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Some Haematological Parameters of Tuberculosis (TB) Infected Africans: The Nigerian Perspective

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Abstract
Tuberculosis (TB) is a major public health problem in Nigeria. This study was aimed at providing information on haematological changes in pulmonary tuberculosis (PTB) infection. One hundred PTB patients aged 15-45 years, attending tuberculosis clinics in Calabar, Nigeria were studied while 70 apparently healthy subjects served as controls. Standard techniques were used for the assays. Results showed significantly lower values of Packed cell volume(PCV), haemoglobin concentration(Hb), mean cell haemoglobin(MCH), mean cell haemoglobin concentration(MCHC) ($p<0.05$) in PTB while mean cell volume(MCV), erythrocyte sedimentation rate (ESR), relative plasma viscosity (RPV), euglobulin lysis time (ELT) and fibrinogen concentration showed significantly higher values in PTB patients than that of control subjects. ESR, RPV and ELT decreased significantly ($p<0.05$) as anti-tuberculosis therapy progressed. PCV, HB, MCV, MCH, MCHC and ESR of PTB patients co-infected with HIV changed significantly ($p<0.05$). Anaemia, increased haemorheologic activity and reduced fibrinolysis occur in pulmonary tuberculosis.
Key words: Pulmonary tuberculosis, haematological variables, haemorheology, fibrinolysis

Introduction

Tuberculosis (TB), one of the oldest known diseases and still a major cause of mortality today, has many manifestations affecting the bone, the central nervous system and many other organ systems, but it is primarily a pulmonary disease. It is caused by a closely-related group of organisms, all of which form the *Mycobacterium tuberculosis* complex. These organisms include *M. tuberculosis, M. bovis, M. africanum, M. microfti and M. Canetti (*Iseman, 2000). Pulmonary tuberculosis (PTB) is a lower respiratory tract infection that is initiated by the deposition of *Mycobacterium tuberculosis* (MTB) contained in aerosol droplets, onto lung alveolar surfaces. The active disease is characterized by a protracted cough, fatigue, loss of weight and appetite and night sweats. Haemoptysis, secondary infection by fungi and permanent lung damage are few of the complications of PTB (Fraser, 1993). Tuberculosis is a major public health problem in Nigeria with an estimated prevalence of 616 cases per 100,000. Nigeria ranks first in Africa and fourth among the 22 high TB burden countries in the world. No fewer than 460,000 cases of tuberculosis are reported annually in Nigeria (WHO, 2008). The co-infection of pulmonary tuberculosis with human immune deficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) has in the recent past compounded the epidemiology, clinical outcome, diagnosis and treatment of the disease worldwide (CDC, 1995).This has led to the different reports made for this condition in Nigeria. Previous study has shown that anaemia, raised erythrocyte sedimentation rate (ESR), peripheral leukocyte count and neutrophilia are the most common haematological manifestations associated with PTB (Devi et al, 2003). Another study carried out in Kano, Northern Nigeria, reported mild anaemia, and raised ESR, leucocytosis, neutrophilia with toxic granulation, thrombocytosis, occasional stickiness of platelets and monocytosis (Nwankwo et al, 2005). In Benin, Southern Nigeria, a study showed significant increase in plasma viscosity and fibrinogen levels and a decrease in platelet count, total white cell count and packed cell volume when compared to values for apparently healthy subjects (Awodu et al, 2007). It is our aim to study the pattern of some haematological parameters in PTB infected subjects in Calabar Nigeria. It is believed that the findings of this study may be useful as indicators of disease progression, cardiovascular risk factors and response to therapy in pulmonary tuberculosis

Materials and Methods

A total of one hundred (100) male and female pulmonary tuberculosis patients aged between fifteen to forty five (15-45) years, attending the Endemic Disease Clinics of University of Calabar Teaching hospital and Dr Lawrence Henshaw Memorial Hospital, Calabar, Nigeria were selected for the study. With approval from the ethical committee of both hospitals, the bio data and medical history of these patients were obtained from their case notes. Subjects who were diagnosed as pulmonary tuberculosis patients based on the presence of the acid fast bacilli in their sputum smear were included while those

who were smear negative were not enrolled in the study. The HIV status of the patients was determined during selection. Seventy (70) age and gender-matched apparently healthy individuals who tested negative with acid fast bacilli in their sputum smear and HIV sero-negative were used as control subjects in this research. They were selected from staff of University of Calabar, University of Calabar Teaching Hospital and residents of Calabar municipality. Informed consent was sought and obtained from all participants.

Six and a half millilitres (6.5 ml) of blood was collected under aseptic conditions with minimal stasis from each subject. 4.5 ml was added to ethylene diamine tetra acetic acid anticoagulant with a concentration of 1.5 mg/ml for determination of packed cell volume (PCV), haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH,) mean cell haemoglobin concentration (MCHC) white blood cell (WBC) and platelet counts, using PCE 2000 automatic cell counter, **e**rythrocyte sedimentation rate by Westergren technique(ICSH,1993) and relative plasma viscosity by Reid and Ugwu(Reid and Ugwu,1987), while the remaining 2 ml was added to 0.22 ml of 3.13% trisodium citrate for euglobulin lysis time test using Haugie (Haugie,1986), method and fibrinogen concentration using kit based on Clauss method (Clauss,1999) purchased from BAUR company USA. The data generated from this work were subjected to student t-test analysis and one-way analysis of variance. A two tailed P-value of < 0.05 was considered indicative of a statistically significant difference.

Results

In this study, some haematological parameters of pulmonary tuberculosis patients in Calabar were investigated. The tests that were performed included measurement of packed cell volume, haemoglobin concentration ,mean cell haemoglobin, mean cell haemoglobin concentration, mean cell volume, erythrocyte sedimentation rate, white blood cell and platelet counts, relative plasma viscosity, euglobulin lysis time and fibrinogen concentration. The results obtained were compared with apparently healthy subjects (controls).

Table 1 shows the mean values of the various parameters that were analysed. The mean values obtained for HB, PCV, MCH and MCHC of PTB patients were significantly lower ($p<0.05$) than values obtained for control subjects. While the mean MCV relative plasma viscosity, euglobulin lysis time and fibrinogen concentration and erythrocyte sedimentation rate of PTB patients were significantly higher ($p<0.05$) than that of control subjects. The WBC and platelet counts of PTP did not change significantly from that observed for the control subjects.

Table 2 shows the means and standard deviations of the haematological variables of pulmonary tuberculosis patients based on duration of therapy. 22 were newly diagnosed patients, 34 had been on treatment for two to eight (2-8) weeks, 21 for nine to twenty (9-20) weeks and 23 for twenty-one to thirty two (21-32) weeks. The erythrocyte sedimentation rate, relative plasma viscosity and euglobulin lysis time parameters decreased significantly ($p<0.05$) as therapy progressed. The haemoglobin concentration increased steadily as the treatment progressed though not significantly. There was no difference ($p>0.05$) among the mean values obtained for white blood cell MCH, MCHC,MCV, platelet counts, packed cell volume and fibrinogen concentration. Table 3 shows the haematological variables of 22 HIV sero-positive pulmonary tuberculosis patients compared with 78 counterparts who were sero-negative for HIV infection. Aside from the WBC, Platelet count, RPV, ELT and Fibrinogen concentration which did not change significantly, the PCV, Hb, MCV, MCH, MCHC and ESR of the sero -positive patients changed significantly ($p<0.05$) when compared with those who are sero-negative.

There was a negative correlation ($r=0.558$; $p<0.05$) between packed cell volume and erythrocyte sedimentation rate and packed cell volume and relative plasma viscosity ($r=0.447$; $p<0.05$) (figs1 and 2).On the other hand, a positive correlation ($r=0.209$; $p<0.05$) between relative plasma viscosity and erythrocyte sedimentation rate was observed (Fig3).

Discussion

Pulmonary tuberculosis (PTB) is a major infectious disease with very high incidence in developing countries and this is expected to rise with the incidence of HIV infection. Severe pulmonary tuberculosis (PTB) is often complicated by deep vein thrombosis (DVT) because of the association between inflammation and haemostatic changes that can result in an acute phase response and a hypercoagulable state. Deep vein thrombosis has been clinically observed and confirmed with laboratory methods in 3–4% of patients with pulmonary tuberculosis (Robson, 1996). Some haematological variables of one hundred (100) pulmonary tuberculosis patients were investigated in this study. While the haemoglobin concentration, packed cell volume, mean cell haemoglobin and mean cell haemoglobin concentration of pulmonary tuberculosis patients (12.71 ±2.15g/dl, 0.36±0.05L/L, 27.93±3.18pg, 34.76±1.55g/l) was significantly lower ($p<0.05$) than that of control

subjects (14.20±1.52g/dl, 0.40±0.04L/L, 29.50±2.38pg, 35.47±1.41g/l), the mean cell volume was (80.15±7.84fl significantly higher than that of control subjects (76.06±4.84fl). Ajayi et al, 2005 reported significant decreases in these haematological parameters of PTB patients. Anaemia, which is a reduction in haemoglobin concentration and by implication PCV, alongside the absolute values (MCV, MCH and MCHC), have been reported in some studies (Robson, 1996, Turken et al, 2002). This anaemia has been classified as normocytic normochromic anaemia with all the characteristics of anaemia of chronic disorders (Nwankwo et al, 2005). Pulmonary tuberculosis being a chronic ailment, impacts on the haemopoietic system leading to a decrease in erythropoeisis. Although Ajayi et al 2005 and Nwankwo et al 2005) reported lower PCV in their studies, our PCV value is higher than the values of their studies. This is probably due to the local diet in Calabar which consists of leafy vegetables and sea foods (fish, snails, crayfish, and periwinkle). These iron-containing foods are available at all seasons as compared to Kano and Benin towns in Nigeria (Nwankwo et al 2005,) where their staple diets consist of food with little or no vegetables and sea foods. This finding thus implies that diet of patients to a greater extent plays an important role in the outcome of their haemoglobin concentration. The PCV of PTB patients correlated negatively with the ESR and RPV (Figures1 &2) thus indicating the relationship between packed cell volume and ESR and RPV.

The ESR and RPV values of PTB patients (44.0±40.0 mm/hr; 2.16±0.62) obtained in this study were significantly higher than control values (8.0±7.8 mm/hr & 1.62±0.31). This agrees with previous findings (Ibeneme et al, 2009, Ajayi et al 2005). ESR is often raised in infections and inflammatory conditions. Increase in ESR and RPV could be attributed to increased production of acute phase proteins often observed in chronic infections and release of proteins by the causative organism (*M. tuberculosis)* into the circulation. Raised plasma viscosity has been reported to cause sluggish flow in microcirculation resulting in insufficient tissue perfusion (Grigoleit et al, 1973). This increase in ESR and RPV implies that there is increased rheology of blood in pulmonary tuberculosis. This is confirmed by a moderate positive correlation ($r=0.209$; $p<0.05$) observed between relative plasma viscosity and erythrocyte sedimentation rate (Fig3). From this observation one may suggest that both erythrocyte sedimentation rate and relative plasma viscosity could be used as sensitive index of plasma protein changes which result from inflammation or tissue damage. The euglobulin lysis time of PTB patients (227.12±64.08 minutes) was significantly higher ($p<0.05$) than the control value (172.77±48.81 minutes). A similar finding has been made (Famodu et al, 2005). A possible explanation is that tuberculosis might enhance the local production and release of pro-inflammatory cytokines, which in turn reduces fibrinolytic activity. A reduction in fibrinolysis can lead to the development of venous thrombosis in these PTB patients.

The fibrinogen concentration of the PTB patients (6.30±2.50 g/L) was significantly higher ($p<0.05$) than control values (3.17±0.55). A possible explanation is that the vascular endothelium could be primed as a result of the interaction between mycobacterial products and the hostmonocyte-macrophage system, which then synthesise large amounts of tissue necrotic factor (TNF-alpha) and interleukin six (IL-6) (Ogawa et al, 1991,Rook and Attiyah,1991). These cytokines induce hepatic acute-phase responses that alter levels of coagulation proteins such as fibrinogen (Andus and Gerok, 1991). Fibrinogen is an acute-phase reactant which increases greatly in inflammatory and degenerative conditions such as PTB. It has been shown that the risk of deep vein thrombosis (DVT) is significantly (four times) higher in patients with a fibrinogen level over 5 g/L (Koster, 1993). In the present study, the fibrinogen level obtained for the patients is 6.30g/L. This implies that PTB patients may develop deep vein thrombosis as a result of the hyperfibrinogenaemia and impaired fibrinolysis observed. In this study, the mean white cell and platelet count of PTB patients was $5.3\pm2.2 \times 10^9$/L and $161.2\pm73.3 \times 10^9$/L and this showed no difference with control values. This result agrees with the mean white cell count ($5.6\pm0.4 \times 10^9$) obtained in the same locality (Ibeneme et al, 2009) and disagrees with leucocytosis and thrombocytosis that have been reported previously(Robson et al, 1996). It is also at variance with a report of decreased white cell and platelet counts in PTB patients (Awodu et al, 2007). However, most of the pulmonary tuberculosis patients enrolled in this study where those undergoing anti-tuberculosis treatment and this may account for the disparity in results.

The rheologic and fibrinolytic activity of PTB patients improves as therapy progresses. The ESR decreased significantly from 67.0± 34.0 mm/hr for newly diagnosed patients to 26.0± 33.0 mm/hr for patients who had been treated for 21-32 weeks ($p<0.05$). Similarly, the relative plasma viscosity decreased significantly from 2.47±0.73 to 1.89±0.38 as therapy progressed. Euglobulin lysis time decreased significantly ($p<0.05$) from 256.46±68.99 minutes at diagnosis to 204.35±42.78 minutes at

more than twenty weeks of therapy. However numerically a decrease in the fibrinogen level from 7.07±2.65 g/L for new patients to 5.93±2.54 g/L after twenty weeks of therapy was observed but this difference was not statistically significant. These findings agree with reports of Turken et al (2002) and Famodu et al, 2005. In their reports a decrease in ESR, plasma fibrinogen and euglobulin lysis time within twelve weeks of anti-tuberculous treatment was observed. Therefore treatment on time reduces the risk of deep vein thrombosis for PTB patients as shown by the improved ESR, RPV, ELT and fibrinogen levels. The Haemoglobin concentration of these patients steadily improved with therapy. Of the 100 pulmonary tuberculosis patients enrolled in this study, 22 were HIV sero-positive giving a 22 per cent prevalence rate while78 were HIV sero-negative. The 22 per cent prevalence rate of HIV co-infection observed in this study is similar to that reported by the National Tuberculosis and Leprosy Control Programme of Nigeria (NTBLCP, 2008). The Hb, PCV and MCHC of the sero-positive patients was significantly lower ($p<0.05$) than that obtained for the sero-negative patients while the ESR of the sero-positive patients was significantly higher ($p<0.05$) than that of the sero-negative patients. These differences may be due to the impact of HIV infection on the alveolar macrophages thereby enhancing the pathogenesis of pulmonary tuberculosis. The mean values obtained for white cell and platelet counts, mean cell volume, mean cell haemoglobin relative plasma viscosity, euglobulin lysis time and fibrinogen of the sero-positive patients were similar ($p>0.05$) to those of the sero- negative patients. In conclusion this study has established significant changes in haematological variables studied in pulmonary tuberculosis patients. While significantly reduced Hb and PCV was common feature in TB patients, significantly increased haemorheologic activity(as represented by raised ESR & RPV) and reduced fibrinolytic activity(as represented by raised ELT) were also observed in pulmonary tuberculosis patients thus pre-disposing them to thrombosis and vascular complications. We therefore suggest the inclusion of relative plasma viscosity, euglobulin lysis time and fibrinogen concentration assays as part of routine test for all pulmonary tuberculosis patients in order to properly monitor response to therapy and prevent venous thrombosis and its complications.

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Table 1
Some haematological parameters of pulmonary tuberculosis patients and control subjects

Parameters	PTB patients n=100	Control n=70	P-value
Hb(g/dl)	12.71±2.15	14.20±1.52	<0.05
MCV(fl)	80.15±7.84	76.06±4.84	<0.05
MCH (pg)	27.93±3.18	29.50±2.38	<0.05
MCHCg/dl)	34.76±1.55	35.47±1.41	<0.05
WBC($x10^9$L)	5.3±2.2	4.9±1.5	>0.05
PCV (L/L)	0.36±0.05	0.40±0.04	<0.05
PLT ($x10^9$L)	161.2±73.1	143.7±51.7	>0.05
ESR (mm/hr)	44.0±40.0	8.0±7.8	<0.05
RPV	2.16±0.62	1.62±0.31	<0.05
ELT(minutes)	227.12±64.08	172.77±48.81	<0.05
FIB (g/L)	6.30±2.50	3.17±0.55	<0.05

TABLE 2

Some haematological parameters of pulmonary tuberculosis patients based on duration of therapy

	New n=22	2-8 weeks n=34	9-20 weeks n=21	21-32 weeks n=23	*P*-value
WBC($\times 10^9$/L)	5.9±2.7	5.6±2.3	4.6±1.4	4.9±1.9	>0.05
Hbg/dl	11.97±2.5	12.0±1.91	13.66±1.81	13.60±1.56	>0.05
MCV(FL)	79.00±7.18	78.30±7.1	81.75±9.29	84.07±8.54	>0.05
MCH(pg)	27.53±3.15	28.84±3.8	28.00±2.48	29.33±3.07	>0.05
MCHC(g/dl)	34.78±1.53	34.27±1.85	34.90±1.25	35.45±1.52	>0.05
PCV (L/L)	0.34±0.04	0.35±0.06	0.39±0.04	0.38±0.04	>0.05
PLT($\times 10^9$/L)	145.9±69.9	166.5±70.8	181.4±79.5	149.5±66.1	>0.05
ESR(mm/hr)	67.0±34.0	48.0±44.0	36.0±35.0	26.0±33.0	<0.05
RPV	2.47±0.73	2.21±0.62	2.12±0.66	1.89±0.38	<0.05
ELT(minutes)	256.46±68.99	229.86±73.17	216.29±51.42	204.35±42.78	<0.05
FIB(g/L)	7.07±2.65	6.47±2.43	5.69±1.97	5.93±2.54	>0.05

TABLE 3

Some haematological parameters of pulmonary tuberculosis patients who are co-infected with HIV

Parameters	sero-positive n=22	sero-negative n=78	P-value
Hbg/dl	11.76±1.80	12.98±2.17	<0.05
MCV(fl)	84.93±9.79	78.80±6.67	>0.05
MCH(pg)	29.20±3.53	27.57±3.00	>0.05
MCHC(g/dl)	32.37±0.96	34.87±1.67.	<0.05
WBC($\times 10^9$/L)	5.30±2.2	5.35±2.2	>0.05
PCV (L/L)	0.34±0.05	0.37±0.05	<0.05
PLT($\times 10^9$/L)	176.9±92.3	156.8±66.0	>0.05
ESR (mm/hr)	86.0±42.0	32.0±30.0	<0.05
RPV	2.11±0.68	2.18±0.61	>0.05
ELT(minutes)	226.86±59.27	227.19±65.37	>0.05
FIB (g/L)	6.68±2.71	6.20±2.38	>0.05

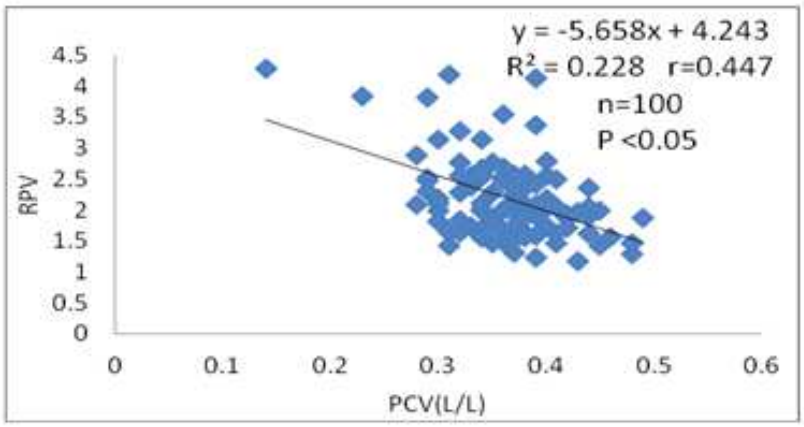

Figure 1 Correlation between packed cell volume and erythrocyte sedimentation rate

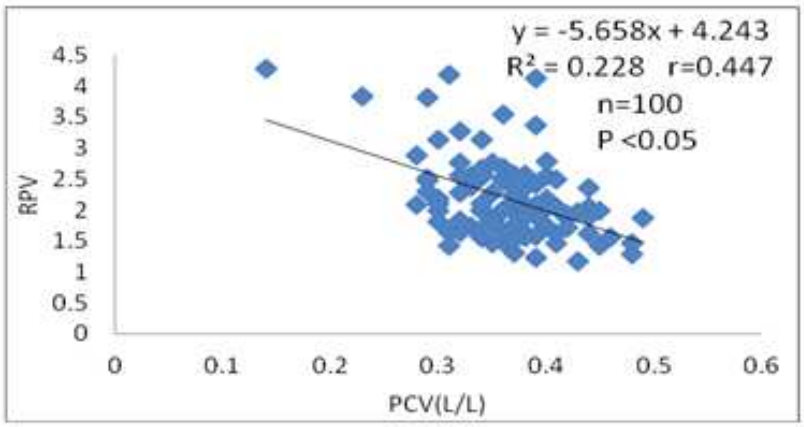

Figure 2 Correlation between relative plasma viscosity and packed cell volume

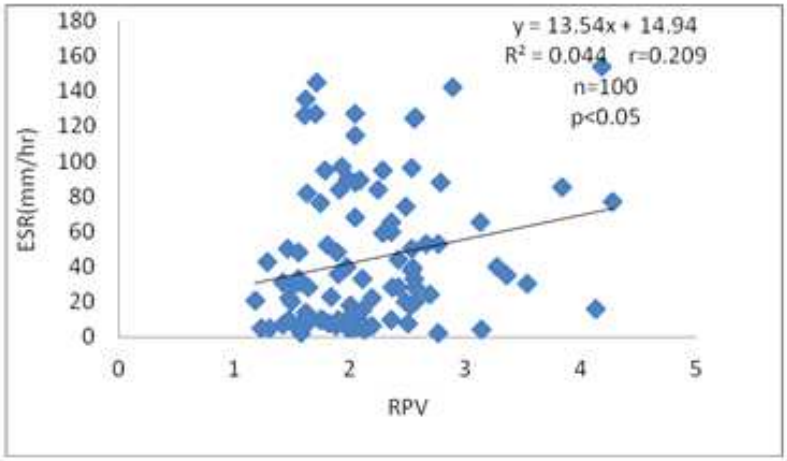

Figure 3 Correlation between relative plasma viscosity and erythrocyte sedimentation rate