Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

The Role of Demographic Factors and IL-32 Levels in Helicobacter Pylori-Associated Diseases

Hussain Abdulhassan Owaid, [1]*, Saad Abdulaziz Atyah Ali, [1]Faez Khalaf Abdulmuhsen, [1]

1 Department of Microbiology, College of Medicine, University of Thi-Qar, 64001, Iraq.

* Corresponding author: Hussain-a@utq.edu.iq

Mobile: +9647732197445

Abstract: Increased production of IL-32 proinflammatory cytokine, has been demonstrated in *Helicobacter pylori*-associated gastric mucosal inflammation. IL-32, a newly characterized cytokine, is thought to be a key mediator in host responses to bacterial infections.

Background: *Helicobacter pylori* (*H.pylori*) was discovered in 1983 and has been identified as the main pathogenic factor for gastric and duodenal peptic ulcers and gastric cancer which is the third most common cause of cancer-related death in the world The bacterium is a micro-aerophilic, curved, gram-negative bacteria, infection causes chronic gastritis. IL32, proinflammatory cytokine secreted by NK cell, macrophage, endothelial cell and monocyte, the studies showed that increase expression of IL-32 is elevated in human gastritis and gastric cancer tissues.

Aim of study: The study aimed to determined the relation of some demographic parameters to gastric disorders due to *H.pylori* and assessment of IL-32 concentration.

Material and methods: The study design is a case - control . was performed in Thi-Qar Province, Iraq, from Nov-2023 to Jan-2024. The diseases was diagnosed by physician and UBT. Serum samples were taken from 180 participants in various age included (120 cases and 60 controls) . The ELISA technique used to detects level IL-32 **.**

Results: The present study showed a significant difference at p. value <0.05, between patients and control group according to sex, the highest percentage of infection in female were 69.17%, also, a significant difference was recorded according to age groups, where the highest percent record in second age group(16-30) 34.17%. With regard residency the study investigated a significant difference was urban scored 70.83% of patients. IL-32 level was showed increased in the patients compared to control group, at p.value <0.05.

Conclusion: *H. Pylori* infection generally associated with demographic data such as sex and age. High increase in the level IL-32 indicated that the gastric *H. pylori* diseases induced strong systemic immune response.

Keyword: Gastric disorders, (*Helicobacter pylori),* IL-32(interlukin-32)

Introduction: *Helicobacter pylori* is a gram-negative, spiral-shaped, microaerophilic bacterial pathogen more than >50% of the world is infection population [1]. In 10 to 20% of infected individuals, the *H. pylori*-induced chronic gastric inflammation progresses to peptic ulcer, gastric cancer, or gastric mucosa-associated lymphoid tissue lymphoma[2]. The pathophysiology of *H.pylori* infection is dependent on complex bacterial virulence mechanisms and their interaction with the host immune system and environmental factors, resulting in distinct gastritis phenotypes that determine possible progression to different gastro-intestinal pathologies [3]. The causative role of the bacterium infection in gastric cancer development presents the opportunity for preventive screen-and-treat strategies [4]

Cytokines are powerful soluble immune mediators that can serve as target indicators for inflammation , however, the accurately measuring them in human samples was proven to be difficult and the objective was to evaluate the dependability of a pro- and anti-inflammatory cytokine [5, 6]. The involvement of cytokines in the development of chronic inflammation, its play a significant role in controlling the features of both the natural and specific inflammatory cellular response in the mucosal lining, they also contribute to the disruptions in stomach physiological responses that are linked to infection, play an important role in controlling the features of both the natural and specific cellular response in the mucosal lining, they also contribute to the disruptions in normal stomach physiological responses that are linked to infection[7]. IL32, is a proinflamatory cytokine secreted by NK cell, macrophage, endothelial cell and monocyte, the studies showed that increase expression of IL-32 is elevated in human gastritis and gastric cancer tissues[8]. The elevated concentration of IL-32 attributed to the ability of *H.pylori* to activate certain cellular pathways, this activation leads to the production of IL-32, which in turn contributes to the development of gastric diseases[8]. In simpler terms, *H.pylori* infection stimulates the production of IL-32 through the activation of NF-kB in a cag-dependent manner, furthermore, IL-32 enhances the NF-kB pathway and promotes the production of other inflammatory cytokines such as IL-12[9]. The study aimed for explain *H.pylori* relation with some demoghraphic and immunological parameterIL_32.

Material and methods : The study aimed to determined the relation of some demographic parameters to gastric disorders due to *H.pylori* and assessment of IL-32 concentration. this study was a case-control study. Patient information include name, age, residence, job, chronic disease and gastric disease in family, the study was done in center of gastro-intestinal disease and laboratory of microbiology department, College of medicine- University of Thi-Qar, in the period from November, 2023 to January,2024.

The case group included (120) patients who were diagnosed with gastric disorders due to H. *Pylori* by the consultant, consisting of (37) males and (83) females, with an age ranging between (3-71) years. The control group included (60) apparently healthy persons consisting of (43) males and (17) females, with an age ranging between (8-52) years.

Blood sample collection : Five milliliters of blood samples were obtained from patients and healthy individuals using a sterile syringe. The samples were placed in gel tubes and centrifuged at 4000 rpm for 5 minutes to separate the serum. The serum was separated into four Eppendorf tubes and stored at -20 ºC. 3.3.2.

Serological Test : Detection of *H. pylori* Ab was done according to manufacture instruction (Ione-China).Feces was collected by sterile container , the *H.pylori* Ag was detected based on manufacture (Ione-China).

Procedure of UBT :A Kit was used to performed UBT(IR-force- China) then the result was read by IR-force spectrometer (IR-Force – China) according mennwal with Kit.

The assay procedure of IL-32 as following :The IL-32 level was determine in serum of paitents and control by using ELISA Kit all steps od detection was done according to mannwal instruction and the microtiter plate was read by microplate reader (BioTec-USA)

Ethical Consideration. :The presented study has been permitted by in Thi-Qar Health Directorate (No.2023208 in 23/10/2023). The patient consent was taken in hospital and clinics.

Statistical analysis :The data of the current study were statistically analyzed by using SPSS (statistical package of social science) version 26, based on both crosstabulation, chi-square and sample T test.

Results:

Descriptive statistical of demographic data for patients with gastric disorder and Control Group1.

The present study was showed a significant difference at p. value <0.05, between patients and control group according to sex, the highest percentage of infection in female were 69.17%, also, a significant difference was recorded according to age groups, where the highest percent record in second age group 34.17%. With regard residency the study revealed a significant difference was urban scored 70.83% of patients. Housewife is the most infected group (36.67) when compared with other occupation with singnificant difference p.value <0.001. Based on the present of chronic disease also, the study showed a significant difference, where the most patients had no chronic disease 80%. According to the family history for paitents , the present study showed the highest percentage of patients had not family history for disease 84.17%, at p. value < 0.05, as in the Table 1.

Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

Table (1): Demographic characteristics of gastric disorders patients and control group

Variable	Categories	Patients		Control		Calx^2 & P. Value
		No.	%	No.	%	
Sex	Male	37	30.83	42	70.00	30.4
	Female	83	69.17	18	30.00	<0.001
Age / Years	1 – 15	17	14.17	6	10.00	20.78
	16 – 30	41	34.17	19	31.67	<0.001
	31 – 45	34	28.33	32	53.33	
	46 – 60	22	18.33	3	5.00	
	≥ 61	6	5.00	0	0.00	
Age Mean ± S. D		34.2 ± 16.2		31.6 ± 10.6		0.198
Residency	Rural	35	29.17	8	13.33	7.71
	Urban	85	70.83	52	86.67	0.005
Jobs	Employed	38	31.67	31	51.67	23.76
	Housewife	44	36.67	7	11.67	<0.001
	Worker	16	13.33	8	13.33	
	Student	18	15.00	14	23.33	
	Child	4	3.33	0	0.00	
Chronic Disease	Non	96	80.00	60	100	22.22
	BP + DM	8	6.67	0	0.00	<0.001
	DM	12	10.00	0	0.00	
	BP	4	3.33	0	0.00	
Family History Of GD	Presence	19	15.83	60	100	106.0
	Absence	101	84.17	0	0.00	<0.001

Detection of *H.pylori* infection by using different tests

The current results were noted a non-significant difference at p. value <0.05, between results of *H. pylori* Ab, Ag, and urea breath test in the gastric disorder patients, which showed 58.33% positive test for *H.pylori* Ab, 59.17% for both *H. pylori* Ag and urea breath test as showed in table 2

Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

Table (3): Detection of *H.pylori* infection by using different tests

Variable	Categories	Patients		Calx^2 & P. Value
		No.	%	
H. Pylori Ab	Positive	70	58.33	2.560
	Negative	50	41.67	0.110
H. Pylori Ag	Positive	71	59.17	3.240
	Negative	49	40.83	0.072
Urea Breath	Negative	71	59.17	3.240
	Positive			0.072

Evaluation IL-32 level in gastric disorders patients and control group

The current results were showed that the concentration of IL-32 increased significantly in the patients compared with control group where records at p. value <0.001, as in Table (4)

Table (4): Evaluation of IL-32 level in GD Patients and Control Group

Immune Parameters	Patients	Control	P. Value
	Mean ± S. D		
IL-32	73.4 ± 42.8	29.6 ± 9.40	<0.001

Evaluation of IL-32 level in GD Patients According to Sex

The present results revealed that the concentration of IL-32 was not scored significant difference in gastric disorder patients according to sex where records at p. value 0.861, as in Table (5)

Table (5): Evaluation of IL-32 level in GD Patients According to Sex

Immune Parameters	Male	Female	P. Value
	Mean ± S. D		
IL-32	74.5 ± 48.0	72.9 ± 40.5	0.861

Discussion: The results showed that the percentage of females among patients was 69.17%, and this is consistent with many previous studies that indicated a higher incidence of this disease in females

compared to males, a study conducted in 2024 which agreement with current study included 120 patients, showed that 69.17% of patients were female, other study agreement with current study showed *H. Pylori* infection was higher in females than in men[10] [11]. A study conducted in KSA was in agreed with current study its revealed according to sex prevalence infection in females more than males[12]. Also another study showed *H. pylori* infection was higher in females than in male [11].The study was conducted in Tehran its inconsistent with this study its revealed no significant between sex [13]. On the other hand a study was in disagreement with current study that showed the percentage of male more than female [14].This difference in infection rates between both sexes may be due to hormonal and genetic factors, in addition to differences in lifestyles and health habits between males and females and the female more exposure to risk factors during prepearing food [15].

The results showed that the most affected age group was the second group(16-30) yrs, our results is agreed with a study conducted in China which revealed that the infection rate is higher in middle age groups[16]. A study was in consistent with current study was showed the age patients more than 36 years the infection increase in this age [11].While, the study conducted in 2016 disagreement with our study it revealed 45% of patient in the highest age group [17]. That may be explained by the fact that this age group is more susceptible to risk factors such as work, the lifestyle of this group is different from other ,as the young in more exposure to the contaminates during their working and contact with the many sources of food that prepare by food handlers in food fast which may increase of disease in this age group [18].

Whilest in the residency, the results showed that the disease is more prevalent in urban area compared to rural erea , which shown in other studies [19] [20] [21]. In the other hand the study conducted in western Australia which disagreed with this study was revealed the prevalence of infection in the rural community was 91%, compared urban area which revealed 60% [22].This is due to the association of urban areas with environmental contamination, crowded, lifestyle, psycholgic conditions economic state and more exposure to different source of food handling.

Regarding occupation, the infection rate among housewives was high in compared with the others. This may be related to the nature of the lifestyle and dietary habits of this group [23]. Other study was agreed with this study it showed the percentage of housewives in patients with positive *H.pylori* is very high compared with other jobs[21]. Large family size is, however generally accepted as being a risk factor for the *H. pylori* infection [24]. And small size family reduces the opportunity for transmission [25].The studies was conducted in the United States of America and Bangladesh, were revealed the crowding in the home was significantly associated with *H. pylori* infection[26].

This study showed the relationship between patients with chronic disease and the frequency of *H. pylori* infection in the current study showed 80% of patient have no chronic disease. There was study consistent with our study it showed the lower rate of *H. pylori* infection with chronic diseases persons [27, 28]. On the other hand , another study revealed that *H. pylori* infection associated patients with chronic disease [29]. The difference between current study and previous studies may due to sample size and the old age

lesser than younger this group was less suffering from chronic diseases like hypertention , 76.6% of patient there average was under 45year [30] .

This study was showed a non-significant difference in the positivity rates of *H. pylori* antibody (Ab), antigen (Ag), and urea breath test results among the gastric disorders patients. These findings are consistent with several studies that have reported similar non-significant differences in the diagnostic performance of these tests for the detection of *H.pylori* infection , a meta-analysis reviewed 59 studies and found of *H. pylori* Ab, Ag, and urea breath were 88%, 92%, and 95% with no significant differences between the tests[31]. Another study was conducted in Tehran, Iran consistent with our study was revealed no significant between positivity tests[32]. A study conducted in France was in agreed with current study it was revealed there is no statistically significant difference between the tests[33]. On the other hand, study conducted in Italy was revealed disagreed with this study was showed a significant difference between this tests[34]. The non-significant difference in the diagnostic methods may depened on size sample or based on the type of gastric disorder aligns with the established association between *H. pylori* infection and specific gastric pathologies.

The current study found that the concentration of IL-32 increased significantly in patients compared to the control group, and this is agreement with study in Japan showed high levels of this immune factors in many immune and inflammatory diseases, also a study showed the levels of IL-32 in human gastric tissues, were elevated in gastritis tissue compared to those in *H. pylori*-negative healthy[8]. Another study consistent with this study it revealed IL-32 level is increase in patients with *H.pylori* infection and its expression is regulated by proinflammatory stimuli, suggesting that IL-32 may play a role in the pathogenesis of *H. pylori*-related to gastritis by activation immune cells [35]. In other hand another study in china showed in disagreed with our study found no statistically significant associations the level of IL-32 between patient with gastric disorder and control group [36]. This increase can be explained by the fact that the IL-32 play an important role in the immune and inflammatory response, and the increase may be an indication of activation of the immune system in these patient

The current study showed no difference in concentration in all of , IL-32 according to sex. This finding is in agreement with where proved there is no significant difference according sex among patient with gastritis disorder[37]. Also according to another study conducted in did not found differ between sex[38]. However this finding disagree where assumed the expression of IL-32 was decreased in female patients with gastric cancer[39].

Conclusion: *H. pylori* infection generally associated with demographic data such as sex and age. High in the level of IL-32 indicated the gastric *H.pylori* infections induced strong systemic immune response.

References:

1. Peleteiro, B., et al., *Prevalence of Helicobacter pylori infection worldwide: a systematic review of studies with national coverage.* Digestive diseases and sciences, 2014. **59**: p. 1698-1709.
2. Takeshima, E., et al., *Helicobacter pylori-induced interleukin-12 p40 expression.* Infection and immunity, 2009. **77**(4): p. 1337-1348.
3. Das, J.C. and N. Paul, *Epidemiology and pathophysiology of Helicobacter pylori infection in children.* The Indian Journal of Pediatrics, 2007. **74**(3): p. 287-290.
4. Ando, T., et al., *Causal role of Helicobacter pylori infection in gastric cancer.* World journal of gastroenterology: WJG, 2006. **12**(2): p. 181.
5. Koelman, L., et al., *Cytokines for evaluation of chronic inflammatory status in ageing research: reliability and phenotypic characterisation.* Immunity & Ageing, 2019. **16**: p. 1-12.
6. Romero-Adrián, T.B., et al., *Helicobacter pylori: bacterial factors and the role of cytokines in the immune response.* Current microbiology, 2010. **60**: p. 143-155.
7. Crabtree, J.E., *The role of cytokine in Helicobacter pylori infection.* Mucosal Immunology Update, 1994. **2**: p. 5-7.
8. Sakitani, K., et al., *Role of interleukin-32 in Helicobacter pylori-induced gastric inflammation.* Infection and immunity, 2012. **80**(11): p. 3795-3803.
9. Mohammed, K.I.A., et al., *Interleukin-32, interleukin-33 and acid phosphatase levels in patients with Helicobacter Pylori.*
10. Metwally, M.A.E.A., et al., *Relationship between Helicobacter Pylori Morphological Forms in Gastric Biopsy and Helicobacter Pylori Stool Antigen.* Benha Medical Journal, 2024. **41**(2): p. 56-65.
11. Hong, W., et al., *Prevalence of Helicobacter pylori infection in a third-tier Chinese city: relationship with gender, age, birth-year and survey years.* Microb Health Dis, 2019. **1**: p. e150.
12. Akeel, M., et al., *Prevalence and factors associated with H. pylori infection in Saudi patients with dyspepsia.* Electronic physician, 2018. **10**(9): p. 7279.
13. Ashtari, S., et al., *The prevalence of Helicobacter pylori is decreasing in Iranian patients.* Gastroenterology and hepatology from bed to bench, 2015. **8**(Suppl1): p. S23.
14. Xia, H., et al., *Demographic and endoscopic characteristics of patients with Helicobacter pylori positive and negative peptic ulcer disease.* Gastric Cancer, 2000. **2**: p. 0.7.
15. Leung, K.K., N. Jawaid, and N. Bollegala, *Gender differences in gastroenterology and hepatology authorship and editorial boards.* Gastrointestinal Endoscopy, 2021. **94**(4): p. 713-723.
16. De Filippis, A., et al., *Gastrointestinal disorders and metabolic syndrome: Dysbiosis as a key link and common bioactive dietary components useful for their treatment.* International Journal of Molecular Sciences, 2020. **21**(14): p. 4929.
17. Breckan, R.K., et al., *The all-age prevalence of helicobacter pylori infection and potential transmission routes. a population-based study.* Helicobacter, 2016. **21**(6): p. 586-595.
18. Zhang, M., et al., *Dietary and lifestyle factors related to gastroesophageal reflux disease: a systematic review.* Therapeutics and Clinical Risk Management, 2021: p. 305-323.
19. Fitzenberger, J., et al., *Urban–rural differences of age-and species-specific campylobacteriosis incidence, Hesse, Germany, July 2005–June 2006.* Eurosurveillance, 2010. **15**(42): p. 19693.
20. Aguemon, B., et al., *Prevalence and risk-factors for Helicobacter pylori infection in urban and rural Beninese populations.* Clinical microbiology and infection, 2005. **11**(8): p. 611-617.

21. Brown, L.M., et al., *Helicobacter pylori infection in rural China: demographic, lifestyle and environmental factors.* International journal of epidemiology, 2002. **31**(3): p. 638-645.
22. Windsor, H.M., et al., *Prevalence of Helicobacter pylori in Indigenous Western Australians: comparison between urban and remote rural populations.* Medical journal of Australia, 2005. **182**(5): p. 210-213.
23. Johnson, C.H., et al., *An evaluation of the epidemiology, management and outcomes for perforated peptic ulcers across the North of England over 15 years: A retrospective cohort study.* International Journal of Surgery, 2019. **64**: p. 24-32.
24. Herbarth, O., et al., *Helicobacter pylori prevalences and risk factors among school beginners in a German urban center and its rural county.* Environmental health perspectives, 2001. **109**(6): p. 573-577.
25. Aguilar, G.R., G. Ayala, and G. Fierros-Zárate, *Helicobacter pylori: recent advances in the study of its pathogenicity and prevention.* Salud publica de Mexico, 2001. **43**: p. 237-247.
26. Alizadeh, A., et al., *Seroprevalence of Helicobacter pylori in Nahavand: a population-based study.* EMHJ-Eastern Mediterranean Health Journal, 15 (1), 129-135, 2009, 2009.
27. Zhou, X., J. Wu, and G. Zhang, *Association between Helicobacter pylori and asthma: a meta-analysis.* European journal of gastroenterology & hepatology, 2013. **25**(4): p. 460-468.
28. Xiong, X., et al., *Helicobacter pylori infection and the prevalence of hypertension in Chinese adults: The Dongfeng-Tongji cohort.* The Journal of Clinical Hypertension, 2020. **22**(8): p. 1389-1395.
29. Huang, M., et al., *Association between Helicobacter pylori infection and systemic arterial hypertension: a meta-analysis.* Arquivos Brasileiros de Cardiologia, 2021. **117**: p. 626-636.
30. Makola, D., D.A. Peura, and S.E. Crowe, *Helicobacter pylori infection and related gastrointestinal diseases.* Journal of clinical gastroenterology, 2007. **41**(6): p. 548-558.
31. Griñó, S.P., J. Such, JA Casellas, M. Niveiro, M. Andreu, J. Sáez, E. Griñó, JM Palazón, F. Carnicer, M. Pérez-Mateo, P, *Comparison of diagnostic methods for Helicobacter pylori infection in patients with upper gastrointestinal bleeding.* Scandinavian journal of gastroenterology, 2001. **36**(12): p. 1254-1258.
32. Pourakbari, B., et al., *Diagnosis of Helicobacter pylori infection by invasive and noninvasive tests.* Brazilian Journal of Microbiology, 2013. **44**: p. 795-798.
33. Monteiro, L., et al., *Diagnosis ofHelicobacter PyloriInfection: Noninvasive Methods Compared To Invasive Methods and Evaluation of Two New Tests.* Official journal of the American College of Gastroenterology| ACG, 2001. **96**(2): p. 353-358.
34. Cosgun, Y., et al., *Evaluation of invasive and noninvasive methods for the diagnosis of Helicobacter pylori infection.* Asian Pacific journal of cancer prevention: APJCP, 2016. **17**(12): p. 5265.
35. Peng, L.-s., et al., *Elevated interleukin-32 expression is associated with Helicobacter pylori-related gastritis.* PLoS One, 2014. **9**(3): p. e88270.
36. Song, X., et al., *Association between interleukin gene polymorphisms and susceptibility to gastric cancer in the Qinghai population.* Journal of International Medical Research, 2021. **49**(5): p. 03000605211004755.
37. Diakowska, D. and M. Krzystek-Korpacka, *Local and Systemic Interleukin-32 in Esophageal, Gastric, and Colorectal Cancers: Clinical and Diagnostic Significance.* Diagnostics (Basel), 2020. **10**(10).
38. Erturk, K., et al., *Clinical significance of serum interleukin-29, interleukin-32, and tumor necrosis factor alpha levels in patients with gastric cancer.* Tumour Biol, 2016. **37**(1): p. 405-12.
39. Liu, Q.-H., et al., *Clinical implications of interleukins-31, 32, and 33 in gastric cancer.* World Journal of Gastrointestinal Oncology, 2022. **14**(9): p. 1808.