MONOCYTE IMMUNE ACTIVATION AND HIV/AIDS DISEASE PROGRESSION AMONG PATIENTS CO INFECTED WITH TB/HIV

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A RESEARCH DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE IN TROPICAL AND INFECTIOUS DISEASES FROM THE UNIVERSITY OF NAIROBI INSTITUTE OF TROPICAL AND INFECTIOUS DISEASES
ACKNOWLEDGEMENT

I am grateful to the one and only true God. The maker and custodian of all knowledge without whom, I would not have been.

I also appreciate my supervisors Prof A O Anzala and Dr. J Oyugi whose patience has been invaluable in bringing this work to this stage. I am grateful to S Koesters for her facilitation and technical training as well as helping with the conceptualization of this project. A big thank you to the Kenya AIDS Control Project laboratory staff for the sound technical training they afforded me. Thank you to the University of Nairobi Institute of Tropical and Infectious Diseases for allowing me to use their laboratories.

I thank God for my family whose support throughout all this has truly been invaluable. May God bless you all according to the riches of His glory.
DECLARATION

I declare that this is my original work with relevant references and it has not been presented for a degree or diploma in any institute.

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LIST OF ABBREVIATIONS AND ACRONYMS

TB - Tuberculosis
MTB - *Mycobacterium tuberculosis*
HIV - Human Immunodeficiency Virus
WHO - World Health Organization
AFBs - Acid fast bacilli
AIDS – Acquired Immune Deficiency Syndrome
PBMCs - Peripheral blood mononuclear cells
CMI – Cell mediated immunity
MR - Mannose receptors
RNA – Ribonucleic acid
TNFα - Tumor necrosis factor alpha
IFNγ - Interferon gamma
MHC II - Major histo-compatibility complex II
NOS - Nitrogen oxide synthase
ZN - Ziehl Neelsen
KNH - Kenyatta National Hospital
MDH - Mbagathi District Hospital
CD – Cluster of differentiation
WHO – World Health Organisation
CR - Complement receptors
HLA DR – Human Leukocyte Antigen DR
LSR II - Life Science Research II flow cytometer
GM CSF – Granulocyte-Macrophage Colony Stimulating Factor
TGFβ – Transforming growth factor beta
FACS - Fluorescence-activated cell sorting
BD ® – Beckton Dickinson
ABSTRACT

**Introduction:** Tuberculosis is an infectious disease caused by the bacillus MTB and is the second leading cause of death from an infectious disease worldwide. Immune activation of T cells and monocytes is a potent mechanism in the immune response to contain or eradicate MTB. The advent of HIV has exacerbated the threat of death due to TB. Among TB patients co-infected with HIV there is a tendency of rapid progression towards AIDS. The immune activation of the T cells and monocytes involved in the cell-mediated immune response to TB may play a role in this rapid progression.

**Objective:** This aim of this study was to determine the relationship between the level of monocyte immune activation and the progression towards HIV/AIDS among patients co-infected with TB/HIV.

**Methodology:** A laboratory based cross sectional study was employed on 101 TB patients from MDH, among whom 33 TB/HIV co-infected patients were selected to provide core data for the study. Their blood was drawn and CD4 count determined, PBMCs isolated, and T lymphocyte and monocyte immune activation determined by flow cytometry. The results for the TB/HIV co-infected group stratified by CD4 count. Spearman’s bivariate correlation was used to determine the strength of association between T cell and monocyte immune activation.

**Results:** The highest level of immune activation was seen among the subjects with CD4 count <200 cells/ul and the lowest among those with CD4 count ≥400 cells/ul. The strongest significant positive correlation between T cell immune activation and TB antigen-specific immune activation was in the CD4 count <200 cells/ul category, while the opposite was true for the CD4 count ≥400 cells/ul category. Also, the only significant correlation between TB antigen-specific immune activation and the level of immune modulation was found among the CD4 count <200 cells/ul category.

**Conclusion:** The results give a strong basis to support the hypothesis that MTB induces monocyte immune activation, which affect CD4+ T lymphocytes and may contribute to more rapid progression to AIDS disease among TB/HIV co-infected patients. These data highlight the need to emphatically warn TB+/HIV- patients to avoid acquiring HIV and to consider periodical evaluation of the immune activation levels among TB/HIV co-infected patients for indicators of good response to disease management.
CHAPTER 1: INTRODUCTION

Tuberculosis (TB) is a major global health problem. It is the second leading cause of death by an infectious disease worldwide. According to the WHO Report on Global TB Control 2011, there were 8.5 million cases with 1.2 million deaths worldwide due to TB in the year 2010. In Kenya, the incidence was 120,000 cases and the prevalence 0.11 with mortality at 5.75% of the incident cases, compared to 5.16% in the year 2009. In line with WHO STOP TB STRATEGY component 6, this underscores the need for intensified research on tuberculosis to unearth information useful in combating the disease (WHO Report Global Tb Control 2011). For purposes of this study the area of focus will be pulmonary TB.

TB is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (MTB) that is transmitted by way of infective respiratory aerosols containing live organisms released into the air when an individual suffering from pulmonary TB coughs. The disease primarily affects the lung causing either a latent or active infection. The active infection may present as pulmonary TB if confined to the lungs, but if it spreads to other organs it can present as extra pulmonary TB. In both latent and active TB infection, the innate and adaptive immune systems interact to control the infection through macrophages, monocytes, lymphocytes and cytokine-mediated inflammation. Therefore the progression of the infection will be determined by the state of the infected individual’s immune system (Kaufmann SHE 20.01, Raja A 2004). Studies by Dye et al. 1999, have found a third of the world's population has been exposed to the organism but development of the active disease depends largely on the state of their immunity.

During infection with MTB, inflammatory processes play a critical role in removal or containment of the pathogen. This inflammatory process involves participation of the cells of the immune system such as the monocytes, macrophages and T lymphocytes. However activation of these cells in individuals co-infected with HIV creates a favorable environment for propagation of HIV. This immune activation is associated with progression to AIDS, under which condition, TB associated morbidity also progresses.

Measurement of immune activation in an individual has been made possible by use of flow cytometry and certain markers found on the surface of immune cells, including T cells and macrophages. Markers of T cell immune activation include CD38, a marker for cell activation proliferation and a facilitator of T cell/endothelial adhesion through interaction with its ligand CD31 on the endothelial cells (Musso T et al. 2001), CD69, a marker for immune-modulation, when cross linked induces production of TGFβ, which regulates inflammation (Sancho D et al. 2005) and HLA DR, an antigen presenter. CD163, a scavenger receptor, CD206, a mannose receptor, and HLA DR, can indicate activation state on monocytic cells.
CHAPTER 2: LITERATURE REVIEW

Tuberculosis (TB) is a major global health problem. It is the second leading cause of death by an infectious disease worldwide. According to the WHO Report on Global TB Control 2011, there were 8.5 million cases with 1.2 million deaths worldwide due to TB in the year 2010. In Kenya alone the incidence was 120,000 cases and the prevalence 0.11 with the mortality at 5.75% of the incident cases compared to 5.16% in the year 2009.

Pulmonary TB is diagnosed by a medical history of a persistent cough for 3 weeks or more, chest pain, coughing up sputum or blood or both, weight loss, chills, fever, night sweats, loss of appetite. It is confirmed by a chest X-ray, microscopic examination of ZN Stained sputum smears positive for AFBs, and cultures positive for MTB.

Individuals suffering from TB are often tested for co infection with HIV by use of antibody tests, and viral load. CD4 counts are also used for routine monitoring and staging of severity of immune suppression as follows:

- Not significant immunosuppression >500/mm³
- Mild immunosuppression 350 – 499/mm³
- Advanced immunosuppression 200 – 349/mm³
- Severe immunosuppression <200/mm³ (Interim WHO Clinical Staging Of HIV/AIDS and HIV/AIDS Case Definitions for Surveillance 2005)

Individuals infected with HIV have a 10% chance of developing reactivation TB within the next year. This arises from the progressive depletion and dysfunction of the CD4 cells affecting monocyte and macrophage function, all of which are key players in protection against MTB.

2.1 Pathogenesis of TB

When an individual suffering from pulmonary TB coughs, they release into the air MTB, which is transmitted by respiratory aerosols containing live organisms. Phagocytosis of MTB by alveolar macrophages is the first event in the host-pathogen relationship that decides outcome of infection. The phagocytosed MTB is degraded and the resulting peptides are presented to T lymphocytes within 2 to 6 wks of infection, cell-mediated immunity (CMI) develops, and there is an influx of lymphocytes and activated macrophages into the lesion resulting in granuloma formation. This restricts the exponential growth of the bacilli. The infected apoptotic macrophages form a caseum.

The bacilli are contained in the caseous centers of the granuloma that may resolve or may remain within the granuloma leading to latent infection, or may be discharged into the airways after enormous increase in number, resulting in necrosis of the bronchi and cavitations, which occurs in active pulmonary TB. The bacilli may also spread from the site of initial infection in the lung through the lymphatics or blood to other parts of the body such as the regional lymph nodes, the kidney and the spine, hence extra pulmonary TB. This is the case among HIV infected patients in whom the risk of disease development within the first year is up to 10% depending on the severity of immunodeficiency. Their immune system is compromised; hence the disease can develop directly after primary infection.
The eradication of the infection from the body, or restriction of the infection by maintenance of the integrity of the granuloma is dependent on the optimum interaction between the two arms of the body’s immune response: the innate and the adaptive immune responses (Raja A 2004).

2.2 The innate immune response
Monocytes and macrophages are the first line of defense against MTB. They phagocytose MTB, degrade it in their phagolysosomes, and present it to the CD4 T cells which then produce cytokines which enhance the protective response against the invading organism and attract more macrophages monocytes and lymphocytes to the site of invasion.

The innate immune response to MTB is primarily mediated by alveolar macrophages, dendritic cells and monocytes, all of which are phagocytic cells. The complement receptors (CR1, CR2, CR3 and CR4), mannose receptors (MR), which are cell surface molecules, and other receptor molecules on the surface of the cell, play an important role in phagocytosis, by binding their respective ligands on the surface of MTB (Schlesinger LS 1996). Macrophage apoptosis is one mechanism used to kill the MTB as it reduces the viability of the microorganism (Molloy A et al. 1994). The MRs on phagocytic cells target Mycobacterial surface glycoprotein, lipoarabinomannan (Schlesinger LS et al. 1994). Once phagocytosed, lysosome fusion occurs and the MTB are subjected to degradation by acidic hydrolases, reactive oxygen intermediates and reactive nitrogen intermediates (Cohn ZA 1963). This killing of the MTB is enhanced by a combination of dihydroxy vitamin D3 and tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) cytokines (Dennis M 1991), which link adaptive immune response to the innate immune response.

Studies by MacDonough KA 1993 and Sabine E 2009 suggest that MTB has developed mechanisms to bypass and survive this offensive by the immune system. These include inhibition of phagolysosome fusion, detoxification of reactive oxygen and nitrogen intermediate molecules produced by the host, repair of damage caused by these reactive intermediate molecules, as well as maintaining neutral intra-bacterial pH despite being in an acidic environment.

2.3 The adaptive immune response
The adaptive immune response to TB is stimulated by the innate immune response through the interaction of CD4+ and CD8+ T lymphocytes of the adaptive immune system and phagocytes of the innate immune system. The adaptive response to TB is characterized mainly as a cell-mediated response. CD4+ and CD8+ effector and memory cells are found in the tuberculous granuloma. A half of these cells are activated as measured by their expression of CD69. These cells act to contain the infection within the granuloma (Muller I et al. 1987). T cells are primed when they recognize MTB antigens presented by phagocyte MHC II in the regional lymph node. This priming is vital to control of the infection (Muller I et al. 1987, Orme IM et al. 1984, Caruso AM et al. 1999).
The activated CD8+ T cells in the granuloma secrete IFN-γ in the granuloma. They also secrete perforins and granulysins upon recognition of dendritic MHC I associated MTB peptides. There is evidence that the MTB are found in the cytoplasm of the infected macrophages and dendritic cells. Therefore killing directed by the CD8+ T cells may have a role to play in the eradication of the infection, and the higher the effector cell to target ratio, the lesser the number of bacteria (Caruso AM et al. 1999, Stenger S et al. 1998).

The MTB proteins in the phagolysosome have access to the macrophage MHC II machinery. They are presented on this molecule to the CD4 T cells which recognize them and are thereby potently activated.

The effector CD4+ T cell's primary function in the granuloma is the production and secretion of chemokines and cytokines. The cytokines TNF-α and IFN-γ work in synergy with other cytokines to enhance macrophage activation. This is based on findings from an experiment on where mice that were MHC II-/- or CD4-/- had severely diminished IFN-γ levels early in the infection while their macrophages had low nitrogen oxide synthase (NOS), levels but in the wild type mice the IFN-γ and NOS levels were normal (Caruso AM et al. 1999). Chemokines of the CCL family attract and activate lymphocytes and monocytes from the peripheral circulation to the site of the infection (Ulrichs T et al. 2006, Russel DG 2007, Flynn JL et al. 2005).

However, it is this activation of the CD4+ T cells that may cause complications among the TB patients co-infected with HIV.

2.4 T cell activation markers in HIV infection

The pathogenesis of HIV infection in humans involves the infection and continuous attrition of CD4+ T cells, which is a major hallmark of HIV progression, leading to immune deficiency and AIDS (Levy J 1993, Pantaleo G et al 1995). According to a study by Bentwich Zvi et al. in 2001, a decrease in CD4 levels and increase in CD8 levels (by number and percentage and CD4:CD8 ratio) correlated more strongly with immune activation of the CD4+ T cells than with plasma viral load or CD4+ T cell proliferation (Leng Q et al. 2001). Increased frequency and turnover of T cells with activated phenotype and increased serum levels of proinflammatory cytokines and chemokines typify this activation of the immune system. The authors used HLA-DR as a marker for cellular immune activation. Another paper by Brenchley et al. identified activated T lymphocytes as HLA DR+ CD38+ (Hellerstein M et al. 1999, Hazenberg MD et al. 1997, Valdez H et al. 1997, Guadalupe M et al. 2003, Brenchley JM et al. 2004, Mehandru S et al. 2004, Brenchley JM et al. 2006).

The strong correlation between infection of CD4 cells by HIV, the immune activation among CD4 cells, and the reduction in CD4 cell levels which correlates with HIV progression, strongly suggests that immune activation plays an important role in progression to AIDS. Findings by Zhou D et al 1999 suggesting that Mycobacterial driven T cell activation might be the underlying mechanism in the enhanced pathogenicity among those co infected with TB/HIV, and findings by Gudo ES et al 2009 suggesting that higher levels of CD4 T cell immune activation may account for faster progression to AIDS, backed this.

While these phenotypes are related to immune activation of lymphocytes, there are changes related to immune activation of monocytes.
2.5 Monocyte/Macrophage markers in TB, HIV, and TB/HIV co-infection

In flow cytometry, macrophage and monocytes are typically described as CD14+ cells. A study conducted by M.D. Sanchez et al. aimed to characterize phenotypes of the monocytes/macrophages during TB. They used CD14 as a marker for the monocytes/macrophages, HLA-DR as a marker for antigen presentation, CD36 and CD163 scavenger receptors for removal of apoptotic cells and CD206 a mannose receptor vital for phagocytosis of MTB. The results were as follows; percentage of CD14+ HLADR+ cells was reduced during active TB. Expression of CD206 and CD163 did not differ from healthy individuals. Infection by MTB caused 10% of the cells from healthy controls and 33% of those from TB patients to die by necrosis. Hence the low expression of HLA DR on CD14+ cells among TB patients suggests an impaired capacity to engulf, process, and present MTB antigens to T cells, which was supported by another study (Sanchez MD et al. 2006, Gercken J et al. 1994).

In earlier experiments, Porcheray et al. elucidated the degree of expression of CD163 and CD206, and revealed that the expression level of these molecules depended on the expression of other inflammatory or of anti-inflammatory molecules, with CD206 being expressed to a greater extent in the presence of inflammatory molecules and to a lesser extent in the presence of anti-inflammatory molecules, while CD163 was expressed to a greater extent in the presence of anti-inflammatory molecules and to a lesser extent in the presence of inflammatory molecules. This highlights the importance of studying more than one aspect of the immune response at on time, as is the goal of this study.
Justification
In 2010, in Kenya alone the incidence was 120,000 cases and the prevalence 0.11 with the mortality at 5.75% of the incident cases compared to 5.16% in the year 2009. To compound this, 44% of the TB patients are co-infected with HIV (WHO Report Global Tb Control 2011). HIV has caused a re-emergence of active TB and increased mortality due to TB. Additionally, the current financial burden of treating TB in Kenya is US$ 6.1 million per year. Linking sentence like: A better understanding of immune correlates of TB disease in HIV-infected individual will help steer vaccine and treatment development, and may provide markers of TB reactivation. Monocytes are among the first line of defense against infection with MTB. However, the activation of monocytes may also have an effect on T lymphocyte activation in the peripheral blood among patients co-infected with TB/HIV that may aid in rapid progression to AIDS. Knowledge gained in this study will also inform the science behind the rapid progression to AIDS among patients co-infected with TB/HIV (reference that TB speeds up HIV disease progression), and open up further areas of study for novel interventions.

Research question
Does monocyte-induced immune activation among individuals co-infected with TB and HIV significantly contribute to T cell activation?

Hypothesis
MTB induces monocyte immune activation, which affects CD4+ T lymphocytes activation.

Objective
This study aimed to determine the levels of monocyte and T cell immune activation among TB patients co-infected with HIV.

Specific objectives
To determine the level of monocyte immune activation among TB/HIV co-infected patients

To determine the level of T cell immune activation among TB/HIV co-infected patients
CHAPTER 3: METHODOLOGY

3.1 Study design
A laboratory based cross sectional study, was carried out on the blood samples of 150 active TB patients aged 18 years and older at the MDH TB clinic, selected on the basis of matching clinical (history, x-ray, signs and symptoms) as well as laboratory profiles (being sputum smear positive for acid fast bacilli (AFBs)) for TB, but not yet on treatment. Patients seeking medical attention at the MDH but not meeting the aforementioned clinical and laboratory criteria for active TB along with those already on treatment for tuberculosis were excluded from the study.

3.2 Sample size
Sample size calculation was determined by the formula \( n = \frac{Z_a^2 \cdot (P \cdot Q)}{L^2} \). Where:

- \( n \) = sample size
- \( Z_a \) = z score for the type I error = 1.96
- \( P \) = the prevalence of tuberculosis in Kenya = 0.11
- \( Q = 1-P \)
- \( L \) = allowable error = 0.05

\[ n = \frac{1.96^2 \cdot (0.11 \times 0.89)}{0.05^2} \]

\[ n = 150 \]

66 (44%) of active TB subjects were HIV positive, representing the fraction of the TB population co-infected with HIV at MDH. The other 56% provided immune data for active TB infection only.

3.3 Sampling method
The subjects were randomly enrolled into the study as they came until the required sample size \( n = 150 \), including the 66 TB/HIV positive patients, was achieved.

Inclusion criteria:

Each patient was diagnosed positive for TB by clinical, radiologic, and/or sputum microscopy criteria.
3.4 Laboratory method

1. PBMC Isolation

By density gradient centrifugation of whole blood as follows;

The blood was centrifuged at 1600 revolutions per minute to remove plasma, cells were diluted 1 in 3 using phosphate buffered saline (PBS) containing 2% fetal calf serum by volume and layered on Ficoll. Further centrifugation at 1600 revolutions per minute with no brakes yielded a clear layer of PBMCs, which was isolated and washed with PBS. The cell pellets were diluted in 4ml R10 media then 50ul was removed, diluted in trypan blue supravital dye and counted in an improved neubauer counting chamber. This allowed for the estimation of the volume of live cell suspension required to obtain 500,000 cells. The formula is the number of cells counted x dilution factor x total volume in ml x 10^4 = total number of cells in 4ml. The cells in the R10 were centrifuged at 1600 revolutions per minute the supernatant poured off and replaced with a volume of R10 according to the formula C1V1 = C2V2; where C1 = concentration of cells, V1 = volume of cells/ml, C2 = final concentration of cells desired, and V2 = volume of R10 to be added to achieve the final concentration.

100ul of cells at the final concentration, which was 5 x 10^5 cells, were transferred into three FACS tubes, two of which were used for surface staining (T cell panel and macrophage panel). The remaining cells were frozen in freezing media.

2. Surface staining of PBMCs

The PBMCs were stained by dyes conjugated to antibodies specific for surface markers on the cells as follows:

Macrophage panel:

<table>
<thead>
<tr>
<th>Surface Marker</th>
<th>Label</th>
<th>Quantity (µl/tube)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14</td>
<td>Phycoerythrin Cyanin7</td>
<td>2</td>
</tr>
<tr>
<td>CD3</td>
<td>V500/AmCyan</td>
<td>2</td>
</tr>
<tr>
<td>CD163</td>
<td>PE</td>
<td>2</td>
</tr>
<tr>
<td>HLA DR</td>
<td>APC</td>
<td>2</td>
</tr>
<tr>
<td>CD206</td>
<td>FITC</td>
<td>2</td>
</tr>
</tbody>
</table>
T cell Panel:

<table>
<thead>
<tr>
<th>Surface Marker</th>
<th>Fluorophore</th>
<th>Quantity (μl/tube)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>Tricolor</td>
<td>2</td>
</tr>
<tr>
<td>CD8</td>
<td>Allophycocyanin Cyanin7 (APC-Cy7)</td>
<td>2</td>
</tr>
<tr>
<td>CD3</td>
<td>V500/AmCyan</td>
<td>2</td>
</tr>
<tr>
<td>CD69</td>
<td>Phycoerythrin (PE)</td>
<td>2</td>
</tr>
<tr>
<td>HLADR</td>
<td>Allophycocyanin (APC)</td>
<td>2</td>
</tr>
<tr>
<td>CD38</td>
<td>Flourescein (FITC)</td>
<td>2</td>
</tr>
</tbody>
</table>

Staining:

An antibody cocktail was made for each panel, 12μl (T cell panel) and 10μl (macrophage panel) of the cocktails were added to each tube of cells. The tubes were covered in foil and left to sit for 30 min at 4°C. Afterward they were centrifuged in 2ml FACS wash at 1600 revolutions per minute. The supernatant was poured off and the cells re suspended in 360μl of FACS wash and 40μl of 10% Para formaldehyde (PFA) after 10 minutes they were ready for acquisition on the LSR II. A third negative control tube was included for each sample and contained no antibody conjugated to fluorophore, but was exposed to the same procedure as the stained tubes.

Compensation beads:

8 tubes were set up and labeled: 1 unstained tube plus one tube for each antibody (fluorochrome). The beads were measured out by diluting 2 drops of negative control beads plus 2 drops of mouse capture beads in 800μl of FACS wash. Beads were mixed and then aliquoted 100 μl to each tube. 2μl of each antibody was added the individual tubes, they were covered in foil, left to sit for 20 minutes at room temperature, then 200 μl of FACS wash was added.

Acquisition and analysis on LSR II® and Flowjo®

This was done as per instructions from the LSR II instrument user manual with final evaluation done using FlowJo® Africa analysis program. Data from the flow cytometer were analyzed in FlowJo® Africa software from Tree Star Inc exported into Microsoft excel® and imported into SPSS® version 17 statistical analysis program. The data were analyzed to determine the mean immune activation levels of the monocytes and CD4+ helper T cells, and to determine the association between the levels of lymphocyte and monocyte immune activation.

Methods for CD4 Enumeration

The Tritest kit from BD® was used for CD4 enumeration. This involved adding 20μl of the staining reagent to the kit testtube, and adding 50μl of whole blood to this, vortexing it and keeping in the dark for 15 minutes. After which 500μl of lysing solution from BD® was added and allowed to lie in the dark for 15 minutes. The cells were then run on the BD® FACSCalibar.
Quality control

All the equipment and reagents used undergo periodic quality control analysis before being certified fit for use. The results obtained in each experiment are compared to those with known properties run under the same conditions. Negative controls consisted of unstimulated PBMCs, and the positive controls consisted of PBMCs stimulated by phytohemagglutinin.

3.5 Ethical approval

The proposal was presented to the Kenyatta National Hospital Ethical Review Committee (KNH-ERC) for approval. Concerns raised were addressed to the committee and the final approval to proceed was obtained.

3.6 Other data collection

The Informed Consent Form and Questionnaire were from an approved ongoing study, within which this study is nested.

Socio-demographic and health status information was gathered from an ongoing study at the MDH bearing records on HIV status, sputum smear Ziehl Neelsen (ZN) results and chest X-ray results. Blood samples were collected from clients into 4ml Sequestrine coated vacutainer tubes. The blood in sequestrine tubes was processed to separate PBMCs for immunophenotyping using the LSR II® flow-cytometer from BD®.
CHAPTER 4: RESULTS

4.1 Study Population
A total of 130 subjects were studied. Among these were 101 patients with active TB and 29 of their close contacts. Of the 101 patients with TB, 33 were co-infected with HIV. Table 4.1.1 summarizes their demographic data. The summary of this study is drawn from data obtained from the patients co-infected with TB/HIV.

Table 4.1.1 A summary of the Study Subjects’ Demographic Data

<table>
<thead>
<tr>
<th></th>
<th>TB Category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB only</td>
</tr>
<tr>
<td>Number of people</td>
<td>68</td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
</tr>
<tr>
<td>Housing Conditions: Single room</td>
<td>44</td>
</tr>
<tr>
<td>Housing Conditions: More than one room</td>
<td>24</td>
</tr>
</tbody>
</table>

4.2 Flow cytometry analysis
4.2.1 CD4 Counts
In this study we were able to determine the CD4 counts for 25 of the patients co-infected with TB/HIV. Based on their CD4 counts, they were divided into three groups. (See table 4.2.1. below)
Table 4.2.1 A Summary of the Details of each CD4 Count Category among the TB/HIV Co-infected (TB+/HIV+) Index Cases

<table>
<thead>
<tr>
<th>CD4 Count Category</th>
<th>Number of Individuals</th>
<th>Mean CD4 Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200 cells/µl</td>
<td>13</td>
<td>69.54</td>
</tr>
<tr>
<td>≥200≤400 cells/µl</td>
<td>8</td>
<td>295.25</td>
</tr>
<tr>
<td>≥400 cells/µl</td>
<td>4</td>
<td>496.75</td>
</tr>
</tbody>
</table>

4.2.2 Immune Activation Markers of TB/HIV Co-infection

Flow cytometry using the LSR II was performed on peripheral blood mononuclear cells (PBMCs), by gating on the two main populations of cells of interest, the CD4+ helper T lymphocytes and the CD14+ monocytes (see figures 4.2.2.1, 4.2.2.2, 4.2.2.3, 4.2.2.4).

Fig 4.2.2.1 The gating strategy for the isolation of CD14+ monocytes. From left to right: gating strategy to isolate singlet cells, large mononuclear cells (marked PBMCs), and CD14+ monocytes.
The gating strategy for the isolation of the markers of TB associated immune activation under anti inflammatory, and under inflammatory conditions, on the CD14+ monocytes top: from left to right; gating strategy to isolate TB associated immune activated CD163+ monocytes (marked as CD163s) under anti inflammatory conditions from the negative control cells, from the test cells, and from the positive control cells. Middle: from left to right; gating strategy to isolate TB associated immune activated CD206+ monocytes (marked as CD206s) under inflammatory conditions from the negative control cells, from the test cells, and from the positive control cells. Bottom: from left to right; gating strategy to isolate non specific immune activated HLA DR+ monocytes (marked as HLA DRs) from the negative control cells, from the test cells, and from the positive control cells.
Fig 4.2.2.3 Gating strategy for the isolation of CD4+ helper T lymphocytes. Top: from left to right; gating strategy to isolate singlet cells, lymphocytes, and T lymphocytes. Bottom: from left to right; gating strategy to isolate CD4+ T lymphocytes (marked as CD4+) from the negative control cells, CD4+ T lymphocytes from the test cells, gating strategy to isolate CD4+ T lymphocytes from the positive control cells. The “negative control” cells in this case were unstained to establish the region of cutoff for both CD8+ and CD4+ T cells.
Fig 4.2.2.4 The gating strategy for the markers of immune activation and immune modulation on the CD4+ helper T lymphocytes. Top: from left to right; gating strategy to identify CD38+ CD4+ T lymphocytes (marked as CD38s) from the negative control cells, gating strategy to isolate immune activated CD4+CD38+ T lymphocytes (marked as CD38s) from the test cells, gating strategy to isolate immune activated CD4+CD38+ T lymphocytes (marked as CD38s) from the positive control cells. Middle: from left to right; gating strategy to isolate immune modulated CD4+CD69+ T lymphocytes (marked as CD69s) from the negative control cells, gating strategy to isolate immune modulated CD4+CD69+ T lymphocytes (marked as CD69s) from the test cells, gating strategy to isolate immune modulated CD4+CD69+ T lymphocytes (marked as CD69s) from the positive control cells. Bottom: from left to right; gating strategy to isolate immune activated CD4+HLA DR+ T lymphocytes (marked as HLA DR) from the negative control cells, gating strategy to isolate immune activated CD4+HLA DR+ T lymphocytes (marked as HLA DR) from the test cells, gating strategy to isolate immune activated CD4+HLA DR+ T lymphocytes (marked as HLA DR) from the positive control cells.
4.2.3 Monocyte Immune Activation Among all the Study Subjects

The levels of monocyte immune activation were determined in two categories; CD163 representing immune activation under anti-inflammatory conditions, and CD206 representing immune activation under inflammatory conditions. Among the TB HIV co-infected subjects (n = 33) CD163 activation was 45% and CD206 activation was 42%. Among the TB only subjects (n = 68) CD163 activation was 31%, and CD206 activation was 28%. Among the contacts (n = 29) CD163 activation was 57%, and CD206 activation was 58%.

Mean TB associated immune activation among monocytes

Fig 4.2.3.1 Mean levels of CD163 and CD206 monocyte immune activation among all the study subjects

4.2.4 CD4+ Helper T lymphocyte immune activation in all the study subjects

The levels of the CD4+ helper T lymphocyte immune activation and immune modulation were determined in contacts (n=29), patients with TB only (n=68), and patients co-infected with TB/HIV (n=33, see figure 4.2.4.1). The levels of immune activation were 69%, 60% and 68%, while the levels of immune modulation were 47%, 29%, and 37% among each group respectively.
Fig 4.2.4.1 Levels of CD4+ helper T lymphocyte immune activation and immune modulation among all the study subjects. From left to right: graph showing the mean immune activation among CD4+ T lymphocytes, graph showing the mean immune modulation among CD4+ T lymphocytes.

4.2.5 Correlation of immune activation between monocytes and lymphocytes among the contacts

The Spearman’s bivariate analysis was used to determine the strength of the association between each of CD163 and CD206 associated monocyte immune activation, and the T lymphocyte expression of CD38 and HLA-DR. The strongest association was due to CD206 associated immune activation even at P value 0.01, despite the P value being set at 0.05, thus emphasizing the strength of the association (n=29 see figure 4.2.5.1).

Fig 4.2.5.1 Mean immune activation, and the correlation of monocyte vs. lymphocyte immune activation among the contacts. From left to right: graph showing the mean lymphocyte and monocyte immune activation among the contacts, graph showing the correlation of mean immune activation between monocytes and T cells.
4.2.5.2 All HIV negative contacts (n=23)

Immune activation among the contacts was further analyzed according to their HIV and latent TB status.

Mean expression of CD206 on monocytes from the TB latent HIV- subjects was 67% and CD163 mean expression was 67%. CD4 Helper T cells CD38+ mean expression was 70%. The Spearman’s bivariate analysis was used to determine the strength of the association between each of CD163 and CD206 associated monocyte immune activation, and the T lymphocyte immune activation. The strongest association was due to CD206 associated immune activation. This was at a P value 0.01 despite being set at P value 0.05, thus emphasizing the strength of the association (see figure 4.2.6.1).

![Mean Immune Activation Amongst HIV- Contacts](image1)

![Correlation of Mean Immune Activation Between Monocytes and Tcells](image2)

Fig 4.2.6.1 Mean immune activation, and the correlation of monocyte vs. lymphocyte immune activation among the HIV negative contacts. From left to right: graph showing the mean lymphocyte and monocyte immune activation, graph showing the correlation of mean immune activation between monocytes and T cells among the HIV negative contacts.

4.2.5.3 All HIV positive contacts (n=2)

Mean expression of CD206 on monocytes from the HIV+ subjects was 57% and CD163 mean expression was 67% of all monocytes. In HIV+ subjects, the mean expression level of CD38 was 58%. The Spearman’s bivariate analysis was used to determine the strength of the association between each of CD163 and CD206 expression on monocytes and the T lymphocyte immune activation markers. It showed a perfect negative association with both CD206 and CD163 associated monocyte immune activations at a P value 0.01 despite the test being set at P value 0.05 thus emphasizing the significance of the association (see figure 4.2.7.1).
Mean immune activation, and the correlation of monocyte vs. lymphocyte immune activation among the HIV positive contacts. From left to right: graph showing the mean lymphocyte and monocyte immune activation, graph showing the correlation of mean immune activation between monocytes and T cells among the HIV positive contacts.

4.2.5.4 Latent TB negative contacts (n=7)

Mean expression of CD206 on monocytes from the TB latent HIV- subjects was 67.53% and CD163 mean expression was 71.32%. Mean expression of CD38 on CD4 Helper T cells was 74%. The Spearman’s bivariate analysis was used to determine the strength of the association between each of CD163 and CD206 and the T lymphocyte immune activation markers. The strongest positive association was due to CD206 associated immune activation at P value 0.05 (see figure 4.2.8.1).

Mean immune activation, and the correlation of monocyte vs. lymphocyte immune activation among the latent TB negative contacts. From left to right: graph showing the mean lymphocyte and monocyte immune activation among the contacts, graph showing the correlation of mean immune activation between monocytes and T cells.
4.2.5.5 Latent TB positive contacts (n=15)

Mean expression of CD206 on monocytes from the TB latent positive (LTBI+) subjects was 58% and CD163 mean expression was 51%. Mean expression of CD38 on CD4 Helper T cells was 79%. The Spearman’s bivariate analysis was used to determine the strength of the association between each of CD163 and CD206 associated monocyte immune activation and the T lymphocyte immune activation. The strongest positive association was due to CD206 associated immune activation at a P value 0.01 despite being set at 0.05, thus the correlation was significant (see figure 4.2.9.1).

Figure 4.2.9.1 Mean immune activation, and the correlation of monocyte vs. lymphocyte immune activation among the latent TB positive contacts. From left to right: graph showing the mean lymphocyte and monocyte immune activation, graph showing the correlation of mean immune activation between monocytes and T cells of latent TB positive contacts.

4.2.6 Immune activation among the active TB index cases

The active TB index cases were grouped according to their HIV status, and then the mean levels of immune activation in each group were determined (see Figure 4.2.10.1). The mean expression of CD206 on monocytes was 33%, the mean expression of CD163 on monocytes was 36%, the mean expression of CD38 on CD4 Helper T cells was 62% and the mean expression of CD69 on CD4 Helper T cells was 31%. N=101.
4.2.7 Correlation of immune activation between monocytes and lymphocytes among the active TB only index cases

4.2.7.1 All active TB only cases n=68

Mean expression of CD206 on monocytes from the TB+/HIV- subjects was 28% and CD163 mean expression was 31%. Mean expression of CD38 on CD4 Helper T cells was 60%. The Spearman’s bivariate analysis was used to determine the strength of the association between each of CD163 and CD206 associated monocyte immune activation and the T lymphocyte immune activation. The strongest positive association was due to CD206 associated immune activation at a P value 0.01 despite being set at 0.05, thus emphasizing the significance of the correlation (see figure 4.2.11.1).
**Mean Immune Activation Amongst TB+/HIV- Index Cases**

Correlation of Mean Immune Activation between Monocytes and T Cells

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**Fig 4.2.11.1** Mean immune activation, and the correlation of monocyte vs. lymphocyte immune activation among the TB only (TB+/HIV-) index cases. From left to right: graph showing the mean lymphocyte and monocyte immune activation, graph showing the correlation of mean immune activation between monocytes and T cells of TB+/HIV- index cases.

**4.2.7.2 TB/HIV co-infected index cases n=33**

Mean expression of CD206 on monocytes from the TB+/HIV+ subjects was 42% and CD163 mean expression was 45%. Mean expression of CD38 on CD4 Helper T cells was 68%. The Spearman’s bivariate analysis was used to determine the strength of the association between each of CD163 and CD206 associated monocyte immune activation and the T lymphocyte immune activation as well as the T lymphocyte immune modulation. For both immune activation and immune modulation, the strongest positive association was CD206 associated at a P value 0.01 despite being set at 0.05, thus emphasizing the significance of the correlation (see figure 4.2.12.1).
Fig 4.2.1 Mean immune activation, modulation, and the correlation of monocyte vs. lymphocyte immune activation and modulation among the TB/HIV co-infected (TB+/HIV+) index cases. From left to right: graph showing mean immune modulation & activation, graph showing correlation of immune activation and immune modulation between monocytes and T lymphocytes among TB/HIV co-infected index cases.

4.2.7.3 TB/HIV co-infected index cases by CD4 count groups

In order to answer the guiding question in this study - which is does monocyte-induced immune activation among individuals co-infected with TB and HIV significantly contribute to T cell activation? - The TB/HIV co-infected index cases were subdivided into groups based on their CD4 count. The data were analyzed within these groups, and then the final results from each group were compared (see table 4.2.13.1).

Table 4.2.13.1 TB/HIV co-infected immune activation and their CD4 counts

<table>
<thead>
<tr>
<th>CD4 COUNT CATEGORIES (cells/μL)</th>
<th>MEAN CD4 COUNT (cells/μL)</th>
<th>MEAN CD4+ T CELL ACTIVATION</th>
<th>MEAN CD163</th>
<th>MEAN CD206</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>69.54</td>
<td>72.98%</td>
<td>52.58%</td>
<td>48.27%</td>
</tr>
<tr>
<td>≥200≤400</td>
<td>295.25</td>
<td>64.64%</td>
<td>55.52%</td>
<td>50.45%</td>
</tr>
<tr>
<td>≥400</td>
<td>496.75</td>
<td>58.73%</td>
<td>31.07%</td>
<td>37.68%</td>
</tr>
</tbody>
</table>
The Spearman’s bivariate analysis was used to determine the strength of the association between each of CD163 and CD206 associated monocyte immune activation and the T lymphocyte immune activation. The same was done to determine the relationship between monocyte immune activation and the T lymphocyte immune modulation. (See table 4.2.13.2 and figure 4.2.13.1).

Table 4.2.13.2 Summary of strength of association between monocyte and lymphocyte immune activation among TB/HIV co-infected cases in CD4 count categories

<table>
<thead>
<tr>
<th>CD4 COUNT CATEGORIES (cells/µl)</th>
<th>CORRELATION OF IMMUNE ACTIVATION RESULT</th>
<th>CORRELATION OF IMMUNE MODULATION RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200 (n=13)</td>
<td>The strongest positive association was CD206. The insignificant positive association was CD163. All were at P value 0.05.</td>
<td>The strongest positive association was CD206 associated. At P value 0.01 despite being set at 0.05</td>
</tr>
<tr>
<td>≥ 200 &lt;400 (n=8)</td>
<td>The strongest positive association was CD206 associated. The insignificant positive association was CD163 associated. All were at P value 0.05.</td>
<td>The strongest positive association was CD206 associated at P value 0.01 despite being set at 0.05. The insignificant positive association was CD163 associated even at P value 0.05.</td>
</tr>
<tr>
<td>≥ 400 (n=4)</td>
<td>A stronger positive insignificant association was CD206 associated. A weaker positive insignificant association was CD163 associated. All were at P value of 0.05.</td>
<td>The strongest positive association was CD163 associated. The insignificant positive association was CD206 associated. All were at P value 0.05.</td>
</tr>
</tbody>
</table>
Fig 4.2.13.1 Graphs summarizing mean immune activation, and the correlation of immune activation/immune modulation in each CD4 count category of TB/HIV co-infected index cases. From left to right: graph showing mean T cell and Monocyte immune activation among each CD4 count category, graph showing the correlation of mean immune activation and mean immune modulation between Monocytes and T cells among each CD4 count category of TB/HIV co-infected index cases.
CHAPTER 5: DISCUSSION

5.1 Discussion
The discussion of results from this study was drawn from 3 patient groups: TB/HIV co-infected patients, active TB patients, and contacts of the patients.

Individuals co-infected with TB/HIV tend to progress towards AIDS and die faster than those with TB only. The major reason suggested for this is the effect of the immune activation interplay between monocytes and CD4+ Helper T cells (Barnes PF et al 1991, Zhou D et al 1999, Gudo ES et al 2009). Therefore, the aim of this study was to determine the relationship between the level of monocyte and T cell immune activation and CD4 count among patients co-infected with TB/HIV. In a cross-sectional study such as this, CD4 count is an indicator of HIV progression status. We thus determine the levels of TB associated monocyte immune activation, the levels of CD4+ T cell immune activation, and demonstrated the strength of association between the levels of monocyte immune activation and levels of CD4+ T cell immune activation.

CD4+ helper T lymphocytes play an important role in pathogenesis in patients co-infected with TB/HIV (Caruso AM et al. 1999). The specific markers under investigation among the CD4+ helper T lymphocytes are CD38 and HLA-DR, markers for longer-term immune activation (Brenchley JM et al. 2006), and CD69, a marker for acute immune modulation (Sancho D et al. 2005). Stimulation negative controls consisted of unstimulated cells, and positive controls of cells stimulated by phytohemagglutinin.

Infection with MTB causes activation and recruitment of monocytes from the peripheral blood to the granuloma (Ulrichs T et al. 2006, Russel DG 2007, Flynn JL et al. 2005). We therefore studied CD14+ monocytes in our infection groups (see figure 4.2.2.3). The specific markers under investigation among the monocytes, were CD163, a TB associated marker for immune activation under anti inflammatory conditions, CD206, a TB associated marker for immune activation under inflammatory conditions, and HLA-DR, a non-specific marker for immune activation (F Porcheray et al; 2005). The assay negative controls consisted of unstimulated PBMCs, and the positive controls consisted of PBMCs stimulated by phytohemagglutinin.

CD4+ T cell immune activation was indicated by CD4+CD38+, CD4+CD69+, and CD4+ HLA-DR+, while monocyte immune activation was indicated by CD163 and/or CD206.

Discussion for contacts group
Among the contacts group, the HIV negative contacts had the highest overall levels of mean immune activation, among both Monocytes and T cells.

The strongest association between monocyte and CD4+ T cell immune activation was CD206 compared to CD38, especially among HIV negative contacts (TB negative). It alludes to the findings of Caruso AM et al. 1999, Stenger S et al. 1998, that immune activation is a part of the
normal response against TB; whether among the TB negative or even TB negative but latent TB positive, and this may be indicative of those whose immune system is able to contain MTB.

The successful maintenance of granuloma integrity is a hallmark of latent TB infection, and it depends on the effectiveness of the cytokines secreted by activated macrophages and T cells. Among these cytokines are the chemokines which activate and attract more immune cells to the site of infection to form the granuloma. Kaufmann 2002, Rabia H et al 2011. Latent TB is therefore an indication of constructive immune activation of the monocytes and CD4+ helper T cells.

**Discussion for active TB only group**

Among the active TB only (TB positive HIV negative category) category, a comparison of the mean T cell and monocyte immune activation revealed the least overall levels of mean immune activation.

Within this group, the strongest association between monocyte immune activation and CD4+ T cell immune activation was CD206 compared with CD163. However, the weakest overall association between CD206 and CD38 was also in this group.

Findings by Rabia H et al 2011, Feng WX et al 2012, attempt to explain that this may be due to the CCL2 genotype single nucleotide polymorphisms 2518 an A/G transition strongly linked with development of latent TB which most often progresses to reactivation TB. Mishra G et al 2012 also found that the CCL5 single nucleotide polymorphism 403G/A was linked with susceptibility to active TB. CCL2 and CCL5 are key chemokines in the activation and recruitment of immune cells from the peripheral blood. The recruited cells are necessary for the successful development of the granuloma which stops spread of MTB from the site of infection.

However these single nucleotide polymorphisms were not tested for in this study and until tested for can only be hypothesized as a possible reason for the overall weakest immune activation seen in the PBMCs of this group.

**Discussion for TB/HIV co-infected group**

Within this group, the strongest association between monocyte immune activation and CD4+ T cell immune activation was CD206 compared with CD163. The second highest overall association between CD206 and CD38 was in this group.

Just as it was among the active TB only group, in spite of CD206 expression being lower than CD163 expression, the degree of association between CD206 and CD38, indicate that immune activation is more likely due to TB-associated inflammation. This is in line with the hypothesis that immune activation due to TB may be responsible for the rapid progress to AIDS among TB/HIV co-infected individuals.
In the TB/HIV co-infected subjects, the highest CD4+ T cell immune activation corresponding to monocyte immune activation was among the <200 cells/µl CD4 count category, while the lowest CD4+ T cell immune activation corresponding to monocyte immune activation was among the >400 cells/µl CD4 count category. Coupled with the fact that the highest CD38 and CD69 levels were seen among the TB/HIV co-infected subjects in the lowest CD4 count category, these data indicate that the possibility of chronic monocyte immune activation among those co-infected with TB/HIV being responsible for the progression towards AIDS may be true as suggested by Zhou D et al. 1999 that Mycobacterial driven T cell activation might be the underlying mechanism in the enhanced pathogenicity among those co-infected with TB/HIV, and as suggested by Gudo ES et al. 2009 that higher levels of CD4 T cell immune activation may account for faster progression to AIDS.

Levy, J., and Pantaleo, G., discovered that HIV weakens the immune system by the progressive depletion and dysfunction of the CD4 cells, thus affecting monocyte and macrophage function, all of which are key players in protection against MTB. This sets the stage for MTB to flourish among the TB/HIV co-infected individuals and in turn causes immune activation which speeds up progression to AIDS in the absence of timely medical intervention. The results showing the highest mean immune activation among the TB/HIV co-infected individuals indicate the desperate attempts of the immune system to contain MTB with the attrition of CD4 helper T cells (Levy J 1993, and Pantaleo G et al. 1995).

5.2 Conclusion
These results are thus far in support of the hypothesis that MTB infection correlates with higher monocyte and CD4+ T lymphocytes immune activation, which may lead to rapid progression to HIV/AIDS disease among TB/HIV co-infected patients. However one extra question comes out of these data. That is; does the CD206-associated monocyte immune activation pose a threat among the TB/HIV co-infected patients? CD206 is more highly expressed under inflammatory conditions in the presence of inflammatory cytokines such as TNFα and IFNy. These cytokines are key players involved in the immune response against MTB and thus CD206 associated immune activation may very well pose a threat among TB/HIV co-infected patients (F Porcheray et al; 2005).

5.3 Recommendation
These results are firmly in support of testing for the immune activation status among TB/HIV co-infected patients along with continued early detection and testing for TB and HIV as is the common practice in Kenya. It is important to notify the public of the personal prevention strategies so as to avoid contracting HIV which would aggravate any latent TB – which is in high prevalence worldwide – causing progression to active TB with possibility of progressing to AIDS and death.

In order to draw and establish firmer conclusions on all the above discussion, it would be necessary for data from greater proportion of total sample size of the study subjects to be
available than was available. It would also be necessary to alter the study design from a cross-sectional one, to a longitudinal prospective study, following up each of the patients for 2 to 3 years from the date of their induction into the study.

This would give a clearer picture after comparison of their immune activation status at different time points. Parameters such as their CD4 count may change over time providing further information when compared to the status of their immune activation. It would also be necessary to include details of their antiretroviral therapy status if available, as well as adding a few more parameters to study such as; the plasma chemokine levels, as well as investigations for chemokine single nucleotide polymorphisms.

Ideally, a policy would been in place so that each time patients visit the health facility, their blood is collected and stored to test immune activation of their PBMCs, which could be compared with the rest of their test results, lending to a huge bank of biological material stored and available for numerous insightful and hopefully useful studies into the immune mechanisms involved in TB.
REFERENCES


4: David Sancho, Manuel Gomez and Francisco Sanchez 2005, CD69 is an immunoregulatory molecule induced following activation, Trends in Immunology Vol 26 No 3 pp 136-140.


30: Gudo ES, Bhatt NB, Bila DR et al 2009; Co-infection by human immunodeficiency virus type 1 (HIV - 1) and human T cell leukemia virus type 1 (HTLV – 1): does immune activation lead to a faster progression to AIDS? BMC Infect Dis. 2009 Dec 222; 9:211


INFORMED CONSENT FORM:
Title: MONOCYTE IMMUNE ACTIVATION AND HIV/AIDS DISEASE PROGRESSION AMONG PATIENTS COINFECTED WITH TB/HIV

Introduction:

Tuberculosis is the second highest killer infection worldwide causing about 8.5 million cases and 1.2 million deaths in the year 2010. It is transmitted through the air when an infected person coughs and the TB bacterium is released into the air. It is a major global public health problem especially in low income countries with a third of the world population infected with TB. It is estimated that in Kenya, TB accounted for 160,000 cases and 7500 deaths in 2010. The prevalence of TB in 2010 was 0.11 and 44% of these were co-infected with HIV. The co-infection with HIV complicates the treatment of the TB patients, and these patients also rapidly progress to AIDS. It is therefore necessary to intensify research to find the reason behind this rapid progression in the hope of finding information that will aid in combating TB thereby reducing deaths due to TB and AIDS among those co-infected with TB/HIV.

The purpose of the study:

This study aims to assess the impact of monocyte immune activation on the progression of HIV/AIDS among TB patients co-infected with HIV.

How do you enroll?

This study is nested in a larger study approved by KNH ethics review committee titled - "use of immune assays to improve the diagnosis of active/latent TB in HIV/TB endemic setting," in which a total of 300 volunteers will be recruited into this study over a period of 6 months comprising:

1. 200 patients with TB diagnosed using a combination of standard diagnostic tools and clinical judgment.
2. 100 healthy individuals with no known history of contact or exposure to TB
3. 100 healthy individuals who have been exposed to patients diagnosed with pulmonary TB.
This study will involve using the information from 150 of the 200 TB patients among whom 84 will be infected with TB only and 66 co-infected with HIV. It will also use information from the contacts of the TB patients.

**Procedure:**
The study will take one visit, during the first visit study procedures will be performed.

**Study Entry Visit**
- At the first visit you will be at the study clinic for about 20 minutes.
- If you agree to join, you will sign or mark 2 copies of the Informed Consent Form confirming that you have been informed about the study and voluntarily agree to take part. One copy is yours to keep and the other will be kept in our confidential study file. If you do not wish to keep your copy, you will sign or mark a form that states you do not want to take it, and we will keep it for you.
- You will be asked questions about your general health and a medical examination will be performed
- 4 mL (about 1 tablespoon) of your blood will be drawn for laboratory tests which include, TB Elisa, TB Elispot to test the production of cytokines, and immune phenotyping to test immune activation status

**What are the Risks and/or Discomforts?**
There may be some risk involved in your personal information being leaked but measures have been taken to ensure this does not happen by limiting access to the information and locking up your records in a secure room to which access is limited to the principal investigator in this study.

There are no anticipated physical risks in allowing your information to be used in this study.

**What are the benefits of study participation?**
There are no direct benefits to you. However, the outcome of this study may help in opening up the field of knowledge concerning rapid progression of those co-infected with TB/HIV to AIDS. Thus allowing for modifications, or emphasizing stricter adherence to the current medical investigations and interventions applied to TB/HIV co-infected patients.

Injuries

We do not expect you to be injured as a result of being in this study. You will not give up any legal rights by signing this consent form.

When can you leave the study?

Your participation in this study is completely up to you. You can leave this study at any time without giving a reason. Withdrawal will NOT compromise any rights you had before entry into the study or influence any current or future medical care you may need. If you leave or are asked to leave the study after lab tests have been done, you may still get your test results from the study site.

How much will it cost?

You do not have to pay to be in the study.

Confidentiality

Your participation in the study, all information collected about you, and all laboratory test results will be available to no one except the study team. You will be identified only by your own unique identity number, which is known only by you and the clinic staff. Your identity will not be disclosed in any publication or presentation of this study.

Contact Numbers

If you have any questions regarding the study or your participation in the study, you can Call Giddings Ochanda, the Principal Investigator, at mobile: 0725 834 358.

If you have a question about your rights as a research volunteer you should contact Prof Guantai, the Chairman of the Ethics Committee at Kenyatta National Hospital, Tel: 726300-9.
I, (name of volunteer)
Of (address)

Agree to take part in the research project entitled: use of immune assays to improve the diagnosis of active /latent TB in HIV/TB endemic setting

I have been told in detail about the study and know what is required of me. I understand and accept the requirements. I understand that my consent is entirely voluntary and that I may withdraw from the research study for any reason, and this will not affect the legal rights I may otherwise have. My questions have been answered to my satisfaction.

Participant: Print Name: .........................................................
Signature/Mark or Thumbprint: ..............................
Date: __/__/__

Person Obtaining Consent:
I have explained the nature, demands and foreseeable risks of the above study to the volunteer and answered his/her questions:

Print Name: ...................................................
Signature: ...................................................
Date: __/__/__

Impartial Witness: (only necessary if volunteer was not able to read and understand the Consent Information Sheet and Informed Consent Document):
I affirm that the Informed Consent Document has been read to the volunteer and he/she understands the study, had his/her questions answered, and I have witnessed the volunteer’s consent to study participation.

Print Name: ....................................................
Signature/Mark or Thumbprint: ...........................................
Date: __/__/__
QUESTIONNAIRE:
MONOCYTE IMMUNE ACTIVATION AND HIV/AIDS DISEASE PROGRESSION
AMONG PATIENTS COINFECTED WITH TB/HIV

TB STUDY
QUESTIONNAIRE

Volunteer ID |___|___|___||___|

Section 1 – Personal Details

Visit Date: [___/___/___ (DD/MMM/YY)], Gender: Male □ Female □

D.O.B: [___/___/___ (DD/MMM/YY)]

Are you available (i.e. resident in Nairobi) □ Yes □ No

Where do you live? ____________________________________________________________ □

Marital status:
□ Single-never married
□ Married (specify if monogamist or polygamist) ________________________________
□ Separated
□ Divorced
□ Widowed
How would you describe your current work situation?

- Unemployed
- Housewife
- CSW
- Casual worker
- Professional worker
- Self employed/Business person
- Student
- Other, please specify _____________________________

How would you describe your living/accommodation conditions

- Single room
- Double Room
- Others (please specify)_________________________________

Section 2—Health

I would like to ask you a few questions about your health:

1. How would you describe your present state of health?

   - Excellent □
   - Good □
   - Average □
2. Have you recently been diagnosed with TB

- No
- Yes \(\Rightarrow\) please give details______________________________

3. Have you recently been exposed to TB?

4. Are you currently taking any TB medicine prescribed by a doctor, or self prescribed?

- No
- Yes

5. If yes, what medication are you taking

6. Were you taken an Xray

- No
- Yes

7. Was your sputum collected:

- No
- Yes
Section 3 – Lifestyle

Now I would like to ask you a few questions about your life-style, some of which are quite private:

Section 4 – Family and Contraception

1. Do you have any children?
   No □ Yes □

2. Are you/is your partner using contraception?
   No □ Yes □

3. If yes are you/your partner using ..... 
   □ Hormonal contraception (oral) 
   □ Hormonal contraception (injection) 
   □ IUD 
   □ Implant 
   □ Male condoms 
   □ Female condoms 
   □ Other (specify) ______________________

Signature of the interviewer: ........................................ Date: ........................................

Signature of reviewer: .................................................. Date: ..................................

Data entered by (sign): ................................................ Date: ..................................
RESULTS:

1: HIV Test results

- Positive
- Negative

2: CD4 Counts.............................counts/ml
**BUDGET**
The price per assay is Kshs 2,500 which includes the prices for all consumables, reagents, and caters for any arising costs. The project is funded by the United States of America President’s Emergency Plan for AIDS Relief

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>QUANTITY</th>
<th>TOTAL PRICE (Kshs)</th>
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<tr>
<td>PBS</td>
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<tr>
<td>RPMI</td>
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<td>STERILE TESTTUBES</td>
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<td>10 ML EDTA TUBES</td>
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