Assessment of some haematological parameters among pre-treatment, 2 months, 4 months and 6 months treatment in pulmonary tuberculosis infected individuals in anambra state university teaching hospital, awka. anambra state, nigeria

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Abstract

This present study was designed to assess the level of some hematological parameters among pretreatment pulmonary tuberculosis infected patients, treatment in 2 months, 4 months, 6 months pulmonary tuberculosis infection and control, non-infected subjects in Anambra State University Teaching Hospital, Awka. Anambra State, Nigeria. A total of 220 pulmonary tuberculosis (PTB) infected subjects aged 18 – 60 were recruited for the study. Hundred (100) subjects were in their pretreatment stage (group B), and 40 each in their 2 months (group C), 4 months (group D) and 6 months (group E) treatment stages. Eighty (80) non PTB subjects served as control. Blood samples collected from the subjects were used for the determination of Haemoglobin concentration (g/dl), packed cell volume (l/l), Mean cell haemoglobin concentration (g/dl), Erythrocyte sedimentation rate (mm/hr), platelet count $(x10^9/l)$, total white blood count $(x10^9/l)$, absolute neutrophil count $(x10^9/l)$, absolute lymphocyte count $(x10^9/l)$ and absolute monocyte count $(x10^9/l)$ using standard laboratory methods for analysis as described by Dacie and Lewis[1]. Tuberculosis diagnosis was carried out using Ziehl Neelsen technique. The result showed that the mean ±SD Haemoglobin concentration and packed cell volume were significantly lower in pretreatment PTB subjects than the Post treatment subjects (P<0.05 in each case). The mean ±SD mean cell haemoglobin concentration showed no significant difference among the groups. The mean ±SD ESR in pretreatment subjects was significantly higher than the control and post treatment subjects. The mean (±SD) platelet count $(x10^9/l)$, TWBC $(x10^9/l)$ and ANC $(x10^9/l)$ were significantly higher while the ALC $(x10^9/l)$ was significantly lower respectively in pretreatment PTB patients compared with the control subjects and post treatment PTB subjects (P <0 .05 in each case). The mean (±SD) AMC $(x10^9/l)$ showed no significant difference when compared among the groups (P<0.05).
The result of the present study shows that haemoglobin and packed cell volume in pulmonary tuberculosis patients were reduced while Erythrocyte is increased. Thus indicating anemia of chronic disease. It also showed that platelet count, total white blood count and absolute neutrophil count are increased while absolute lymphocyte count is decreased in pulmonary tuberculosis infection.

Keywords: Pulmonary tuberculosis, Haemotological Parameters.

Acronyms

AIDS: Acquire Immune Deficiency Syndrome
AFB: Acid Fast Bacilli
DOTS: Directly Observed Short Course Therapy
ESR: Erythrocyte Sedimentation Rate
HIV: Human Immunodeficiency Virus
WHO: World Health Organization
MDR-TB: Multidrug resistance tuberculosis
MTB: Mycobacterium tuberculosis
TB: Tuberculosis
PTB: Pulmonary Tuberculosis
PCV: Packed cell volume
WBC: White blood cell

Background

Tuberculosis is a chronic infectious bacterial disease caused by Mycobacterium tuberculosis(MTB) complex which commonly affects the lungs but can affect any other parts of the body. Mycobacterium tuberculosis is an obligate, acid fast, slender, slow growing, and rod like bacteria. The commonest source of infection is an untreated pulmonary tuberculosis patient who is sputum smear positive.

Pulmonary tuberculosis (PTB) is a common disease in developing countries and efforts have been made to diagnose patients presenting hematological picture.[1]Pulmonary tuberculosis spread through the air when people who have an active Mycobacterium tuberculosis infection cough, sneeze of spit.[2,3] It can also be got through eating meat infected with tuberculosis and drinking unpasteurized milk.

Pulmonary tuberculosis infection is common in Nigeria at an incidence rate of 297 per 100,000 population.[4] Tuberculosis accounts for 1.8 million deaths and is the world's greatest infections killer of man and the leading cause of death among people with HIV/AIDS.[5] Other contributing factors are poverty, crowded living conditions in some homes, schools, prison, drug abuse, health workers, treatment failures, and insufficient funding for tuberculosis control programs.[6] The emergence of multiple drug resistant strains (MDR-TB) have also contributed to this new epidemic with from 2000 to 2004, 20% of tuberculosis cases being resistant to standard treatment.[4] Haematological abnormalities have been associated with tuberculosis.[7]

Few studies have been reported on the effects of pulmonary tuberculosis on hematological parameters especially in Nigerian literature. Significant abnormal hematological findings such as anemia, high Erythrocyte sedimentation rate, leukocytosis, and neutrophilia have been reported in patients with pulmonary tuberculosis.[8] Studies have reported high prevalence of anemia in patients with pulmonary tuberculosis. The precise mechanism of anemia in PTB patients is not clearly known, however, anemia due to inflammation as well as that of iron deficiency has been implicated. Factors such as decrease in red cell survival and reduced erythropoietin response by the bone marrow erythroid cells are also known to cause anemia. The possibility of poor nutrition has also been suggested to be the cause of anaemia in PTB patients. Haemoglobin concentration and pack cell volume were significantly lower in females than males, probably due to heavy menstrual period. Drug resistance TB is an emerging public health problem in Nigeria. The national MDR-TB survey data recorded 2.9% among the new cases and 14.3 among the retreatment cases. WHO estimated MDR-TB rate of 3.0% among new smear positive cases and 10.1% among re-treatment cases. (WHO Report 2013).

Studies have also documented an increase in platelet counts, Erythrocyte sedimentation rate (ESR), anaemia and Lymphopenia in pulmonary tuberculosis patients [8]

The objectives of this research is therefore to explore avenues to aid in the improved understanding of the disease entity which will enhance the diagnosis and treatment of PTB infected persons by determining the effects of pulmonary tuberculosis on some haematological parameters.

Methods

A total of two hundred and twenty pulmonary tuberculosis infected patients aged 18 – 60 years were recruited for the study from the DOTS center of General Hospital, Awka. Based on sputum smear for AFB positive by Ziehl Neelsen's stain. One hundred (100) were in their pre-treatment stage, and 40 each in their 2 months treatment stage, 4 months treatment stage and 6 months treatment stage respectively.

The TB positive subjects were identified based on sputum smear for AFB positive by Ziehl Neelsen stain technique which relies on the principle that M. tuberculosis is acid fast and stains red due to mycolic acids (fatty acids) in the cell wall which form a complex with carbol fucsin (an arylmethane dye) and cannot be removed by the acid in the decolorizing reagent [9].

Three millimeters (3.0mls) of blood was collected from all the participants for the analysis of the parameters. Hemoglobin concentration, packed cell volume and Erythrocyte sedimentation rate were performed using method described by Dacie and Lewis. Hemoglobin concentration were assayed by the cyanmethaemoglobin method which relies on the principle that when blood is diluted in Drabkin's fluid it lyses the red cells and converts hemoglobin to cyanmethaemoglobin. The absorbance of this solution is read in a colorimeter at a wavelength of 540nm.

Packed cell volume were assayed by the microhaematocrit method which relies on the principle that when ant coagulated blood in a glass capillary tube of specific length, bore size and wall thickness is centrifuged in a microhaematocrit centrifuge at a high speed of 1200rpm a constant packing of the red cells is obtained. The height of the column of red cells is taken as the packed cell volume.

Erythrocyte sedimentation rate was performed using the Westergreen method which is based on the principle that when citrated blood is left undisturbed in a vertically positioned westergreen pipette. The red cells aggregate, stack together to form rouleaux which sediment through the plasma. The Erythrocyte sedimentation rate indicated by the length of the column of clear plasma above the red cells is measured in mm.

Mean cell hemoglobin concentration (MCHC), the concentration of hemoglobin in g/dl of packed red cells was estimated from hemoglobin concentration (g/dl) and packed cell volume (l/l) as described by Cheesbrough as follows:-Hb (g/dl) divided by PCV (l/l).

Blood cells count (platelet count, total white cell count, white blood cell differential count) was performed using method described by Dacie and Lewis [1].

Platelet count was estimated based on the principle that when whole blood is diluted in a filtered solution of ammonium oxalate reagent the red cells are lysed leaving platelets which are counted microscopically using an improved Neaubauer ruled counting chamber and the number of platelet per liter of blood calculated.

Total white cell count was estimated based on the principle that when whole blood is diluted in Turk's solution, the red cells are lysed leaving the white cells to be counted. The white cells are microscopically counted using an improved Neaubauer ruled counting chamber and the number of WBC per liter of blood calculated.

White blood cell differential count was performed on a Leishman stained thin blood film made from a drop of blood sample using x100 objective of the microscope. The differential cell count was done by the longitudinal method using the mechanical differential white cell counter. The

percentage of each cell type was expressed as a decimal fraction and the absolute number of each white cell type obtained by multiplying it with the total white cell count.

Statistical method

Results generated in this study were tabulated using excel with statistical analysis done using SPSS package. The variables were expressed in mean and standard deviation. The student's t-test and ANOVA were used. A P-value of less than 0.05 (P< 0.05) was considered statistically significant.

Results and discussion

This present study was designed to assess the level of some hematological parameters among pre-treatment pulmonary tuberculosis infected patients, treatment in 2 months, 4 months, 6 months pulmonary tuberculosis infection and control, non-infected subjects at Anambra State University Teaching Hospital, Awka in Anambra State, Nigeria. A total of 220 pulmonary tuberculosis (PTB) infected subjects aged 18 – 60 were recruited for the study. Hundred (100) subjects were in their pre-treatment stage (group B), and 40 each in their 2 months (group C), 4 months (group D) and 6 months (group E) treatment stages. Eighty (80) non PTB subjects served as control. Blood samples collected from the subjects were used for the determination of Hemoglobin concentration (g/dl), packed cell volume (l/l), Mean cell hemoglobin concentration (g/dl), Erythrocyte sedimentation rate (mm/hr), platelet count $(x10^9/l)$, total white blood count $(x10^9/l)$, absolute neutrophil count $(x10^9/l)$, absolute lymphocyte count $(x10^9/l)$ and absolute monocyte count $(x10^9/l)$ using standard laboratory methods for analysis as described by Dacie and Lewis[10]. Tuberculosis diagnosis was carried out using Ziehl Neelsen technique.

The result of the present study shows that hemoglobin and packed cell volume in pulmonary tuberculosis patients were reduced while Erythrocyte is increased. Thus indicating anemia of chronic disease. It also showed that platelet count, total white blood count and absolute neutrophil count are increased while absolute lymphocyte count is decreased in pulmonary tuberculosis infection.

The mean ±SD hemoglobin concentration g/dl and packed cell volume (l/l) were significantly lower in pre-treatment subjects compared with control and post treatment subjects (P<0.05 in each case). However, the mean ±SD mean cell hemoglobin concentration (g/dl) was not significantly different (P > 0.05) compared among the groups (Table 1)

The mean ±SD platelet count $(x10^9/l)$, total white blood count $(x10^9/l)$ and absolute neutrophils count $(x10^9/l)$ were significantly higher in pre-treatment subjects compared with control and post treatment subjects (p <0 .05 in each case). The mean ±SD absolute lymphocytes count $(x10^9/l)$ were significantly lower in pretreatment subjects compared with control and post treatment subjects (p <0 .05 in each case). However the mean ±SD absolute monocytes count was not significantly different (P >0 .05) compared among the groups (Table 2)

Sex differentiation was observed in control subjects with respect to hemoglobin concentration, packed cell volume and platelet count (Table 3)

Significant drop of hemoglobin concentration and pack cell volume was observed in pre-treatment PTB patients. This is an indication that PTB patients are susceptible to anemia. Studies have reported high prevalence of anemia in patients with pulmonary tuberculosis [2,3]. The precise mechanism of anemia in PTB patients is not clearly known , however, anemia due to inflammation as well as that of iron deficiency has been implicated[11,12]. Factors such as decrease in red cell survival and reduced erythropoietin response by the bone marrow erythroid cells are also known to cause anemia. The possibility of poor nutrition has also been suggested to be the cause of anemia in PTB patients [10]. Hemoglobin concentration and pack cell volume were significantly lower in females than males, probably due to heavy menstrual period.

In the current study, a significant increase in Erythrocyte sedimentation rate was observed. This could be attributed to the chronicity of PTB infection. The findings are fully supported by earlier reports specifying higher ESR values for PTB patients [1,7]. The study concludes that PTB infection predisposes to anemia of chronic disease and raised Erythrocyte sedimentation rate.
Platelet counts were found higher in pretreatment PTB patients as compared with control and post treatment subjects. The findings are in agreement with the earlier reports [1]. The cause for the observed thrombocytosis in PTB cases might be attributed to an immune phenomenon due to production of platelets antibodies and to reactive myeloid hyperplasia [1]. Thrombocytosis was significantly higher in the females than in males, reason not known, but increase production following blood loss due to menstrual flow have been suggested.
Our findings also show significantly increased levels of total white blood count before treatment as compared to the control and post-treatment subjects indicating leucocytosis in Nigeria patients suffering from PTB. Neutrophil composition in PTB patients was found higher as compared to the normal healthy Nigerians and post treatment PTB subjects. Lymphocyte composition in PTB patients was found lower as compared to the normal healthy Nigerians and post treatment PTB subjects. The findings are in agreement with the earlier reports [7,].

Recommendation

The present study showed that there was no significant difference in absolute monocyte count in pretreatment, post treatment and controls subjects. Therefore we recommend that pre-treatment baseline level in these parameters are obtained prior to treatment of PTB patients in order to monitor the progress of treatment.

Tables and figures

Table 1: Mean ± SD Hematological parameters among control subjects (group A), pre-treatment PTB patients (group B) and pulmonary tuberculosis patients in their 2-month (group C), 4-month (group D) and 6-month (group E) treatment.

Groups	HB (g/dl)	PCV(l/l)	MCHC(g/dl)	ESR(mm/hr)
A – Control(n = 80)	13.53±1.29	0.40± 0.04	33.34± 3.43	5.95± 3.0
B– Pre-treatment(n = 100)	9.71±1.14	0.29±0.37	33.81±0.88	94.05±16.10
C–2-month treatment(n= 40)	13.50±0.92	0.40± 0.03	33.54± 0.57	9.33± 2.11
D–4-monthtreatment(n=40)	13.41±0.85	0.40± 0.28	33.62± 0.40	7.20± 2.52
E–6-monthtreatment(n=40)	13.21±0.88	0.39± 0.28	33.63± 0.46	6.95± 2.41
F(p)value	195.04(0.00)	174.88(0.00)	0.71(0.58)	1.38(0.00)
A vs B p value	0.00	0.00	0.76	0.00
A vs C p value	1.00	0.10	0.99	0.00
A vs D p value	0.97	0.98	0.95	0.13
A vs E p value	0.48	0.58	0.94	0.29
B vs C p value	0.00	0.00	0.22	0.00
B vs D p value	0.00	0.00	0.45	0.00
B vs E p value	0.00	0.00	0.55	0.00
C vs D p value	0.99	0.10	0.95	0.00
C vs E p value	0.59	0.69	0.93	0.00
D vs E p value	0.84	0.87	1.00	0.99

Key = (p<0.05)

Table 2: Mean ± SD Blood cells count among control, non-pulmonary tuberculosis subjects (group A), pretreatment pulmonary tuberculosis patients (group B) and pulmonary tuberculosis patients in their 2-month (group C), 4-month (group D), and 6-month (group E) treatment.

Groups	Platelet C.(x1^9/l)	TWBC (x 10^9/l)	Abs.Neut. C(x 10^9/l)	Abs.Lym.C. (x 10^9/l)	Abs.Mon.C. (x10^9/l)
A – Control(n = 80)	219.71 ±62.16	4.74 ±0.73	5.16 ±23.31	2.10 ± 0.38	0.037 ± 0.05
B–Pretreatment(n=100)	372.85 ±114.25	11.51 ± 8.87	7.94 ± 1.89	1.57 ± 0.76	0.046 ± 0.08
C–2-month treatment(n=40)	260.25 ±57.97	6.19 ±1.46	4.06 ± 0.88	3.03 ± 0.74	0.022 ± 0.05
D–4-month treatment(n=40)	228.90 ±37.25	4.91 ± 0.70	3.72 ±0.41	2.16 ±0.34	0.026 ±0.04
E–6-month treatment(n = 40)	245.52 ±42.41	4.64 ±0.67	3.68 ±0.59	2.97 ±0.32	0.026 ±0.04
F(p)value	10.40 (0.00)	26.05 (0.00)	1.43 (0.00)	118.35 (0.00)	1.78 (0.13)
A vs B p value	0.01	0.00	0.00	0.00	0.92
A vs C p value	1.00	0.88	0.99	0.38	0.43
A vs D p value	0.82	0.76	0.88	0.93	0.63
A vs E p value	0.99	0.94	0.88	0.32	0.65
B vs C p value	0.00	0.00	0.00	0.00	0.20
B vs D p value	0.00	0.00	0.00	0.00	0.32
B vs E p value	0.00	0.00	0.00	0.00	0.33
C vs D p value	0.07	0.49	0.34	0.49	0.99
C vs E p value	0.80	0.51	0.28	0.56	1.00
D vs E p value	0.24	0.42	1.00	0.11	1.00

Key = (p<0.05)

Table 3: Mean ± SD HB (g/dl), PCV(l/l), MCHC (g/dl), and ESR (mm/hr), Platelet Count (x10^9/L), total white blood count-TWBC (x10^9/L), absolute neutrophil count – ANC (x10^9/L), absolute lymphocyte count – ALC (x10^9/L),absolute monocyte count – AMC (x10^9/L) , compared between male and female control subjects.

Parameters	Male (n = 44)	Female (n = 36)	F(p)value
HB (g/dl)	14.24 ± 1.23	12.67 ± 0.69	10.67 (0.002)
PCV (l/l)	0.431 ± 0.04	0.37 ± 0.2	8.47 (0.003)
MCHC (gldl)	33.53 ± 0.04	33.11 ± 5.09	2.86 (0.095)
ESR (mm/hr)	6.45 ± 3.12	5.33 ± 2.84	0.33 (0.56)
Platelet Count(x10^9/l)	225.95 ± 60.88	305.44 ± 389.15	5.85 (0.018)
TWBC (x10^9/l)	4.74 ± 0.67	4.74 ± 0.80	0.45 (0.504)
Abs.Neut.Count(x 10^9/l)	2.53 ± 0.52	4.38 ± 34.74	4.85 (0.31)
Abs.Lym.Count(x 10^9/l)	2.11 ± 0.38	2.10 ± 0.39	0.04 (0.841)
Abs. Mon.Count(x10^9/)	0.037 ± 0.05	0.038 ± 0.04	1.67 (0.199)

Key=(P < 0.05)

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