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Article

Hematological and Analytical Study to Evaluate Age-Dependent Immunological Differences Between Active and Chronic Salmonella Typhi and Paratyphi Infections in Nineveh Governorate, Iraq

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Abstract: Background: Typhoid and paratyphoid fever constitute serious public health problems both at a worldwide level and within the territory of Iraq, especially because of the pathogenic bacteria's ability to establish a chronic infection through adaptation in the gallbladder. Hence, this study attempted to identify the physiological and immunological differences in Nineveh Governorate, Iraq, due to age being a critical parameter for differentiating the active from the chronic phase of the infection. Methods: The study involved 100 samples (80 active and chronic cases and 20 as the control group) divided into three age classes. White blood cells (WBC), granulocytes, and hemoglobin (Hb) were measured using a complete blood count (CBC). In addition, packed cell volume (PCV) was evaluated. Results: The results showed proportional differences between the active and chronic phases, significantly dependent on the patient's age. Patients above 40 years of age had higher levels of WBC and granulocytes, increasing up to 177% for typhoid and 123% for paratyphoid cases. On the other hand, Hb fell to 79%, while PCV fell to 83%, showing that normocytic normochromic anemia occurs in the elderly. Conclusions: It was concluded that combining hematological parameters constitutes a very efficient way to diagnose the disease, due to the fact that there were more acute and sharp changes during the active phase than in the chronic one.

Keywords: Typhoid fever, Salmonella Typhi, hematological indicators, chronic infection.

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Introduction

The infection known as typhoid fever due to Salmonella Typhi is considered one of the most pressing global health concerns, especially in areas where there is insufficient health care. The threat posed by the bacteria is in its exceptional ability to adapt in the host body by showing signs of either an active infection or chronic disease, where the microorganism usually stays in the gallbladder, turning the patient into a carrier [1]. Global statistics show that about 11-21 million people suffer from typhoid fever yearly, leading to more than 200,000 deaths each year [2].

In Iraq, according to recent research, there has been a growing number of people between 15 and 45 years who have been infected. According to Al-Obaidi [3], a study conducted in northern Iraq revealed that approximately 65% of the cases had hematological complications, including anemia and thrombocytopenia. Hameed [4], revealed that changes in lifestyle and environment in urban settings have led to the development of 3-5% of the acute infections into chronic carrier states.

Salmonella infection causes many physiological and immunological changes. Once the bacteria have entered the body, the body reacts by producing more WBCs and stimulating the granulocytes in order to fight off the effects of the bacterial toxins [5]. However, this mechanism does not work equally among all people, because age is crucial when assessing the level of immunological and hematological alterations in a person. According to some research, older people may react more strongly to the infection due to "immunosenescence" [6].

Furthermore, anemia is directly related to both active and chronic infections due to the destabilization of red blood cells' membranes and suppression of bone marrow caused by the action of bacterial toxins [7]. Recent research, such as Kaur & Jain [8], states that it is crucial to understand the alterations caused by the pathogen in order to differentiate the infection stages. Active infections cause acute inflammation, which is expressed in increased numbers of granulocytes. Chronic infections usually cause physiological problems that affect the formation of red blood cells. According to Mohammed & Issa [9], a comparison study in Iraq revealed an increased number of neutrophils in typhoid cases compared to non-infected individuals. On the other hand, Chen et al. [10] analyzed the "hematological fingerprint" in different ages and found that older people experience a slow but more damaging inflammatory reaction to red blood cells; hence, the lower levels of hemoglobin in people above 40 years. This research is geared toward analyzing the immunological differences among the infected patients with Salmonella in the governorate of Nineveh.

Materials and Methods

Study Population and Sample Collection

This study was conducted between October 2025 and March 2026 across several main hospital outpatient clinics and primary diagnostic laboratories in Nineveh Governorate, Iraq. A total of 100 human blood samples were collected, comprising a Study Group of 80 patients diagnosed with active or chronic Salmonella infections (Salmonella enterica serovars Typhi and Paratyphi) and a Control Group of 20 healthy age-matched individuals with no recent history of febrile illness or enteric infection. The subjects ranged in age from 20 to over 40 years and were stratified into three age groups: Group I (20–30 years), Group II (31–40 years), and Group III (>40 years).

Inclusion and Exclusion Criteria

Inclusion criteria comprised patients presenting with clinical manifestations of enteric fever (persistent high fever, abdominal pain, hepatosplenomegaly, or confirmed chronic carrier status via serological/stool screening) who provided informed consent.

Exclusion criteria included patients with pre-existing hematological disorders (such as sickle cell anemia, thalassemia, or leukemia), autoimmune diseases, chronic liver or renal failure, active tuberculosis, pregnant women, and individuals currently receiving immunosuppressive therapy or recent blood transfusions.

Ethical Approval

Ethical approval for this study was formally granted by the Institutional Review Board (IRB) / Ethics Committee of the University of Telafer (Approval No. UOT-ETH-2022-1042, dated September 18, 2022). Written informed consent was obtained from all participating patients or their legal guardians prior to sample collection, in accordance with the principles of the Declaration of Helsinki.

DNA Extraction and PCR Amplification

Bacterial genomic DNA was extracted from 200 µL of whole blood using a commercial blood DNA extraction kit (ReliaPrep™ Blood gDNA Miniprep System, Promega, USA) following the manufacturer's protocol. Molecular identification of *S. typhi* and S. *paratyphi* was performed by targeting the invA gene (common for *Salmonella* genus) and specific flagellin genes (fliC-d for S. Typhi and fliC-a for S. Paratyphi A) using published primers (invA-F: 5′-

ATCAGTACCAGTCGTCTTATCTTGAT-3', invA-R: 5'- TCTGTTTACCGGGCATACCAT -3'; fliC-d-F: 5'-ACTCAGGCTTCCCGTAACGC-3', fliC-d-R: 5'-GGCTAGTACACACAAACCC-3') [11, 12]. The PCR reaction mixture (25 µL total volume) contained 12.5 µL of 2X Master Mix (Biorad, USA), 1.0 µL of each forward and reverse primer (10 pmol/µL), 3.0 µL of template DNA (~30 ng), and 7.5 µL of nuclease-free water. Thermal cycling conditions consisted of an initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 94°C for 45 s, primer annealing at 58°C for 45 s, and extension at 72°C for 1 min; with a final extension step at 72°C for 7 min. Amplicons were visualized by 1.5% agarose gel electrophoresis stained with ethidium bromide under a UV transilluminator.

Laboratory Tests

A complete blood count (CBC) test was conducted on blood samples using an automated hematology analyzer. The measured parameters included Hemoglobin (Hb, g/dL), Packed Cell Volume (PCV, %), Total White Blood Cell count (WBC, 10^9/L), and Granulocyte percentage (Granulocytes %, including neutrophils). PCV was additionally evaluated using standard mathematical derivation [13]:

PCV (%) = Hemoglobin (g/dL) × 3.3

Statistical Analysis

The statistical analysis was carried out using the SPSS package. Data were presented as Mean ± SE. One-way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test were applied to evaluate significant differences among age groups and controls at a significance level of $P < 0.01$.

Results

Total White Blood Cell Count (WBC)

Older patients in the study had a higher WBC count (Table 1). The 40 + age group had a mean WBC of 8.84 ± 0.376 × 10^9/L, approximately 177% higher than the control group mean of 4.99 ± 0.187 × 10^9/L. Older patients in the S. Paratyphi study (Table 2) also had increased WBC counts. The 40 + age group had a mean WBC of 7.78 ± 0.272 × 10^9/L, approximately 156% above the control mean of 6.64 ± 0.164 × 10^9/L. Patients in the 20-30 age group had means of 7.01 ± 0.168 × 10^9/L (140% control) and 6.83 ± 0.129 × 10^9/L (132% control), respectively.

Table1. Comparison of Total White Blood Cell Count (WBC) by Age Group in Typhoid Cases

Age Group	Mean ± SE (10^9/L)	Percentage (%)	Change
Control	4.99 ± 0.187 c	100%	–
20–30 years	6.07 ± 0.560 c	122%	+22
30–40 years	7.32 ± 0.122 b	147%	+47
>40 years	8.84 ± 0.376 a	177%	+77

Different letters within the same column indicate statistically significant differences between age groups and the control group at a probability level ($P < 0.01$) according to Duncan's test.

Table 2. Comparison of Total White Blood Cell Count (WBC) by Age Group in Paratyphoid Cases

Age Group	Mean ± SE (10^9/L)	Percentage (%)	Change
Control	4.99 ± 0.187 c	100%	–
20–30 years	6.64 ± 0.164 b	133%	+33
30–40 years	7.05 ± 0.089 b	141%	+41
>40 years	7.78 ± 0.272 a	156%	+56

Different letters within the same column indicate statistically significant differences between age groups and the control group at a probability level ($P < 0.01$) according to Duncan's test.

Percentage of Granulocytes

The percentage of granulocytes increased with age (Tables 3 and 4). Patients in the 20-30 age group had mean granulocyte counts of 65.74 ± 0.874% and 60.25 ± 0.177%, respectively, while patients

in the >40 age group had mean counts of 71.98 ± 1.234% and 74.18 ± 2.540%. These means represented 109% and 120% of the control, respectively, and 123% and 122% of the control, respectively.

Table 3. Comparison of Granulocyte Percentage (Granulocytes %) by Age Group in Typhoid Cases

Age Group	Mean ± SE (%)	Percentage (%)	Change
Control	60.25 ± 0.177 d	100%	–
20–30 years	65.74 ± 0.874 c	109%	+9.1
30–40 years	68.5 ± 0.4 b	114%	+14
>40 years	71.98 ± 1.234 a	120%	+20

Different letters within the same column indicate statistically significant differences between age groups and the control group at a probability level (P < 0.01) according to Duncan's test.

Table 4. Comparison of Granulocyte Percentage (Granulocytes %) by Age Group in Paratyphoid Cases

Age Group	Mean ± SE (%)	Percentage (%)	Change
Control	60.25 ± 0.177 c	100%	–
20–30 years	67.24 ± 0.581 b	112%	+12
30–40 years	68.72 ± 0.243 b	114%	+14
>40 years	74.18 ± 2.540 a	123%	+23

Different letters within the same column indicate statistically significant differences between age groups and the control group at a probability level (P < 0.01) according to Duncan's test.

Hemoglobin (Hb) Levels and Packed Cell Volume (PCV)

Hemoglobin levels diminished with patient age in both types of infection ($p < 0.01$). With typhoid fever (Table 5), the average Hb level in the 20-30 year age group was 13.53 g/dL which decreased to 11.87 g/dL in the >40 year age group (79% of control value). Correspondingly, PCV in the typhoid fever group also decreased to 39% (from control PCV of 49%). With paratyphoid fever (Table 6), Hb levels in the >40 year age group (78% of control value) reduced to 11.79 g/dL, and PCV also reduced to 38%.

Table 5. Effect of Age Group on Hemoglobin (Hb) Levels and Anemia Index in Typhoid Cases

Age Group	Mean ± SE (g/dL)	Percentage (%)	Change	PCV (%)
Control	**14.96 ± 0.209 d**	**100%**	**–**	**49**
20–30 years	**13.53 ± 0.058 c**	**90%**	**-10**	**45**
30–40 years	**12.73 ± 0.151 b**	**85%**	**-15**	**42**
>40 years	**11.87 ± 0.274 a**	**79%**	**-21**	**39**

Different letters within the same column indicate statistically significant differences between age groups and the control group at a probability level (P < 0.01) according to Duncan's test.

Table 6. Effect of Age Group on Hemoglobin (Hb) Levels and Anemia Index in Paratyphoid Cases

Age Group	Mean ± SE (g/dL)	Percentage (%)	Change	PCV (%)
Control	**14.96 ± 0.209 a**	**100%**	**–**	**49**
20–30 years	**13.72 ± 0.170 b**	**91%**	**-8**	**45**
30–40 years	**12.75 ± 0.403 b**	**85%**	**-15**	**42**
>40 years	**11.79 ± 0.432 c**	**78%**	**-21**	**38**

Different letters within the same column indicate statistically significant differences between age groups and the control group at a probability level (P < 0.01) according to Duncan's test.

Typical Differences of Infection Caused By S. Typhi and S. Paratyphi

When the characteristics of infection caused by S. Typhi and S. Paratyphi (Table 7) were compared, infection by S. Paratyphi in the 20-30 year age group caused a slight increase in total WBC

(6.64 x 10^9/L) and relative granulocyte (67.24%) counts as compared to infection caused by S. Typhi (5.98 x 10^9/L and 65.74%). Likewise, in the >40 year age group, infection caused by S. Typhi increased total WBC count (8.84 x 10^9/L) and relative granulocyte (74.18%) while infection caused by S. Paratyphi (7.78 x 10^9/L and 71.98%) slightly reduced PCV to 38%.

Table 7. Comparison between Hematological Indicators (WBC, Granulocytes, Hb, PCV) in Typhoid and Paratyphoid Patients by Age Group

Age Group	Indicator	Typhoid	Paratyphoid	More Severe
20–30 years	WBC (10^9/L)	6.07	6.64	Paratyphoid
20–30 years	Granulocytes (%)	65.74	67.24	Paratyphoid
20–30 years	Hb (g/dL)	13.53	13.72	Paratyphoid
20–30 years	PCV (%)	45	45	Equal
31–40 years	WBC (10^9/L)	7.32	7.05	Typhoid
31–40 years	Granulocytes (%)	68.5	68.72	Paratyphoid
31–40 years	Hb (g/dL)	12.73	12.75	Close
31–40 years	PCV (%)	42	42	Equal
>40 years	WBC (10^9/L)	8.84	7.78	Typhoid
>40 years	Granulocytes (%)	71.98	74.18	Paratyphoid
>40 years	Hb (g/dL)	11.87	11.79	Typhoid
>40 years	PCV (%)	39	38	Paratyphoid

Severity evaluation: The "More Severe" column indicates the greater magnitude of the physiological response (increase or decrease) for each age group for typhoid versus paratyphoid infection at the level of significance ($p < 0.01$). Equality and proximity: Equality means that for a given parameter, the two types of infection being compared are identical. Closeness means that for a given parameter, the two types of infection being compared are not different from each other at a level of significance ($p > 0.01$).

Discussion

The higher leukocyte counts in older patients (> 40 years) with typhoid fever may represent a defensive response of the bone marrow to sepsis due to circulating endotoxins. This is supported by Al-Jubouri's report [14], which indicated that S. typhi stimulates leukocyte formation. Paratyphoid fever, which is less severe, caused leukocytosis to the same extent as S. typhi in older patients (156%). It is generally acknowledged that S. Typhi and Paratyphi stimulate an increased immune response, which is in line with Roberts et al.'s report on age-related changes [6].

Increased levels of granulocytes in different age groups reflects an increased inflammatory response. Bacteremia stimulates a neutrophil response. Therefore, increased neutrophil counts indicates that S. Typhi and Paratyphi infections are of a systemic nature [5]. In older patients (> 40 years), the neutrophil response to S. Paratyphi was 123% and to S. Typhi was 120% infection. This supports Khan [15] and Chen et al. reports [10], that S. Paratyphi predominantly stimulates an early, intensive granulocyte response.

Several studies reported that S. Typhi and Paratyphi infection result in infection-related anemia. Endotoxins may result in reduced erythrocyte survival. In conjunction with minor mucosal and intestinal bleeding, these may stimulate suppression of erythropoiesis. This accounts for the relative, but similar reductions in Hb and PCV in older patients. Consequently, the anemia in these patients is normocytic and normochromic, and not due to iron deficiency [7, 16].

S. Typhi infection, in comparison with S. Paratyphi infection, stimulated a greater leukocyte response, as supported by previously published studies. S. Paratyphi infection predominantly suppressed the erythrocyte indices and induced a more severe granulocyte response in older patients [9, 14]. These data point to the relationship that patient age affects host hematological changes during the course of enteric fever.

Conclusion

Leukocytosis occurring as part of the systemic inflammatory response to Salmonella enterica serotypes Typhi and Paratyphi is more pronounced in older (40 years and above) patients (177% and

78% rise, respectively, from baseline) compared to children and young adults (up to 40 years of age). Among the older patients, Typhi infection causes a greater rise in absolute granulocytes (120%), compared to Paratyphi infection (123%). P. Paratyphi infection can cause more suppression of red blood cell lineage (i.e. anemia) compared to Typhi infection. Anemia is seen among both older adults as well as children and young adults. The anemia is normally Functional (i.e. due to infection), and therefore can be treated with antibiotics.

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