.
4. Kronsteiner, B.; Bassaganya-Riera, J.; Philipson, C.; Viladomiu, M.; Carbo, A.; Abedi, V. and Hontecillas, R. (2016). Systems-wide analyses of mucosal immune responses to *Helicobacter pylori* at the interface between pathogenicity and symbiosis. Gut microbes, *7*(1), 3-21.
5. Ansari, S. and Yamaoka, Y. (2020). Role of vacuolating cytotoxin A in *Helicobacter pylori* infection and its impact on gastric pathogenesis. Expert Review of Anti-infective Therapy, 18(10), 987-996.
6. Hatakeyama, M. (2017). Structure and function of *Helicobacter pylori* CagA, the first-identified bacterial protein involved in human cancer. The Proceedings of the Japan Academy, Series B.93(4): 196-219.
7. Kao, C. Y.; Sheu, B. S. and Wu, J. J. (2016). *Helicobacter pylori* infection: An overview of bacterial virulence factors and pathogenesis. Biomedical Journal, 39(1), 14-23.
8. Blaser, M. J. (2006). Who are we? Indigenous microbes and the ecology of human diseases. EMBO Reports, 7(10), 956-960.
9. Campuzano-Maya, G. (2016). Helicobacter pylori and Hematologic Diseases. World Journal Gasteroenterology, 2016, 33-9.
10. Hershko, C.; Hoffbrand, A. V.; Keret, D.; Souroujon, M.; Maschler, I.; Monselise, Y. and Lahad, A. (2005). Role of autoimmune gastritis, *Helicobacter pylori* and celiac disease in refractory or unexplained iron deficiency anemia. Haematologica, *90*(5), 585-595.

11. Karvar, S.; Karch, H.; Frosch, M.; Burghardt, W. and Gross, U. W. E. (1997). Use of serum-specific immunoglobulins A and G for detection of Helicobacter pylori infection in patients with chronic gastritis by immunoblot analysis. Journal of clinical Microbiology.1997; 35(12), 3058-3061.
12. Ng.; R. H.; Brown, B. A. and Valdes Jr.;R. (1983). Three commercial methods for serum ferritin compared and the high-dose" hook effect" eliminated. Clinical Chemistry, 29(6), 1109-1113..
13. SAS, J. (2012). Statistical Analysis System, v. 10.0. 2. *Cary, North Carolina. USA*.
14. Abood, R. S.; Abood, A. S. and Saleh, G. M. (2016). Detection of anti. *Helicobacter pylori*. World Journal of Bioscience, 4, 123-126.
15. Sayar, R.; Shirvani, J. S.; Hajian–Tilaki, K.; Vosough, Z. and Ranaei, M. (2019). The negative association between inflammatory bowel disease and *Helicobacter pylori* seropositivity. Caspian Journal of Internal Medicine, 10(2), 217-222.
16. Rana, R.; Wang, S. L.; Li, J.; Wang, Y. X.; Rao, Q. W. and Yang, C. Q. (2017). *Helicobacter pylori* infection: A recent approach to diagnosis and Management. Journal Biomedicine, 2(1), 45-56.
17. Gisbert, J. P. and Pajares, J. M. (2002). Stool antigen test for initial *Helicobacter pylori* diagnosis and for confirmation of eradication after therapy. Medicina Clinica, *118*(11), 401-404.
18. Ali, S. S.; Abd Elnabi, M. K.; Alkherkhisy, M. M.; Hasan, A.; Li, F.; Khalil, M.; and El-Zawawy, N. (2022). Exploring the potential of Cinnamomum zeylanicum oil against drug resistant Helicobacter pylori-producing cytotoxic genes. Journal of Applied Biomedicine, 20(1) 1-7.
19. Idowu, A.; Mzukwa, A.; Harrison, U.; Palamides, P.; Haas, R.; Mbao, M. and Njom, H. (2019). Detection of Helicobacter pylori and its virulence genes (cag A, dup A, and vac A) among patients with gastroduodenal diseases in Chris Hani Baragwanath Academic Hospital, South Africa. BMC Gastroenterology, 19(1), 1-10.
20. Epplein, M.; Zheng, W.; Xiang, Y. B.; Peek, R. M.; Li, H.; Correa, P. and Shu, X. O. (2012). Prospective study of *Helicobacter pylori* biomarkers for gastric cancer risk among Chinese men. Cancer Epidemiology and Prevention Biomarkers, 2*1*(12), 2185-2192.
21. Elhakeem, A. and Mohammed, A. A. (2019). *Association between Helicobacter pylori* Infection and Iron Deficiency in Sudanese Population (Doctoral dissertation, Sudan University of Science & Technology).
22. Hou, B.; Zhang, M.; Liu, M.; Dai, W.; Lin, Y.; Li, Y. and Wang, G. (2019). Association of active *Helicobacter pylori* infection and anemia in elderly males. BMC infectious Diseases, *19*(1), 1-9.
23. Lafta, S. A., & ALFaisal, A. H. M. (2017). Association of Helicobacter pylori infection and gastric cancer. Iraqi Journal of Biotechnology, 16(1):1-7.
24. Amer, A. N., Hassan, J. G., & Al-Saimary, I. E. (2017). The possible cancer risk factor of *Helicobacter pylori* infections in immunocompromised children. Iraqi Journal of Biotechnology, 16(3):12-17.
25. ALMohaidiP, A. M. S., ZyaraP, A. M., AlrumaidhP, S. Z. F., & AbassP, M. (2015). Association among family history and some microbial infectious (Helicobacter pylori IgG and Hepatitis B and C Virus) as Risk Factors for Atherosclerosis in Iraqi Patients. Iraqi Journal of Biotechnology, 14(2), 84-92.
26. Asl, D. H.; Farivar, T. N.; Rahmani, B.; Hajmanoochehri, F.,;Razavi, A. N. E.; Jahanbin, B. and Peymani, A. (2019). The role of transferrin receptor in the *Helicobacter pylori* pathogenesis; L-ferritin as a novel marker for intestinal metaplasia. Microbial Pathogenesis, *126*, 157-164.