Original Article

Haematological Manifestations in Children with Extended Drug-Resistant Salmonella Typhi Infections in a Tertiary Care Hospital

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ABSTRACT

OBJECTIVE: To analyze the haematological parameters in children affected by extended drug-resistant salmonella typhi (XDR S. typhi) infections in a Tertiary care hospital.

METHODOLOGY: This Descriptive Cross-Sectional study was carried out at the Department of Haematology at PNS SHIFA Hospital, Karachi, from November 2021 to October 2022. Seventy children with XDR S. Typhi on blood culture were selected using a consecutive sampling technique. The study focused on haematological parameters, i.e., haemoglobin (Hb), WBC count, and platelet count. Descriptive statistics were expressed as mean ±SD, and the Chi-square test was applied. A p-value ≤0.05 was considered statistically significant.

RESULTS: Out of the total of 70 children (under 12 years of age) included in the study, 41 (58.6%) were male and 29 (41.4%) were female. The mean age was 8.17±2.92 years. Anaemia was observed in 51% percent of cases. Leucopenia and thrombocytopenia were noted in 44% and 54% of cases. Seventeen children had bicytopenia, while pancytopenia was seen in only two children.

CONCLUSION: XDR S. Typhi infection causes significant cytopenias. Therefore, patients with a history of sustained fever with cytopenias should be worked up for possible XDR S. Typhi infection.

KEYWORDS: XDR Salmonella typhi, drug-resistant S. Typhi, High-grade fever, thrombocytopenia, leukopenia, blood culture test.

INTRODUCTION

Salmonella typhi is a gram-negative flagellated bacterium that spreads through the feco-oral route, i.e. using contaminated water and food as the infected individuals shed the pathogen in their feces[1]. Typhoid fever is the clinical manifestation of Salmonella infection; it is characterized by sustained fever in a stepladder pattern and other non-specific features like headache, decreased appetite, nausea, constipation, or diarrhea. Children under five may present with diarrhea, febrile seizures, nausea, and neurological manifestations[2]. Despite continuous efforts to eradicate this disease, Typhoid fever remains a significant health hazard worldwide. WHO estimates 11-21 million cases and 128,000-161,000 annual deaths from typhoid fever globally, occurring more commonly (more than 70%) in South Asia. Pakistan is among the top five countries and has been declared endemic for Salmonella typhi infection[3].

Children are affected most. In Pakistan, the reported incidence of typhoid fever in children between 5 and 15 years of age is 573.2/ 100,000 per year, and for the age group of 2-4 years, it is 413/100,000 per year[4]. Vaccines have aided in the prevention of typhoid fever. However, they are not recommended for children under two years of age[5]. Typhoid fever, if left untreated, can have severe life-threatening complications; the most common complication that can prove fatal is intestinal perforation; such complications are more common in underprivileged countries due to a lack of resources that leads to delay in diagnosis[6]. Effective antibiotics reduced mortality in the pre-antibiotic era to 10-30%, to <1%. In Pakistan, it is <2% [7]. However, the excessive and unjustified use of antibiotics has produced resistance among salmonella strains.

Chloramphenicol, trimethoprim-sulfamethoxazole, and ampicillin were the first-line drugs for typhoid fever. Multidrug-resistant salmonella strains are resistant to first-line drugs. Second-line drugs include fluoroquinolones and third-generation cephalosporins. The emergence of resistance to first and second-line drugs (extended drug resistance) has been a matter of concern; these strains are sensitive to only azithromycin and meropenem. Extended drug-resistant (XDR) Salmonella was first reported in Hyderabad in 2016. WHO has estimated that over 14,000 XDR salmonella cases have been reported in Pakistan[8]. Blood culture and culture of bone marrow aspirate are gold standard laboratory investigations for

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diagnosing enteric fever. Bone marrow culture remains uncommon due to the invasiveness of the procedure. Widal test, which detects the antibodies against the O and H antigen of S. Typhi, has widely been used in low-resource countries. Widal and other serological tests are obsolete due to insufficient sensitivity and specificity[9,10]. Typhoid fever can cause cytopenia. Thrombocytopenia is most commonly reported but can also result in anaemia, Leucopenia, or, less commonly, pancytopenia[11].

The increasing number of cases of XDR salmonella is alarming for our country. It is the need of an hour to diagnose and manage all the cases of typhoid fever. Patients presenting with continuous fever should promptly be investigated for possible typhoid fever. In this study, we will evaluate the possible haematological changes observed in patients with XDR salmonella typhi infection.

METHODOLOGY

This descriptive cross-sectional study was conducted at the Haematology Department PNS SHIFA Hospital, Karachi, after approval and ethical clearance from the Institutional Review Board (PNS SHIFA SEC-491/5252). The study duration was from November 2021 to October 2022. After a thorough literature search, we calculated a sample size 31 via the WHO calculator. We kept the margin of error at 5%, a confidence interval at 95%, and a typhoid fever prevalence of <2% in Pakistan.[7] Sampling was done using the non-probability consecutive sampling technique. A maximum number of available participants (70) during the study period were recruited. All children under 12 with positive culture for extended drug-resistant Salmonella typhi were included in the study. Patients above the age of 12 and children having co-morbidities that can cause cytopenias were excluded from the study. Our study only focused on cases with XDR S. typhi; blood cultures positive for XDR Salmonella paratyphi were not included.

Before enrolling these patients in the study, we obtained their consent, and the confidentiality of the patients was ensured. A detailed history and complete physical examination were performed.

Seventy children with a positive culture for XDR Salmonella typhi on the BD BACTEC automated blood culture system were recruited for the study. A 3 ml venous blood sample was taken in an EDTA container and gently and slowly mixed to ensure anticoagulation from these available cases. After providing appropriate quality control, a complete Blood count (CBC) was done on an automated CBC analyzer (Sysmex Kx-21). Microscopic examination of blood films was done to exclude clumping of platelets and agglutination of RBCs.

The study focused mainly on haematological parameters, i.e. haemoglobin (Hb), hematocrit, platelet count, total leucocyte count (TLC), and neutrophil to lymphocyte ratio. Cytopenias were considered as Hb < 12 g/dl in males and 11 g/dl in females, TLC < $4x10^9$/l, and platelet count <$150x10^9$/l. Data was analyzed using Statistical Package for Social Sciences (SPSS) 21.0. Descriptive statistics were expressed as mean ± standard deviation (Mean ± SD), and categorical data were presented in terms of frequency and percentage. These variables were compared, and the Chi-square test was applied to non-resistant and XDR typhoid cases. A p-value ≤0.05 was considered statistically significant.

RESULTS

Seventy children were included in the study; 41 (58.6%) were male and 29(41.4%) were female. All the children were under 12 years of age. The mean age was 8.17±2.92 years. Age and gender distribution are shown in **Table I**. **Table II** shows the hematological parameters of all XDR salmonella typhi infection cases.

Table I: Demographic Characteristics of the Patients (n=70)

Characteristics	Mean ± SD
Age	8.17±2.92 years
Gender	n (%)
Male	41 (58.6%)
Female	29 (41.4%)
Age Group	n (%)
≤ 5 Years	18 (25.7%)
6 – 10 Years	30 (42.9%)
>10 Years	22 (31.4%)

Table II: Hematological Parameters of Suffering from Resistant Typhoid (n=70)

Parameters	Frequency (%)	
Haemoglobin	Anemia	Normal
	36 (51.4%)	34 (48.6%)
Haematocrit	Low	High/Normal
	39 (55.7%)	31 (44.3%)
Total Leukocyte Count	Leucopenia	High/Normal
	31 (44.3%)	39 (55.7%)
Platelet Count	Thrombocytopenia	Normal
	38 (54.3%)	32 (45.7%)
Neutrophil to Lymphocyte Ratio	Low	High
	46 (65.8%)	24 (34.2%)
Bicytopenia	**Present n (%)**	**Absent n (%)**
	17 (24.3%)	53 (75.7%)
Pancytopenia	**Present n (%)**	**Absent n (%)**
	2 (2.8%)	68 (97.2%)

DISCUSSION

Extended drug-resistant Salmonella typhi (XDR S. typhi) infection is highly prevalent in Pakistan. The disease pattern and pathophysiology of typhoid fever are complex. The symptomatic phase of the disease follows the initial incubation period of 7-14 days; during the first week, increasing bacteremia causes sustained fever. Rose spots and splenomegaly characterize the second week, while the patient is most likely to develop complications in the third week[12]. Diagnosing typhoid fever promptly to avoid the dreaded complications is very important.

The widal test was the most widely used investigation for typhoid fever. It is based on the agglutination of antibodies against the O and H antigens of S. typhi. Widal tests can give false negative results early in the course of the disease and false positive results in patients with previous exposure or a history of vaccination. Similarly, typhidot is primarily used to diagnose typhoid fever by detecting the IgG and IgM antibodies against the outer membrane proteins. Typhidot also lacks sensitivity and specificity. Molecular diagnostic techniques like nucleic acid amplification testing have yet to flourish in diagnosing typhoid fever. Blood culture is the gold standard laboratory investigation for the diagnosis of enteric fever. A sample for blood culture is ideally taken before starting antibiotics. Unfortunately, this practice is uncommon in our setup. Obtaining a blood culture after starting antibiotics causes decreased chances of pathogen detection.

In our study, most of the patients were in the age group of 6-10 years. This age group is particularly vulnerable to infections that are transmitted through the feco-oral route due to the consumption of unhygienic street food. Similar age distribution has been reported in a previous study by Abiduzzaman MF *et al.*[13]

Gender distribution in our study is also comparable to previous studies, i.e., male predominance. One prior study by Ahdi SG *et al.*[14] attributed the male predominance to the fact that parents are more eager to seek medical advice for their male children. Among the haematological parameters, in our study, anaemia had a strong association with XDR salmonella infection, with an overall percentage of 51.4%. Tashfeen S 2021[15] compared the frequency of anemia among Non-resistant and XDR S. typhi patients to 37.7% and 63.6%, respectively. Similar results were obtained by Dhilon SP et al.[16] in which anemia was seen in 47.8% of cases. Abro et al. reported anemia in 61.3% of patients with typhoid fever. Anemia in typhoid fever occurs due to bone marrow suppression, as demonstrated in some studies, but as cases with XDR S. typhi infection are more prone to develop complications, anemia in such cases may also be secondary to intestinal bleeding or perforation.

Total leucocyte count was also analyzed in our study, and 31 children (44.3%) showed Leucopenia; 4 children (5.7%) had leukocytosis, while the remaining children (50%) had normal leucocyte count. Prayudha R 2021[17] their study estimated that 28% of children had Leucopenia. This finding was common among children with a history of short hospital stays, and administration of ceftriaxone also had an association with Leucopenia.

65.8% of patients in our study also showed a low Neutrophil to Lymphocyte ratio (NLCR); this is partly due to neutropenia that causes a relative lymphocytosis, as stated by Gaffar A 1992[18] On the contrary, Raza SK 2022[19] concluded that NLCR has a higher value among typhoid patients owing to increased neutrophil margination in bacterial infections.

Thrombocytopenia was seen in 54.3% of cases in our study. The number of cases with thrombocytopenia is higher in our study than in previous studies. In a study by Tengli MB 2017[20] low platelet count was seen in only 10% of cases.

Seventeen children (24.3%) also showed bicytopenia in our study; this could partially be related to the fact that laboratory investigations could not rule out nutritional deficiencies due to poor resources. Children of this age group are prone to develop iron deficiency; thus, their haemoglobin levels are below the required level for age and gender.

Pancytopenia was observed in only two children in our study; the likely cause of pancytopenia is bone marrow suppression. Subhan M 2017[21] in a study, performed a bone marrow biopsy of 48 patients with pancytopenia in typhoid fever. Hypocellular marrow was reported in 10.6% of cases, and 28.6% showed dysplastic changes. However, hemophagocytosis was a common feature in all the cases; this explains the pathophysiology of cytopenias in typhoid fever.

Our study aimed to determine the degree of cytopenia observed in patients with XDR S. Typhi infection. Compared to previous studies, we observed a higher percentage of cases of cytopenias as most of the studies were based on non-resistant strains of S. typhi. As demonstrated in the results, patients with a history of sustained fever and cytopenias should be suspected of having typhoid fever.

This study was single-centred and had a small number of patients. The reporting was incomplete, and travel histories were not explored. The genetic characteristics of resistant isolates were not studied. The population studied was the same ethnic group with limited resources and time. A widespread study is recommended for more reliable results.

CONCLUSION

Haematological parameters are significantly influenced by XDR S. Typhi infection, with variations ranging from monocytopenia to pancytopenia. The possibility of XDR S. Typhi infection should be considered while investigating patients with suggestive clinical presentation and cytopenias on CBC.

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AUTHOR CONTRIBUTION

Tahir N: Acquisition, drafting, analysis, interpretation of data for the study
Khattak SAK: Concept, design, analysis, interpretation of data for the study
Jamal N: Revision of data for intellectual content production
Shaikh GM: Revision of data / statistics and final approval of the version to be published
Ahmed R: Revision and proofreading
Ahmed W: Revision and proofreading

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