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Studies on Some Haematological and Immunological Indices of Tuberculosis Patients

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Abstract
The study aimed at determining some of the haematological and immunological indices of TB infected patients. The study was a cross-sectional study conducted at the Infectious Diseases Hospital (IDH), Kano. A total of 196 TB patients were purposely selected for the study. Sputum samples were collected and processed using standard mycobacteriological procedure for the presence of Mycobacterium tuberculosis complex (MTBC). Blood samples were collected and blood cell count was conducted using automated cell counter and ESR values were determined using Wintrobe's methods. Also, the CD4+ and CD8+ cell count were determined using flow cytometry. The HIV status of the studied subjects was obtained from their medical records. The result revealed that most of the studied TB subjects were aged 15-44 years with more males infected than females and that 12.25% of the patients were co-infected with HIV. The mean haematological and immunological values of the various groups of the studied subjects varied significantly with that of the control (healthy subjects). Compared with the mean Hb and PCV values of the control subjects, the $TB^{+}HIV^{-}$ and $TB^{+}HIV^{+}$ groups recorded significantly lower values. The mean ESR values of $TB^{+}HIV^{+}$ was found to be significantly higher than that of the control subjects while the $TB^{+}HIV^{-}$ reported significantly lower values. All the groups studied with the exception of $TB^{-}HIV^{-}$ recorded lower WBC counts compared with the control with $TB^{+}HIV^{+}$ recording the lowest significant counts ($p<0.05$). The $TB^{+}HIV^{-}$ and $TB^{+}HIV^{+}$ groups recorded significantly higher neutrophil count while the monocyte and basophil counts were both significantly higher in $TB^{+}HIV^{-}$ groups. The study further reported that the studied groups recorded significantly lower lymphocyte counts and lower CD4+ and CD8+ cells count compared with the control with $TB^{+}HIV^{+}$ groups recording significantly lower counts. The study recommends continues assessment of TB patients and those co-infected with HIV for effective management.
Keywords: **Tuberculosis, Haematological, Immunological, Parameters.**

INTRODUCTION
Tuberculosis (TB) mainly caused by *Mycobacterium tuberculosis* remains an infectious disease with devastating consequences on the infected individuals. Globally, an estimated 10.0 million people fell ill with TB in 2018 (WHO, 2019). Nigeria accounted for 4% of the TB burden and together with other eight countries accounted for two thirds of the global total WHO (2019).

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Being it a prototype of infection that involves a complex mechanism of immune interaction, TB leads to numerous pathological changes in the body of the infected individuals. Notably, the hematological and immunological indices play a vital role in the assessment of the health status of the infected. Javed *et al*. (2018) noted that TB is a chronic bacterial infection affecting many organs of the body not only the lungs but seriously also affects the hematopoietic system with TB patients co-infected with other disorders more prone to death than TB alone.

Complete blood count (CBC) is one of the most common blood test that is used in the diagnosis of hematological abnormalities that provides important information about the kinds and numbers of cells in the blood, especially red cells, white cells and platelets (Elyass *et al*, 2019). Normally it involves red blood cells count (RBC), white blood cells count (WBC), haemoglobin estimation (Hb), hematocrite, red blood cells indices, platelets count, white blood cells differential, erythrocyte sedimentation rate (ESR) and reticulocyte count. Studies by Akhigbe *et al*. (2019) and Akpan *et al*. (2012) showed significantly lower values of Packed cell volume (PCV), Hb concentration, mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) ($p<0.05$) in PTB, while mean cell volume (MCV), ESR, relative plasma viscosity (RPV), euglobulinlysis time (ELT) and fibrinogen concentration showed significantly higher values in PTB patients than that of control subjects which were aggravated in co-infection with HIV.

The interaction of *M. tuberculosis* with the host involves a multifaceted complex immune mechanism that results in pathological changes that leads to induction of inflammation which is either rapid and involves host pattern recognition with immediate launch of an emergency intensive cross-talk or is protracted and characterized by delayed onset of host defense (Rohini *et al*., 2016; Dorhoi *et al*., 2012). Beside macrophages which are the most abundant host cells at sites of infection and implicated in both disease control and progression, are the T lymphocytes especially CD4+ and CD8+ T cells which produce various cytokines including IFN-γ and display cytolytic activity against mycobacteria infected cells (Huang *et al*. 2018; Weiss and Schaible, 2015). Yao *et al*. (2014) demonstrated that CD4+ T cells play an important role in sustaining multiple CD8+ T cell effector functions in primary *M. tuberculosis* infection. Aminu and Habib (2019) demonstrated that the mean CD4+ and CD8+ cells counts varied significantly ($p<0.05$) among the different groups of the study and that compared to the control (healthy subjects).

Javed *et al*. (2018); Yasin *et al*. 2015; Akpan *et al*. (2012) stated that determination of immunological and hematological parameters become essential for further consideration of supportive care and other treatment options that could enhance TB treatment outcome as well as help in diagnosis and prognosis of the disease. Studies by Doherty and Andersen (2005) suggested that the magnitude of the immune response to TB infection reflects the magnitude of the bacterial load as such, immunological profiling of the TB patients especially CD4+ cell counts becomes very important in the assessment of disease progression.

Reviewed literature indicated that information on haematological and immunological parameters of TB patients especially CD4+ and CD8+ cell counts were not adequately documented in recent published works in the Kano state. Therefore, ∓ the study aimed at determining some of the haematological and immunological indices of TB infected patients. This study will assist Clinicians in the management of TB infected patients.

MATERIALS AND METHODS

The study was a cross-sectional study conducted at the Infectious Diseases Hospital (IDH), Kano. Ethical approval for the study was obtained from the Research Ethics Committee of Kano State Hospitals Management Board. An informed consent was also sought from the participants of the study prior to sample collection.

All Subjects with documented history of PTB irrespective of HIV status and who provided consent were included in the study. A total of 196 TB patients were purposely selected for the study.

Sputum samples were collected and processed according to standard mycobacteiological procedures described by National Tuberculosis and Leprosy Control Programme (NTBLC) SOP Manual (2011) and National Committee for Clinical Laboratory Standards (NCCLS) (2011). The collected sputum samples were liquefied with N-acetyl L-cystein (NALC), decontaminated with NaOH solution and neutralized with Phosphate Buffered Saline (PBS) solution and then centrifuged. The sediments were then smeared on a clean grease free glass slide and after air drying and heat fixing, the smeared slide was stained by hot Ziehl-Neelsen (ZN) staining technique for the detection of acid fast bacilli (AFB). The sediments were also cultured on Lowenstein Jensen (LJ) egg medium. The cultured medium was incubated at 37^{o}C for up to six weeks.

Isolates obtained from the cultured samples were confirmed as *M. tuberculosis* complex (MTBC) using the SD BIOLINE TB Ag MPT64 Rapid test according to manufacturer's specifications (SD Bioline Kit, Standard Diagnostics, Inc., Korea, 2015) which was a rapid immunochromatographic identification test for the *M. tuberculosis* complex (MTBC) that uses mouse monoclonal anti-MPT64. For each sample four colonies obtained from 4 weeks culture were suspended in 200µl of the extraction buffer which is made up of TB Ag MPT 64 assay diluent (supplied together with the test kit). The cassette was then removed from the foil pouch and placed on a flat dry surface thus exposing the sample well and for each sample 100µl of it was added into the sample well. After 15 minutes of sample application, the appearance of two colour (purple) bands ("T" test band and "C" control band) within the result window was considered a positive result. Samples that were confirmed as being MTBC were then used for further analysis.

Exactly 5mls of venous blood was drawn aseptically from anterior cubital vein by means of vacutainer syringe from the selected participants and transferred into vacutainer tube containing

potassium ethylene diamine tetra acetic acid (K_2EDTA) as described by Cheesbrough (2006). Then 2.5mls of the blood samples were tested on automated cell counter (manufactured by Sysmexcorporation Kobe, Japan) with complete profile including Hb, hematocrit, total white blood cells count and differential among others. Another 2.5mls of blood specimen was used for ESR measurement by Wintrobe's method.

The patient's CD4+ and CD8+ cell count were determined from the blood samples using flow cytometry (Becton-Dickson FACSCalibur, San Jose CA).Four (4) mls of venous blood was drawn into vacutainer tube containing potassium ethylene diamine tetra acetic acid (K_2EDTA) as described by Cheesbrough (2006). All the samples collected were processed within four hours and analyzed on the same day. Two sets of Tri-Test reagent kits: one containing CD3/CD4/CD45 and the other containing CD3/CD8/CD45 were used. Phycoerythrin (PE) tagged against monoclonal antibody (MoA) CD4, Allophycocyanin (APC) tagged against MoA CD8, Peridinin-chlorophyll protein (Per CP) tagged against MoA CD45, and Fluorescein isothiocyanate (FITC) tagged against (MoA) CD3, were used for absolute count of CD4+ and CD8+ lymphocytes respectively.

Blood samples were also collected from thirty (30) healthy volunteers and subjected to haematological and immunological analysis as described above.
It is noteworthy to mention that the HIV status of the patients was obtained from their medical records.

Data generated from the study were presented using percentages and analyzed using students' unpaired t-test. A p value of 0.05 or less was considered significant.

RESULTS

The results of the study revealed that of the 196 studied patients 135 (68.87%) were males and 61 (31.12%) were females (Table 1). Table 1 further shows that out of the 196 patients, 144 (73.47%) had TB only, while 24 (12.25%) patients were co-infected with TB and HIV, 5 (2.55%) were found to have only HIV and 23 (11.73%) were identified as negative to both HIV and TB.

Table 1: Sex Distribution of the Studied Subjects according to TB/HIV Status

TB/HIV Status	Number studied		Gender			
			Males		Females	
	No	(%)	No	(%)	No	(%)
TB^+HIV^-	144	(73.48)	96	(66.67)	48	(33.33)
TB^+HIV^+	24	(12.24)	19	(79.17)	5	(20.83)
TB^-HIV^+	5	(2.55)	5	(100)	0	(0)
TB^-HIV^-	23	(11.73)	15	(65.22)	8	(34.78)
Total	**196**		**135**	**(68.87)**	**61**	**(31.12)**

Table 2 shows that most of the 196 studied patients were aged 15-24 years (23.47%), 25-34 years (37.24%) and 35-44 years (16.84%) (Table 2). Specifically, Table 2 revealed that among the TB^+HIV^- group majority of the patients i.e. 80.43% and72.60% patients were aged 15-24 years and 25-34

years old with the exception of one patient aged less than 5 years and 12 other patients aged greater than 55 years. Among the TB^+HIV^+ group, most of the patients were aged 15-24 years (13.04%) and 25-34 years (19.70%) (Table 2). Among the TB^-HIV^+ group, 4 (12.12%) patients were aged 35-44 years (Table 2). Also, among the TB^-HIV^- most of the patients (32.23%) were aged 45-54 years old (Table 2).

Table 2: Age Distribution of the Studied Subjects

Age (years)	TB cases studied No	(%)	TB^+HIV^- No	(%)	TB^+HIV^+ No	(%)	TB^-HIV^+ No	(%)	TB^-HIV^- No	(%)
<15	1	(0.5)	1	(100)	0	0	0	0	0	0
15-24	46	(23.47)	37	(80.43)	6	(13.04)	0	0	3	(6.52)
25-34	73	(37.24)	53	(72.60)	14	(19.70)	0	0	6	(8.22)
35-44	33	(16.84)	22	(66.67)	3	(9.09)	4	(12.12)	4	(12.12)
45-54	31	(15.81)	19	(61.29)	1	(3.22)	1	(3.22)	10	(32.23)
>55	12	(6.12)	12	(100)	0	0	0	0	0	0
Total	**196**		**144**	**(73.47)**	**24**	**(12.24)**	**5**	**(2.55)**	**23**	**(11.73)**

Table 3 revealed that the mean haematological and immunological values of the various groups of the studied subjects varied significantly with that of the control (healthy subjects). Compared with the mean Hb value of 15.05g/dl of the control subjects, the TB^+HIV^-, TB^+HIV^+ and TB^-HIV^- groups recorded significantly lower values, with TB^+HIV^+ recording the lowest mean value of 10.55g/dl ($p<0.05$) (Table 3). Similarly, compared with the mean PCV value of 52.53% of the control subjects, the TB^+HIV^-, TB^+HIV^+, TB^-HIV^+ and TB^-HIV^- groups recorded significantly lower values with TB^+HIV^+ recording the lowest mean value of 32.87% ($p<0.05$) (Table 3).

The mean ESR value of 10.57mm/hr of the TB^+HIV^- was found to be significantly lower than that of the control subjects (15mm/hr), while that of TB^+HIV^+ (17.90mm/hr) and TB^-HIV^- (21.96mm/hr) groups were significantly higher than of the control subjects ($p<0.05$) (Table 3). It is noteworthy to report that, the mean Hb and ESR values of the TB^-HIV^+ group of 11.42 g/dl and 27.60mm/hr were found to be insignificantly lower and higher than that of the control subjects (Table 3).

Table 3 shows that although only the TB^-HIV^+ group recorded significantly lower WBC count of $3.00x10^9/l$ compared with the $4.69x10^9/l$ of the control subjects ($p<0.05$), still the other groups also recorded lower counts compared with the control subjects. Specifically, TB^+HIV^- recorded WBC count of $4.63\ x10^9/l$ while TB^+HIV^+ recorded a count of $3.54\ x10^9/l$. On the other hand, the TB^+HIV^-, TB^+HIV^+ groups recorded significantly higher neutrophil count of 78.22% and 74.39% compared with 57.53% of the control subjects (Table 3). Of interest is that even the TB^-HIV^- group recorded significantly higher WBC count of 81.76% compared with the control subjects (Table 3). Among the 4 studied groups only the TB^+HIV^- group recorded significantly higher basophil and monocyte counts of 0.16% and 0.67% respectively ($p<0.05$), and none of the groups recorded any eosinophil count (Table 3). Table 3 further shows that the mean lymphocyte count of all the

groups studied were significantly lower than that of the control subjects (41.29%) and apart from the $TB^{-}HIV^{-}$ group, the lowest count of 24.80% was recorded from $TB^{+}HIV^{-}$.

Table 3 revealed that the CD4+ cell counts of all the groups studied were lower compared with that of the control subjects except for the $TB^{-}HIV^{-}$ (912.46 cells/µl) which was insignificantly high. The $TB^{+}HIV^{+}$ group recorded the lowest significant CD4+ cell counts of 412.08 cells/µl compared with the control (725.66 cells/µl) ($p<0.05$).Similarly, the $TB^{+}HIV^{-}$ and $TB^{-}HIV^{+}$ group recorded insignificantly lower counts of 630.40 cells/µl and 686.00 cells/µl. Table 3 further indicated that with the exception of $TB^{-}HIV^{-}$ group which recorded a CD8+ cell count of 612.66 cells/µl, the CD8+ cell count of the other 3 groups studied were lower compared with that of the control (418.10cells/µl). The $TB^{+}HIV^{+}$ group recorded the lowest significant count of 256.86cells/µl compared with the control ($p<0.05$). Similarly, the $TB^{+}HIV^{-}$ and $TB^{-}HIV^{+}$ group recorded insignificantly lower counts of 372.28cells/µl and 384.00cells/µl compared with the control subjects.

Table 3: Mean Values of Haematological and Immunological Indices of the TB/HIV Studied Subjects.

TB/HIV Status	Haematological and Immunological mean values										
	HB (g/dl)	**PCV (%)**	**ESR (mm/hr)**	**WBC x10⁹/l**	**NEU (%)**	**BASO (%)**	**EOS (%)**	**MON (%)**	**LYM (%)**	**CD4+ cells/µl**	**CD8+ cells/µl**
$TB^{+}HIV^{-}$	11.72*	35.69*	10.57*	4.63	78.22*	0.16*	0	0.67*	24.80*	630.40	372.28
$TB^{+}HIV^{+}$	10.55*	32.87*	17.90*	3.54	74.39*	0.42	0	0.42	32.65*	412.08*	256.86*
$TB^{-}HIV^{+}$	11.42	35.5*	27.60	3.00*	74.40	0	0	0	26.80*	686.00	384.00
$TB^{-}HIV^{-}$	10.87*	40.81*	21.96*	5.40	81.76*	0	0	0.09	17.62*	912.46	612.66
Control	**15.05**	**52.53**	**15**	**4.69**	**57.53**	**0**	**1.3**	**0.02**	**41.29**	**725.66**	**418.10**

* Value differs significantly with those of the control subjects ($p<0.05$)

DISCUSSION

The findings of this study clearly support the WHO report that more males were infected with TB than females as 68.87% of the studied cases were found to be males. The WHO (2019) reports that TB affects people of both sexes in all age groups but the highest burden is in men (57%) aged ≥15 years. Genuine biological differences in male and female susceptibility to *M. tuberculosis* due to sex steroid hormones, the genetic makeup of the sex chromosomes, and sex-specific metabolic features were some of the reasons that accounted for the excess of male pulmonary TB cases which is witnessed in all regions of the world, and almost in all countries (Neyrolles and Quintana-Murci, 2009; Davila *et al*., 2008). Similar observations were reported by Akhigbe *et al*. (2019) with 72.4% of their study subjects being males. In contrast to the observation of the present study Obeagu *et al*. (2019); Yasin *et al*. (2015) reported that 48.67% and 42% of their study subjects were males. Yasin *et al*. (2015) expounded that the higher percentage of women seen in their study could be attributed to less accessibility of females to hospitals, least health diagnostic facilities at door steps, communal living life style of females in rural settings and illiteracy in female population in the society coupled with the fact that women also remain neglected in the society in terms of treatment as they are mostly confined to household, maximizing the chances of more contacts with infected carrier.

The findings of the present study are also consistent with WHO's observation that majority of TB cases occur among people aged >15 years (WHO, 2019). Aminu and Habib (2019) opined that this may not be unconnected with the fact that patients in this age category are physically active and may be engaged in various sectors of life working experiences as such becoming more exposed to acquire and develop TB.

The findings of this study revealed that 12.25% of the patients were co-infected with TB and this is higher than the reports by WHO (2019) which revealed that 8.6% of TB people are co-infected with HIV worldwide but lower than 19.1% of TB/HIV co-infection reported in Nigeria by NTBLCP (2014). Adejumo *et al*. (2017) in Lagos state reported that higher proportion (21.6%) of their study patients were co-infected with TB and HIV which was associated with older age group, retreatment cases, extrapulmonary TB and poor treatment outcomes and concluded that there is need to put measures in place to improve treatment outcomes of TB/HIV co-infected patients. Ranti *et al*. (2016) also reported higher rates of HIV/TB co-infection of 29.27% in Ogun State and reiterated the need for researches to be conducted to provide more insight into the epidemiology of co-infection in order to better address the dual burden of HIV and TB among tuberculosis patients in Nigeria. Other studies by Javed *et al*. (2018) reported lower rates of 2.55% of TB/HIV co-infection among their patient in Punjab, Pakistan. It is interesting to note that 11.73% of the studied cases were identified as $TB^{-}HIV^{-}$ and this were earlier documented as TB positive cases. Aminu and Tukur (2016) stated that although so many factors might account for the observed discrepancy in result presentation, yet there is need to conduct and report the TB diagnosis with expertise and caution by the appropriate authority lack of which could lead to inappropriate administration of anti-TB drugs which is detrimental to the health of the patient and encourages development and spread of drug resistance.

The findings of this study revealed that that the mean haematological and immunological values of the various groups of the studied subjects varied significantly with that of the control (healthy subjects). Compared with the mean Hb and PCV values of the control subjects, the $TB^{+}HIV^{-}$ and $TB^{+}HIV^{+}$ groups recorded significantly lower values with $TB^{+}HIV^{+}$ groups recording the lowest mean values. This clearly support the earlier observations that the hematopoietic system is another organ seriously affected by tuberculosis that manifest into a variety of hematological abnormalities/changes which might act as a marker for the diagnosis, prognosis and response to therapy (Yasin *et al*., 2015). Studies by Elyass *et al*. (2017) also reported that they recorded significantly lower Hb and PCV values of 9.93% and 31.3% among their study subjects compared with the control group, although their observations are still lower than the reports of this study. Rohini *et al*. (2016) expounded that the mean serum haemoglobin level in their study group was found to be less by nearly 1.4-fold reflecting anemia which may largely be due to chronic inflammation. Obeagu *et al*. (2019) reiterated that the low levels of Hb and PCV may be the reason for anaemia which is a major complication in tuberculosis.

Just like the reports of this study, other reports by Javed *et al*. (2015); Okafor *et al*., (2013); Akpan *et al*. (2012) indicated that there were significant reduction in Hb and PCV values in the patients especially those co-infected with HIV. This is not surprising as the presence of HIV in an infected TB person leads to profound deficiencies with various manifestations on their health status. Chapel and Haeney (1993) stated that the haematopoeitic progenitor cells are among other cells that are affected by HIV.

Erythrocyte sedimentation rate is considered as a diagnostic tool in many bacterial infections and also as an indicator of disease severity in PTB (Javed *et al*., 2015). ESR has also been reported to be raised in infections and inflammations which could be linked to elevated synthesis of acute phase proteins usually seen in chronic infections and release of proteins by *Mycobacterium tuberculosis* into the circulation **(**Obeagu *et al*., 2019). The above assertion was supported in the present study which reports that the mean ESR values of $TB^{+}HIV^{+}$ was found to be significantly higher than that of the control subjects although the $TB^{+}HIV^{-}$ reported significantly lower values. This clearly indicates that the presence of infections adversely affects the ESR value. Other studies reported significantly higher ESR values among their study subjects (Elyass *et al*., 2019; Yasin *et al*., 2015). It is noteworthy to mention that compared with normal ESR value of 3-7mm/hr, the control subjects in this study recorded higher values of 15mm/hr. Obeagu *et al*. (2019) expounded that increase in ESR values could result from inflammation, stage of the disease, immune status, nutritional status and reduced PCV, while Naim (2019) explained that factors that could lead to decrease in ESR values apart from nutrition intake include health care behavior and environmental health behavior. The fact that the $TB^{-}HIV^{-}$ also reported The fact that the $TB^{-}HIV^{-}$ also recorded significantly lower Hb and PCV values and higher values of ESR compared with the control further emphasized that this group of patients could actually be suffering from other infections although not documented in their medical history but erroneously identified as TB positive.

The low number of lymphocytes in the peripheral blood of the groups of the studied subjects is indicative of the fact that TB infection is controlled by lymphocytes. Moreover the effect of HIV as witnessed among the $TB^{+}HIV^{+}$ group further reduces the body's peripheral lymphocytes and this is not surprising as the CD+ cells which are subset of T lymphocytes are infected in the course of HIV as well as the major effector cells that counteract TB infections.

The findings of this study are consistent with early documented works (Obeagu *et al*., 2019; Ifeyinwa *et al*., 2013; Akpan *et al*., 2012) which revealed lower WBC counts compared with the control. The findings of this study revealed that all the groups studied with the exception of $TB^{-}HIV^{-}$ recorded lower WBC counts compared with the control with $TB^{+}HIV^{+}$ recording the lowest significant counts ($p<0.05$). The immunodeficiency status of $TB^{-}HIV^{+}$ may likely be the reason for them recoding the lowest WBC counts. Of interest is that even the $TB^{-}HIV^{-}$ group recorded significantly higher WBC count compared with the control subjects indicating a serious infection state. Rohini *et al*. (2015) expounded that in tuberculosis, WBCs increases during infection, due to the increased polymorphonuclear leukocytes and macrophages as part of the body's immune

defense mechanism to combat the invading bacterial population Ifeyinwa *et al*. (2013) also pointed out that anti-tuberculosis treatment may account for the disparity in result between the TB patients and the control group.

The fact that $TB^{+}HIV^{-}$ and $TB^{+}HIV^{+}$ groups recorded significantly higher neutrophil count compared with that the control subjects indicated a serious infection state. Additionally, the monocyte and basophil counts were both significantly higher in $TB^{+}HIV^{-}$ groups indicated that these cells may play a role in the containment of TB infection. However, the effect of HIV when co-infected with TB reduced their number (as in $TB^{+}HIV^{+}$) or even in some cases ($TB^{-}HIV^{+}$) no count was recorded.

Central to immunity against TB infection and disease beside the innate mechanisms, are the role played by CD4+ T cells. The findings of this study revealed that the CD4+ T cell counts of all the groups studied were lower compared with that of the control subjects except for the $TB^{-}HIV^{-}$ which was insignificantly high. The observation of the study shows that even in the absence of HIV infection, the $TB^{+}HIV^{-}$ group still recorded insignificantly lower CD4+ T cell counts compared with the control indicated that the CD4 + T cells plays a role in the course of TB infection. Earlier studies have documented that containment of TB infection is dependent on T-cells particularly the CD4+ cells which was an outcome of MHC class II presentation of mycobacterial antigens that result from *M. tuberculosis* residing within macrophages (Perreau *et al*., 2013; Kaufmann, 2013). Caruso *et al*. (1999) and Flynn *et al*. (1992) demonstrated that mice lacking MHC class II molecules or CD4+ T cells, therefore lacking T-helper cell activity were susceptible to infection even with the avirulent Bacillus Calmette Guerin strain of *M. bovis*. They further demonstrated that activation of these cells make them respond by cell division and production of lymphokines such as interferons, interleukins, tumor necrosis factor and chemo-attractant chemokins, with lymphokines further acting as local hormones controlling the growth, maturation, function and behavior of other immune cells (CD8+, B-cells) as well as monocytes, tissue macrophages and dendritic cells.

The fact that compared with the control the CD4+ cell count was significantly lower among the TB+HIV+ indicated that there was a profound depletion of CD4+ cells when TB is co-infected with HIV infection. This observation support earlier reports that infection of CD4+ T cells by HIV leads to their reduction and automatically affect their ability to contain tuberculosis infection by interfering with the ability of macrophages to produce gamma interferon (IFN-γ) (key activating agent that triggers antimycobacterial effects) and less granuloma formation suggesting low production of TNF-α by macrophages (Murray, 1999; Alexandra *et al*., 1998; Flesh and Kauffman, 1987; Rook *et al*., 1986). Wilkinson and Pasvol (1995) also revealed that macrophages are unable to produce lysozyme or α-1 antichymotrypsin, indicative of disrupted functions.

The study further observes that the $TB^{-}HIV^{+}$ group recorded insignificantly lower CD+ cell count (630.40 cells/μL) compared to the control and this suggests that this group might belong to HIV

patients categorized in the early infection group or stage A1 with CD4+ cell count >500 cells/µL. The observation that $TB^{-}HIV^{-}$ reported higher CD+ cell count compared with control although not significant indicated that this group of patients may be suffering from other infection whose defense may not necessarily involve cell mediated immunity in form of CD4+ cell activation/participation.

Flynn *et al.* (1992) demonstrated that both CD4+ and CD8+ T cells are required for immunity to virulent *M. tuberculosis* as studies revealed that mice that lack CD8+ cells are highly susceptible to *M. tuberculosis* infection. The primary function of CD8+ cells in TB appeared to be lysis of the infected cells and production of cytokine and the cytolytic activity was associated with granulysin-a lytic peptide that lyses infected macrophages (Murray, 1999). The observations of this study revealed that just like the CD4+ cell counts, the $TB^{+}HIV^{-}$ and $TB^{+}HIV^{+}$ group recorded lower CD8+ cells counts compared with the control with $TB^{+}HIV^{+}$ recording lower significant counts and this support earlier observation that CD4+ cells control the activities of other cells including CD8+cells. The study also noted that CD8+cells likely contributes to overcoming mycobacterial infection as the mean count reported by $TB^{+}HIV^{-}$ although lower did not differ significantly from that of the control subject.

CONCLUSION

The findings of this study revealed that most of the studied TB subjects were aged 15-44 years with more males infected than females and that 12.25% of the patients were co-infected with HIV. The mean haematological and immunological values of the various groups of the studied subjects varied significantly with that of the control (healthy subjects). Compared with the mean Hb and PCV values of the control subjects, the $TB^{+}HIV^{-}$ and $TB^{+}HIV^{+}$ groups recorded significantly lower values. The mean ESR values of $TB^{+}HIV^{+}$ was found to be significantly higher than that of the control subjects while the $TB^{+}HIV^{-}$ reported significantly lower values. All the groups studied with the exception of $TB^{-}HIV^{-}$ recorded lower WBC counts compared with the control with $TB^{+}HIV^{+}$ recording the lowest significant counts ($p<0.05$). The $TB^{+}HIV^{-}$ and $TB^{+}HIV^{+}$ groups recorded significantly higher neutrophil count while the monocyte and basophil counts were both significantly higher in $TB^{+}HIV^{-}$ groups. The study further reported that the studied groups recorded significantly lower lymphocyte counts and lower CD4+ and CD8+ cells count compared with the control with $TB^{+}HIV^{+}$ groups recording significantly lower counts. The study recommends continues assessment of TB patients and those co-infected with HIV for effective management.

Conflict of interest

None.

REFERENCES

Adejumo, O. A., Daniel, O. J., Otesanya, A. F., Adegbola, A. A., Femi-Adebayo, T., Bowale, A., Adesola, S., Kuku, O. O., Otemuyiwa, K. O., Oladega, S. N., Johnson, E. O., Falana, A. A., Dawodu, O., Owuna, H., Osoba, G., &Dacosta, A. (2017). Factors Associated with TB/HIV Co-Infection among Drug Sensitive Tuberculosis Patients Managed in a Secondary Health

Facility in Lagos, Nigeria. *African Journal of Infectious Diseases*, **11**(2): 75-82. https://doi.org/10.21010/ajid.v11i2.10

Akhigbe, R. O., Ugwu, A. C., Ogolodom, M. P., Ihua, N., Maduka, BU, *et al*. (2019). Evaluation of Chest Radiographic Patterns and Its Relationship with Hematological Parameters in Patients with Pulmonary Tuberculosis in Lagos Metropolis, Nigeria. *Health Science Journal,* **13** (1):628. doi: 10.21767/1791-809X.1000628

Akpan, P. A., Akpotuzor, J .O. and Akwiwu, E. C. (2012). Some Haematological Parameters of Tuberculosis (TB) Infected Africans: The Nigerian Perspective. *Journal of Natural Sciences Research*, **2** (1): 50-56. www.iiste.org ISSN 2225-0921

Alexandra O. S., Francis, K. L., Rula, F., Philippe, H. and Andre, N. (1999). Human Immunodeficiency Virus Infection Alters Antigen-Induced Cytokine Responses in Patients with Active Mycobacterial Diseases. *The Journal of Infectious Diseases*, **177**: 1554–62.

Aminu, A. I. and Habib, N. L. (2019).Studies on some Immunological Profile of Patients Infected with Tuberculosis attending Infectious Diseases Hospital. *Bayero Journal of Pure and Applied Sciences (Special Conference Edition November 2019)*, 12 (1): 5-12 http://dx.doi.org/10.4314/bajopas.v12i1.12S

Aminu, A.I. and Tukur, A.D. (2016):Detection Of Multidrug Resistant Tuberculosis (MDR-TB) Among Rifampicin-Resistant TB Patients using Line Probe Assay (LPA) in Kano, Nigeria *Bayero Journal of Pure and Applied Sciences*, **9** (2): 1-8http://dx.doi.org/10.4314/bajopas.v9i2.1

Caruso, A. M., Serbina, N., Klein, E., Triebold, K., Bloom, B. R. and Flynn, J. L. (1999). Mice deficient in CD4 T cells have only transiently diminished levels of IFN-gamma, yet succumb to tuberculosis. *Journal of Immunology*, **162** (9): 5407-5416. https://pubmed.ncbi.nlm.nih.gov/10228018/.

Chapel H. and Haeney, M. (1993). *Essential of Clinical immunology* (3rded.). London: Blackwell Scientific Publications.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. 5th ed. Cambridge: Cambridge University Press.

Davila, S., Hibberd, M. L., Dass, R. H., Wong, H. E. E., Sahiratmadja, E., Bonnard, C., Alisjahbana, B., Szeszko, J. S., Balabanova, Y., Drobniewski, F., van Crevel, R., van de Vosse, E., Nejentsev, S., Ottenhoff, T. H. M. AandSeielstad, M. (2008). Genetic Association and Expression Studies Indicate a Role of Toll-Like Receptor 8 in Pulmonary Tuberculosis. *PLOS Genetics,* **4** (10): e1000218 https://doi.org/10.1371/journal.pgen.1000218.

Doherty, T. M. and Andersen, P. (2005). Vaccines for Tuberculosis: Novel Concepts and Recent Progress. *Clinical Microbiology Reviews*, **18** (4): 687-702. doi: 10.1128/CMR.18.4.687-702.2005.

Dorhoi, A., Nouailles, G., Jo¨rg1, S., Hagens, K., Heinemann, E., Pradl, L., Oberbeck- Mu¨ller, D., Duque-Correa1, M. A., Reece, S. T., Ruland, J., Brosch, R., Tschopp, J., Gross, O. and Kaufmann, S. H. E. (2012). Activation of the NLRP3 Inflammasome by *Mycobacterium*

tuberculosis is Uncoupled from Susceptibility to Active Tuberculosis. *European. Journal of Immunolology*, **42**: 374–384. http:// www.eji-journal.eu

Elyass, M., Abdullah, A., Yaseen, N., Elnour, A. M. and Samuel. (2017) Hematological Parameters among Tuberculosis Patients under Treatment Attending Kosti and Kenana Teaching Hospitals in White Nile State, Sudan. *American Journal of Research Communication*, **5** (6): 58-94. www.usa-journals.com, ISSN: 2325-4076.

Flesh, I. and Kaufmann, S. (1987). Mycobacteria Growth Inhibition by Interferon-gamma Activated Bone Macrophages and Differential Susceptibility among Strain of *Mycobacterium tuberculosis*. *Journal of Immunology*, **138**: 4407-13.

Flynn, J. L., Goldstein, M. M., Triebold, K. J., Koller, B. and Bloom, B. R. (1992). Major Histocompatibility Complex Class 1 Restricted T cell are Required for Resistance to *Mycobacterium tuberculosis* Infection. *Proceedings of the National Academy Science USA*, **89** (24):12013-12017. doi:10.1073/pnas.89.24.12013.

Huang, L., Nazarova, E. V., Tan, S., Liu, Y. and Russell, D. G. (2018). Growth of *Mycobacterium tuberculosisin-vivo* Segregates with Host Macrophage Metabolism and Ontogeny. *Journal of Experimental*, **215** (4): 1135–1152. https://doi.org/10.1084/jem.20172020

Ifeyinwa M. O., Aniekan-Augusta O. E., Etim, A. O. and Akpan, P. A. (2013). Some Haematological Parameters of Tuberculosis Infected Nigerians: A Study in Calabar – Nigeria. *Global Journal of Pure and Applied Sciences*, *19*: 95-100. **DOI:** http://dx.doi.org/10.4314/gjpas.v19i1.12

Javed, I., Javed, M. T., Mahmood, Z., Riaz, M., Iqbal, R. and Rasul, A. (2018). Hematological Profiling of Tuberculosis Infected and Co-Morbid Patients: A Study Carried out in Central Punjab, Pakistan. *European Journal of Inflammation*, **16**: 1-6. doi: 10.1177/2058739218818684 journals.sagepub.com/home/eji

Kaufmann, S. H. (2013). Tuberculosis Vaccines: Time to Think about the Next Generation.*Seminars in Immunology Journal,***25** (2): 172-81. Doi:10.1016/j.smim.2013.04.006.

Murray, P. J. (1999). Defining the requirements for immunological control of mycobacterial infections. *Trends in Microbiology*, **7** (9): 366-372. DOI: https://doi.org/10.1016/S0966-842X(99)01567-X.

Naim, M. (2019).Result Analysis of Erythrocyte Sedimentation Rate (ESR) For Tuberculosis (TB) Patient with Anti-Tuberculosis Drug. *International Journal of Science and Healthcare Research* **4**(2): 143-148. *www.ijshr.com.*

National Committee for Clinical Laboratory Standards (NCCLS).Susceptibility of Mycobacteria, Norcadia, and Actinomycetes 2nd ed. Tentative Standards M24-T2 Wayne, P.A.7.

National Tuberculosis and Leprosy Control Programmes (NTBLCP) (2011): Manual on Technical SOPs for Tuberculosis Laboratories in Nigeria.

National Tuberculosis and Leprosy Control Programmes (NTBLCP) (2016): Nigeria 2013 NTBLCP Annual report. Federal Ministry of Health, National Tuberculosis and Leprosy Control Programmes (2014) Nigeria 2013 NTBLCP Annual Report http://stoptbng.org/wp-content/uploads/2014/08/2013-ANNUAL-REPORT-NATIONAL-TUBERCULOSIS-LEPROSY-CONTROL-PROGRAMME.pdf .

Neyrolles, O. and Quintana-Murci, L. (2009). Sexual Inequality in Tuberculosis. *PLoS Medicine*, **6** (12):e1000199. DOI:10.1371/journal.pmed.1000199

Obeagu, E. I., Okoroiwu, I. L., Nwanjo, H. U. and Nwosu D. C. (2019). Evaluation of Haematological Parameters of Tuberculosis Patients in Umuahia. *European Journal of Pharmaceutical and Medical Research*,**6** (7): 693-699.www.ejpmr.com

Okafor, I. F., Eyo, A. O., Okon, E. A. and Akpan, P. A. (2013). Some Haematological Parameters of Tuberculosis Infected Nigerians: A Study in Calabar – Nigeria. *Global Journal of Pure and Applied Sciences*, **19**: 95-100. http://dx.doi.org/10.4314/gjpas.v19i1.12

Perreau, M., Rozot, V., Welles, H. C., Belluti-Enders, F., Vigano, S., Maillard, M., Dorta, G., Mazza-Stalder, J., Bart, P., Roger, T., Calandra, T., Nicod, L. and Harari, A. (2013). Lack of *Mycobacterium tuberculosis*–specific interleukin-17A–producing CD4+ T cells in active disease. *European Journal of Immunology*, **43**: 939–948 DOI: 10.1002/eji.201243090.

Ranti, K. O. Atilola O. G, Babalola T. V., Komolafe O. I. (2016). Prevalence of HIV infection among Tuberculosis Patients in a Teaching Hospital in South-west Nigeria: A Four-year Retrospective Study. *HIV & AIDS Review,***15**: 136–140. http://dx.doi.org/10.1016/j.hivar.2016.11.001 1730-1270/

Rohini, K., Bhat, M. S., Srikumar, P. S. and Kumar, A. M. (2016). Assessment of Hematological Parameters in Pulmonary Tuberculosis Patients. *Indian Journal of Clinical Biochemestry,***31** (3): 332–335. doi 10.1007/s12291-015-0535-8

Weiss, G. and Schaible, U. E. (2015).Macrophage defense mechanisms against intracellular bacteria. *Immunological Reveiws*, **264** (1): 182–203. http://doi.org/10.1111/imr.12266

Wilkinson, R. J. and Pasvol, G. (1995). Tuberculosis, HIV, Hormones and Children. *Journal of the Royal College of Physicians London*, **29** (20:86-88

World Health Organization (WHO) (2019): Global Tuberculosis Report, Executive Summary. **W**HO/CDS/TB/2019.23. https://www.who.int/tb/publications/global_report/GraphicExecutiveSummary.pdf?ua=1.

Yao, S., Huang, D., Chen, C. Y., Halliday, L., Wang, R. C. and Chen, Z. W. (2014). CD4+ T Cells Contain Early Extrapulmonary Tuberculosis (TB) Dissemination and Rapid TB Progression and Sustain Multieffector Functions of CD8+ T and CD32 Lymphocytes: Mechanisms of CD4+ T Cell Immunity. *Journal of Immunology*, **192**: 2120-2132. http://www.jimmunol.org/content/192/5/2120.

Yasin, A., Alaa, M. H. and Haithym, A. (2015). Hematological Changes in Pulmonary Tuberculosis. *Al-AzharAssiut Medical Journal*, **13** (4) Suppl-2: 224-229.http://www.aamj.eg.net/journals/pdf/2825.