HEMATOLOGICAL PARAMETERS AND ITS ASSOCIATION WITH TYPHOID INFECTION AMONG TYPHOID SUSPECTED INDIVIDUALS AT SHANAN GIBE HOSPITAL, JIMMA, SOUTH WEST ETHIOPIA

BY: -

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A THESIS SUBMITTED TO SCHOOL OF MEDICAL LABORATORY SCENCE, FACULTY OF HEALTHY SCIENCES, INSTITUTE OF HEALTH, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF MASTER OF DEGREE IN CLINICAL LABORATORY SCIENCE, SPECIALITY IN HEMATOLOGY AND IMMUNOHEMATOLOGY

FEBRUARY, 2020

JIMMA ETHIOPIA

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULITY OF HEALTH SCIENCES

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FEBRUARY, 2020

JIMMA, ETHIOPIA

ABSTRACT

Background: *Salmonella species are easy to multiply in the blood stream and affects all system of the body. Salmonella infection is one of the major public health problems worldwide. Hematological abnormalities are common in typhoid patients, anemia, leucopenia, neutrophilia and thrombocytopenia are the commonest one. Thus a specific diagnosis and determination of hematological parameters are required for initiating and monitoring of the disease.*

Objective:*To determine hematological parameters and its association with typhoid infection patientsat Shanan gibe hospital Jimma,Southwest Ethiopia.*

Methodology: *Hospital based cross-sectional study was conducted from January 30, 2019 to August 30, 2019 at Shanan gibe hospital which is a generalized hospital located in Jimma city. The study was conducted among 394 subjects suspected of typhoid fever whom selected consecutively. Socio-demographic data were collected by structured questionnaire.Four milliliter (4ml) of EDTA anticoagulated whole blood was collected by venous sample collection method for Complete blood count, Blood film, and Widal test. Complete blood count was done byHumacount 30^{TS}/80^{TS}automatic hematology analyzer,Germany,humangmbh campony, where as widal test was performed by using the "O and H" antigen reagent on direct slide method and tube titration. Demographic data were edited, coded, and entered into Epi-data version 3.1 and exported to statistical package for social sciences (SPSS) version 20 for analysis by using of t-test and chi-square in addition to descriptive statistics.*

Result:*A total of 384/394 (97.5% response) cases of suspected typhoid fever were taken for final analysis,out of which Widal test titer reactive were 41(10.7%) and 343 (89.3% negative. There were 226 (58.9%) males and 158(41.1%) females.the participants in this study with the mean ± SD age were 26.31±12.93 years. In this study, there were significant mean difference of WBC, (p=0.04), RBC (p=0.00), Hb (p= 0.00), platelet count (p < 0.000), neutrophil counts (p = 0.03), mixed count (p=0.006) and MCV (p=0.04) among typhoid confirmed patients as compared to typhoid negative patients. Additionally, significant association of hematologic parameters were WBC, RBC, HGB, PLT, Neutrophil, and mixed (p<0.05) observed.Maximum number of the typhoid suspected patients were seen with leucopenia, 201(52.3%), anemia198 (51.6%) and neutrophilia, 159(41.1%); besides, patients also presented with 134(35.7%) lymphopenia, 134(34.9%) thrombocytopenia76 (19.8%) neutropenia, 105(27.3%) midgranulopenia, 62(16.1%) mid-granulocytosis, 70(18.2%) lymphocytosis, 51(13.3%) leukocytosis and thrombocytosis18 (4.7%) were observed respectively.*

Conclusion and recommendation: *Typhoid fever has significantly associated with hematological parameters therefore complete blood count is important. The major abnormalities observed in typhoid suspected patients were leucopenia, anemia, thrombocytopenia, neutrophilia and also mid granulopenia. Hematological change of WBC, platelet, neutrophil, mixed and hemoglobin may give as aclue for diagnosis of typhoid fever and used as valuable aids in patient's clinical management.*

Key words:*Ethiopia, Hematological parameter, Typhoid fever*

ACKNOWLEDGEMENT

I would like to express my sincere and deepest appreciation to my Advisors Dr. Tilahun Yemane and Mr. Wondimagegn Adissu for their valuable advice and constructive comments. I would also like to extend my thanks to Jimma University, Institute of Health and School of Medical Laboratory Science for giving me this opportunity. My appreciation also goes to clinical nurses at study area; all study participants, for their voluntarily participation, and medical laboratory staff member of Shanan Gibe hospital for their friendly collaboration and support me at every aspect. Finally, I am also grateful to my sponsor organization, Wollega University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATION AND ACRONYM

CBC: Complete blood count

EDTA: Ethylenediamine tetra acetic acid

ESR: Erythrocyte sedimentation rate

Hb: Hemoglobin

Hct: Hematocrit

IRB: Institutional review board

MCH: Mean corpuscular hemoglobin

MCHC: Mean corpuscular hemoglobin concentration

MCV: Mean corpuscular volume

PLT: Platelet

RBC: Red Blood Cell

SD: Standard Deviation

SPSS: Statistical package software for social science

SOP: Standard operating procedure

WBC: White blood cells

CHAPTER ONE: INTRODUCTION

1.1. Background

Typhoid fever is a systemic bacterial infection caused by gram negative rods. It is an acute infection of the blood and intestinal systems caused by the bacterium *Salmonella typhi(S.typhi)*. It is acquired through the ingestion of contaminated water or food. The disease is mainly associated with low socioeconomic status and poor hygiene, with human beings the only known natural hosts and reservoir of infection(1).

The bacteria are entering through food or water that is contaminated. They attach themselves to the intestinal epithelium where they penetrate lamina propria and sub mucosa, *S. typhi*are engulfed by monocytes (2). Bacteria have host defense evasion mechanism that enables them to resist intracellular killing and continue to multiply (3)**.**

The organisms survive and multiply within the mononuclear phagocytes and characterized by a rapidly developing infiltration which is associated with necrosis of the upper mucosa in large areas of the terminal ileum and colon (4).The bacteria are phagocytosed and have the ability to survive and multiply within macrophages of the lymphoid follicles, liver and spleen. Once the bacteria multiply to a certain threshold, in addition to their virulence and the host response, bacteria are released and sequestered into the blood stream. Following release into the bloodstream, they invade secondary sites such as the Peyer's patches of the terminal ileum, liver, spleen, bone marrow or the gall bladder. Bacteria that are excreted in the bile re-invade the intestinal wall or are passed on through the feces. Cell counts of patients with acute TF gave a median bacteria concentration of 1 bacterium per ml of blood (about 66 percent of which are inside phagocytic cells) and about 10 bacteria per ml of bone marrow. This indicates that the bone marrow has a higher concentration of bacteria and is a better site than blood for detection of S. Typhi in patients. S. Typhi infection has been shown to induce local and systemic immune responses in humans, but this provides incomplete protection against relapse and reinfection(4)

The course of disease is divided into four phases each lasting a week. In first week the temperature rises slowly with relative bradycardia, malaise, headache and dry cough. In the second week there is prostration with high fever and bradycardia followed by delirium and abdominal rashes in every 3rd patient also abdominal pain with constipation/diarrhea may occur. Hepatosplenomegaly and positive widal test occur in this week.By the end of third week if no serious complications are there the fever subsides slowly and gradually. In this disease multisystem are involved leading to various complications including hematological complications. Hematological manifestations in form of anemia, leucopenia and thrombocytopenia are well known in enteric fever(5).

Clinical manifestation of typhoidfever is variesfrom a mild illness with low grade fever, headache, constipation, extreme fatigue, joint pain, splenomegaly, inflammation of the intestine with the formation of intestinal ulcers, a characteristic rose-spot eruption on the abdomen. These are associated with some physiological changes in affected persons and these changes form part of the pathophysiology of the disease. Clinical diagnosis is difficult. In the absence of laboratory confirmation, any case of fever of at least 38 °C for 3 or more days is considered suspect if the epidemiological context is suggestive. Depending on the clinical setting and quality of available medical care, some 5–10%of typhoid patients may develop serious complications,the most frequent being intestinal hemorrhages or peritonitis due to intestinal perforation(6)Isolation of the causative bacteria in TF patients by culture remains the gold standard for diagnosis. Culture is the most reliable way of detecting typhoid in infected patients, and usually by blood culture, but bone marrow culture has a greater sensitivity. However, in most developing countries a serological test known as the Widal test is most commonly applied(7).

Laboratory confirmation should always be sought for clinically suspected cases. Confirmation by culture (or validated molecular methods, as available) is essential as typhoid fever, paratyphoid fever and other invasive salmonellosis can present as a non-specific febrile illness, and current serological tests lack diagnostic specificity. Confirmation is essential to assess the proportion of enteric fever caused by these different organisms, determine antimicrobial susceptibility and do molecular epidemiology studies. Blood culture is currently the preferred laboratory method in most endemic settings for the diagnosis of enteric fever and invasive nontyphoidal Salmonella

infections. While bone marrow culture has been shown to be approximately 50% more sensitive than blood culture, it is an invasive procedure that is impractical in most endemic settings and not appropriate for public health surveillance. Bone marrow specimens may still be submitted for culture when clinically indicated, for example, if other reticuloendothelial infection or malignancy is suspected. The test's enhanced sensitivity may also be useful in selected patients who have been heavily treated with antimicrobials. While blood culture is the most common method for laboratory confirmation, it has several limitations including relatively poor sensitivity, particularly when only one sample is collected and there is extensive use of antimicrobials prior to health centre or hospital presentation. As such, many clinically suspected enteric fever cases may lack laboratory confirmation and be culture-negative. Stool culture is not recommended for the diagnosis of acute enteric fever. A brief period of asymptomatic faecal shedding typically occurs following Salmonella infections; a subset of these patients will progress to long-term, asymptomatic carriage. Stool culture may thus be used for the detection of chronic carriers and to monitor faecal shedding in patients following acute typhoid fever. Although serologic tests are commonly used in many settings, current evidence suggests that these tests are limited by poor sensitivity and inadequate specificity, and so are inappropriate for use in routine surveillanceSeveral investigational serologic assays appear to show promise, but these are not commercially available at this time. All microbiology laboratories reporting Salmonella data should have an external quality assurance and quality control system implemented at all stages, including: h The minimum standard recommended for blood culture confirmation is to use a semi-automated system that will support isolation of common pathogens associated with vaccine preventable diseases. h Biochemical testing algorithms used for bacterial identification should be able to at least differentiate between Salmonella serovars Typhi, Paratyphi A and Salmonella spp. (not serovar Typhi or Paratyphi A)(8).

Hematological abnormalities are the abnormality of hematological parameters those selected that are related to the blood and blood forming organ which consist of red blood cells (RBCs), hemoglobin (Hb) and hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) total white blood cells (WBCs) and WBC differentials which uses in the diagnosis and monitoring of the disease (9)The hematological changes which are common in typhoid fever include; anemia, leucopenia,

eosinopenia, thrombocytopenia, elevated erythrocyte sedimentation rate (ESR) and chronic disseminated intravascular coagulation(10).

The occurrence of anemia, leucopenia, thrombocytopenia and eosinopenia is attributed to invasion of hematopoietic organs such as lymph nodes, spleen, tonsils, bone marrow by *S. typhi* causing depression of hematopoiesis. The bone marrow of typhoid patients shows myeloid maturation arrest, decrease in number of erythroblasts and megakaryocytes with increased phagocytic activity of histiocytes(11). Those abnormalities can occur when the complication happen to intestinal hemorrhage due to bleeding in congested payers patches. It is done to ascertain the degree of changes in some hematological parameters of typhoid patients as it is a multi-systemic disease. These changes may be attributed to suppression of bone marrow activity and haemophagocytosis which are the major attacking mechanism of *S.typhi*in typhoid patients (12).

The invasion of the organs by *S. typhi*which can also depress the rate of hematopoiesis may also explain the observed anemia and thrombocytopenia seen in typhoid patients. Most acute and chronic typhoid fever cases usually lead to anemia and thrombocytopenia, which results the generation of spontaneous bruises and prolonged bleeding lesions in the intestinal tract with the consequent danger of hemorrhage and intestinal wall perforations (13).

Absolute eosinopenia in the proper clinical settings can give a strong clue to the diagnosis of enteric fever. It could be used as marker in strongly suspected cases of enteric fever to promptly initiate therapy particularly in setting of limited diagnostic resources (14).

Generally, typhoid fever is associated with leucopenia and this is serving as a diagnostic aid and anemia is also involved. Neutrophilic leukocytosis is a feature of complicated typhoid fever and that lymphopenia is associated with typhoid because of typhoid fever, specific mediators released by cells which act on bone marrow to increase proliferation of neutrophils (15).

1.2.Statement of the problem

Typhoid fever is a major public health concern in many developing countries of the world as well as developed countries. It is endemic in the tropics and the second most common cause of fever next to malaria. It is continued to be important of causes illness and death, particularly among children and adolescents in south-central and southeast Asia (1).

According to World Health Organization (WHO) 2000 census report typhoid fever caused an estimated 21.7 million illnesses and 2,17, 000 deaths approximately per year (16) and estimates from the worldwide incidence of typhoid fever is approximately 16 million cases annually, with >600,000 deaths, of which seven million cases occur annually in South East Asia. Management of typhoid fever still requires more efforts (2).

As typhoid develops, the predominant symptom is fever. Usually have anorexia, nausea, and vomiting; in severe cases, constipation or bloody diarrhea will occur, and besides anemia, which is a common finding. Usually neutropenia and thrombocytopenia are observed in typhoid fever. It has been attributed to increased marginalization and defective granulopoeisis(17).

Elevated neutrophil (neutrophilia), with relatively low lymphocyte counts (lymphopenia), the involvement of neutrophil in the primary immune response to acute infection with *S. typhi*is usually associated with neutrophilia and relative or absolute lymphopenia (18).

Enteric fever causes thrombocytopenia and leucopenia and other hematological manifestation, such as anemia and bicytopenia. Early diagnosis and treatment may decrease morbidity and mortality (19).Thrombocytopenia and anemia are also supposed. It is typically seen as a complication during the course of typhoid fever (20). The magnitude of anemia observed during typhoid reported that (61.3%)(10).

A significant decrease in platelet levels observed. It suggests that platelets' activation could be a major factor when antibody level increases. Platelets were reported to be activated by some particulate factors like bacteria. Once activated, it undergoes viscous metamorphosis that leads to intravascular thrombus formation which is a prelude to disseminated intravascular coagulation.

The decrease in platelet observed could be either due to decreased production of platelets by the bone marrow during acute infection or in part sequestered during an enlarged spleen (21).

Eosinopenia is often absolute might be present in 70-80% cases,Eosinophils normally account for only 1 to 3% of peripheral blood leucocytes. In acute bacterial infection of infants, zero absolute eosinophil count was also reported; eosinopenia is described in adult and pediatric patients with typhoid fever. In children infected with enteric fever was diagnosed who reported eosinopenia in 72 % among pediatrics patients diagnosed with enteric fever(22) and found absolute eosinopenia in 73% cases of enteric fever. They observed that patients positive blood cultures had a peripheral blood smear of more than 1% eosinophil, suggesting a possible relationship between bacteremia and low eosinophil(14). The mechanism of decreasing the eosinophil count is considered to be, in part, secondary to sequestration of circulating eosinophils, mainly resulting from a chemotactic substance, C5a.eosinopenia has been reportedly observed in 72–77% of patients with enteric fever(23)(24).

The hematological changes obtained in the different study reported that erythropoiesis and myelopoesis were depressed as indicated by decrease hemoglobin, red blood cell and white blood cell counts. Typhoid fever can lead to cytopenia of variable degree, coagulopathies and hypo or hyper cellular bone marrow. In more severe and chronic cases, leucopenia is accompanied by thrombocytopenia, anemia, and reduced hemoglobin and hematocrit (25). Even though typhoid fever are significant effect on hematological parameters, there is no adequate studies related the associated effects of typhoid fever on hematologic parameter in Ethiopia. Therefore, the aim of this study will be carried out to determine the basic hematologic abnormality happened during typhoid infection and to give some clue for diagnosis.

1.3.Significance of the study

This study will provide information on the hematologic abnormality and for identifying the association during a typhoid infection in the study area.

Conducting this study will use to promote the diagnosis of an infected patient; it gives a clue for diagnosis of typhoid fever infected individuals towards the early detection and management of infection and ultimately for decreasing the burden of the disease particularly in the current study area. Additionally, there is no previous updated data on this finding in the study area. It serves as a base line for other study, can give information for policy makers, and also will give direction for physician and other concerned bodies about the basic hematologic changes and association of it to the disease.

CHAPTER TWO: LITERATURE REVIEW

2.1.Literature review

Typhoid fever is more common and remains an important public health problem in the world today particularly in developing countries and causes significant hematological changes (26).Study has been conducted on hematologic abnormality happened during the typhoid infection in different country the following result were obtained.

A prospective study conducted by *Kakaria A et al.* At Kasturba Hospital Mumbai, India in 2005 to 2006 on fifty enteric fever patients showed that low hemoglobin was found in 42.9% of cases. Leucopenia &Leucocytosis was seen in 21% & 10% patients respectively. Thrombocytopenia was seen in 30.9%(27).

The other conducted from 2006 to January 16, 2007, at an endemic area of Enugu Urban-Nigeria by *Okafor,A.* on hematological alterations due to typhoid fever showed that the mean differences between hematological values obtained for typhoid patients were compared with those of non-typhoid individuals. It was found that typhoid fever led to a statistically significant leucopenia, which is in the reduction of white blood cells (WBC) red blood cells (RBC) blood platelet counts as well as hemoglobin contents. In more severe and chronic cases, leucopenia is accompanied with thrombocytopenia, anemia, and hypohaemoglobinaemia and reduced hematocrit(13).

According to the study conducted in 2010 at Vom Christian Hospital and DadinKowa General Hospital in Plateau State, Nigeria. on hematological changes associated with *S. typhi by A. Dangana et al.* showed there was a significant decrease in the Hb, and WBC and also lymphocytes with reticulocytes count was significantly higher and a relatively higher in neutrophils as against those of apparently healthy control individuals, but there was a significant increase in monocytes and eosionophils, but there was no significant difference in basophiles count (15).

The study was conducted 2013 at King Edward Medical University, the hematological changes associated with typhoid fever by UnaizaQamar&JaveriaAijaz Out of 150 patients, 58 (38.6%) were found to have pancytopenia. Anemia was found in 92 patients (61.33%). Leucopenia was

found in78(52%) patients neutropenia 48(32%),neutrophilia12(23%),lymphopenia12(8%),lymphocytosis18(12%),monocytosis46(30.67%),thrombocytopenia 37.3% (17).

The other study conducted in 2014 by Ifeanyi et al 42 typhoid patients and 22 non-typhoid apparently healthy individuals who attended University Health Services Department of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Changes in some hematological parameters of typhoid patients and non-typhoid individuals showed that no significant difference($P>0.05$) in the mean values of total white blood cell count, monocyte and eosinophil counts of the typhoid patients and the non-typhoid individuals. But there were significant difference in the mean values of PCV, neutrophil and lymphocyte counts of the typhoid patients and the non-typhoid apparently healthy individuals respectively (28).

According to the study conducted 2015 at Kathmandu Valley, Nepal on Hematological parameters of culture positive patients Enteric fever done by Rana B et al, showed that a culture positive cases caused a significant decrease in total white blood cell, blood platelet count, lymphocyte, packed cell volume, eosinophils and hemoglobin ($p < 0.05$) compared to healthy controls. Similarly, enteric fever led to significant increase in neutrophil and monocyte count in enteric fever patients ($p < 0.05$). Anemia was found in 23.52 %.Leucopenia was found in 7.84% patients, Leucocytosis in 2.94%, neutrophilia in 28.43%, lymphocytopenia in 18.62%, monocytopenia in 47.05%, absolute eosinopenia in 93.13%, thrombocytopenia in 23.52% and thrombocytosis in 1.96%(25).

Another Study done in 2015 at Khaja Banda Nawaz Institute of Medical Sciences and Teaching Hospital Kalaburgi, India by Shilpa V. Uplaonkar et al ,on hematological profile in typhoid fever, Among 130 cases of suspected typhoid fever were taken, out of which 58 (44.61%) were positive for typhoid fever and 72(55.38%) were negative. In majority of the positive cases, neutrophilia was seen in 23 cases (39.65%), anemia in 20 cases (34.48%),Leucocytosis in 13 cases (22.41%) and thrombocytopenia in 10 cases (17.24%)(29).

Another study conducted in 11 Nov 2015 at LLR Hospital Kanpur who presented to Emergency Pathology Lab, GSVM Medical College Kanpur by Shrivastava K, Vahikar S and MishraV

Showed 121 patients diagnosed with typhoid fever of which 43 patients were admitted patients who had severe systemic manifestations and complications like hepatomegaly. Sex ratio was found to be almost equal with 50.5% male and 49.5% female patients. Maximum cases in male patients (32.7%) were seen in 11-20 yrs. age group while in females most common age group was 21- 30 yrs of age (38.3%). The differential count mean neutrophil percentage was 28% and mean lymphocyte percentage was 59% and mean eosinophil percentage was 2% Thrombocytopenia was observed in 39.7%. Leucopenia in 11.6% of cases and anemia was noted in 58.7% of total cases (12).The other study conducted in 2015 at mayiladuthuria, Nagapattinam, India, by Anusuya*et al* showed significant decrease in the WBC, HB,PLT,Neutrophil, lymphocyte observed compared with healthy individuals(21).

According to a comparative study conducted in 2016, at Word Mission Hospital, Aba, Abia State, Nigeria by Ozougwu, J.et al. on the hematological changes associated with male typhoid fever patients showed that Red blood cell was decreased and the difference was statistically significant at ($p < 0.05$). Similarly, HB content was decreased, both were statistically significant at ($p < 0.05$) on the other hand, WBC was decreased from while PLT was decreased and also the difference was statistically significant at ($p < 0.05$)(30).

The study conducted in 2016 by Modi R at Ahmedabad, Gujarat teaching hospital, India, showed that Leukopenia was seen in 11.2% and leucocytosis in 17.4% patients. Lymphocytosis was observed in 70.4% patients. The most common symptoms were fever, abdominal pain, vomiting, and anorexia, and cough, hepatomegaly, and splenomegaly(31).

A cross-sectional study conducted in 2017 at India to study hematological profile of Enteric fever patients, by Dr Neeraj Lata, showed on 100 patients attending OPD and IPD this study 46% of the patients were blood culture proven typhoid fever patients. Male children of school going age group were most commonly affected by typhoid fever. They found anemia, leucopenia,and thrombocytopenia in 47.8%, 6.5% and 21.5% of patients. Lymphocytosi s was seen in 10.9 % of patients (32). Thus, the current study is trying to look at these contradictory concepts in this new study setting, in shanan gibe hospital, Jimma southwest Ethiopia and identify the hematological abnormality present in typhoid fever in the study area.

CHAPTER THREE: OBJECTIVES

3.1. general objective

To determine hematological parameters and its association with typhoid infection among the patients suspected for typhoid fever at Shanan Gibe Hospital, Jimma,southwest, Ethiopia from January 30,to August 30, 2019.

3.2. specific objectives

- To determine hematologic parameters among typhoid suspected individuals at Shanan gibe hospital.
- To compare the hematological parameters of typhoid positive and typhoid negative patients in typhoid suspected individuals at Shanan gibe hospital.
- To identify the association of hematological parameters with typhoid infection in typhoid suspected patients at Shanan gibe hospital.

CHAPTER FOUR: METHODS

4.1. Study area

The study was conducted at Shanan Gibe Hospital which is located in Jimma city. Jimma is the largest city in south-western Ethiopia Found at 357 km from Addis Ababa. It is Oromia Region and is surrounded by Jimma Zone. It has an altitude of 1, 780 meters above sea level. Prior to the 2007 census, Jimma was reorganized administratively as a special Zone (33). Shanan Gibe Hospital is a generalized hospital established by oromia regional health bureau (ORHB) in 2004 E.C-.in Jimma zone with a catchment population of 1,088,707 from the town and nearby Woredas like:Dedo,Seka ,Kersa and ManaWoreda. Since its establishment it is widely serving the community in different service areas; Emergency outpatient services**,** Outpatient services, Inpatient services, Maternal and child health services, Labor and delivery services, Operation services, TBL/TB-HIV/MDR-TB treatment services, Comprehensive ART care services, medical services, Ophthalmologic services**,** Dental care and treatment services**,** Psychiatric services.

4.2. Study Design and Study Period

Hospital based cross sectional study was conducted from January 30, to August 30, 2019.

4.3. Population

4.3.1. *source population*

All individual attending at Shanan gibe hospital during the study period were considered as sources of population.

4.3.2. Study population

All patients who were coming to Shanan gibe hospital with typhoid fever symptoms during the study period were the study population.

4.3.3.Studysubject

Selected individual patients suspected for typhoid fever and mate the inclusion criteria were taken as the study subjects.

4.3.4. Sample size determination and sampling technique

4.3.5. Sample size determination

The sample size was determined based on the single population proportion formula. Since the prevalence of hematologic abnormality of typhoid infected individual in Ethiopia was not obtained, the prevalence of anemia in typhoid infected from the study conduct in India was (34.48%) used (28). The number of samples of typhoid suspected patients to be included in the study was calculated based on the following single population proportion formula,

$$n = \frac{Z^2 PQ}{d^2}$$

With a 95% confidence interval, 5% margin of error and an estimated Thus, the total sample size was 347. To calculate the final sample size, considering non-response rate (10%), the final sample size for this study was 394.

Where:

N = minimum sample size

Z α/2= 95% confidence interval (1.96)

P = Estimated prevalence rate (34.48%)

d = Marginal of sampling error

4.3.6. Sampling technique

Consecutive sampling technique was used until the required sample size was attained.

4.4. Eligible criteria

4.4.1. Inclusion criteria

- All clinically suspected cases of typhoid fever with any age and sex were included
- Patients volunteer to participate in the study.

4.4.2. Exclusion criteria

- Individuals who have known chronic disease status
- Individuals who had positive results for the screening tests of blood film for malaria and intestinal parasite.
- Pregnant women.

4.5. Study variables

4.5.1. Dependent variable

- Hematological parameters

4.5.2. Independent variables

- ✓ Socio-demographic variable; Age, Sex, Residence
- ✓ Typhoid fever
- ✓ occupational status
- ✓ Educational level

4.6. Data collection tools and procedures

Demographic data were collected by using interviewer administered pre-designed questioners. The questionnaire have four part which includes; socio-demographic characteristics, behavioral and nutritional status, clinical features and history of chronic disease. Hematologic parameters were performed in the laboratory interpreted by senior staff of the hospital and the widal, titration and blood film was also performed.

4.6.1. Sample collection

The sample collected from the study participants were processed by following the SOP of each test at Shanan gibe hospital.

- ✓ **Blood sample collection and processing: -**About 4 ml of blood sample were collected into EDTA anticoagulant tubes. Whole blood was used for hematological analysis and identification of hemoparasite whereas; plasma was used for serological test. Hematological and serological analysis was processed according to the SOP.

✓ Stool specimen was collected in a clean, dry container and processed according to the SOP.

4.6.2. Laboratory analysis

✓ **Hematological analysis**

Four (4) ml of EDTA anticoagulant whole blood was used for complete blood count: Hb, RBC, red cell induces; total WBC count, WBC differential count and PLT count. Complete blood counting was done by Huma count 30^{TS}/80^{TS} hematology analyzer Germany. The Huma count 30^{TS}/Huma count80^{TS} hematology analyzer is determines 22 hematology parameters and fully automated 3- differential cell counters designed for in vitro diagnostic use developed for small clinics and point of care lab offices (Annex V:I).

✓ **Peripheral smear:** Blood film was prepared for hemo-parasite examination (Annex V:II).

✓ **Stool examination test**: Direct wet mount test was done for parasitological examination.(Annex V:III)

✓ **Widal test analysis:** plasma was used for Widal test and titration whole blood was centrifuge at 3000 rpm. For 5 min and perform using the Widal test kit containing O and H antigens of S. ty*phi* (Annex V: V).The Widal test is simplest, over utilized, and it is diagnostic investigation tool available in the laboratories. The widal test was performed with standardized kits (BEACON Diagnostics,India). Plasma samples of patients were screened with a slide agglutination test which measures agglutinating antibodies against the lipopolysaccharide 'O' and protein flagellar 'H' antigens of S. *typhi.* Serial dilution of plasma starting at a dilution of 1:20

were made with 0.9% saline and examined for visible agglutination. Appropriate positive and negative plasma were included.

- ✓ **Testing Procedure and interpretation of results**

The main principle of widal test is that if homologous antibody is present in patients serum/plasma, it will react with respective antigen in the reagent and gives visible clumping on the test card and agglutination in the tube. The two major antigens used in the test are "H" and "O" antigens of *S.Typhi*. "O" antigen is a somatic antigen and "H" antigen is flagellar antigen.During infection antibodies are produced in patient's sera against these antigens. Antigens specifically prepared from this organism are used in the agglutination test to detect the presence of antibodies in patients' sera which are elucidated in response to infection by these bacteria. There are some agglutinins that are produced in the patient's serum/plasma.

Before starting the experiment, bring all reagents to room temperature and mix well, mark the circles of slides as PC (Positive control), NC (Negative control), O and H, as per antigen solutions used for testing.Add 1 drop of positive control (25µl) into the circle marked as PC of given glass slide.Then add 1 drop of negative control (25µl) into the reaction circle marked as NC.Add 1 drop of test sample (25µl) into each reaction circle labeled as O and H according to given antigen solution.Add 1 drop of Antigen solution of *S.typhi*'H' into PC and NC circle each. Mix well with using new mixing stick for each circle.to circles labeled as O and H in which test samples has been added, add antigen solutions of *S. typhi*'O' and *S.typhi*'H' and Mix the content of each reaction circle uniformly with a separate mixing stick.Rock the glass slide gently (approximately for one minute) and observe for agglutination. (AnnexV:V)

- ✓ **The of tube titration method test**

Prepare 2 sets of test tubes for individual antigen. Each set contains 1- 6 tubes.Add 1.9 ml of 0.85% sterile saline to tube no. 1 of each antigen set.to tube no. 2-6 of all sets add 1 ml of physiological saline. to tube No. 1 of all sets add 0.1 ml of test sample to be tested and mix well. Transfer 1 ml of the diluted plasma sample from tube No. 1 to tube No. 2 and mix well. Transfer 1 ml of the diluted plasma sample from tube No. 2 to tube No. 3 and mix well. Continue this serial dilution till tube No. 5 in each set of antigen. Discard 1.0 ml of the diluted plasma from tube No.5 of each set. So the dilutions of the plasma sample from tube No. 1 to 5 respectively in each antigen set are 1:20,1:40,1:80, 1:160, 1: 320. Tube no. 6 is negative control with 0.85%

sterile saline. To one set i.e. from tube no.1- 6 add 50 µl of *S.typhi*'O' antigen. In second set i.e. from tube no.1- 6add 50 µl of *S.typhi* 'H' antigen. Mix well, cover and incubate these tubes overnight at 37^0C (approximately 18 hours).After incubation dislodge the sediment and observe for agglutination.(AnnexV:V)

Result interpretation

Antibodies present in the sample react with the antigen suspension to give clearly visible agglutination which can be seen through naked eye. The antibody titre of the test sample is its highest dilution that gives a visible agglutination.Agglutinin titre equal to 1:320 is considered as significant infection and low titres indicate absence of infection

4.7. Statistical analysis

All the data from the questionnaires and laboratory results were coded and checked for completeness. Data were entered into EPI-DATA version 3.1. Then the data exported to SPSS version 20 for analysis. Descriptive analysis such as mean, standard deviation, percentage and cross tabulation was used to summarize the data. Independent-sample T Test was computed to compare the continuous variables, and Chi- square (X^2) analysis was conducted to identify the categorical variables with selected hematologic abnormality. Significant association was set at a p-value less than 0.05. The result of the study was discussed and compared with different literatures on hematologic parameters and its associations with typhoid. Finally, recommendations were forwarded based on the findings.

4.8. Quality assurance

To ensure the quality of data, training was given to data collectors prior to data collection. We used a standard operating procedure (SOP) for pre-analytical, analytical, and post-analytical procedures implemented during hematological tests measurement, serological and parasitological test. All samples were analyzed by one machine. Manufacturer Instructions was followed to maintain equipment performance and reagent expired date. Control reagents were used to check the accuracy and precision of the results for the test. Repeated analysis of randomly selected specimens for reproducibility check (delta check) was carried out to evaluate instrument performance consistently and accurately. Two BSC Nurse and two laboratory technologists were recruited as data collectors and physicians from the ward of Shanan gibe hospital was supported me to collect especially the clinical data of the patients. Besides, the collected data was checked for completeness and internal consistency by principal investigator. The questionnaire was translated to the local language. The results of all laboratory examination were recorded on standardized report format carefully and attached to questionnaire according to subject's unique identification number.

4.9. Ethical consideration

Data collection were carried out after approval of the research proposal by the institutional review board (IRB) of Jimma University, Institute of health and support letter from post graduate program directorate was submitted to the concerned body for all organizations. Written informed consent was obtained from the study participants. After getting all permission from all responsible body, the data collector informs the patients by reading or giving to read the information sheet which is translated to patients' language about the objectives of the study, risk, benefits and asking question related to the study. Confidentiality was maintained using of identification numbers instead of individual names. Finally, patient's laboratory result was printed and submitted to OPD for appropriate intervention.

4.10. Plans for dissemination of result

The finding of this study will be submitted to Jimma university, institute of health, faculty of health sciences, school of medical laboratory science. The findings of this study will be also published in peer reviewed scientific journals. It will also be presented on different scientific forums.

4.11. Operational definitions

Hematologic parameters: the selected parameters like Red Blood Cells (RBCs), total white blood cells (WBCs), and Platelet, in differential neutrophil, lymphocyte and mixed, in red cell indices mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean concentration hemoglobin (MCH) included in this study.

Typhoid suspect: The individual who have clinical sign and symptom of typhoid fever confirmed by the physician and sent to the laboratory from clinical site for confirmatory test.

Typhoid positive: The patients who have sign and symptom of typhoid fever and confirmed with laboratory test result of Widal test both slide positive and titer equal to 1:320 was included as positive result.

CHAPTER FIVE: RESULTS

5.1. socio-demographic characteristics

A total of 394 patients suspected for typhoid fever were included in the study. From those 384 study participants, whose data was completed were included for final statistical analysis to identify of hematological abnormality and its association with typhoid infection among typhoid suspected patient. The rest of 10 (2.5%) participants were excluded from analysis because of data incompleteness during data clarifying. The mean ± SD age of the participant was 26.31±12.93 ranging from 4 to 71 year. More than half of the patients, 226 (58.9%) were males and 158(41.1%) were females and 211 (54.9%)of the participants were a rural resident and 112(29.2%) were students, 71 (18.5%) house wife, 70(18.2%) merchant, 228 (59.4%) had educated secondary and above and the majority of affected typhoid patients was between the age group of 15-30 (56.1%) in this study and most of the male affected with typhoid relatively higher than that of female participants.(Table 1).

Table 1: Socio-demographic characteristics of typhoid suspected individuals at Shanan gibe hospital, January 30, to August 30,2019, Jimma, southwest Ethiopia.

Variable	Category	Sex		Total(No) %
		Male(No) %	**Female(No)%**	
Age-Group (years)	1-14	47(57.3)	35(42.7)	81(21.4)
	15-30	89(49.2)	92(50.8)	181(47.1)
	31-45	65(72.2)	25(27.8)	90(23.4)
	>46	25(80.6)	6(19.4)	31(8.1)
Total		**226(58.9)**	**158(41.1)**	**384(100)**
Residence	Urban	120(69.4)	53(30.6)	173(45.1)
	Rural	106(50.2)	105(49.8)	211(54.9)
Total		**226(58.9)**	**158(41.1)**	**384(100)**
Education	Illiterate	12(80.0)	3(20.0)	15(3.9)
	Primary(1-8)	78(55.3)	63(44.7)	141(36.7)
	Secondary and above	136(59.6%)	92(40.4)	228(59.4)
Total		**226(58.9)**	**158(41.1)**	**384(100)**
Occupation	Student	67(59.8)	45(40.2)	112(29.2)
	Govt Employee	18(72.0)	7(28.0)	25(6.5)
	House wife	0(0)	71(100)	71(18.5)
	Merchant	54(77.1)	16(22.9)	70(18.2)
	Farmer	51(89.5)	6(10.5)	57(14.8)
	Other	36(73.5)	13(26.5)	49(12.8)
Total		**226(58.9)**	**158(41.1)**	**384(100)**

5.3. Clinical presentations

Concerning their clinical presentation at the time of diagnosis, the majority of the participants were febrile for at least 4 days and had a headache. The majority of patient were 324 (84.4%) presented withfever greater than 4 days, headache 313 (81.5%) and loss of appetite 300 (78.1%); otherwise the low grade of 311 (81.0%) with weakness and fatigue, 264 (68.8%) sweating, 198 (51.6%) diarrhea, 91 (23.7%) vomiting 90(23.4%) dry cough, 45(11.7%) muscle ache 33 (8.6%), nausea and 8(2.08%) swollen abdomen was presented respectively.So that, in this study fever, headache, weakness and fatigue, loss of appetite and sweating were the major sign and symptom of typhoid infection observed.(Table 3)

Table 2: Clinical sign and symptom observed among typhoid suspected patients at shanan gibe hospitalJanuary 30, to August 30,2019, Jimma, southwest Ethiopia.

Clinical Sign/Symptom	Present No (%)	Absent No (%)
Fever	324(84.4%)	60(15.6%)
Headache	313(81.5%	71(18.5%)
Weakness and Fatigue	311(81.0%)	73(19.0%)
Loss of Appetite	300(78.1%)	84(21.9%)
Sweating	264(68.8%)	120(31.3%)
Diarrhea	198(51.6%)	186(48.4%)
Vomiting	91(23.7%)	293(76.3%)
Dry Cough	90(23.4%)	294(76.6%)
Muscle Ache	45(11.7%)	339(88.3%)
Nausea	33(8.6%)	351(91.4%)
swollen abdomen	8(2.08%)	376(97.92%)

5.5. Comparative selected hematological parameters with typhoid fever.

Total WBC and Differentials count

The comparative hematological changes associated with typhoid fever patients with regards to WBC of the study subjects was statistical significant difference between typhoid positive (Mean=4.29, SD=4.18) and typhoid negative (Mean=5.74, SD=4.42). Similarly, the neutrophil and mixed result of the study subjects was statistical significant difference between typhoid positive (Mean= 56.42, SD=22.67) andtyphoid negative (Mean=63.39, SD=17.25) and mean=6.96, SD=6.91 and mean =8.85, SD=5.75 respectively. This indicatesWBC, neutrophil and mixed of typhoid positive was lower than that of typhoid negative participants.

Red blood cell and red blood cell induces count

The comparative hematological changes associated with typhoid fever patients with regards to RBC of the study subjects was statistical significant difference between typhoid fever positive (Mean=2.46, SD=1.12) and typhoid negative (Mean=3.70, SD=1.36).this indicates RBC of typhoid positive was lower than that of typhoid negative participants.The hemoglobin changes associated with typhoid fever showed that significantly difference between typhoid positive patients (mean =8.40, SD=2.52) was lower than that of typhoid negative participants (mean=11.85, SD=3.84).The red cell induces change associated with typhoid fever patients with regard to MCV and RDWcof the study subject was statistical significant differencebetween TPp (mean =82.44, SD=18.34) was lower than that of TNp (mean= 87.95, SD=8.56), (mean=11.98,SD=2.00)was lower than that of TNp(mean=13.38, SD=1.95)respectively.

Platelet count (plt)

The comparative hematological changes associated with typhoid fever patients with regards to the platelet of the study subjects was a statistical significant differencebetween typhoid positive (Mean=127, SD=61.82) and typhoid negative (mean=243.7,SD=122.15).This indicates the platelet count of typhoid positive was lower than that of typhoid negative patients. (Table 5).

Table 3: Mean comparison of Hematologic parameters with typhoid positive and negative participants at Shanan gibe hospital, January 30,to August 30,2019, Jimma, southwest Ethiopia

Parameters	Typhoid positive	Typhoid negative				
	Mean± SD	Mean± SD	MD	t	df	p-value
WBC $\times 10^3$/ µl	4.29±4.18	5.74±4.42	-1.450	-1.899	382	0.04
RBC $\times 10^6$/ µl	2.46±1.12	3.70±1.36	-1.249	-5.659	382	0.00
Platelet count$\times 10^3$/ µl	127.5±61.82	243.87±122.15	-6.005	-116.38	382	0.00
Neutrophil (%)	56.42±22.67	63.39±17.25	-6.584	-2.222	382	0.03
Lymphocyte (%)	31.76±20.22	28.11±16.6	3.650	1.298	382	0.21
Mixed (%)	6.21±6.48	8.85±5.75	-2.744	-2.644	382	0.006
Hemoglobin (g/dl)	8.40±2.52	11.85±3.84	-5.605	-3.445	382	0.00
Hematocrit (%)	29.38±8.71	37.59±8.05	-6.334	-8.361	382	0.001
MCV (fl)	82.44±18.34	87.95±8.56	-3.321	-5.510	382	0.001
MCHC (g/dl)	32.97±3.63	33.94±3.11	-1.845	-0.968	382	0.066
MCH (Pg)	29.5±5.28	30.73±3.53	-1.915	-1.199	382	0.056
RDVc(%)	11.98±2.00	13.38±1.95	-1.400	-4.340	382	0.000

Key:SD=standard deviation, MD=mean difference, T=t-test, df= degree of freedom, WBC=white blood cell,RBC=red blood cell, MCV=mean corpuscular volume, MCHC= mean corpuscular hemoglobin concentration, MCH=mean hemoglobin concentration,RDVc= red cell distribution width coeficitent variation.

5.6.Hematological parameters associated with typhoid fever

Selected Hematological parameters were entering into Chi- square for identify the parameters which significantly associated with typhoid fever and know the predictor variables for typhoid fever.Therefore White blood cells, red blood cellsand plateletobservedin this study was significantly associated with typhoid feverat p-value <0.05. Similarly neutrophil,mixed andhemoglobin also significantly associated with typhoid fever patients at p- value< 0.05.The MCV and RDW also significantly associated with typhoid infection,however,the lymphocyte,MCHC and MCH presented in our finding was insignificant associated with typhoid infection p>0.05.(Table 6).

Table 4: Association of hematological parameters with typhoid suspected patients at Shanan gibe hospital, January30, to August 30, 2019, Jimma, southwest Ethiopia

Hematological parameters		Positive No (%)	Negative No (%)	X^2	df	p-value
WBC	Normal	3 (2.3)	129(97.7)	15.634	2	0.00
	Low	32(15.9)	169(84.1)			
	High	6(11.8)	45(88.2)			
Neutrophil	Normal	5(3.4)	144(96.6)	22.388	2	0.00
	Low	19(25)	57(75.0)			
	High	17(10.7)	142(89.3)			
Lymphocyte	Normal	20(10.6)	169(89.4)	3.642	2	0.16
	Low	10(7.8)	119(92.2)			
	High	11(16.7)	55(83.3)			
Mixed	Normal	2(0.9)	215(99.1)	64.387	2	0.00
	Low	32(30.5)	73(69.5)			
	High	7(11.3)	55(88.7)			
RBC	Normal	6(3.2)	184(96.8)	22.295	1	0.00

	Low	35(18.0)	159(82.0)			
Hb	Normal	3(1.6)	183(98.4)	31.076	1	0.00
	Low	38(19.2)	160(80.8)			
Hct	Normal	13(5.1)	244(94.9)	25.723	1	0.0
	Low	28(22.0)	99(78.0)			
MCV	normal	22(7.5)	272(92.5)	21.206	2	0.00
	Low	14(17.7)	65(82.3)			
	High	5(45.5)	6(54.5)			
MCHC	normal	28(8.9)	285(91.1)	5.700		0.06
	Low	10(17.2)	48(82.8)			
	High	3(23.1)	10(76.7)			
MCH	normal	25(9.3)	244(90.7)	3.223	2	0.2
	Low	11(16.9)	54(83.1)			
	High	5(10.0)	45(90.0)			
RDW_c	normal	5(2.2)	220(97.8)	40.746	2	0.00
	Low	32(22.5)	110(77.5)			
	High	4(23.5)	13(76.5)			
Platelet	Normal	1(0.4)	235(99.60	74.754	2	0.00
	Low	39(29.3)	94(70.7)			
	High	1(6.7)	14(93.3)			

Key: DF=degree of freedom, X^2= chi-square, Normal=between the reference range, low=below the reference range, high =greater than the reference range, WBC=white blood cell, RBC=red blood cell, HB=hemoglobin,Hct= Hematocrit,MCV=mean corpuscular volume, MCHC= mean corpuscular hemoglobin concentration, MCH=mean hemoglobin concentration, RDVc= red cell distribution width coeficitent.

NB: the reference range is categorized according to the study conductedon establishment of the hematological parameters on apparently health population of south west Ethiopia(34).

5.7. Selective hematological abnormalities presented in typhoid suspected individuals

Out of the total number of study participants, the majority of hematologic abnormality observed were leucopenia, 201(52.3%), anemia198 (51.6%), neutrophilia, 160(41.4%) and 131(34.1%) thrombocytopenia whereas, 137(35.7%) presented with lymphopenia76 (19.8%) neutropenia, 105(27.3%) mid-granulocytopenia 62(16.1%) mid granulocytosis 70(18.2%) lymphocytosis, 51(13.3%) leukocytosis and 18(4.7%) was thrombocytosis respectively.Asa result the hematologic abnormality presented in this study was leucopenia, anemia, neutrophilia with mild lymphopenia, thrombocytopenia and low number of mixed were the parameters more observed with typhoid patients.

Table 5: Hematologic abnormalities of observed among typhoid suspected individual at Shanan gibe hospital, January 30,to August 30,2019, Jimma, southwest Ethiopia

Parameters	Numbers	Percentage (%)
Leucopenia	201	52.3
Anemia	198	51.6
Thrombocytopenia	134	34.9
Lymphopenia	137	35.7
Neutropenia	76	19.8
Mid- granulopenia *	105	27.3
Leucocytosis	51	13.3
Thrombocytosis	18	4.7
Lymphocytosis	70	18.2
Neutrophilia	160	41.4
Mid-granulocytosis**	62	16.1

*Key: * and **= mid granules indicates the mixation of the three of hematological parameters which is monocyte, eosinophil and basophil.*

CHAPTER SIX: DISCUSSION

Typhoid fever is endemically posing significant health problem in developing countries where there are high risk factors. Likewise; in Ethiopia, typhoid fever is considered to be highly endemic and represented by high morbidity and mortality each year (35). Anemia, leucopenia neutrophilia and thrombocytopenia were common findings in this study.

In this study, (10.7 %) of plasma examinations were observed to have positive titer in both O and H antigens equal to or greater than 1:320µl that was considerable serological widal titer reactive and 89.3% was non-reactive test. In our studymales were more affected than females which are agree with the study done at India and Nepal by *Kakaria A et al.* and Rana B et al (25)(27).

The major characteristic presenting the clinical features of typhoid in our study was fever, headache, loss of appetite, weakness and fatigue, sweating, diarrhea and vomiting. Otherwise typhoid fever presented with low grade of dry cough, muscle ache and nausea was also presented with the typhoid patients this is agreed the study reported by *Kakaria A et al.* which is fever 100%, anorexia36%, headache 26%, diarrhea 28%, vomiting 44%, cough (5%)(27)(36).The other study conducted inIndia by *Jeeyani HN et al.* was showed that the patient with high-grade fever was 94.6 %, vomiting and cough were the most common associated symptoms seen in 47.6%, and 54.6% patients respectively(37).

In present study the major hematologic abnormality observed in typhoid suspected patients were leucopenia201(52.3%),anemia198(51.6%) and neutrophilia 159(41.1%);whereas,137(35.7%)presented with lymphopenia,134(34.9%) thrombocytopenia ,76(19.8%) neutropenia,105(27.3%)midgranulocytopenia 62(16.1%) midgranulocytosis,70(18.2%)lymphocytosis,51(13.3%) leucocytosis and 18(4.7%) thrombocytosis were reported respectively.

In our study the prevalence of leucopenia was 201 (52.3%) which was agree with the result reported by UnaizaQamar&JaveriaAijaz, *et al* showed 52% (17). The study conducted in India in 2017, by SPS Dhillon, *et al*(32), reported the prevalence of leucopenia was 6.5%, and by Sajjad,*et al*(5) lecopenia reported 7.29%, which was lower than the present study. The other study conducted at India teaching hospital by modi*R,et.al*(31) leucopenia was seen 11.2% and the study reported by Rana B et al(25), showed that 8% respectively which is lower than that of

our result. Another result reported by *Kakaria A, et al* a prospective study conducted at India showed thatleucopenia was 21% lower than that ours (27).

Anemia was reported 51.6% which was higher than the study done by SPS Dhillonet al, reported by *Rana B, et al* in Nepal in 2015 and by *Shilpa V. et al*shows that 47.8%, 23.52 % and 34.4% respectively (32)(25)(29) and lower than the study done by Sajjad et al and Unaiza Q &Javeria A. *et al* which showed the prevalence of anemia 67.70% and 61.33%(5)(17).Another result reported by *Kakaria A et al* anemia was 42.9%, which is lower than my study (27).

In present study Thrombocytopenia was reported (34.9%) which was greater than the study done at India by SPS Dhillon*et al ,* by Shilpa V. U plaonkar et alshowed 21.5% (32)and 17.24% (29).the study reported by *Qamar&JaveriaAijazet al,* showed 37.3%(17) which is higher than our present result. Thrombocytosis reported (1.96%) conducted by Rana B,*et al*(25) which lower than the present study reported which is 4.7%.

The study conducted at India teaching hospital by Modi R *et.al*, showed Lymphocytosis was 70.4% which are higher than our results which as observed 18.2% and lower than the study reported by*Qamar&JaveriaAet al*, lymphocytosis 12% and other prevalence of lymphocytosis reported by Sajjad *et al* showed 10.9%(17)(31).The study conducted by Qamar U, Aijaz lymphopenia 12 (8%),(17). Rana B PS lymphopenia in 18.62%,(25) which is lower than that of the presented study reported lymphopenia 137 (35.7%).

The study conducted by Modi R *et.al* and reported by *Shilpa V. et al* showed that the prevalence of Leucocytosis was 17.4% and 22.41% patients which are higher than 13.3% that of my study (31)(29). Another result reported by *Kakaria A et al* a prospective study conducted at India showed that Leucocytosis was 10% respectively which is similar with our result reported (27).And also Leucocytosis in 2.94%, was reported by *Rana B PS* (25) which is

In this study the prevalence of neutropenia was 19.8%, which is lower than the result reported by *Qamar&JaveriaAet al*, showed 32%; on the other hand the neutrophilia was reported 23% which is lower than our result which is 41.4% . the other result reported by Shilpa V. U plaonkar *et al*39.65% and by *Rana B PS* neutrophilia in 28.43%(17)(29)(25).

Generally, the overall probable reason of variation of the results with my study might be due to sample size, geographical area and study design.

In our study white blood cells, red blood cellsand platelet observedin this study was significantly associated with typhoid fever at p-value <0.05. Similarly neutrophil,mixed andhemoglobin also significantly associated with typhoid fever patients at p- value< 0.05.The MCV and RDW also significantly associated with typhoid infection however,the lymphocyte,MCHC and MCH presented in our finding was insignificant associated with typhoid infection p>0.05

There was a significant mean difference p<0.05 in the red cell, Hb, WBC and neutrophil which is agrees the result reported by *A. Dangana et al* (15) *and* by *Okafor,A* (13).the mixed and MCV was observed P<0.05 significant mean difference.

The result of the present study also seen that leucopenia, thrombocytopenia, anemia which is similar that of the finding reported in Nigeria by *Okaforet,al*(13).
In this study WBC, Hb, neutrophil, mixed and platelet was the associated for typhoid fever which is agree with *A. Dangana et al.* Significant decrease in Hb, and WBC and also lymphopenia neutrophilia was reported(15), but in this study lymphocyte was not significant associated. The other study conducted at India by Anusuya*et al*showedsignificant decrease in the WBC, HB, PLT with compared to healthy individuals which is similar to our study (21).

Here, we report that a statistically significant decrease in the, total white blood cell count and hemoglobin (p<0.05) in enteric fever patients compared to the negative for typhoid fever, which is agree with the study conducted at Nepal by Rana*et al*. In this study lymphocyte was insignificantly associated with typhoid infected patients which is disagree with the study conducted at Nepal by Rana*et al*showed that lymphocyte significantly associated with typhoid fever(25).

CHAPTER SEVEN: LIMITATIONOF THE STUDY

- ✓ The study used a 3 part differential, which will not give all the 5 differentials WBC parameters separately
- ✓ Perform blood/stool culture for diagnosis of typhoid fever to prevent false positive result should be recommended for Shanan gibe hospital,as the slide agglutination technique is not specific

CHAPTER EIGHT: CONCLUSION AND RECOMMENDATION

8.1. CONCLUSION

Typhoid fever is a major causes study area.The diagnosis of typhoid fever by signs and symptoms can help laboratory results. Typhoid fever has been significant effect on some hematological parameters; on the basis of our finding we may conclude that the observed mean variations of hematological parameters were significantly decreased between the typhoid positive patients compared with typhoid negative except lymphocyte, MCHC and MCH. The major abnormality observed in our study was anemia, leucopenia, thrombocytopenia, neutrophilia, lymphopenia and mid granulopenia. Likewise this parameters;(WBC,RBC,PLT, HB, Neutrophil, mixed,MCV and RDW) were showed significantly associated with typhoid fever. Therefore, it may lead to an increased clinical suspicion during diagnosis. This study appears to be large evidence based on hematological parameters in typhoid patients to explain influence of typhoid incidence. Typhoid fever is a curable when it treated at early stage.

8.2. RECOMMENDATIONS

Based on this finding the following recommendations are forwarded.

- As a result complete blood count should be ordered early by the clinicians for proper diagnosis and treatment.
- Further researches is needed by performing the gold standard test for diagnosis, and reinforce the conclusion of this study, because there are various difficulties associated with the disease admitted with fever.
- Hematologic abnormality of typhoid infected patients observed in my study could be used as valuable aids in patient's clinical management.

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ANNEX-I: INFORMATION SHEETS

Information sheet in English version

This information sheet is prepared for individuals who volunteer to participate in the study. The detailed explanation about what will be undertaken in the study is presented as follows and it is after reading the description that informed consent is obtained.

Title of the project:-"hematological paraneters and its association with typhoid fever among typhoid suspected individuals attending at shanan gibe hospital Jimma, south west Ethiopia."

Name of Principal Investigator:-Ayantu Endalew

Advisors:

1. Dr.TilahunYemene (MD, Ass.prof)

2. Mr. Wondimagegn Adissu (BSc, MSc)

Name of the Organization: Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences.

Introduction: This information sheet was prepared for the aim of explaining the research that you are asked to join with research participant. This information Sheet describes about the research.

Description and Purpose of the study:-Clinical laboratory are an important tool for identifying abnormal hematologic results of clinically suspected typhoid individuals and for ultimately guiding those patients to diagnoses. The determination of hematological abnormality is very crucial to improve quality of health care as well as for diagnosis patient or aid the patient with suffering from typhoid infection. Therefore this study will be carried out to determine "hematological parameters and its association among typhoid suspected individuals shanan gibe hospital Jimma, south west Ethiopia.

- Procedures: **-**If the patient was agreed to take part in the study, clinical nurse was given verbal and/or written information about the study and patient was signed on consent form. Patients were kindly requested to give the correct information about them. Then Blood sample (4ml) and stool sample was collected

  The blood sample was analyzed for CBC, for screening of hemoparasite, and widal test.

  Risks and discomforts:-During sample collection we will follow Standard operational procedures. The blood drawing may cause minor pain, at the place where blood is taken. However, this pain will no longer appear.

  Benefits: -. If you are participating in this study, there may not be direct benefit to you but your participation is likely to help us an important input to find the hematologic diagnostic predictors during typhoid infection, early control and management the patients and if the medical examination reveals any abnormalities that need immediate treatment, your doctor will be notified about the result.

  Incentives and payment for participating in the study: You will not be provided with any direct incentives for your participation in this study. But the cost for your medical examination would be covered.

  Confidentiality:-Any information obtained during this study will be kept confidential. This is assured by avoiding use of any identifier and information will be recorded with code number and it will not be revealed to anyone except your physician and the principal investigator.

  Voluntary participation: - Participation on this study is voluntary and you have the right to refuse participation at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Your decision will not put you at risk any present or future medical care. You may ask questions now and in the future if you do not understand something that is being done contact the investigator. For the success of the study, I will be asking you to give the correct answer for the respective questions. Thank you very much for your assistance.

I.I.unkaAfaanOromooIbsaHirmaattotaqo'annotiif

Gucni kun warren qu'anniichatti fedhaan hirmataniif yoo ta'u haalli adeemsa qu'annichaa irra ga d ibsamee booda wanti itti aanu hunduifa godhama.

Matadureequ'anna:-Qorannonbu'alaaboraatorijiijjiramadhiigaa warren mallattoo typhoid agarsiisanirrattihospital shanangibee Jimma naannokibbalixaItiopiyaattikanhoojetamudha

Maqaaqorattuu:-AyyantuuIndaaluu

Gorsitoota:Dr.TilahunYemene (MD, Ass.Prof)

2. Mr. Wondimagegn Adissu (Bsc, Msc)

Dhimmiqu'annicha:-Bu'aa laboratory kanneenmallatto typhoid agarsiisanirrattirakkoondhiigaaisaanmudatuilaaluufikanyaaliiftahumurteessuuykngargaaruaddaba asuunakkayaaladhukkubsataafistaheogeessaafayyaattikaraaagarsiisuuf nu garaggara. bu'aanisaawarranaannotiifistahedhukkubsataafbaay'eeguddaadha.

Haalaadeemsaqu'annicha:- yooqorannoo kana irrattihirmaachuufwaliigalte, qorattootakeessaanamnitokkoykn ogeessi fayyaa waa'eeqorannichaaafaaninyknbarreeffamaansiifkennu; akkasumas uunka waliigalteemallatteessitusiifkennu.

Waa'eekeeodeeffannoosirriiakkanuufkennitukabajaansigaafachaa,Qu'annaa kana irrattifedhaanhirmaachuufmallattookeessaninwaannuufibsitaniif,ragaawwanarmaangadikanneenn ifudhanna. Dhiiga 4ccniifudhanna

- Kanarmaanolittifudhatamequ'annichaqofaaftahuuisaaniiibsina.

Sodaa fi miidhaaqabu:-Seeraafnaamuusawal'aansafayyaawaanhordufnuufwantinamasodaachisuhinjiru, haata'uumaleeyeroodhigafuudhannudhukkubbiinxiqqoobakkalilmoonseenteettidhaga'aamumala ,ta'usbattalumattibadaykndafeeisindhiisuudandaha.

Faayidaaqu'annichaa fi kaffaltiihirmaataafgodhamu: Qu'annichibu'aaguddaasabaafbuusainnistajaajiladhugaaakkaargatantaasisa.

Qu’annichairrattihirmaachuunkaffaltiitokkollehinqabu .haatahumaleebu’aanqorannoowaantabattalumattideebi’uuftajaajilawaldhansaargachuuniidandahu.

Iccitiiqu’annichaa:-
Wantootniqu’annichaanargamanhundiicciitineegamu.Akkasumasragaaleenfudhatamanhundinumaqaakeesaniinosoohintaanelakkoopsakoodiiaddata’eenwaangalmaahuuf kanas kanbeeku warren ragichaguraanqofadha.

Mirgafedhaanhirmaachu:-Qu’annoo kana irrattihirmaachunfedhaguutuukeeqofata’uusaabeekteeyeroobarbbaaddeettidhiisuakkadandeessumirgaguutuuqabda. qu’annoo kana irrattihirmaachu fi hirmaachudhiisuunkeegarafuuladuraaftajaajilaargattuirrattirakkootokkolleesittihinfidu.

Qu’annichailaalchiseegaaffileeqabdanhundayeroobarbaaddanittiteessooolittibareefameenabbaaqu’annichaagaafachuniidandeessu.
Ittiaanseequ’annokanaafhirmaannaagootanifgalannikeenyaguddaadha.

Galatoomaa!!!!!

I.*i.i. የጥናቱተሳታፊዎችመረጃቅፅ(የአሚኛግልባጭ*

ይህየሚጃቅጸየተዘጋጀውበዚህጥናትለሚሳተፉፍቃደኛሰዎችይሆናል፡ ፡ ፍቃደኝነ ታቸውእንደሰትእንደሚወስደከተገ ለፀበኋላየሚከናወኑትተግባራትበግልፅይብራራሉ፡ ፡

የጥናቱርዕስ፡ -የአንጀትተስቦበሽታበደምካይየሚሳየውለውጥ

የተመራማሪስም - አያንቱእንዳለዉ

አማካሪ፡ : **1.Dr.Tilahun Yemene (MD, Ass.Prof)**

2. Mr. Wondimagegn Adissu (Bsc, Msc)

የጥናቱአላማ -

የአንጀትተስቦበሽታበደምካይየሚሳየውለውጥማወቅለታማሚዎቹይህንነትጉልህአስተዋፆአለው፡ ፡ እንዲሁምሊተለየ ያየዩየህክምናምርምርስራዎችበጣምጠቃሚናይሆናልስለዚህይህየምርምርስራየአንጀትተስቦበሽታበደምካይየሚ ያሳየውለውጥበጂማከተማበሚገኙየህክምናተቋምማላይበሚከተናገዱየሳንባነቀርሳታማሚዎችላይይሰራል፡ ፡

የጥናቱዝርዝርሂደት፡ -

በጥናቱለተሳተፉፍቃደኝነትዎንበፊርማዎስለገለፁልንእንዲሁምከራሳቸውቃልበኋላየሚከተሉትንመረጃዎችናናሙ ዎችአንወስዳለን ;;

- ከራስዎአንደበትየአስርደቂቃቃለመጠይቅይደረግለታል
- 5 ሚሊሌትርየደምናሙናይወሰዳል ;;
- የደምናሙናውለመለየደምምርመራይወላል፡ :

ስጋትናጉዳት፡-

ህክምናውየሚደስገደደወንየአሰራርሂደትስለምንከተልለየጋጥምየማሉውየህመምስሜቱበጣምአነስተኛነው፡ ፡ በሆ ንምየደምናሙናውበሚወሰደበትጊዜትንሽየህመምስሜሊያጋጥምይችላል፡ ፡ ነገርግንህመሙበአጭርጊዜይጠፋል፡ ፡

ሊያስገኛቸውየሚችሉትጥቅሞችናየካሳክፍያ፡ -

ይህጥናትለህብረተሰብጠቃሚመረጃወማካንያቱምየጥናቱውጤቱታካሚውትክክለኛህክምናእንዲያገኝይረደዋል፡ ፡ በ

ዚህጥናትውስጥበመሳተፍዎበጥሬገ ንዘብየ ሚደረግየ ካሳክፍያአይኖርም ነ ገ ርግንየ ምርምሩውጤቱበወቅቱየ ሚገኙ ሆንበምርምሩውጤቱመሠረትአስፈላጊውየ ህክምና እርዳታይጠቀማሉ፡ ፡

የ ጥናቱምስጢራዊነ ት፡ -

ማንኛውምበጥናቱየ ሚ ገኙመረጃዎችበሚስጥርይጠበቃሉ፡ ፡ የ ሚሰጡንመረጃዎችበመለየ ሚቀመጡትከእርሶዎስምጋርሳይ ሆንለጥናቱተብሎበሚሰጠውሰው ቁጥርሲሆንጥናቱንከሚያካሄዱትባለሞያዎችበስተቀርማንምሊያውቅአይችልም፡ ፡ የ እርስዎንማንነ ትበሚ ያጋልጥመልኩየ ተዘጋጀውመረጃበፊርማዎየ ተረጋገ ጠፍቃድሳናገ ኝይፋአናደርግም፡ ፡ ይህጥና ትሳይንሳዊመረጃእንደመሆኑመጠንበወረቀትታትሞበውጣውይምበሚዲያበነ ገ ርየ እርሶዎስምበምንምመልኩአይጠቀስም ፡ ፡

በፍቃደኝነ ትየ መሳተፍመብት፡ -

በዚህጥናትውስጥየ ሚኖርዎትተሳትፎሙሉበሙሉበፍቃደኝነ ትላይየ ተመሠረተይሆናል፡ ፡ በማንኛውምጊዜይህንንጥናት የ ማቋረጥመብትዎሙሉበሙሉየ ተጠበቀነ ው፡ ፡ በጥናቱበመሳተፍዎወይምከጥናቱበማ ለልዎምክንያትበአሁኑወይምየ ወ ደፊትየ ህክምናእርዳታዎላይምንምአይነ ትተፅዕኖአይኖረውም፡ ፡ ከዚህበፊትሲያገ ኙትከነ በሩትጥቅሞችላይአንዳችነ ገ ርአይጎ ልቦትም፡ ፡

ስለጥናቱማንኛውንምአይነ ትጥያቄካሉትበማንኛውምጊዜአሁንምሆነ ወደፊትከላይበተጠቀሰውአድራሻየ ጥናቱንባለቤ ትማነ ጋገ ርይችላሉ፡ ፡ ለዚህጥናትስኬታማነ ትጥያቄዎቹንበትክክልበመመለስእንድትተባበሩስልበትህትናእየ ጠየ ኩ ስለትብብርዎልባዊምስጋናአቀርባለሁ፡ ፡

በጣምእናመሰግናለን!!

ANNEX-II: CONSENT FORMS

i. Consent forms (English version)

Participant Code Number_____

Participant full name _____

I am informed fully in the language I understand about the aim of the above-mentioned study. I understood the purpose of the study entitled with "hematological parameters of typhoid fever among typhoid suspected individual attending at shanan gibe hospital Jimma south western, Ethiopia." I have been informed that blood samples will be taken and there will be a minimal risk during sample collection. In addition, I have been told all the information collected throughout the study process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or withdraw from the study.

Agree _____ Not agree_____, Therefore I give my consent freely for my participation in this study.

Participant's Name _____signature _____ Date_____

Investigator's Name _____signature _____Date_____

i.i .UunkaaWaliigaltee (AfaanOromoo)

Lakk.AddaaHiirmaataa_____

MaqaaguutuuHiirmaataa_____

Aniihiirmaataanmaqaankiyyaarmaanolittiibsamekaayyoonqo'annoo"Qorannonbu'aalaaboraatori "rakkoodhiigayeroomallattooleengolfaanamarrattimullatuargamusafaruu. " jedhuurattihoojjatamuufafaannaagaluunodeefannoogahaaargadheenjira. Odeefannoonfayyaa fi naamuusnidhiigaaskaraamiidhaa/rakkoohingeesisneenakkafuudhatamuhubadheenjira. Dabalataaniisodeefannoonnarraaargamanhunduuicciitiinakkaqabamannattihiimameera. Gaaffileegaafatamuufdeebiikennudhiisuu, hiirmachuudhiisuu fi yeroonbarbaadettiaddaankutuuakkandanda'uuhubadheenjira. Kana gochuukiyyaanis ammasta'egarafuuladuraaffayyadamummaatajaajilafayyaakiyyaairrattirakkoontokkolleeakkahinu umamneenaafgaleejira.

Waliigaleera_____ waliihingallee_____

Kanaafuuqoorannoo kana irattifeedhiinnanhirmaadha.

Maqaa hiirmaataa_____Mallattoo_____Guyyaa_____

Maqaa qoo'ataa_____Mallattoo_____Guyyaa_____

Ragaa

1. Maqaa_____Mallattoo_____Guyyaa_____
2. Maqaa_____ Mallattoo_____Guyyaa_____

i.i.i. የስምምነት ትቅፅ (የአሚኖግልባጭ

የተሳታፊዉልዩመለያቁጥር _____

የተሳታፊዉክም_____

እኔስሜልላይየተጠቀሰዉተሳታፊ“የአንጅትተስቦበሽታበደምላይየሚሳየዉላዉጥ”
በሚልርዕስበታሰበወሚኖምርላይበሚባኝቋንቋበቁመጃአግኝቻለሁ፡፡የህክምናመረጃናየደምናጭምንምአይነት ጉዳትብሞደርስመልከእንደሚሰድተረድቻለሁ፡፡በተፈጩምየሚሰዱሞችወምመረጃዎችብሚጥርእንደሚዘተነግሮኛል፡፡እንደህምየምየቀዳመረጃያለመፈጠናበጥናቱያለመፈተኩጥናቱበሞችወምመቅትራሴንማግለልእንደምችልየተገለፀልኝሰሆንይህንንምፀሞርረጌወደፊትምህ አሁንየማ ኛችወየህክምግልጋለፆችእንደሚጓደሉብኝተረድቻለሁ፡፡

እስማማለሁ_____ አልስማማም_____

በመሆኑምለዚህምርምርለመሳተፍወስናለሁ፡፡

የተሳታፊስም_____ፊርማ----------ቀን ----------

የተመራሚስም----------ፊርማ----------ቀን ----------

ምስክሮች

1.ስም----------ፊርማ----------ቀን ----------

2.ስም----------ፊርማ----------ቀን----------

ANNEX III: QUESTIONNAIRE

JIMMA UNIVERSITY, INSTITUTE OF HEALTH, FACULTY OF HEALTH SCIENCES, SCHOOL OF MEDICAL LABORATORY SCIENCE.

INSTRUCTIONS: This questionnaire contains a question, which are pertinent to the research objectives. You are kindly requested to answer all as much as possible and carefully by filling the blank spaces and encircling one appropriate choice from the alternatives given. Participant Identification Participant serial number _____

Identification code_____

S.No	Question	Response	Comment
Section I. SOCIO-DEMOGRAPHIC CHARACTERISTICS			
1.	Age	**_____years**	**/-----/**
2.	Sex	Male……1 female……2	/------/
3.	Residence	Urban……..…..…1 Rural……...2	**/-----/**
4.	Educational status	No education……1 Primary(1-8)…….2 Secondary ……..…...3 College and above…….4	**/-----/**
5.	Occupation	Student ……1 Gov't employee……2	**/-----/**

		Housewife…….....3 Merchant……..4 farmer……..…….…5 Other …….6	
Section IIBEHAVIORAL CHARACTERISTICS AND NUTRITIONAL STATUS			
6.	Do you habit of eat red meat and animal Products?	Yes……..……1 No……...2	**/---/**
7.	Do you eat green vegetables per week?	Yes……..……1 No……..…….2	**/----/**
8.	Do you eat fruits per week?	Yes……..……1 No……..…….2	**/----/**
9.	Do you drink coffee	Yes……..……1 No……..…….2	**/---/**
10.	Do you drink Tea after meal?	Yes……..……1 No……..…….2	**/…/**
Section III clinical feature			
1.	Do you have fever	Yes……..…..……1 No……..…….2	**/----/**
2.	Do you have Headache	Yes……..……1 No……..…….2	**/---/**
3.	Do you have Weakness and fatigue	Yes……..……1 No……..…….2	**/---/**
4.	Do you have Sweating	Yes……..……1 No……..…….2	**/---/**

5.	Do you have dry cough	Yes……..……1 no	**/---/**
6.	Vomiting	Yes……..……1 No……..…….2	**/---/**
7.	Nausea	Yes……..……1 No……..…….2	**/---/**
8.	Do you have Loss of appetite	Yes……..……1 No……..…….2	**/---/**
9.	Do you have Diarrhea	Yes……..……1 No……..…….2	**/---/**
10.	Do you have any Muscle aches	Yes……..……1 No……..…….2	**/---/**
11.	Do you have Extremely swollen abdomen	Yes……..……1 No……..…….2	**/---/**
Section IV Have you exposed to the following disease			
1.	History of chronic illness 1. TB 2. DM 3. Hypertension 4. HIV 5. Other _____	Yes……..……1 No……..…….2	**/---/**

Laboratory result format

Code no_____

Laboratory requesting and recording format for widal test and hematologic parameter

1. Serological test

- ✓ Widal slide test _____
- ✓ Titration result_____
  1:20, 1:40, 1:80, 1:160, 1:320…
- NB; 1:320 considered as reactive_____

2. Hematology

CBC	Unit	Reference range	Observed result
RBC	$\times 10^6$/ µl	4.26-6.68	
HCT	%	36.86-51.59	
HGB	g/dl	12.06-18.76	
MCV	fl	77.3-98.82	
MCH	Pg	24.86-33.58	
MCHC	g/dl	32.06-36.5	
RDWc	%	12.46-17.56	
Total WBC	$\times 10^3$/ µl	3.31-11.6	
Neutrophil	%	40-70	
Lymphocyte	%	20-40	
Mid granules(mixed)	%	4.5-12.5	
Platelet count	$\times 10^3$/ µl	164.0-444.5	

ANNEX-V: LABORATORY PROCEDURES

V, I. Complete blood counting (CBC) by huma count 30^{TS}/huma count80^{TS} hematology analyzer

A CBC (complete blood count), also known as a FBC (full blood count), is usually the first test requested by physicians to assess general patient health. The CBC can be used to detect a wide range of pathological states including anemia, infection and hematological malignancy, as well as for the monitoring of cancer patients undergoing chemotherapy.

PRINCIPLE:

The huma count 30^{TS}/huma count80^{TS} hematology analyzer are fully automated 3- differential cell counters designed for in vitro diagnostic use developed for small clinics and point of care lab offices. It is bench top hematology cell counters. They implement the so-called coulter-method for counting cells passing through a small aperture, and measure the hemoglobin content of red blood cells. The analyzer features a color graphical display module with large touch screen. The software allows sending result to an external printer (via USB port), to the 58mmbuilt-in thermal printer module.

Its internal memory is capable of storing 10000 records with full histogram, and individual patient data. QC measurements are also stored in separate data base. The software operating the instrument is easy to upgrade using a USB pen-drive. The instrument allows connecting to a host computer for uploading records stored in the memory through a USB (slave) port. Archiving and restoring of records to and from USB pen-drive is also possible.

The huma count 30^{TS} can process 30 samples, huma count80^{TS} can process 80 samples per hour in 3-part differential mode. Samples can have individual sample data, and additional parameters. You can print results to an external or to built-in printer. The user can customize the report format. The analyzer determines the following 22 hematology parameters, including 3-part WBC differential,froma25µlwholebloodsample:WBC,LYM,MID,GRA,LYM%,MID%,GRA%,HGB,RBC,HCT,MCV,MCH,MCHC,RDWcv,RDWsd,PLT,PCT,MPV,PDWcv,PDWsd,P-LCC,P-LCR(38).

REAGENTS AND MATERIALS:

A. Supplies

1. Distilled water
2. Gauze, plastic lined wipes
3. Humacount reagent
4. EDTA anticoagulant tube/(Vaccutainer tube)
5. Cotton Swab
6. Vaccutainer needle with holder
7. 70% Ethanol alcohol or similar antiseptic
8. Tourniquet
9. Glove

Specimen type: EDTA anticoagulated blood.

A. Specimen Volumes required:

i. Four (4) ml of EDTA anticoagulant of whole blood is required for analysis the result.

B. Unacceptable specimens including those listed below must be rejected:

i. Clotted samples or those containing clots

ii. Grossly hemolysis samples.

Specimen stability: collected in EDTA anticoagulant, a well-mixed whole blood specimen and run within two hours after collection, provides the accurate results.

Huma count reagent

Use only reagent supplied by human with the analyzer, otherwise accuracy cannot be guaranteed.

- HC-DILUENT (Cat.No.17400/11):

Isotonic saline solution, used to dilute whole blood samples and to rinse the flidic system between measuring procedures.

- HC-LYSE(Cat.No.17400/22):

Create hemolysate for 3-part WBC differential and for total WBC and HGB.

- HC –CLEANER (Cat.No.17400/31): For cleaning process of the fluidics.
- HC-Control (Cat.No.17400/40): Stabilize blood cell control.
- HC-calibrator (Cat.No.17400/50): Stabilize blood cell calibrator.

Technical operation

As the cell counter is fully automated instrument, operating requires minimal training or technical support. Operator instruction is reduced to the following:

- ✓ Perform a blank measurement in case the instrument is not used for a specific time.
- ✓ Enter sample and /patient data.
- ✓ Insert the sample to be analyzed into the sample holder.
- ✓ Print the result either one-by-one, or in groups by selecting records from the data base.
- ✓ Perform simple weekly maintenance.

Method of measurement

I. Photometric light absorbance method

A lysed blood sample (WBC) dilution is analyzed for hemoglobin concentration based on its stable chromogen content. The lyse reagent lyses the red blood cells causing them release cellular hemoglobin. The hemoglobin concentration is measured by taking a photometric reading across the huma count 30^{TS}/humacount80^{TS} WBC chamber. An HGB result is calculated as the difference between a blank and a sample measurement with and without illumination to reduce the effect of liquid refraction and incident light.

All human branded reagents are cyanide free, and thus are environment friendly. However, some reagents from other manufacturers may contain cyanide. In that case cyanide and any other chemical composition formed using cyanide is environmentally dangerous.

II. Volumetric impedance method

It determines cellular concentrations and volume distributions of cells by detecting and measuring changes in electrical impedance when particles suspended in conductive liquid pass through a small aperture. A constant direct current flows between the electrodes on both sides of aperture. Each cell passing through the aperture causes a change in the electrical impedance of the conductive blood cells suspension (diluted blood).this impedance change is detected by the humacount30^{TS}/80^{TS}electronics and converted to an electrical voltage pulse. The number of pulses is proportional to the number of particles in the diluted sample.

Intensity of each voltage pulse is proportional to the volume of the particle. The volume distribution diagrams of the particles are displayed as the WBC, RBC and PLT histograms measured in femtoliter (fl, μm^3) units. Electronic discrimination by size allows separation of platelets and erythrocytes and white blood cell populations. Discriminators are indicated by dotted vertical lines on the histograms(38).

III. 3-part differential analysis method

Humacount 30^{TS}/80^{TS} is a 3-part differential hematology analyzer. It uses a WBC lytic process that allows the instrument to simultaneously count and size- differentiate the WBCs. There are three elements controlling where the different cell types fall in the 3-population WBC histogram:

- Chemical formulation and concentration of the lytic reagent:

It controls how different WBC types are differentially lysed. Concentration of the lytic reagent controls the rate of the lysing (shrinking) process.

- Cell type and maturation of the cell types present for analysis:

Different WBC types and their grade of maturation have different sensitivities to the lytic reagent. Different WBC types lyse at different rates:lymphocytes are the most sensitive, while band neutrophils and segmented neutrophils are the least sensitive. Eosinophil, basophils, immature granulocytes and blasts also have a cell wall membrane that is more sensitive to the lytic process there by moving them into the mid -size cell population following the lytic process and at the time, these cells are counted and sized.

- Time window in the lytic process that the cells are counted and sized:

The lytic process is a dynamic reaction, not a static reaction. Therefore, the count and sizing time window used during the lytic process has been optimized for performance. Data of particles are used to present a size distribution histogram of the WBC populations. Since size distribution is the only data available for the WBC histogram, there is no enough resolution to accurately differentiate more than three distinct WBC populations.

Quality control

The quality control menu allows the operator to monita or day-to-day reproducibility, and accuracy of humacount30^{TS}/ humacount 80^{TS}. Before performing the QC measurements the target values and tolerance ranges for each parameter must be entered for the QC material (control blood) is possible to use. Target values of the control material should be sent only once, when you start using the given lot. Resetting parameters deletes previous QC results of that level. Material any change in the QC material setting deletes previous QC result. It is strongly recommended to print QC results prior to changes.

Always follow the manufacturer's instructions for warming and mixing of the QC material before use. Instructions are listed in the package insert instruction sheet. Keep track of the expiration and open bottle stability of the QC material. Most QC materials (controls) are shipped with non-piercable hard caps. Remove the cap before running the control (30TS/80^{TS}humacount machine manual)(38).

V, II.Blood film preparation

Principle: Eosin and methylene blue in the solution contains various azure compounds such as thiamine and its methylene derivatives the staining reaction is oxidative. Therefore, the oxygen in water will initiate the reaction and run the stock stain (39).

Materials, Reagents &Equipment's

- Clear microscope slide
- Microscope
- Staining jars (dish)
- Lens paper
- Methyl alcohol
- Immersion oil
- Buffer (ph7.1-7.2)
- Giemsa stock solution
- Timer
- Drying rack

Sample: One to two drop of whole blood from EDTA anticoagulated venous blood.

Evaluation of well-stained thin film:

The back ground should be clean and free from debris; the colour of erythrocytes is a pale green pink.

The combination of malaria parasites is a deep purplish red and cytoplasm a clear purplish blue.

Stippling should Shaw up as schuffner's dots in erythrocytes containing *P.vivax* or *P.ovale,* and *Mauree's* spots in erythrocytes containing the larger ring forms of *P.falciparum.*

Evaluation of well-stained thick film:

Malaria parasites are well defined with deep-red chromatin and pale purplish blue cytoplasm. In *P.vivax* and *P.ovale* infections the presence of schuffner's stippling in the ''ghost'' of the host erythrocyte can be seen especially at the edge of the film.

PROCEDURE:

Fixing the thin film.

1. When the films are completely dray, fix only the thin film by dipping if in absolute methanol for approximately 30 seconds. Care must be taken not to fix any point of the thick film.

2. Allow the film to dray.

Staining the thick and thin films.

1. Gently pour 3% or 10% Giemsa working solution in the staining jar.

2. Put the slides in a rack inside the staining jar; the slides should be fully submerged /covered with the stain.

3. Stain for 30-45 minutes and 10-15minutes for 3% and 10% Giemsa working solutions, respectively.

4. Pour clean water gently in to the jar to float off the iridescent scum on the surface of the stain. Alternatively, gently immerse the whole jar in a vessel filled with clean water.

5. Gently pour of the remaining stain, and rinse slides again in clean water for a few seconds. Pour water off.

6. Wipe the back of each slide with paper towels.

7. Dray the slides in a vertical position with the thin film down wards.

- **Focusing and scanning the blood film:**

1. Place the BF on the microscope stage, switch on the light and adjust the light source optimally by looking through the ocular and the 40x objective.

2. Place the drop of immersion oil on the dray stained slide. To avoid cross contamination, ensure that the immersion applicator never touches the slide.

3. Slowly change to the oil immersion objective, and a thin film of oil will form between the slide and the lenses.

4. Adjust the light source optimally by looking through the 10x ocular (eyepiece) and the 100x objective and use the fine adjustment knob to focus the lens should not be allowed to touch the slide.

5. Examine the slide start at the left end of the thick film and begin reading at the periphery of the field and finish at the other end. When the field is read, move the slide right to examine adjacent fields.

Examining the thick blood film:

- Scan the thick film under oil immersion objective (100x) and ascertain whether a smear is positive or negative.
- If positive, determine all species and stages present in the slide.
- Read a minimum of 200 oil immersion fields before declaring a slide negative. If time permits, scan the whole thick film.

Examining the thin blood film:

1. When species is doubtful on the thick film, or mixed infections are suspected, a careful examination of the parasite morphology should continue on the thin smear for verification.

2. If deferent hemo-parasites are observed, it should be recorded.

Quality control:

1. Follow proper sample collection procedures.

2. Glass slides must be clean and free from grease.

3. Thick films and thin films must be prepared properly and draying the blood films.

4. Do not dry exposed to direct sun light.

5. Too thin a film may not have adequate quantity of blood for detection of parasites.

6. Blood film spread unevenly on a grease slide makes examination difficult.

7. Thin film too long, leaves less space for thick film.

8. When fixing the thin film, take care the thick film do not touch by methanol.

9. Wet slides are warped together and the slides stick to one another.

10. Never add a pinch of EDTA powder directly to the sample tubes. High concentration of EDTA leads to shrinking of RBC and destroys the structure of WBC and platelet

11. Quality control of Giemsa stain is performed for every batch of the stain prepared and quality control results documented using the quality control form.

12. Working solution of Giemsa should be changed at least every 8 hours.

13. Check pH of buffered water, and add appropriate correcting fluid (39).

V, III. Parasitological Examination of Faeces

- **Principle:** Microscope slides made from patient specimens can be examined under low and high power for the presence of parasites.

Reagents and equipment:

- Normal Saline
- Glass slides
- Cover slips
- Pipettes
- Gloves
- Microscopes

- **Sample:**

Ask the patient to pass the stool sample directly into a waxed cardboard or a plastic cup with a tight fitting lid. Collection of sample in a match box or on plant leaves is not a satisfactory method.

About 20-40 grams of well-formed stool or 5-6 table spoonfuls of watery stool will suffice for a routine examination.

- **Microscopic examination:** It is the simplest and easiest technique. A wet mount can be prepared directly from faecal material or from the concentrated specimens. The basic types of wet mounts that should be made from each sample include:

Saline wet mount: It is used to detect worm eggs or larvae, protozoan trophozoites and cysts. Additionally, it can reveal the presence of RBCs and WBCs.

Procedure:

- Apply the patient's sample to a small area on a clean microscope slide.
- Immediately before the specimen dries, add 1 or 2 drops of saline with a pipette. Mix with pipette tip.
- Cover the specimen with a cover slip.
- Examine the specimen with the low power objective (10x) and low light.

- Examine the entire cover slip for motile flagellates. Suspicious objects can be examined with the high power (40x) objective.
- Ova, cysts, trophozoites and adult worms can be identified as per their characteristic features.

➢ **Quality control:** Check the saline. It should be clear with no visible signs of contamination.

➢ **Limitations:** If the specimen is left at room temperature or held at refrigerator temperature for > 1 hour, the organisms will round up, lose their motility, and eventually die(40).

V,V. Widal test

Principle

The main principle of widal test is that if homologous antibody is present in patients serum/plasma, it will react with respective antigen in the reagent and gives visible clumping on the test card and agglutination in the tube. The two major antigens used in the test are "H" and "O" antigens of *S.Typhi*. "O" antigen is a somatic antigen and "H" antigen is flagellarantigen.During infection antibodies are produced in patient's sera against these antigens. Antigens specifically prepared from this organism are used in the agglutination test to detect the presence of antibodies in patients' sera which are elucidated in response to infection by these bacteria. There are some agglutinins that are produced in the patient's serum/plasma (41).

Materials Required

- ✓ Sterile test tubes.
- ✓ 0.85% sterile saline.
- ✓ Micropipettes, Tips.
- ✓ centrifuge
- ✓ Salmonella typhi'O' Antigen
- ✓ Salmonella typhi'H' Antigen
- ✓ negative control
- ✓ plasma Sample

Procedure for test tube method

1. Before starting the experiment, bring all reagents to room temperature and mix well.

2. Prepare 2 sets of test tubes for individual antigen. Each set contains 1- 8 tubes.

3. Add 1.9 ml of 0.85% sterile saline to tube no. 1 of each antigen set.

4. To tube no. 2-8 of all sets add 1 ml of physiological saline.

5. To tube No. 1 of all sets add 0.1 ml of test sample to be tested and mix well.

6. Transfer 1 ml of the diluted plasma sample from tube No. 1 to tube No. 2 and mix well.

7. Transfer 1 ml of the diluted plasma sample from tube No. 2 to tube No. 3 and mix well. Continue this serial dilution till tube No. 7 in each set of antigen.

8. Discard 1.0 ml of the diluted plasma from tube No.7 of each set.

9. So the dilutions of the plasma sample from tube No. 1 to 7 respectively in each antigen set are 1:20,1:40,1:80, 1:160, 1: 320, 1:640, 1: 1280.

10. Tube no. 8 is negative control with 0.85% sterile saline.

11. To one set i.e. from tube no.1- 8 add 50 µl of Salmonella typhi 'O' antigen.

12. In second set i.e. from tube no.1- 8 add 50 µl of Salmonella typhi 'H' antigen.

14. Mix well, cover and incubate these tubes overnight at 37^0C (approximately 18 hours).

15. After incubation dislodge the sediment and observe for agglutination ((41)(42)(43).

- **Evaluation of the result**
  - ✓ During the infection of Salmonella the human body responds to the antigenic stimulus and as a result corresponding antibodies are produced.
  - ✓ When the test sample is treated with colored and attenuated Salmonella antigen suspensions,
  - ✓ The antibodies present in the sample react with the antigen suspension to give clearly visible agglutination which can be seen through naked eye.
  - ✓ The antibody titre of the test sample is its highest dilution that gives a visible agglutination.
  - ✓ Agglutinin titre greater than 1:320 is considered as significant infection and low titres indicate absence of infection (44).
- **Procedure of slide method :**

1. Before starting the experiment, bring all reagents to room temperature and mix well.

2. Mark the circles of slides as PC (Positive control), NC (Negative control), O and H as per antigen solutions used for testing.

3. Add 1 drop of positive control (25µl) into the circle marked as PC of given glass slide.

4. Then add 1 drop of negative control (25µl) into the reaction circle marked as NC.

5. Add 1 drop of test sample (25µl) into each reaction circle labeled as O and H according to given antigen solution.

6. Add 1 drop of Antigen solution of *Salmonella typhi*'H' into PC and NC circle each. Mix

Well with using new mixing stick for each circle.

7. to circles labeled as O and H in which test samples has been added, add antigen solutions of *Salmonella typhi*'O' and *Salmonella typhi*'H'.

8. Mix the content of each reaction circle uniformly with separate mixing stick.

9. Rock the glass slide gently (approximately for one minute) and observe for agglutination.

Material and reagent required

- ✓ Glass Slide
- ✓ Disposable Mixing Sticks
- ✓ Positive control
- ✓ Negative control

- **Evaluation of the Result:**

After mixing the test sample with Antigen Solution, Positive control, Negative control separately

Observe for the agglutination reaction

- **Quality control for widal test**

✓ Widal tube test kit should be done according to the kit instructions.

- ✓ Allow all reagents to reach room temperature before use.
- ✓ Do not dilute any of the kit reagents.
- ✓ Do not inter mix the reagents.
- ✓ Do not freeze any of the kit reagents.
- ✓ Ensure that the latex reagent, positive and negative controls are stored in refrigerator (2-8oC) ((41)(42)(43).

APPROVAL SHEET

JIMMA UNIVERSITY SCHOOL OF GRADUATE STUDIES

SCHOOL OF MEDICAL LABORATORY SCIENCES

DEPARTMENT OF HEMATOLOGY AND IMMUNOHEMATOLOGY

As thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance by **: AYANTU ENDALEW**, entitled: **"The hematological parameters and its association with typhoid infection among the patient suspected for typhoid fever at Shanan gibe hospital,Jimma, Ethiopia".**

1st advisor Dr. TILAHUN YEMANE (MD, Ass.prof) **…..…… ……...**

Signature Date

2nd advisor Mr. WONDIMAGEGN ADDISU (BSc, MSc) **…… ……..**

Signature Date

As members of the Examining Board of the Final M.Sc. Thesis Open Defense, we certify that we have read and evaluated the thesis prepared by **Ayantu Endalew** entitled: **"Thehematological parameters and its association among the patient suspected for typhoid fever at Shanan gibe hospital Jimma,Ethiopia"** and examined the candidate. We recommend that the thesis could be accepted as fulfilling the thesis requirement for the Degree of Masters of Science in hematology and immunohematology.

Approval by Assessor **Signature** **Date**

Lealem Gedefawe (MSc, Ass, prof) _____ _____

Head of the Department **Signature** **Date**

Lealem Gedefawe (MSc, Ass. prof) _____ _____