

Hematological and Immunological Alterations, in Individuals, with Typhoid Fever; an Investigation Comparing CCL5 and IgA Levels Using ELISA Assays

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Abstract: Typhoid fever continues to be a public health concern, in parts of the world caused by *Salmonella enterica* subspecies I serovar Typhi. The objective of this study was to investigate whether there are any changes in immunological parameters among individuals with typhoid fever. This research was conducted as a case control study in September 2022. A total of 30 adults (above 15 years old) diagnosed with typhoid fever were included, alongside a number of adults for comparison. In this study we examined the levels of human CCL5 Elisa and human IgA. Out of the patients 87.50% tested positive for IgM antibodies while 18.75% showed positivity for both IgM and IgG antibodies; intriguingly only one sample displayed positivity solely for IgG antibodies. Moreover, compared to the control group TG levels were significantly increased LDL levels exhibited an increase but not significantly different from control values (whereas HDL levels were notably decreased). Additionally, both CCL5 and IgA showed elevations, in patients compared to the control group.

Keywords: typhoid fever, *Salmonella enterica*, CCL5, IgA.

Introduction

In many regions of the world, typhoid fever poses a severe threat to public health because over 16 million cases are reported each year. Patients with typhoid fever with an infection caused by *Salmonella enteric* subspecies Typhi (*S. typhi*) usually present with fever, headache, stomach pain, and generally lethargic demeanor. Typhoid fever is an infectious disease caused by the *Salmonella Typhi* bacteria that is conveyed orally. Usually, the culprits are contaminated food and water. *S. typhi* bacteria may live in freshwater and groundwater, including sewage, making them significant typhoid etiological agents^(13,14).

Transmission of typhoid fever can also occur as a result of defecation in public areas. In most developing nations, uncontaminated cut fruits that have been left out for a while are a major source of foodborne illness^(1,5). Human gastrointestinal disorders and severe systemic infections such as typhoid fever are caused by the Gram-negative bacterium *Salmonella typhi*. The causative agent of human typhoid disease is *Salmonella typhi*. *S. typhi* attaches to and invades the M and epithelial cells that line the distal ileum during infection. *S. typhi* must navigate a variety of challenging extracellular and intracellular settings in order to thrive in its human host⁽²⁾. The most prevalent immunoglobulin class in humans is by far immunoglobulin (Ig)A. Approximately 3-5 g of IgA are produced daily by plasma cells, which is significantly more than all other isotypes combined. IgA differs from other Ig classes in that it has distinct characteristics since it has many receptors and different glycosylation patterns and molecular shapes. IgA has the ability to both create and diversify commensal microbiota in the gut and kill pathogens^(2,3).

Serum IgA plays a dual role, triggering either pro-inflammatory or anti-inflammatory signaling pathways. In humans, IgA exists as two closely related subclasses, IgA1 and IgA2, that differ by 13 additional amino acids in the hinge region of the IgA1 molecule, while this difference might explain the increased susceptibility of IgA1 to bacterial proteases, ^(4,11) whereas WBC decreased and PLT increased significantly ($P \leq 0.05$) in patients. The intense and varied interactions between microorganisms and humans shaped, and are still shaping, the different resistance mechanisms of the host against pathogens, as well as distinct parasite and pathogen evasion strategies. In this context, cytokines are some of the most important components of innate and adaptive immune responses during infection ^(8,18).

Small cell signaling molecules called cytokines are in charge of stimulating and controlling immune responses ^(7,19). Since they have chemotactic action and are in charge of leucocyte migration from blood to tissues during inflammatory responses, a class of cytokines is known as chemokines. In addition to cell migration and the onset of autoimmune disorders, chemokines and their receptors are implicated in a number of other biological processes ^(6,20,21).

The aim of the present study was to determine whether some hematological and immunological parameter changed in typhoid fever patients.

Materials and Methods:

Study design:

This was a case control study conducted in September 2022 .

Study area:

Study conducted in Najaf city in Iraq

Study population:

The current study consisted of 15 adults (over the age of 15) who had type typhoid and an equal number of adults who were healthy. A standardized questionnaire was used to gather each participant's demographic information, including gender and age.

Inclusion criteria

Every patient with a typhoid fever diagnosis was part of the study. Typhoid infection was examined at in patients who suffered from a fever for longer than a week. For the study arms, only patients who showed positive for typhoid fever were included.

Exclusion criteria

Individuals who had started taking antibiotics before visiting the hospital for care were not included. The study excluded patients with other major systemic illnesses, including ongoing alcoholism, recent history of drug intake that could change blood-profile parameters, history of liver disease, renal disease, hematologic disorders, malaria, and other conditions. In order to rule out the confounding conditions mentioned above, comprehensive clinical histories were gathered.

Sample collection

Samples were collected using five milliliter disposable syringes. Each participant's venous blood sample (3–5 mL) was subjected to a complete blood count (CBC). The obtained samples were placed in EDTA-filled universal containers, and serum samples were utilized for lipid profiles and ELISA analysis.

Ethical permit

The Pathological Analysis Department received and accepted the project proposal. This study was carried out in accordance with guidelines and standards for reporting diagnostic accuracy.

Human CCL5 ELISA kit:

Principle: This kit comes with a set of calibration standards. By monitoring the calibration standards and samples simultaneously, the optical density versus CCL5 concentration standard curve is created. The concentration of CCL5 in the samples is then ascertained by comparing their optical density (OD) to the reference curve.

Human IgA Elisa kit

Principle: This kit comes with a set of calibration standards. By monitoring the calibration standards and samples simultaneously, the optical density versus IgA concentration standard curve is created. The concentration of IgA in the samples is then ascertained by comparing their optical density (OD) to the reference curve.

Immunological Rapid Test

IgG and IgM antibodies were detected using a typhoid test kit; approximately 50 μ L of test serum was placed to the sample pad, and it was left for 10 minutes. IgM-positive patient samples displayed a colony band against IgM or IgG against IgG; in the case of mixed antibodies, both IgM/IgG test bands will show positive results in addition to the control band.

Statistical analysis

A statistical analysis was performed on the obtained data. The data were described using mean \pm standard deviation, and the results of the independent-sample t test were utilized to infer the difference between the two groups.

Results

Thirty-one samples with probable typhoid were enrolled in this investigation, without regard to age or gender. Subjects' ages ranged from 15 to 85 years, and their serum samples from suspected patients were examined to check for typhoid fever IgM and IgG antibodies. The test showed that 87.50% of patients had positive IgM antibodies, 18.75% had positive IgM Pulse IgG antibodies, and surprisingly, only one sample had positive IgG antibodies. The results are shown in graphical form in figure (1).

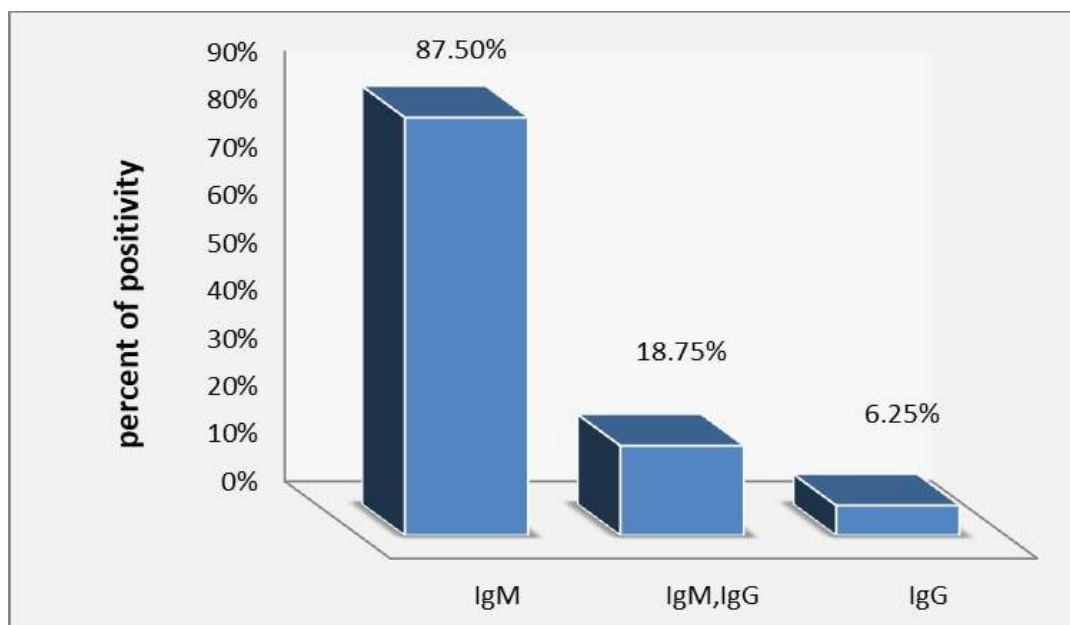


Figure 1: Incidence of immunoglobulin (IgG and IgM) in Typhoid fever patients

* In the present study blood HB, RBC, HCT, Neutrophil, Eosinophil, Monocyte, Age were not change significantly ($P \geq 0.05$) in patients (13.21 ± 1.68 , 4.542 ± 0.76 , 38.6 ± 4.56 , 56.7 ± 11.50 , 2.975 ± 2.0768 , 7.327 ± 1.473 , 45.7333 ± 1.473) respectively as compared with the control group (12.58 ± 5.42 , 4.342 ± 0.57 , 39.1 ± 5.42 , 9.47 ± 0.6788 , 22.989 ± 0.5531 , 0.693 ± 0.6817 , 6.82 ± 1.011 , 175536)

± 0.9554), whereas WBC decreased and PLT increase significantly ($P \leq 0.05$) in patients ($7.78284 \pm 1.05419, 263.166 \pm 105.25$) respectively as compared with the control group ($8.68125 \pm 1.53577, 22.25 \pm 24.61$).

Table (1) : The mean \pm standard deviation for hematological parameters.

Parameters	Patients Number (30)		Control Number (30)		P-value	
	Mean	SD	Mean	SD		
WBC	7.782	1.054	8.681	1.535	0.0061	S
HB	13.21	1.68	12.58	5.42	0.8897	N S
Platelet	263.166	105.25	22.25	24.61	0.0395	S
RBC	4.542	0.76	4.342	0.57	0.7940	N S
HCT	38.6	4.56	39.1	5.42	0.9296	N S
Neutrophil	56.7	11.50	43.86	22.989	0.5531	N S
Eosinophile	2.975	2.076	2.24	0.693	0.6817	N S
Age	45.733	19.480	44.562	17.553	0.9554	N S
Monocyte	7.327	1.473	6.82	1.011	0.7235	N S

* Also in the present study TG, LDL, HDL were ($179.4033 \pm 137.507, 144.786 \pm 24.47799, 25.6544 \pm 7.02624$), TG was increased significantly as compared with control (144.786 ± 24.47799), where was LDL increased but not significantly as compared with control (132.155 ± 0.3084), whereas the HDL was decreased significantly as compared with control (3.825448 ± 0.3084).

Table (2) : The mean \pm standard deviation for lipid profile

Lipid profile	Patients Number (30)		Control Number (30)		P-value	
	Mean	SD	Mean	SD		
TG	179.403	137.507	144.786	24.477	0.00001	S
LDL	145.222	44.005	132.155	30.327	0.3518	N S
HDL	25.654	7.062	33.345	3.825	0.0009	S

*In the present study CCL5, IgA were increased in significantly ($p < 0.05$) in patients ($448.529 \pm 87.526, 61.041 \pm 38.328$) respectively as compared with the control group ($61.041 \pm 38.328, 1.530 \pm 1.009$).

Table (3) : The mean \pm standard deviation for some imunological parameters

Parameters	Patients Number(30)		Control Number (30)		P-value	
	Mean	SD	Mean	SD		
Human CCL5	448.529	387.526	6.525	5.113	0.0028	S
Human IgA group	61.041	38.328	1.530	1.099	0.0072	S

Discussion

Typhoid fast serology testing has been used to diagnose both the acute and chronic stages of typhoid disease. Test is inexpensive, single-use, disposable, simple to do and comprehend, doesn't require any additional equipment or training, and works in combination with a blood culture and viral typhoid diagnosis.

The production of toxins in the bone marrow, which is the site of myelopoiesis during bacterial metabolism, can be blamed for the observed decrease in the value of WBC in patients with typhoid fever. In the current study, HDL, LDL, and TG showed significant statistical differencing in Typhoid fever have IDA compared to control to determine typhoid fever WBC. The existence of

WBCs is crucial for assisting in the body's ability to rid itself of an infection. Consequently, the body's capacity to fight infection is diminished by low WBC levels, often known as leukopenia (9,16).

Patients with typhoid fever had a neutrophil level of 56.7 ± 11.50 , whereas those without the illness had a level of 43.86 ± 22.989 . Neutrophils constitute a significant portion of white blood cells (WBCs) in the body and are essential for oxygen-dependent toxicity in the eradication of bacteria during infection defense. As a result, in reaction to the existence of an infection, such as in typhoid fever, there is usually an increase in neutrophil counts. This indicates that typhoid fever did not significantly affect neutrophils in this study (9). TG, LDL, and HDL This study found that TG levels increased and HDL and LDL levels decreased, with the latter being caused by lipid oxidation in typhoid patients. The intricacy of lipid anisotropy during *Salmonella typhi* infection is highlighted by this study (15).

IgA Additionally, the current study has shown increases in IgA immunoglobulins. The current study's notable increase in serum immunoglobulins associated with typhoid fever is in line with earlier research that showed these antibodies to have unique properties that make them useful in the fight against intracellular infections like *Salmonella* (12,17). CCL5 When faced with bacterial infections, macrophages often up-regulate genes implicated in M1 polarization, which include those that encode chemokines like CCL2, CCL5, and cytokines like TNF (10).

Conclusion

The research focused on examining how common and related factors of typhoid fever were, among individuals in Iraq who were suspected to have the disease. The primary discoveries showed that a majority of patients had tested positive for IgM antibodies, which suggests ongoing infection. Additionally the study found that typhoid fever had an impact on aspects of the patients health including blood cell count, platelet count, lipid profile and cytokine levels. Overall this investigation offered insights into the epidemiology and underlying processes of typhoid fever, within this region.

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