Original article

Impact of HIV and multidrug resistant tuberculosis co-infection on CD4+ and CD8+T cells among patients attending Aminu Kano Teaching Hospital, Kano, Nigeria

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Background: human immunodeficiency virus (HIV) infection triggers massive depletion of cluster of differentiation type 4 (CD4) T cells and is a known cause of developing active pulmonary tuberculosis (TB). *Mycobacterium tuberculosis* (*Mtb*) infection also has a negative impact on the immune response to HIV, accelerating the progression from HIV infection to AIDS. **Aim of the work**: is evaluation of plasma level of CD4+ and CD8+ T cells in multi drug resistant tuberculosis (MDR/ TB) co infected with HIV. **Materials and methods**: This is a case-control study. A total of 130 participants were consecutively selected for the study. The target populations were male and female adults aged 18 to 70 years who were attending Institute of Human Virology Nigeria (IHVN) Clinic, Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria. We evaluated the CD4+ and CD8+ T cells levels in each group by flow cytometry and analyzed the differences. **Results:** The mean CD4+ T cell count in both treatment naïve-patients and treatment-experienced patients, lower than similar values in apparently healthy control. We found a significant (P<0.011) decreased of plasma levels of CD4+ T-cells count in MDR-TB/HIV co-infected treatment-naïve patients compared to MDR-TB monoinfected treatment-naïve patients. However, CD8+ T-cell counts was not statistically (*p*>0.05) different between MDR-TB/HIV co-infected treatment naïve-patients and MDR-TB monoinfected treatment naïve-patients. **Conclusion**: Investigation revealed significantly lower CD4+ T cells in MDR-TB/HIV co-infected treatment-naïve patients compared with MDR-TB monoinfected treatment naïve patients, suggesting a more advanced immunodeficiency in co-infected patients. In MDR-TB/HIV co-infected treatment-naïve individuals, MDR-TB additionally contribute to reduction in CD4+ T cell counts.

Introduction

The syndemic interaction between the human immunodeficiency virus (HIV) and multidrug-resistant tuberculosis (MDR-TB) epidemics has had deadly consequences around the world [1]. World health organization (WHO) has recommended the rapid molecular test called Xpert MTB/RIF assay which gives the results within two hours in addition to detecting rifampicin resistance and being more accurate than sputum smear microscopy for diagnosis [2]. As a regulator of the immune response, T-helper (Th) cells activate other lymphocytes from the immune system, such as B cells [3]. T-helper cell activation requires two signals: the first from the binding of antigen receptors on the surface of T cells with the majorhistocompatibility complex (MHC) class II antigen complex on antigen presenting cell (APC) and the second derived from interleukin 1 [3]. T-helper cells also activate cytotoxic T cells, whose main function is to kill all non-self cells). Cytotoxic T cells can be distinguished from Th cells because they have CD8 antigens and can recognize foreign antigens with MHC class I profiles. CD4 proteins bind to MHC class II molecules, and CD8 proteins bind MHC class I molecules to antigen presenting cells (APCs). Thus, both CD4 and CD8 cells participate in creating the MHC–antigen complex. The negative immunologic impact on T-cell function conferred by HIV heightens the risk of

tuberculosis (TB) disease progression. The presence of HIV significantly influences tuberculosis (TB) pathogenesis, clinical presentation, and management [3]. Persons with untreated HIV, because of immunosuppression, may be particularly susceptible to acquiring *Mycobacterium tuberculosis (Mtb)* infection (including MDR-TB) upon exposure in settings of poor infection control and have been shown to rapidly progress to TB disease [4]. MDR-TB is a type of TB that is resistant to the two most effective first line drugs; Rifampicin and Isoniazid. MDR-TB results from primary infection or may develop in the course of a patient's treatment. Higher rates of unsuccessful treatment outcomes, including death, occur among patients with HIV and MDR TB [4]. CD4$^+$ T cells along with CD8$^+$ T cells make up most T-lymphocytes. The CD4$^+$ T cells carry out multiple functions, ranging from activation of the cells of the innate immune system, B-lymphocytes, and cytotoxic T cells, as well and also play critical role in the suppression of immune reaction [5].

Study design
This is a case control study that was conducted among MDR-TB co-infected with HIV patients.

Study areas
The study was conducted in Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria.

Study subjects
Group 1: a total of 30 MDR-TB/HIV co-infected patients were divided into group1a (treatment naive

) and group1b (treatment experienced) 15 patients each.

Group 2: a total of 40 MDR-TB monoinfected patients were divided into group 2a (treatment naive) and group 2b (treatment experienced) 20 patients each.

Group 3: a total of 20 drug susceptible tuberculosis (DS-TB) co-infected with HIV treatment-experienced patients.

Group c: apparently healthy control

Sample size determination

Sample size is calculated using the formula below [6].

$$n = \frac{r+1}{r} \quad \frac{(SD)^2 (Z_\beta + Z_\alpha)^2}{d^2}$$

n = desired sample size

SD = Standard deviation from previously published data.

r = Ratio of control to case, 1 for equal number of case and control

d = expected mean difference between case and control based on previously published studies.

Z_β = Standard normal variate for power 80% is 0.84

Z_α = Standard normal variate at 5% type 1 error is 1.96

$$n = \frac{r+1}{1} \times \frac{(SD)^2 (Z_\beta + Z_\alpha)^2}{d^2}$$

$$= \frac{1+1}{1} \times \frac{(4.19)^2 (0.84 + 1.96)^2}{(3)^2}$$

$$= 2 \times \frac{17.5 \times 7.84}{9}$$

$$= 2 \times 15.2$$

$$= 30$$

Therefore, minimum of 30 subjects was required in the case as well as in the control group.

Ethical approval

The study design and protocol were approved by the Ethics and Research Committee of Aminu Kano Teaching Hospital (AKTH), Kano. The research was carried out in accordance with the 1964 declaration of Helsinki concerning the ethical principles for medical research involving human subjects.

Informed consent

Written informed consent was obtained from all study participants before enrolment.

Sputum samples collection and processing

The participants were counseled about sputum production and given wide mouthed sputum containers to produce sputum for Xpert MTB/RIF assay. The lid of sputum collection container was unscrewed, 2 volumes of Gene Xpert sample reagent was added into the 1 volume of sputum sample container (2:1 v/v), vigorously mixed and incubated for 15 minutes at room temperature. The samples were liquefied completely and no clumps of sputum are visible [7].

Blood samples collection and processing

From each selected subject, a venous blood specimen was collected using a sterile vacutainer blood specimen bottles, holder and needle. Two milliliter of the blood specimen was collected into a sterile ethylene diaminetetra acetic acid (EDTA) vacutainer blood specimen bottle, and used to determine HIV-status and for the enumeration of $CD4^+$ count and $CD8^+$ T cell count within 3 hours of the blood sample collection.

Laboratory analysis

Detection of *MTB* and rifampicin resistance (MDR-TB)

Detection of *MTB* and rifampicin resistance was carried out using Gene Xpert MTB/RIF technique (Cepheid 904 Caribbean Drive Sunnyvale, CA 94089-1189 USA) according to the manufacturer's instruction and standard operating procedure [2,7]. The Gene Xpert system is a fully automated nested real-time polymerase chain reaction (PCR) system, which detects *MBT* complex DNA in smear positive and negative sputum samples. It simultaneously identifies mutations in the *rpoB* (gene encoding β-subunit of RNA polymerase and associated with resistance to rifampicin) gene, which is associated with rifampicin resistance. The Gene Xpert system consist of the instrument, a computer, a barcode scanner and requires single-use disposable Xpert MTB/RIF cartridges that contain assay reagent. The specimen is transferred into the Xpert MTB/RIF cartridge and entered into the Gene Xpert instrument. By starting the test on the system software, the Gene Xpert automates nucleic acid amplification, detection of the target sequence and result interpretation. The primers in the Xpert MTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair 'core' region. The probes

are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with resistance to RIF. Two volumes of sample reagent were added to 1 volume of sample (ratio of 2:1) and the lid is closed again. The sputum collection container is shaken vigorously 20 times. It is incubated for 5 minutes at room temperature. The specimen is shaken again vigorously 20 times, incubated for another 10 minutes. The samples liquefied completely and no clumps of sputum are visible. Using a sterile transfer pipette, the liquefied sample is aspirated into the transfer pipette. The cartridge lid is opened. The sample is transferred into the open port of the Xpert MTB/RIF cartridge. The cartridge lid is closed firmly. In the Gene Xpert system window, create test clicked. The scan cartridge barcode dialog box appeared. The barcode on the Xpert MTB/RIF cartridge was scanned using the barcode reader. The create test window appeared. The patient's information is taken. Start Test clicked. The instrument module door opened with the blinking green light and the cartridge loaded. The door is carefully closed. The test started and the green light stopped blinking. The system released the door lock at the end of the run. The results displayed in the "view results" window of the Gene Xpert machine and recorded in the TB laboratory register.

HIV screening test

The HIV screening test was carried out using the WHO screening criteria for developing countries which entails the use of a parallel testing algorithm for serological testing of HIV antibodies in the patient's sera using a combination of three (3) different screening methods, in a stepwise order for the detection of HIV-1 and HIV-2 in the blood [8].

Rapid HIV Screening Test (Screening 1)

Human immunodeficiency virus screening 1 was performed using Determine HIV-1 and 2 kit (manufactured by Abbot Japan Co ltd. Tokyo, Japan). The procedure was described by the manufacturer. Briefly, 50µl of plasma sample from participants were applied to appropriately labeled sample pads. After 15 minutes of sample application, the results were read. The inherent quality control of the kit validates the results. Two visible red lines occurring in the region labeled control and test represents HIV seropositive reaction while a single red colour in the region of control validates the test kit. Absence of red line in the test region represents HIV seronegative reaction.

Rapid HIV Screening Test (Screening 2)

The HIV screening 2 was carried out using Uni-Gold HIV 1 and 2 test kit (manufactured by Trinity Biotech Plc Co Wicklow Ireland). The procedure was described by the manufacturer.

Two drops (60µl) of the plasma sample was added over the sample pad carefully. This was followed by the addition of two drops (60µl) of the wash reagent to the sample port. The result as indicated by the appearance of one or two pink/red bars was read 10 minutes after the addition of the wash reagent.

Rapid HIV Screening Test (Screening 3 or Tie Breaker)

The Tie breaker or HIV screening 3 test was performed when the results of the screening I and II was indeterminate (discordant). STAT- PAK HIV 1and 2 assay test kit (manufactured by Chembio diagnostic system INC Newyork, USA). This method utilizes immobilized antigen for the detection of antibodies to HIV 1 and 2 in the human plasma. The procedure was as described by the manufacturer of the kit. In brief, 50µl of plasma sample was dispensed into appropriately labeled sample wells, then three drops of running buffer was added drop-wise into the appropriately labeled sample wells. The results of the test were read at 10 minutes after the addition of the running buffer. This method had inherent quality control that validates the results. The presence of two pink lines in the region of test sample and control indicates HIV seropositive reaction while a single pink line at the control region indicates HIV seronegative reaction. HIV seropositive results using these two methods were used to confirm participants presenting with HIV infection.

CD4$^+$ and CD8$^+$ T cell count determination:

CD4$^+$ and CD8$^+$ T CELL Count was done by flow cytometry method of Cassens et al. [9], using Cyflow Counter manufactured by Partec, Munster, Germany, using BD FACS count CD4 and CD8 reagents from BD Biosciences (Becton Dickinson Company) following the instruction manual provided by the manufacturer and standard operating procedure. Fifty (50) µl of whole blood in Ethylene diamine tetra acetic acid (EDTA) anti-coagulant was dispensed into a partec test tube and 10 µl of CD4/CD8 PE antibody was added. The

reaction mixture was incubated in the dark for 10-15 minutes. After incubation, 800 µl of the already prepared diluted buffer (Xn 0.09% NaN3) was added to each reaction tube and vortexed. The partec tubes containing these reactions were plugged in position in the Cyflow SL Green (Partec Germany) which has already been connected to flow max software, CD4 count template data file and CD4 count instrument. The test was run on the Cyflow for 90 seconds. The results were displayed as histogram and printed. The CD4/CD8+T cell count was read.

Statistical analysis

Variables were expressed as frequencies and percentage. Statistical analysis was performed using statistical package for social sciences (SPSS) software version 23.0 (SPSS Inc. Chicago, IL, USA, 2020). ANOVA and student t test was used to find the association between two or more variable. P-values of less than or equal to 0.05 was considered statistically significant.

Results

Socio-demographic characteristics

Majority of the study population were male (66.92%) with only (33.08%) female, most of them are married (56.92%) followed by single (29.23%). Majority of the patients in the study group were under the age group of 18-29 (46.67%). Most of them attained secondary school level (46.92%) of education and self-employed (50.00%) (**Table 1**).

Effects of HIV on plasma level of CD4$^+$ T cell count and CD8$^+$ T cell count in MDR-TB

Immunological status was assessed using CD4$^+$ T-cell counts. **Table 2** show that, the mean CD4$^+$ T cell count in both treatment naïve-patients (group 1a and group 2a) (318.67±271.70 cells/µl and 622.65±279.10 cells/µl respectively) and treatment-experienced patients (group 1b, group 2b and group 3) (438.00±224.59 cells/µl, 781.30±254.07 cells/µl and 253.35±176.61 cells/µl respectively) were significantly ($p<0.05$) lower than similar values in apparently healthy control (group c) (912.58±173.67 cells/µL). We found a significant ($p<0.011$) decreased of plasma levels of CD4$^+$ T-cells count in MDR-TB/HIV co-infected treatment-naïve patients (group 1a) (318.67±271.70 cells/µl) compared to MDR-TB monoinfected treatment-naïve patients (group 2a) (622.65±279.10 cells/µl) (**Table 3**). However, CD8$^+$ T-cell counts was not statistically ($p>0.05$) different between MDR-TB/HIV co-infected treatment naïve-patients (group 1a) (318.67±271.70 cells/µl) and MDR-TB monoinfected treatment naïve-patients (group 2a) (622.65±279.10 cells/µl) (**Table 3**).

Effect of antiretroviral and anti-tuberculosis treatment on plasma level of lymphocyte subpopulation

We found that there was no statistical difference of the plasma level of lymphocyte subpopulation measured between MDR-TB/HIV co-infected treatment-naïve patients (group 1a) and MDR-TB/HIV co-infected treatment-experienced patients (group1b) (**Table 4**). We found that the mean plasma level of CD4$^+$ T-cell count was insignificantly lower in the MDR-TB/HIV co-infected treatment-naïve patients (318.67±271.70 cells/µL), than the MDR-TB/HIV co-infected treatment-experienced patients (438.00±224.59 cells/µL) ($p >0.05$). The mean plasma level of CD4+ T cell count were decreased in MDR-TB/HIV co-infected treatment-experienced patients than in MDR-TB/HIV co-infected treatment-naïve patients but not statistically difference (**Table 5**). The production of CD4+ T cell in patients with MDR-TB/HIV co-infected treatment-naïve patients, MDR-TB treatment-naïve patients, MDR-TB/HIV treatment-experienced patients, MDR-TB monoinfected treatment-experienced patients and DS-TB co-infected with HIV treatment-experienced were lower compared with the group of apparently healthy controls (**Table 3** and Figure 1 to Figure 4).

Effect of anti tuberculosis treatment on plasma level of lymphocyte subpopulations on MDR-TB monoinfected patients

We also measured the plasma level of lymphocyte subpopulation between MDR-TB monoinfected treatment-experienced patients (group 2b) and MDR-TB monoinnfected treatment-naïve patients (group 2a) to see if any of this lymphocyte subpopulation showed a difference as a result of anti-TB treatment. Statistically significant differences (P>0.05) were not observed in the mean CD4$^+$ T cell count and CD8$^+$ T cell count between treatment-naïve patients (group 2a) and treatment-experienced patients (group 2b) (Table 5).

Comparism between MDR-TB co-infected with HIV and DS-TB co-infected with HIV

The results in **table (6)** showed that the plasma level of CD4$^+$ T cell count and CD8$^+$ T cell count in MDR-TB/HIV co-infected treatment-experienced patients (group 1b) (438.00±224.59 cells/µl and 354.20±104.17 cells/µl respectively) and DS-TB co-infected with HIV treatment-experienced patients (group 3) (253.35±176.61 cells/µl and

321.70±172.57 cells/µl respectively) were significantly lower ($p<0.05$) than similar value in apparently healthy control (group c) (912.58±173.67 cells/µl and 584.40±170.85 cells/µl respectively). The mean plasma levels of CD4+ T cell count and CD8+ T cell count in DS-TB co-infected with HIV treatment-experienced patients (group 3) was lower compared with similar values in MDR-TB co-infected with HIV treatment-experienced patients (group 1b) but not statistically difference.

Correlation between pairs of variables

The correlation between the subpopulation of lymphocyte are shown in **Table 2**. There was weak positive correlation between CD4+ T cell count and CD8+ T cell count in MDR-TB/HIV co-infected treatment-naïve group ($r = 0.39$); CD4+ T cell count were negatively correlated with CD8+ T cell count in MDR-TB monoinfected treatment-naïve group (r = -0.102) **(Figure 3)**. There was weak positive correlation between CD4+ T cell count and CD8+ T cell count in MDR-TB/HIV co-infected treatment-experienced group ($r = 0.269$); There was strong positive correlation between CD4+ T cell count and CD8+ T cell count in DS-TB/HIV co-infected treatment-experienced group (r = 0.768). CD4+ T cell count were positively correlated with CD8+ T cell count in MDR-TB monoinfected treatment-experienced group ($r = 0.564$) **(Figure 4)**.

Table 1. Socio demographic MDR-TB/HIV co-infected group.

Characteristics	Number of Subjects	Percentage (%)
Gender	**130**	
Male	87	66.92
Female	43	33.08
Age (years)	**90**	
18 – 29	42	46.67
30 – 49	32	35.56
50≤	16	17.78
Marital Status	**130**	
Married	74	56.92
Single	38	29.23
Widowed	7	5.38
Divorced	11	8.46
Educational Level	**130**	
No formal	19	14.62
Primary	27	20.77
Secondary	61	46.92
Tertiary	23	17.69
Employment Status	**130**	
Civil Service	14	10.77
Self employed	65	50.00
Student	18	13.85
Unemployed	33	25.38

Table 2. Plasma levels of CD4⁺ T cells, CD8⁺ T cells in studied groups.

Variables	Group 1a (n=15)	Group 1b (n=15)	Group 2a (n=20)	Group 2b (n=20)	Group 3 (n=20)	Group c (n=40)
CD4(cells/µl)	318.67 ± 271.70^{a1}	438.00 ± 224.59	622.65 ± 279.10	781.30 ± 254.07^{a2}	$253.35 \pm 176.61^{a5\ a6}$	912.48 ± 173.67^{a4}
CD8(cells/µl)	392.80 ± 194.14^{b1}	354.20 ± 104.17^{b2}	536.40 ± 306.39	420.50 ± 356.73	321.70 ± 172.57^{b3}	584.40 ± 170.85

KEY: Values are mean ± standard deviation; n = number of Subjects; MDR-TB = multidrug resistant tuberculosis; DS-TB = drug susceptible tuberculosis; HIV = human immunodeficiency virus; CD4 = cluster of differentiation type 4 CD8 = cluster of differentiation type 8; ART=antiretroviral therapy; ATT=anti-tuberculosis therapy; BMI = Body Mass Index; Significant differences: **a¹** (P<0.01)= Group 1a versus Group 2a; **a²** (P<0.001)= Group 2b versus Group 3; **a⁴** (P<0.001)= Group 1a versus Group c; **a⁵** (P<0.01)= Group 1b versus Group c; **a⁶** (P<0.04)= Group 3 versus Group c; **b¹** (P<0.001)= Group 1a versus Group c; **b²** (P<0.001)= Group 1b versus Group c; **b³** (P<0.011)= Group 3 versus Group c; **c¹** (P<0.041)= Group 1a versus Group c; **c²** (P<0.02)= Group 2a versus Group c; **c³** (P<0.05)= Group 2a versus Group 2b by Tukey-Kramer Multiple Comparisons Test.
GROUP 1a = MDR-TB co-infected with HIV ATT and ART treatment-naïve patients.
GROUP 1b = MDR-TB co-infected with HIV ATT and ART treatment-experienced patients.
GROUP 2a = MDR-TB ATT treatment naïve-patients.
GROUP 2b = MDR-TB ATT treatment-experienced patients
GROUP 3 = DS -TB co-infected with HIV ATT and ART treatment-experienced patients
Group c = Apparently Healthy control.

Table 3. Effect of HIV on plasma level of lymphocyte subpopulations on MDR-TB.

Parameter	**MDR-TB/HIV** (n=15)	**MDR-TB** (n=15)	*p*-value
Treatment-naïve Mean ± SD	**Treatment-naive** Mean ± SD		
CD4+ T cell (cells/µl)	318.67±271.70	622.65±279.10	0.011
CD8+ T cell (cells/µl)	392.80±194.14	536.40±306.39	0.087

KEY: Values are mean ± standard deviation; n = number of Subjects; CD4 = cluster of differentiation type 4 CD8 = cluster of differentiation type 8; ART = antiretroviral therapy; ATT = anti-tuberculosis therapy; SD = standard deviation; MDR-TB = multidrug resistant tuberculosis; HIV = human immunodeficiency virus; p-values of less than or equal to 0.05 was considered statistically significant.
MDR-TB/HIV = MDR-TB co-infected with HIV ATT and ART treatment-naïve patients.
MDR-TB = HIV negative MDR-TB ATT and ART treatment-naive patients.

Table 4. Effect of antiretroviral therapy and anti-TB therapy on lymphocyte subpopulations.

Parameters	**MDR-TB/HIV**	**MDR-TB/HIV**	*p-value*
Treatment-naïve Mean ± SD (n=15)	**Treatment-experienced** Mean ± SD (n=15)		
CD4⁺ T cell (cell/ml)	318.67±271.70	438.00±224.59	0.485
CD8⁺ T cell (cell/ml)	392.80±194.14	354.20±104.17	0.326

KEY: Values are mean ± standard deviation; n = number of Subjects; SD = standard deviation; CD4 = cluster of differentiation type 4 CD8 = cluster of differentiation type 8; ART=antiretroviral therapy; ATT=anti-tuberculosis therapy; MDR-TB = multidrug resistant tuberculosis; HIV = human immunodeficiency virus; p-values of less than or equal to 0.05 was considered statistically significant.

Table 5. Effect of anti-TB therapy on lymphocyte subpopulations.

Parameters	MDR-TB Treatment-naïve Mean ± SD	MDR-TB P-value Treatment-experienced Mean ± SD	
CD4+ T cell (cell/ml)	622.65±279.10	781.30±254.07	0.686
CD8+ T cell (cell/ml)	536.40±306.39	420.50±356.74	0.514

KEY: Values are mean ± standard deviation; n = number of Subjects; CD4 = cluster of differentiation type 4 CD8 = cluster of differentiation type 8; ART=antiretroviral therapy; ATT=anti-tuberculosis therapy; MDR-TB = multidrug resistant tuberculosis; p-values of less than or equal to 0.05 was considered statistically significant.

Table 6. Comparison between MDR -TB/HIV and DS -TB/HIV Treatment-experienced.

Parameter Mean ± SD	MDR-TB/HIV Treatment-experienced Mean ± SD	DS-TB/HIV Treatment-experienced	*P-value*
CD4+ T cell (cell/ml)	438.00±224.59	253.35±176.61	0.370
CD8+ T cell (cell/ml)	354.20±104.17	321.70±172.57	0.069

KEY: Values are mean ± standard deviation; n = number of Subjects; SD = standard deviation; CD4 = cluster of differentiation type 4 CD8 = cluster of differentiation type 8; ART = antiretroviral therapy; ATT = anti-tuberculosis therapy; MDR-TB = multidrug resistant tuberculosis; DS-TB = drug susceptible tuberculosis; HIV = human immunodeficiency virus; p-values of less than or equal to 0.05 was considered statistically significant.

Figure 1. Data are expressed as mean standard deviation. CD4+ T cell count was significantly higher in the control group (group c) compared with all the other groups.
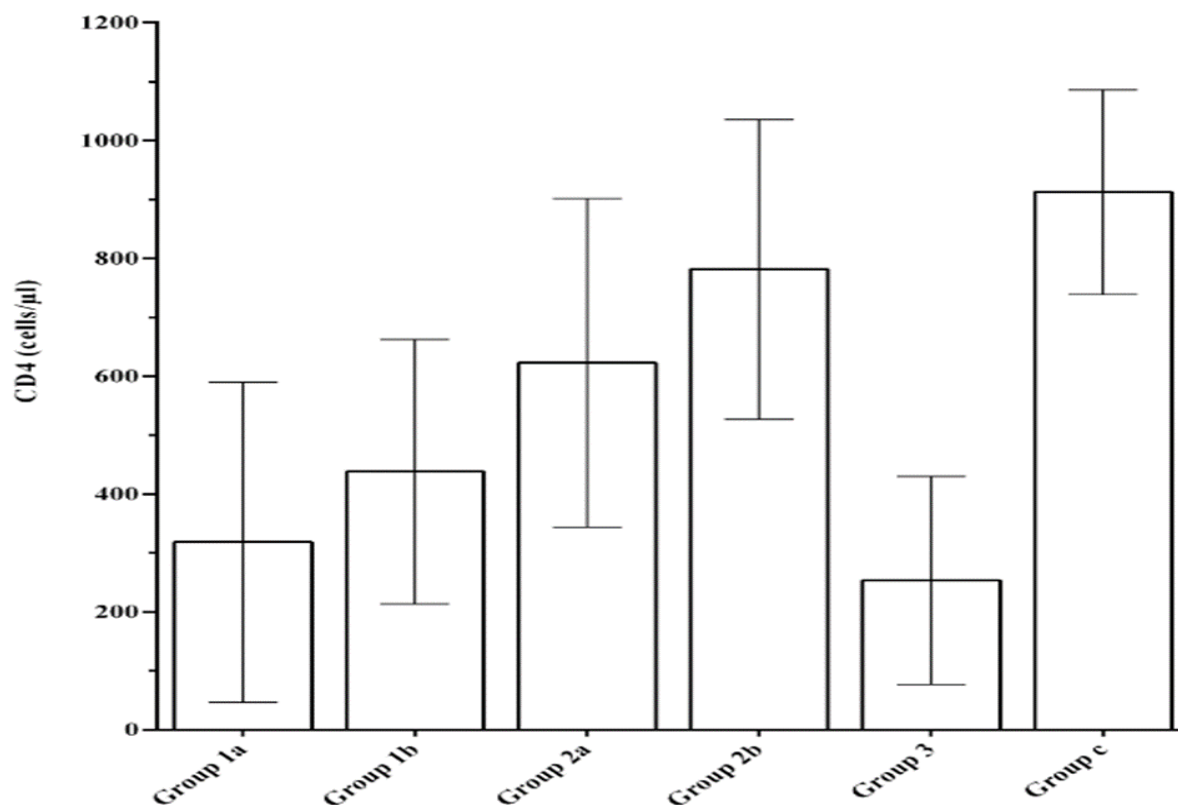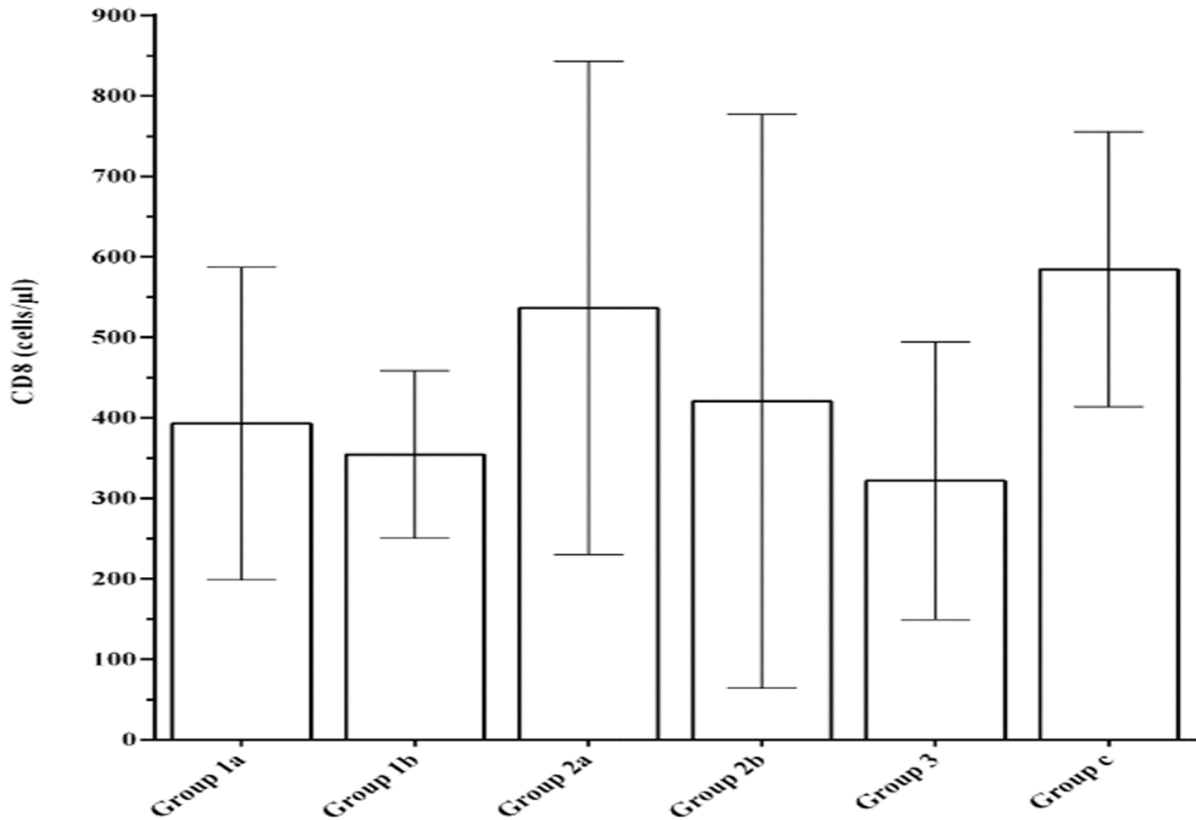
Figure 2. Data are expressed as mean standard deviation. CD8^{+} T cell count was significantly higher in the control group (group c) compared with all the other groups.

Figure 3. Correlation between pairs of variables. Positive correlation were found between CD4^{+} T cell count and CD8^{+} T cell count in MDR-TB/HIV co-infected treatment-naïve Group ($r = 0.39$); negative correlation were found between CD4^{+} T cell count and CD8^{+} T cell count in MDR-TB monoinfected treatment-naïve Group (r = -0.102).
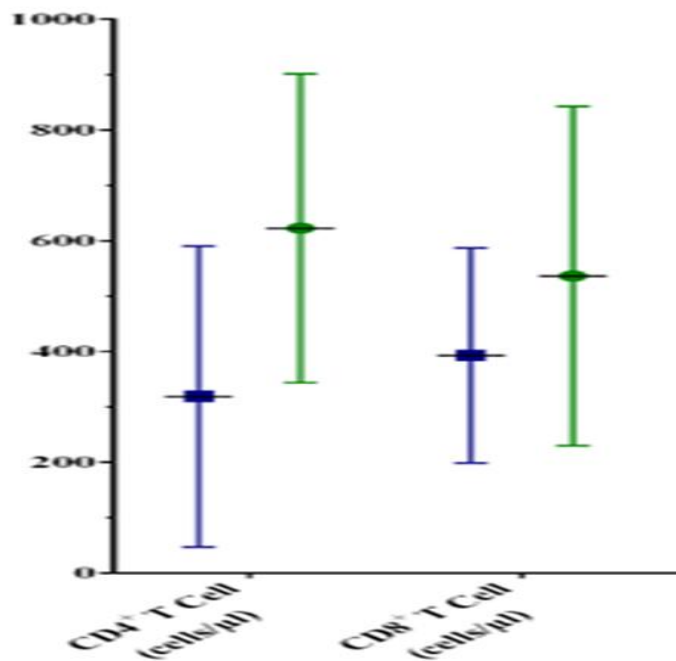
Figure 4. Correlation between pairs of variables. Positive correlation were found between CD4$^+$ T cell count and CD8$^+$ T cell count in MDR-TB/HIV co-infected treatment-experienced group ($r = 0.269$); Positive correlation were found between CD4$^+$ T cell count and CD8$^+$ T cell count in MDR-TB monoinfected treatment-experienced group ($r = 0.564$); positive correlation were found between CD4$^+$ T cell count and CD8$^+$ T cell count in DS-TB/HIV co-infected treatment-experienced group (r = 0.768).
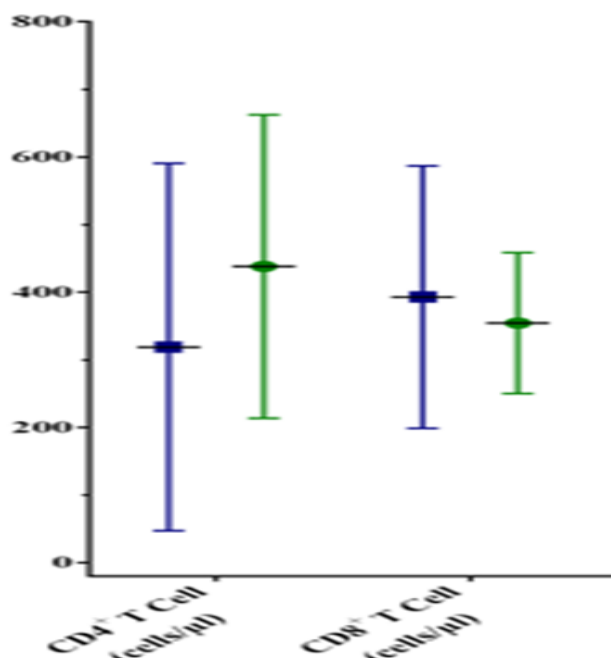
Discussion

In the current study, the finding of 35.24±1.53 years as means age for MDR-TB co-infected with HIV patients. These findings are consistent with reports of previous studies of **Cherono et al.** [10] who indicated that TB/HIV is known to affect reproductive age group of between twenty five to forty five years because these are individuals who are sexually active hence the transmission of HIV is very high. Marital status is an important risk factor when exploring the patterns of MDR-TB/HIV co-infection. Married had higher proportion (56.92%) of co-infection than single (43.08%) counterpart. These results are in agreement with the finding of **Cherono et al.** [10]. Married couple is at high risk of infection especially if one partner is unfaithful. Although patients in all education levels were predisposed to MDR-TB/HIV co-infection. Secondary education had higher proportion (46.92%). Contrary to the findings of the study are the findings conducted in Zambia by **Muyunda et al.**[11] who reported higher percent among primary school level. Contrary to the findings of the study are the findings of other researchers that found that patients with high

education level, with high chances of being employed in skilled occupations which attracts huge salaries are likely to have multiple partners compared to those with unskilled occupations having attained low education level [11]. This indicates that availability of disposable income can lead to unhealthy behaviours that result in transmission of HIV which destroy CD4$^+$ T cells increasing the susceptibility to TB. Male had higher co-infection (66.92%) than females (33.08%). This is in contrast with the finding of **Cherono et al.** [10] in Kenya who found greater prevalence of TB/HIV co-infection amongst females. The type of occupations also plays a very important role on HIV-TB confection. Most of them were self-employed (50.00%) followed by unemployed (25.38%). People with low income earning casual jobs are highly susceptible to the co-infection than those with high income generating and stable jobs.

In our study, we found that the levels of CD4$^+$ T cell count in peripheral blood of MDR-TB/HIV co-infected treatment-naïve patients and MDR-TB monoinfected treatment-naïve patients were significantly ($p<0.05$) lower than those in the apparently healthy control group. CD4$^+$ T cell count

in MDR-TB/HIV co-infected treatment-naïve patients was significantly lower compared with MDR-TB monoinfected treatment-naïve patients. This is in conformity with the study conducted by **Nosik et al.** [12] and **Mbow et al.** [13] who found significantly lower CD4+ T cell count in co-infected subjects than TB monoinfected subjects. Suggesting a more advanced immunodeficiency in co-infected patients. The plasma levels of CD4+ T cell count in DS-TB/HIV co-infected treatment-experienced patients was decreased compared to MDR-TB/HIV co-infected treatment-experienced patients but not statistically different. CD4+ T cells depletion is known to occur in TB patients not infected by HIV[14]and become normalized following anti tuberculosis treatment (ATT) [14].CD4+ T cell count was increased in MDR-TB/HIV co-infected treatment-experienced patients compared to MDR-TB/HIV co-infected treatment-naïve patients. This is in agreement with **Nosik et al.** [12] who reported treatment increased CD4+ T cell count.The aim of treatment is to reduce the viral load, and this make the CD4+ T cell count increases so that the body's ability to fight infections improves.

CD8+ T cell count was lower in MDR-TB/HIV co-infected treatment-naïve-patients compared to MDR-TB monoinfected treatment-naïve patients. This is in contrast with the study conducted by **Mbow et al.** [13] who found higher CD8+ T cell count. Patients with AIDS exhibit relative increase in the CD8+ T cells subtype. CD8+ T cell count was decreased in MDR-TB/HIV co-infected treatment-experienced patients compared with MDR-TB/HIV co-infected treatment-naïve patients. This concurs with **Cartwright et al.** [15]. Viral load and CD8+ T cell counts of HIV-infected patients were suppressed on ART. **Wong et al.** [1] hypothesized that due to the negative impact of HIV-1 on CD4+ cells, their pool decreases and consequently, there are fewer CD4+ cells [1].

Conclusion

The study revealed significantly fewer CD4+ T cells in MDR-TB and HIV co-infected patients compared with MDR-TB monoinfected subjects, suggesting a more advanced immunodeficiency in co-infected patients. There is an association of TB with the acceleration of immunodeficiency. There was significant decreased CD4+ T cell count among DS-TB/HIV co-infected treatment-experienced patients compared with the MDR-TB/HIV co-infected treatment-experienced patients.

Recommendation

Based on the findings from this study, it is recommended that: Our study was case control; further studies should be conducted to include larger sample size. Recombinant human interferon gamma interleukin 2 (IL-2) should be used to improve immunity status among MDR-TB/HIV co-infected patients.

Conflicts of interests

The authors declare that there is no conflict of interestsregarding the publication of this paper.

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