Prevalence of significant bacteriuria among symptomatic and asymptomatic homozygous sickle cell disease patients in a tertiary hospital in Lagos, Nigeria

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Abstract

Background: Patients with sickle cell disease have an amplified vulnerability to urinary tract infection, because of abnormally dilute and alkaline urine, which favors bacterial proliferation. This is due to altered blood flow in the renal vasculature, which causes papillary necrosis and loss of urinary concentrating and acidifying ability of the nephrons. Asymptomatic bacteriuria is common, but the prevalence in populations varies widely with age, gender, sexual activity and the presence of genitourinary abnormalities. The aim of this study was to determine the prevalence of significant bacteriuria in symptomatic and asymptomatic sickle cell patients in Lagos.

Materials and Methods: This was a cross-sectional study of patients attending the sickle cell clinics of Lagos State University Teaching Hospital, Ikeja. Single voided aseptically collected mid-stream urine was obtained from each patient and all samples processed immediately, were sent for urinalysis and culture. Isolates were considered significant if there were $\geq 10^5$ colony forming units per milliliter (CFU/ml) with two or less isolates, doubtful significance if $\leq 10^5$ CFU/ml. Significant isolates were selected for identification. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS, Inc., Chicago, Ill).

Results: A total of 100 consenting participants were recruited into the study. The mean age was: 23.42 ± 8.31 years and a range of 14-50 years. Only 9% (9/100) had significant bacteriuria while 44.4% (4/9) participants who had significant bacteriuria were asymptomatic. *Escherichia coli* was isolated in 66.6% (6/9) participants who had significant bacteriuria while *Klebsiella oxytoca, Klebsiella pneumonia* and *Staphylococcus aureus* (11.11%) was isolated in each of the remaining three participants.

Conclusions: Significant bacteriuria is found in only one-tenth of sickle cell patients, nearly half of the participants who had significant growth had asymptomatic bacteriuria.

Key words: Asymptomatic bacteriuria, prevalence, screening, sickle cell disease patients, significant bacteriuria

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Introduction

Sickle cell disease (HbSS) results from inherited abnormality of hemoglobin structure. The abnormal hemoglobin has valine substuiting glutamine at position six of the beta-globin chain.[1] The hemoglobin variant, sickle-cell hemoglobin produced, polymerizes at low oxygen tension, causing the characteristic sickle deformity of the red cells, increased mechanical fragility, shortened survival and chronic hemolytic anemia.[2]

HbSS is common in Nigeria with prevalence values ranging from 2% to 3% of the 140 million population[2] and is associated with increased frequency and severity of infections, especially with encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenza* as well as *Salmonella* species and *Escherichia coli*.[3] Reported in patients with HbSS is an amplified vulnerability to urinary tract infection (UTI) because of abnormally dilute and alkaline urine, which favors bacterial proliferation. This is due to altered blood flow in the renal vasculature, which causes papillary necrosis and loss of urinary concentrating and acidifying ability of the nephrons.[4] This predisposes them to recurrent UTI and subsequent renal damage.

"Asymptomatic bacteriuria" or asymptomatic urinary infection, is isolation of a specified quantitative count of bacteria in an appropriately collected urine specimen obtained from a person without symptoms or signs referable to urinary infection.[3] The microbiologic definition is usually greater than or equal to 10^5 colony forming units per milliliter (CFU/ml) of the same organism or organisms in two consecutive voided urine specimens for asymptomatic women, a single, clean-catch, voided urine specimen with 1 bacterial specie isolated in a quantitative count of 10^5 CFU/ml identifies bacteriuria in asymptomatic men, or a single catheterized urine specimen with one bacterial specie isolated in a quantitative count of 10^2 CFU/ml identifies bacteriuria in women or men[3,4] while for other authors, a single voided urine specimens for asymptomatic women and men is sufficient.[5-7]

The normal genitourinary tract is sterile, apart from the distal urethra. Asymptomatic bacteriuria occurs following the ascension of bacteria after the adherence of uropathogens to the bladder mucosa, up the urethra into the bladder, sometimes with subsequent ascension to the kidneys. Bacteria isolated from the urine of patients with asymptomatic bacteriuria usually originate as colonizing flora of the gut, vagina or peri-urethral area.[8]

Asymptomatic bacteriuria is common, but the prevalence in populations varies widely with age, sex, sexual activity and the presence of genitourinary abnormalities.[4] Uncomplicated UTIs occur most often in young healthy adult women and are easy to treat. However, in other patient groups, UTIs can have a complicated course, are more difficult to treat and often recur. Complicated UTIs occur most commonly in patients with abnormalities of the genitourinary tract. However, other conditions such as age over 65 years, treatment with immunosuppressive drugs, the presence of human immunodeficiency virus-infection and diabetes mellitus also predispose to an enhanced susceptibility for the development of a UTI with a complicated course.[9,10] There is a need to determine the prevalence of significant bacteriuria in symptomatic and asymptomatic sickle cell patients in Lagos.

Materials and Methods

Study population
This was a cross-sectional study of sickle cell patients attending the adult sickle cell clinics of Lagos State University Teaching Hospital (LASUTH), Ikeja. The clinic attends to between 15 and 20 HbSS patients on Mondays and Thursdays. In LASUTH, patients from age 14 years are referred from pediatric sickle cell clinic to adult clinic as there is no special sickle cell clinic for those between the ages of 14 and 18 years. During the study period between April and October 2012, all consenting patients who gave written and verbal informed consent and satisfied the study's inclusion criteria were recruited consecutively into the study. With the aid of a pre-tested structured questionnaire, data such as the socio-demographic and UTIs symptoms i.e. dysuria, hematuria, frequency, nocturia, fever, urgency, abdominal pain and hesitancy were collected from participants.

Ethics
The research was approved by the Ethics Review Committee of LASUTH.

Inclusion criteria
All consenting HbSS patients attending the clinic.

Exclusion criteria
1.   Non-consenting HbSS patients and
2.   All Haemoglobin SC patients.

Sample collection
A single voided aseptically collected mid-stream urine (MSU) was obtained from female and male patients and all samples sent on the same day to Microbiology Department for Urinalysis and Culture.

Laboratory analysis
Samples were processed immediately. Urinalysis was performed on aliquots of MSU specimen using Combi 10SL Uriscreen test strips. Bacterial culture was performed by streaking 0.001 ml of mid-stream collected urine with a calibrated loop on 5% sheep blood agar and cysteine

lactose electrolyte deficient agar plates. These agar plates were incubated at 35°C ± 2°C for 24 h under aerobic conditions. Isolates were considered significant if there were ≥10⁵ (CFU/ml) with two or less isolates, doubtful significance if ≤10⁵ CFU/ml. Mixed growths, in any count, of more than two organisms were considered to be contaminated. Significant isolates were selected for identification. API 20E (BioMerieux) was used for identifying the gram-negative bacilli while the morphology on plates and biochemical reactions were used in identifying the gram-positive cocci.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS, Inc., Chicago, Ill). The continuous variables were given as means ± standard deviation. The Pearson Chi-squared test was used to test for association between discrete variables. P value was considered to be statistically significant when <0.05.

Results

A total of 100 consenting participants were recruited into the study, the socio-demographic data presented in Table 1. Only 9% (9/100) participants had a history of dysuria while 91% (91/100) were asymptomatic. Only 4% (4/100) had pus discharge from the genital tract while majority 96% (96/100) had no pus discharge from the genital tract. None of the participants (100%) had hematuria. About half 49% (49/100) had a fever in the past 2 weeks while 51 of 100 (51%) had no fever in the past 2 weeks. About 30% (30/100) of the participants were on antibiotics at the time of this study and were excluded during data sub analysis. Only 40% (40/100) of the participants were sexually active.

Urinalysis of the samples [Table 2] showed 3% (3/100) tested positive to leucocytes and nitrates each while 7% (7/100) tested to blood, 19% (19/100) tested positive to protein, 2% (2/100) tested positive to glucose and ketones, 26% (26/100) tested positive to urobilinogen while 2% (2/100) tested positive to bilirubin.

Only 12.85% (9/70) had significant bacteriuria [Table 3]. *E. coli* was isolated in 66.6% (6/9) participants who had significant bacteriuria while *Klebsiella oxytoca, Klebsiella pneumonia* and *Staphylococcus aureus* (11.11%) was isolated in each of the remaining 3 participants.

Only 44.4% (4/9) participants who had significant bacteriuria were asymptomatic i.e., had no dysuria, hematuria, fever, urgency, abdominal pain, nor hesitancy. One was a male while three females. The four participants had *K. Oxytoca, E. coli, S. aureus* and *K. pneumonia* isolated each from their urine. Three of the participants with symptomatic bacteriuria had a fever in the past 2 weeks while two had dysuria.

There was no significant association between the isolates and history of fever in the past 2 weeks, $P = 0.583$, similarly no significant association existed between dysuria and the isolates $P = 0.306$.

Discussion

UTIs are among the most prevalent infectious diseases with a substantial health and financial burden on society.

Table 1: The socio-demographic data

Parameters	
Age (years)	
Minimum	14
Maximum	50
Mean	23.42±8.31
Gender	
Males	44/100 (44)
Females	56/100 (56)
Marital status	
Married	11/100 (11)
Single	86/100 (86)
Separated	3/100 (3)
Educational level	
Primary	6/100 (6)
Secondary	55/100 (55)
Tertiary	39/100 (39)

Table 2: Urinalysis findings of the study population

Parameters	Positive %	Negative %
Blood	7	93
Pyuria	3	97
Nitrite	3	97
Protein	19	81
Glucose	2	98
Ketones	2	98
Bilirubin	2	98
Urobilinogen	26	74

Table 3: The prevalence of bacteriuria and asymptomatic bacteriuria

Significant growth (%)	9/70 (12.85)
No significant growth (%)	91/100 (87.41)
Asymptomatic bacteriuria (%)	4/9 (44.4)
Symptomatic bacteriuria (%)	5/9 (55.5)
Isolates in asymptomatic bacteriuria (%)	
Escherichia coli	6/9 (66.66)
Klebsiella oxytoca	1/9 (11.11)
Klebsiella pneumonia	1/9 (11.11)
Staph. Aureus	1/9 (11.11)

About 40% of the study participants were sexually active, it is well- established that both hereditary and behavioral factors such as sexual intercourse and use of diaphragm,[11,12] determine the risk of having urinary infection and whether it will be symptomatic or not is determined by the virulence of the organism.[13]

Despite prevalence of significant bacteriuria (12.85%) reported in HbSS patients in this study, a high prevalence of 44.4% of asymptomatic bacteriuria was noted among those who had significant bacteriuria, Asymptomatic bacteriuria prevalence of 10.9% was reported amongst HbSS patients in Jamaica[14] and 14.6% in Lagos, Nigeria.[15] The high prevalence of asymptomatic bacteriuria obtained in this study compared with the Jamaican and previous Lagos study could be accounted for by different methodologies used. Whilst this study used the recommendations of Makuyana *et al.*,[5] Stein and Fünfstück[6] and Hooton *et al.*[7] in which a single clean-catch, MSU specimen was considered adequate in diagnosis for females, in the other two studies, female participants had two consecutive voided urine specimens cultures as recommended by Infectious disease Society of America guidelines.[4] Several studies have consistently confirmed patients with asymptomatic bacteriuria are at increased risk of symptomatic UTI and the latter being an important contributor to the development of chronic renal failure, hypertension and toxemia of pregnancy.[7,13] A positive urine culture even without symptoms is therefore considered potentially harmful.

However, with a 12.85% prevalence of significant bacteriuria and a high prevalence of asymptomatic bacteriuria reported in this study, none of the participants gave a history of gross hematuria while only 7% of them tested positive to blood when their urine samples were subjected to urinalysis. Lewis *et al.*[16] reported 0% of hematuria among HbSS patients. Sickling of red blood cells results in increased blood viscosity, microthrombi formation and papillary necrosis causing structural changes leading to hematuria.[17] Hematuria may originate from either kidney; although, a preponderance of left sided renal bleeding has been observed.[17]

Nearly, 19% prevalence of proteinuria obtained in this study is similar to 19.4% reported by McKie *et al.*[18] and 22.3% reported by Iwalokun *et al.*[15] amongst sickle cell patients in Lagos.

Abdu *et al.*[19] reported a proteinuria prevalence of 28% among adult HbSS patients in Nigeria they concluded based on other parameters done that proteinuria is a marker of renal insufficiency and routine screening for proteinuria may help detect those at increased risk of renal disease. Falk and Jennette[20] reported that generally, proteinuria occurred in 20-30% of patients with HbSS.

E. coli was the most common bacteria (66%) isolated in this study, it was also the most common isolate among Jamaicans[15] and previous study among Nigerians[7] 33% and 39.3% respectively. A wide range of other bacteria; however, may be isolated such as *Klebsiella oxytoca, E. coli, S. aureus* and *K. pneumonia.*

Patients with structural or functional abnormalities of the urinary tract, often with foreign material *in situ* and repeated antimicrobial exposure, frequently have other *Enterobacteriaceae* and gram-negative organisms, such as *Pseudomonas aeruginosa.* Gram-positive organisms including *Enterococcus spp* and coagulase-negative *Staphylococcus* other than *Staphylococcus saprophyticus* may be isolated more frequently from patients with asymptomatic compared with symptomatic infection.[8]

Almost all, three of four patients who had significant bacteriuria in this study were females; this is in keeping with previous studies, which reported a much higher prevalence in females due to the nature of their anatomy[21-23] compared with males. A limitation of this study was a lack of concurrent similar data collections in HbAA controls. The demonstration of a difference in prevalence of significant bacteriuria in patients with HbSS and non-HbSS patients would provide better population-specific recommendations.

Conclusion

Significant bacteriuria is found in only one-tenth of sickle cell patients, nearly half of participants who had significant growth had asymptomatic bacteriuria.

Acknowledgment

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