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Serum inflammatory cytokine IL-4 and IL-18 Levels in Patients with Typhoid Fever in Baghdad

Muna Sabah Dawood[1], Khetam Habeeb Rasool[1]*, Nadheema Hammood Hussein[1]

[1]Department of Biology, College of Science, Mustansiriyah University, Baghdad – Iraq.

*Corresponding Author: Khetam Habeeb Rasool

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ABSTRACT: Background: Gram-negative Salmonella enterica serotype typhi, or S. typhi, is intracellular bacteria belong to Enterobacteriaceae family that infect humans and cause Salmonellosis (typhoid fever), or (enteric fever), this disease is a major public health problem and a life-threatening bacterial infection in developing countries. Every year, there are thought to be between 12 and 33 million cases of typhoid fever, which leads to over 600,000 deaths. Specific immunity both humoral and cellular specific immune response activated to control Salmonella infection. This study aimed evaluate the function of IL-4 and IL18 levels as a diagnostic aid for this infection. **Material and methods**: Ninety suspected patients with typhoid fever diagnosed by specialist according to sign and symptom and thirty apparently healthy control where enrolled during the study period from September 2023 to February 2024. Blood was taken from both groups (5ml) and serum were gathered using centrifugation (3000 rpm for 5 min) and kept frozen in three Eppendorf for detection of serum IgM, IgG, IL-4 and IL-18 levels. **Results**: the result showed that out of 90 blood samples only 60 samples were positive to salmonella infection (confirmed by detecting specific salmonella IgM and IgG antibodies using ELIZA kits Sunlong biotech \China). Serum levels of IL-4, and IL18 (pg/ml) was measured in positive samples using ELIZA kits (Cloud-clone corp \USA). There is a significant increase in the level of IgM (0.33 ± 0.13) (mg\dl) and IgG (0.27 ± 0.1) (mg\dl) as compared to the control group in patients (0.16±0.03 and 0.12±0.03) (mg\dl) respectively with $P < 0.01$, and there was a significant increase in the average IL-4 (334.00 ± 87.49) (pg/ml) and IL-18 level in patients (380.44 ± 68.95) (pg/ml) compared to the control group (83.40 ± 17.44 and 189.66 ± 70.40) (pg/ml) respectively with $P < 0.001$. The results indicated a direct correlation between IL-4 and IL-18 serum levels with high level of serum IgM antibody compared to their levels in control group.

Keywords: Salmonella enterica, typhoid fever, IgM, IgG, IL-4 and IL-18 levels

1. INTRODUCTION

Typhoid fever is a systemic disease that causes acute inflammation of the intestine and is associated with Gram-negative bacteria of Salmonella enterica serovar Typhi (S. Typhi) [1]. Millions of patients worldwide suffer from an undifferentiated febrile sickness each year as a result of the disease's transmission through the fecal-oral route, which crosses the intestinal epithelium and spreads to systemic and intracellular locations [2]. Gram-negative Salmonella typhi is an intracellular bacillus belonging to the Enterobacteriaceae family. It can infect and colonize people, resulting in a variety of clinical signs include intestinal fever, bacteremia, and gastroenteritis. Some strains of salmonella are referred to as non-typhoidal, however the typhoidal serovars (Typhi and Para typhi A) that cause enteric fever are exclusive to humans. Typhimurium and Enteritidis serovars of Salmonella are widely distributed among hosts and are mostly responsible for gastroenteritis [3] . An epidemiological study conducted in Iraq revealed that a large number of people dying every year due to the deficiency of fresh water and contamination of rivers by sewage and factories [4, 5]. The most common serovars of Salmonella enterica are Typhimurium (S. Typhimurium) and Enteritidis (S. Enteritidis) prevalent serovars in the United States accounting for 41.5% of all outbreaks. Approximately 60% of all Salmonella outbreaks globally are caused by these two serovars [6]. An estimated 12–33 million instances of typhoid fever, also

known as enteric fever, occur annually, with an estimated 600,000 deaths from this potentially fatal bacterial infection [7], and it is among the most ancient illnesses that have followed humankind's arrival on this planet. It continues to be a worry for world health. Researchers have established that humans are the only hosts for typhoid fever, which they first caught several millennia ago [1] and claims more than 180,000 lives annually [8, 9]. The disease can linger for three to four weeks, and between 12 and 30 percent of people die from it [10]. When culture testing is available, blood culture is still the practical reference standard for diagnosing typhoid fever; however, the development of innovative diagnostic methods is a top priority. Through elevated levels of circulating proinflammatory and anti-cytokines in typhoid patients, clinical studies have demonstrated that S. typhi infection triggers both an intestinal mucosal and systemic humoral and cellular immune response, which is responsible for regulating and clearing S. typhi infection [11, 12]. Additionally, every strain of Salmonella is pathogenic and capable of invading and surviving in human cells, which makes them stand out when they target nonphagocytic human cells [13]. To enter the host cell, this characteristic promotes and takes advantage of phagocytosis. Thus, S. typhi infection spreads because of its capacity to elude the immune system, reach the gallbladder, establish a biofilm there, and enter a state of stasis, which allows it to elude the body's defenses without causing any symptoms [14]. When bacterial infections, such as typhoid fever infection, occur, cytokines are crucial in controlling inflammation. Mast cells, T-helper 2, basophils, and eosinophils generate the cytokine interleukin-4 (IL-4), which has a strong regulatory function in immunity because it helps leukocytes survive bacterial infections, stimulates B-cell development, and binds to growth factors for B cells [15]. 18 interleukins (IL-18) human IL18 gene 1 encodes this particular protein. It is sometimes referred to as IGF-1 (interferon-gamma inducer). Because it promotes inflammation, IL-18 is a pro-inflammatory cytokine that influences the immune response [16]. The aim of this study was to determine the levels of IL-4 and IL-18 in patients who have a S.typhi infection compared to control group.

2. Material and Methods

2.1 Group of Study

This study was performed on ninety out patients suspected to be infected with typhoid fever at Ibn Albalady Hospital, Imam Ali Hospital, and National center for Educational Laboratories between September 2023 to February 2024. out of these, 60 patients (31 female and 29 male patients; mean age, (24.78±16.53) years were included. For comparison,30 apparently healthy individuals (13 female and 17 males; mean age, (23.90 ± 18.89 years) were included in the study as a control group.

2.2 Blood Collection

Each person has had five milliliters of blood extracted using a sterile syringe into a sterile tube. The blood is then allowed to clotte at room temperature before being centrifuged for five minutes at 3000 rpm. Serum separation which collected in three Eppendorf tubes for determination of IgM, IgG, IL-4and IL-18 levels.

2.3 Measurement of IgM, IgG, IL4 and IL18 serum levels

The level of IgM and IgG (mg/ml),using ELIZA kits sunlong biotech \China. IL-4 and IL-18 (pg/ml),using ELIZA kits cloud-clone corp \USA were determined in serum from both controls and patients utilizing the enzyme-linked immunosorbent assay (ELIZA) in a ccordance with the manufacturer's instructions.

2.4 Statistical analysis

The statistical software SPSS-19 was used to analyze the data. Simple frequency, percentage, mean, and standard deviation measurements were used to display the data. Probability value that refers to statistically significant as P <0.05, statistically very significant as P <0.01. While probability value that refers to non-statistically significant as P <0.05, the level of IgG, IgM (mg\dl), IL-4 and IL-18 tests in the current study were express as mean ± S.D. The t-test was utilized to determine the significance of the correlation between two quantitative variables, and Pearson correlation was computed for that purpose. With a value of less than 0.3 signifying no connection, 0.3-<0.5 representing weak correlation, 0.5-<0.7 representing moderate strength, and >0.7 representing strong correlation, the correlation coefficient value (r) can be either positive (direct correlation) or negative (inverse correlation).

ROC curve (receiver operating characteristic curve), this graph was used to discriminate whether IgM, Igg, IL-4 and IL-18 are biomarkers to disease or not. The value for AUC ranges from 0 to 1. In addition, classification into five class: AUC= 0.5 = No discrimination, AUC= 0.5-0.7 = Poor discrimination, AUC= 0.7-0.8 = Good discrimination, AUC= 0.8-0.9= Very good discrimination and, AUC= >0.9 = Excellent discrimination.

3. Results

3.1 Patients' characteristics

The demographic and laboratory parameters of Typhoid fever patients and healthy controls enrolled in this study are described in Table 1.

Patients group include 29 men (48.3%) and 31 women (51.7%) and in the control group, there were 17 (56.7%) male and 13 (43.3%) female. The medium age of patients and control we (re 24.78± 16.53) years, and (23.90 ± 18.89) years, respectively.

Table 1. - Characteristics of patients and controls included in the study

Characteristic	Patients	Control	p-value
Group N (%)	60	30	0.06 NS
Age (year) Mean± S.D.	24.78±16.53	23.90±18.89	0.82 NS
Sex: Female N (%)	31(51.7)	13(43.3)	0.04*
Male N (%)	29 (48.3)	17(56.7)	
%= Percentage, p-value= Probability value, S.D. = Standard Deviation, *= significant) <0.05(, NS= non-significant.			

The mean of patients' antibody serum level of Salmonella specific (IgM and IgG) detected by ELIZA were (0.33±0.13, 0.16±0.03) (mg\dl) compared to control (0.27±0.1 & 0.12±0.03) (mg\dl), respectively, with a significant P<0.01. Table2

Table 2. - Antibody serum level (IgM and IgG) in study groups

Group	N	IgM level (mg\dl) Mean± S.D.	IgG level (mg\dl) Mean± S.D.	Reference Range	P-Value
Patients	60	0.33±0.13	0.27 ±0.1	Up to 0.22	<0.01**
Control	30	0.16±0.03	0.12±0.03		
P-value= Probability value, S.D. = Standard Deviation, **= highly significant (<0.01).					

The mean serum levels of IL-4 and IL-18 (pg/ml) showed a significant increase in-patient compared to control (334.00±87.49) (pg/ml) versus (83.40±17.44) (pg/ml) and (380.44±68.95) (pg/ml) versus (189.66±70.40) (pg/ml) respectively with P<0.01 as demonstrated in Table 3.

Table 3. - IL-4 and IL-18 serum levels in study groups

Group	**N**	**IL4 (pg/ml) Mean± S.D.**	**IL18 (pg/ml) Mean± S.D.**	**P-Value**
Patients	**60**	**334.00±87.49**	**380.44±68.95**	<0.01**
Control	**30**	**83.40±17.44**	**189.66±70.40**	
Frequency, p-value= Probability value, S.D. = Standard Deviation, **= very significant) <0.01)				

The correlation between IL-4 with IgM, IgG in Typhoid fever patients revealed there was a highly significant positive correlation (P<0.001, r = 0.493) and the correlation of IL-18 with IgM, IgG in Typhoid fever patients revealed there was a highly significant positive correlation of IgM (P<0.001, r = 0.405) But in IgG showed there is no significant differences, additionally there is no correlation (P<0.980, r = 0.003) Table 4:

Table 4. - The Correlation of IL-4 and IL-18 with IgM, IgG level

Correlations(r)		IgM level (mg\dl)	IgG level (mg\dl)	IL4(pg/ml)	IL18(pg/ml)
IgM level (mg\dl)	*r*	1	0.059	0.493(**)	0.405(**)
	p		0.655	0.001	0.001
IgG level (mg\dl)	*r*	0.059	1	0.444(**)	0.003
	p	0.655		0.001	0.980
IL4(pg/ml)	*r*	0.493(**)	0.444(**)	1	0.090
	p	0.001	0.001		0.492
IL18(pg/ml)	*r*	0.405(**)	0.003	0.090	1
	p	0.001	0.980	0.492	
** Correlation is significant at the 0.01 level (2-tailed). (r) correlation coefficient value, P-value= Probability value.					

The diagnostic significance of IL-4 and IL-18 levels in patients with Typhoid fever.

Receiver operating characteristic curve analysis confirmed the AUC of IL-4 and IL-18 were 1.000 additionally 0.970, in that order, the asymptotic significant (P-value) for IL-4 and IL-18 was (P<0.001), considered a potential biomarker in Typhoid fever patients. As shown in Figure 1:

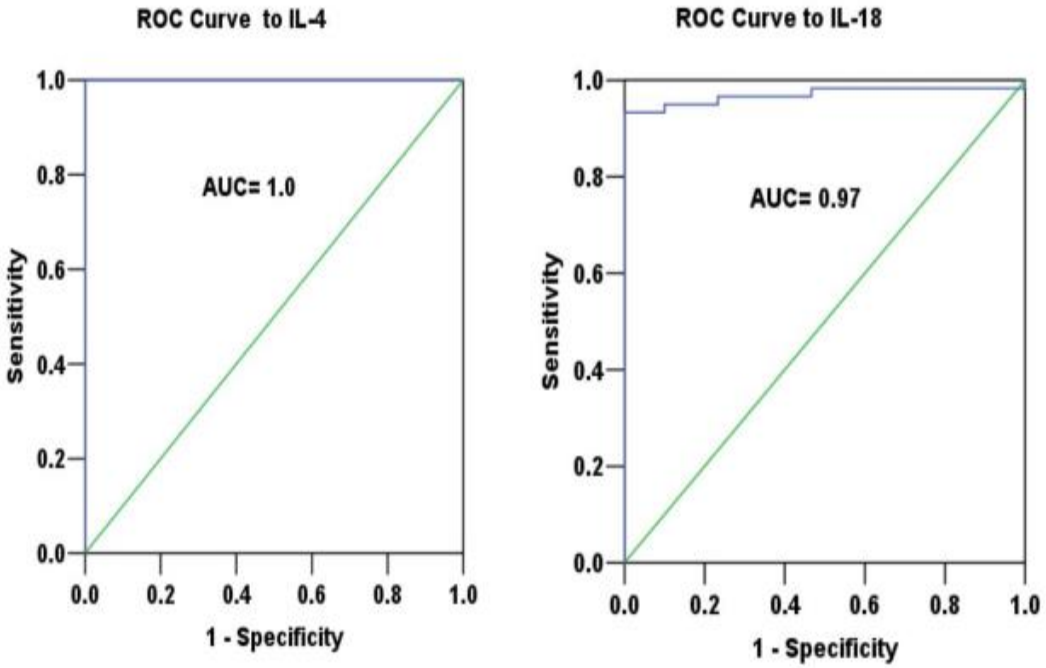

FIGURE 1. - Receiver Operating Curve (ROC) analysis of the IL-4 and IL-18 for predicting Typhoid fever infection, AUC = Area under Curve

4. Discussion

Foodborne disease outbreaks are frequently linked to Salmonella, an intracellular opportunistic bacterium. Public health is seriously threatened by its widespread circulation [17]. bacterial infection requiring immune response that presents as cellular immunity and its effectors the body's reaction to bacterial infections is largely dependent on innate and adaptive immunity [18]. The first line of protection against many bacteria, including Typhi, is innate immunity.

Following an infection with S. typhi, during the initial phases of infection, the innate immune system employe several mechanisms to ensure the survival of the host. These mechanisms include the recruitment of inflammatory cells to the infection site, the activation of inflammatory cells, the prevention of bacterial replication, and the production of

cytokines [19, 20].Th2 cells produce interleukin-4 (IL-4) which enhances humoral immunity and supports B cell development and differentiation.

The severity of a disease may affect cytokine response. IL-4 is also known to be produced by T helper 2 cells, and it possesses dual important functions for Th2 response, that it is responsible for several functional characteristics of Th2 cell effector, and it plays the role as principal inducer of the subsequent differentiation of precursor cells to become Th2 cells [21]. Th1-inducing cytokine (IL-18) has many biological similarities to other cytokines and is essential for the host's defense against intracellular infections by activating T cells at different stages in typhoid patients than in the healthy control group.

As shown in table 3 the group of typhoid patients had higher serum levels of IL-4 and IL-18 (334.00±87.49 and 380.44±68.95 pg/ml, respectively) compared to the group of healthy controls, and these variations were statistically significant ($P \leq 0.01$).

The concentrations of IL-4 and IL-18 in typhoid patients found higher in this study when compared to the control group which indicates that if IgM is high in patients infected with S.typhi ,it will lead to an increase in the levels of IL-4 and IL-18 in order to stimulate the immune system to respond and eliminate the disease-causing agent, S.typhi. This result agrees with the result of [22-24] were Increased neutrophil response to bacterial infection demonstrated the critical function of IL-4 receptor signaling in immune response type 2. Also results support that IL-18 are implicated in the pathogenesis of typhoid fever and agrees with the result of [25]. whose findings indicate that typhoid compared to controls, patients' IL-18 levels were higher [26].

The current study's findings demonstrate that IL-4 and IL-18 levels in typhoid fever patients compared to the control group were significantly higher. Additionally, the ROC analysis verified the existence of a superior region, which may help patients with Typhoid fever have more options for diagnosis as well as care. Furthermore, when comparing cases to controls, there seems to be a favorable link between IL-4, IL-18 levels and antibody IgM (mg\dl) level.

5. Conclusion

In patients infected with S. typhi, the levels of IL-4 and IL-18 rise in proportion to the length and severity of the illness. This highlights the crucial function of these cytokines in combating S. typhi infection.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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