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Original Research Article

Some Hematological Parameters among Patients with Pulmonary Tuberculosis – Khartoum State

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Abstract: Tuberculosis is a highly prevalent chronic infectious disease caused and it's still a common disease in develop countries and still a major cause of mortality today , can affect many part of the body like lung and bone this defect can lead to defect in most of blood component. The aim of the study is to measure some of hematological parameters among Sudanese patients affected with pulmonary tuberculosis in Khartoum state. During the period between April to August 2013. 40 patients with pulmonary tuberculosis (25 males and 15 females) and 20 healthy controls (14 males and 6 females) were included 2.5 ml of blood was collected from patients and controls into EDTA containers then CBC Test was carried out immediately after collection, were carried out (Hb, RBCs count, HCT, MCV, MCH, MCHC, TWBCs, platelet count and differential leucocyte count) using Hematological analyzer (Sysmex KX-21N). Data obtained were analyzed using software program statistical package of social science (SPSS.V.11.5). The results in pulmonary tuberculosis patients when compared with control showed that there was significant lower values in Hemoglobin (Hb), Red Cell Count (RBCs) and Packed Cell Volume (PCV) (P= 0.00) and normal Means MCV (83.4±2), Mean MCH (30.1±4.3) and MCHC (36.1±1.1) in T.B patients compared with controls group. Total leukocyte count (TWBCs) and absolute Monocyte count was found both significant increase (P=0.00) compared to controls. The absolute Neutrophil count showed significant increase in T.B patients (P. 0.008) compared with control. And the most type of anemia was normocytic normochromic anemia. The significant increase in platelet count (Thrombocytosis) was showed in T.B patients (P. 0.00) compared with controls group. Moderate normocytic normochromic anemia , leuckocytosis especially neutrophilia with monocytosis and moderate thrombocytosis was found in T.B patients compared to controls group and other further studied is needed in this field.
Keywords: Tuberculosis, chronic infectious disease, Hemoglobin, Total leukocyte count

INTRODUCTION:

Tuberculosis is a highly prevalent chronic infectious disease caused by Mycobacterium tuberculosis. Pulmonary tuberculosis (PTB) is still a common disease in develop countries[1]. One third of world population is infected and approximately 3 million people die annually from pulmonary tuberculosis[2]. The following people are at high risk for active tuberculosis: elderly, infants and people with weaken immune system, for instance, AIDS. The risk for contracting TB increases if one is in frequent contact with people living with the disease, poor nutrition and living in crowded or unsanitary living condition [3]. The following factors may increase the rate of TB infection in a population: Increase in HIV infections, increase in number of homeless people (poor environment and nutrition) and the appearance of drug-resistant strains of TB [3].

Test that may be used in the diagnosis of tuberculosis include sputum examination for acid – fast bacilli ,chest CT scan , chest X-ray , cultures and tuberculin skin test however nucleic acid amplification test and PCR test are used for diagnosis of tuberculosis [4]. Tuberculosis prevention and control takes two parallel approaches. First people with tuberculosis and their contacts and then treated in the second approach children are vaccinated to protect them from tuberculosis infection[5].

In other concept, hematological profile are those parameters of the blood forms (red blood cells, white blood cells and platelets) with normal range or value as reference for any value to know whether or not they are normal[6]. Therefore there is the need to know those parameters of the blood which are abnormal in patients living with pulmonary tuberculosis and few of red and white cell indices would be considered. It is true that when one gets infected with pulmonary

tuberculosis there is some level of changes which occurs in the blood because it is a bacterial infection that it is secrete some substance that effect of some hematological parameters in active pulmonary tuberculosis anemia and iron deficiency erythropoiesis was observed there was a closed correlation between acid-fast bacilli in sputum and abnormal hematological value [7-9].

Hematological abnormalities in pulmonary tuberculosis:

Pulmonary tuberculosis may produce reversible abnormalities of the peripheral blood. Hematological abnormalities have been associated with tuberculosis and response drug therapy. However the changes in response to therapy have not fully been determined in pulmonary tuberculosis patient living developing countries[10].

Anaemia:

Anaemia is define as qualitative or quantitative deficiency of hemoglobin which normally carries oxygen from the lung to the tissue [11]. some of causes of anemia reduce hemoglobin production , reduce DNA synthesis , reduce stem cell production, Bone marrow infiltration, infection, increase red cell destruction and acute or chronic blood loss [12]. The mild to moderate anemia that is often observed In patient with infection , inflammatory or neoplastic disease that persist for more than 1-2 months is called anemia of chronic disease. Anemia has been reported in 16 % to 94 % in patient with pulmonary tuberculosis. All chronic infections including tuberculosis can cause anemia. Typically anemia develops during the first 1-2 months of illness and there after does not progress[13]. The hematocrit usually maintained between 0.25 and 0.40 l/l but significantly lower values observed in 20-30 % patients [14]. This is particularly likely in syndrome associated with increased levels of interleukin 6. Interleukin 6 produces a delusional anemia: expansion of the plasma volume resulting in a reduced hematocrit or hemoglobin concentration without changing in the circulating red cell mass [15].

Various theories of pathogenesis have been suggested in TB-associated anemia, but most Studies have shown suppression of erythropoiesis by inflammatory mediators as a cause of anemia. The observation that patients with TB - associated anemia display an absence of bone marrow iron[16]. Suggests that iron-deficiency is a possible cause of anemia in patients with TB. Hypoferremia can be caused by tumor necrosis factor (TNF)-alpha, interleukin (IL)-1 and IL-6, which are T helper (TH) 1 cytokines. In a cell-specific fashion, TNF-alpha and IL-1 enhance iron uptake and ferritin synthesis [17]. In addition, inflammatory cytokines such as interferon- gamma can stimulate nitric oxide production in addition to their effects on iron metabolism; inflammatory cytokines also influence erythropoietin production and erythroid progenitor cell proliferation. Tumor necrosis factor-alpha and IL-1 inhibit erythropoietin production by mechanisms independent of the hypoxia-inducible factor (HIF)-1 pathway, although a role for an increase in intracellular iron cannot be excluded. Interestingly, IL-6 increased erythropoietin production in hepatocytes but appeared to inhibit it in the kidney. The overall net effect is a limited increase in erythropoietin production that is inappropriately low for the degree of anemia a phenomenon that is common to infectious, inflammatory, and neoplastic disorders[18].

The erythrocytes usually are normocytic and normochromic, however, hypochromic and microcytosis may be observed. The initial MCV was found reduced in subjects with untreated tuberculosis, but the level was not as low as that found in iron deficiency. Microcytosis (mean corpuscular volume (MCV) less than 80Fl) was observed in 2-8% of patient with anemia of chronic disorder [19].

However other more recent studies report a frequency of 20-40 % .In various studies, hypochromasia was observed in 23-50 % of patient with chronic infection . Another distinction from iron deficiency is that hypochromic typically proceeds microcytosis in the anemia of chronic disorders but typically follows the development of microcytosis in iron deficiency , Macrocytic anemia is rarely associated with pulmonary tuberculosis. Anemia attributable to folate or vitamin B12 deficiency is infrequently identified, although decrease in serum folic acid level is documented in as much as 30 percent with pulmonary tuberculosis [14].

White blood cell changes:

Changes in leukocyte parameter are often considered among the hall mark of infection. These include changes in cellular morphology and number. Fishman demonstrated that, differential white cell count was usually normal except when the tuberculosis disease was advanced and active. Although changes did occur in relative numbers of lymphocyte, monocyte and polynorphonuclear leukocyte, this did not prove useful either as clinical or prognostic indexes [20].

Leukocytosis:

The extent of WBC count may vary depending on etiologic agent, severity of infection and host factors. Most limited bacterial infection are associated with a WBC count of 12- 14 x 10^9 / L. Massive infection may produce leukumoid reaction with

elevation in WBC count 150-200x 10^9/L [22]. Various hematologic malignancies may be mimicked by the response to infectious agent among these, a picture that appears like chronic mylogenous leukemia (CML) is probably most common .A very high WBC count with immature cells may accompany infection when underlying disorder is present, such as malignancy, rheumatoid disorder or glomarulonephritis. Disseminated tuberculosis may manifest with WBC count of up to 200* 10^9 / L and a moderate left shift[20].

Neutrophilia:

Neutrophilia (or neutrophil leukocytosis) describes a high number of neutrophil granulocytes in blood. Neutrophils are the primary white blood cells that respond to a bacterial infection, so the most common cause of neutrophilia is a bacterial infection, especially pyogenic infections. Neutrophils are also increased in any acute inflammation, so will be raised after a heart attack other infarct or burn [21]. Neutrophila usually accompanies the leukocytosis and left shift .A relative or absolute neutrophilia is documented in 29-57 percent of patients with tuberculosis [7].

Lymphocytosis:

Lymphocyte count of less than 1X 10^9/ L are common in acute bacterial, fungal, viral, and protozoan infection, chronic infection with tuberculosis, histoplasmosis and brucellosis. Alteration In the number of B and T cell may also occur in bacterial, fungal and viral disease, in addition to, HIV, with which they are classically associated[20]. Absolute or relative decrease in peripheral lymphocyte count has been documented in patients with pulmonary tuberculosis[22].

Monocytosis:

Monocytosis may be present in certain bacterial infection including active tuberculosis. The role of monocyte in tuberculosis has been studied intensively. The cell plays an important part in the cellular reaction to the tubercle bacillus. The phospholipids of the organism are partly degraded within the monocyte and macrophage and cause the transformation of these cells to epitheliod cells. Monocyte is thus chief cell in new tubercle formation where there is a high monocyte – macrophage turn over. This activity reflected in the blood, with monocytosis regarded as evidence of active extension of the tuberculosis infection[23].

The monocyte to lymphocyte ratio is useful. The normal ratio is about 0.3 to 1 or less, in active tuberculosis, the number of circulating monocyte may equal or exceed the number of lymphocyte. A ratio of 0.8 to1.0 or higher indicates active exudation and an unfavorable prognosis.

Thrombocytosis:

Platelets are considered to be pulmonary immune cells, because they possess many of the classical features of immune cells and participate in the pathogenesis of some pulmonary diseases [24]. Platelets have been suggested to play a role in the inflammatory response, including defense against bacteria have been suggested to play a role in the evolution of inflammatory response against mycobacterium [24]. Various inflammatory cells, cytokines and mediators are involved in the formation of granulomatous lesions encountered in tuberculosis. Of variety of cytokines, interleukin-6 (IL-6) has been known to promote platelet production [8]. Reversible mild thrombocytosis is seen in about 52 percent of patients with severe pulmonary tuberculosis. The elevated mean platelet count of untreated patients decreases and normalizes with successful treatment of pulmonary tuberculosis [25].

Tuberculosis (T.B):

Pulmonary tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis. Other mycobacteria can also produce pulmonary tuberculosis these include Mycobacterium africanum and mycobacterium bovis. Usually the patients who have cavity lesion are an important source of infection. These patients are usually sputum smear positive. Coughing produce tiny infectious droplets, usually 3000 droplet nuclei and these can stay in the air for a long period of time[26].

The first genuine success in immunizing against tuberculosis was developed from attenuated bovine-strain tuberculosis by Albert Calmette and Camille Guérin in 1906. It was called "BCG" (Bacillus of Calmette and Guérin). BCG vaccine was first used on humans in 1921 in France, but it was not until after World War II that BCG received widespread acceptance in the USA, Great Britain, and Germany. The primary cause of TB, Mycobacterium tuberculosis, is a small aerobic non-motile bacillus. It divides every 16 to 20 hours, an extremely slow rate compared with other bacteria, which usually divide in less than an hour[27].

Signs and Symptoms:

When the disease becomes active, 75% of the cases are pulmonary TB that is TB in the lungs. Symptoms include chest pain, coughing up blood, and a productive , prolonged cough for more than three weeks. Systemic s symptoms include fever, chills , night sweats, appetite loss , weight loss , pallor, and often a tendency to fatigue very easily [28] . In the other 25% of active cases, the infection moves from the lungs, causing other kinds of TB, collectively

denoted extra pulmonary tuberculosis. This occurs more commonly in immunosuppressed persons and young children. Extrapulmonary infection sites include the pleura in tuberculosis pleurisy, the central nervous system in meningitis, and the lymphatic system in scrofula of the neck, the genitourinary system in urogenital tuberculosis, and bones and joints in Pott's disease of the spine An especially serious form is disseminated TB, more commonly known as miliary tuberculosis. Extrapulmonary TB may co-exist with pulmonary TB as well [29].

Pathogenesis of Tuberculosis:

Mycobacterium tuberculosis is characterized by a complex cell wall rich in mycolic acid together with peptidoglycan and arabinogalactin, a complex polysaccharide molecule that surrounds the cell membrane. Many cell wall components are of pathogenic significance. Lippoarabinomannan (LAM) stimulates monocyte inflammatory activity principally by binding to the $CD4^{+}$ receptor, also the binding site for bacterial lipopolysachrid [30].

The principle tissue immune response in tuberculosis is the formation of granulomas comprising cell of the monocyte lineage, including multinucleated giant cell and T lymphocyte. In initial stage of immune response, neutrophils are present; whereas more advanced disease is characterized by caseous necrosis and eventually deposition of calcium. After inhalation, mycobacterium tuberculosis is phagocytosed by the alveolar macrophage. Pulmonary surfactant protein may enhance the process of phagocytosis. The phagocytosing macrophage initiates the host immune response. Phagocytosis involves the compliment receptor CR1, CR2, CR3 as well as mannose receptor and adhesion molecule. Tuberculosis replicates within the cell by blocking fusion of phagosome and lysosome. Mycobacterium have several mechanism to block the formation of phagolysome formation, including inhibition of Ca^{2+} signals and Blocking recruitment and assembly of the proteins which mediated Phagosome lysosome fusion which prevent the acidification of vacuole. Phagocytosis is potent stimulus to gene expression and secretion of pro inflammatory cytokines such as tumer necrosis factor (TNF) , interleukin (IL) 1, IL6. The known consequences of TNF secretion include fever and cachexia, two prominent symptoms of tuberculosis. TNF also have role in granuloma formation and is formed at the site of human infection. At early stage of infection, cellular recruitment to the granuloma is essential, and macrophage derived chemokins are important in the process[31].

Diagnosis of Tuberculosis:

Tuberculosis is diagnosed definitively by identifying the causative organism (Mycobacterium tuberculosis) in a clinical sample (for example, sputum or pus). When this is not possible, a probable - although sometimes inconclusive [5]. diagnosis may be made using imaging (X-rays or scans) and/or a tuberculin skin test (Mantoux test). The main problem with tuberculosis diagnosis is the difficulty in culturing this slow-growing organism in the laboratory (it may take 4 to 12 weeks for blood or sputum culture). A complete medical evaluation for TB must include a medical history, a physical examination, a chest X-ray, microbiological smears, and cultures. It may also include a tuberculin skin test, a serological test. The interpretation of the tuberculin skin test depends upon the person's risk factors for infection and progression to TB disease, such as exposure to other cases of TB or immunosuppressant[5]. Tuberculin tests have the disadvantage of producing false negatives, especially when the patient is co-morbid with sarcoidosis, Hodgkin's lymphoma, malnutrition, or most notably active tuberculosis disease. The newer interferon release assays (IGRAs) overcome many of these problems. IGRAs are in vitro blood tests that are more specific than the skin test. IGRAs detect the release of interferon gamma in response to mycobacterial proteins such as ESAT-6. These are not affected by immunization or environmental mycobacteria, so generate fewer false positive results .There is also evidence that the T-SPOT.TB IGRA is more sensitive than the skin test. Diagnosis of TB has also been done with use of various radiotracers using nuclear medicine methods, which not only detects but also locates tubercular infection[32].

New TB tests are being developed that offer the hope of cheap, fast and more accurate TB testing. These include polymerase chain reaction assays for the detection of bacterial DNA. The development of a rapid and inexpensive diagnostic test would be particularly valuable in the developing world[33]. The acid – Fast(Ziehl-Neelsen)smear is rapid and inexpensive test that can be performed with a minimum of equipment and is very specific for mycobacteria.

Previous Study:

Tuberculosis (TB) is a highly prevalent chronic infectious disease caused by Mycobacterium tuberculosis, an aerobic intracellular binding bacterium (bacillus); because of this characteristic it prefers tissues which are always in contact with high oxygen levels, as in the lung [13]. The below study was done to know Hematological abnormalities among patients with pulmonary tuberculosis around the world but in Sudan till now no document have been found. Case control study was done in Saudi Arabia to investigations hematological changes and abnormalities associated to pulmonary tuberculosis patients. a total of fifty proven pulmonary tuberculosis patients (30 males and 20

female Saudis) were included, a mild anemia was observed in 18 out of 30 male PTB patients (60%) and 9 out of 20 female patients (45%). The MCV in male patients (83.28 fL) was also lower than in the normal males (86 fL). In the study, anemia occurred in 60% male PTB patients and 45% female patients. The blood cell morphology showed normocytic normochromic in 80% of the patients, while only 20% PTB patients had microcytic and hypochromic RBC. Such patients had correspondingly lower MCV and MCH values. Platelets counts were found higher both in male and female untreated PTB patients as compared with the normal values for Saudi population. However, the neutrophil count was relatively higher in the female patients as compared to the male PTB patients[34]. Another study was done in India to evaluate the hematological parameters in pulmonary tuberculosis patients who are positive for Mycobacterium tuberculosis bacilli in sputum. One hundred patients of fresh pulmonary tuberculosis with sputum positive for acid fast bacilli (AFB) were included and AIDS patients, disseminated tuberculosis and patients receiving ATT drugs were excluded in this study .In the study Anemia was seen in 74% of patients. In spite of the infection, 71 patients had a normal leukocyte count. Leucocytosis as a response to infection was observed in 26 patients. Three patients had leucopenia. Thrombocytosis was observed in 24 patients while thrombocytopenia was observed in 9 patients [35].

Other research carried out on Hematological profile of patients with pulmonary tuberculosis in Ibadan, Nigeria, it was found that the hematological indices of sixty two pre-treatment, sputum-smear-AFB positive pulmonary tuberculosis patients were examined. Haematocrit, white cell count and differentials and erythrocyte sedimentation rates (ESR) were estimated by manual methods. Statistically significant hematologic abnormalities found include high erythrocyte sedimentation rate (ESR), anemia occurred in 93.6%, leukocytosis in 22.3%, neutrophilia in 45.2% and lymphopaenia in 4.8% of the patients. Thrombocytosis occurred in 12.9%, while 8% had thrombocytopenia. None of the patients had leucopenia and only 8.4% had lymphocytosis[36].

Rationale:

Pulmonary tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis or TB (short for tubercles bac hematological parameter[26]. In Sudan there is no document have been found about the study, and the aim of study to investigate the changes of some hematological parameters among patients affected with pulmonary tuberculosis in Khartoum state. The study can be help to avoid complication of the disease and reduce rate of morbidity and mortality. Then the study may be useful as indicators of disease progression.

Objectives:

General Objective:

- To measure some hematological parameters among tuberculosis patients in Khartoum state, 2013.
- To measure hemoglobin, RBCs, PCV, red cell indices count in pulmonary tuberculosis patients and compared with normal individual
- To measure WBCs, absolute Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil count among pulmonary tuberculosis patients and compared with healthy individual.
- To count platelets among pulmonary tuberculosis patients and compared with normal individual.
- To determine morphological abnormalities in blood cells among pulmonary tuberculosis patients compared with normal individual.
- To determine the most common type of anemia in pulmonary tuberculosis patients.
- To determine the severity of anemia among pulmonary tuberculosis patients.

MATERIAL AND METHODS:

Study design:

This is analytical case control study , enrolled between March 2013 and June 2013 to measure of some hematological parameter among pulmonary tuberculosis patients whom visit the district hospital (Abuanjh Teaching Hospital) in Khartoum state.

Study population:

Covered Forty new cases with pulmonary tuberculosis patients not treated (25 male and 15 female), and were diagnosed by Ziehl – Neelsen stain for acid-Fast bacilli (+ve reaction) and Twenty healthy volunteers selected as controls, these volunteers are Negative (-ve reaction) Ziehl – Neelsen stain for acid-Fast bacilli and free from dieses or medication therapy in the last month before sample collection.

Inclusion Criteria:

- New cases of patients with pulmonary tuberculosis not treated diagnosed by Z-N stain for acid-Fast bacilli (+ve reaction).

Exclusion Criteria:

Were as follows:

- Pulmonary tuberculosis patients whom drug resistance.
- Pulmonary tuberculosis patients with HIV.
- Pulmonary tuberculosis patients whom receive drug.
- Pulmonary tuberculosis patients with any other complication and blood malignant or systemic disorder.

All above excluded from study population because it's actually changes and affect hematological parameters to that we don't know the causes of this change or affect from pulmonary tuberculosis or from other causes.

Sample Size:

Forty new cases pulmonary tuberculosis patients chosen by non probability sampling (25 males and 15 females) and control group of twenty healthy individuals have been selected.

Sampling:

Most of the blood samples are collected during Outpatient Department visit. 2.5 ml of venous blood was collected from each patients and controls via the antecubital vein using a plastic syringe with minimum stasis, into commercially prepared concentrations of sequestrene Ethylene Di-amine Tetra-acetic Acid (EDTA) bottles. Each sample was mixed gently and thoroughly to prevent cell lysis and clotting of blood, then determines complete blood counts (CBC) within 2 hours of collection while the remainder of 2.5 ml of blood was used to prepare thin blood film for blood cells morphology. full blood count analysis done on the same day of collection using Sysmex KN-21 N,(manufactured by Sysmex corporation Kobe, Japan) a three- part auto analyzer able to run 19 parameters per sample including hemoglobin concentration, packed cell volume, red blood cell concentration, mean corpuscular hemoglobin, mean cell volume, mean corpuscular hemoglobin concentration, white blood cells and platelet values and the reference value of these parameters see appendix-6 [37, 38].

Tools of Data Collection:

Data were collected by using proper personal interview questionnaire to the patients, included age, gender, occupation, present and past history of disease. All procedures were conducted after getting permission for informed consent from TB patient and Institutional Ethical Committee in Abo Anjah Hospital.

Methods of hematology analyzers Sysmex (KX2-1N):

Principally sysmex analyzer is based on the electronic resistance (impedance) detection method for counting and sizing recognition of the leukocytes erythrocyte, and platelet using three hydraulic systems for, WBC, RBC, platelet and hemoglobin, and display the results on the liquid crystal displayer (LCD) with histogram and printed out the results in thermal paper. The analysis was performed by using automated hematology analyzers Sysmex (KX2-1N) using EDTA anti coagulated blood fresh venous blood sample. For each sample of blood the following hematometric variables: red blood cells (RBC), hematocrit (PCV), hemoglobin (Hb),mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) absolute neutrophils count (ANC) and absolute lymphocytes count (ALC) were determined in an automated hematological counter and the reference value of hematological parameters show in appendix-6 [39].

Reagents and Materials:

Commercial close system reagents were provided by Sysmex KX-21N operators and Consist of:

- Cell pack and Stromatolyser: diluents and lysing reagent for use in Sysmex.
- Detergent and Cell cleaner: use for cleaning solution to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex Automated Hematology Analyzers, see appendix-5[39].

Principle and procedure of Sysmex KX-21N:

Measurement of blood cells (RBCs, WBCs, & Platelet),and hematological concentration were measured obtained by aspiration of small volume of well mixed K3 EDTA blood by sample probe and mixed with isotonic diluents in nebulizer .Diluted mixture aspiration was delivered to RBCs aperture both for providing information about RBCs and Platelet based on the cell size . Particles of 2to 20 fl counted as platelet. Above 36 fl was counted as reamed cell. Some portion of aspiration mixture induced in to WBCs both in which hemolytic reagent (Stromatolyzer) was added automatically to measure hemoglobin concentration in build calorimeter, based on cyanomethemogolobin method (HICN). Blood cell were counted size information was generated in triplicate pulses according to electronic conductivity , and translated into digital number using in build calculator programmed and designed for that RBCs ,WBCs count . Three parameters were directly measured and displayed on (LCD). Other values of red cell indices, platelet, and leukocyte differential and absolute count were calculated from given information and automated constructed histograms. The results printed out according to the setting mode .commercial close system reagent were provided by Sysmex KX- 21 operator and consists of cell pack , stromatolyser , detergent and cell cleaner and the reference value of hematological parameters show in appendix-6 [39] .

Preparation and Stain thin Blood Film:

Principle:

Manual rack method and Ral 555 kit stain were used. Firstly the slide was dipped 5 seconds in bottle(1) and drained on filter paper and dipped 5 seconds in bottle (2) contain eosin acidic dye ,which gives color to a basic component and surface solution drained in filter paper and dipped 5seconds in bottle(3) which contain methylene blue dye, a basic dye, which gives color to an acidic component then the slide was rinsed in distilled water and was lat to air dry then examined

under microscope .These dye differentiate the different component of blood cells as show in figure1.

Procedure:

Clean labeled slide was placed on flat bench then one drop of well mixed EDTA blood was added, spreader slide was possessed at angel 45 degree and moved back to touch blood drop, then the spreader was bushed smooth with little pressure to make and ideal thin blood film compose (head, body and feathered sharp tail) and let to dry and do all stain stabs as show below [37].

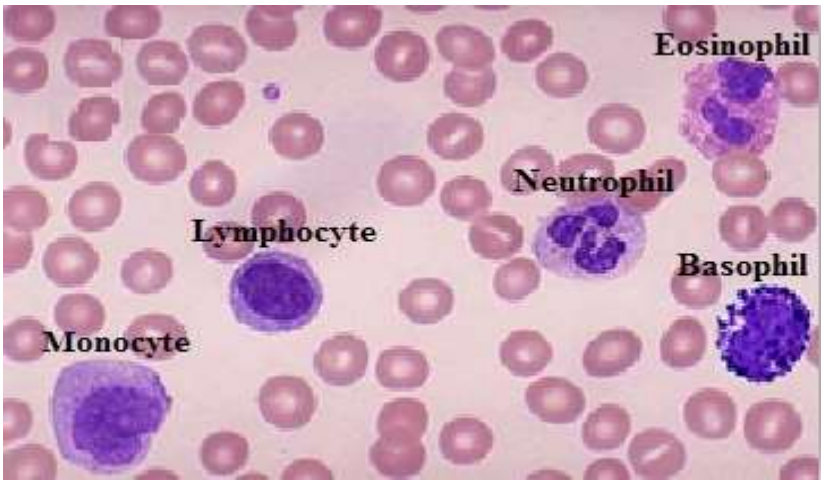

Fig-1: Normal Blood Cells Morphology [37].

Ethical Consideration:

The selection of subjects and the collection of specimens from the subjects were done after prior notice and approval from all protocols involved, was obtained and informed the aim of the study.

Data Presentation:

Data obtain analyzed by Statistical Package of Social Science (SPSS.version -11.5) software program and obtain mean and p.values by Independent – sample T test. The results presented in form of tables and figures.

RESULTS:

Demographic details:

The study was conducted to measure of some of hematological parameters among pulmonary tuberculosis patients whom visit Abo Anjh hospital in Khartoum state were investigated .The result were compared with apparently healthy subjects (controls). The study included sixty (60) subjects, 40 patients selected were already diagnosed as acid- fast bacilli positive by Z-N smear stain with mean age 41 years, 25 male (62.5%) and 15 female (37.5%) and 20 healthy volunteers were selected as controls, these volunteers are Z-N Stain Negative and free from dieses or medication therapy in the last three month before sample collection the mean age of control was 39 years as show in **Table1**.

Table2 Show the mean ± SD of Hemoglobin level in T.B patients was (9.6 ± 2.01g/dl) and in control group the mean of Hemoglobin was (14.06 ± .80 g/dl) ,when compared with controls group showed significant decrease in hemoglobin level (P.value = 0.00) . the mean of Red Blood Cell in T.B patients was (3.7± .64 $x10^6$ /ul) and in control group was (4.7 ± .77 $x10^6$ /ul) also showed significant decrease when compared with controls (P.value = 0.00), the packed cell volume was found significant decrease in T.B patients mean (31.0± 6.2%) compared with control group (39.4 ± 2.7 %) ,the mean of Mean Cell Volume was found in study group (82.7 ± 8.7 fl) and in control group was (83.4± 2 fl) when compared with controls group showed no significant difference wich is normal as controls (P.value= 0.78), the mean of Mean Cell Hemoglobin was found in study group (26.2 ± 4.3 pg) and in control group was (30.1 ± 1.2 pg) was found significant different (P.value = 0.00) , the mean of Mean Cell Hemoglobin Concentration was found in study group (30.6± 2.1 g/dl) and in control group was (36.1± 1.1 g/dl) , when compared with control showed decrease MCHC in patients with significant difference (P.value = 0.00) .

Table 3 show the mean ± SD of Total leukocyte count in T.B patients was (10±3.9 $x10^9$/L) and in control group was (6.7±1.9 $x10^9$/L) was found significant increase total leukocyte count when compared with controls group (P.value =0.00).the absolute neutrophils count in study group was found increase in count when compared with controls found significant different (P.value=0.008) ,the mean of patients (6.8±3.9 $x10^9$/L) and mean in control group was (3.9 ±1.5 $x10^9$/L), the mean of absolute lymphocytes count in study group was found (1.8±0.85 $x10^9$/L) and in control group was (2.2±0.57$x10^9$/L) with no significant difference (P.value=0.06), the

absolute monocytes count was increase in T.B patients when compared with controls and theirs significant difference (P-value0.00),the mean of absolute Eosinophils count in study group was found ($0.23\pm0.17x10^9$/L) and in control was (0.11 ± 0.1 $x10^9$/L), the mean of absolute Basophiles count in study group was (0.04 ± 0.06 $x10^9$/L) and in control group was ($0.15\pm0.03x10^9$/L).when compared absolute eosinophil and basophil count of patients to controls was found no significant difference (P.value>0.05).

Table-4 Show the mean ± SD of platelet count in study group was found (425.5 ± 22.9 $x10^9$/L) and in control group was (268.7 ± 12.1 $x10^9$/L) which is significant increase platelet count in T.B patients compared with control group (p.value=0.00).

Table-1: Frequency of Gender & Mean of Age among Study population:

	Frequency	**Percent%**	**Mean of years** ± SD
Male	25	62.5	41.2±7.3
Female	15	37.5	42.4±7.9
Total	40	100.0	41.7±7.3

Table-2: Some of Hematologic Parameters among Study Population:

Parameter	Variable	Number	9.67 ± 2.01	P.values
Hb (g/dL)	PTB patient	40	14.06± 0.8	0.00
	Control	20		
RBC($x10^6$/ul)	PTB patient	40	4.682± 0.37	0.00
	Control	20	31.02± 6.2	
PCV (%)	PTB patient	40	39.41± 2.69	0.00
	Control	20	82.75± 8.7	
MCV (FL)	PTB patient	40	83.45± 2.02	0.78
	Control	20	26.23± 1.2	
MCH (Pg)	PTB patient	40	30.16± 4.3	0.00
	Control	20	30.66± 2.1	
MCHC (g/dL)	PTB patient	40	36.14±1.1	0.00
	Control			

* The mean difference is significant at the P.value ≤ 0.05

* PTB (Pulmonary Tuberculosis Patient)

Tables-3: Total WBCs and Absolute Leukocytes Count in T.B patients:

Parameter($x10^9$/L)	Variable	Number	Means ± SD	P.values
TWBCs	PTB patient	40	10.06±5.09	0.00
	Control	20	6.71±1.9	
Neutrophil	PTB patient	40	6.84±3.9	0.008
	Control	20	3.92±1.5	
Lymphocyte	PTB patient	40	1.83±0.8	0.06
	Control	20	2.23±0.5	
Monocyte	PTB patient	40	1.14±0.89	0.00
	Control	20	0.39±0.16	
Eosinophil	PTB patient	40	0.23±0.17	0.29
	Control	20	0.11±0.1	
Basophile	PTB patient	40	0.04±0.06	0.12
	Control	20	0.015±0.03	

* The mean difference is significant at the P.value ≤ 0.05

* PTB (Pulmonary Tuberculosis Patient)

Table-4: Platelet Count among Study population:

Parameter **$x10^9$/L**	Variable	Number	Means ± SD	P.values
platelet	PTB patient	40	425.4±145	0.00
	Control	20	268.7±54.2	

The mean difference is significant at the P.value ≤ 0.05

* PTB (Pulmonary Tuberculosis Patient)

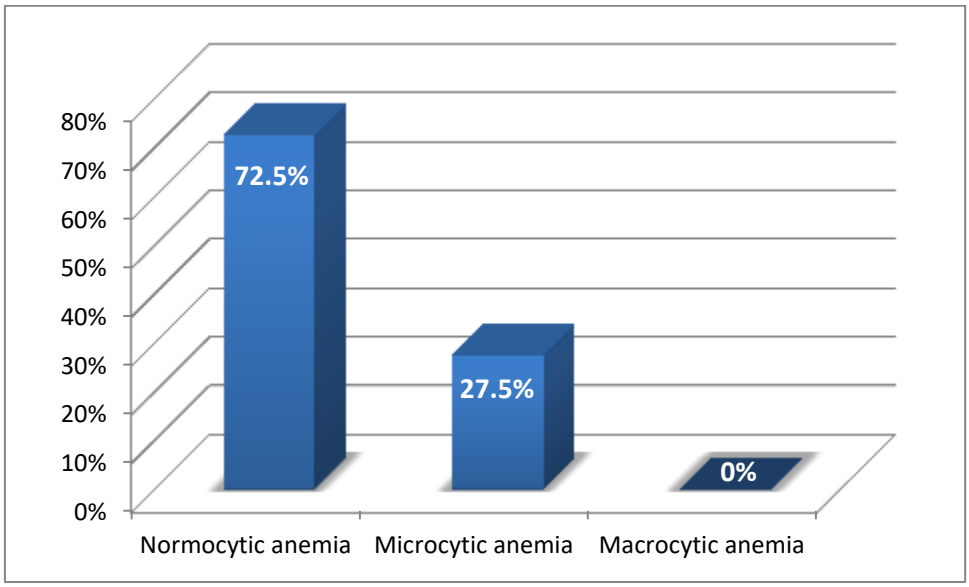

Fig. -2: Type of anemia among study population:

Type of anemia was found in patients:

The definition of anemia is degrease in hemoglobin concentration less than 13 g/dl in men and 12 g/dl in women anemia was identified in 34(85%) patient at the time of diagnosis of tuberculosis . 22 (64.7%) men and 12 (35.3%) women had anemia, normocytic anemia was the most common, and was identified in 29 (72.5%) of patient and microcytic anemia was next common, 11(27.5%) patients were identified with microcytic as show in figure 2.

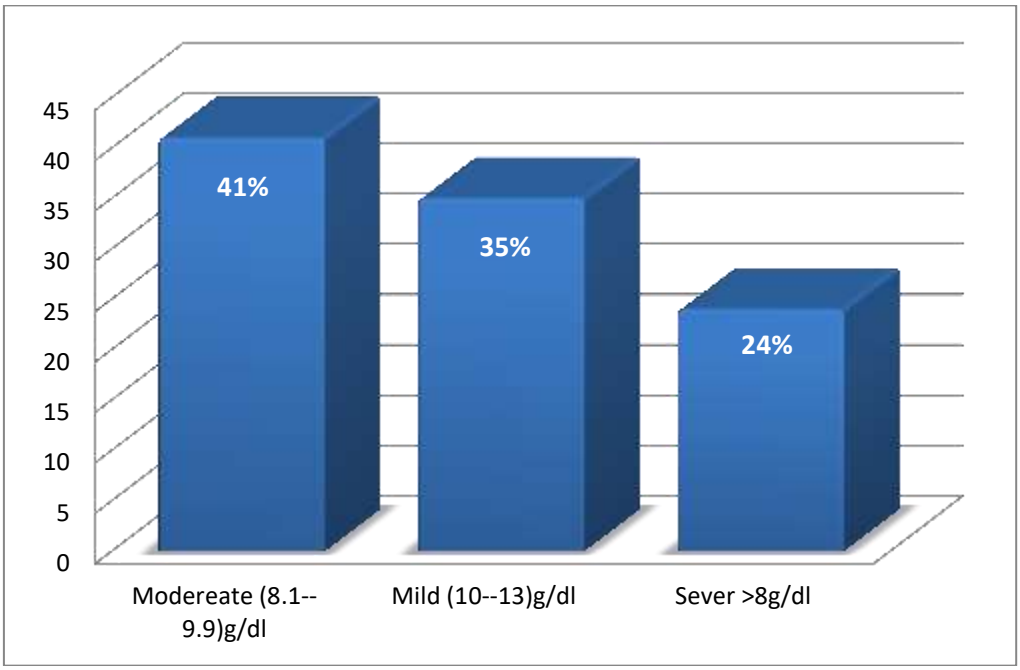

Fig.3: Severity of anemia among study population

The severity of Anemia among pulmonary tuberculosis patients:

The severity of anemia was determined as the result of hemoglobin level mild Hb (10-13) g/dl, moderate Hb (8.1–9.9) g/dl and sever >8 g/dl. There was found moderate anemia in 15(41 %) of total patients and 9 (36 %) in male, 6 (40 %) in female, the mild anemia was found 13 (35%) of total patients and 13(32.5%) in male 7 (23%) in female, the severe anemia was found in 9 (24%) of total patients**.** as showed above in figure 3.

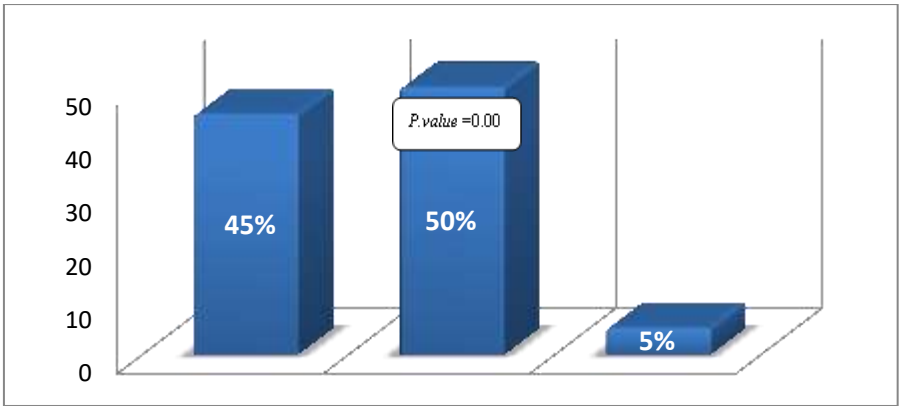

Fig.4: Total leukocytes count among study population

Total leukocytes count in study population:

In 40 patients, 2(5%) had total leukocytes count below 4.0 x10^9/L .18 (45%) had normal total leucocytes count and 20 (50%) above 11.0 x10^9/L.

Absolute Neutrophil Count (A.N.C) in study population:

As showed 40 patients, 2 (5%) had A.N.C below 2 x10^9/L, 15 (38%) had normal A.N.C and 23 (58%) had A.N.C above 7 x10^9/L.

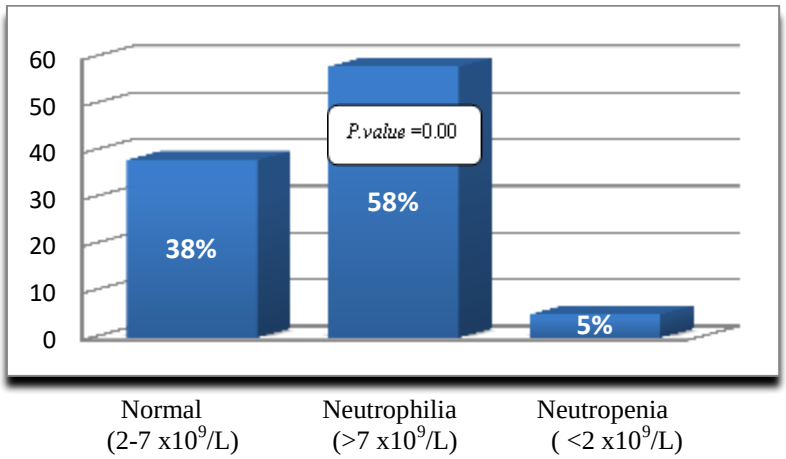

Fig. 5: Absolute Neutrophil Count (A.N.C) among study population:

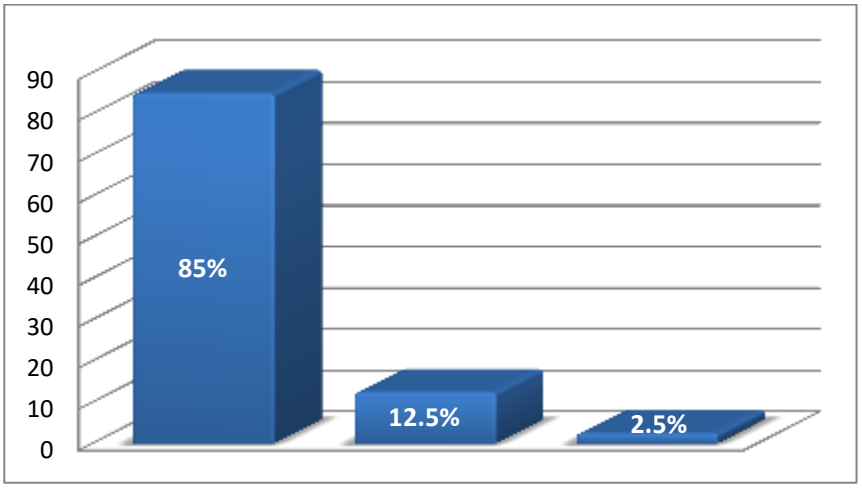

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Fig. 6: Absolute Lymphocyte Count among Study Population:

Absolute Lymphocyte count in Study population:

40 patients, 5(12.5%) had absolute lymphocyte count below1x10^9/L, 34 (85%) had normal absolute lymphocyte count and 1(2.5%) had absolute lymphocyte count above 3 x10^9/L

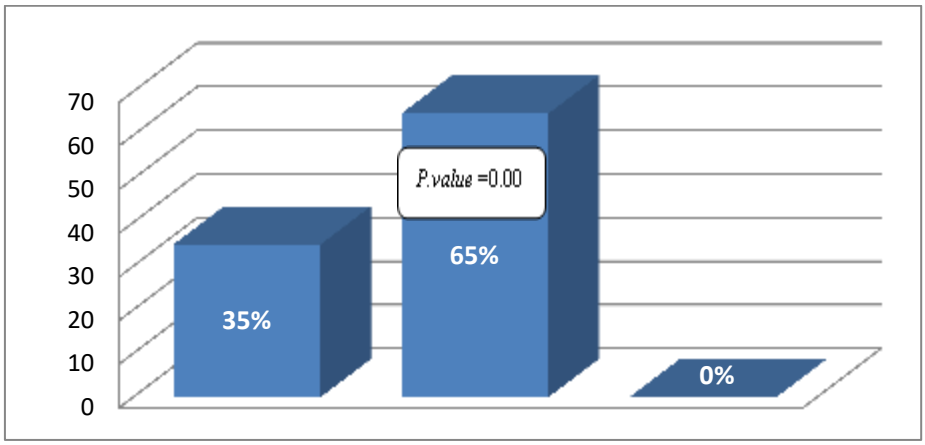

Fig. 7: Platelet Count in Studied among Study Population

Platelet Count in Study population:

In this study out of the 40 patients, 14 (35%) had normal platelet count and 26 (65%) had platelet count above 400 x10^9/L (thrombocytosis).

DISCUSSION:

Tuberculosis (TB) is a highly prevalent chronic infectious disease caused by Mycobacterium tuberculosis, an aerobic intracellular binding bacterium (bacillus); because of this characteristic it prefers tissues which are always in contact with high oxygen levels, as in the lung .in which single patient may have devastating effects on tuberculosis control program by infecting large number of people[1].

To that study was conducted to measure of some hematological parameter i.e. complete blood count of RBC and associated indices, WBC and absolute differential leukocyte count and platelets .The result obtained from the study carried on 40 pulmonary tuberculosis patients not treated in Abu Anjh Hospital in Khartoum state and 20 healthy volunteers whom not received any kind of therapy or have any malignant or chronic disease throughout the last three months from date of investigation.

Anemia was occurred (85%) in study population, (64.7%) of men and (35.3%) of women was found had anemia, these findings agree with reports by Yaranal *et al.* [35] who reported the possible cause of anemia in patient with pulmonary tuberculosis production of some cytokines that impair red cell production in the marrow. IL-1 and TNF-alpha inhibit the production of erythropoietin. The severity of anemia was determined as the result of hemoglobin level in which (41%) of total patients have moderate anemia and (35%) have mild anemia and only (24%) of patients have severe anemia the result finding was agree with study carry out by Al-Omar *et al.* [34]. The most type of anemia was showed predominantly normocytic anemia in patients and the second common type of anemia is microcytic anemia in patients, this study agree with study done by Al-Omar *et al.* [34]. And also similar to study done in India by Yaranal et *al.* [35].

Leuckocytosis in patients with pulmonary tuberculosis was increase in number count compared with controls group and show significantly difference (p=0.00) as response to infection, and leuckopenia also was documented in study patients (5%), These results similar with study done in Nigeria by Olaniyi *et al* [36].

Neutrophilia was documented in (58%) of patients in means of ANC (6.8±3.9) x10^9/L when compared with controls group show significant difference (P=0.00) the result agree with study done by Olaniyi *et al.* [36] and also similar with Olaniyi *et al.*[36] who reported neutrophilia associated with acute inflammation and bacterial infection. Monocytosis was reported in T.B patients was found significant difference (P.values =0.00) compared with controls group. Because monocyte plays an important part in the cellular reaction to the tubercle bacillus. The phospholipids of the organism are partly degraded within the monocyte and macrophage and cause the transformation of these cells to epitheliod cells. Monocyte where there is a high monocyte – macrophage turn over. This activity reflected in the blood, with monocytosis regarded as evidence of active extension of the tuberculosis infection [23]. Highly significant increase in platelet count in patients with pulmonary tuberculosis when compared with

healthy individual (p.value=0.00). The thrombocytosis was reported and no patient had thrombocytopenia in study population these findings agree with study done by Yaranal *et al.;* [35] and disagree with him and with Olaniyi *et al.;* [36]. In thrombocytopenia, who reported thrombocytopenia in the study population. was documented Platelets have been suggested to play a role in the inflammatory response, including defense against bacteria and in the evolution of inflammatory response against mycobacterium, Various inflammatory cells, cytokines and mediators are involved in the formation of granulomatous lesions encountered in tuberculosis. Of variety of cytokines, interleukin-6 (IL-6) has been known to promote platelet production.

CONCLUSION:

This study was concluded:

- Moderate normocytic normochromic anemia was found in the majority of pulmonary tuberculosis patients.
- Total WBCs count significantly increased in pulmonary tuberculosis patients with significant increase in absolute neutrophil and monocyte count compared to controls group.
- The majority of pulmonary tuberculosis patients were significantly moderate increase platelet count (Thrombocytosis) compared to the controls group.

Recommendation:

This study recommended:

- That physicians treat patients suffering from pulmonary tuberculosis not only of pulmonary tuberculosis but underlining hematological disorders as a result of pulmonary tuberculosis.
- Further studies should also focus on finding the extent of damage caused by these abnormal changes as a co-infection with pulmonary tuberculosis. This will help establish whether these changes significantly affects the progress of pulmonary tuberculosis or the vice versa in pulmonary tuberculosis patients, it will help establish the relation between pulmonary tuberculosis and abnormal hematological profile in pulmonary T.B patients.

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