Predictors, Haematological and Cytokine profiles in Tuberculosis-HIV Coinfection patients during TB treatment and HAART in Coastal Region of Kenya

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Abstract

***Background**: Tuberculosis and HIV Co-infection has become a major public health problem worldwide. Tuberculosis has become the major cause of death in HIV positive patients.HIV infection is characterised by CD4+ lymphocyte depletion manifested through the loss of the immune response capacity. The impact of simultaneous infections on the immune parameters is still not fully explored. **Aim:** This study was aimed to determine the predictors and estimates of T cell subsets including cytokine profiles of TB-HIV co-infection patients after initiation of dual therapy in Coastal Region, Kenya. **Study design**: Hospital and laboratory based cross-sectional study was carried between May 2012 and November 2014 at Coast General Referral hospital, Tudor, Port-Reitz, Mlaleo, Likoni and Mikandani districts and Sub-districts hospitals. **Methodology**: Tuberculosis was diagnosed following standard clinical bacteriological and radiological procedures. Sputa from 500 tuberculosis suspects underwent mycobacteriologic evaluation using Ziel Nelsen smear microscopy, Lowestein and Jensen and BACTEC MGIT 960 culturing. Consenting participants were screened for HIV infection by enzyme -linked immunosorbent assay. CD4+, CD8+cells/µl were analyzed using a BD FACS Count Flow cytometer. These parameters were measured at the time of HAART initiation (30 days after onset of TB treatment) and at the follow-up visits after 30, 60 and 90 days. **Results:** In Total, 210/500 (42%) of the suspects had Mycobacterial disease and 78/210 (37.1%) were HIV infected 53.8% females and 46.2% males. Age (OR=2.06; 95% CI: 1.1-3.00; p<0.003), marital status (OR=3.54; 95% CI: 2.01-4.65;p<0.0001), baseline CD4 count (OR=3.23; 95% CI: 1.23-8.34; p<0.0001) and WHO clinical stage at presentation (OR=3.84; 95% CI:1.45-7.63; p<0.003) remained significant predictors after adjustment for confounding. Increase of CD4+T cell counts and suppression of HIV viral load were observed for all patients under HAART and TB treatment. Higher values of IFN-γ, IL-2, IL-4, IL-6, IL-10 were observed from the baseline to two months after treatment initiation, whereas reduced levels of TNF-α were observed between 60 and 90 days of HAART. **Conclusion:** Lower CD4 lymphocyte count, substance exposure and WHO clinical stage at presentation were found to be predicting factors for co-infection. Independent of the immunosuppressant profile at baeline, patients under HAART were able to recover the CD4+T cell counts, control viral replication and immune activation parameters overtime.*

Key words: Tuberculosis-HIV co-infection; predictors; immunological markers; Cytokine ; Mombasa, Kenya.

1. Introduction

Tuberculosis (TB) and human immune deficiency virus (HIV) are two major public health problems in many parts of the world. There were approximately 36.3 million people living with HIV in 2013 [WHO, 2013]. In sub-Saharan Africa including Kenya, the HIV and AIDS infection has contributed significantly to the rising levels of tuberculosis incidence. As the greatest killer worldwide due to single infectious agent, TB is just the second to HIV/AIDS.

People with HIV infection are increasingly infected with TB because HIV weakens their immune system. HIV/AIDS is one the most risk factor for the development of tuberculosis [Antonucci *et al*., 2004]. Patients with TB infection co-infected with HIV, have a 20-30 times higher risk of developing tuberculosis disease during their lives, than TB infected person without HIV infection [Dye *et al*., 2009]. In immune competent individuals with TB infection the lifetime risk of developing active TB disease is 10% in contrast with TB infected patient co-infected with HIV where the annual risk of developing TB disease is (5-8%) [Korenromp, *et al*., 2003]. Tuberculosis is the most common opportunistic infection (OI) in HIV/AIDS patients in developing countries. Tuberculosis is the common pre AIDS opportunistic infection and accounts for about 40% of all presentations seen in HIV patients in Haiti [Yassin *et al*., 2006 , Bock *et al*., 2007] . Other common presentation is the wasting syndrome, which includes weight loss of more than 10% of normal weight and prolonged fever or diarrohea. The wasting syndrome is also associated with TB and more often the symptoms of TB are misattributed to HIV/AIDS [Corbett *et al*., 2003]. Tuberculosis can occur at any stage of $CD4^+$T cells depletion but it is common during the early stage when the $CD4^+$T cells is relatively normal [Tsegaye *et al*., 2002].

In Haiti for example, 56% of the TB patient infected with HIV were diagnosed when $CD4^+$T cells were >350/microlitre, 23% and 12% of the patients infected with HIV has TB at the $CD4^+$T cell levels of 200-350/microlitre and <200/ microlitre, respectively [Nwachukwu *et al*., 2010]. HIV-1 infection is characterized by profound immune suppression associated to inappropriate immune activation [Appay and Sauce, 2008].
In general, after initiating highly active antiretroviral therapy (HAART), a viral suppression to undetectable levels, increase of CD4+ counts and remarkable clinical improvement are observed [Mocroft *et al*., 2007]. Moreover, cellular and soluble markers of immune activation are strong predictors of HIV disease progression [Zhang *et al*., 2006]. CD4+ and CD8+ T cell subsets expressing CD38+ and/or HLA-DR+ are overrepresented as hallmark of immune activation in HIV positive patients associated or not with other pathogens [Mahan *et al*., 2010]. Tuberculosis infection enhances local HIV-1 replication in-vitro. Cytokines produced during TB infection may result in activation of lately HIV infected cells with virus. Expression and induction of virus replication, increased IL-2, IL-6 and TNF- & (TH-2 type cytokine) generated by infection with TB may be responsible for the increase in the viral load [Lederman *et al*., 1998, Sharma *et al*., 2009]. HIV infection is associated with a profound deregulation of the immune system and alterations in the cytokine profiles. TNF- & also plays a pivot role in HIV-1 pathogenesis, being found in increased levels in acute and chronic HIV-1 infections [Lawn *et al.*, 2001]. T helper type 2 (Th2) cell activation and increased interleukin-4 (IL-4) production have been associated with poor clinical outcome after HIV treatment [Subramanyam *et al*., 2004]. It is recognized that joint TB-HIV interventions will require additional funding to improve both TB and HIV program performance and coverage and antiretroviral treatment. Despite multiple studies demonstrating TB and HIV infections, there are no studies showing the profile of cellular markers along concomitant therapy for both diseases. It is essential to determine the predictors and estimates of activated T cell subsets including cytokine profiles of TB-HIV co-infection during treatment for both diseases and the impact of the CD4 immuno suppression levels at baseline for trends of such parameters.

2. Materials and Methods

2.1 Study area

The study was conducted in Mombasa County which has a population of 1,031,266 by the year 2013. The population is steadily growing due to rural-urban migration and immigration from unstable countries. The total area Mombasa is 109 $Km^{2\ with}$ about 60% of the people living overcrowded informal settlements in the form of shelters. The region has rapid population growth and is characterized by low socio-economic indicator. This creates huge demands on health facilities and inability to keep pace with the environment, continued economic prosperity, public health and quality of life of residents. Tuberculosis and HIVAIDS are the leading causes of deaths in the area representing 50%.

2.2 Study site

The study was done at done at Ganjoni clinic, Coast provincial General hospital (CPGH), Mlaleo Health and Mikindani Health centers, Likoni, Portreizt and Tudor district hospitals. These hospitals provides inpatient and outpatient services, including care and treatment for TB and HIV/AIDS patients with ARTs.

2.3 Study design

This was hospital and laboratory based descriptive cross-sectional study carried out between May 2012 to November 2014.

2.3.1 Inclusion and exclusion criteria

Those suspected of having TB and resident in Mombasa County for at least six (6) months, not on anti-TB chemotherapy and consented to participate in the study were recruited. The exclusion criteria included Tuberculosis suspects who had not lived in Mombasa County for the last six (6 months), resistance to rifampicin, CD4 counts above 350 cells/mm^3 for patients with pulmonary tuberculosis and those unwilling to participate in the study.

2.3.2 Sampling frame and follow-up

Coastal region was purposively sampled because of high cases of TB and HIV co-infection. The sampling frame consisted of all the public health facilities within the study area. After the selection of the study sites, each was allocated a proportionate number of study subjects based on the level of health care delivery system and the average client attendance in the past one month before embarking on the study. To minimize bias in selecting study subjects, consecutive sampling was used hence every alternate TB suspect who satisfied the inclusion criteria. HAART was initiated 30±10 days after initiation of tuberculosis therapy and included two reverse transcriptase nucleoside analogs plus efavirenz (600mg or 800mg). Follow up visits were performed at 30, 60 and 90 days (end of TB treatment) and after initiation of HAART. Blood samples were collected at baseline (15 days after initiation of HAART) and at all follow-up visits to study the plasmatic immune profile.

2.4 Collection of demographic data

Study participants were interviewed about presence clinical manifestations of TB. Socio-demographic characteristics and other related risk factors were collected using structured questionnaire by trained nurses and physical examination was done by physicians.

2.4.1 Collection of sputum

A specialist medical doctor working in the TB clinic performed the necessary clinical and diagnostic work. Diagnostic was made based on the combined evaluation of clinical, radiological and laboratory features. Three sputum specimens (spot, early morning, spot) were collected from 500 TB suspects under the supervision of trained and competent medical staff. The patients were advised to rinse their mouth twice with water before producing the specimen and this helped to remove food and any contaminating bacteria in the mouth. They were instructed to take two breaths, coughed vigorously and expectorated the material in into the sterile 50ml blue cap screw-capped bottle. This process allowed sputum to be produced from deep in the lungs. The TB suspects were asked to hold the sputum container close to the lips and spit into it gently after a productive cough. Sputum samples were decontaminated using the modified Petroff´s method and concentrated by centrifugation at 3000g for 15 minutes. At the peripheral laboratory, the standard Acid-fast (AFB) direct smear microscopy using Ziehl-Neelsen (ZN) staining was done on the initial sputum to confirm TB diagnosis of suspected patients. A second sputum specimen was then collected which was refrigerated at 4^oC and transported to the Central reference Laboratory (CRL) weekly for culture. The safety for research assistants and healthcare workers during collection and handling of sputum specimens was censured by observing the WHO guideline.

2.4.2 Collection of blood samples and screening for HIV

A total of 500 participants consented phlebotomy for HIV testing. Blood samples were tested for HIV antibodies according to the Kenyan national testing algorithm for voluntary counselling. Blood samples were delivered in vacutainer Brand STERILE interior ethylene diamine-tetra-acetic acid (EDTA) tubes and used for HIV test, complete blood cell (CBC) counts and for CD4+T cell counts. Screening for HIV infection was done by screening serum/plasma by using Determine HIV1/2 (Abott laboratories, Japan co. LTD), Capillus HIV1/2 (The Trinity Biotech, Ireland) and Unigold H1/2 (Trinity Biotech, Ireland) rapid test kits and positives confirmed with the enzyme linked immunosorbent assay.

2.4.3 CD4+T Cell Counts and Viral Load Evaluation

The puncture area was identified, swabbed with alcohol and blood sample collected by well trained phlebotomists. Blood samples were collected by venipuncture using a 10ml syringe and 21g needle which were all sterile.

Whole blood samples were collected in 4.5ml BD K2E vacutainer (lavender cap) and mixed adequately with the EDTA by gently inverting up and down for at least ten times to avoid clotting. Blood was processed within 24 hrs after collection. The test vial was vortexed upside down and then upright for 5 seconds each to ensure even mixing. Fifty microliter (50ul) of blood was added to the vial using the back pipetting technique with an electronic pipette and vortexed again for 5 seconds before incubating in the dark for a period of 1 hour at room temperature. At the end of the incubation period, the product was fixed with a 50µl fixative solution and vortexed to ensure even mixing. The reagent tubes were placed onto the holder beneath the probe and allowed the machine to read the CD4+ sample. Plasma samples were obtained by centrifugation within four 4 hours of blood collection and aliquots were stored at -70 C freezer until use for viral load and cytokine measurements. Quantification of the plasma viral load was determined for TB-HIV co-infection patients at each visit.

2.4.4 Immunoassays for Cytokines

Plasma level of cytokines (IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α) were measured using the Cytometric Beads Array kit (CBA, BD Biosciences, San Diego, CA, USA) using a FACS Calibur cytometer. The reagents and samples were prepared according to the manufacturer instructions.

2.4.5 Microscopic examinationsand isolation of *Mycobacterium tuberculosis*

Sputum smears were examined for acid-fast bacilli (AFB) after staining following ZN method. The degree of ZN smear positivity was quantified as 1+ for 10-100 AFBs per 100 fields, 2+ for 1-10 AFBs per field (50 fields) and 3+ for >10 AFBs per field (20 fields). For less than 10 AFBs per 100 fields, the exact number of AFBs was indicated. A suspect was considered to be ZN smear positive if at least one specimen was positive. Using sterile 1ml disposable pipette, 0.5ml of the sediment (sputum) obtained after centrifuging was inoculated on two Lowenstein Jensen (LJ) slopes/ or MGIT, one with glycerol (0.75%) and one without glycerol, but supplemented with 0.5% pyruvate. The caps were labelled with lab serial number of the specimen and named as 1 and 2. All Lowenstein-Jensen media slopes were incubated at 37^0C. The slopes were examined weekly for any visible growth and negative culture tubes discarded after 8 weeks.

2.4.6 Screening for Malnutrition

All participants were screens were assessed for malnutrition using body mass index (BMI). Normal nutrition status was defined as BMI>18.5/kg/m^2, mild malnutrition as BMI of 17.5-18.4kg/m^2, moderate malnutrition as BMI of 16-17.4kg/m^2 and severe malnutrition as BMI<16 kg/m^2.

2.4.7 Haematological Assay

Haematological parameters: haemoglobin (Hb), haematocrit (PCV), white blood cell count (WBC), platelet count (PLT) were determined using the automated blood analyzer cell-Dyn 1800 (Abbot Laboratories Diagnostics Division, USA).

2.4.8 Case definitions

Smear positive PTB was defined as one or more sputum smear examinations positive for acid fast bacilli (AFB). Smear-negative PTB was also defined as three sputum smear examinations negative for AFB but with clinical and radiographic consistent abnormalities with active tuberculosis.

2.5 Data management and analysis

Demographic data were confidentially obtained from the TB suspects by clinicians / nurses running the chest clinics. Results of ZN smear microscopy, culture, and HIV tests were confidentially sent to the respective clinicians / nurses. Provisions of these data were made available to the clinicians/ nurses for the purpose of managing the patients. Data was recorded on questionnaires, register books, ELISA reader print-outs and species evaluation sheets. The data was coded, entered into MS Excel 8.0 and processed using a statistical package for social sciences (SPSS) version 16.5 software for windows. The chi-squared (χ^2) test was used to compare categorical data and logistics regression to analyze multivariate data.

Univariate odds ratio (OR) and 95% interval (CI) were calculated to assess predictors with regard to TB and HIVco-infection. The strength of an association. *p* values of <0.05 were considered statistically significant.

2.5.1 Ethics Statement

This protocol was approved by Kenyatta University Ethical Review Committee (No PKU018/115). It was approved by the ministry of education, Science and Technology (MOEST). Clearance was also obtained from respective County health authorities and hospital administrations. The study was conducted in accordance with the declaration of Helsinki and all patients who accepted to participate in the study signed an informed consent form. Code numbers rather than names were used to identify candidates in order to maintain confidentiality.

Results

3.1 Socio-demographic characteristics of the TB suspects

A total of 500 participants suspected of having TB were enrolled in this study at the study sites, 271 (54.2%) males and 229 (45.8%) females. Their ages were between 18 and 80 years and median age being 32 years. The majority (42.4%) of the participants were in the 25-34 age-group, followed by those in the 35-44 (24.4%), 18-24 (19.2%), 45-54 (9.8) and 55+ (3.4%) age groups respectively. Almost 85% had attained secondary and college of education while 14.6% primary level. Over a half (54.2%) of the participants were married while (38.4%) were not married (Table 1).

Table 1: Socio-demographic Characteristics of the TB suspects (n=500)

Characteristic	Groupings	Female (n=229)	Male (n=271)	Total (n=500)
Age in Years	18 – 24	44 (19.2%)	52 (19.2%)	96 (19.2%)
	25 – 34	101 (44.1%)	115 (42.4%)	216 (43.2%)
	35 – 44	53 (23.1%)	69 (25.5%)	122 (24.4%)
	45 – 54	21 (9.2%)	28 (10.3%)	49 (9.8%)
	55 +	10 (4.4%)	7 (2.6%)	17 (3.4%)
	Mean age	**32.84 ± 9.90**	**32.75 ± 9.64**	**32.79±9.75**
Education level	College	77 (33.6%)	82 (30.3%)	159 (31.8%)
	No Education	1 (0.4%)	1 (0.4%)	2 (0.4%)
	Primary	39 (17.0%)	34 (12.6%)	73 (14.6%)
	Secondary	112 (48.9%	154 (56.8%)	266 (53.2%)
Marital Status	Divorced	2 (0.9%)	4 (1.5%)	6 (1.2%)
	Married	122 (53.3%)	159 (58.7%)	281 (56.2%)
	Unmarried	89 (38.9%)	103 (38.0%)	192 (38.4%)
	Widowed	16 (7.0%)	5 (1.9%)	21 (4.2%)
Employer	Government	55 (24.0%)	66 (24.4%)	121 (24.2%)
	Jobless	59 (25.8%)	66 (24.4%)	125 (25.0%)
	House wife	6 (2.6%)	27 (10.0%)	33 (6.6%)
	Others*	13 (5.7%)	34 (12.6%)	47 (9.4%)
	Farmer	15 (6.6%)	9 (3.3%)	24 (4.8%)
	Self Employed	79 (34.5%)	67 (24.7%)	146 (29.2%)
	Student	2 (0.9%)	2 (0.7%)	4 (0.8%)

***Other occupations include daily labourers, commercial sex workers and house hold servants**

3.2 Prevalence of HIV among newly diagnosed tuberculosis patients

A total of two hundred and ten TB patients were tested for HIV virus and 39.7% (78/210) were sero-positive and the mean age was 35.16 ± 0.71. Mean age for males was 36.12 ± 1.06 and females 34.19 ± 0.94. Over all males constituted 46.2% and females 53.8% of the HIV-cases. There was significant difference between the TB-HIV co-infection rate and age (χ^2 =3.391, df=4; p< 0.01). The majority (44.9%) of the TB-HIV co-infection cases were in the 35-44 years age bracket followed by 25-34 (23.1%) and 45-54 (20.5%) and 55+ (7.7%) years age brackets. There was no significant difference in the TB-HIV co-infection rate between gender, females being more vulnerable (OR=1.70, 95% CI: 0.347-1.240; p=0.062**).** Logistic regression analysis comparing the association of TB with age-group revealed females in the 35-44 and 45-54 age-groups to be significantly affected compared to males (Table 2).

Table 2: TB-HIV co-infection and gender-age distribution (n=78)

Age in Years	Male (n=36)	Female (n=42)	OR (95% CI)
18-24	1 (1.3%)	2 (2.6%)	1.00 **
25-34	9 (11.5%)	9 (11.5%)	0.57 (0.26-1.60)
35-44	16 (20.5%)	19 (24.4%)	2.58 (0.57-3.65)
45-54	6 (7.7%)	10 (12.8%)	0.3(0.23-2.56)
55+	4 (5.1%)	2 (2.6%)	0.92 (0.18-4.57)
Mean Age	**36.12 ± 1.06**	**34.19 ± 0.94**	**35.16 ± 0.71**

OR=Odds ratio; CI= Confidence interval; **1.00=Reference exposure

3.3 HIV sero prevalence among TB patients by marital status

HIV prevalence was significantly higher among the widowed 46.2%, married 40.3%, divorcees 33.3% and in single group 30.7% respectively. The association between HIV-TB co-infection and marital status was statistically significant (χ^2=2.338, df=3; p< 0.01). Another predictor of TB and HIV co-infection among this group was contact with commercial sex workers. Out of ten (10) patients who had history of contact with commercial sex workers, eight (10.2%) were HIV positive and two (2.6%) HIV-negative (Table 3)

Table 3: HIV sero prevalence among TB patients by marital status (n=210)

Marital Status	HIV + (n=78)	HIV - (n=132)	Total
Married	48 (40.3%)	71 (59.7%)	119 (56.7%)
Single	23 (30.7%)	52 (69.3%)	75 (35.7%)
Widowed	6 (46.2%)	7 (53.8%)	13 (6.2%)
Divorced	1 (33.3%)	2 (66.7%)	3 (1.4%)
Inference		χ^2=2.338, df=3, p<0.01	

3.4 Predictors associated with *Mycobacterium tuberculosis* and HIV co-infection

Results from the bivariate logistic regression analysis of predictor's associated with tuberculosis and HIV co-infection are given in Table 4. There was a highly significant association between female sex and *M. tuberculosis* and HIV co-infection (OR=3.15; 95% CI: 2.01-4.65; p<0.001). The risk of TB increased significantly with age. TB co-infection was significantly associated with marital status, WHO clinical stage and CD4 cell count. These three factors remained significant predictors of TB co-infection. Compared to married people, the single, divorced or widowed people were at higher risk of TB and HIV co-infection. There was also a more than 3-fold likelihood of TB co-infection among patients presenting with WHO stage IV disease as compared to those presenting with stage I stage disease(OR=3.84, 95% CI:2.16-9.63, p<0.001). In addition, the risk of *M.tuberculosis* and HIV co-infection among patients with a CD4 count lower than 200 CD4+T lymphocytes/mm^3 was more than that of patients with count higher than 600 CD4+T lymphocytes/mm^3 (OR=3.231, 95% CI; 23-8.34, p<0.001). There was a doubling of risk for TB co-infection among patients who were widowed as compared to single patients. In addition, the risk of TB co-infection among patients who are employed was more than double that of unemployed patients. The results also revealed substance abuse associated with TB-HIV co-infection included alcohol (OR=7.15, 95% CI: 957-23.5, p<0.002) and intravenous drug injectors (OR=4.37,95% CI: 1.34-9.58, p<0.003).

Table 4: Predictors of *M. tuberculosis* and HIV co-infection among participants in Coastal Region, Kenya

Characteristics:	TB-HIV co-infection (78)	NO HIV (TB alone-132)	OR (95% C.I)	P-value
Age in Years				
18-24	3 (10.3%)	26 (89.7%)	1.000****	
25-34	18 (22.2%)	63 (77.8%)	1.12 (1.41-7.88)	0.001
35-44	35 (57.4%)	26 (42.6)	2.06 (1.1-3.00)	0.003
45-54	16 (55.2%)	13 (44.8%)	2.13 (1.02-2.03)	0.41
55+	6 (60%)	4 (40%)	0.11 (0.06-1.50)	0.21
CD4 cell count (cells/mm^3				
<200	50 (98.0%)	1 (2.0%)	3.23 (1.23-8.34)	0.0001
200-399	27(25.0%)	81 (75.0%)	1.75 (2.63-5.32)	0.002
400-599	1 (2.0%)	50 (98.0%)	1.31 (1.51-3.20)	0.004
>600			1.000****	
Gender				
Male	36 (34.3%)	69 (65.7%)	1.000****	
Female	42 (40.0%)	63 (60.0%)	3.54 (2.01-4.65)	0.001
Marital Status				
Divorced	1 (33.3%)	2 (66.7%)	1.76 (1.83-2.03)	0.004
Married	48 (40.3%)	71 (59.7%)	1.000****	
Single	23 (30.7%)	52 (96.3%)	1.41(1.01-1.71)	0.045
Widowed	6 (46.2%)	7 (53.8%)	2.53 (1.43-4.32)	0.82
Employer				
Unemployed	20 (48.8%)	21(51.2%)	2.14 (1.15-4.02)	0.0001
Self Employed	42 (41.2)	60 (58.8%)	1.000****	
Formal Employment	15 (22.7%)	51 (77.3 %%)	0.84 (0.45-1.63)	0.034
Student	1 (100%)	0 (0%)	0.46 (0.18-1.11)	0.543
WHO clinical stage				
I	35 (30.4%)	80 (69.6%)	1.000****	
II	20 (33.3%)	40 (66.7%)	1.41 (1.12-7.63)	0.31
III	18 (66.7%)	9 (33.3%)	2.13 (1.27-5.10)	0.003
IV	5 (62.5%)	3(37.5%)	3.84 (2.16-9.63)	0.0001
Substance Exposure				
Smoking	15 (27.8%)	39 (72.2%)	1.000****	
Alcoholic	40 (36.1%)	71 (63.9%)	7.15 (0.7-23.5)	0.002
Injection drugs	14 (48.3%)	15 (51.7 %%)	4.37 (1.34-9.58)	0.003
No exposure	9(56.3%)	7 (43.7%)	1.67 (0.56-3.21)	0..050

3.5 Haematological status of Tuberculosis and human immunodeficiency virus Co-infection

The mean absolute CD4+T count in males and females combined was 276.44±142.71 cells/mm^3. Mean in males was 265.12.±158.35 cells/mm^3 and females 289.64±128.67 cells/mm^3. The mean CD8+T cells in males were 780.19±288.07 cells/mm^3 and females 802.98±247.96 cells/mm^3. The mean CD4+T cells count in males was lower than for females but the difference was not statistically significant (t=0.754, df=76, p>0.05). The mean haemoglobin levels in males and females combined was 11.09±9.44 gm/dl and the in males were 12.51±13.74gm/dl and females 9.88±1.69 gm/dl respectively. The difference was not statistically significant (t=1.23, df=76, p>0.05). On nutritional status assessment using BMI, the mean BMI for the participants was 18.77±2.67 kg/m^2. The mean BMI for males was 18.55±2.55 kg/m^2 and females 18.97±2.76 kg/m^2 but it was not statistically significant (p>0.05). Significantly more tuberculosis HIV co-infected patients were malnourished (χ^2=7, df=1; p<0.05) as compared with HIV positive tuberculosis negative patients (Table 5)

Table 5: Haematological status of TB and HIV Co-infected patients (N=78)

Gender	N	Median	2.5th-97.5th	Mean ± SD	95% CI	P-Value
Absolute CD4 T Cells						
Male	36	283.5	169.00 - 368.50	265.12.±158.35	236.06 - 343.22	t=0.75
Female	42	249.5	168.00 - 349.50	289.64±128.67	225.02 - 305.22	df=76
Total	78	254.5	168.75 - 362.50	276.44±142.71	244.26 - 308.61	p=0.45
Absolute CD8 T Cells						
Male	36	786.5	462.00 - 983.50	780.19±288.07	682.73 - 877.66	t=0.375
Female	42	811.5	699.50 - 972.25	802.98±247.96	725.71 - 880.25	df=76
Total	78	786.5	628.75 - 362.50	796.46±265.69	732.56 - 852.36	p=0.708
Absolute CD4/CD8 T Cells Ratio						
Male	36	0.365	0.217 - 0.472	0.339±0.320	0.315 - 0.532	t=1.103
Female	42	0.308	0.223 - 0.441	0.356±0.216	0.289 - 0.423	df=76
Total	78	0.327	0.223 - 0.468	0.387±0.269	0.326 - 0.448	p=0.273
Absolute CD3 T Cells						
Male	36	1182	975.00 - 1415.25	1187.56±365.68	1063.83 - 1311.28	t=0.529
Female	42	1145	965.25 - 1342.00	1146.12±326.20	1044.47 - 1247.77	df=76
Total	78	1166	970.00 - 1348.00	1165.24±343.32	970.00 - 1348.00	p=0.598
Haemoglobin						
Male	36	10.5	9.125 - 11.500	12.51±13.74	7.859 - 17.158	t=1.230
Female	42	10.1	8.725 - 11.125	9.88±1.69	9.355 - 10.407	df=76
Total	78	10.2	9.05 - 11.23	11.09±9.44	9.05 - 11.23	p=0.233
BMI in Kg/M2						
Male	36	19.4	16.725 - 20.100	18.55±2.55	17.69 - 19.41	t=0.703
Female	42	18.5	17.125 - 20.550	18.97±2.76	18.11 - 19.83	df=76
Total	78	18.5	16.88 - 20.50	18.77±2.67	16.88 - 20.50	p=0.484

CD4+=Cluster differentiation T-lymphocyte no.4; CD8+=Cluster differentiation T-lymphocyte no.8; BMI=Body mass index; SD=Standard deviation; CI=Confidence interval; P-value=level of marginal significance

3. 6 Changes in the immune activation markers along HAART and TB treatment

There was a significant increase in the $CD4^+$ cell counts and decrease of viral load as observed in the first days of treatment. The later periods did show an increase or decrease trends. A decreasing trend of plasma concentration of gamma-interferous (IFN-*Y*) was observed over time. Except for TNF-α and IL-2 that did not change along treatment, other cytokines discrete variations were observed.

Table 6: Changes in the virological and immunological profiles including immune activated T-Cell subsets and cytokines in TB-HIV patients during TB treatment.

Variable	Follow up visits			
	Base line	**30 days**	**60 days**	**90 days**
$CD4^+$T cell	276.4(244 – 308)	295(251 – 320)	307(149 – 370)	350(158 – 420)
HIV-Viral load	5.7(4.6 – 6.0)	2.8 (1.9 – 2.9)	1.9(1.6 – 2.0)	1.8(1.8 – 1.8)
HLA-$DR^+/CD3^+$	40.2(26 – 62)	46(29 – 63)	43(27 – 55)	25(21 – 58)
$CD38^+/CD8^+$	95(73 – 99)	87(70 – 95)	83(74-93)	75(60 – 86)
IFN-γ(pg/ml)	1.8(0.0 – 10.4)	2.1(0.0 – 11.5)	1.3(0.0 – 6.9)	0.3(0.0 – 5.1)
TNF-α(pg/ml)	2.4(0.0 – 3.4)	2.5(0.0 – 3.5)	2.1 (0.0 – 3.1)	2.1(0.0 – 2.6)
IL-2 (pg/ml)	11.1(8.1 – 12.8)	10.9(8.2-14.6)	11.0(8.6 – 14.4)	10.5(8.4 – 12.3)
IL-6 (pg/ml)	4.1(1.5 – 9.2)	5.0(2.3-11.7)	3.4(1.5 – 6.3)	2.3(0.7 – 5.0)
IL-10 (pg/ml)	2.9(0.2 0 5.8)	3.1(0.4-6.9)	2.4(0.7 – 4.8)	2.0(0.3 – 3.5)
IL-4(pg/ml)	4.1(1.2 – 8.2)	4.9(2.0-9.1)	5.7(1.4 – 8.0)	4.5(0.6 – 8.5)

Values are expressed as median (IQR): $CD4^+$T cells count (cells/mm^3); HIV-Viral load (log^{10} copies/ml; HLA-DR+; %HLA-DR+ molecule on $CD3^+$ T cells; $CD38^+$: IFN-γ: interferon-gamma; TNF-α Tumor necrosis factor – alpha; IL-interleukin.

4. Discussions

The ever-increasing prevalence of pulmonary tuberculosis in Kenya has been made worse by increasing incidence of HIV/AIDS. In this current study, we noted higher prevalence of TB and HIV co-infection (37.1%) which was higher than the global prevalence of 14.8% during the same period [WHO, 2013].

Females dominated males in TB-HIV co-infection with 58.3% of the cases. However, findings of the present study indicate that HIV infection does not alter the prevalence of TB among the gender. The findings of the present study were also in agreement with the DLTLD´s annual report of 2013 [DLTLD, 2011], which indicated increasing TB burden in Kenya attributed to the concurrent HIV/AIDS epidemic, which presents special challenges. Women are more likely to have lowered immunity probably because of stress produced by their biological, economic and cultural roles as care givers. The finding of high rate TB-HIV co-infection in females compared to males also reflect the general notion that females are involved in risky behaviours like prostitution due to poverty in this population. The vaginas large surface area of susceptible tissue and micro trauma during intercourse makes women more physiologically vulnerable [Hill *et al*., 2006].In this present study, the degree of the infection varied significantly with age and the highest number occurred in the age group 35-44 years (OR=2.58, 95% CI:0.57-3.65, $p<0.001$). The significant association between age and TB-HIV co-infection was also observed by Dobler *et al*., 2008. Similarly, these findings agree with a study from Ukraine [Vander *et al*., 2005] which showed a high frequency of infection in patients with <50 years.

By affecting this age-group so heavily, HIV/AIDS is not only hitting adults in their most economically productive years but also removing the very people who could be responding to the infection crisis. This means that HIV/AIDS will continue to adversely affect socio-economic development in resource-poor countries for many years to come. In Africa a cross-sectional study of adult tuberculosis patients admitted into the DOTs program of one of Nigeria's University teaching hospitals showed sero prevalence of 14.9% [Vander *et al*., 2005] while an earlier report from Zimbabwe showed a co-infection rate of 6.5% [Barnejee et al., 2008]. A study in Tanzania found that HIV prevalence among newly diagnosed tuberculosis patients was 15% [Ngowi *et al*., 2008,].

Compared to single TB patients, widowed and married TB patients showed high TB-HIV co-infection prevalence in this study and the variation was significant ($p<0.001$). The odds of HIV infection for widowed and divorced patients was about 5 to 4 times higher than the odds of single patients (OR=5.2, 95% CI; 1.39-15.8) and (OR=4, 95% CI; 1.16-14.5) respectively, showing that being married and widowed are the strongest independent predictors for HIV infection in TB patients. Contrary to findings in this study, in Jemikalajah *et al* [2009] study, seroprevalence of TB-HIV co-infection was higher among unmarried males and those living singly than male tuberculosis patients living with their spouses.

We found that sex, age, marital status and education level were independently associated with increased risk of TB infection in people living with HIV. Current studies have reported that the risk of *M. tuberculosis*/HIV co-infection increases with male sex [Getahun *et al*., 2010, Fenner *et al*., 2011] and age [Awoyemi *et al*., 2002] which differs from our findings which shows female sex were greatly affected. On the other hand, current studies have shown that the risk TB and HIV co-infection is higher among people who are immunocompromised or have CD4 cell count below 200 cells/mm^3 [Hwang *et al*.,2013]. Our results were consistent with these findings. Multivariate analysis indicated that previous TB infection and CD4 cell count less than 350 cells/mm^3 substantally increased the risk of TB in people living with HIV. In this study tuberculosis and HIV co-infected patients had significantly lower CD4+ T-Cells and Leukocytes (276±142 cells/litres) suggesting that a combination of tuberculosis and HIV/AIDS causes a more serious depletion of CD4+T cells compared to tuberculosis patients without HIV infection and HIV/AIDS without tuberculosis. The levels of CD4+T cells were significantly low and ratio inverted in comparison to healthy subjects (controls).

[Mocroft *et al*., 2007] reported that each person has a unique level of CD4+T cell numbers that is reflective of his/her immunocompetence to protect him/her against the development of clinical symptoms. The findings in this study were also consistent with the report of [Tarbasi *et al*., 2011] where CDT+T count was >200 cells/mm^3 but contrast with the report of [Tegbaru *et al*., 2011, Affussimet *et al*., 2011]. Meanwhile, there were some similarities between the present results and the findings of [Yassin *et al*., 2006] but differed in the sense that the respondents for this study were all 18 years and above while those in the other study were all children. The decrease in CD4+ T cells correlate with the severity of both HIV/AIDS and tuberculosis due to the reduction of the cellular immunity against *Mycobacterium tuberculosis* and human immunodeficiency virus [Rob *et al*., 2007].

H IV positive individuals with CD4+ T <200 cells/µl blood are more susceptible to tuberculosis than HIV positive individuals with >500 CD4+T cells/µl/blood, regardless of anti-retroviral therapy [Appay and Sauce, 2008]. The combination of the low cellular immunity in tuberculosis and HIV/AIDS has been associated with malnutrition in this current study. The decrease in CD4+ T cells correlate with the severity of both HIV/AIDS and tuberculosis due to the reduction of the cellular immunity against *Mycobacterium tuberculosis* and human immunodeficiency virus [Patel *et al*., 2007]. In this study, combination of tuberculosis and HIV/AIDS co-infection caused serious depletion of both CD4+T cells and leukocytes leading to the rejection of null hypothesis hence there was a significant difference between TB-HIV co-infection and immunohaematoogical cells ($p<0.05$). Cytokines are important immuno modulating agents of the immune system. IFN-γ and TNF-α have key roles in TB-HIV co-infection and are associated with inflammatory granuloma organization, immune protection against intracellular mycobacteria and cell mediated immunity [Subramanyam *et al*., 2004]. Decrease in plasmatic IFN-γ levels were observed in our study all along the treatment, however TNF-α and IL-2 levels did change during the follow up, inspite of CD4+T cell recovery and viral control. Lower IL-2 and TNF-α plasmatic levels were observed in our study than those reported by others [Tadokera *et al*., 2011].

These differences can possibly be due to the employed methodological approaches for cytokine measurement. IL-2-6 plays a role in the development of the inflammatory response during immune restoration and can act as a marker for persistent immune activation [Stone *et al*., 2002]. More ever, this cytokine is also considered as a biomaker for IRIS in TB-HIV co-infection. High levels of IL-6 were observed in this study at baseline, followed by a significant reduction 30 days after HAART and after wards. In general, significant recovery of CD4+ T cell counts and control of viral replication were obtained along the follow-up treatments [Graziosi *et al*., 1996].
Moreover, decrease of immune activated T cell subsets CD38+/CD8+ and CD3+/HLA-DR were observed after HAART initiation, resulting in a satisfactory recovery of the cellular immune response, in accordance with other studies [Blanc *et al*., 2011, Pean *et al*., 2011].

5. Conclusions

Our results indicate that independent of the immune suppression profile, HIV-TB patients under tuberculosis treatment and HAART are able to recover the CD4+T cell counts and control viral replication over time. Lower CD4 lymphocyte count, substances abuse and WHO clinical stage at presentation were found to be predicting factors for co-infection.

Competing Interest

Authors have declared that no competing interests exist

Authors´ contribution: This work was carried out in collaboration between all others. Author SAY collected the data, did statistical analysis and drafted the manuscript. MFO and RRS initiated the study and made major contributions to the study design. All authors read and approved the final manuscript.

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