Assessment of Haematological Parameters of Pulmonary Tuberculosis Patients with and Without HIV Infection Attending Two Secondary Health Facilities in Jigawa State

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Abstract: TB and HIV form a lethal combination, as each fuels the progress of the other in the infected patients. This study assessed the haematological parameters of pulmonary tuberculosis (PTB) patients with and without HIV infection attending the two secondary health facilities in Jigawa State, Nigeria. A total of 150 PTB patients visiting the two secondary health facilities were randomly selected for the study. 5ml of venous blood was drawn aseptically with the help of sterile syringe using vein puncture technique and transferred into EDTA bottle to avoid coagulation. 2ml of the EDTA blood was transferred in to plane test tube for the haematological analysis using automated Haematological Analyser (Abacus Junior 380) while the remaining 3ml of the blood was placed into a Western green tube up to the zero mark for Erythrocyte sedimentation rate (ESR) determination. The data was analysed using SPSS version 20, One-way ANOVA was used to check the significant difference among the new, follow-up and Multi drug resistant TB (MDR-TB) groups of patients. 136 (90.67%) patients were HIV negative and 14 (9.33%) were HIV positive, 95 (63.33%) were males and 55 (36.37%) were females. Among the studied population 56 (37.33%) and 94 (62.67%) were new and follow-up PTB patients respectively. In this study, there were significantly lower mean values at (P>0.05) of White blood cells, lymphocytes, neutrophils (M&D), granulocytes, Red Blood Cells, packed cells volume, and Platelets counts among PTB-HIV co-infected patients when compared with PTB patients. Of the PTB patients, 27.94% were Leukopenic, 25.7% Lymphopenic, 26.47% neutropenic, 30.88% anaemic and 20.59% thrombocytopenic. On the other hand, of the PTB-HIV co-infected patients, 64.29% were leukopenic, 35.71% lymphopenic, 26.47% neutropenic, 21.43% anaemic, and 14.29% thrombocytopenic. This study demonstrated high prevalence of leucopenia, followed by neutropenia and anaemia, lymphopenia, and thrombocytopenia. There was also high ESR values among more than two-third of the 150 PTB patients. The study also revealed that, females were more co-infected with HIV 64.29% than the males with 35.71%. HIV co-infection worsens haematological abnormalities of PTB patients. Knowledge of these haematological parameters will enhance the overall management of the PTB patients with regard to monitoring the disease progression and response to antimicrobial chemotherapy as they will serve as useful indicators for treatment success or failure. It is recommended that all newly diagnosed and follow-up PTB and PTB-HIV co-infected patients should be exposed to haematological counts to monitor their immune status.

Keywords: Pulmonary Tuberculosis, Human Immunodeficiency Virus, Haematological Parameters, New TB Patients, Follow up TB Patients, Erythrocyte Sedimentation Rate
1. Introduction

Tuberculosis, commonly known as TB, is a bacterial infection that usually affects the lungs but could also affect all parts of the body, with exception of nails and hair. The causative organism Mycobacterium tuberculosis was first discovered in 1882 by Robert Koch. The physiology of M. tuberculosis is highly aerobic and requires high levels of oxygen. The M. tuberculosis genome was sequenced in 1998 [1]. Unlike HIV (Human Immunodeficiency Virus), TB is completely preventable and treatment is a fraction of the cost of medications used to treat HIV. When a person is infected with HIV, he/she is at an increased risk of also contracting TB due to his/her low immune status caused by the virus. HIV co-infection with TB can also mean an accelerated progression to AIDS. Tuberculosis can be latent or active. Close to one-third of the world’s population has dormant or latent TB. TB continues to be the top infectious killer worldwide, claiming over 4,500 lives a day [2]. In 2018, 10 million people fell ill with TB and 1.5 million died from the disease [2]. 2018’s Global TB report ranked Nigeria 4th in terms of TB burden which makes it among the six countries that accounted for 60% of the new cases of TB. The country is also classified among countries with high burden for TB, TB/HIV and Multidrug-resistant TB (MDR-TB) and currently ranked 6th and 1st in Africa [3].

HIV continues to be a global public health issue, having claimed more than 35 million lives so far [4]. Nigeria has the second largest HIV epidemic in the world [5]. HIV and TB are deadly combination, as each fuels the progress of the other in infected patients [6]. Persons who are infected with HIV are prone to TB disease than those without HIV infection. The demonstrable changes that PTB, and/or PTB plus HIV positive patients have in their haematological values showed varied pictures as a result of the phase of the infection and the causative agents, M. tuberculosis and HIV [7]. Haematological parameters are the blood forms (Red Blood Cells, White Blood Cells, and Platelets) with normal range as reference for any value to know whether or not they are normal [8]. Haematological abnormalities are common in PTB patients [9].

There are limited studies conducted on the haematological parameters of pulmonary tuberculosis patients with and without HIV in infection in developing countries, especially in Nigeria. There is scarcity of data in Nigeria that assessed the progression to AIDS. Tuberculosis can be latent or active. Terms of TB burden which makes it among the six countries currently ranked 6th in Africa [3].

2. Materials and Methods

2.1. Study Area

The study areas were Hadejia and Kafin Hausa towns in Hadejia Emirate, North-Eastern part of Jigawa State. Hadejia town which is the capital of the emirate has a projected population of 139,400 people as at 21st March, 2016 [10]. The town lies to the north of the Hadejia River, and is upstream from the Hadejia-Nguru wetlands, an internationally important ecological and sensitive zone. Hadejia is located at Latitude: 12°27’59”N and Longitude 10°3’40”E and it is 361m above Sea level with an area of 32.0Km². Kafin Hausa town is the capital of Kafin-Hausa Local Government Area and is located at Latitude: 12°14’27.6”N and Longitude 9°54’46.8”E. It has a projected population of 357,200 as at 21st March, 2016 [10].

2.2. Study Design, Sample Size and Population

The study design was both across sectional and descriptive design. A total of 150 new and follow-up pulmonary tuberculosis patients with and without HIV infection were randomly selected for the study. A patient was classified as pulmonary tuberculosis patient if all or one out of the two sputum samples collected in the laboratory were or was positive for Acid Fast Bacilli (AFB), chest X-ray and clinical symptoms were suggestive of the disease, or his/her sputum sample was detected for PTB during Gene-Xpert techniques.

The study population was categorized as those who had only PTB and PTB-HIV co-infection, new and follow-up PTB patients, the follow-up patients were also classified as those at intensive phase of treatment and those at their continuation phase of treatment.

2.3. Exclusion Criteria

PTB patients with bleeding manifestations, chronic inflammatory disease, pregnant or breast feeding women were excluded as these clinical conditions may also alter the patient’s haematological parameters.

2.4. Samples Collection and Processing

About 4-5ml of venous blood was drawn aseptically with the help of sterile syringe and transferred in to EDTA bottle to avoid coagulation. 2ml of the EDTA blood was transferred in to a plane test tube for the haematological analysis using the Automated Haematological Analyser (Sysmex, KX-21N) in one of the health facility and (Abacus Junior 380) in the other one. The remaining 3ml of the blood was drawn in to a Western Green Tube up to the zero mark for Erythrocyte Sedimentation Rate (ESR) determination. The tube was placed vertically in an ESR stand for one hour and the reading for each patient was recorded.

2.5. Data Analysis

The data was analysed using Statistical package of Social Science (SPSS. version 20) software and One-way analysis of variance (ANOVA) was used to check the significant
difference among the new, follow-up, and Multi drug resistant PTB groups of patients. SAS version 9.1 software was also applied in the analysis of data among PTB with or without HIV co-infection, to determine Correlation matrix for all the assessed parameters, age and gender to check the possible relationship between the variables using One-way analysis of variance. Duncan's Multiple Range Test (DMRT) was used to separate means where differences existed.

2.6. Ethic Statement

An ethical approval with reference number: MOH/SEC. 3/S/711/I/21 dated 5th July, 2018 was obtained from Jigawa State Ministry of Health, Dutse and written permission from Medical Directors of the two Secondary health facilities dated 20th July, 2018 and 10th July, 2018 respectively was obtained. Informed consent of all the participants was obtained and were given the option to opt out any time they desire to do so during the work.

Table 1. Characteristics of the study population of the two secondary facilities.

| Characteristics | n=150 | n=136 (90.67%) | n=14 (9.33%) |
|-----------------|-------|----------------|--------------|
| Age group (years) |       |                |              |
| 08 - 17 | 12 (8.00%) | 12 (8.82%) | 0 (00.00%) |
| 18 - 27 | 34 (22.67%) | 31 (22.79%) | 3 (21.43%) |
| 28 - 37 | 45 (30.00%) | 42 (30.88%) | 3 (21.43%) |
| 38 - 47 | 30 (20.00%) | 24 (17.65%) | 6 (42.86%) |
| 48 - 57 | 14 (09.33%) | 12 (08.82%) | 2 (14.28%) |
| 58 - 67 | 09 (06.00%) | 09 (06.62%) | 0 (00.00%) |
| 68 - 77 | 05 (03.33%) | 05 (03.68%) | 0 (00.00%) |
| 78 – 87 | 01 (00.67%) | 01 (00.74%) | 0 (00.00%) |
| Gender |       |                |              |
| Male | 95 (63.33%) | 90 (66.18%) | 5 (35.71%) |
| Female | 55 (36.67%) | 46 (33.82%) | 9 (64.29%) |
| PTB Status |       |                |              |
| New Patients | 56 (37.33%) | 50 (36.76%) | 6 (42.86%) |
| Follow-up Patients | 94 (62.67%) | 86 (63.24%) | 8 (57.14%) |
| Follow-up Patients n=94 |     | n=86 | n=8 |
| Intensive Phase Patients | 43 (45.74%) | 40 (46.51%) | 3 (37.50%) |
| Continuation Phase patients | 51 (54.26%) | 46 (53.49%) | 5 (62.50%) |

3. Results

3.1. Characteristics of the Study Participants

A detail of the characteristics of the study participants is presented in Table 1. 136 (90.67%) were HIV negative and 14 (9.33%) were co-infected with HIV in addition to the PTB infection. 95 (63.33%) were males and 55 (36.67%) were females, among the males 90 (94.74%) have only PTB infection while 5 (5.26%) have both PTB and HIV infections. 46 (83.64%) of the females have only PTB infection while 9 (16.36%) have both infections. 56 (37.33%) and 94 (62.67%) were PTB new and Follow-up patients respectively, out of the 94 follow-up PTB patients, 43 (45.74%) were at Intensive Phase of their treatment while 51 (54.26%) were at the Continuation Phase of their treatment. Among the 43 Intensive Phase PTB patients, 3 (6.28%) were PTB/HIV co-infected patients while the rest have only PTB disease. As for those in Continuation Phase of treatment 46 (90.20%) and 5 (9.80%) have PTB and PTB/HIV infections respectively. The mean age of the participants was 48±10 years.

3.2. Haematological Profiles of PTB/HIV Negative and PTB/HIV Positive Patients

The haematological profiles of the participants displayed in Table 2 revealed that the mean value of total White Blood Cells (WBC) count was 8.11 × 10³/L for PTB/HIV negative patients and 5.26 × 10³/L for PTB/HIV positive patients, Lymphocytes (LYMP) count was 2.29 × 10³/L for PTB/HIV negative and 1.93 × 10³/L for PTB/HIV positive patients, MID count was found to be 1.11 × 10³/L for PTB/HIV and 0.70 × 10³/L for PTB/HIV co-infected patients, Granulocytes (GRA) count was 4.48 × 10³/L and 2.80 × 10³/L for PTB/HIV negative and PTB/HIV positive patients respectively. Red Blood Cells (RBC) count was 5.23 × 10¹²/L for PTB/HIV negative patients and 5.15 × 10¹²/L for PTB/HIV positive patients while 46.65% and 44.37% were found as Packed Cells Volumes (PCV) of the PTB/HIV negative and PTB/HIV positive patients respectively. Mean (red) cell volume (MCV) count was 93.93 (fl) for PTB/HIV negative patients and 86.68 (fl) for PTB/HIV positive patients, 26.84 (pg) and 25.59 (pg) were recorded as the Mean Cell Haemoglobin (MCH) of the PTB/HIV negative and PTB/HIV positive patients respectively while the Mean Cell Haemoglobin Concentration (MCHC) count was found to be 29.89 (g/dl) for PTB/HIV negative and 29.09 (g/dl) for PTB/HIV positive patients. The Platelet (PLT) count was 317.16 × 10³/L and 291.50 × 10³/L for PTB/HIV negative and PTB/HIV positive patients respectively. Mean Platelet Volume (MPV) was 9.98 for PTB/HIV negative and 9.79 for PTB/HIV positive patients, and finally the mean Erythrocyte Sedimentation Rate (ESR) was found to be 44.45 mm/hr for PTB/HIV negative patients and 52.57 mm/hr for PTB/HIV positive patients at (P>0.05).
3.3. Haematological Disorders Among PTB Patients with and Without HIV Infection

The profile of haematological disorders of PTB patients with and without HIV infection is shown in Table 3. Among the 136 PTB/HIV negative group 38 (27.94%) have low and high WBC counts each, while in the 14 PTB/HIV co-infected patients 9 (64.29%) and 2 (14.29%) have low and high WBC counts respectively. Lymphocytes count of the PTB/HIV negative shows that, 51 (37.50%) have high count beyond the normal range while in the PTB/HIV positive patients 3 (21.43%) have low count and 22 (16.18%) have high GRA counts of PTB/HIV negative group of patients reflected 28 (20.59%) out of 136 to have low platelets count and 41 (30.88%) with high readings outside the normal range values while in the case of PTB/HIV positive patients 3 (12.43%) have low and high PCV results each. Platelets counts of PTB/HIV negative group of patients reflected 28 (20.59%) out of 136 to have low platelets count and 41 (30.15%) with higher counts, while among the PTB/HIV positive patients 5 (35.71%) have low lymphocytes count and only 1 (7.14%) had higher values. MID count among the same group showed that 36 (26.47%) and 74 (54.41%) have low and high values respectively, while among the PTB/HIV positive patients 9 (64.29%) have low MID count and 5 (35.71%) have high count beyond the normal range. Granulocytes count of the PTB/HIV negative shows that, 51 (37.50%) have low count and 22 (16.18%) have high GRA count as the PTB/HIV positive group have 9 (64.28%) with low and 2 (14.29%) having higher values.

For RBC count of PTB/HIV negative patients, 35 (22.74%) have low count and 48 (35.29%) have high count than the normal range while in the PTB/HIV positive patients, 5 (35.71%) and 6 (42.86%) have low and elevated RBC counts respectively. PCV results of the Patients having only PTB infection showed 42 (30.88%) with anaemia and 30 (22.06%) with high readings outside the normal range values while in the case of PTB/HIV positive patients 3 (12.43%) have low and high PCV results each. Platelets counts of PTB/HIV negative group of patients reflected 28 (20.59%) out of 136 to have low platelets count and 41 (30.15%) with higher counts, while among the PTB/HIV positive patients 2 (14.29%) have low count and 4 (28.57%) high count. In the case of ESR 86 (63.24%) of the PTB/HIV negative patients have high rate of sedimentation above the normal values while 10 (71.43%) PTB/HIV positive patients also have high rate.

Table 3. Haematological Parameters among the Pulmonary Tuberculosis Patients.

| Parameters  | PTB (%) | PTB/HIV Negative (%) | PTB/HIV positive (%) |
|-------------|---------|----------------------|-----------------------|
| WBC (x10^3/L) | 5.00 – 10.00 | 40 (26.67%) | 38 (27.94%) | 2 (14.29%) |
| High         |         | 63 (42.00%) | 60 (44.12%) | 3 (21.42%) |
| Normal       |         | 47 (31.33%) | 38 (27.94%) | 9 (64.29%) |
| Low          | 1.30 – 4.00 | 13 (8.67%) | 12 (8.82%) | 1 (7.14%) |
| LYMP (x10^3/L)|         | 97 (64.67%) | 89 (65.44%) | 8 (57.14%) |
| High         |         | 40 (26.66%) | 35 (25.74%) | 5 (35.71%) |
| Normal       |         | 79 (52.67%) | 74 (54.41%) | 5 (35.71%) |
| Low          | 0.59 – 0.70 | 26 (17.33%) | 26 (19.12%) | 0 (00.00%) |
| MID (x10^3/L)| 2.50 – 7.50 | 45 (30.00%) | 36 (26.47%) | 9 (64.29%) |
| High         |         | 24 (16.00%) | 22 (16.18%) | 2 (14.29%) |
| Normal       |         | 66 (44.00%) | 63 (46.32%) | 3 (21.43%) |
| Low          |         | 60 (40.00%) | 51 (37.50%) | 9 (64.28%) |
| GRA (x10^3/L)| 4.00 – 5.50 | 54 (36.00%) | 48 (35.29%) | 6 (42.86%) |
| High         |         | 56 (37.33%) | 53 (38.97%) | 3 (21.43%) |
| Normal       |         | 40 (26.67%) | 35 (25.74%) | 5 (35.71%) |
| Low          |         | 36.00 – 52.00 | 30 (22.06%) | 3 (21.43%) |
4. Discussion

This study revealed that, females are more co-infected with HIV (64.29%) than the males with 35.71% possibly due to their inadequate awareness of the mode of transmission of the two disease causing microorganisms and also probably due to the practice of polygamy which sometimes give room for a single infected household man to infect two or more wives in their matrimonial rooms/homes, and other factors like unfaithfulness of some of the men that travel far away for business or hand works for months and later bring back home the HIV to their innocent wives. An attempt was made to study some of haematological parameters among the said patients with and without HIV infection. Various pathogeneses have been suggested in TB associated anaemia, but most studies showed infection of the haematopoietic progenitor cells, effect of treatment on erythropoiesis and folate activity, nutritional deficiencies and malabsorption, absence or depletion of bone marrow iron, and suppression of erythropoiesis by inflammatory mediators as a potential explanation for TB-HIV related anaemia [15]. For instance, it has been reported that mild to moderate anaemia is common during chronic inflammatory infections, including TB [17]. The high percentage of anaemia may due to the influence of proinflammatory cytokines, such as Interleukin-6 (IL-6) and TNF-a, which may in turn impair erythropoiesis [18].

In this study, the WBC showed the level of Leucopenia to 27.94% and 64.29% among PTB and PTB/HIV co-infected patients respectively, these values were higher than 6% and 14% leucopenia of PTB and PTB/HIV co-infected patients reported in Northwestern Ethiopia by [9]. These observed differences might be due to HIV co-infection. Leucopenia in HIV infection might be due to decrease in bone marrow production of granulocyte progenitor cells [19].

The mean value of MID count of PTB/HIV negative patients was higher as compared to PTB/HIV co-infected patients in this study (1.11±0.43 and 0.70±0.56) with 54.41% and 35.71% prevalence respectively. Since the subjects included in this study were those with active tuberculosis, polymorphonuclear leukocytes (neutrophils) may be increased as part of the immune defense mechanism to defend the M. tuberculosis infection. Low MID count (26.47% and 64.29%) in PTB/HIV negative and PTB/HIV positive patients respectively may be due to consequences of the combined effect of hypersplenism and marrow granulopoietic failure.
mediated by different cytokines and malnutrition [20].

The other haematological parameter assessed in this study was platelet count. The result showed a statistically significant higher platelet count in PTB/HIV patients when compared to PTB/HIV co-infected patients (317±0.98 versus 291±0.03). The finding is supported by studies in Babylon province and Kirkuk city, Iraq [21, 22], and Pakistan [19], and the values are higher when with 111.9 recorded in Calabar, Nigeria [23]. Also 30% of the study population have high platelets count, this was lower when compared with studies conducted in Dhaka, Bangladesh with 80% [16] and East London by Morris et al., 1989, that recorded 52% elevated platelet count, and the result is higher when compared with 8% in Hungary [24]. These differences may be attributed to the reactive thrombocytosis which was found in a number of clinical situations including infectious diseases such as pulmonary tuberculosis due to increased thrombopoietic factors such as IL-6 which is released by inflamed cell as an inflammatory response [18]. The secretion of IL-6 in PTB patients will stimulate the production of platelets [25]. Some studies also reported the presence of autoantibody complexes as being responsible for the mild decreased platelet count in PTB/HIV infection [26].

The prevalence of thrombocytopenia among PTB patients was 20.00% while it was 14.29% among PTB/HIV co-infected patients. Different mechanisms such as immune mechanisms, bone marrow fibrosis, direct megakaryocyte infection, and hypersplenism had been implicated as possible causal factors for thrombocytopenia in PTB/HIV co-infected patients [27].

High ESR values were found in both PTB and PTB/HIV co-infected patients (44.45±0.71 and 52.57±3.12) with 64% of the whole study population having high ESR values. 44.45mm/hr and 52.57mm/hr values are lower when compared with research conducted in Calabar, Nigeria with the mean ESR values of 104.2mm/hr [23], Ludhiana city, India by [28] who found 66.86mm/hr ESR values among TB patients and 64.3mm/hr found in Pakistan by [29]. 64% is also low when compared with the result of [16] who recorded 93.33% of the TB patients having high values above the normal range. ESR value usually increased with the pulmonary tuberculosis [30]. Elevated ESR to a different level is one of the indicators of the severity of disease and as a prognostic tool. This might be due to alterations in the plasma proteins [31] which in turn affect ESR values. The higher ESR values could also be as a result of inflammation and degenerative changes (these are general features of PTB) due to increase in production of acute phase protein, reduced albumin, often observed in chronic infections and release of proteins by the causative organism into the circulation [32].

5. Conclusion

In this study, different haematological abnormalities in pulmonary tuberculosis with or without HIV infection were observed. There was high prevalence of leucopenia, followed by neutropenia (MID) and anaemia, lymphocytopenia and thrombocytopenia in sequential order, the MCH and MCHC low values were very prevalent among the PTB patients, there was also high ESR values among more than two-third of the whole pulmonary tuberculosis patients studied. Leucocytes (WBCs), Lymphocytes, MID (Neutrophils, Eosinophils, and Basophils), granulocytes, Erythrocytes, packed cells volume, and platelet counts showed statistical significant difference between pulmonary tuberculosis with and without HIV patients. A high ESR value was recorded in all the study participants. The study also pointed out that females were more co-infected with HIV (64.29%) than the males with 35.71%. Assessment of haematological parameters can be used as an indicator in the diagnosis and follow-up of pulmonary tuberculosis patients with or without HIV for various haematological disorders such as anaemia, increased ESR, thrombocytosis, thrombocytopenia and leucopenia. In addition, knowledge of these haematological pictures will enhance the overall management of the PTB patients with regard to monitoring the disease progression and response to antimicrobial chemotherapy as they will serve as useful indicators for treatment success or failure.

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