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IMMUNOPHENOTYPING OF HIV PATIENTS CO-INFECTED WITH *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Human immunodeficiency virus and *Mycobacterium tuberculosis* have been linked with immune suppression leading to an impaired immune response to pathogenic infections. Several studies on HIV, TB, HIV/TB co-infection were previously focused on the estimation of immunological damage to various human tissues. This present study attempt to immunophenotype the $CD4^+$ and $CD8^+$ cells counts of HIV, TB and HIV/TB subgroups bearing in mind age and gender relationships of the subjects. A cross-sectional random sampling method was used for 400 subjects. Flow cytometer was used for immunophenotyping of $CD4^+$ and $CD8^+$ cell counts. HIV diagnosis was performed using the National Algorithm utilizing Determine, Stat Pak and Uni-Gold rapid test kits. while gene-Xpert was used for identification *Mycobacterium tuberculosis*. The result observed showed a significant variation in the ages of HIV, TB and HIV/TB categories and also in their gender ($P < 0.001$ and $P < 0.002$ respectively). Chronologically, Older HIV patients from (30 - 59) had significantly lower $CD4^+$ count with the least counts found within (60 - 69) age group. A higher significant decrease was also observed between the mean $CD4^+$ and $CD8^+$ counts of HIV, TB, HIV/TB categories ($P < 0.005$ and $P < 0.012$ respectively). A Post Hoc test of a homogeneous subset was used to ascertain which of the group is responsible for the significant difference in the mean $CD4^+$ and $CD8^+$ counts of HIV, TB, HIV/TB categories. Result revealed that the mean $CD4^+$ counts of (HIV and HIV/TB, TB and HIV/TB) groups and mean $CD8^+$ counts of (HIV and TB, HIV and HIV/TB) groups contribute higher to the significant decrease ($P < 0.001$) observed that

the mean $CD4^+$ categories of (HIV and TB) and the mean $CD8^+$ of (TB and HIV/TB) groups which was not significant (P > 0.0603). Since older HIV patients from (30 - 69) showed lower $CD4^+$ count and the least count found within 60 - 69 age group and since the $CD4^+$ cell counts decrease with increasing age of the study participants. Developing program targeting older subject from 30 - 69 age group and applying methods with high productivity, sensitivity and specificity will help in faster medical treatment in the countries with high TB, HIV/AIDS and HIV/TB prevalence and in general population.

KEYWORDS: Immunophenotyping, HIV, TB, HIV/TB, $CD4^+$ and CD8.

INTRODUCTION

Human Immunodeficiency Virus (HIV) and *Mycobacterium tuberculosis* (MTB, TB) are common medical disasters ravaging human race (WHO, 2016). HIV/TB co-infection is one of the major public health burdens globally. According to the World Health Organization (WHO), nearly 15% of individuals diagnosed with MTB were HIV positive and a total of 360,000 deaths were attributed to MTB-HIV co-infection alone (Maria *et al*., 2015). Clinical evidence suggests that MTB is the most common opportunistic infection causing exacerbation of viral load and diminished CD4+ count in HIV patients. The possibility of acquiring *Mycobacterium tuberculosis* infection is 20 - 37 times greater in HIV positive patients than non-infected individuals (Guinn and Rubin, 2017), and HIV remains a significant risk factor for *M. tuberculosis* (Rohan and Indra 2015). *Mycobacterium tuberculosis* remains a leading cause of death in people living with HIV (Daria *et al*., 2014). The World Health Organization (WHO) estimates that there are about 10.4 million new cases and 1.8 million deaths from TB each year. One-third of these new cases (about 3 million) remain unknown to the health system, and many are not receiving proper treatment (Bloom *et al*., 2017). About 13% of tuberculosis cases worldwide were co-infected with HIV, and approximately 430,000 deaths in HIV-infected persons in 2011 were due to tuberculosis (Agneta *et al*., 2014). HIV co-infection with TB remains a major problem in Sub-Saharan Africa (Agneta *et al*., 2014). The incidence of *M. tuberculosis* remains high in Africa and Asia and sub-Saharan Africa (Mukhtar *et al*., 2014). *Mycobacterium tuberculosis* stimulates conventional and unconventional T-cell subsets (Teresa *et al*., 2014). The predominant response is mediated by naturally restricted, peptide-specific Th1 type $CD4^+$ T cells and $CD8^+$ cytotoxic T lymphocytes (CTLs), which are crucial for protective immunity in murine models of tuberculosis (Yang *et al*., 2018). T-cell deficiency increases susceptibility to

disease in animal models (Marcos *et al*., 2015). The severity of *Mycobacterium tuberculosis* in HIV positive patients is a good indication of the importance of $CD4^+$ T-cells in control of tuberculosis. $CD8^+$ T-lymphocytes play an important role in the effectiveness of T-cell immune reaction (Yang *et al.*, 2018), by excreting cytokines such as IFN-γ and IL-4 which modulate the balance of Th1 and Th2 cells in the lungs of patients with pulmonary tuberculosis (Patrizia *et al*., 2017). $CD4^+$ and $CD8^+$ T-cells have many effector functions that facilitate anti mycobacterial activities and $CD8^+$ T-cells respond by stimulating immune cells to provide protective immunity to the system (Yang *et al*., 2018).

Functional $CD4^+$ and $CD8^+$ T-cell subsets have been describe based on single-cell cytokine (interferon γ [IFN-γ]/interleukin 2 [IL-2]/tumor necrosis factor α [TNF-α]) signatures. These are differentially impacted by disease stage, Mycobacterial load, and treatment (Katrina *et al*., 2013). This means that certain subsets may serve as biomarkers of disease activity, pathogen burden, or treatment response. However, till date there are evidence of scarcity of data on the various cell surface marker phenotype of these subsets of *M. tuberculosis* infection, which is integral in the characterization of T cells, denoting memory status, disease site homing, survival, and activation of cellular reaction (Drain *et al*., 2018). Changes in bacteria main function defined memory cell response associated with varying antigen load in other disease models (Prezzemolo et al., 2014), and many studies insinuate that *M. tuberculosis* specific cells existing in active tuberculosis are mainly of effector memory phenotype (Chiacchio *et al.*, 2014).

Several reports have suggested measuring $CD4^+$ cells of the *M. tuberculosis*-specific TNF-α-only secreting cells serving as a precise biomarker of active tuberculosis (Chiacchio *et al*., 2014). It was recently found that primate CD8+ T cells and their effector function are critical for controlling *M. tuberculosis* infection and TB lesions (Philana *et al*., 2015), but sustaining $CD8^+$ T-cell responses relies on helper function of $CD4^+$ T cells (Yao *et al*., 2014). Consistently, the depletion of TNF-α+ $CD8^+$ T cells by anti-TNF-α immunotherapy may contribute to reactivation of TB in humans (Shao *et al*., 2016). Thus, immunological studies of correlation between *M. tuberculosis* co-infection and $CD4^+$ and $CD8^+$ T cells in HIV-infected humans will ultimately help define anti-TB immunity and mechanisms of the T-cell population. This study will help us explain the biological and metabolic difference in disease management. The disparity in age and gender differences has not been elucidated, investigating the specific activity will highlight the age levels of HIV, TB HIV/TB. There is a

conflicting result background patterns of gender disparities in HIV, HIV and TB co-infection observed from previous studies. These form the basis of this study.

MATERIALS AND METHODS

STUDY AREA

This study was carried out at the Federal Teaching Hospital (FETHI), IdoEkiti. Nigeria. The Hospital serves an estimated population of 107,000 and also cover a large geographical area boarding the northern part with an estimated area of 6353 $km^2$2453 square miles. FETHI was upgraded in 2006 to serve as a referral hospital for HIV/AIDS, diagnosis and treatment and had remained a center of excellence for *M. tuberculosis* and also offers a free treatment and management of subjects with HIV and those on antiretroviral therapy in Ekiti State. The hospital is currently serving neighboring states such as Osun, Kogi, and Kwara etc.

STUDY DESIGN

A total of four hundred (400) subjects were recruited and their samples analyzed in this study. These subjects were grouped into three categories; 150 HIV infected subjects, 150 individuals infected with *M. tuberculosis* and 100 HIV individuals co-infected with *M. tuberculosis*, all the subjects that participated in this research were on HAART, and anti-Tb Drugs. All patients seen were included in this work.

SAMPLE COLLECTION

Six milliliter (6mls) of whole blood were collected from each subject for $CD4^+$ and $CD8^+$ cell count. The remaining sample was used for HIV status screening on the subjects.

Sputum Sample Collection Procedures

Sputum samples were collected from the individual subjects early in the morning before eating or drinking. The subjects were instructed to breathe in and out deeply 2 to 4 times, to give a series of low deep coughs to raise sputum from the lungs and deposit material into the sterile container. The containers was immediately covered with a screw cap cover to prevent leakage, and then labeled with the date and time of collection. The sputum specimens was also collected from subjects for three days and each morning with three containers respectively.

Identification of *Mycobacterium tuberculosis* (MTB) Genexpert techniques as described by Somily *et al*., (2016).

Principle of the PCR

The gene expert identify DNA sequence specific for *M. tuberculosis* and rifampicin resistance by polymerase chain reaction. The test is based on the Cepheid geneXpert system were a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT). The Xpert MTB/RIF purifies and presents *M. tuberculosis* bacilli in sputum samples, isolates genomic material from the captured bacteria by sonication and successively amplifies the genomic DNA by PCR and identifies all the clinically relevant rifampicin resistance-inducing mutations in the RNA polymerase beta (rpoB) gene in the *M. tuberculosis* genome in a real-time setup using fluorescent probes called molecular beacons. Results are obtained from unprocessed sputum sample in 2 hours.

Determination of HIV Seropositivity

The method used in this study was the National Algorithm for HIV screening utilization Determine, Uni-Gold and Stak Pak kits (Ogbonnaya *et al*., 2015).

Principle of Flow Cytometer (Adan *et al.*, 2017)

The principle of flow cytometer is based on the passage of cells in single file to the laser beam so as to be detected, counted and sorted. Cell components are fluorescently labeled and then excited by the laser to emit light at varying degree of wavelengths. The fluorescence is then measured to determine the amount and type of cells present in a sample. Thousands of particles per second can be analyzed as they pass through the liquid stream. A beam of laser light is directed at a hydrodynamically-focused stream of fluid that carries the cells. Many detectors are precisely placed around the stream near the fluid as it passes through the light beam. One of these detectors is in line with the light beam and is used to measure forward scatter (FSC). Another detector is placed perpendicular to the stream and is used to measure Single Scatter (SSC). Humanimmunodeficiency virus (HIV) infection is characterized by a decrease, and eventually, a depletion of $CD4^+$ T-lymphocytes (helper T cells). By using immunophenotyping, patient specimens are tested for the proportion of lymphocytes that are T cells, B cells, natural killer (NK) cells, $CD4^+$ T cells (helper T cells), and $CD8^+$ T cells (suppressor/inducer T cells). This is done by incubating anti-coagulated whole blood with monoclonal antibodies to the various cellular antigens that identify specific cell populations (phenotypes) and then lysing the blood to remove red blood cells. The antibodies are conjugated to fluorescent tags that emit light at a certain frequency when excited by a laser beam. The specimens are then analyzed in flow cytometer to determine the proportion of

cells with a particular phenotype (that emit light at the right wavelengh). The absolute count of a full lymphocyte subset profile $CD4^+$ and $CD8^+$ was determined in four tubes with TriTEST by calculating the ratio of regional events for each subset to bead events using the BD Biosciences-developed software, A precise quantity of whole blood is added to the tubes, and the lymphocytes are stained with TriTEST monoclonal antibodies (mAb).

STATISTICAL ANALYSIS

Data collected were subjected to SPSS Version 16.0 (IBM, 2016) statistical analysis using the chi-square and students't-test. Data was significant when $P \leq 0.05$

RESULTS

Table 4.1: Age and Gender distributions of Human immunodeficiency virus-seropositive subjects, *M. Tuberculosis* positives, and HIV Subjects Co-infected with *M. Tuberculosis*.

Age group (in years)	HIV (%) N = 150	TB (%) N = 150	HIV/TB (%) N = 100
10 – 19	11 (7.3)	2 (1.3)	0 (0.0)
20 – 29	13 (8.7)	23 (15.3)	2 (2.0)
30 – 39	33 (22.0)	44 (29.3)	38 (38.0)
40 – 49	56 (37.3)	36 (24.0)	40 (40.0)
50 – 59	29 (19.3)	22 (14.7)	20 (20.0)
60 – 69	8 (5.3)	23 (15.3)	0 (0.0)
	Chi square = 56.747, p value ***<0.001***		
Gender			
Male	59 (39.3)	87 (58.0)	57 (57.0)
Female	91 (60.7)	63 (42.0)	43 (43.0)
	Chi square = 12.539, p = ***0.002***		

Table 4.2: Age and Gender variations of CD4+ levels of Human immunodeficiency virus- seropositive Subjects, *M. Tuberculosis* positives, and HIV Co-infected with *M. Tuberculosis*.

Age group (in years)	HIV N = 150	TB N = 150	HIV/TB N = 100	F	p-value
	{(Mean ± SD) (Cells/µL)}	{(Mean ± SD) (Cells/µL)}	{(Mean ± SD) (Cells/µL)}		
10 – 19	761.91 ± 255.84	237.00 ± 0.000		25.128^t	**<0.001**
20 – 29	672.08 ± 230.54	521.83 ±234.24	489.00 ± 0.00	31.662	**<0.001**
30 – 39	284.88 ±163.18	379.32 ±197.01	308.21 ± 130.36	12.357	**<0.001**
40 – 49	263.90 ± 115.73	269.92 ± 110.47	256.70 ± 97.46	0.440	0.644
50 – 59	236.90 ± 105.73	241.00 ± 132.96	168.25 ± 82.74	15.001	**<0.001**
60 – 69	176.13 ± 56.35	283.13 ± 156.76		7.867^t	**<0.001**
Gender					

Male	285.14 ± 158.25	359.49 ± 185.03	278.88 ± 120.64	10.745	<**0.001**
Female	365.22 ± 254.88	307.13 ± 200.67	242.49 ± 123.70	10.507	<**0.001**

t – Independent t-test

Table 4.3: Age and Gender variations CD8+ levels of Human immunodeficiency virus-seropositive Subjects, *M. Tuberculosis* positives, and HIV Co-infected with *M. Tuberculosis.*

Age group (in years)	HIV N = 150	TB N = 150	HIV/TB N = 100	F	p-value
	{(Mean ± SD) (Cells/µL)}	{(Mean ± SD) (Cells/µL)}	{(Mean ± SD) (Cells/µL)}		
10 – 19	358.64 ± 103.91	205.00 ± 0.000		18.109[t]	**<0.001**
20 – 29	325.85 ± 64.24	300.09 ± 44.52	213.00 ± 0.00	83.079	**<0.001**
30 – 39	280.42 ± 75.51	314.18 ± 130.94	262.37 ± 57.82	7.817	**<0.001**
40 – 49	347.87 ± 211.51	300.53 ± 131.74	339.45 ± 57.82	3.843	**0.022**
50 – 59	288.41 ± 50.82	295.95 ± 49.47	268.75 ± 56.40	8.474	**<0.001**
60 – 69	694.38 ± 383.47	266.78 ± 59.57		13.495[t]	**<0.001**
Gender					
Male	319.86 ± 138.40	290.10 ± 89.94	301.14 ± 123.78	2.404	0.092
Female	351.24 ± 209.88	307.35 ± 117.96	283.35 ± 68.96	6.600	**0.002**

t – Independent t-test

Table 4.1 shows a higher significant variation in both age and gender of HIV, TB and HIV/TB subjects in the three study categories ($P < 0.001$ and $P < 0.002$ respectively). Also Male subjects had greater percentage of TB and HIV/TB (58.0%), (57.0%) than Female subjects which had (42.0% and 43.0%). Greater number of females were infected with HIV (60.7%) than Male (39.3%) subjects. The preponderance of the subjects infected with HIV, TB, and HIV/TB fell within 30 to 49 years age bracket.

Table 4.2 compared the difference mean $CD4^+$ counts of HIV, TB, HIV/TB subjects studied. Results obtained showed higher significant decrease in both age and gender respondents ($P < 0.001$), except subjects within the age bracket of 40 - 49 years which was not significant ($P > 0.644$). Chronologically, Older HIV patients from (30 - 59) had significantly lower $CD4^+$ count with the list counts found within (60 - 69) age group.

Table 4.3 also compared the difference mean $CD8^+$ counts of HIV, TB, HIV/TB groups and there were higher significant decrease in all the three group and also in female subjects ($P < 0.001, 0.022, 0.002$) except male subjects which was not significant ($P > 0.092$).

Table 4.4: Mean $CD4^+$ and $CD8^+$ levels of Human immunodeficiency virus-seropositive Subjects, *M. Tuberculosis* Subjects, and HIV Co-infected with *M. Tuberculosis.*

Variable	HIV N = 150 {(Mean ± SD) (Cells/µL)}	TB N = 150 {(Mean ± SD) (Cells/µL)}	HIV/TB N = 100 {(Mean ± SD) (Cells/µL)}	F	p-value
Mean CD4+	333.72 ± 224.79	337.50 ± 192.84	263.23 ± 122.69	5.371	**0.005**
Mean CD8+	338.90 ± 185.20	297.35 ± 102.63	293.49 ± 103.74	4.485	**0.012**

Table 4.5: Tukey Post Hoc HSD Test for the Mean $CD4^+$ and $CD8^+$ of Human immunodeficiency virus-seropositive Subjects, *M. tuberculosis* positives, and HIV Subjects Co-infected with *M. tuberculosis.*

(I)	(J)	/(I – J)/	p-value
$CD4^+$			
HIV	TB	3.78	0.876
HIV	HIV/TB	70.49	**<0.001**
TB	HIV/TB	74.27	**<0.001**
$CD8^+$			
HIV	TB	41.55	**<0.001**
HIV	HIV/TB	45.41	**<0.001**
TB	HIV/TB	3.86	0.772

Table 4.4 shows the comparison between mean $CD4^+$ and $CD8^+$ counts of HIV, TB, HIV/TB groups studied. Results obtained showed a higher significant variation in the mean $CD4^+$ and $CD8^+$ counts of the three study categories ($P < 0.005$ and $P < 0.012$ respectively).

In Table 4.5, A Post Hoc test of a homogeneous subset was used to ascertain which of the group is causing the higher significant variation recorded in the mean $CD4^+$ and $CD8^+$ counts of the HIV, TB, HIV/TB category. Result obtained showed that the mean $CD4^+$ counts of (HIV and HIV/TB, TB and HIV/TB) groups and mean $CD8^+$ counts of (HIV and TB, HIV and HIV/TB) groups was responsible for higher significant variation ($P < 0.001$) observed than the mean $CD4^+$ categories of (HIV and TB) and the mean $CD8^+$ of (TB and HIV/TB) groups was not significant.

DISCUSSION

Several T-lymphocytes are involved in the inflammatory response against TB and HIV such as interleukin 2 [IL-2], tumor necrosis factor α [TNF-α], $CD4^+$ T cells, and $CD8^+$T cells. Among all, $CD4^+$ T cells and $CD8^+$ T cells play a critical role in immune response against TB and HIV (Cecilia *et al.*, 2014). In the present study, an attempt has been made to immunophenotype the circulating $CD4^+$ and $CD8^+$ cells counts of HIV, TB and HIV/TB

subgroups. Result recorded in this study showed a significant variation in the ages of HIV, TB and HIV/TB categories and also in gender ($P < 0.001$). This findings is in agreement with the earlier studies by Genene and Solomon (2016) who recorded significant variations in gender disparity of HIV infection. Greater number of Female were infected with HIV (60.7%) than Male (39.3%) subjects in this study. Female vulnerability to HIV infection might be aggravated by their inability to negotiate safe sex through condom use. A study in South Africa noted that the gender power imbalance significantly affected female ability to suggest condom use to their partners (Taha *et al.*, 2018). In general, the basic background patterns of gender disparities in HIV infection observed from previous studies are generally consistent, but the higher risk of HIV infection among women than men persists, despite men exhibiting multiple sex partners (Hemmige *et al.*, 2012). Women are particularly at risk of rape or sexual assault in conflict situations. Lack of data on gender disaggregated on TB and HIV are not routinely collected or reported, making it difficult or impossible to determine sex differences or gender dynamics in TB. For example, our study revealed that higher number of male subjects were infected with TB (58.0%) and HIV/TB (57.0%) than female subjects which had (42.0% and 43.0%). A predominant increase in TB/HIV patients was reported in male subjects than female with a positive difference of 88.8%, compare to 69% for females (Matilda *et al.*, 2017). In another study in Albania (Balla *et al*., 2016), (88%) of TB and HIV patients were males. In Albania, more men than women are diagnosed with HIV and TB. This gender difference could result from under-diagnosis among women, differences in sociocultural causes or various issue related with access in healthcare system and services (Balla *et al*., 2016).

The Mean age $CD4^+$ and $CD8^+$counts of HIV, TB and HIV/TB categories also showed a higher significant decrease and also in gender ($P < 0.001$), except subjects within the age bracket of 40 - 49 years and Male Subject which was not significant ($P > 0.644$ and $P > 0.092$) respectively. Chronologically, Older HIV patients from (30 - 59) had significantly lower $CD4^+$ count with the least counts found within (60 - 69) age group. Results on investigations of age-associated differences in $CD4^+$ T-cell response for HIV-infected adults on HAART have been inconsistent in the context of cART (Silverberg *et al*., 2007). The progression from Latently infected TB (LTBI) to Pulmonary (PTB) might be due to $CD4^+$ T-cell depletion driven by *M. tuberculosis*-specific $CD4^+$ and $CD8^+$ T-cell function resulting from pathological impairment (Amelio *et al*., 2018). This shows that HIV-infected individuals might be harboring reduced *M. tuberculosis*-specific $CD4^+$ T-cell frequencies

associated with significant changes in *M. tuberculosis*-specific $CD4^+$ T-cell cytokine profiles. Although active anti-viral therapy (ART) reduces opportunistic infections in HIV-infected patients (Cingolani *et al*., 2011), the increased risk of TB conferred by HIV infection does not appear to be significantly diminished by ART. An immune competent TB and HIV co-infected adult men and women on anti-tuberculosis therapy - with or without ART, were found to be associated with age differential rise in $CD4^+$ of TB patients on concurrent anti-tuberculosis and antiretroviral therapy. For patients on simultaneous TB and ART therapies Similar results have been reported by Dodd *et al*., (2016) who found that TB contributes to the decrease in $CD4^+$ T-cell count during TB/HIV co-infection. previously Amara *et al*., (2015) had examined Age and gender relation to $CD4^+$ T-cell and reported a higher significant difference in HIV/TB co-infected adults receiving antiretroviral therapy (cART). Our findings is in agreement to the above work which reported higher significant difference in age and gender of TB and HIV/TB subjects. The age-related $CD4^+$ recovery rate of subjects is depended on gender and nutritional status (Amara *et al*., 2015). Previous studies carried out in HIV/TB infected subjects not receiving ART show variable results with regard to change in $CD4^+$ counts following ATT (Swaminathan *et al*., 2008). HIV infection clearly increases susceptibility to TB (Lewinsohn *et al*., 2003), but relative importance of $CD4^+$ T cell depletion in HIV infection or both have not been elucidated. Recent mechanistic studies in non human primates have demonstrated that $CD4^+$ T cells are clearly needed to control TB infection and sustain multi-effector function of $CD8^+$ T cells and other immune cells (Yao *et al*., 2014). Although, research that demonstrate $CD8^+$ T cells protection against TB is lacking in humans and controversial in mice (Lewinsohn *et al*., 2003), primate $CD8^+$ T cells have shown to be significant for immunity against TB (Chen *et al*., 2009). The mean $CD4^+$ and $CD8^+$ counts of HIV, TB, HIV/TB groups was also compared in this study and there were significant variation in the mean $CD4^+$ and $CD8^+$ counts of study categories ($P < 0.005$ and $P < 0.012$ respectively). A Post Hoc test of a homogeneous subset was used to ascertain which of the group is causing the significant variation recorded in the mean $CD4^+$ and $CD8^+$ counts of the study category. Result obtained showed that the mean $CD4^+$ counts of (HIV and HIV/TB, TB and HIV/TB) groups and mean $CD8^+$ counts of (HIV and TB, HIV and HIV/TB) groups contributed higher to the significant decrease ($P < 0.001$) observed than the mean $CD4^+$ categories of (HIV and TB) and the mean $CD8^+$ of (TB and HIV/TB) groups which was not significant. The pattern of this finding is suggesting a higher advanced immunodeficiency in co-infected patients. this is similar to the work of (Moustapha *et al*., 2013), who recorded a significantly fewer mean $CD4^+$ and $CD8^+$ T-cell counts in TB and

HIV co-infected patients compared with single-infected subjects with either TB or HIV alone, Ddo *et al*., (2003) also reported that TB contribute to the decrease in $CD4^+$ T-cell count during TB/HIV co-infection. Several studies have reported an involvement of TB in the acceleration of immunodeficiency and increased virus replication in HIV infection (Rodrigues *et al*., 2002; Morris *et al*., 2003).

CONCLUSION

It has remained unknown whether $CD8^+$ T cells contribute to anti-TB immunity against *M. tuberculosis* co-infection or active TB in HIV-infected persons. The devastating impact of HIV/TB co-infection is increasing with little supplementary effort toward management and treatment of such individual. Developing program targeting older subject from 30 - 69 age group and applying methods with high productivity, sensitivity and specificity will help in faster medical treatment in the countries with high TB, HIV/AIDS and HIV/TB prevalence and in general population.

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