IL-6 and TNFα Genetic Mutations and Immunological Response in Salmonellosis

Zina Murshd Kadim [1], Nahla Ghazi Mohammed Al Loza [2,4*], Teba Habeeb Sayfe [3]

Abstract

Background: Understanding the genetic and immunological aspects of *Salmonella* infection is essential for effective management and prevention strategies. This study aimed to investigate the prevalence of *Salmonella* IgM and IgG antibodies, as well as the concentrations of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα), in patients with suspected *Salmonella* infection compared to healthy controls. Additionally, genetic mutations in the IL-6 and IFNα genes were explored. Methods: Between March and November 2023, blood samples were collected from 60 patients visiting Baghdad Teaching Hospital and 30 healthy individuals serving as the control group. ELISA assays were performed to measure *Salmonella* IgM and IgG levels, while IL-6 and TNFα concentrations were determined. Genetic mutations in the IL-6 (rs1800799) and IFNα (rs2430560) genes were analyzed using molecular techniques. Results: The mean levels of *Salmonella* IgM and IgG did not significantly differ between males and females (p = 0.21 and p = 0.58, respectively). Similarly, there were no significant gender differences in IL-6 and TNFα concentrations (p = 0.55 and p = 0.55, respectively). Mutations were observed in the IL-6 gene (rs1800799), resulting in variations from the wild AA variant to AG, AG, GG, GG, GG, AG, AG, GG, AG, and GG. Additionally, mutations were detected in the IFNα gene (rs2430560), with the wild TT variant changing to TA, TA, TA, AA, AA, TA, TA, AA, TT, and AA. Conclusion: This study provides insights into the immunological and genetic aspects of *Salmonella* infection. The lack of significant gender differences in antibody levels and cytokine concentrations suggests a similar immune response between males and females. The identification of genetic mutations in the IL-6 and IFNα genes highlights the genetic variability associated with *Salmonella* infection and warrants further investigation into their clinical implications.

Keywords: Salmonellosis, genetic mutations, immunological response, IL-6, TNFα

Significance | This study demonstrated the prevalence of mutations in IL-6 and IFNα genes in patients with Salmonella infection compared to healthy controls. Additionally, it explores the immunological response through IL-6 and TNFα concentrations.

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1. Introduction

Salmonella is a well-known pathogen that typically causes infection, known as salmonellosis, through the ingestion of contaminated raw or undercooked meat, poultry, eggs, or dairy products, as well as through drinking unpasteurized milk. The incubation period for salmonellosis can range from 6 hours to 6 days (Jin et al., 2017). Individuals with salmonellosis often experience symptoms resembling stomach flu. While salmonellosis is generally non-life-threatening, it can lead to serious complications in vulnerable populations, including infants, young children (Shakya et al., 2019), the elderly, transplant recipients, pregnant women, and immunocompromised individuals.

Salmonella enterica serotypes, particularly typhi and, to a lesser

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extent, paratyphi A, B, and C, are the primary agents responsible for typhoid fever or enteric fever, a potentially fatal multi-systemic illness (Mohan et al., 2015). Salmonella belongs to the Enterobacteriaceae family, is motile, and can cause various gastrointestinal infections, the most severe being typhoid fever. This disease manifests in a spectrum ranging from mild diarrhea with low-grade fever to severe, life-threatening conditions. Classic symptoms of typhoid fever include malaise, fever, diffuse abdominal pain, and constipation (Allos and Blaser, 2010). If left untreated, typhoid fever can progress to delirium, obtundation, intestinal hemorrhage, bowel perforation, and death within a month of onset. Survivors may suffer from long-term neuropsychiatric complications. The term "typhoid" is derived from the ancient Greek word for "cloud," reflecting the severe and prolonged neuropsychiatric impacts of untreated illness.

Annually, typhoid fever affects approximately 21 million people worldwide and causes about 161,000 deaths (Blaser, 2010). Over the years, the causative agent has developed increasing resistance to antibiotics, with extensively drug-resistant (XDR) typhoid reported in Pakistan in 2016 (Fitzgerald and Nachamkin, 2011). Currently, only three classes of antimicrobial agents—azithromycin, carbapenems, and tigecycline—remain effective against these resistant strains. Until 2018, two vaccines were available and licensed in various parts of the world for typhoid fever: the Vi-capsular polysaccharide parenteral subunit vaccine (Vi-PS) and Ty21a, a live attenuated oral vaccine (Vivotif, Crucell Vaccine, Leiden, Netherlands) (Horneman and Ali, 2011). However, these vaccines are not recommended for children under 2 and 6 years old, respectively, due to their moderate effectiveness and short-term protection (Seas et al., 1996; Wang et al., 2006).

The advent of new conjugate typhoid vaccines has marked significant progress. These vaccines bind Vi-polysaccharides to carrier proteins to stimulate helper T-cells and drive immunological memory. The Vi tetanus toxoid conjugate vaccine (Vi-TT, Typbar-TCV, Bharat Biotech, Hyderabad, India) has been shown to be effective and safe in both children and adults (Qadri et al., 2021). Our study aimed to detect mutations in the IL-6 and IFNα genes, which could provide further insights into the pathogenesis and potential treatment strategies for Salmonella infections.

Materials and Methods

Study Design and Sample Collection:

This case-control study was conducted at Baghdad Teaching Hospital in Baghdad City from March to November 2023. During this period, 60 blood samples were collected from patients presenting with symptoms suggestive of salmonellosis, and an additional 30 samples were collected from healthy individuals to serve as a control group.

ELISA:

The presence of *Salmonella Typhi*-specific IgM and IgG antibodies was determined using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. The procedure involved several steps: blood samples were centrifuged to separate the serum, which was then used for ELISA testing. Microtiter plates were coated with *Salmonella Typhi* antigen and blocked with a protein solution to prevent non-specific binding. Serum samples were added to the wells and incubated to allow binding of any specific antibodies present. A secondary antibody conjugated to an enzyme was added to bind to the IgM or IgG, followed by a substrate solution to produce a color change measured spectrophotometrically.

Primers:

Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNFα) levels were also measured using the ELISA technique, following a protocol similar to that described for antibody detection. To investigate potential genetic factors, specific primers were used for PCR amplification and sequencing of target gene regions. The primers for rs2430561 were Forward: TGTAAAACGACGGCCAGTCGTTGCTCACTGGGATTT and Reverse: CAGGAAACAGCTATGACCATGTCTTCCTTGATGGTCTC; for rs1800797, the primers were Forward: TGTAAAACGACGGCCAGTCAGTGAAACAGTGGTGAAGA and Reverse: CAGGAAACAGCTATGACCTTGTGGAGAAGGAGTTCATAG. The sequences were determined using a Sanger sequencer.

Statistical analysis:

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20. The statistical methods included descriptive statistics to summarize the demographic and clinical characteristics of the study population, and the t-test to compare the mean levels of antibodies and cytokines between the patient and control groups. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant. These methods allowed for a comprehensive analysis of serological responses, cytokine levels, and genetic variations associated with salmonellosis in the study population.

Results

The data in Table 1 showed that the Mean±S.E of Salmonella IgM in patients was 2.01±0.15, which was significantly higher compared to the control group, which had a Mean±S.E of 0.11±0.03 ($P<0.001$). Similarly, the Mean±S.E of Anti-Salmonella IgG1 in patients was 33±0.08, which was markedly higher than the control group's 0.09±0.03 ($P<0.001$).

In Table 2, it was demonstrated that the Mean±S.E of IL-6 in patients was 60.68±3.46, which was significantly elevated compared to the control group's 5.62±0.37 ($P<0.001$). Additionally, the Mean±S.E of TNFα in patients was 22.85±1.18, which was significantly higher than the control group's 4.92±0.32 ($P<0.001$).

Table 1. Comparison of the mean Anti Salmonella IgM, IgG between the study groups

Parameter	Groups	Concentrations (Mean±S.E)	P value
Salmonella IgM	Control	0.11±0.03	<0.001**
	Patients	2.01±0.15	
Salmonella IgG	Control	0.09±0.03	<0.001**
	Patients	1.33±0.08	

Table 2. A comparison of the research groups' mean levels of IFNα and IL-6

Parameter	Groups	Concentrations (Mean±S.E)	P value
IL-6	Control	5.62±0.37	<0.001**
	Patients	60.68±3.46	
TNFα	Control	4.92±0.32	<0.001**
	Patients	22.85±1.18	

Table 3. Distribution of anti-Salmonella IgM and IgG mean by gender

Parameter	Patients gender	Concentrations (Mean±S.E)	P value
Salmonella IgM	Male	1.83±0.17	0.21 NS
	Female	2.21±0.25	
Salmonella IgG	Male	1.29±0.11	0.57 NS
	Female	1.37±0.11	

Table 4. Distribution according to gender for meaning IL-6, IFNα

Parameter	Patients gender	Concentrations (Mean±S.E)	P value
IL-6	Male	58.81±4.67	0.58 NS
	Female	62.67±5.19	
TNFα	Male	22.16±1.64	0.55 NS
	Female	23.58±1.72	

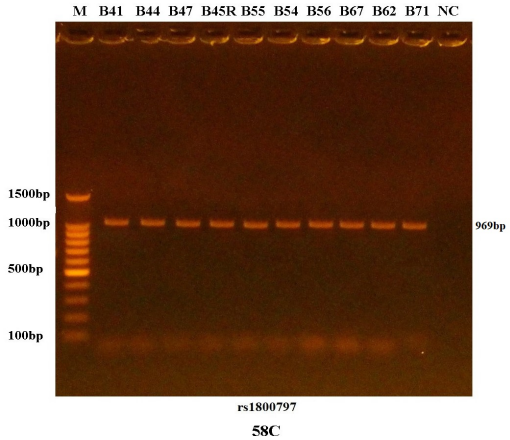

Figure 1. The rs1800797-specific gene sections in the human sample were amplified, separated, and electrophoretically separated on a 1.5% agarose gel before being stained with Ethidium Bromide M: 100 bp ladder markers. Lanes C1–A34 had 969 bp, similar to products of PCR

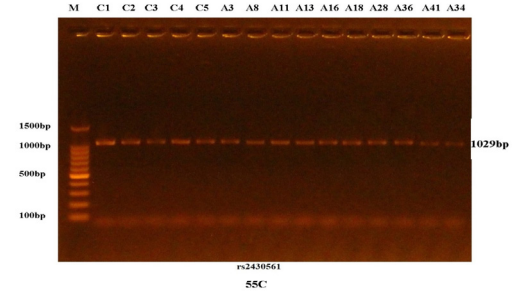

Figure 2. The rs2430561 specific gene regions in the human sample were amplified, segregated, and stained with Ethidium Bromide M: 100 bp ladder markers using 1.5% agarose gel electrophoresis. Lane C1-A34 is comparable to 1029 bp PCR products.

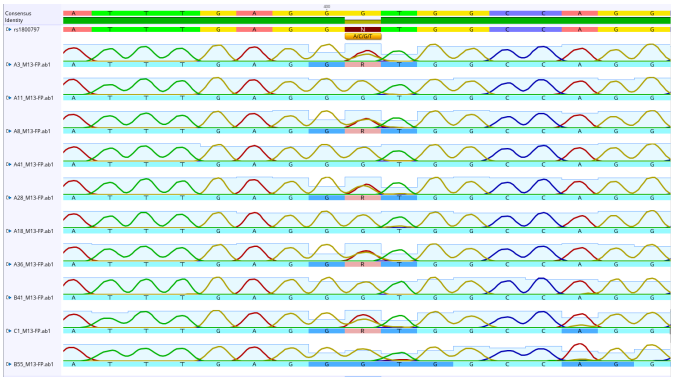

Figure 3. Analysis of IL6 gene rs1800797 SNP using Sanger sequencing. Only one "G" peak denotes homozygous G alleles. Single "A" peaks signify alleles that are homozygous. The "G" and "A" peaks signify heterozygous G/A alleles.

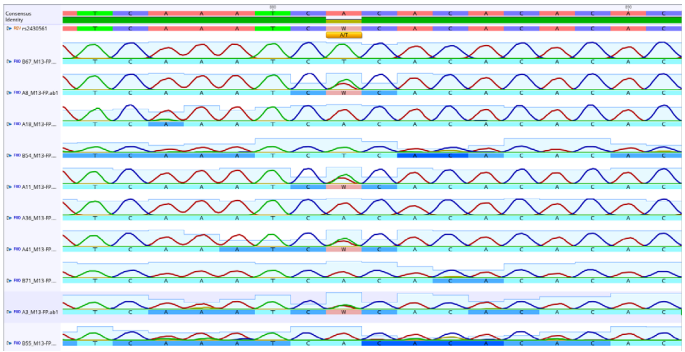

Figure 4. Sanger sequencing analysis of the IFNα gene rs2430561 SNP. T homozygous alleles are indicated by single "T" peaks. Homozygous alleles are indicated by single "A" peaks. T/A heterozygous alleles are shown by the presence of "T" and "A" peaks.

Table 5. Variation of wild SNPs of IL-6 gene ID 3569

IL6 GENE ID 3569	
SNPs	**rs1800799**
Wild	**AA**
Variation	**A>G**
Samples	
3	AG
8	AG
11	GG
13	GG
18	GG
28	AG
36	AG
41	GG
43	GG
54	AG
55	GG
62	GG
C1	AG
C2	AG
C3	AG
C4	GG
C5	AG

Table 5. Variation of wild SNPs of IFNα gene ID 3458

IFNα GENE ID 3458	
SNPs	**rs2430560**
Wild	**TT**
Variation	**T>A**
Samples	
3	TA
8	TA
11	TA
13	TA
18	AA
28	AA
36	TA
41	TA
43	TT
54	AA
55	TT
62	AA
C1	TA
C2	TA
C3	AA
C4	AA
C5	TA

According to Table 3, it was found that the Mean±S.E of Salmonella IgM was 1.83±0.17 in males and 2.21±0.25 in females, with no significant difference (P=0.21). The Mean±S.E of Salmonella IgG was 1.29±0.11 in males and 1.37±0.11 in females, also showing no significant difference (P=0.57).

Table 4 revealed that the Mean±S.E concentration of IL-6 was 58.81±4.67 in males and 62.67±5.19 in females, with no significant difference (P=0.58). The Mean±S.E concentration of TNFα was 22.16±1.64 in males and 23.58±1.72 in females, again with no significant difference (P=0.55).

In Table 5, it was indicated that a mutation occurred in the IL-6 gene ID 3569 at SNP rs1800799, where the wild-type AA was altered to AG, AG, GG, GG, GG, AG, AG, GG, AG, GG, GG in the patient group compared to the control group.

Table 6 showed that a mutation occurred in the IFNα gene ID 3485 at SNP rs2430560, where the wild-type TT was altered to TA, TA, TA, TA, AA, AA, TA, TA, AA, TT, AA in the patient group compared to the control group.

Discussion

Salmonella Typhi has been recognized as a serious pathogen, causing high fever, body aches, and recurrent infections. According to the results of this study, there were highly significant differences between those acutely infected with Salmonella and the control group. The Mean±S.E of Salmonella IgM in patients was 2.01±0.15 compared to 0.11±0.03 in the control group (P<0.001). These findings were consistent with Chiwan et al. (2023), who reported significantly higher concentrations of anti-IgM antibodies in acutely infected individuals.

The Mean±S.E of Anti-Salmonella IgG in patients was 1.33±0.08, significantly higher than the control group's 0.09±0.03 (P<0.001). This result aligned with Verma et al. (2022), who observed a noticeable increase in IgG concentrations in individuals with chronic Salmonella typhoid infection. Verma et al. (2022) attributed this to the bacterium's ability to reside within white blood cells, making it difficult to treat with conventional methods.

The Mean±S.E of IL-6 in patients was 60.68±3.46, compared to 5.62±0.37 in the control group, with a highly significant difference (P<0.001). These results were in harmony with Fu-Ch. (2010), who investigated the effects of PJ 34 on Salmonella-induced enterocyte IL-6 production. Fu-Ch. (2010) found that PJ 34 enhanced IL-6 production in infected Caco-2 cells, mediated through ERK and NF-κB pathways, but not through p38 MAPK, JNK, or PI3K/Akt pathways. This indicated that PJ 34 could protect intestinal epithelial cells against invasive salmonellosis by upregulating IL-6 production.

The study also found that the Mean±S.E of TNFα in patients was 22.85±1.18, significantly higher than the control group's 4.92±0.32 (P<0.001). Pham et al. (2020) demonstrated that TNF signaling limited M2 granuloma macrophage polarization, thereby restricting *Salmonella Typhi*murium persistence. Neutralization of TNF shifted granuloma macrophages towards an M2 state, increasing bacterial persistence, partially dependent on SteE activity.

No significant differences were found in the prevalence of *Salmonella typhi* between infected males and females. The Mean±S.E of Salmonella IgM was 1.83±0.17 in males and 2.21±0.25 in females (P=0.21), while the Mean±S.E of Salmonella IgG was 1.29±0.11 in males and 1.37±0.11 in females (P=0.57). These findings matched those of Najib et al. (2021) and Chiwan et al. (2023).

The study also revealed a mutation in the IL-6 gene ID 3569 at SNP rs1800799, where the wild-type AA was altered to AG, AG, GG, GG, GG, AG, AG, GG, AG, GG, GG in the patient group compared to the control group. Guo et al. (2020) showed similar polymorphisms in the IL-6 gene sequence associated with Salmonella infection.

Additionally, a mutation occurred in the IFNα gene ID 3485 at SNP rs2430560, where the wild-type TT was altered to TA, TA, TA, TA, AA, AA, TA, TA, AA, TT, AA in the patient group compared to the control group. Calenge et al. (2010) reported several genetic mutations in the necrosis factor genes, indicating that Salmonella infection leads to significant tissue damage (Calenge et al., 2010; Zghair et al., 2023).

Conclusion

This study showed significant immunological and genetic findings in patients with Salmonella infections. The Mean±S.E of Salmonella IgM and Anti-Salmonella IgG were significantly higher in patients compared to the control group, indicating a robust antibody response during infection. Elevated levels of IL-6 and TNFα in patients further underscored the inflammatory response to Salmonella. No significant differences in antibody levels or cytokine concentrations were observed between males and females. Genetic analysis revealed notable mutations in the IL-6 gene ID 3569 at SNP rs1800799 and in the IFNα gene ID 3485 at SNP rs2430560 in patients. These mutations suggest a potential role in the pathogenesis of salmonellosis and may offer new insights for therapeutic targets. The findings support the need for continued research into the immunological and genetic factors influencing Salmonella infections, which could lead to improved treatment strategies and outcomes.

Author contributions

Z.M.K., N.G.M.A.L., T.H.S. conceptualized, drafted, wrote, reviewed and edited the article.

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None

Competing financial interests

The authors have no conflict of interest.

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