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Hematology Parameter Based on Tubex TF® Color Scale Result in Typhoid Fever Patients

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Abstract

BACKGROUND: Infection with Salmonella enterica subspecies serovar Typhi and Paratyphi A, B, and C causes typhoid and paratyphoid fever. This is a major source of mortality and morbidity, especially in youngsters. Typhoid fever frequently causes non-specific symptoms. Symptoms that appear obstructively are comparable to those seen in other infectious illnesses and have similar clinical manifestations. Complications can be avoided with early detection and treatment.

AIM: This study will look at the hematology parameter profile to determine whether there are any hematological differences between patients with high and low antibodies.

METHODS: The research was conducted in Bengkulu, Indonesia, in June 2020 until July 2021. Subject collection was done according to inclusion and exclusion criteria. Subjects have given informed consent. The research included a total of 39 subject patients. Demographic data were collected using a questionnaire and 3 mL of blood was taken. Blood was examined for Tubex TF® and hematological examination. Statistical analysis used independent t-test and performed with SPSS.

RESULTS: The research included a total of 39 subject patients. Most were male and most were aged 21–25 years. The most common symptom felt by the subject was gastrointestinal complaints. The analysis was carried out based on the color scale value of the Tubex TF® which was divided into two groups, 4 color scale groups and 6 color scale groups. Hematological parameters of hemoglobin, hematocrit, WBC, platelets, monocytes, lymphocytes, and neutrophils showed no significant difference. However, color scale group 6 showed lower value than 4 color scale group.

CONCLUSION: Hematological indicators in typhoid fever were not substantially changed in Tubex TF®-positive typhoid fever patients. Clinicians can offer the same therapy in instances with typhoid fever, despite having differing positive Tubex TF® scores.

Introduction

Typhoid and paratyphoid fever are also known as enteric fever. This disease is caused by infection with *Salmonella enterica* subspecies serovar Typhi and Paratyphi A, B, and C [1]. *S. enterica* is classified into six subspecies, consisting of 1500 serotypes from subspecies 1, which is the main cause of infection in humans by *Salmonella bacteria* [2].Some countries with clean water facilities and inadequate environmental sanitation are typically endemic locations for typhoid and paratyphoid fever. This is notably prevalent in South and Southeast Asia, as well as Sub-Saharan Africa. This is a leading cause of death and morbidity, particularly among children [3], [4]. About 10–15% of hospitalized patients are estimated to have complications. At risk, the incidence of these complications increases at the age of the child and when it is too late to give antibiotic therapy [5], [6].

A meta-analysis study discovered a greater overall incidence of problems than previously reported, as well as a clear relationship between the length of symptoms before admission and the probability of major consequences [4], [5]. Non-specific symptoms often appear in typhoid fever. Symptoms that arise obstructively resemble other infectious diseases and show similar clinical symptoms. Hence, the differential diagnosis caused by other infectious diseases must be considered so that the clinician can make a correct and appropriate diagnosis [7]. Symptoms of typhoid fever begin with complaints in the digestive tract and then with non-specific symptoms of other organ systems, causing various complications [7]. Enteric fever is seen with an alternating pattern of ups and downs. Fever is especially felt at night. In addition, it is accompanied by symptoms of headaches and abdominal pain [7].

The process of bacterial immunopathogenesis begins with the recruitment of lymphocytes and

mononuclear cells due to the proliferation of Payer patches then induces necrosis and causes ulceration of the intestine. Endotoxin in *Salmonella typhi* bacteria has an important role in pathogenesis. The presence of lipopolysaccharide in cell finding causes endotoxinemia, causing vascular hyperactivity and release of catecholamines, causing focal necrosis and bleeding [8].

Early diagnosis and appropriate treatment will prevent complications. At present, the incidence of death is decreasing, but the complications that occur are increasing [5]. If the diagnosis is delayed until the 3rd week, the patient will be more likely to develop complications, anorexia and weight loss will occur. There may be an increase in perforation in the gastrointestinal tract over time so that it will increase the pressure on the abdomen and cause peritonitis [7]. The clinical approach to cases of typhoid fever is very important. If the patient lives in an area of poor water hygiene or there is a history of travel from an endemic area characterized by a fever of more than 3 hairs with abdominal complaints, it will lead to clinical typhoid fever. In the 1st week, the diagnosis is difficult to establish so that a laboratory approach will help in establishing the diagnosis [9].The aim of this study was to investigate the hematology parameter profile in typhoid fever patients to help clinicians make a diagnosis and so on to see the hematological trend of differences between patients with high and low antibodies.

Materials and Methods

Geographical location

This research was carried out at Bengkulu City, Bengkulu, Indonesia. Medical Faculty and Health Science Laboratory Universitas Bengkulu and microbiology medical faculty Universitas Indonesia were used for sample collection and assay. Patients were recruited for the study after informed consent was given.

Study design

This is a case–control study. This study was conducted on patients in clinic and hospital in Bengkulu city. All of 39 patients were included in the study. Inclusion criteria: The age range 16–60 years old. Both sexes were included in the study. Patients with the complaints of fever and other gastrointestinal tract symptoms of typhoid fever were investigated for typhoid. Diagnosis was confirmed by Tubex TF®. All patients with Tubex TF® positive were selected by color scale score (2, 4, 6, and 8). Exclusion criteria for subject are pregnant and have major systematic illness such as liver disease, hematology disorders, and immunocompromised disease.

Data collection

Informed agreement was obtained, and a full clinical history was gathered to rule out the confounding conditions described above. In addition, the symptoms of the present illness were inquired about (typhoid). A questionnaire was used to collect information from people selected for the study, which included age, gender, socioeconomic position, and education. Symptoms are also inquired about and recorded on the questionnaire.

Collection of blood sample

Each patient had 3 ml of venous blood collected from them using a 5 ml disposable syringe for the CBC and typhoid test. Blood samples were taken from the subjects following conventional techniques. Samples were collected and deposited in EDTA universal containers. Following coagulation, the serum sample was utilized for the Tubex TF® test, while blood (plasma) samples were used to determine the whole blood count. Patients who tested positive for typhoid fever after the tests were enrolled for the research.

Tubex TF® detection

All samples were subjected to the Tubex TF® test (IDL Sweden). Amount of 45 μl of Tubex TF® test brown reagent was applied to the reaction well strip provided with the kit. After that, 45 μl of the patient's serum sample was added and incubated for 2 min. Following incubation, 90 μl of blue reagent was added, and the well strip was taped shut. After 2 min of shaking, the well was put on the Tubex TF® test color scale. After 5 min, the reading was obtained by comparing the color to the Tubex TF® test color scale (colors ranging from clear pink (negative) to bright blue (positive) are assigned scores of 0 and 10, respectively). Scores of 4 were considered positive [10].

Hematology test

Using the hematology analyzer (Mindray BC 3600 Plus®, P.R.China), whole blood was used to assess leukocyte count, erythrocyte count, hemoglobin, hematocrit (HTC), platelet count, and the proportion of lymphocytes, monocytes, neutrophils, eosinophils, and basophils.

Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS Software, IBM, USA) for Windows version 22.0 and GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). The Chi-square test was performed to assess the difference in categorical variables between two groups.

p < 0.05 was deemed significant. The independent t-test was used to compare the differences in numeric variables (hemoglobin, hematocrit, platelets, and WBC) between the two groups. p < 0.05 was considered statistically significant.

Results and Discussion

Demography distribution and clinical manifestation of subject

A total of 39 subject patients were enrolled into the study. The mean age ± SD of the patient under the study was 32.41 ± 11.15 years. The number of male subjects was 23 (59%) subjects and female 16 (41%) subjects. There was no significant difference between two groups. The subject was collected the data in 1–3 days febris. The dominant age in this study was 21–25 years old (Table 1).

Table 1: Frequency demography distribution of subject

Characteristic	N = 39	%
Sex		
Male	23	59
Female	16	41
Age		
15–20	4	10.25
21–25	10	25.64
26–30	6	15.38
31–35	6	15.38
36–40	4	10.25
41–45	3	7.6
46–50	3	7.6
51–55	2	5
56–60	1	2.5

The main complaints experienced by the subject were fever, nausea, vomiting, cephalgia, myalgia, arthralgia, and diarrhea. These complaints were experienced by almost all subjects (>80%). On physical examination, only a small proportion (<10%) found typhoid tone, hepatomegaly, and splenomegaly. Epigastric pain was found in most of the subjects (84.6%) (Table 2).

Table 2: Frequency clinical manifestation of subject

Characteristic	N = 39	%
Febris	39	100%
Nausea	35	89.7
Vomitus	33	84.6
Cephalgia	33	84.6
Myalgia	35	89.7
Arthralgia	32	82.1
Dyspnea	2	5.1
Fatigue	13	33.3
Typhoid tongue	3	7
Epigastric pain	33	84.6
Hepatomegaly	2	5.1
Splenomegaly	1	2.51
Diarrhea	33	84.6

Typhoid fever examination was carried out using the Tubex TF® test. The results obtained are in the form of scoring. A score of 0–3 is negative for typhoid fever, while 4–8 is positive. The examination obtained in this study did not get a score of 8. The distribution of Tubex TF® scores was 4 and 6. The following Figure 1 shows the results of the Tubex TF® examination.

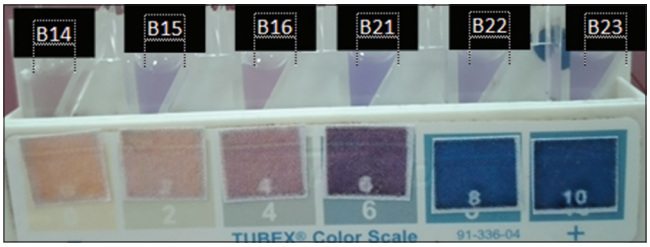

Figure 1: Tubex examination results. Quantitative; B14=2, B15=6, B16=4, B21=6, B22=6, B23=6. Qualitative; B14=Negative, B15, B16, B21, B22, B23=Positive

Hematological parameters different based on Tubex TF® score

Tubex examination results are categorized based on the Tubex TF® score. The groups are divided into 4 and 6 color scale groups. Furthermore, statistical analysis of hematology parameters will be calculated based on 4–6 color scale groups. Analysis of the relationship between hemoglobin values using the Mann–Whitney U-test between 4–6 color scale groups found no significant difference. However, the trend of the results shows that the mean value of 4 color scale groups is lower than 6 color scale groups. The mean value of 4 color scale groups is 14.33 ± 1.717 g/dl, while the mean value of 6 color scale groups is 21.23 ± 2.370 g/dl.

The value of hematocrit, leukocytes, and platelets also did not show a significant difference. However, the mean value of 4 color scale groups is higher than 6 color scale groups. The mean value of hematocrit 4 color scale group is 41.09 ± 5.035 while 6 color scale group is 39.13 ± 7.736. The leukocyte parameter shows that 6 color scale groups have a lower mean leukocyte (4313 ± 2293) than 4 color scale groups (5204 ± 2156). This event also applies to the platelet parameter, namely, the mean thrombocyte value is lower in 6 color scale groups (121.563 ± 79.502) compared to 4 color scale groups (131.739 ± 62.749).

Based on the independent t-test analysis, the comparison of monocyte, lymphocyte, and neutrophil values did not show a significant difference. The mean value of monocytes in the 6 color scale groups showed a higher value (7.2 ± 4.3) compared to the 4 color scale groups (4.5 ± 4.3). Likewise with the mean value of neutrophils, the mean value of neutrophils in the 6 color scale groups was higher (66.71 ± 8.425) than the 4 color scale groups (65.09 ± 10.23). However, the lymphocyte value in the 6 color scale group was lower (21.36 ± 7.218) than the 4 color scale group (25.23 ± 8.712) (Table 3).

Table 3: Comparison hematology parameter based on Tubex TF® score

Parameter hematology	Tubex TF® score		p
	4	6	
HAE	14.33 ± 1,717	21,23 + 2,370	0.1932[x]
HT	41.09 ± 5.035	39,13 + 7,736	0.342[y]
WBC	5204 ± 2156	4313 ± 2293	0.2245[y]
PLT	131.739 ± 62.749	121.563 ± 79.502	0.658[y]
MONO	4.5 ± 4.3	7.2 ± 4.3	0.461[y]
LYMP	25.23 ± 8.712	21.36 ± 7.218	0.175[y]
NEUT	65.09 ± 10.23	66.71 ± 8.425	0.729[x]

[a]Mann–Whitney U-test. [b]Independent t-test. HAE: Hemoglobin concentration, HT: Hematocrit, WBC: White blood cell, PLT: Platelet count, MONO: Monocyte, LYMP: Lymphocyte, NEUT: Neutrophil.

Discussion

Tubex TF® is a quick and sensitive test for detecting *S. typhi* in individuals with acute typhoid fever. The examination is founded on a semi-quantitative calorimetric test known as the inhibitory magnetic binding immunoassay [10]. Tubex TF® has a high sensitivity of roughly 76% and a specificity of 96–99% when compared to the typhidot and Widal test techniques [11].

One of the infectious diseases with non-specific symptoms is typhoid fever. Patients usually complain of enterocolitis at 12–48 h of incubation. The initial complaints felt by the patient were fever in the bacteremia phase, nausea, and vomiting, then the longer it was accompanied by diffuse abdominal pain, bloating, anorexia, and diarrhea, or also complaints of flu-like symptoms [12]. Symptoms of typhoid fever appear after 1 week of ingestion. The fever in typhoid fever goes up and down, the fever is felt at night, then it goes down the next day, this is called the step ladder pattern. Most patients with typhoid fever have gastrointestinal complaints. In the presence of hypertrophy of the payer patch, constipation may also occur. This is also seen in the results of this study. The dominant clinical symptoms are gastrointestinal symptoms.

Anemia, leukopenia, eosinophilia, thrombocytopenia, and disseminated intravascular coagulation are hematological disorders caused by typhoid fever. Important mechanisms in the process of hematological abnormalities are pressure on the bone marrow and hemophagocytosis [13], [14]. In this study, there was a decrease in the value of hemoglobin, platelets, and leukocytes in subjects with the Tubex TF® 6 color scale group compared to the Tube TF® 4 color scale group. The leukocyte value is in line with the previous studies which tend to be normal, but in this study, the 6 color scale group had a lower leukocyte value. Leukopenia is also found but only in a minority of cases. Abro *et al.* found 4% leukopenia [15], Ahmet *et al.* found 18% [16] and Rasoolinad *et al.* found 11.2% leukopenia [17]. Anemia, leukopenia, eosinophilia, thrombocytopenia, and disseminated intravascular coagulation are hematological disorders caused by typhoid fever. Important mechanisms in the process of hematological abnormalities are pressure on the bone marrow and hemophagocytosis. *Salmonella* is a Gram-negative bacteria that can release toxins in the bone marrow which is the main organ for myelopoiesis. This can cause a decrease in hemoglobin and leukocytes. The presence of invasion of people during the pathogenesis process such as the spleen, tonsils, bone marrow, and lymph nodes is the main cause of the decrease in the hematological value [4].

In this study, there was a decrease in the value of platelets in subjects with the Tubex TF® 6 color scale group compared to the Tubex TF® 4 color scale group. A study showed that 40% of patients had thrombocytopenia [15], in this study, all subjects had platelet values below 150,000/mm^3. The mean value of platelets decreased significantly in men compared to normal individuals, the increased antibody was suspected to be the cause of platelet activation [18]. Bacteria or soluble chemokines such as toxins have been reported to activate platelet function. Once activated, there will be disseminated intravascular coagulation which begins with the formation of an intravascular thrombus [19].

Salmonella is a Gram-negative bacterium that can cause toxins to be released in the bone marrow, which is the primary organ for myelopoiesis. This might result in a drop in hemoglobin and leukocytes. The major cause of the decline in hematological value is the existence of invasion of individuals throughout the pathogenesis process, such as the spleen, tonsils, bone marrow, and lymph nodes [4]. The results of the study from Shilpa *et al.* obtained data that there was anemia in 34% of patients with enteric fever [20]. Kakaria *et al*. reported that incidence anemia in typhoid fever cases is 42.9%, accordingly the study said that hemoglobin can decrease gradually during infection process [21]. In cases of typhoid fever, severe anemia is rare unless there is heavy bleeding in the digestive tract or hemolysis or other comorbidities [18].

Anemia, hemophagocytosis, and bone marrow suppression are abnormalities seen in typhoid fever. This is a major factor causing hematological disorders. Changes in the bone marrow by typhoid fever cause changes in body systems that lead to decreased levels of neutrophils, red blood cells, and platelets [18].

Conclusion

Hematological parameters in typhoid fever were not significantly different in typhoid fever-positive patients based on the results of the color scale value of the Tubex TF® examination. This does not indicate the significance of the scores on the Tubex examination. So that clinicians can provide the same treatment in cases of typhoid fever although based on different positive scores.

Recommendation

Diagnostic methods that can differentiate patient severity are necessary for effective and efficient treatment in early phase.

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