Journal of Immunological Techniques in Infectious Diseases

Review Article A SCITECHNOL JOURNAL

Assessment of Hematological Parameters in Typhoid Fever

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Received date: 10 January, 2022, Manuscript No. JIDIT-22-51459;

Editor assigned date: 12 January, 2022, PreQC No. JIDIT-22-51459 (PQ);

Reviewed date: 27 January, 2022, QC No. JIDIT-22-51459;

Revised date: 14 March, 2022, Manuscript No. JIDIT-22-51459 (R);

Published date: 21 March, 2022, DOI: 10.4172/ 2329-9541.1000323.

Abstract

Typhoid fever acute and unspecified infection of the reticuloendothelial system caused by *Salmonella typhi*, and causes substantial hepatic complications and biochemical changes. Currently, the diagnostic test is isolation of bacteria from blood, stool, and rarely urine, but serologic tests are still commonly used. There is still a need to introduce rapid and reliable test for typhoid fever. The main purpose of this study was to determine the hematological variations in adult typhoid patients as compared to healthy control. A total of 50 patients and 50 healthy individuals were enrolled in this research, and variations in hematological factors were studied. Hematological parameters were found deranged in typhoid fever including hemoglobin (low, 10.97 ± 0.88), hematocrit (low, 37.72 ± 1.40), ESR (high 45.08 ± 13.42), platelet count (high 588840 ± 97185), WBCs count (high 38267 ± 22279), neutrophil percentage (high, 73.56 ± 9.96), lymphocyte percentage (low, 21.24 ± 10.08), and NLCR (high, 5.14 ± 4.00) as compared to healthy control group. This differentiating pattern is easy to obtain by minimal invasive procedure and can be used for typhoid infection diagnosis.

Keywords: Typhoid; Leukocytosis; NLCR; Thrombocytosis; He matological parameters; Diagnosis

Abbreviations: LFTs: Liver Function Test; CBC: Complete Blood Count; EDTA: Ethylene Diamine Tetra Acetic acid; SIM: Sulphur Indole Motility agar; TSI: Triple Sugar Iron agar; NLCR: Neutrophil to Lymphocyte Cell Ratio; ESR: Erythrocyte Sedimentation Rate; ROC: Receiver Operating Characteristic; AUC: Area Under the Curve; WBCs: White Blood Cells

Introduction

Typhoid fever is a general bacterial infection triggered by a gram-negative *bacterium Salmonella enteric* subspecies *enterica* serotype typhus (*Salmonella typhi*), and it is communicable disease that can be transferred orally. It is caused by consuming contaminated food and impure water; by the urine or feces of infected carrier [1]. When a person is infected with typhoid he seems to have fever, abdominal pain, relative bradycardia, headache, and splenomegaly [2]. Classical duration of disease is 4 weeks and after this duration condition start changing to normal. In the first week acute symptoms like toxemia, constipation and high fever occur, and severe condition like diarrhea in the second week, and more severe and highly attention gaining conditions splenomegaly, bone marrow findings and some other complications such as perforation and intestinal hemorrhage are seen in the third week [3].

Typhoid is a chief reason of morbidity and mortality throughout the world, causing an approximately 16.6 million new infections and 600,000 deaths per year. In Asia almost death rate is 80% and in Pakistan morbidity of typhoid is relatively high and needs public health interventions. Hot months have higher incidence of typhoid, and now although incidence of typhoid fever has been decreasing, but sporadic outbreaks continue to occur still [4-6]. It is estimated that number of new cases of typhoid is around 27 million and it happen with mortality around 200,000 mostly in South, Central, and Southeast Asia [6]. In typhoid disease transmission is human host-limited and high hazard for disease is more prevalent in low-and center salary based nations where typhoid causing Salmonella is endemic, and that have poor sanitation system, and absence of access to safe food and water [7]. The major way of spreading this infection is contamination with human faces and the typical vehicle is sullied water, nourishment, desserts, crude organic products, vegetables, squeezes, and contact with contaminated carriers or patients [8].

Literature Review

This disease has incubation period of 7 to 14 days and the most predominant symptom is fever [9]. It is seen that most of the serotypes of typhoid infections are detected purely on clinical grounds and treated on the base of presumptions. Laboratory capability is limited in many areas where there this disease is endemic and it makes difficult to diagnose typhoid fever clinically, as the presentation of this disease is diverse and usually similar to those observed in other febrile illnesses [10]. In laboratory typhoid fever diagnosis requires purification and then identification of Salmonella enteric serotype Typhi. Isolated Salmonella from blood, urine or stool is the most trustworthy mean of confirming the infection, and the most standardized diagnostic method is blood culture and it can be positive in 60-80% of the case [11,12]. However, in our country, patients often take antibiotics before proceeding for laboratory investigations, so the bacteria isolated from the blood cultures is only 40 to 60% of the typhoid cases. Possibility of blood culture positivity decreases with the time being; it is highly positive in first week but after that it goes low positive and becomes negative in the fourth week [13]. Stool culture is likewise an essential aid for diagnosing typhoid fever; it might be performed to certain when blood culture is negative. Culture of the upper gastrointestinal tract utilizing a duodenal string can be important yet the procedure is ineffectively endured by youthful youngsters [12,14].

Hematological derangements are common in typhoid fever, which can be used to diagnose and assess the prognosis of this condition [15,16]. So in this study our aim is to compare the hematological findings among typhoid patients and healthy individuals, and come up with some differentiating parameters that can be used as diagnostic markers for typhoid fever.

Materials and Methods

Sample collection and processing

In this study 100 individuals were included; 50 with typhoid fever and 50 healthy individuals as control group. This study contains confirmed typhoid patients, and typhoid test was performed to confirm typhoid fever. Venous blood of these 100 subjects was collected into both Ethylene Diamine Tetra Acetic acid (EDTA) containing vacationers for plasma and plain vacationers without anticoagulant for serum. Individual patient consent was not taken because all data was retrospectively retrieved through the Laboratory Information System (LIS). EDTA anti coagulated blood samples were used for analyzing full blood count while the serum samples were used for diagnosis of typhoid fever in patients. We centrifuged the blood samples without anticoagulant at 6000 rpm for 10 min at 4ºC, and the serum was aliquot to reduce the breakdown of the proteins in the serum, the then immediately specimens were placed and remained frozen at -20°C till further processing [17,18].

Blood culture and antimicrobial susceptibility testing

For blood culture technique, 5 ml of patient's blood was inoculated to blood culture bottle filled with blood culture medium, and were incubated at 37°C for next 7 days. After 48 and 72 hours by manual method, the broth was subculture on MacConkey agar and blood agar. Next day gram stained was used for isolates and these isolates were recognized by standard biochemical methods (Urease, TSI, SIM and Citrate). As a result, presence of typhus strains known as positive and if there is no typhus strain present it will be known as negative. Modified Kirby-Bauer disk diffusion method was the method used for antimicrobial susceptibility investigation. Strains are resistant to trimethoprim and ampicillin, and following antimicrobial agents (zone size for sensitive) were used: gentamicin (15 mm), tetracycline (19 mm), ampicillin (17 mm), ceftriaxone (21 mm), chloramphenicol (18 mm), ofloxacin (16 mm), ciprofloxacin (21 mm), nalidixic acid (19 mm), and norfloxacin (17 mm) [19,20].

Rapid diagnostic test

Serum was evaluated by using TyphiDot (CTK) rapid diagnostic kit. Patient serum, plasma, or heparinized whole blood is used to find out the antibodies IgG or IgM *via* TyphiDot technique.

Infection markers

Aero set 2.0 analyzer is an instrument which uses fully automated enzyme-linked immunoassay technique for the measurement of CRP levels in the patient's serum (Abbott Diagnostics, Santa Clara, CA, USA). For counting of White Blood Cells (WBCs), neutrophil and lymphocyte percentage, platelet count, and RBCs parameters System XE-2100 hematology analyzer (System Corporation, Kobe, Japan) was used. The Neutrophil to Lymphocyte Cell Ratio (NLCR) was calculated by dividing the neutrophil percentage with lymphocyte percentage [7]. Hematological parameters which were included are hemoglobin, hematocrit, platelet, WBCs, Erythrocyte Sedimentation Rate (ESR), lymphocyte and neutrophil percentage, and NLCR.

Statistical analysis

Statistical analysis of our study was performed using Minitab 19. Graphical presentation and descriptive analysis was performed for all parameters. Descriptive statistics are calculated for this data in the format of mean of the data and standard deviation (mean ± SD) of the data.

Results

A total of 50 patients and 50 healthy individuals were enrolled in this study at Allied Hospital Faisalabad. Blood samples were prepared and processed for further analysis. Fever, anemia, relative bradycardia, toxic and sick look, abdominal tenderness, splenomegaly, hepatomegaly, and jaundice were the predominant clinical signs at the time of sample collection in typhoid patients. The results of hematological derangements which we got in our study in typhoid patients are; decreased hemoglobin, thrombocytosis, leukocytosis, lymphocytopenia, and raised Erythrocyte Sedimentation Rate (ESR) and neutrophil count. In all cases rate of illness and recovery rate was determined by the sequential evaluation of physical examination, hematological and biochemical parameters. Progress towards health from the disease was monotonous and all the patients were discharged in good health.

There was no huge difference in the statistical values of HB in typhoid fever as compared to healthy individuals. The hemoglobin level was found slightly low in typhoid patients (mean ± SD, 10.97 ± 0.88) *Vs.* healthy control group (12.63 ± 1.129), and the hematocrit level was found low in typhoid patients (37.72 ± 1.40) *Vs.* healthy control group (44.52 ± 2.54). Significant difference was noted in the ESR values of those affected with typhoid fever in comparison to control group. The mean value of ESR in typhoid patients and healthy control group was (45.08 ± 13.42) and (10.46 ± 4.315), respectively.

The platelet count was high in typhoid patients (588840 ± 97185) in comparison to healthy group (271600 ± 76529). Similarly, the WBC count in the study was significantly high (38267 ± 22279) in comparison to healthy control group (6562 ± 1645), and a WBC count above 11000 is termed leukocytosis. In the study, 48/50 patients had a WBC count above 11000.

The neutrophil percentage was high in typhoid patients (73.56 ± 9.96) *Vs.* healthy group (53.06 ± 9.26), and in this study it was seen significantly lower percentage of lymphocyte; (21.24 ± 10.08) and (31.82 ± 6.317) in typhoid patients and healthy control group, respectively. There was a significant difference in the SD, minimum and maximum values of NLCR parameter; high in typhoid fever (5.14 ± 4.00) as compared to healthy group (1.74 ± 0.47).

Discussion

Typhoid fever is an infectious disease of acute sickness, and is a complex process which passes through many stages (from low infection rate to high infection rate) of infection [21,22]. During the incubation time of 7-14 days of disease patient remain asymptomatic and during these period bacteria get hold on the host cell and infect the macrophages and spread throughout the reticuloendothelial system. After onset of symptoms in first week, the temperature elevates progressively following bacteremia, and then in the second week abdominal pain, raised spots, and splenomegaly begins to develop. Complications occur in the third week and are characterized by further powerful intestinal inflammatory response with associated necrosis which might result in hemorrhage and perforation. History tells us that more than a century ago serological approaches were used to diagnose typhoid fever and it begins with the development of the Widal test [12,21,23,24].

Typhoid fever patient can have multiple changes in physiological condition and usually hematological changes are anemia, thrombocytopenia, Disseminated Intravascular Coagulation (DIC), and eosinophilia. It is observed that hematological changes occur due to bone marrow suppression, and hemophagocytosis, and in our routine practice some old markers like C-Reactive Protein (CRP), WBCs and neutrophil count are still beneficial infection markers to diagnose the typhoid fever [25,26].

As in this study our aim was to get differentiating hematological parameters in typhoid fever patients, so lots of derangements in hematological parameters were found in comparison to healthy control. Average platelets count was found increased significantly in patients of typhoid fever than control group in accordance with a previous report by Elisa et al. in typhoid patients [27]. Similarly, ESR values were found high in these typhoid patients. In 2015 same finding; high values of ESR have been reported in 100% of cases of Salmonella myocarditis [28,29]. Hemoglobin levels were decreased from normal value in patients in accordance with the previously report [30].

We found that leucocyte counts were high in typhoid patients as compared to healthy control. Further in differential leucocyte count, mean neutrophil percentage was high and mean lymphocyte percentage was low in typhoid fever patients. Previously in numerous stressful conditions it was observed that similar increase in neutrophil counts and a decline in lymphocyte counts have been seen. The reasons behind increased neutrophil counts are deamination and delayed apoptosis of neutrophils, and stimulation of stem cells *via* growth factors, while the mechanisms behind lymphocytopenia are redistribution of lymphocytes and imagination inside the lymphatic system and characterized by enhanced apoptosis [26,36-37]. Wyllie et al. found the medical value of lymphocytopenia as an indicator to diagnose bacteremia in emergency patients, and came to the point that lymphocytopenia is predictor of bacteremia in typhoid fever patients [31]. Lymphocytopenia can be used in the diagnosis of typhoid infection.

In recent researches, the NLCR parameter has been discovered as a very useful and simple indicator in numerous clinical situations, and has been studied in colorectal cancer, lung cancer, orthotropic liver transplantation, cardiovascular medicine and the value correlate well with overall and the cancer-specific survival [38-44]. First time this marker was used by Goodman et al. to assess its diagnostic potential in appendicitis, and they came with the point that the NLCR is a more sensitive than leukocytosis alone for differential diagnosis of appendicitis [26,38]. Recently, this parameter has been used as a simple infection marker to predict bacteremia in infectious emergency patients as compared to WBC count, neutrophil, and CRP level [33]. Further, NLCR was found increased in patients admitted with *Salmonella Typhi* infection and our study complements to this outcome by showing that this indicator is of much importance in patients with typhoid fever and it can help to diagnose typhoid fever and afterwards assessing prognosis and severity of typhoid fever. NLCR is an easy parameter to implement just using already available parameters of CBC (WBC count, neutrophil and lymphocyte percentages), and calculating this parameter is very easy and one more benefit of this parameter is, it does not require separate testing, so this NLCR has even more advantage in diagnosing typhoid. Moreover, the ratio of neutrophil and lymphocyte counts-referred to as the NLCR-has even higher value in diagnosing typhoid fever.

Our study has some limitations, and first of these is that this is a single hospital based study and in Faisalabad city only, so the reported findings in our study must be validated in other localities also. Secondly, some recently used infection markers like neutering, procalcitonin, and pro-adrenomedullin were not used in our study, due retrospective data collection for this study. Third, these findings should be explored in a separate prospective validation on large sample size. Fourth, there are lots of other causes for lymphocytopenia in addition to infection, such as malnutrition; induced apoptosis or effected lymphocyte maturation *via* bone marrow hypoplasia, so in our study nutritional aspect was also not judged as a confusing feature for lymphocytopenia [45,46]. Fifth, positive blood cultures were used as the gold standard for diagnosis, however, blood culturing are error prone, especially, due to taking improper blood amount and the sampling time in relation to start of antimicrobial treatment [47]. And also adherence to Standard Operating Procedures (SOPs) for blood sampling has to be evaluated in prospective validation study.

Conclusion

In conclusion, typhoid fever affects hematological parameters significantly. WBCs count, ESR and platelet count were above the normal range. Hemoglobin and hematocrit was below the normal value. Neutrophil percentage was found high and lymphocyte percentage was found low in typhoid fever as compared to healthy control, resulting in high NLCR in typhoid fever patients. This differentiating pattern of hematological parameters is easy to obtain by minimal invasive procedure and can be used for the diagnosis of typhoid fever.

Statement of Author Contributions

Syed Kashif Raza and Hina Jawaid participated in the design, interpretation of the studies and analysis of the data and write-up of the manuscript. Hina Javaid collected the data and Syed Kashif Raza supervised the study.

Acknowledgements

Authors are thankful to Hafiz Mohsin and Basharat, for their kind cooperation in sample collection. Our special thanks are due to all typhoid patients who voluntarily gave their blood sample for this research project.

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