

DETECTION OF BACTERIURIA AMONG HUMAN IMMUNODEFICIENCY VIRUS SEROPOSITIVE INDIVIDUALS IN OSOGBO, SOUTH-WESTERN NIGERIA

O. A. Olowe^{1,*}, B. B. Ojo-Johnson², O. B. Makanjuola³, R. A. Olowe¹ and V. O. Mabayoje⁴

¹Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, P.M.B. 4400, Osogbo, Nigeria

²Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria

³Department of Medical Microbiology and Parasitology, University College Hospital, U.C.H, Ibadan, Oyo State, Nigeria

⁴Department of Haematology and Blood Transfusion, College of Health Sciences, Ladoke Akintola University of Technology, LAUTECH, P.M.B. 4400 Osogbo, Osun State, Nigeria

Received: December 4, 2014; Accepted: January 20, 2015

Human immunodeficiency virus-positive individuals are at increased risk of both asymptomatic and symptomatic urinary tract infections. The aim of this study was to determine the prevalence of asymptomatic bacteriuria (ASB) in HIV-positive individuals, its associated factors including any correlation with the CD4 count of the patient, and the antibiotic susceptibility pattern of the isolated organisms. Midstream urine and blood samples were collected from 242 consenting HIV-positive patients who were attending routine follow-up clinic during the six-month period of the study. Microscopy, culture, and antibiotic susceptibility testing of the samples were carried out following standard protocols, and CD4 counts were also determined. Fifty one (21.1%) of the 242 individuals had significant bacteriuria. The predominant organism was *Klebsiella* spp. (35%) followed by *Escherichia coli* (31%). Prevalence of bacteriuria was higher in the women. Low CD4 counts and young age were significantly associated with the presence of bacteriuria. ASB prevalence is high in this population and related to the CD4 count level.

Keywords: bacteriuria in HIV, CD4 count, antibacterial, Nigeria

Introduction

Immunocompromised individuals including those with HIV and AIDS are at particularly high risk of infections including urinary tract infections (UTIs) [1].

Urinary tract infections are very common infections. They are usually bacterial in origin, might be complicated by frequent recurrences, and may also present as more severe manifestations such as pyelonephritis and sepsis especially in individuals at risk [2, 3].

Asymptomatic bacteriuria (ASB), the presence of a significant quantity of bacteria, of $\geq 10^5$ colony-forming units per milliliter (CFUs/ml), in the urine of a patient without symptoms or signs of a UTI, has been said to be associated with increased risk of symptomatic urinary tract infection [4–6].

ASB is a frequent finding, varying in prevalence from as low as 1% in healthy premenopausal women to 100% in those on long term indwelling catheterization [7, 8]. It is apparently benign and of no clinical significance in healthy individuals. In some individuals, however, such as pregnant women, it is associated with significant ad-

verse outcomes; hence, the recommendation that screening for ASB and treatment should be carried out among them [4].

The prevalence of asymptomatic bacteriuria in HIV-positive individuals varies depending on the population studied [6, 9–11]. De Pinho et al., for example, reported a prevalence of 3% in asymptomatic HIV-positive individuals and 13% in those with AIDS [10].

Although the current recommendation is not to screen for or treat ASB in this population, many reports have indicated an increased risk of bacteriuria and UTI in HIV [9].

Low CD4 counts have been said to put HIV-positive patients at higher risk of bacteriuria [6]. Hoepelman et al. also noted a similar finding [12].

Nigeria has a high burden of HIV infection with the 2013 prevalence estimate as 3.2% [13], and considering its large population size, this constitutes a significant population of individuals who are at risk of UTI and other complications following ASB.

In view of the recognized importance of ASB in pregnant women, several studies have explored this condition among HIV-positive pregnant women [6, 9, 11]. There is,

* Corresponding author: Dr. Olubenga Adekunle Olowe; Department of Haematology, College of Health Sciences, P.M.B. 4400 LAUTECH Osogbo, Osun State, Nigeria; E-mail: olowekunle@yahoo.com

however, limited data especially in this environment, on the occurrence of ASB in the general population of HIV-positive individuals. This study, therefore, had as its aim to determine the prevalence of asymptomatic bacteriuria in HIV-positive individuals as well as to determine its associated factors including any correlation with the CD4 count of the patient. We also determined the antibiotic susceptibility pattern of the isolated organisms.

Methodology

This study was carried out in a six-month period from January 2013 to June 2013 in Osogbo, a capital city in South Western, Nigeria. The Ladoke Akintola University Teaching Hospital Osogbo (LAUTECH), where participants were recruited, provides both tertiary and secondary health care including HIV/AIDS care and support.

Approval for the study was sought and obtained from the Ethics Review Committees of LAUTECH Teaching Hospital.

A total of 242 nonpregnant HIV-positive individuals receiving free HIV care at the HIV treatment center (IHVN) in LAUTECH were recruited. Consecutive attendees on routine clinic visit who consented within the study period were enrolled.

Sample size was determined based on the prevalence obtained from a similar study [11] resulting in a minimum sample size of 202.

Procedure

Informed consent was obtained from the patients before inclusion in the study. The nature of the study, the minimal risk involved in the procedure, and advantage of the research were explained to the patients. Those who were on antibiotics at the time of recruitment were excluded from the study.

Questionnaire was administered to each individual to obtain demographic and clinical information including age, sex, and marital status.

Blood and urine samples were collected from all participants. Appropriately labeled universal bottles were given to each participant for the collection of clean catch midstream urine specimen following explanation of the procedure for sample collection. In addition, 5 ml of venous blood was collected from the antecubital fossa of each patient into ethylenediaminetetraacetic acid (EDTA) bottles. All samples were immediately sent to the laboratory for processing.

Rescreening for HIV-1 and HIV-2 to confirm HIV status of those recruited was also done after counseling the participants. This was carried out using rapid test kits, Determine™ HIV-1/2 (Abbott Laboratory, IL, USA). The test results were confirmed with the ImmunoComb® II HIV 1 and 2 (Bispot kit PBS Organics and Israel 2005), according to manufacturer's instructions.

Specimens were first examined macroscopically, and thereafter, microscopic examination was done by examination of wet preparation for white blood cells, red blood cells, and urine sediments. Each sample was then cultured on cysteine lactose electrolyte deficient (CLED) agar and chocolate agar and incubated aerobically at 37 °C for 18–24 h. [14]. Diagnosis was made based on significant bacteriuria which is greater than or equal to 10⁵ colony forming units per milliliter (CFU/ml) of the same organism. Colonial morphology, Gram stain, and standard biochemical tests were used to identify the organisms. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control organisms.

Antimicrobial susceptibility testing was done using the Modified Kirby Bauer disc diffusion test according to Clinical Laboratory Standards Institute (CLSI) guidelines for antimicrobial susceptibility testing of the isolates for the following antibiotics: chloramphenicol, amoxicillin, cefuroxime, augmentin, gentamycin, sparfloxacin, cotrimoxazole, ciprofloxacin, and erythromycin.

The CD4 count was determined from serum samples following the manufacturer's instructions.

Data analysis

All data were entered onto the Statistical Package for the Social Sciences (SPSS) version 20 software which was also used for analysis. Descriptive statistics were used for initial data exploration. Chi-squared (χ^2) test was used to explore relationship between categorical variables, and the level of significance was set at 95%.

Results

Two hundred and forty-two HIV-positive individuals were enrolled. The age range was 20–65 years with a mean 37.8 (± 9.2) years. The female population predominated as there were 56 male (23.1%) and 186 (76.9%) females. The CD4 counts ranged from 49 to 1082 cells/mm³ with a mean count of 346.8 (± 197.4) cells/mm³ (Table 1).

Table 2 shows the results of culture of the urine samples. The organisms isolated in samples with significant bacteriuria are as shown. Samples from 51 (21.1%) individuals yielded significant bacteriuria with a colony count of >10⁵ CFU/ml; the other 191 samples (78.9%) had either no growth or no significant growth. Bacterial monocultures were obtained in all patients with significant bacteriuria (Table 3).

The enteric gram-negative organisms were the most frequently isolated organisms (72.6%). The predominant organism was *Klebsiella* spp. (35.3%) composed of 17 *Klebsiella pneumoniae* and only one *Klebsiella oxytoca*. This was closely followed by *E. coli* (31.4%), and *Proteus* spp. (two *Proteus mirabilis* and one *Proteus vulgaris*) accounted for about 6%. *Enterococcus faecalis* was the least isolated organism (2%).

Table 1. Characteristics of participants

Age group (years)	Male (%)	Female (%)	Total (%)
20–29	3 (1.2)	40 (16.5)	43 (17.8)
30–39	21 (8.7)	87 (36.6)	108 (44.6)
40–49	22 (9.1)	40 (16.5)	62 (25.6)
50–59	9 (3.7)	14 (5.8)	23 (9.5)
60–69	1 (0.4)	5 (2.1)	6 (2.5)
	56 (23.1)	186 (76.9)	242 (100)
CD4 count			
<200	13 (5.4)	56 (23.1)	69 (28.5)
200–500	28 (11.6)	86 (36.4)	116 (47.9)
>500	15 (6.2)	42 (17.4)	57 (23.6)
	56 (23.1)	184 (76.9)	242 (100)

Table 2. Organisms isolated on culture

Organism	No. (%)
<i>Escherichia coli</i>	16 (31.4)
<i>Klebsiella</i> spp.	18 (35.3)
<i>Proteus</i> spp.	3 (5.8)
<i>Staphylococcus aureus</i>	13 (25.5)
<i>Enterococcus faecalis</i>	1 (2.0)
Total	51 (100)

A significant association was found between age and the presence of bacteriuria with younger individuals at higher risk ($p = 0.02$). Those with low CD4 counts also had a significantly higher prevalence of bacteriuria ($p < 0.0001$). There was no significant association with the female gender although the prevalence was higher in the female population (23.7% vs. 12.5%, $p = 0.07$).

Table 4 shows the antibiotic susceptibility pattern of the isolates to tested antibiotics. There was a high prevalence of multidrug resistance to some of the antibiotics

used especially to amoxicillin, augmentin, cotrimoxazole, and moderate resistance to erythromycin and cefuroxime.

Discussion

We found a high prevalence of asymptomatic bacteriuria in our study population, with at least one in five of them having this condition. This result appears to be much higher than those reported in other parts of the country and globally as most studies report prevalence of 3–15% [6, 9–11]. Only a few studies such as that by Ojoo et al. have recorded such high prevalence as ours [15]. Another study in this environment also reported a high prevalence of 18% among HIV-positive pregnant women [16].

Compared with HIV-negative population within the same region, it appears that the prevalence of ASB is similar to what was obtained in our study population. A community-based study on HIV-negative individuals in the same region also found a prevalence of 22.6%,

Table 3. Patients characteristics associated with significant bacteriuria

Characteristics	Significant bacteriuria			χ^2	p value
	Yes (%)	No (%)	Total		
Age				5.5	0.02
<40 years	39 (25.8)	112 (74.2)	151 (62.5)		
40 years and above	12 (13.2)	79 (86.8)	91 (37.6)		
Sex				3.2	0.07
Male	7 (12.5)	49 (87.5)	56 (23.1)		
Female	44 (23.7)	142 (76.3)	186 (76.9)		
CD4 count				16.0	<0.0001
<200	26 (37.7)	43 (62.3)	69 (28.5)		
200 and above	25 (14.5)	148 (61.2)	173 (71.5)		

Table 4. Antibiotics susceptibility testing of the isolates

Antibiotics pattern		CHL	SPX	GEN	CIP	AMX	AUG	CXM	COT	ERY
<i>Klebsiella</i> spp.	S	13 (72.2)	11 (61.1)	14 (77.8)	13 (72.2)	6 (33.3)	5 (27.8)	NT	5 (27.8)	NT
	R	5 (27.8)	7 (38.9)	4 (22.2)	5 (27.8)	12 (66.7)	13 (72.2)	NT	13 (72.2)	NT
<i>Escherichia coli</i>	S	4 (25.0)	10 (62.5)	14 (87.5)	15 (93.8)	9 (52.9)	6 (37.5)	NT	5 (31.3)	NT
	R	12 (75.0)	6 (37.5)	2 (12.5)	1 (6.3)	7 (47.1)	10 (62.5)	NT	11 (68.8)	NT
<i>Proteus</i> spp.	S	1 (33.3)	2 (66.7)	3 (100.0)	1 (33.3)	0 (0)	1 (33.3)	NT	0 (0)	NT
	R	2 (66.7)	1 (33.3)	0 (0.0)	2 (66.7)	3 (100.0)	2 (66.7)	NT	3 (100.0)	NT
<i>Staph. aureus</i>	S	NT	NT	NT	10 (76.9)	1 (7.7)	5 (38.5)	1 (7.7)	4 (30.8)	7 (53.8)
	R	NT	NT	NT	3 (23.1)	12 (92.3)	8 (61.5)	12 (92.3)	9 (69.2)	6 (46.2)
<i>Enterococcus</i>	S	NT	NT	NT	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	NT	NT	NT	1 (100.0)	1 (100.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100)

Key: CHL – chloramphenicol, SPX – sparfloxacin, GEN – gentamycin CIP – ciprofloxacin, AMX – amoxicillin, AUG – augmentin, CXM – cefuroxime, COT – cotrimoxazole, ERY – erythromycin; NT – not tested

which is comparable to our prevalence of 21.1% [17]. Similar findings were also reported by Widmer et al. and Gugino et al., as they did not find any difference in the prevalence of ASB between their study population of HIV-positive women and HIV-negative controls [9, 18].

Klebsiella spp. was the most prevalent organism, slightly higher in prevalence than *E. coli*. This differs from usual findings in studies on both HIV-positive and -negative individuals where *E. coli* usually predominates as the causative organism [7, 10, 11]. *Klebsiella* spp. is more commonly isolated in hospitalized individuals; it is therefore probable that HIV-positive patients are at higher risk from infections due to hospital associated pathogens. A similar finding with predominantly nosocomial pathogens was also reported by Gugino et al. [18]. The usual trend in ASB is for the prevalence to be higher in the female population [17], and our study also reported similar finding. We found the prevalence of ASB to be higher in women than men in keeping with reported trend which is attributed to the proximity of the urethra to the anus and its short length [19]. We, however, could not establish a statistically significant association and, therefore, cannot conclude such an association in this group of HIV-positive individuals.

The prevalence of asymptomatic bacteriuria in the younger age group was almost twice as high as what was found in the older age group. This might be related to the sexual activity of the younger age group which is a recognized predisposition to bacteriuria and UTI [20]. It should also be borne in mind, however, that the older age group, especially the elderly, are also at risk of bacteriuria [17, 20, 21]. It has been observed that those with low CD4 counts (<200 cell/mm³) are at significantly higher risk of asymptomatic bacteriuria and are therefore more likely to develop UTI. We also report the same as we were able to demonstrate a strong correlation between low CD4 counts and the presence of ASB in keeping with the high risk of opportunistic and other infections that result from immune suppression. A reason that has been proposed for this is that, in HIV/AIDS, the impaired immunity that occurs makes it

easier for bacterial pathogens to adhere to the urinary epithelium [10]. Ezechi et al. in their study on the risk factors for ASB in HIV-positive pregnant women also had similar findings in their study population of pregnant women. Furthermore, they found high viral load, low hemoglobin, and previous UTI to be associated with ASB [16]. We, however, did not explore these parameters in our study.

Treatment of ASB in pregnancy has been seen to reduce the incidence of adverse outcome such as pyelonephritis and low birth weight infants [9]. Although the usefulness of treating asymptomatic bacteriuria in HIV-positive individuals is presently doubtful, we sought to determine the antibiotic susceptibility pattern of these isolates, due to reports that persons with HIV usually have urinary tract infections due to multidrug resistant organisms [15]. Our study corroborates this as most of the organisms showed moderate to high level resistance to commonly used first line antibiotics. Of note is the remarkably high resistance of most of the isolated organisms to the penicillins, which might be related to the empiric use and probably abuse of penicillins in the treatment of UTI and many other infections in this environment. Such individuals are therefore likely to develop symptomatic urinary tract infections for which treatment options will be a challenge.

Conclusion

Asymptomatic bacteriuria is common in HIV-positive individuals and shows strong correlation with the level of CD4 count. Young individuals appear to be at increased risk and may therefore benefit from close monitoring in order to treat symptomatic infections early.

References

- Schönwald S, Begovac J, Skerk V: Urinary tract infections in HIV disease. *Int J Antimicrob Agents* 11(3–4), 309–311 (1999)

2. Barber AE, Norton JP, Spivak AM, Matthew AM: Urinary tract infections: current and emerging management strategies. *Clin Infect Dis* 57(5), 719–724 (2013)
3. Kayima JK, Otieno LS, Twahir A, Njenga E: Asymptomatic bacteriuria among diabetics attending Kenyatta National Hospital. *East Afr Med J* 73, 524–526 (1996)
4. Nicolle LE, Bradley S, Colgan R, Rice KC, Schaeffer A, Hooton MT: Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Clin Infect Dis* 40, 643–654 (2005)
5. Nelius T, Filleur S, Nelson JS (2011): Asymptomatic Bacteriuria: Significance for Different Patient Population, *Urinary Tract Infections*, ed. Dr. Peter Tenke, InTech, ISBN: 978-953-307-757-4, available from: <http://www.intechopen.com/books/urinary-tract-infections/asymptomatic-bacteriuriasignificance-for-different-patient-population>
6. Banu A, Jyothi R: Asymptomatic bacteriuria in HIV positive individuals in a tertiary care hospital. *J HIV Hum Reprod [Internet]* 1(2), 54 (2013)
7. Nicolle LE: Asymptomatic bacteriuria – important or not? *N Engl J Med* 343, 1037–1039 (2000)
8. Colgan R, Nicolle LE, McGlone A, Hooton TM: Asymptomatic bacteriuria in adults. *Am Fam Physician* 74, 985–990 (2006)
9. Widmer TA, Theron G, Grove D: Prevalence and risks of asymptomatic bacteriuria among HIV-positive pregnant women. *South Afr J Epidemiol Infect* 25(1), 28–32 (2010)
10. De Pinho AM, Lopes GS, Ramos-Filho CF, Santos OR, De Oliveira MP, Halpern M, Gouvea CA, Schechter M: Urinary tract infection in men with AIDS. *Genitourin Med* 70, 30–34 (1994)
11. Awolude OA, Adesina OA, Oladokun A, Mutiu WB, Adebole IF: Asymptomatic bacteriuria among HIV positive pregnant women. *Virulence [Internet]* 1(3), 130–133 (2010)
12. Hoepelman AI, van Buren M, van den Broek J, Borleffs JC: Bacteriuria in men infected with HIV-1 is related to their immune status (CD4+ cell count). *AIDS* 6(2), 179–184 (1992)
13. <http://www.unaids.org/en/regionscountries/countries/nigeria>, accessed on 24/10/2014
14. Collee JG, Mackie TJ, McCartney JE (1996): Mackie & McCartney Practical Medical Microbiology, Churchill Livingstone, New York
15. Ojoo J, Paul J, Batchelor B, Amir M, Kimari J, Mwachari C, Bwayo J, Plummer F, Gachihi G, Waiyaki P, Gilks C: Bacteriuria in a cohort of predominantly HIV-1 seropositive female commercial sex workers in Nairobi, Kenya. *J Infect* 33, 33–37 (1996)
16. Ezechi OC, Gab-Okafor CV, Oladele DO, Kalejaiye OO, Oke BO, Ekama SO, Audu RA, Okoye RN, Ujah IAO: Prevalence and risk factors of asymptomatic bacteriuria among pregnant Nigerians infected with HIV. *J Matern-Fetal Neonat Med* 26(4), 402–406 (2013)
17. Olowe OA, Makanjuola OB, Olabiyi KO, Akinwusi PO, Alebiosu CO, Isawumi MA, et al.: Asymptomatic bacteriuria among elderly and middle-aged rural community-dwellers in South-Western Nigeria. *Infect. Drug Res* 6, 55–58 (2013)
18. Gugino L, Russo T, Wactawski-Wende J, Goodnough SL, Tristram DA, Mylotte J: Asymptomatic bacteriuria in human immunodeficiency (HIV)-infected women. *Prim Care Update Ob Gyns* 5(4), 146 (1998)
19. Iduoriyekemwen NJ, Sadoh WE, Sadoh AE: Asymptomatic bacteriuria in HIV positive Nigerian children. *J Med Biomed Res* 11(1), 88–94 (2012)
20. Hermann V, Palma P, Geo MS, Lima RS: Urinary tract infections: pathogenesis and related conditions. *Int Urogynecol J* 13, 210–213 (2002)
21. Ariathianto Y: Asymptomatic bacteriuria – prevalence in the elderly population. *Aust Fam Physician* 40, 805–809 (2011)