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CD4 Profile and Relationship with Bacterial Isolates from the Blood of HIV-1 Patients attending Art Clinic at a Tertiary Healthcare Institution in Southwest, Nigeria

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Abstract

The types of bacterial species present in the blood of HIV-1 positive individuals attending the Antiretroviral Therapy (ART) clinic of a Federal Medical Centre in Southwest Nigeria was evaluated in relation to the patients' CD4 profile. Blood samples were collected from the antecubital regions of five hundred (500) confirmed HIV Patients attending the ART clinic. The collected samples were subjected to standard microbiological techniques for the isolation and identification of bacterial species present in the sample while their CD4 populations were determined using standard technique. The bacterial isolates with the highest frequency of occurrence in the group of patients with severe immunosuppression (≤ 200 cells/mm3) was Salmonella typhimurium while Enterobacter aerogenes, Streptococcus pneumoniae and Shigella dysenteriae dominated the population of bacterial isolates from the blood of HIV patients with CD4 counts of 201-300, 300 - 400 and 300 – 500 cells/mm3 respectively. Sociodemographic and socioeconomic parameters such as age, occupation and nutrition alongside ART were observed to affect the CD4 profile of the patients.

Keywords: HIV; CD4 profile; ART; Bacterial species

Introduction

Human Immunodeficiency Virus (HIV) destroys CD4 T-cells. This destruction makes it possible for some microorganisms which ordinarily will not cause infections in healthy individuals to initiate infections at specific sites in the host, due to weakened immunity. Most of these implicated organisms are the normal microbial flora of the body [1].

The normal CD4 count for a healthy individual ranges from 500 to around 1200 cells/mm^3 and above. HIV infection however causes it to reduce gradually until it gets to a value $\leq$ 200 cells/mm^3 that ushers in AIDS. To checkmate this, antiretroviral therapy (ART) is administered to HIV infected patients when CD4 count falls to around 350 cells/mm^3 because of the occurrence of opportunistic infections at this stage [2]. However, administration of ART can be earlier than this in infected individuals with earlier onset of acute retroviral syndrome, pregnancy or constitutional symptoms or other concerns which require urgent medical attention. Some of the factors that play significant roles in the management of HIV infection include age, occupation, presence of pathogens, other disease conditions, lifestyle of patients, drug adherence, nutrition etc. [3].

Bacterial infections are critical in the progression of HIV to AIDS. Complications especially from the group, Enterobacteriaceae include pneumonia, diarrhoea, haemolytic-uremic syndrome (HUS), dysentery, necrotizing enterocolitis, salmonellosis and a variety of nosocomial infections [4]. Most of these bacterial pathogens infecting specific sites in a host are also potential viable sources of bacteremia especially in immunocompromised individuals, which could be sequel to failure on the part of the infected individual to present for early diagnosis, poor diagnosis, microbial resistance or ineffective treatment [5].

This study therefore seeks to investigate the relationship between the CD4 counts of HIV-1 positive patients and the concomitant mixed bacterial populations in their blood juxtaposing the various peculiar factors involved in their immune reconstitution.

Materials and Methods

Prior to commencement of the research in May, 2015, an ethical clearance was got from the ethical review committee of the Federal Medical Centre, Owo, Ondo State, Nigeria where the blood samples for the investigation was carried out. Moreover, a well – structured, close-ended questionnaire was administered to each recruited HIV-1 positive patient at the ART clinic. Five Hundred (500) clinically confirmed HIV-1 patients were sampled for the study with 32.4% participants being males and 67.6% being females across all age groups. Approximately 90% of the patients were under Antiretroviral Therapy while about 10% were Non - Antiretroviral Therapy Patients.

Retroviral screening of recruited participants was conducted using the National serial algorithm for HIV testing. The algorithm series is Determine - Unigold - Stat pak (tiebreaker). The blood sampling (non-repeat) from HIV-1 infected patients for CD4 evaluation and bacteriological isolation processes were carried out with standard operating procedures provided by the United States Agency for International Development (USAID) and standard bacteriological procedures following the methods of the Clinical and Laboratory Standards Institute, 2007 respectively.

Two milliliters (2 ml) of the collected blood was introduced into 5ml Brain-Heart infusion broth and were incubated at 37ºC for 24 hrs before culturing on suitable agar plates for bacterial growth [6]. The bacterial isolates from each plate were sub cultured for pure colonies at 37ºC for 18-24 hours. The isolates were identified by Gram staining, biochemical tests, sugar fermentation, motility test and molecular characterization techniques of Barraquio et al. [7].

Data analysis and interpretation of results

Statistical Package for Social Sciences Version 17.0 (SPSS Inc.,

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Chicago, IL) was used for all data. Descriptive statistics were used in the computing of the results in order to give lucid representations of the data analysed. The Chi-square (X^2) test was used to determine significant differences and effects based on the comparison with the probability values for statistical outcomes.

Results

The most frequently encountered bacterial species associated with the group of HIV patients with severe immunosuppression (≤ 200 cells/mm^3) was *Salmonella typhimurium* (Table 1). However, *Enterobacter aerogenes, Streptococcus pneumoniae* and *Shigella dysenteriae* dominated the population of bacterial isolates from the CD4 groups in the categories of high risk and low risk immunosuppressed patients (201-300, 300 - 400 and 300 – 500 cells/mm^3 respectively). *Enterobacter aerogenes* was also the most frequently encountered in HIV patients with CD4 counts between 500 and 600 cells/mm^3 while *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most frequently encountered bacterial species in patients with CD4 range between 601and 800 cells/mm^3 (Table 1).

The CD4 profile of HIV -1 patients according to their age grouping revealed that the patients within the age group 40-49 years had the best record of immune reconstitution in accordance with their CD4 counts, having CD4 values in the region of 401 - 500 cells/mm^3 to 1201cells/mm^3 and above more than any other group in the study (Figure 1).

Considering the relationship between CD4 counts and ART status of patients, it was observed that the larger portion of patients in this group have the highest CD4 values in the study. However, the group within which most of them have higher and better CD4 counts (401-500 cells/mm^3 – 1201 cells/mm^3 and above) were the non-ART category of Patients (62.5%) as against the outcome obtained within the ART (48.2%) category in this study (Figure 2).

The rate and frequency of fruit intake by each patient revealed that those who had fruit intake more frequently in their diets had better and higher CD4 values than their counterparts with less intake of fruits in their diets (Figure 3).

The Occupational divisions of HIV-1 patients were considered in accordance with their CD4 profile, it was evident that the group with highest frequency of occurrence and overall highest CD4 values was the group of the Artisans. However, considering each group there was higher values of CD4 counts (401-500 cells/mm^3 – 1201 cells/mm^3 and above) within the public servant group than any other group of patients involved in this study (Figure 4).

Discussion

This study investigates the relationship between the CD4 counts of HIV-1 positive patients and the concomitant bacterial populations in their blood and the effect of various factors such as fruit intake and ART on the CD4 counts.

The high frequency of occurrence of *Salmonella typhimurium* in the HIV patients with CD4 counts of ≤200 cells/mm^3 might likely be due to severe destruction of the immune cells that plays significant role in conferring immunity against this pathogen which are the T-lymphocytes. This is in agreement with reports from Mshana et al. [8].

The high occurrence of bacterial populations in the 601-800 cells/mm^3 was likely due to the condition referred to as Immune Reconstitution Inflammatory Syndrome (IRIS). This is a condition at which the CD4 count of an infected patient rises as a result of immune restoration through initiation or re-initiation of antiretroviral therapy, but the condition is accompanied with the onset of bacterial infections. This agrees with a report from Shelburne et al. [9].

Patients within the age group of 40-49 years are grouped within the actively productive population in any society and are likely to be more knowledgeably adherent to prescriptions on how to live healthy and achieve immune reconstitution quickly. This agrees with reports from Rudy et al. [10] and Fisher et al. [11].

Majority of HIV-1 Patients who have not commenced antiretroviral treatment have relatively high CD4 values than their counterparts with lower CD4 values due for commencement of ART this shows that there is reasonably a slow rate of disease progression which could be due to early diagnosis, effective chemotherapy and close monitoring. However, the overall high CD4 values obtained from patients who were on ART, is adduced to the immune reconstituting effects of antiretroviral therapy. This is logically supported by a publication from CDC [12].

Fruit intake on more frequent basis noticed within the patients has a close link with high CD4 values in HIV-1 Patients. Patients who frequently included fruits in their diets especially on daily and twice-daily bases, had higher CD4 values in the study. This is due to the beneficial role of vitamins in warding off infections in the body. This is

	CD4 (cells/mm^3) (%)											
Bacterial isolates	**01-100**	**101-200**	**201-300**	**301-400**	**401-500**	**501-550**	**551-600**	**601-800**	**801-1000**	**1001-1200**	**1201 and above**	**Total**
Escherichia coli	0(0.0)	1(3.45)	7(24.1)	6(20.7)	3(10.3)	3(10.3)	3(10.3)	6(20.7)	0(0.0)	0(0.0)	0(0.0)	**29**
Klebsiella pneumoniae	4(7.1)	6(10.7)	7(12.5)	11(19.6)	7(12.5)	0(0.0)	2(3.6)	10(17.9)	3(5.4)	2(3.6)	4(7.1)	**56**
Salmonella typhi	1(6.7)	2(13.3)	2(13.3)	3(20.0)	2(13.3)	1(6.7)	3(20.0)	1(6.7)	0(0.0)	0(0.0)	0(0.0)	**15**
Salmonella typhimurium	4(17.4)	4(17.4)	3(13.0)	2(8.7)	1(4.3)	2(8.7)	2(8.7)	3(13.0)	1(4.3)	1(4.3)	0(0.0)	**23**
Staphylococcus aureus	2(7.7)	2(7.7)	5(19.2)	5(19.2)	2(7.7)	3(11.5)	0(0.0)	3(11.5)	2(7.7)	0(0.0)	2(7.7)	**26**
Streptococcus pneumoniae	2(9.1)	1(4.5)	2(9.1)	6(27.3)	2(9.1)	1(4.5)	0(0.0)	6(27.3)	1(4.5)	1(4.5)	0(0.0)	**22**
Pseudomonas aeruginosa	2(5.7)	4(11..4)	6(17.1)	3(8.6)	5(14.3)	2(5.7)	4(11.4)	4(11.4)	2(5.7)	3(8.6)	0(0.0)	**35**
Shigella dysenteriae	4(7.0)	5(8.8)	12(21.1)	6(10.5)	9(15.8)	6(10.5)	4(7.0)	9(15.8)	2(3.5)	0(0.0)	0(0.0)	**57**
Proteus mirabilis	2(15.4)	2(15.4)	1(7.7)	0(0.0)	2(15.4)	1(7.7)	1(7.7)	1(7.7)	0(0.0)	3(23.1)	0(0.0)	**13**
Enterobacter aerogenes	1(5.9)	1(5.9)	5(29.4)	1(5.9)	1(5.9)	1(5.9)	1(5.9)	5(29.4)	0(0.0)	0(0.0)	1(5.9)	**17**

X^2= 1.426E2. P = 0.212.
The result is not statistically significant.

Table 1: Types of bacteria isolated from HIV-1 patients based on CD4 profile.

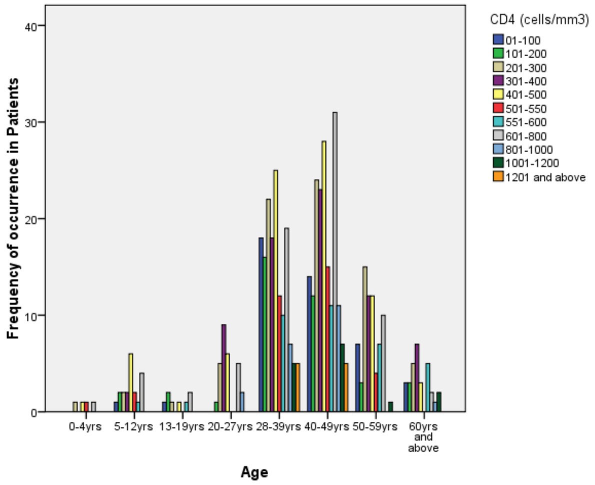

Figure 1: The CD4 profile of Patients according to their age grouping. X^2 = 65.083. P = 0.644. The result is not statistically significant.

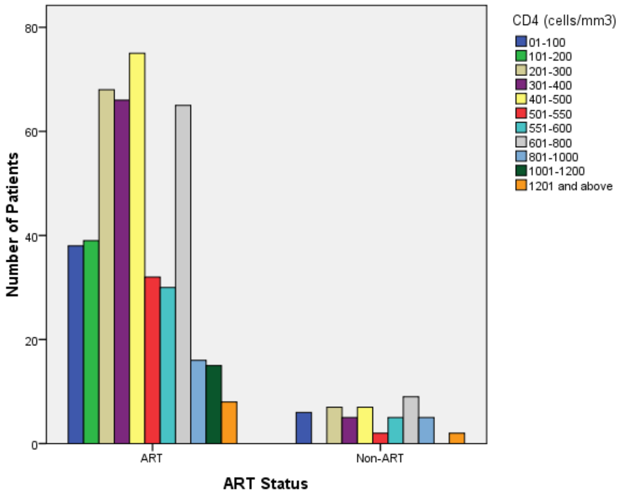

Figure 2: The CD4 Profile of HIV-1 Patients based on the commencement of antiretroviral treatment.
X^2 = 15.328. P = 0.008. The result is statistically significant.

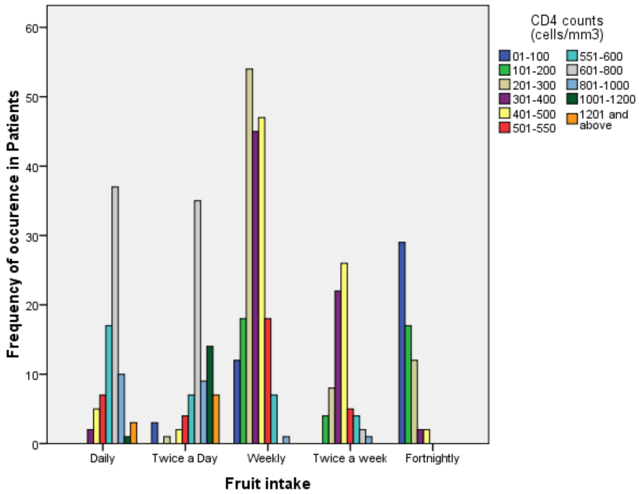

Figure 3: The CD4 profile of HIV-1 Patients according to their rates of fruit intake.
X^2 = 5.981E2. P= 0.000. The result is statistically significant.

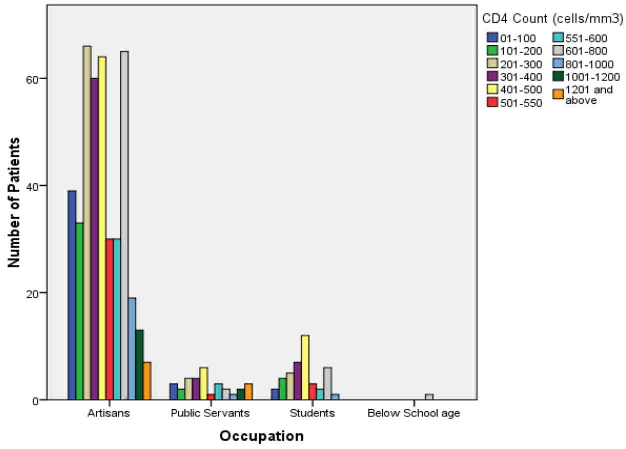

Figure 4: The CD4 profile of patients in accordance with their occupational stratification.

X^2 = 27.942, P = 0.574. The result is not statistically significant.

in accordance with a report by Keservani et al. [13].

The vast majority of HIV-1 infected patients were Artisans, the overall set of highest CD4 values from the study came from the group. However, more members in the public service group had higher CD4 values than the rest members in the same group. This outcome is higher in this group than it occurred in other groups with similar results. This is traceable to the level of education and exposure among members in the group to counseling, and hence imbibing a culture that enhances better life expectancy. This is in agreement with a report from Vermeire et al. [14].

Conclusion

This study has investigated and ascertained that specific bacterial species occurring in HIV Patients are prominent in the group of patients with severe immunosuppression while some other bacterial isolates can accompany immune restoration in a condition known as immune reconstitution inflammatory syndrome (IRIS). Decrease in disease progression and immune reconstitution depend largely on a concatenation of factors such as CD4 profile, nutrition, occupational stratification, commencement and adherence to ART in order to live a healthier life and attain better life expectancy.

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