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COMPARATIVE STUDY OF ASYMPTOMATIC BACTERIURIA AMONG HIV SERO-POSITIVE AND HEALTHY INDIVIDUALS IN PORT-HARCOURT, NIGERIA

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ABSTRACT

The study aimed to analyse the incidence of Asymptomatic bacteriuria in Human immunodeficiency virus (HIV) sero-positive and sero-negative individuals and the spectrum of bacterial uropathogens among these 2 populations in Port-Harcourt, Nigeria. Both clean-catch, early morning urine and blood specimens were obtained from the HIV-positive individuals attending University of Port-Harcourt Teaching Hospital (UPTH) and Braithwaite's Memorial Specialist Hospital in Port-Harcourt. Same specimens were equally collected from HIV sero-negative individuals. The urine specimens were semi-quantitatively analyzed by culturing on Cysteine lactose deficient agar and the pure cultures identified by means of API 20E strips. Statistical analyses were carried out using students' package for science students version 20.0 (USA). Of the 800 urine samples; 400 from each group, bacteriuria was diagnosed in HIV-positive subjects (203/400, 50.75%) and control group (96/400, 24.00%). The highest bacterial isolates from HIV and control groups were *Escherichia coli* (24.63%, 43.75%) trailed by *Klebsiella* species (20.69%, 10.42%), *Pseudonomas* species (16.75%, 22.92%), *Proteus* species (10.84%, 4.17%), *Staphylococcus aureus* (2.46%, 16.67%) and *Candida albicans* (1.97%, 2.08%) respectively. *Enterobacter* species *and Providencia* species, *Alcaligenes* species and *Enterococcus faecalis* with prevalence rates of 12.81%, 7.88%, 0.99% and 0.99% respectively were isolated only from HIV group. The difference in the levels of the CD4 and haemoglobin is statistically significant ($p < 0.05$) in the two groups. The study revealed that individuals who are positive for HIV are favorably to asymptomatic bacteriuria. Broader array and more divergent uropathogens were isolated among them than in healthy individuals.

KEYWORDS: HIV, bacteriuria, haemoglobin, infection.

INTRODUCTION

Urinary tract infections (UTIs) are said to occur where exist pathogenic organisms in a quantified amount in a sample of urine aseptically obtained. A urine sample culture is declared positive for UTI once it displays a distinct bacterial colony count equal or above 10^3 colony-forming units/µl of urine (Nicolle, 2008). Asymptomatic bacteriuria (ASB) is defined as the presence of the identical species of bacteria on an aseptically obtained mid-stream urine to the tune of 10^5 CFU/ml or above with no presence of any signs of urinary tract infections (Harding, *et al.*, 2003). Globally, UTIs are among the infections frequently implicated in community settings. *Escherichia coli* is the most fingered causative agents (Subedi & Pudasini, 2017).

Urinary tract infection (UTI) is usually characterized by symptoms ranging from flank pain, lower back pain, frequent micturition, sepsis and even death (MacLean, 2001; Betsy, 2002). Vulnerable populations favorably disposed to UTIs include mostly women, especially in pregnancy, children and aged patients (Nicolle, 2008; Ovalle, 2001; Jackson *et al*., 2004; Nelson, 2015).

Certain circumstances such as spinal cord injuries, urinary catheters, diabetes, multiple sclerosis, immunodeficiency as well as primary urologic anomalies might also predispose some people to infections (Mladenovic, *et al*., 2015; Foxman, 2003).

Asymptomatic bacteriuria being a common occurrence, varies from 1% in healthy pre-menopausal women to as high as 100% among patients with long term indwelling catheters (Nicolle, 2000). Asymptomatic bacteriuria in healthy individuals, does not have any clinical implication but may be grave in some individuals, especially pregnant women. Many of them may come down with symptomatic bacteriuria or acute pyelonephritis (Guberman, *et al.*, 2007). Numerous studies documented that the incidence of UTIs in HIV-infected persons is evidently linked to the immune state measured by estimating the immune T-cells (Banu & Jyothi, 2013). Screening for asymptomatic bacteriuria is recommended for this group of immunocompromised individuals (Nicolle, *et al.*, 2005). Our study, therefore, is to compare the incidence of asymptomatic bacteriuria

in HIV-positive and the healthy control group and determine its linked influences.

MATERIALS AND METHODS

HIV clinics of the University of Port Harcourt Teaching Hospital and Braithwaite's Memorial Specialist Hospital located at Obio/Akpor and Port-Harcourt Local Government Areas of Port-Harcourt, Nigeria were the institutions where the study took place. Eight hundred (800) consented subjects; 400 from each from HIV-positive and HIV-negative groups were enrolled for this case control study which was carried out over a year period. The HIV group included HIV sero-positive persons irrespective of sex, age, and whether exposed to highly active antiretroviral therapy (HAART) or not while the control group comprised of persons who tested negative to HIV-1/2 confirmation test done to ascertain their HIV status.

Inclusion criteria

All adult (>18 years old) HIV-positive persons without current antibiotics therapy and willing to participate.

Exclusion criteria

Individuals not enrolled in this study include pregnant women, people who had symptomatic bacteriuria, catheterized or on antibiotic treatment.

Sample collection

Confirmation of the HIV status of the subjects was done using Alere Determine HIV-1/2 combo Kit before samples were collected from the HIV-positive and HIV-negative individuals. After collection, the samples after properly labeled were transported without delay to the Microbiology Laboratory unit of the University of Port-Harcourt Teaching Hospital for analyses. If processing was delayed, the samples were refrigerated at 4^{o}C. Also collected were blood samples for evaluation of the haemoglobin level and $CD4^{+}$ T-lymphocyte cells count. Questionners that provided personal details were filled by the counseled participants.

Methodology

The urine specimens were semi-quantitatively analyzed by culturing on cysteine lactose electrolyte deficient (CLED) agar and incubated aerobically at 37^{o}C overnight. The pure bacterial colonies on the agar were counted with the aid of electronic counter machine (Lapiz, Indian). The number of bacteria estimated as CFU/ml of urine was determined by multiplying the colonies counted by 500 since the calibrated wire loop (KD surgicals, Indian) used was 2mm in diameter and delivered 0.002ml (1/500 ml) of urine. Hundred thousand 100,000 (10^5) CFU per mL was recorded as significant bacteriuria. Characterization of the significant colonies were done using the colonial appearance on the plate, Gram staining reaction and biochemical tests. The $CD4^{+}$ cells of the participants were analyzed using Sysmex CD4 easy count kit on Partec (Sysmex) Cy Flow Counter (Germany).

Hundred thousand 100,000 (10^5) CFU per ml of urine was recorded as significant bacteriuria. The cultures with significant growth were characterized using Analytical profile index API 20E strips. The isolates were tested against single antibiotic impregnated discs containing; Cephalexin 30µg, Cefuroxime 30µg, Ceftriaxone 30µg, Ceftazidime 30 µg, Cefotaxime 30µg, Cefpodoxime 10µg, Cefepime 30µg, Meropenem 10µg, Gentamycin 30µg, Ciprofloxacin 5µg, Nitrofurantoin 300µg, Cotrimoxazole 25µg, and Augmentin 30µg using modified Kirby Bauer diffusion method for antimicrobial sensitivity. Interpretation was done by measuring the diameter of zones of inhibition around the antibacterial discs and categorizing them as susceptibility; intermediate and resistant using the CLSI standard table.

Ethical Approval

Ethical Committees of the hospitals where specimens were collected and processed approved the study. The subjects also gave written consents.

RESULTS

The incidence of the uropathogens were significantly difference ($P < 0.05$) between the two studied groups with HIV group having more bacterial growth. Out of the 800 urine samples altogether; 400 samples each from the 2 groups, asymptomatic bacteriuria was diagnosed from HIV sero-positive (203, 50.75%) and sero-negative (96, 24.00%) individuals (Table 1). Both HIV and control groups showed higher prevalence of *Escherichia coli*, (24.63%) and (43.75%); closely followed by *Klebsiella species* 20.69% and 10.42%; *Proteus species* 10.84% and 4.17% respectively in descending order. *Staphylococcus aureus* 16.67% *and Pseudomonas species* 22.92% (16.67% and 22.92%) occurred more in the control group than in HIV group (2.46% and 16.75%) respectively. *Enterobacter species* 12.81%, *Providencia species* 7.88% *Alcaligenes species* 0.99% and *Enterococcus faecalis* 0.99% were isolated only from HIV subjects. Collectively, more uropathogens isolated were higher in HIV group than in the healthy subjects except *Staphylococcus aureus* 16.6% which had higher incidence rate in the control group comparatively (Table 2).

Table 1: Bacteriuria in Control and HIV subjects.

Bacteriuria	Control	Subjects	OR (95% CI)
No	304 (76.00%)	197 (49.25%)	***3.2 (2.4 - 4.4)*** ***p= 0.0001***
Yes	96 (24.00%)	203 (50.75%)	
Total	**400 (100.0%)**	**400 (100.0%)**	

OR: Odds Ratio, CI: confidence interval,

Likelihood of bacteriuria increases if OR is >

Table 2: Distribution of Isolates in HIV-Positive and Control Subjects.

Isolate	Subjects	Control	OR (95% CI)
Enterococcus faecalis	2 (0.99)	0 (0.0%)	NA
Alcaligenes species	2 (0.99)	0 (0.0%)	NA
Staphylococcus aureus	5 (2.46)	16 (16.67%)	0.1 (0.04 – 0.3)
Providencia species	16 (7.88)	0 (0.0%)	NA
Proteus species	22 (10.84)	4 (4.17%)	2.7 (0.9 – 8.3)*
Enterobacter species	26 (12.81)	0 (0.0%)	NA
Pseudomonas species	34 (16.75)	22 (22.92%)	0.6 (0.3 – 1.2)
Klebsiella species	42 (20.69)	10 (10.42%)	2.2 (1.0 – 4.6)*
Escherichia coli	50 (24.63)	42 (43.75%)	0.4 (0.2 – 0.7)
Candida albicans	4 (1.97)	2 (2.08%)	0.9 (0.1 – 5.2)
Total	**203 (100.0%)**	**96 (100.0%)**	

OR: Odds Ratio, CI: confidence interval,
Likelihood of bacteriuria increases if OR is >1
**OR is statistically significant (p < 0.05)*

Table 3: Distribution of Isolates by Age and Gender.

GROUPS	Age Group (years)	Female	Male	Chi-square (p-value)
Hiv Subjects	20 -30	22 (16.18)	14 (20.90)	0.68 (0.4078)**
	31 – 40	68 (50.0)	15 (22.39)	14.1 (0.0001)*
	41 – 50	31(22.79)	27 (40.30)	6.71 (0.0094)*
	51 -60	8 (5.88)	9 (13.43)	3.35 (0.0678)**
	61 – 70	7 (5.15)	2 (2.99)	0.49 (0.4816)**
	Total	**136 (100.0)**	**67 (100.0)**	
Control Subjects	20 -30	23 (46.00)	16 (34.78)	1.24 (0.2635)**
	31 – 40	17 (34.00)	22 (47.83)	1.89 (0.1682)**
	41 – 50	4 (8.00)	4 (8.70)	0.01 (0.9019)**
	51 -60	3 (6.00)	2 (4.35)	0.13 (0.7159)**
	61 – 70	3 (6.00)	2 (4.35)	0.13 (0.7159)**
	Total	**50 (100.0)**	**46 (100.0)**	

********Difference is not statistically significant p> 0.05; ********Difference is statistically significant p< 0.05*

Table 4: Haemoglobin and CD4 count in study subjects with Bacteriuria.

	Parameter	Uropathogen Present	Uropathogen Absent	T-Test
HIV Subjects	Haemoglobin (g/dl)	11.3±1.2	11.2±12	0.1366**
	CD4 count (cells/ml)	416±206	418±401	0.9634**
Control	Haemoglobin (g/dl)	13.8±1.5	14.0±0.8	0.2311**
	CD4 count (cells/ml)	570±181	496±146	0.3552**

**Difference between both groups is statistically insignificant (p > 0.05)

Bacteriuria was more among age group 31-40 years (72.39%) and 21-30 years (80.78%) in the HIV and healthy groups respectively. Females were more infected with bacteriuria than men among all age groups except in control group where age group 31-40 years (47.83%) was seen to have higher infections than males (34.00%) (Table 3). The $CD4^+$ T- lymphocyte cell count and the haemoglobin levels evaluation showed control subjects (514±158 cells/ml and 13.9±1.0g/dl) having higher values than HIV subjects (416±206 cells/ml and Hb 11.3±1.2 g/dl) correspondingly (Table 4).

DISCUSSION

Asymptomatic bacteriuria is more frequent in HIV sero-positive subjects (50.75%) in comparison to healthy individuals in this study (24.00%) with significant difference (P < 0.05). Adjusted odds ratio (AOR) is above 1 and statistically significant (p < 0.05) in *Proteus* species as well as *Klebsiella* species infections which showed that the infection is more pronounced in test group as a result of HIV. These findings were corroborated by a study in Benin city, Nigeria that reported HIV and Acquired immunodeficiency syndrome (AIDS) as possible causes of higher occurrence of UTIs in younger people (Ibadin *et al*. 2006). Bacteriuria being more pronounced in HIV group is in consonance with recent study that reported by Akinsete and Ezeaka, (2018) who reported prevalence of 24.7% and 8.2% in children positive for HIV and healthy children respectively in Lagos, Nigeria. Nonetheless, the

results obtained when compared to the findings of Inyang-Etoh *et al*., (2009 were higher. They reported 25.3% and 13% from HIV-positive group and the healthy groups respectively.

Escherichia coli was more preponderance in both groups; 24.63% in the test and 43.75% in the control groups. The results tallied with a recent study in Ethiopia by Marami, *et al*., (2019) and Kanu, *et al*., (2017) in Nigeria. Both researches posited that Escherichia *coli* (38.1%) and (68.18%) respectively caused more UTIs. However, our results disagreed with some studies that reported other organisms as the principal aetiologic agents of UTIs. In Nigeria, Muoneke, *et al*., (2011) and Oko, *et al*., (2017) described *Staphylococcus aureus* 24.5% and (29%) respectively as commonest isolates causing UTIs. The higher incidence of *Escherichia coli* in this study may be due to the endowment of diverse virulence genes; the utmost vital one being the fimbrae.

The study also showed more divergent infecting bacteria amongst the HIV-positive individuals; ten (10) as against six (6) amid the healthy control group. *Enterobacter* species, *Providencia* species *Alcaligenes* species and *Enterococcus faecalis* were isolated only form HIV-positive group. These were in addition to *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, *Proteus* species *Staphylococcus aureus* common to the two groups. These findings agreed with a report that broader array of organisms was often isolated from HIV-positive persons (Hrbacek, *et al*., 2010).

Generally, statistical analysis showed insignificant variance between infection and gender ($p > 0.05$) even though females had more infections than males. Notwithstanding, statistical significance difference ($p < 0.05$) between bacteriuria and gender could be seen among age groups 31-40 years (50%) and 41-50years (22.79%). The findings agreed with other studies globally with reports of higher infection rates in females than males. Subedi & Pudasaini, (2017) doumented that the highest prevalent rate of UTI (33.3%) among ages 21-30 years in Nepal. The reasons for higher prevalence amid this middle-aged people is the high sexual activities (Demilie, *et al*., 2012; Frank-Peterside, *et al*., 2013).

Higher levels of haemoglobin and CD4 T-lymphocyte cell values were recorded among non-HIV group than in HIV-positive participants though with insignificant difference ($p < 0.05$). These findings were not surprising as most participants 365/400 (91.25%) were HAART-users which depicts high level of immunity as shown in their CD4+ values. These results were in consonance with the findings from other studies (Obirikorang & Yeboah, 2009; (Nacoulma, *et al*., 2007; Berhane, *et al*., 2004). HIV infection advancement is monitored with the levels of the haemoglobin and CD4 T-lymphocyte cells (Paton, *et al*., 2006) It is a known fact that CD4 counts decrease with the low haemoglobin levels (Anastos, *et al*., 2004; Costello, *et al*., 2005) and further project treatment efficacy (Gange, *et al*., 2003).

CONCLUSION

In conclusion, *Escherichia coli* has been implicated as the most causative agents of bacteriuria amongst the HIV and non-HIV infected individuals in Port-Harcourt, Nigeria. Asymptomatic bacteriuria with broader array of uropathogens was found more in PLHIV than healthy individuals. We share the view that these immunocompromised persons be periodically screened and treated for bacteriuria to avoid development of more grave conditions.

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