Immunophenotyping of Human Immunodeficiency Virus-infected Patients with Mycobacterium Tuberculosis Co-infection

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Authors’ contributions

This work was carried out in collaboration between all authors. This study was undertaken by author AFC who did the laboratory work, analysis and manuscript writing. The research interest was initiated by author BD, while author AO developed the aspect that has to do with MTb co-infection. All the authors read the manuscript and made their contributions.

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ABSTRACT

Human immunodeficiency virus (HIV) and Mycobacterium tuberculosis (MTb) infections are two human dilapidatory illnesses globally, but more worrisome is how to diagnose them and monitor disease progression in patients effectively. This study determines the reliability, sensitivity and affordability of the Flow cytometer in counting immune cells. It will enhance the effective diagnosis, monitoring of therapy and disease progression in HIV-infected patients with MTb co-infection in Port Harcourt. A total of 88 individuals (42 males and 46 females) were studied in three groups (healthy =13, HIV-infected =67 and HIV/MTb co-infected=8). The mean age of the patients is 33.5±10.2 (range is 18-52). CD4 cells were highest in healthy subjects (820 cells/ul) and lowest in HIV/MTb co-infected patients (220 cells/ul). CD8 cells count was highest in HIV-infected patients (1020 cells/ul) and lowest in healthy subjects (440 cells/ul). In the HIV-infected group, the CD4

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cells count for the treatment-naive patients is the highest (490 cells/ul) and those on treatment
lowest (280 cells/ul), while the CD8 cells count in the men-having-sex-with-men (MSM) subgroup is
the highest (1260 cells/ul) and those undergoing treatment lowest (940 cells/ul). The CD4:CD8 is
4:1 for healthy, 1:4 for HIV/MTb co-infected and 1:2 for HIV only infected participants respectively.
There is a general increase in CD4 cells count for every decrease in CD8 cells count and vice
versa. The Flow cytometer, being a useful immunologic diagnostic tool with distinct features and
use on direct current was employed to count the immune cells in this study, and was thus,
recommended for this environment.

Keywords: Flow cytometer; Neubauer; Mycobacterium tuberculosis; GeneXpert; AIDS; tuberculosis.

ABBREVIATIONS

ART: Anti-retroviral therapy; TNP: Treatment-naive patients; N-IMC: Non-immunocompromised;
IMC: Immunocompromised; MSM: Men having sex with men.

1. INTRODUCTION

The list of medical disasters in the world will be
incomplete without the human immunodeficiency
virus (HIV). Since its discovery, HIV/AIDS
threatens the human race [1]. The virus consists
of two subtypes, HIV-1 and HIV-2, the latter
being less pathogenic [2]. AIDS was discovered
in 1981, to the dismay of the scientific community
[3]. It was first noticed in Nigeria, in the South-south town of Calabar [4].

The Mycobacterium tuberculosis (MTb) has on
its own done immense harm to the human race, but
this has been worsened by the emergence of
HIV. MTb infection reduces the survival time of
AIDS patients by suppressing their immune
system, and accounts for one third of AIDS-
associated deaths globally [5].

Individuals infected by either HIV or MTb are
stigmatized, even by loved ones. This is due to
the dilapidatory condition associated with these
microbial infections. The cosmopolitan nature of
Port Harcourt city and its hosting of multi-national
companies make it a hub for both business and
social activities, and led to the influx of people of
diverse nationalities, which through unguarded
social interactions, has led to increased
transmission of these microbes.

The immune cells play a crucial role in the
protection of the body against invaders. This is
by the recognition of invader substances and
particles as foreign to the host and then, secrete
molecules that either neutralize, metabolize or
eliminate it. Since CD4+ and CD8+ T-cells are
vital cells in both HIV and MTb infections, the
early diagnosis of HIV infection and subsequent
treatment with anti-retroviral drugs, would
enhance the immunocompetence of the HIV-
infected person, and also delay the process of
inactive MTb infection becoming active TB
disease.

In Nigeria, CD4+ T-cells assay is the mainstay
for the determination of the immune status in HIV
patients, Viral load assays are rarely used. CD4+
T-cells (mainly) and CD8+ T-cells are crucial in
the management, monitoring and response to
therapy of HIV infection, thus, a means of
estimating the accurate absolute counts of these
immune cells and their ratio is important.

In HIV/AIDS transmission, adolescents are more
vulnerable [6]. They are also more predisposed
to new HIV infections [7], about 40% in Africa [8:
9; 2]. Similarly, for MTb, its transmission to a
susceptible individual, requires just a single
sneeze with up to 40,000 droplets of the bacilli
[10]. Currently, for TB control in developing
countries, radiology and microscopy are the most
sensitive, reliable and affordable methods for
active TB diagnosis, but this is different for high
income, low prevalence countries [11].

At present, a definitive treatment exists for TB, a
multi-drug regimen, but is absent for HIV
infection. For HIV infection, palliative
medications, and social and lifestyle adjustments
are the mainstay. This implies that in HIV and
MTb co-infection, the patient will be required to
consume large doses of drugs on a daily basis,
and compliance may be a challenge. Due to this,
the Directly Observed Therapy-short course
(DOT-SC) regimen for TB is introduced, when TB
is implicated.

MTb and HIV have a mutual interaction towards
devastation of the host, when they co-exist. They
also expedite the dilapidatory action of each other, accounting for one of the greatest causes of mortality and morbidity globally. Several efforts have been employed to eradicate or curtail the over-bearing burden they place on the human population, but more still needs to be done. In this regard, effective diagnosis and monitoring of the disease progression, of which immunophenotyping of the immune cells is a major technique, is unavoidable.

Immunophenotyping is the characterization of immune cells, and Flow cytometry is one of the techniques employed. The Flow cytometer aids the rapid diagnosis and monitoring of cellular biomarkers, especially, in countries where capital is a major hindrance in diagnosing and instituting treatment. The equipment is also used to measure the serum antibody titer [12], and permits the simultaneous evaluation of the presence of different cytokines that can describe different subsets of T-cells as having polyfunctional profile [13]. These polyfunctional T-cells are linked with protective immunity towards MTb. The technique consumes less time, is less laborious, and cost-effective, and coupled with its high sensitivity, reliability, portability and likelihood to be used on direct current, is better, compared to other methods, such as Vral load assay (VLA), hemocytometer and Nucleic Acid Testing (NAT).

This technique may not be the most cost-effective and reliable, when compared to other techniques like viral load assay, but in a country like ours, where capital is a major setback for most of the patients, and where the government makes both the procedure and reagents available to the individuals to test their HIV status at no cost, it comes in readily as the most sensitive and reliable. It is this sensitivity and reliability of the Flow cytometer that this study aims to address and consolidate.

2. METHODS

This cross-sectional study was conducted in the University of Port Harcourt Teaching Hospital (UPTH) between September, 2015 to February, 2016 in the Anti-retroviral (ARV) and Tuberculosis clinics. Three groups of participants (healthy, HIV infected and HIV/MTb co-infected), comprising eighty eight (88) persons were studied, with the HIV-infected group having three sub-groups (MSM, TNP and ART). Persons with co-morbid conditions that affect the immune cells, sick individuals, and persons unwilling to sign the consent form were excluded. Participants were between 18 to 55. Written ethical approvals were obtained from the ethical units of both the UPTH and the University of Port Harcourt.

Two types of specimen (blood and sputum) were obtained from the participants, depending on whether they are healthy (control), HIV-infected or HIV/MTb co-infected. Sputum samples of coughing (TB-suspected) HIV sero-positive participants were screened using the GeneXpert technique (by Cepheid Inc, Sunnyvale USA), in the TB Reference laboratory. On confirmation of MTb infection, which now implies an HIV/MTb co-infection, their intravenous blood samples were obtained and taken to the HIV research laboratory for analysis.

For HIV analysis, intravenous blood samples were obtained from the median cubital vein with a vaccutainer needle attached to a pressurized ethylene diamine tetra acetic acid (EDTA) vaccutainer tube. The later was put in an automixer for about five minutes, for proper mixing of the blood. With the aid of an automatic pipette 0.2 ml each of blood was taken and introduced into two test tubes. Then 0.1 ml of CD4 antibody reagent was introduced into one test tube while the same amount of CD8 antibody reagent was introduced into another tube. Each test tube was put into a benchtop Flow cytometer (Cyflow, by Partec, Germany) to analyze and display the number of cells.

General HIV screening was done for healthy subjects using Determine HIV 1/2 test strips, and confirmed with a Confirm HIV 1/2 confirmation test kit.

The results were statistically analyzed with statistical package for social sciences (v.11.0) and Microsoft excel (2009 version).

3. RESULTS AND DISCUSSION

3.1 Results

In the first table (Table 1), the age distribution and frequency of the study participants based on gender and microbe infectivity is presented. 88 persons participated in the study, 42 (47.7%) were males while 46 (52.3%) were females. The standard deviation is 33.5 +10.2, while the average age of the participants is between 23 and 43 years.
The average CD4 counts for healthy (820 cells/ul), HIV infected (380 cells/ul) and HIV/MTb co-infected (220 cells/ul) individuals, are presented in Fig. 1, while Fig. 2 presents the average CD8 counts for the three groups (healthy; 440 cells/ul, HIV infected; 1020 cells/ul and HIV/MTb co-infected 860 cells/ul). The CD8 cell counts of HIV infected persons are the highest compared to the other groups while that of the healthy individuals are the lowest.

In Fig. 3 a multiple bar chart shows the CD4:CD8 ratio of healthy individuals (840:440 cells/ul), HIV infected patients (400:1020 cells/ul) and HIV/MTb co-infected patients (240:860 cells/ul). The CD4:CD8 ratios were 2:1, 1:3 and 1:4 for the three groups respectively.

Table 1 displays the mean CD4 and CD8 cell counts of the three participating groups (healthy, HIV infected and HIV/MTb co-infected) based on gender. The healthy participants had 845 ±184 cells/ul and 455 ±247 cells/ul as the CD4 and CD8 cell counts for males, while the female counterparts had 876 ±216 cells/ul and 517 ±149 cells/ul respectively as their immune systems function at different levels.

<table>
<thead>
<tr>
<th>Sex</th>
<th>n (88) (%)</th>
<th>Groups: n (88) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td>Healthy</td>
</tr>
<tr>
<td></td>
<td>42 (47.7)</td>
<td>HIV</td>
</tr>
<tr>
<td></td>
<td>7 (8.0)</td>
<td>HIV/TB</td>
</tr>
<tr>
<td>Female</td>
<td>46 (52.3)</td>
<td>24 (27.3)</td>
</tr>
<tr>
<td></td>
<td>6 (6.8)</td>
<td>43 (48.8)</td>
</tr>
<tr>
<td>Total</td>
<td>(88) (100)</td>
<td>67 (76.1)</td>
</tr>
<tr>
<td>Ages (year)</td>
<td></td>
<td>8 (9.1)</td>
</tr>
</tbody>
</table>

Fig. 1. Average CD4 counts in three groups (healthy; HIV-infected and HIV/MTB co-infected) ANOVA showed average CD4 of HIV and HIV/TB co-infected persons was significantly different (p < 0.05) when compared to CD4 of healthy individuals.

Fig. 2. Average CD8 counts in the three groups (healthy; HIV-infected and HIV/MTb co-infected) ANOVA showed average CD8 of HIV and HIV/TB co-infected persons was significantly different (p < 0.05) when compared to CD8 of healthy individuals.
Fig. 3. Ratio of CD4 to CD8 counts in the three groups (healthy; HIV-infected and HIV/MTb co-infected)

T-test showed CD4 to CD8 ratios was statistically significant ($p < 0.05$) among the different groups.

Table 2. Mean CD4 and CD8 counts of the three groups (healthy; HIV-infected and HIV/MTb co-infected) based on gender

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average CD4 ($\pm SD$): in cells/ul</th>
<th>Average CD8 ($\pm SD$): in cells/ul</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Healthy</td>
<td>845±184</td>
<td>876±216</td>
</tr>
<tr>
<td>HIV</td>
<td>382±195</td>
<td>403±253</td>
</tr>
<tr>
<td>HIV/TB</td>
<td>187±133</td>
<td>247±98</td>
</tr>
</tbody>
</table>

Fig. 4. Mean comparison of the CD4 counts in HIV positive patients

No significant difference ($p > 0.05$) was observed when CD4 count was compared between the different groups of HIV positive individuals.

cells counts. The HIV infected group had their CD4 and CD8 for males as 382 $\pm$195 cells/ul and 1128 $\pm$492 cells/ul, while the females of this category had 403 $\pm$253 cells/ul and 936 $\pm$ 563 cells/ul respectively. Similarly, the CD4 and CD8 cells count were 187 $\pm$133 cells/ul and 784 $\pm$342 for the males and 247 $\pm$98 and 932 $\pm$73 for the females of the HIV/MTb co-infected group.

A comparison of the CD4 cells counts for HIV positive individuals is presented in [Fig. 4]. For MSM, it is 360 cells/ul, while it is 280 cells/ul and 490 cells/ul for those on treatment and treatment-naive patients respectively. Those on treatment had the lowest mean. Similarly, the mean CD8 counts for the HIV groups presented in [Fig. 5] shows that the MSM group had 1260 cells/ul, treatment-naive group 1100 cells/ul and 940
cells/ul for those on treatment. As in the CD4 analysis, those on treatment had the lowest mean (of 940 cells/ul).

Fig. 6 presents the CD4 to CD8 ratio in HIV positive participants. In the MSM group, the ratio is 340:1280 cells/ul (1:4), for the TNP, 460:1060 cells/ul (depicting 1:2) and 320:920 cells/ul, with a ratio of 1:3 for those on treatment.

Table 3 presents the association of HIV category of participants based in relation to gender and CD4 status. It considers two major features that are associated with CD4 cells count. Non-immunocompromised (N-IMC) persons, with a CD4 cells count >200 cells/ul and immunocompromised persons, with a CD4 cells count <200 cells/ul. All the categories presented in the table have a CD4 count >200 cells/ul, and are thus, N-IMC.

3.2 Discussion
CD4 and CD8 cells are very vital cells both in HIV and MTb infections, just as they are in other disease conditions that suppress the host's immune system. The cells also have an interplay that is very important in the immune status of healthy, HIV-infected and HIV/MTb co-infected persons, as observed in the present study. The result show that both cells are crucial in HIV and MTb infections.
Table 3. Analysis of association of HIV category based on gender and CD4 status of the participants

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N-IMC</td>
<td>IMC</td>
<td></td>
<td>N-IMC</td>
<td>IMC</td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>84.67</td>
<td>15.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ART</td>
<td>66.67</td>
<td>33.33</td>
<td></td>
<td>64.71</td>
<td>35.29</td>
<td></td>
</tr>
<tr>
<td>TNP</td>
<td>87.50</td>
<td>12.50</td>
<td></td>
<td>90.00</td>
<td>10.00</td>
<td></td>
</tr>
</tbody>
</table>

The CD8 cells count could be used to ascertain the health status of an individual, in situations where CD4 techniques or reagents are inadvertently unavailable or are in limited supply.

This study re-affirms the mean age (23-44 years) that is at high risk of contracting the HIV virus. The age range correlates with that obtained from an earlier study by [14]. It is also similar to the result presented (25-43 years) in another study by [15]. This implies that the mean age of persons that are at a high risk of contracting the HIV infection is 23 and 44 years. This age correlates with the sexually active period of human life.

The mean CD4 count for males and females (861 cells/ul) in this study falls in the same range with the mean CD4 of 828 cells/ul obtained in a similar study [16]. This is similar to the mean CD8 count obtained from this study (486 cells/ul) for healthy participants which falls within the same range as the value (435 cells/ul) obtained by another study [16]. This is quite expected, as studies have shown that CD4 cells number is meant to be twice that of the CD8 cells number in healthy individuals. Similarly, the higher values of CD4 cell counts noted in this study (876 cells/ul and 845 cells/ul for females and males respectively) correlates with that shown by another study by [17], in which females and males had values of 920 cells/ul and 782 cells/ul respectively. It confirms the fact that CD4 cells number is twice higher than that of CD8 cell numbers in healthy persons. This is affirmed in studies from other African countries [18-20].

The mean CD4 cells count of HIV infected patients obtained in this study (217 cells/ul) was also not different from that obtained from an Asian study (200 cells/ul) [21].

This study concerns HIV/MTb co-infected patients, thus, the frequency of HIV infected patients (75) in the whole studied sample was calculated to be 10.67% (8/75 X 100). Those with only HIV infection was 89.33%, while the others with HIV/MTb co-infection was close to the 12.3% obtained by [22], although, different from that from another study [23]. It simply indicates that the prevalence of HIV/MTb co-infection over the years is on the decline.

Generally, the CD4 cells number of HIV infected individuals on treatment was low. This is because, most times, these cells would have relatively come down before anti-retroviral therapy is commenced. The CD4 cells number of male HIV patients is also more reduced compared to that of their female counterparts of the different categories. This reduction could be due to the more stressful lifestyle of males and also to the fact that males are more exposed to environmental conditions that predisposes to immunosuppressive illnesses. Similarly, the CD8 cells number in the male HIV patients are higher than that of the female HIV-infected group, while the CD8 cells of healthy males and HIV-MTb co-infected patients are lower than that of the healthy female counterparts.

In this study also, the MSM subgroup were observed to have the lowest CD4 cells count. This is quite unexpected, as one would expect that the HIV/MTb co-infected group should (due to the combined condition that reduces CD4 cell numbers) be. The underlying immunology is for future study. TNP had a considerably high CD4 cells count, the reason they are not placed on treatment.

In the course of the study, it was observed that most people infected by the virus (mostly) and atimes, the bacilli, only know their status accidentally, during clinic visits for other conditions. This was linked to either lack of education, awareness or ignorance of the presence of the virus, which according to Edewor N [24] are some of the challenges faced by people living with HIV/AIDS.

The Flow cytometer and the reagents used for the immune cells analysis may not be the cheapest or most sensitive method available, but in most developing countries the margin in the exchange rate is high and thus, not feasible to most of the citizens, to enable them access this technique, as is the case of where the study was conducted. Due to this, the government takes responsibility (with the assistance of other non-governmental organizations, such as World Health Organization and United States AIDS) for the cost of diagnosis and management of
patients infected by these microbes, thus, lessening the burden on them.

4. CONCLUSION

HIV infection attempts to extinct the human race. It intends to achieve this by several mechanisms, that include mutation, which would enable it resist the host's immune response to achieve its devastating action. This action is worsened by its co-existence with MTb, which concommitantly poses a greater disaster on the immune system and makes the host succumb to death faster. At its focus are the immune cells.

Of a truth, both CD4 and CD8 T-cells are vital in their ability to confer immunity, more so, in persons that have HIV infection and also those with an MTb co-infection. Although, other factors may play a role in the acquisition of the HIV, the anatomy of both males and females is intrinsically implicated, and has made females more prone to the acquisition of the HIV.

The HIV has greatly burdened the human race, but while scientists battle with the thought of eradicating or ameliorating its effect, the impact of MTb set in to worsen its course and further increase its dilapidating effect on both humans and the economy of the world. Amidst all these, is the challenge of how to properly and effectively diagnose the infection, moreso, at a reliable, sensitive, but cost-effective rate.

The scientific community has contributed extensive man power and resources at making sure that these microbes are eradicated or their effect ameliorated. Most times the materials (equipment and drugs) are non-available or too expensive for individuals in low-income countries. Several methods/techniques were developed for its diagnosis and monitoring, but the cost of obtaining these has also deprived people in most regions of the world where the economy is constrained, like ours, from accessing it. The invention of the Flow cytometer has bridged the gap between the rich and economically-stable countries and the poor and economically-less stable countries. This is because the later group can now effectively diagnose and monitor patients with HIV infection only and also those with MTb co-infection. The overbearing and devastating effect the HIV and its co-infection with MTb poses on the human population can be effectively controlled by a highly reliable and sensitive approach of diagnosing and managing the diseases. This begins with a technique that has these features. The Flow cytometer, is one of such equipment used for the counting of the immune cells, and is very vital not only in HIV infection but also in disease conditions like diabetes mellitus and leukemia. This study has proven this, especially for HIV infection and also in its co-infection with MTb. This study has shown that the equipment is sensitive and reliable. Its use is, thus, advocated in these disease conditions, that deeply implicates the immune cells. It (the Flow cytometer) should, therefore, form a component of the diagnostic and management protocol for these disease conditions, as its output is highly reliable and is cost-effective for this environment.

This study consolidates the fact that the Flow cytometer is not only vital, but an indispensible equipment in HIV immunology only, management of HIV/MTb co-infection and in the monitoring of the serum immune cell numbers of those that are undergoing treatment for these conditions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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