Original Article

The role of TNF-α as a marker of apoptotic response in Iraqi patients infected with *Helicobacter pylori*

Shahad Ahmed Hussein [1,2,*], Zeana S. Al-Hindi [2]

[1] Department of Medical Microbiology, Hammurabi College of Medicine, University of Babylon, 51002, Hillah, Iraq.
[2] Department of Biology, College of Science for Women, University of Babylon, Babylon, Iraq.
[*] Corresponding author: shahad.hannon.gsci160@student.uobabylon.edu.iq.

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Abstract

Background: Tumor necrosis factor is one of the host genetic variables that influence susceptibility to illnesses associated with *Helicobacter pylori* infections. **Objectives:** Determination of apoptosis response in *H. Pylori* patients by an immunological marker TNF-α level. **Materials and Methods:** The study involved (110) of infected *H. pylori* and healthy from Nov, 2023 to Jan, 2024 from the Digestive center in Merjan Medical City. The levels of TNF-α were measured using the enzyme-linked immunosorbent assay. **Results:** The results showed the highest rate of infection in males (56.4%) compared to females (43.6 %). The incidence of *Helicobacter pylori* were distributed in different blood groups [B, O, A, and AB]. [O] Group had the highest incidence of rate (34.5%). According to the data, the percentage was lowest in rural areas (40.9%) and highest in urban (59. 1%). The present investigation showed, only female granulocytes demonstrated significant ($p \leq 0.05$) immune cell counts about (4.8086 ± 1.59457 pm/ml), whereas male total immune cell counts had significant as (7.9621 ± 2.60552pm/ml). Immunological tests for patients showed increased TNF-α concentration at varied age groups in comparison to the control group and the age group with the largest concentration of them (41.28529 ± 6.169497pg/ml). One-way ANOVA shows a non-significant difference under ($p \leq 0.05$). **Conclusion:** The present investigation pointed to the correlation subject between TNF-α and WBC, result found there is an inverse correlation between them in patients with the present relationship in control, which ensures an important of immune regulation. Those achieve the aim of our study as can help comprehend the process by which *Helicobacter pylori* cause cell death and how it relates to the pathophysiology of stomach illness.

Keyword: TNF-α, apoptotic response, *Helicobacter pylori*, ELISA

Introduction

Helicobacter pylori infection is still very common. In 2015, an estimate of 4.4 billion individuals worldwide is infected with *Helicobacter pylori*. Due to the spread of infection, over 80% of patients do not exhibit any symptoms, making this a sizable proportion that may not be statistically recorded [1]. Among the most widespread and persistent long-term bacterial illnesses over time associated with stomach sickness is *Helicobacter pylori*, which was first identified by Marshall and Warren in 1983 and is well-known for its rapid adaptation to the gastrointestinal environment [2]. *H. pylori* can create biofilms and transition from a spiral to a coccid form that may be viable but is not cultivable [3] . There is currently a lack of specific data and statistics in relation to the prevalence of the bacterium, based on the few research projects carried out in Iraq. According to studies done in Iraq, the prevalence of *H. pylori* varied from 11.3% to 71.3% [4]. Males were more likely than females to get infections

in both the teenage and adult populations. Given that stomach adenocarcinoma—the most fatal consequence of *Helicobacter pylori* infection-predominates in men; gender imbalance *H. pylori* infection is a fascinating subject [5]. Patients with peptic ulcers may benefit from measuring their cytokine levels as a useful indicator of tissue damage. Stomach ulcer development as a result of TNF-α's chemotactic effect on T and B cells. As a result, apoptosis causes the typical adult will experience a 50–70 billion cell loss every day. The apoptotic system is frequently impacted by *H. pylori* infection, and many infections have either pro- or anti-apoptotic, or occasionally both, effects [6]. TNF plays a major role in regulating the sudden, intense inflammation that might result from various infections including Gram-negative bacteria. Due to the attach lipopolysaccharide from *Helicobacter. pylori* on macrophages, which triggers the production of a significant amount of TNF-α and subsequently initiates the inflammatory responses during infection [7]. Maintaining the balance between intestinal cell proliferation and death is required to stop tumors from forming and TNF-α is essential role in this regard [8] .TNF-α contributes to the genesis and development of tumors, particularly gastric cancer, via modulating many signaling pathways [9]. At the same time bacterial genome encodes a multitude of virulence factors which are critical to a broad spectrum of disorders. There is a significant genetic variance in *H. pylori*. Aside from the exterior envelope sac, the two most researched separating cytotoxin-associated gene and cytotoxin gene A (vacA) are genes linked to virulence [10].

This research aims to track the apoptotic pathway using TNF-α as a biological marker in H. pylori patients to obtain an understanding of the mechanisms underlying H. pylori–induced apoptosis and its implications in the pathogenesis of gastric disorders.

Materials and Methods

This study included 110 patients of different ages undergoing at *Helicobacter pylori* at the Medical Merjan City and Digestive and Liver disease center from November 1, 2023 to January 15, 2024, after recording information about patients. Taken 2ml whole blood to make CBC to ensure immune cells and ABO test to detect type blood groups. Immunological study the study contributed utilizing the ELISA method in accordance with the manufacturer's instructions to measure the TNF-α concentration of both infected and healthy.

Statistical Analysis:

Using statistical analysis to analyze the U.S. Census (SPSS, 26), the results were examined using the least significant difference test (Chi-square and the T-test) less than the $P \leq 0.05$ significance threshold, as well as the test of less significant differences and the design random full-scale analysis of variance Table analysis of variance (One way-ANOVA Table) [11].

Ethical Approval:

Ethical consent for each patients had obtained verbal agreement before any samples were taken. Approved by Babylon University's Women's College of Sciences Research Ethics Committee, the study design was approved at (date in 8\10\2023 with the number 7/M.Sc.).

Results

The study was carried out) November 2023 and January 2024) on 110 people suffering *H. pylori* cause discomfort at a rate of 43.6% for females and 56.4% for males of various ages and sexes. Concerning residency, the current study revealed that of the patients who took part, 35 (56.5%) reside in an urban region and 27 (43.5%) in a

rural one. Even though the control group's *H. pylori* proportion is high, 30 patients (62.5%) live in metropolitan areas, compared to the control group's percentage of 18 (37.5%) in rural areas Table 1.

Table 1: Percentage of *H. pylori* according to sex and residency.

Sex					
Study population	**Male**	**Female**	**Total**	**P value (P≤0.05)**	**ODD (C195%)**
Patient	34 (54.8%)	28 (45.2%)	62	.365 NS	0.867 (0.405-1.857)
Control	28 (58.3%)	20 (41.7%)	48	.087 NS	
Total	62 (56.4%)	48 (43.6%)	110 (100%)	.191 NS	
Residency					
Study population	**Urban**	**Rural**	**Total**	***P value* (P ≤ 0.05)**	**ODD (CI95%)**
Patient	35 (56.5%)	27 (43.5%)	62	.191 NS	0.778 (0.360-1.680)
Control	30 (62.5%)	18 (37.5%)	48	.657 NS	
Total	65 (59.1%)	45 (40.9%)	110 (100%)	.522 NS	

Difference that is not significant at the 0.05 level is indicated by a chi-square test.

High significant deference found between all age groups patients, which ranged from 15-58 years in average 32.91± 10.31 year. The highest percentage of infection was found in the age range 26-36 years, and then 15-25 years, lastly the lowest incidence in the age group 37-47 showed in Figure 1.

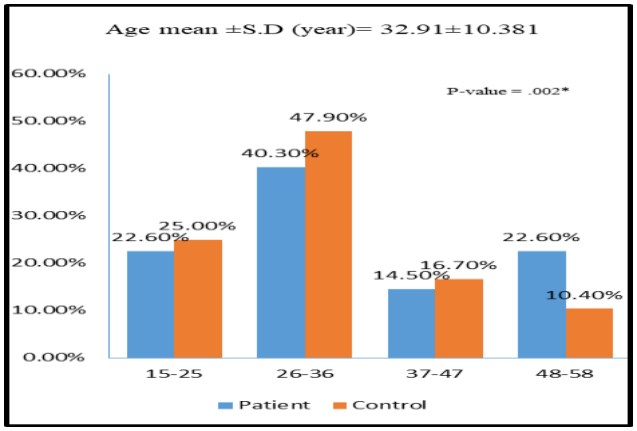

Figure 1: Distribution of infection according to the age group.

Helicobacter pylori infection and the distribution of ABO blood groups revealed that the blood groups with the highest positivity rates for *H. pylori* were O, B, A and AB, with 37.1%, 27.4%, 22.6%and 12.9% (Table 2). Consequently, blood type among them, blood group AB had the lowest prevalence and O the highest of individuals with *H. pylori* infection. (Chi-square test results show a significant difference at the 0.05 level). In the case of patients who are not affected, the trend is identical, with O ranking highest and AB ranking lowest.

Table 2: Distribution *H. pylori* according to blood group.

Study population	O*	A	B	AB	Total	*P value* (P ≤ 0.05)
Patient	23 (37.1%)	14 (22.6%)	17 (27.4%)	8 (12.9%)	62	0.0041
Control	15 (31.3%)	14 (29.2%)	13 (27.1%)	6 (12.5%)	48	0.0282
Total	38 (34.5%)	28 (25.5%)	30 (27.3%)	14 (12.7%)	110 (100%)	0.016

* Chi-square test results show a significant difference at the 0.05 level

The current study's results, obtained via the use of ELISA, demonstrated a substantial (P ≤0.05) rise in TNF-α level of concentration for all patients' age groups as compared to control population. The age range with the greatest values of this study was 37–47 years old while the low incidence in the age group15-25 years, Table 3.

Table 3: The concentration of TNF in study population according to age group.

Age group	Co. of TNF (pg/ml) Mean ± Std. Deviation		P value (p ≤ 0.05)
	Patient	**Control**	
15-25	33.61901 ± 11.824833	35.29164 ± 7.212061	.722 NS
26-36	34.87992 ± 13.862411	36.13330 ± 11.800449	.822 NS
37-47	75.96290 ± 30.260977	40.43187 ± 10.915419	.490 NS
48-58	38.09699 ± 16.785108	35.93330 ± 6.722280	.786 NS
Total	41.28529 ± 6.169497	36.66442 ± 9.843065	.585 NS
P value (p ≤ 0.05)	.141 NS	.792 NS	
One way – ANOVA yields non -significant difference under p ≤ 0.05.			

The current study's results indicated that the highest proportion of WBC was found in males 7.9621± 2.60552*, while females 7.6603 ± 2.11073 had the lowest percentage. The proportion of immune cells was similar in both sexes, but only a significant correlation was observed in female granulocytes, Table 4.

Table 4: Evaluation of White Blood Cell in patients group according to sex

WBC type	Concentration (pm/ml) Mean ± Std. Deviation	
	Male	Female
Total WBC (10^3\µL)	7.9621 ± 2.60552 *	7.6603 ± 2.11073
Lym. (10^3\µL)	2.4244 ± .82487	2.1279 ± .69811
Gran. (10^3\µL)	4.6368 ± 1.73555	4.8086 ± 1.59457 *
Neu. (10^3\µL)	3.7362 ± 1.64043	4.0617 ± 1.75536
P value	.026 *	.042 *
* Significant difference under one-way p ≤ 0.05 – ANOVA		

The correlation study revealed an inverse association in age groups 26-36 in neutrophil -.439*, with significant correlation (p≤0.05). There is an inverse connection between WBC, Lym, and Neu in the patient age group of 37–47 -.001–.019 and -.126, respectively. Although not statistically significant, there is an inverse in the age groups of 15–25 for WBC and, 26–36 only for Lym, 37–47 for WBC, Gran, and Neu, and finally 48–58 for WBC and Gran in the non-infected patients.

Table 5: Correlation between TNF and WBC in study population

Correlations							
Study population	Age Group			WBC. (10^3\µL)	Lym. (10^3\µL)	Gran. (10^3\µL)	Neu. (10^3\µL)
Patients	15-25	TNF-α (pg\ml)	r.	.110	-.216-	.018	.300
			Sig.	.709	.458	.950	.298
			N	14	14	14	14
	26-36	TNF-α (pg\ml)	R	-.394-	-.019-	-.310-	-.439*-
			Sig.	.051	.930	.132	.028
			N	25	25	25	25
	37-47	TNF-α (pg\ml)	R	-.001-	-.019-	.072	-.126-
			Sig.	.999	.962	.854	.746
			N	9	9	9	9
	48-58	TNF-α (pg\ml)	R	.263	.184	.222	.275
			Sig.	.364	.530	.446	.342
			N	14	14	14	14
Control	15-25	TNF-α (pg\ml)	R	-.117-	-.687-	.097	.048
			Sig.	.782	.060	.820	.911
			N	8	8	8	8
	26-36	TNF-α (pg\ml)	R	.323	-.019-	.366	.272
			Sig.	.240	.945	.180	.327
			N	15	15	15	15
	37-47	TNF-α (pg\ml)	R	-.317-	.345	-.516-	-.243-
			Sig.	.541	.502	.295	.643
			N	6	6	6	6
	48-58	TNF-α (pg\ml)	R	-.291-	.217	-.231-	.241
			Sig. (2-tailed)	.634	.726	.709	.696
			N	5	5	5	5

*. At the 0.05 level, the correlation is significant (2-tailed).
(-): Mean correlation exhibiting an inverse relationship
R: Pearson Correlation

Discussion

The current study of prevalence of *Helicobacter. pylori* infection worldwide is relatively similar between genders, with approximately 42.7% in women and 46.3% in men [12]. The study's results do not statistically differ significantly from one another. According to the present study, which supported a prior study, rural populations had significantly healthier diets and higher levels of physical activity than their city counterparts. The authors assert that these differences result from the greater standard of living that people in rural areas enjoy [13].The current results disagreed with those of an earlier study. The results show that increasing the age to 57 years old and above increases the incidence of infected cases at a time when the prevalence of infection is decreasing. Consequently, numerous investigations have revealed that an infection of *H. pylori* can affect individuals of any age. However, there is a direct link between growing older and the prevalence of *Helicobacter. pylori* infection in a

community[14] . The current research confirms the results of another study, which showed a strong connection between *Helicobacter. pylori* infection and ABO blood type, with type O being more likely to be infected and type AB being less likely to be. Information from other researchers that suggests blood group O is more vulnerable to *H. pylori* supports our findings [15]. The results, however, are at odds with other previous studies that found an increased risk of *H. pylori* infection was not associated using the O blood types [16]. Although the correlation between the ages of patients infected with *H. pylori* and the amount of TNF-α in the blood, no statistically significant differences were found. *H. pylori* groups that are negative, which is in line with this investigation. The findings of this investigation, along with one by Van, showed that the pathogen has no discernible effect on TNF-α levels [17]. The notable increase in serum TNF-α levels was observed in patients with gastric tumors positive for *H. pylori* .This study is in line with earlier findings that individuals with stomach cancer and *H. pylori* infection had greater serum TNF-α levels [18]. These results suggest that peripheral WBC count may be a helpful marker for assessing the degree of *Helicobacter pylori*-induced gastric mucosal inflammation, which is thought to function as a mediator between gastric cancer and *Helicobacter. pylori* infection [19]. It is yet unknown what physiological mechanisms cause patients with *H. pylori* infection to have elevated WBC counts, which in turn raises their risk of cancer. It has been demonstrated that *Helicobacter. pylori* infection increases the production of an inflammatory cytokines from stomach mucosal epithelial cells, involved interleukin-8, tumor necrosis factor and interleukin-6 [20]. It is currently unknown how TNF affects each type of cell that exists in the stomach mucosa of people who have *H pylori* infection. However, as has been it is believed that TNF can cause gastric epithelial cells to undergo apoptosis, based on a variety of reports [21]. Reduced membrane surface TNF-R expression on gastric epithelial cells and increased TNF-R release from these cells regulated TNF-induced apoptosis. In fact, the receptor's extracellular domain is assumed to shed as part of the host defense response. This raises the reduces the quantity of binding sites on the cell surface and is a binding protein for circulating TNF, preventing attachment to receptors on the cell surface [22].

Conclusion

The current study looked at the association between WBC and TNF-α and found that there is an inverse correlation between them in patients compared to controls, highlighting the importance of immune regulation. By providing insight into the process by which *Helicobacter pylori* causes apoptosis and its relationship to the pathophysiology of stomach illnesses, they achieve the goal of our work.

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