Original Article

Bacteriuric Profiles and CD4 Counts of Human Immunodeficient Virus (HIV) Seropositve Individuals Seeking Care in a Nigerian Health Facility

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Background: A bacterial count of ≥10^5 CFU/mL is used to define significant bacteriuria. However, lower counts have been identified in symptomatic individuals with compromised immune status. The relationship between CD4 cells count and significant bacteriurial threshold was investigated in HIV sero-positive and sero-negative individuals in a healthcare setting in Jalingo - Nigeria.
Methods: A cross sectional hospital- based study that included 220 HIV/AIDS positive and 180 HIV/AIDS negative individuals was conducted. A mid-stream clean catch urine and 3mLs of blood samples were collected from each subject. Significant bacteriuria was detected by the method of Leigh and William (1964) while the blood samples were assayed for CD4 cell counts using Partec cyflow counter.
Results: Of the 220 urine samples obtained from HIV/AIDS sero-positive subjects, 143 (65%) yielded bacterial growth compared to 82(45.6%) of the 180 obtained from sero-negative or control group (p= 0.000096). Though higher percentage of significant bacteriuria (≥10^5 CFU/mL) was observed among 62(43.4%) of the 143 culture positive test subjects compared to 33(40.2%) of the 82 control, the statistical analysis is not significant (p=0.056). Overall, 23(16%) of the 143 HIV/AIDS test subjects were symptomatic for UTIs, compared to 12(14.6%) of the 82 control participants (p=0.51). Among those with significant bacteriuria, symptomatic cases were recorded in 13/62(21%) test subjects and 8/33(24%) control subjects (p=0.029). Symptomatic UTIs were observed only among those with bacteria count of ≥10^4 CFU/mL in the control subjects, in the test subjects however, subject exhibited symptoms even at low bacterial count of ≤10^3 CFU/mL.
Conclusion: CD4 cell levels may have little or no effect on bacteriuria, but with CD4 count of ≤ 200 cells/mm^3, test subjects are more likely to exhibit symptoms of UTIs even at a low bacterial count.

Key words: Urinary Tract Infections (UTIs), Significant bacteriuria, CD4 Cells, Symptomatic UTI, HIV/AIDS

Introduction

Urinary tract infection (UTI) is defined by a bacterial count of ≥10^5 CFU/mL of a single species or multiple microorganisms in a single or two consecutive well voided mid-stream clean catch urine specimen (s). This threshold defines UTIs in both immunocompetent and immunocompromised individuals with HIV/AIDS. Urinary tract infections are very common infections and might be complicated by frequent recurrences, and more severe manifestations such as pyelonephritis and sepsis especially in individuals at risk (Barber *et al*., 2013, Kayima, *et al.*, 1996). HIV seropositive individuals have compromised immunity as a result of viral attack on the CD4 cells, causing a reduction in their numbers, which gradually result to AIDS (CD4 count ≤ 200 cells/mm^3), this in turn reduces the host's resistance to infections (Milan, 2006, Prescott *et al.*, 2008, Charles, 2011 & Nelius, *et al.*, 2011). Urinary tract infection represents a considerable health

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problem amongst HIV infected patients, and has been shown to be more common in patients with advanced form of infection compared with early HIV infection (Evans *et al*., 1995).

Nigeria has the second largest HIV epidemic in the world (NACA, 2015). Although HIV prevalence among adults is remarkably small (3.1%) compared to other sub-Saharan African countries such as South Africa (19.2%) and Zambia (12.9%), the size of Nigeria's population means 3.5 million people were living with HIV in 2015 (UNAIDS, 2016), and this constitutes a significant population of individuals who are at risk of UTIs (Olowe, 2015). Located in the North Eastern part of Nigeria, Taraba State has one of the highest HIV/AIDS prevalence rates of the country. It has many high- risk settings and behavioural attitudes that increasingly enhance the spread of the virus (Oruonye, 2011). The state has a location disadvantage with respect to HIV/AIDS as it shares boundary with Benue and Plateau states in the north central zones which have the highest rates of HIV/AIDS in the country. In addition, it is bounded on the eastern part by Bamenda, a region in the Republic of Cameroon which also has high HIV/AIDS prevalence. (Obioha, 2008).

This study therefore investigated the relationship between CD4 cell count and bacteriuria among HIV seropositive individuals in a healthcare facility of Jalingo, the Taraba State Capital in Nigeria.

Methods

Study Subjects

Patients seeking medical attention at the Government House Clinic of Jalingo in Taraba State – Nigeria, were recruited between February and July 2016. Two hundred and twenty HIV seropositive individuals comprised of 66 males and 154 females, and 180 HIV seronegative individuals; 72 males and 108 Females were recruited into the study.

Ethical Clearance

Ethical approval was obtained from the Taraba State Ministry of Health. The consent of each subject sought following explanation of the overall aim of the study. Those who accepted to participate in the study gave their consent through duly signed consent forms. All data gathered from participants were treated confidentially in accordance with the ethics governing medical research.

Sample Collection and Processing

Urine and blood samples were obtained from each subject. An early morning mid-stream clean-catch urine was voided by each participant into a sterile wide-mouthed plastic screw capped tube. Prior to this, subjects were instructed on how to ensure aseptic sample collection or to prevent contamination of the urine samples by normal vaginal, perineal and anterior urethral normal flora. Three millilitres of blood was collected from each subject through venipuncture into an EDTA container. All samples were labelled appropriately and processed immediately without delay.

Urine Culture and Enumeration

Using a calibrated nickel wire loop (Pro-lab diagnostics, Canada), 0.001mL of urine sample was inoculated onto Cysteine Lactose Electrolyte deficient (CLED) agar and Blood agar (BA) (Antech Laboratories, USA). Agar plates were incubated at 37 °C for 18-24 hours, following which they were examined for bacterial growth. Bacteria were enumerated by counting the colonies on the culture plates and converted to a count per milliliter, known as colony-forming units (CFU/mL). The 5% blood agar was prepared from the nutrient agar by adding five millilitres of blood to 100mL of molten agar cooled to 45-50 °C.

Analysis of Whole Blood Cells

CD4 cells count was analysed using Partec cyflow counter made in Germany, and result reported as absolute count of CD4 Cells per milliliters (CD4 cells/ mm^3). Blood samples from the control group were screened for HIV prior to inclusion using Alere Determine HIV-1/2 rapid test kits.

Statistical Analysis

Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) data software version 18, and Statistical computing software version 3.1.2. Correlation analysis was performed with significance level set at 0.05. A p-value of less than 0.05 was considered statistically significant, and null hypothesis was rejected. The lower the p-value, the stronger the relationship.

Results

Significant Bacteriuria

The CD4 cell counts for seropositive subjects ranged from <100 to 800 cells/mm^3. Of the 220 subjects in this category, the urine samples of 143 subjects yielded bacterial growth. Specifically, 35 (24.5%), 46 (32.2%) and 62 (43.4%) of samples yielded 10^3, 10^4 and 10^5 CFU/mL respectively. A total of 117 of the 143(81.2%) subjects with bacterial growth had CD4 cell counts of <100-300. Among test subjects with CD4 counts of ≤200, 98/143(68.5%) had bacteriuria with 29/35 (83%), 34/46 (74%) and 35/62(56.5%) having bacterial counts of 10^3, 10^4, and 10^5 CFU/mL respectively. At CD4 cell count of >400 cells/mm^3, the number of subjects with bacterial growth significantly dropped. Though the higher percentage of the test subjects with bacteriuria (a count of 10^5CFU/mL) occurred in 62(43%) of the test subjects compared to 33(40%) in the control subjects, the statistical analysis is not significant (p=0.056) (Table 1, 2a and 2b).

Table 1. Bacterial growth in sero-positive and sero-negative participants based on CD4 cell-count

CD4 cell count	Sero-positive		Sero-negative	
	No. (%) examined	No. (%) with bacterial growth	No. (%) examined	No. (%) with bacterial growth
<100	75 (34.1)	48 (64.0)	0 (0)	0 (0)
101-200	63 (28.6)	50 (79.4)	0 (0)	0 (0)
201-300	29 (13.2)	19 (65.5)	0 (0)	0 (0)
301-400	27 (12.3)	16 (59.3)	9 (5.0)	6 (66.7)
401-500	11 (5.0)	5 (45.5)	60 (33.3)	29 (48.3)
501-600	5 (2.3)	1 (20.0)	66 (36.7)	25 (37.9)
601-700	7 (3.2)	3 (42.9)	26 (14.4)	13 (50.0)
701-800	3 (1.4)	1 (33.3)	14 (7.8)	7 (50.0)
>800	0 (0)	0 (0)	5 (2.8)	2 (40.0)
TOTAL	**220**	**143 (65.0)**	**180**	**82 (45.5)**

CFU/mL- Colony Forming Unit Per milliliter, TBG= Total bacterial growth, TNE= Total no Examined, 10^3 CFU/mL, 10^4 CFU/mL and 10^5 CFU/mL= bacteriuria at different bacterial count.

Table 2(a). Bacterial count of sero-positive participants in relation to their CD4 cell-count

CD4 cell count	No. (%) examined	No. (%) with bacterial growth	No. (%) with bacterial count		
			≤10^3cfu/mL	10^4cfu/mL	≥10^5cfu/mL
<100	75 (34.1)	48 (64.0)	16 (33.3)	17 (35.4)	15 (31.3)
101-200	63 (28.6)	50 (79.4)	13 (26.0)	17 (34.0)	20 (40.0)
201-300	29 (13.2)	19 (65.5)	3 (15.8)	3 (15.8)	13 (68.4)
301-400	27 (12.3)	16 (59.3)	3 (18.8)	6 (37.5)	7 (43.8)
401-500	11 (5.0)	5 (45.5)	0 (0)	2 (40.0)	3 (60.0)
501-600	5 (2.3)	1 (20.0)	0 (0)	0 (0)	1 (100)
601-700	7 (3.2)	3 (42.9)	0 (0)	1 (33.3)	2 (66.7)
701-800	3 (1.4)	1 (33.3)	0 (0)	0 (0)	1 (100)
TOTAL	**220**	**143 (65.0)**	**35 (24.5)**	**46 (32.2)**	**62 (43.4)**

CFU/mL- Colony Forming Unit Per milliliter, 10^3 CFU/mL, 10^4 CFU/mL and 10^5 CFU/mL= bacteriuria at different bacterial count.

Table 2(b). Bacterial count of sero-negative participants in relation to their CD4 cell-count

CD4 cell count	No. (%) examined	No. (%) with bacterial growth	No. (%) with bacterial count		
			≤10^3CFU/mL	10^4CFU/mL	≥10^5CFU/mL
301-400	9 (5.0)	6 (66.7)	1 (16.7)	3 (50.0)	2 (33.3)
401-500	60 (33.3)	29 (48.3)	9 (31.0)	8 (27.6)	12 (41.4)
501-600	66 (36.7)	25 (37.9)	8 (32.0)	7 (28.0)	10 (40.0)
601-700	26 (14.4)	13 (50.0)	3 (23.1)	4 (30.8)	6 (46.2)
701-800	14 (7.8)	7 (50.0)	2 (28.6)	2 (28.6)	3 (42.9)
>800	5 (2.8)	2 (40.0)	1 (50.0)	1 (50.0)	0 (0)
TOTAL	**180**	**82 (45.5)**	**24 (29.3)**	**25 (30.5)**	**33 (40.2)**

CFU/mL- Colony Forming Unit Per milliliter, 10^3 CFU/mL, 10^4 CFU/mL and 10^5 CFU/mL= bacteriuria at different bacterial count.

Bacteriuric Profile and CD4 Count

Among the test subjects, CD4 cells counts ranged from <100 to 800 cells/mm^3; with a total of 138 (62.7%) having CD4 counts of ≤ 200 cells/mm^3, followed by 56 (25.5%) subjects having cells counts of 201-400 cells/mm^3. Bacterial growth occurred in 98/138 (71%) and 35/56 (62.5%) of these subjects. CD4 cells counts above 400 occurred in 26, with 10 (38.5%) had bacteriuria. Of those with CD4 cells count of less than 200 cells/mm^3, 29/35, 34/46 and 35/62 subjects yielded bacterial count of 10^3, 10^4 and 10^5 CFU/mL respectively. (Table 2a).

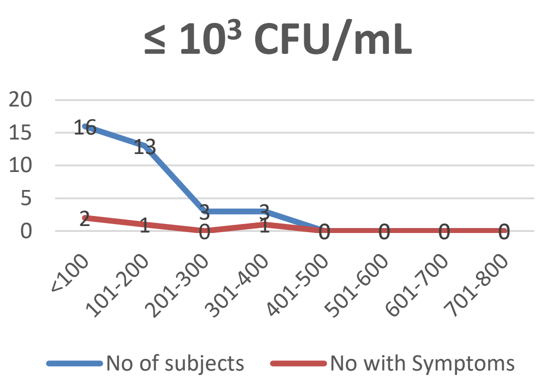
In the 180 control subjects examined, 126 had CD4 cells count between 400 and 600 cells/mm^3, urine samples of 54/126 (43%) yielded bacterial growth.

Similarly, 17/24, 15/25 and 22/33 of the samples had 10^3, 10^4 and 10^5 CFU/mL respectively. (Table 2b)

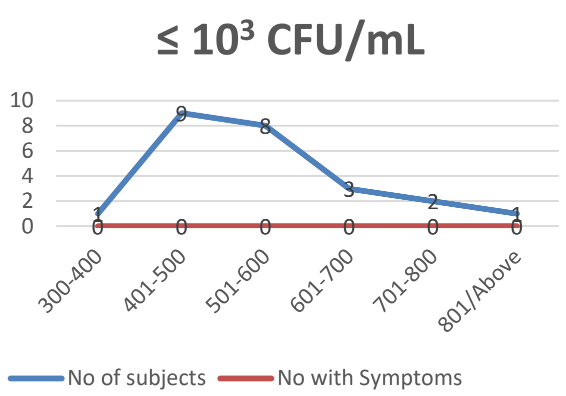
Of the 35, 46 and 62 test subjects with 10^3, 10^4 and 10^5 CFU/mL examined respectively, 29 (83%), 34 (74%) and 35 (56.6%) had CD4 cell counts of ≤200 cells/mm^3. (Table 2a). Furthermore, of the 4, 6 and 13 symptomatic test subjects, in each of these categories, 3 (75%), 2 (33%) and 9 (69%) had CD4 cells ≤200.

In the control group, CD4 cell counts ranged between 300 and above 800 cells/mm^3. A total of 82 culture positive samples yielded 24, 25 and 33 bacterial count of 10^3, 10^4 and 10^5 CFU/mL respectively. However, number of symptomatic cases recorded among control subjects were 0/24(0%) with 10^3 CFU/mL, while 4/25 (16%) and 8/33 (24%) of subjects were symptomatic in the 10^4 and 10^5 categories respectively. Of the 82 control subjects examined, 54 (66%), had CD4 cells count between 400 and 600 cells/mm^3. Eight of the 12 symptomatic subjects were also in this category. The slight difference however, was not statistical significant (within test subjects P=0.37), (Test vs Control P= 0.4). This indicates a lack of significant association between CD4 cells level and bacteriuria. (Figure 1,2 and 3).

A



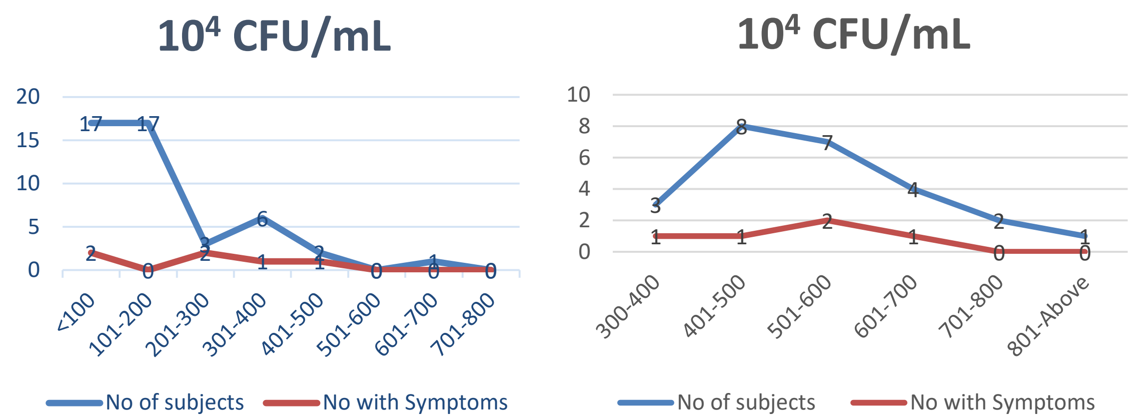
B



A-Test Subjects, B-Control Subjects, CFU/mL- Colony Forming Unit Per Millilitre

Figure 1: Symptomatic Bacteriuria of Test and Control Subjects at Bacterial Count of ≤10^3 CFU/mL

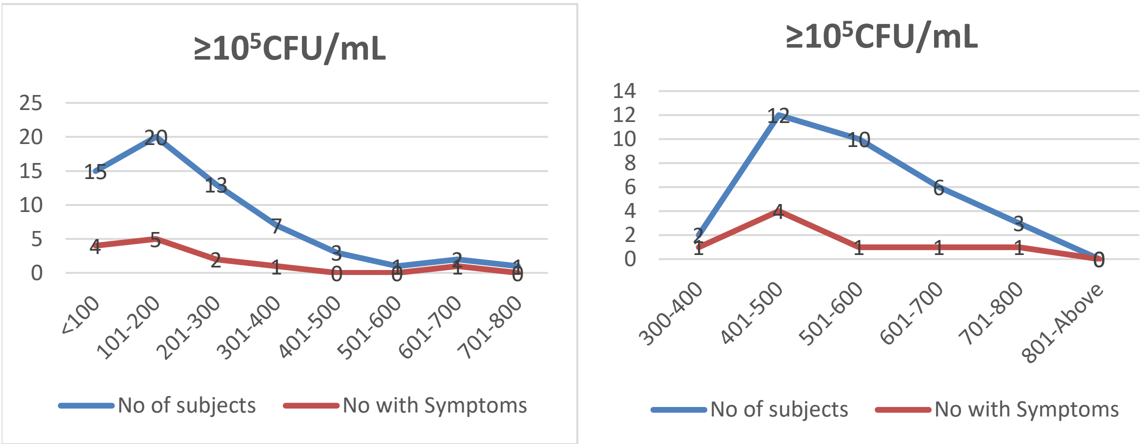
A B



A-Test Subjects, B-Control Subjects, CFU/mL- Colony Forming Unit Per Millilitre

Figure 2: Symptomatic Bacteriuria of Test and Control Subjects at Bacterial Count of 10^4 CFU/mL.

A B



A-Test Subjects, B-Control Subjects, CFU/mL- Colony Forming Unit Per Millilitre

Figure 3: Symptomatic Bacteriuria of Test and Control Subjects at Bacterial Count of $\geq 10^5$ CFU/mL

Symptomatic UTI and CD4 Cells Profile

Symptomatic UTIs was observed both in the test and control groups as shown in figure 1,2 and 3. Overall, 23 (16%) of the 143 bacteria- positive test subjects had symptoms of UTIs, compared to 12 (15%) of the 82 control participants, statistical analysis showed no significant association (p=0.51). However, comparison among those with significant bacteriuria shows that 13 (21%) of the 62 test subjects and 8 (24.2%) of the 33 control were symptomatic of UTIs. Data obtained in this study also showed that symptomatic UTI decreased as the CD4 cells count increased in both test and control subjects. In the test subjects, 11.4% of those with bacterial count of 10^3 CFU/mL were symptomatic. In the control subjects however, no symptomatic cases were recorded at lower bacterial count (10^3 CFU/mL). (Figure 1). Statistical analysis showed a significant association between symptomatic bacteriuria (with a significant count) and CD4 cells level (P= 0.029). (Figure 1,2 and 3).

Discussion

Immunocompromised individuals are prone to different opportunistic infections; very common among them are upper/lower respiratory tract infections e.g. tuberculosis, candidiasis, chronic pneumonia, progressive multifocal leukoencephalop -athy, systemic mycosis infections and diarrhea (Ajay, 2017 & Debasu, 2013). Few other studies have also identified urinary tract infections as a common infection in immunocompromised individuals (Barber *et al*., 2013, Schönwald *et al.,* 1999, Kayima *et al.,* 1996). This study attempts to establish a defined relationship between bacteriuria and the level of CD4 cell count among immunocompromised individuals particularly HIV/AIDS. The prevalence rate of significant bacteriuria in this study was 19.5% and 18.3% in test and control subjects respectively. This is very similar to the study carried out by Olowe, 2015 in the south-western part of Nigeria where he reported a prevalence rate of 21.1%. The difference in this study however, was not statistically significant; suggesting a no significant association between bacteriuria and the host CD4 level (p=0.056). Several studies have also shown a lack of association; for example, the study of Akimbami *et al.*, (2013) in Lagos, Nigeria, they noticed that bacteriuria was not affected by the CD4 level among HIV infected women. Omoregie and Eghafona, (2010) in a study on the effect of UTIs on the prevalence of anaemia among HIV patients in Benin City Nigeria, observed that bacteriuria was not dependent on the CD4 count less than 200 cells/mm^3. Banu & Jyothi, 2013 reported no correlation between bacteriuria with CD4 counts. Ojoo *et al.*, (1996) had earlier reported a lack of significant association between bacteriuria and CD4 count. Wilmer *et al*., (2010) state "CD4 cells count level of < 200 cells/mm^3 did not influence the occurrence of asymptomatic bacteriuria". The authors noted that the prevalence of bacteriuria did not vary among HIV/AIDS seronegative and seropositive individuals when they studied the prevalence and risk of asymptomatic bacteriuria among HIV-positive pregnant women in South Africa. Gugino *et al*., (1998) also state "there was no significant difference in prevalence of bacteriuria between groups … the prevalence of bacteriuria was not different between CD4 counts of $\leq$ 200 and $\geq$200". Serkadis *et al*., 2014 examine ART users and non-ART users and reported no statistical significant difference in the prevalence of significant bacterial growth between the groups.

However, in other related studies, occurrence of bacteriuria was found to be associated with CD4 cells level and/or viral load (Hoepelma *et al.*, 1992 & Njunda, *et al*., 2008, Ezechi *et al*., 2013, Olowe *et al*., 2015, Adoum *et al*., 2016).

It is reasonable to accept the hypothesis that bacteriuria may not be depended on CD4 level because; careful studies have shown that immunity to UTI results from complex interactions of the host factors and the invading organism. Defenses against UTIs include urine pH, flushing effects of the urine, high salt concentration, chemical barriers secreted by the epithelia cells lining the urinary tracts, secretary IgA, activities of lactoferrin (an iron binding protein that sequester iron, starving invading bacteria of iron leading to a decrease in growth rate). The prostate gland in males secretes an infection fighting substance; the lining epithelial cells of the urinary tracts also secrete antimicrobial peptides which directly kill bacteria. The mucous lining of the bladder contains antimicrobial substances which help to eliminates invading pathogens (Milan, 2006, Morgyn, 2009 & Bankole *et al*., 2011). These defensive mechanisms are independent of the host CD4 level, that partly explains why individuals with CD4 cell count less than 100 cells/mm^3 could have a sterile culture. Though lack of exposure to UTI causing agents could also be responsible. This suggest that the host CD4 level may have little or no effect on bacteriuria.

Cases of symptomatic UTIs increased with increase in bacterial count among the study subjects, the test subjects exhibited symptoms of UTIs even at a low bacterial count (≤10^3 CFU/mL); in the control subjects however symptoms were seen only among those with bacterial count of at least 10^4 CFU/mL and above. Manifestation of symptoms of UTIs even at a low bacterial count among the test subjects could likely be due to weaken immunity.

Conclusion

Comparing the occurrence of bacteriuria and CD4 count, it shows that bacteriuria might not be influenced by the host CD4 cells level despite a slight difference in the prevalence level. Since subjects with bacterial count of 10^3CFU/mL in the test subjects exhibited symptoms of UTIs, the need to set the diagnostic threshold at this bacterial load is strongly advised.

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