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Asymptomatic bacteriuria among HIV positive pregnant women

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Key words: asymptomatic bacteriuria, HIV positive, pregnant women, prevalence

The prognostic significance of asymptomatic bacteriuria resides in the observation that the prevalence is, relatively, high in persons with certain medical conditions, such as diabetes mellitus and pregnancy. This prevalence might, even, be higher in patients with human immunodeficiency virus infection. Hence, this study set out to determine the prevalence of asymptomatic bacteriuria among symptom free and newly enrolled HIV infected pregnant women attending PMTCT unit of Antiretroviral Clinic of University College Hospital, Ibadan, Nigeria between 1st May and 30th September 2007. Information was obtained on the socio-demographic characteristics of the subjects, CD4 count and viral load. Microbial culture was carried out on aseptically collected urines from the patients. Statistical analysis was done with SPSS 12 package. There were 161 analyzable samples from the participants. The mean age and gestational age at presentation of participants was 30.49 ± 4.3 years and 27.3 ± 3.2 weeks, respectively with modal parity of 2. Twenty-five (15.5%) of the urine samples significantly grew bacteria. The CD4 cells were significantly lower and the viral loads significantly higher (250.52 vs. 355.57 cells/mm³; 88,731 vs. 55,384 copies/ml; p = <0.0000) for the urine culture positive patients. *Escherichia coli* were isolated in 48%, *Proteus* in 16.0%, *Klebsiella* in 8.0% and *Staphylococcus aureus* in 28.0% of the urine positive cultures. The study showed that the prevalence of asymptomatic bacteriuria among PLWHAs is high. The microbial isolate from the urine samples were not different from those of HIV-negative patients.

Introduction

Asymptomatic bacteriuria (ASB) is defined as the quantitative growth of bacteria, greater than or equal to 10⁵ colony forming units per milliliter urine of the same organism, on aseptically collected midstream urine specimens, in the absence of symptoms of urinary tract infection.¹⁻³

The prognostic significance of ASB resides in the observation that persons with ASB in certain medical conditions, such as diabetes mellitus and pregnancy, are at increased risk of pyelonephritis and renal impairment.⁴⁻⁶ If asymptomatic bacteriuria is not treated in pregnancy up to 40% of the patients will develop urinary tract infection (UTI) and approximately, 25–30% of women will develop acute pyelonephritis.⁷ It has been estimated that treatment of asymptomatic bacteriuria would lead to approximately a 75% reduction in the incidence of pyelonephritis.⁷ Asymptomatic bacteriuria has been associated with preterm delivery, fetal loss and preeclampsia.⁸ Today, screening for asymptomatic bacteriuria have become standard of care in some obstetric units to prevent these complications.

The prevalence of ASB in normal pregnant population has been quoted to range from 2–10%⁹⁻¹¹ and in Nigeria a prevalence of 4–14.1% have been documented.¹² Prevalence has been found to be, generally, increased with age, sexual activity and parity. However, higher prevalence have been found in specific clinical conditions like anatomic and functional urinary

tract abnormalities, lower socio-economic status, anaemia and immunosuppressive state like in sickle cell trait, diabetes mellitus¹³⁻¹⁵ and, possibly, human immunodeficiency virus (HIV) infection.

Human immunodeficiency virus (HIV), a chronic infection associated with progressive immune dysfunction, appears to increase risk for developing significant bacteriuria in patients.¹⁶ In HIV infection co-morbidity with other organisms is common and this may impact on the pregnancy outcome in these patients. Such organisms may include those of asymptomatic bacteriuria.

The diagnosis of ASB is based on isolation of microorganisms with a colony count >10⁵ organisms per millilitre of urine in a clean-catch specimen.¹⁶ The patient should be instructed to clean the vulva area from front to back to avoid contamination of the urine sample.⁷

In all studies of asymptomatic bacteriuria, *Escherichia coli* is the most common organism associated with bacteriuria, representing at least 80% of isolates, with other gram-negative rods and certain gram-positive organisms including *Staphylococcus saprophyticus* and enterococci occasionally being isolated.¹⁷

To date there is no data to describe the prevalence of ASB among HIV infected pregnant women in our environment. This study was undertaken to estimate the prevalence and possible risk factors for ASB in pregnant women undergoing care in an HIV prevention, treatment, care and support program in a tertiary center in Nigeria.

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Table 1. Socio-demographic characteristics of asymptomatic bacteriuria positive and negative patients

Characteristics	Culture positive	Culture negative	p value
Mean age (years)	30.52	30.46	0.9823
Type of marriages (n/%)			
Monogamy	17 (68)	100 (73.5)	0.9523
Polygamy	8 (32)	36 (26.5)	0.8469
Mean gestational age at presentation (weeks)	27.4	29.1	0.9972
Modal parity (n)	2.0	2.0	0.2474
Mean packed cell volume (%)	28.16	27.96	0.8526

Results

A total of 165 urine samples were collected. However four samples were excluded from analysis—two patients were on antibiotic treatment, one sample was wrongly labeled and one sample was processed but found to be contaminated—leaving 161 analyzable samples. A total of 25 cases of asymptomatic bacteriuria were seen out of 161 HIV positive obstetric patients screened during the study period. This gives prevalence of asymptomatic bacteriuria of 15.5% among these patients. There was no case of more than one bacteria isolated. However, four of the urine specimen had, in addition to bacteriuria, *Candida albicans* isolated.

Table 1 shows the sociodemographic characteristics of the patients. The mean age and gestational age at presentation of participants was 30.49 ± 4.3 years and 27.3 ± 3.2 weeks (30.52 years and 30.46 years; 27.4 weeks and 29.1 weeks, respectively, for patients with and without significant bacteriuria respectively) and the modal parity was 2 for patients with or without significant bacteriuria with a range of 0–3. Forty-one percent of the participants were traders, 85.7% were of Yoruba tribe and 60.9% were Christian. One hundred and fifteen, representing 71.4% of the participants, had at least secondary education.

Table 2 shows the laboratory results of the participants. While the packed cell volume was not significantly different between those with and without significant bacteriuria (28.16% and 27.96% respectively), the mean value was generally low at 28.06%. The CD4 counts were significantly lower among the patients with significant bacteriuria compared with those without significant bacteriuria ($250.52 \text{ cells/mm}^3$ vs. $355.57 \text{ cells/mm}^3$; $p < 0.0001$ at 95% CI). Similarly, the viral loads of the patients with asymptomatic bacteriuria was significantly higher compared with those without asymptomatic bacteriuria (88,731 copies/ml vs. 55,384 copies/ml; $p < 0.0001$ at 95% CI).

There were 32 patients with abnormal midstream urine results. Twenty five samples grew identifiable bacteria of which four were with mixed candida growth (2 each for *Klebsiella species* and *E. coli*). This gave a prevalence of asymptomatic bacteriuria of 15.5% among this group. There were seven culture results of pure candida growth. Twelve of the 25 positive samples for bacteria (48%) grew *E. coli* in significant quantity. This was the commonest organism cultured in this study. Other urinary tract pathogens identified in this study included: *Staphylococcus aureus*

(28%), *Proteus mirabilis* (16%) and *Klebsiella species* (8%). All of these organisms were grown in pure cultures (Fig. 1).

Discussion

The prevalence of asymptomatic bacteriuria was 15.5% among the studied HIV possible pregnant women accessing the PMTCT interventions at the ART program at the University College Hospital, Ibadan. This rate of asymptomatic bacteriuria is higher than the range generally quoted for non-pregnant and pregnant women.^{9,10} This can be associated with the fact that women included in this study were recruited from a tertiary hospital which serves as a referral centre for most ill patients and, as such, may represent a population with more severe disease.

The use of a clean-void midstream method of collection and quantitative urine cultures to differentiate infection from contamination is well established.¹⁻³ This method has been shown to provide at least 80 percent likelihood that a voided midstream urine specimen will provide the necessary sample to detect high colony count if present.^{4,5}

The infecting organisms identified in this study are in keeping with commonly isolated bacteria in other studies of asymptomatic bacteriuria.^{4-6,13-15}

In this study 48% of 25 cultures showing significant bacteriuria grew *E. coli*. These numbers, though considerably lower than those reported in similar studies,^{2,3} still follow the pattern of *E. coli* being responsible for majority of the microbes implicated in ASB. The 28% isolated staphylococcus aureus might indicate the risk of opportunistic infections to which HIV positive patients are particularly prone to. The possibility of nosocomial infection as shown in a similar study in non-pregnant women²⁴ can explain this finding.

Opportunistic infections (OIs) are important co-morbidities in PLWHAs¹⁸ and they influence the morbidity and mortality due to HIV infections. This study showed that in seven of the patients without bacteriuria and four patients with bacteriuria, there was growth of candida in the urine. This highlights the need for early screening and also the need to increase awareness in healthcare providers, in order to improve decision making regarding prophylaxis for prevention and appropriate therapeutic intervention.

There are few studies with which to compare our data on risk factors. In other studies prevalence of asymptomatic bacteriuria was found to be higher with clinical conditions like diabetes mellitus and sickle cell disease due to their immunosuppressive nature. It is not, therefore, surprising the high prevalence found in this study.^{6,14,19,20}

The present study demonstrated a significant and independent association between the presence of ASB and both CD4 counts and viral load. These results imply that the more morbid the patients the higher the risk of asymptomatic bacteriuria and, possibly, the more vulnerability to bacterial and other opportunistic infections.

Maternal age or type of marriage has no effect on the prevalence of asymptomatic bacteriuria and parity had no influence either unlike other studies.^{21,22} There was no correlation between

Table 2. Laboratory results

Laboratory parameter	Patients with positive bacteriuria	Patients with negative bacteriuria	p value
	25 (15.5%)	136 (84.5%)	
Urine Bacteria Culture	<i>E. coli</i>	12 (48%)	
	<i>Staph. aureus</i>	7 (28%)	
	<i>Proteus mirabilis</i>	4 (16%)	
	Klebsiella Spp.	2 (8%)	
Candida-associated culture	Candida only	7	
	Candida mixed with <i>E. coli</i>	2	
	Candida mixed with Klebsiella	2	
Packed cell volume	28.16%	27.96%	0.317
CD4	250.52 cells/mm ³	355.57 cells/mm ³	<0.0001
Viral Load	88,731 copies/ml	55,384 copies/ml	<0.0001

the packed cell volume of the patients and ASB. The generally low packed cell volume of the patients may reflect the known haemopoietic suppressive effects of HIV infection.^{23,24}

In conclusion, this study has shown a high prevalence of ASB among HIV positive pregnant women. However, the result is similar to those seen in patients with other immune suppressive clinical conditions like diabetes mellitus and sickle cell disease.^{19,20} The risk factors for ASB in this patients were low CD4 counts and high viral load.

Methodology

The participants comprised a group of HIV positive pregnant women managed in the PMTCT arm of the antiretroviral therapy (ART) clinic of University College Hospital (UCH), Ibadan. This Antiretroviral treatment programme provides services for HIV positive men, women and children. The PMTCT programme provides health education, pre- and post-test counseling, provision of antiretroviral drugs for pregnant women and infants, ongoing counseling before and after birth, infant feeding services, determination of status for infants at birth, 6 weeks, follow-up of the mother-infant pairs and provision of care and support for the entire families affected by HIV. The study was conducted among consenting patients in research protocol on Safety, Adherence and Effectiveness of Antiretroviral Therapy in Pregnant HIV-Infected Nigerians between 1st May and 30th September 2007. This protocol, which was approved by the Institutional Review Committee of University College Hospital and University of Ibadan, Nigeria, included screening for genitourinary infections as part of the pre-assessment evaluations prior to enrolment into treatment or care arm of the program.

Aseptically collected midstream urine samples (MSUs) were obtained from the symptom free attendees of clinic and sent on

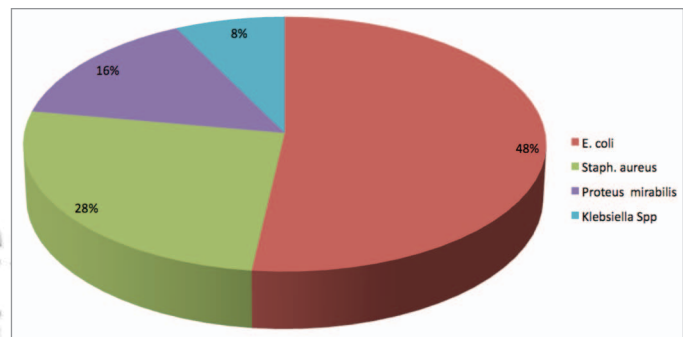


Figure 1. Microbial isolate from the urine specimen.

the same day for microbiological culture. Samples were immediately processed. Routine microscopy of centrifuged urine sediments was done and culture was on Cysteine-Lactose-Electrolyte-Deficient (CLED) and blood Agar plate. There was incubation of inoculated agar plates at 37°C for 24 hours and then identification of suspected pathogens was by biochemical and sugar utilization tests. Antibiotic sensitivity testing was by disc diffusion technique. Isolates were considered significant if there were $\geq 10^5$ colony forming units/mL (CFU/mL) with 2 or less isolates; doubtful significance if 10^4 – 10^5 CFU/mL; insignificant if $< 10^4$ CFU/mL. Mixed growths, in any count, of more than two organisms were considered to be contaminated.

Blood samples were also taken for the patients CD4 counts, viral load, packed cell volume along with other tests for Pre-assessment for eligibility for antiretroviral treatment or PMTCT services which include haematologic studies, blood chemistry studies, liver function tests, serum lipid studies and blood sugar levels. A proforma was designed to obtain information on the socio-demographic characteristics of the participants.

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