

Haematologic Indices in Pulmonary Tuberculosis with or without HIV Co-Infection in South Eastern Nigeria

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ABSTRACT:

To evaluate the changes in haematologic indices in patients with pulmonary tuberculosis (PTB) with or without Human Immune Deficiency Virus (HIV) co-infection in South Eastern Nigeria. The study population included 116 subjects (60 = males; 56 = females), recruited from 2 study centers: mile 4 Hospital Abakaliki Ebonyi State and Nnamdi Azikiwe University, Teaching Hospital Nnewi, Anambra State, both in Nigeria. PTB + HIV (n = 20); PTB infection (n = 27) and HIV seropositive (n = 28). The PTB and HIV negative; control subjects were 41 (n = 41). Blood samples collected from subjects in Ethylene diamine tetra acetic acid (EDTA) container were used for the analysis of the Haematological cells count, packed cell volume (PCV) and Haemoglobin estimation using routine methods as described (Dacie and Lewis, 1984). HIV screening was done with Stat pak kit and confirmatory test by Western blot method. Erythrocyte sedimentation rate (ESR) was by Westergren method. Haemoglobin estimation (Hb), packed cell volume (PCV) values were significantly lower in patients with PTB (11.27±1.62 g/dl, 0.35±0.04 l/l) compared with control values (13.67±1.46 g/dl 0.41 ± 0.05 l/l) (p < 0.05). Patients with HIV seropositive showed significantly low PCV values of (0.36 ± 0.04 l/l) compared with the control subjects (0.41 ± 0.05 l/l) (p < 0.05). PTB patients showed higher TWBC counts (6062.5 ± 1481.8310⁹/l) when compared those with HIV infection (3841.38±735.58 x 10⁹/l) as well as normal control value (4363.64±551.66 x 10⁹/l) (p < 0.05). Male and female values compared in this work showed no significant difference (p > 0.05). The results showed that the effect of PTB and HIV infection have caused some haematological deregulation. It also showed that sex has little or no effect on the studied parameters.

Keywords: Pulmonary tuberculosis (PTB); Human ImmunoDeficiency Virus (HIV) and Hematologic Indices

INTRODUCTION: The past few years have witnessed a world wide resurgence of pulmonary tuberculosis (PTB) due to Human Immuno Deficiency Virus (HIV co-infection (WHO, 2005). Tuberculosis is an old disease of man and one of the most widespread and persistent disease of this century (1910 – 2013) (WHO, 2005). Some reports over the past several years have indicated that while tuberculosis problem is on the wane in most developed countries, the disease still ranks as a major health problem in the developing countries; in general and in Africa in particular (WHO, 1982, 2008). It has been reported that Human Immunodeficiency Virus (HIV) pandemic has caused an increase in the number of patients with PTB particularly in African and South East Asia (Raviglone *et al*, 1995). A variety of factors have been identified as being predictive of a greater risk of death in PTB patients for example, inadequate treatment, and late diagnosis of the disease, multidrug resistant, tuberculosis and HIV infection as well as advanced age (Connolly *et al*, 1999). Extra pulmonary tuberculosis (EPTB) is also on the rise with approximately ten percent (10%) of the cases involving the bone and the joints (Putong *et al*, 2002). Patients with PTB may present with a variety of rheumatic symptoms and signs even without evidence of direct musculoskeletal or local involvement PTB and systemic lupus erthematosus (SLE) share many symptoms such as fever, myalgias, arthralgia/arthritis, rash and multi organ involvement (Watts *et al*, 1996).

Nigeria is a country with high incidence of tuberculosis. A recent report on the incidence in Nigeria shows there is a prevalence rate of 19.5%. (Jemikalajah *et al*, 2009). Hence, there is need to review modalities of the implementation of various components involved in control of TB. The aim of this study is to establish the importance of haematologic parameters such as Hb, PCV, ESR, WBC, platelet count, Differential white cell count in monitoring and management of PTB with or without HIV-co-infection.

MATERIALS AND METHODS: This study was conducted at the chest clinics of Mile 4 Abakaliki, Ebonyi State and Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, both in Nigeria. Patients who have tested positive for *Mycobacterium tuberculosis*, by Ziehl-Neelson (Z-N) stain of the sputum were recruited. A total number of 116 subjects were recruited, (60 = males, 56 = females) from the two treatment centers: Mile 4 Hospital Abakaliki (47) Nnamdi Azikiwe University Teaching Hospital, Nnewi Campus (69). These comprised, 20 patients with pulmonary tuberculosis and HIV – co-infection (PTB + HIV), (males 10, female = 10), 27 patients with pulmonary tuberculosis (PTB) without HIV co-infection (PTB); (males = 15, females = 12), 28 patients with HIV seropositive (males = 14, females = 14), 41 apparently healthy control subjects which comprises staff and students of the two institutions, (males = 21 females = 20).

The following haematologic parameters were carried out on EDTA anticoagulated blood samples collected from the subjects: Haemoglobin (Hb), Packed Cell Volume (PCV), Total white blood cell count (TWBC), Platelet count (PLT), Erythrocyte sedimentation rate (ESR), Differential white blood cell count; neutrophils (neut), lymphocytes (lymph), eosinophils (eosino), monocytes (mono) and basophils (baso).

Haemoglobin determination was done by cyanmethaemoglobin method, whereby haemoglobin was converted to haemoglobin cyanide (HICN) and colour change.

Blood samples collected in EDTA container were used for the analysis of the Haematological cells count and Haemoglobin estimation using standard method as described in (Dacie and Lewis 1984). HIV screening and confirmatory tests were done using Stat Pak and western blot kits respectively. Stat Pak Assay-ChemBio Diagnostic system USA, Western Blot technique for confirmation using Qualicode TM HIV kit (Immunitics, Boston USA)

TEST METHODS: Haemoglobin determination was done cymethaemoglobin method. The packed cell volume was determined using the microhaematocrit standard method whereby EDTA blood was separated in, 4 layers (red cells, platelets, white blood cells and plasma). The red cell layer was read with a microhaematocrit reader and calculated as percentage of the whole blood. The total white blood cell count was done using Turk's solution as the diluents and was counted manually using haemocytometer (Improved Neubauer counting chamber). The erythrocyte sedimentation rate (ESR) was done by Westergren method. The differential white blood cell count was done using the manual method. The thin film stained by the Romanowsky method, was examined using oil immersion objective lens (X100mm) while the proportion of the white cell types were expressed in percentage. All the tests procedures above were as described by Dacie and Lewis (1984).

STATISTICAL ANALYSIS: Data obtained were analyzed using student t-test and ANOVA. The variables were expressed in mean \pm standard deviation (\pm SD) then compared. The significant difference was regarded as $P < 0.05$

RESULTS: Table I shows the mean \pm SD comparison of haematologic changes in PTB, PTB + HIV, HIV seropositive patients and normal control subjects. The between comparisons among the four group: mean \pm SD Hb g/dl for PTB is 11.60 ± 1.96 , PTB + HIV = 11.29 ± 1.6 , HIV seropositive patients = 11.50 ± 1.15 , Normal control 13.67 ± 1.46 . This showed no statistical difference ($F = 1.059$; $p > 0.05$). However, mean \pm SD PCV l/l for PTB; $.35 \pm .07$, PTB + HIV; 0.35 ± 0.04 , HIV seropositive; 0.36 ± 0.05 , and normal control; 0.41 ± 0.05 showed statistical significant difference among the four groups ($f = 5.07$, $p < 0.05$). The mean \pm SD ESR mm/hr compared among PTB (68.63 ± 31.81), PTB + HIV (75.00 ± 43.59), HIV seropositive patients (43.14 ± 42.37), and control showed significant difference ($F = 3.35$, $p < 0.05$). Moreover, mean \pm SD WBC $\times 10^9$ /l in PTB (6062.5 ± 1481.83), PTB + HIV (4422.22 ± 1851.88), HIV seropositive (3841.38 ± 735.58) and normal control (4363.64 ± 551.66) compared showed significant difference.

Also, the mean \pm SD Neutrophil % in PTB (50.63 ± 17.95); PTB + HIV (38.89 ± 17.90), HIV seropositive (37.93 ± 15.54) and normal control (33.41 ± 8.99), the mean \pm SD Lymphocyte % in PTB (40.88 ± 19.08), PTB + HIV (56.33 ± 17.90), HIV seropositive (55.28 ± 16.15) and normal control (60.77 ± 8.16) respectively compared showed statistical significant difference. See table 1. However, the mean \pm SD monocyte in PTB (3.21 ± 1.97); PTB + HIV (3.75 ± 2.49), HIV seropositive (4.50 ± 2.81) and control (3.57 ± 1.63) when compared were not statistically significant ($F = 1.12$; $p > 0.05$). The mean \pm SD compared within the groups for Hb. g/dl in PTB and control showed statistical significant difference ($p < 0.05$). However, mean \pm SD of the following groups PTB and PTB + HIV, HIV seropositive and control compared applying post Hock, showed no statistical significant difference ($p > 0.05$).

The mean \pm SD compared within the groups applying post Hock for PCV l/l showed significant statistical difference ($p < 0.05$) between PTB and control, HIV seropositive and control, PTB + HIV and control. However, the mean \pm SD post Hock comparison between PTB and PTB + HIV, HIV seropositive and PTB + HIV and PTB and PTB + HIV respectively, showed no significant statistical difference ($p > 0.05$).

Also, the mean \pm SD post Hock comparison of WBC $\times 10^9$ /dl showed that PTB and HIV, PTB and normal control and HIV seropositive and normal control were statistically significant ($p < 0.05$). The statistical comparison between PTB and PTB + HIV, HIV seropositive and PTB + HI, then PTB + HIV and normal control groups in WBC $\times 10^9$ /dl show statistically no significant difference ($p > 0.05$).

The mean \pm SD neutrophil % compared between the following: PTB and HIV, PTB and PTB + HIV, HIV seropositive and normal control then PTB + HIV and normal control were in each case, not significantly different ($p > 0.05$). Also, the mean \pm SD neutrophil % compared by post Hock analysis between PTB and normal control showed statistical significant difference ($p < 0.05$).

The mean \pm SD Lymphocyte % compared between PTB and HIV, PTB and PTB + HIV, HIV seropositive and PTB + HIV, HIV seropositive and control, then PTB + HIV and control show no significant difference ($p > 0.05$). However, mean \pm SD comparison of lymphocyte % between PTB and normal control show statistical significant difference ($p < 0.05$). Mean \pm SD lymphocyte % comparison within all the groups: PTB and HIV seropositive, PTB and PTB + HIV, PTB and normal control HIV seropositive and PTB + HIV, HIV seropositive and normal control showed no significant difference ($p > 0.05$ in each case). (See table 1).

The mean \pm SD platelet count ($\times 10^9/\text{dl}$) values in PTB ($223, 166.67 \pm 56, 056 - 39$), HIV seropositive ($179,666.67 \pm 39831.381$), PTB + HIV ($191,000.00 \pm 63,493.53$) and control ($178, 545.45 \pm 34, 617.26$), were compared using ANOVA and showed significant mean difference ($F = 4.81, p < 0.05$). The mean \pm SD comparison of platelets between PTB and HIV seropositives, showed higher significant value in PTB ($p < 0.05$). Also the mean \pm SD platelets count showed higher significant value in PTB compared with the controls ($p < 0.05$). However, the mean \pm SD platelet count compared between PTB and PTB + HIV, HIV seropositive and PTB + HIV also between HIV seropositive and control and PTB + HIV and control showed no significant difference in values ($p > 0.05$) in each case.

The mean \pm SD ESR mm/hr values in PTB (75.54 ± 36.71), HIV seropositive (46.89 ± 41.65); PTB + HIV (112.75 ± 32.07), and control subjects (11.18 ± 9.75) compared using ANOVA presented statistically significant difference ($F = 24, 13; p < 0.05$). Mean \pm SD comparison of values in ESR using tests showed that PTB, HIV seropositive and PTB + HIV, ESR mm/hr values were significantly higher than the values of ESR mm/hr in the control subjects ($p < 0.05$) in each case. Also, PTB compared with HIV seropositive ESR mm/hr values showed higher significant difference ($p < 0.05$). Also the comparison of the PTB + HIV compared with PTB showed statistically significant difference ($p < 0.05$).

The mean \pm SD for MCHC values in PTB (32.64 ± 1.90), HIV seropositives (32.84 ± 1.26), PTB + HIV (33.16 ± 1.95) and control (33.40 ± 1.32) compared showed no significant difference ($F = 2.03; p = 0.11$). Moreover, further inter - comparison of values in parameter (MCHC) in PTB and HIV seropositive PTB and PTB + HIV, PTB and control showed similar mean values ($p > 0.05$) in each case.

Table 2, Compared the mean \pm SD, Haematological changes in different groups PTB, HIV seropositive, PTB + HIV and normal control, between male and female (in each case) using student's t test.

The mean \pm SD Hb g/dl in PTB compared between male (11.76 ± 1.95) and female (11.06 ± 1.60) showed no statistical significant difference ($p > 0.05$). Also, mean \pm SD PCV l/l compared between male ($0.35 \pm .07$) and female (0.34 ± 0.04) showed no significant difference ($p > 0.05$). Mean \pm SD WBC $\times 10^9/\text{l}$. ESR mm/hr, in PTB compared between male ($6.25 \pm 1.39, 87.67 \pm 40.13$) respectively and female ($4.76 \pm 1.06; 64.70 \pm 31.00$) respectively were both not significantly different in values ($p > 0.05$). Moreover, the mean \pm SD neutrophil % and lymphocyte % in PTB male and female patients compared showed no statistical significant difference ($p > 0.05$ in each case).

The mean \pm SD Hb g/dl; PCV l/l, platelet $\times 10^9/\text{l}$, WBC $\times 10^9/\text{l}$, ESR mm/hr, Neutrophil % and Lymphocyte %, in PTB + HIV infected males and females compared (in each case), were statistically similar in value ($p > 0.05$). Also mean \pm SD Hb g/dl; PCV l/l, WBC $\times 10^9/\text{l}$, ESR mm/hr, Neutrophil % and Lymphocyte % values, compared between control male and female (in each case) resulted in statistically no significant difference ($p > 0.05$); On the other hand, mean \pm SD, platelet count $\times 10^9/\text{l}$ in PTB male (225.54 ± 54.4) compared with female (125.6 ± 38.47) showed higher significant difference ($p < 0.05$). However, mean \pm SD monocyte in PTB, compared between male (1.82 ± 1.40) and female (3.91 ± 2.43) show significantly lower difference ($p < 0.05$).

DISCUSSION: There was a significantly lower haemoglobin level in PTB patients with and without HIV co-infection in this study. This may be due to decreased erythrocyte lifespan, impaired marrow response or impaired flow of iron (fe) from macrophages to the plasma in iron (fe) cycle metabolism. This decrease in haemoglobin may also be due to a non-immune mechanism that develops secondary to granulomatous infiltration of the bone marrow. This finding is in agreement with those of (Das *et al* 2003; Lawson *et al* 2008; Mugusi *et al* 2009; and Ursavas *et al* 2010); who suggested a mild to moderate anaemia that frequently accompanied infections, inflammatory and neoplastic diseases. The anaemia may reflect reticuloendothelia iron block by disease entity and in HIV disease; partly due to suppression of erythropoiesis by cell to cell interaction (Lemoha *et al* 2004; Mugasi *et al* 2009 and Ursavas *et al*, 2010).

The mean ESR in PTB and PTB + HIV patients was significantly high when compared with that of control subjects. The reason may be due to alterations in the plasma proteins as may be the case in different entities (Ursavas *et al*, 2010). Since the erythrocyte sedimentation rate is a non-specific reaction which may be compared with the body temperature, pulse rate and the leukocyte count, it is a measure of the inflammatory response or reaction in a disease entity.

Hence an accelerated erythrocyte sedimentation rate (ESR) is seen in patients with PTB and PTB + HIV and HIV seropositive patients when compared with control.

So an accelerated erythrocyte sedimentation rate (ESR) may serve as a guide to the progress of disease that has already been recognized and this being particularly true in regard to pulmonary tuberculosis (Bozoky *et al*, 1999 and Ursavas *et al*, 2010).

The total white blood cell count (WBC) of patients with PTB + HIV and PTB were significantly higher than those of the control subjects in this study. The reason for these alterations in the blood leukocyte concentration which in this case was due to neutrophilia is an indication of recurrent continuous inflammatory response. This will usually change to lymphocytosis during chronic inflammatory response, the patients used for this study were those with active tuberculosis and the findings were in agreement with those of (Jadoon *et al* 2004 and Ursavas *et al*, 2000). The total WBC in patients with HIV seropositive in this study showed mild leucopenia. This observed difference, might be due to the causative agent, HIV, a lentivirus, when compared with that of PTB, caused by *Mycobacterium tuberculosis*. Another reason, for the leucopenia in HIV infection might be due to decrease bone marrow production of granulocyte progenitor cells (CFUGM), colony forming unit granulocyte – monocyte from the bone marrow of patients with HIV infection (Costello *et al*, 1999).

The result also showed a significant increase in monocyte count in patients with PTB and PTB + HIV. The monocyte, a known phagocytic cell is expected to be increased in active tuberculosis infection. Hence, this finding is in agreement with of previous authors (Bozoky *et al*, 1997).

There was no significant difference in lymphocyte count of patients with PTB and PTB + HIV, when compared in each case with control. This is not in agreement with previous report by Bozoky *et al*, (1997) who reported mild lymphocytosis in PTB with or without HIV co-infection. The location where this study was done may have contributed to this different observation.

The MCHC result in this study showed low normal results; hence there is no significant difference between the PTB, HIV seropositive and PTB + HIV with the normal control results. Although, anaemia is recorded for the patients but the type is anaemia of chronic disorder, hence, normocytic – normochronic. This is in agreement with previous report of Treacy *et al* (1984); who reported a mild anaemia in HIV/AIDS patients. It does not agree with the report of severe anaemia with erythroid hypoplasia in HIV patients with disseminated mycobacterium avium intracellular (MAI) infection (Gardener *et al* 1987).

The platelets result in this study, showed significant difference between the groups. The count in PTB is significantly higher than those of HIV seropositive, normal control and PTB + HIV. The mean \pm SD platelet count showed no significant difference in values between PTB + HIV, HIV seropositive and control groups. This is in agreement with previous report by Pust *et al* 1974, which showed there was mild thrombocytosis in tuberculosis. The result is also in agreement with previous report by Costello *et al* (1999) which showed there is mild thrombocytopenia in male and female patients with HIV. Some authors have reported presence of auto-antibody complexes as being responsible for the mild decreased platelet count in HIV infection (Mugusi *et al*, 2009).

The Haematologic changes in PTB + HIV, PTB and HIV seropositive patients showed varied pictures due to the stage of the infection and the causative agents. *Mycobacterium tuberculosis* and Human Immune deficiency virus.

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Table 1: HAEMATOLOGICAL CHANGES IN PTB, HIV, PTB + HIV AND NORMAL CONTROL SUBJECTS.

SUBJECT	Hb g/dl	PCV l/l	ESR mm/hr	WBC $\times 10^9/l$	Platelet $\times 10^9/l$	Neut. %	Lymph %	Mono %	MCHC
1, PTB (n=32)	11.60 ± 1.96	0.35 \pm 0.07	75.54 \pm 36.71	6062.5 \pm 1481.83	223166.67 \pm 56056.39	50.63 \pm 17.95	40.88 \pm 19.08	3.21 \pm 1.97	32.64 \pm 1.90
2, HIV (n=58)	11.51 ± 2.15	0.36 \pm 0.05	46.89 \pm 41.65	3841.38 ± 735.58	179666.67 \pm 39831.38	37.93 \pm 15.54	55.28 \pm 16.15	4.50 \pm 2.81	32.84 \pm 1.26
3, PTB+HIV (n=20)	11.27 ± 1.62	0.35 \pm 0.04	112.75 ± 32.07	4422.22 \pm 1851.88	191000.00 \pm 63,493.53	38.89 \pm 17.95	56.33 \pm 17.90	3.75 \pm 2.49	33.16 \pm 1.95
4, Control (n=40)	13.67 ± 1.46	0.41 \pm 0.05	11.18 \pm 9.75	4363.64 ± 551.66	178545.45 \pm 34617.26	53.41 ± 8.99	60.77 \pm 8.16	3.57 \pm 1.63	33.40 \pm 1.32
F (p) Value	1.059 0.37	5.07 0.003	24.13 0.043	15.25 0.00	4.81 0.004	4.34 0.007	5.54 0.002	1.12 0.35	2.03 (0.11)
1 vs. 2 p Value	0.41	0.96	0.06	0.00	0.02	0.11	0.074	0.36	0.38
1 vs. 3 p Value	0.97	0.99	0.07	0.15	0.60	0.42	0.22	0.95	0.91
1 vs. 4 “	0.007	0.044	0.00	0.002	0.01	0.01	0.005	0.94	0.06
2 vs. 3 “	0.37	0.86	0.00	0.80	1.96	1.0	1.0	0.89	0.81
2 vs. 4 “	0.74	0.007	0.00	0.33	1.00	0.56	0.40	0.52	0.92
3 vs. 4 “	0.009	0.017	0.00	1.0	0.95	0.82	0.89	0.97	0.36

Table 2: Haematologic Changes in Different Groups: PTB, PTB + HIV, HIV Seropositive and Normal Control compared between sexes (male and female) using student's t tests.

S/N	SUBJECT	Hb g/dl	PCV l/l	WBC x10 ⁹ /l	ESR mm/hr	Mono %	Neut %	Lymph %	Platelets x10 ⁹ /l
1	PTB – HIV male (n=30)	11.76 ± 1.95	0.35 ± 0.07	6.25 ± 1.39	87.67 ± 40.13	1.82 ± 1.40	50.87 ± 16.66	41.40 ± 16.77	244.33±46.39
2	PTB – HIV female (n=20)	11.06 ± 1.60	0.34 ± 0.04	4.76 ± 1.06	64.70 ± 31.00	3.91 ± 2.43	39.70 ± 19.92	54.00 ± 22.39	233.55±67.16
3	PTB–HIV male/female P-value	0.97	0.99	0.09	0.74	0.03	0.82	0.79	0.69
4	PTB + HIV male (n=10)	10.36 ± 5.1	0.33 ± 0.05	5.46 ± 1.24	131.00 ± 74.20	1.67 ± 1.53	43.00 ± 21.42	51.60 ± 40.85	164.08±33.07
5	PTB + HIV female (n=6)	12.80 ± 0.53	0.37 ± 0.02	4.30 ± 0.61	60.00 ± 34.64	3.67 ± 3.06	30.67 ± 14.19	67.00 ± 12.12	184.58±42.68
6	PTB + HIV male/female	0.16	0.85	0.66	0.63	0.32	0.96	0.87	0.19
7	HIVseropositive male (n=24)	13.12 ± 1.84	0.39 ± 0.05	3.68 ± 0.60	32.92 ± 37.50	3.42 ± 2.71	33.83 ± 15.41	60.42 ± 16.78	225.40±54.5
8	HIV seropositive female (n=30)	11.24 ± 1.54	0.34 ± 0.04	4.0 ± 0.90	54.73 ± 46.44	3.83 ± 3.27	40.67 ± 16.47	51.60 ± 16.10	125.60±38.47
9	HIV male/female P.Value	0.14	0.17	0.96	0.87	0.74	0.95	0.86	0.02
10	Control male (n=22)	14.78 ± 1.05	0.43 ± 0.04	4.11 ± 0.45	7.73 ± 5.62	2.64 ± 1.69	33.27 ± 7.76	61.00 ± 4.45	169.09±36.61
11	Control female (n=18)	12.56 ± .83	0.38 ± .04	4.54 ± .04	14.64 ± 11.91	4.18 ± 1.54	33.54 ± 10.49	60.55 ± 10.95	188.00±31.30
12	Control male / female P Value	0.001	0.06	.71	.67	0.08	1.00	1.00	0.16

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