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Prevalence and antimicrobial sensitivity of asymptomatic bacteriuria among sickle cell anaemia patients in Aminu Kano Teaching Hospital, Nigeria

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Abstract: *Introduction:* Urinary tract infection (UTI) is a common cause of morbidity in children with sickle cell anaemia (SCA) and may present as asymptomatic bacteriuria. Asymptomatic bacteriuria is often under-diagnosed and may lead to long term renal complications.

Aim: This study compared the prevalence of asymptomatic bacteriuria, bacterial isolates and their antimicrobial sensitivity in children with and without SCA seen at Aminu Kano Teaching Hospital.

Subjects and Methods: This cross-sectional study recruited 139 children with SCA in stable state and 139 children without SCA aged 1-15 years. All children were tested for asymptomatic bacteriuria using clean catch or mid-stream urine samples. Children whose samples yielded significant bacteriuria (10^5 cfu/ml) had a repeat urine culture within a week to confirm asymptomatic bacteriuria.

Results: Both study groups had a mean age of 4.25 years \pm 3.04, and a male: female ratio of 1.4:1. The

prevalence of asymptomatic bacteriuria was higher in children with SCA(5.8%) compared to children without SCA (1.4%). Asymptomatic bacteriuria was more common among females in both study groups. The most common isolate in both groups was E. coli. Majority of the isolates were sensitive to quinolones, gentamycin and nitrofurantoin but resistant to commonly used antibiotics like amoxicillin and cotrimoxazole.

Conclusion: Asymptomatic bacteriuria is more common in children with SCA than those without SCA. Majority of isolated pathogens showed low susceptibility to ampicillin, amoxicillin, cotrimoxazole and nalidixic acid, this implies that the use of these drugs for empirical or first line treatment for UTI in children with SCA should be avoided.

Keywords: Urinary tract infection, Asymptomatic bacteriuria, Children, Sickle cell anaemia,

Introduction

Urinary tract infection (UTI) is a common cause of febrile illness in children and contributes to childhood morbidity and mortality globally especially in the developing countries.^{1,2}The term UTI simply implies the presence of organisms within the urinary tract and this encompasses both symptomatic and asymptomatic infections.³Asymptomatic urinary infection or asymptomatic bacteriuria has been defined as the finding of positive cultures with 10^5 colony forming units per mL of urine (CFU/ml) of the same organism in two consecutive urine specimens, in the absence of urinary symptoms.⁴The majority of patients with asymptomatic bacteriuria may not develop symptomatic UTI or adverse consequences, however this is not the case in certain conditions such as pregnancy, renal transplant and sickle cell disease.⁵⁻⁸

In sickle cell anaemia (SCA), there is an increase susceptibility to bacterial infections due to impaired immunological function.⁹The causative agents of some of these bacterial infections include those implicated in UTIs such as *Escherichia coli* and *Klebsiella* spp.¹⁰ Studies have reported an increase in the prevalence of UTI in children with SCA when compared to their counterparts without the disease, and also highlighted the clinical significance of asymptomatic bacteriuria in SCA.^{11,12}In addition to recurrent UTIs, other renal manifestations of SCA include sickle cell nephropathy, impaired urinary concentrating ability, acute kidney injury and chronic kidney disease (CKD).¹³ Repeated UTIs may lead to renal scarring and when coupled with compromised kidney function, puts the kidney at risk of further kidney damage.¹⁴

This study was designed to determine and compare the

prevalence of asymptomatic bacteriuria in children with and without SCD, and to assess the antibiotic sensitivity pattern of the isolated organisms.

Subjects and Methods

This cross-sectional study was conducted at Aminu Kano Teaching Hospital (AKTH) from July to September 2018. A total of 139 children with SCA (1 to 15 years) in stable state were enrolled as subjects and 139 aged and sex matched children without SCA as controls. Subjects and controls were enrolled consecutively in the Paediatric Sickle Cell Clinic and the Paediatric Outpatient Department (POPD) respectively.

Ethical approval for the study was obtained from the Aminu Kano Teaching Hospital Health Research Ethics Committee, while written consent from the guardians and assent in children > 7 years were obtained from the participants. Children who had fever, history suggestive of recurrent UTI, use of antibiotics during the preceding 2 weeks and those who declined consent were excluded from the study.

History was taken from the primary care givers and children who were old enough to respond to the researcher, and a systemic physical examination was performed looking for evidence of symptoms and signs associated with kidney disease.

Spot midstream urine sample was collected from each child into a sterile universal container with 1.8% boric acid. Before collecting the midstream urine in females, the perineum was cleaned with sterile water from anterior backwards, with the labia separated. In males, the glans penis and the urethral orifice were also cleaned with sterile water, with the prepuce retracted in the uncircumcised. Urine was collected into two sterile universal bottles; urine sample were collected at the same time of the day (early morning urine) throughout the data collection. Urine samples were examined immediately and where samples could not be worked on immediately; they were stored in the refrigerator at 4–8°C for not more than 12 hours. Each urine sample was examined with the Combi 10 urinalysis test strip to determine its protein, leucocyte esterase, and nitrite content. Positive reactions were measured in accordance with the manufacturer's guidelines.

Urine for microscopy was centrifuged at 2000 rpm for 5 min, the supernatant was discarded, and a wet preparation made from the sediments and examined under a 40x objective lens of a microscope for pus cells, red cells, and casts. Greater than 10 pus cells per high power field was regarded as significant pyuria.^{1,8} A loop calibrated to deliver approximately 0.001 ml urine was used for inoculation on cystine lactose electrolyte deficient and MacConkey agar plates. All plates were incubated at 37°C for 24 h for colony counts and reported as colony forming units (cfu) per milliliter.^{8,9} Thereafter, bacterial identification was done by standard laboratory methods.

Only samples that yielded pure bacterial growth of 10^5 cfu/mL were regarded as yielding significant bacteriuria. Counts between 10^4 and 10^5 were repeated while counts $<10^4$ cfu/mL were regarded as negative. Mixed growths (growth of more than one species in a sample) especially growth of normal skin flora, picked up during urine collection were regarded as contaminants and therefore disregarded. Second urine samples were collected from children with significant bacteriuria within seven days, and those whose second urine samples yielded significant bacteriuria were regarded as having a symptomatic bacteriuria.

Organisms were identified using standard identification techniques. Antimicrobial sensitivity was performed on significant isolates by the disc and diffusion method of strokes,¹⁰ using oxoids multi disc (Oxoid Ltd, Basingstoke, Hampshire England) with the following antibiotics amoxicillin, cotrimoxazole, nitrofurantoin, nalidixic acid, ciprofloxacin, ofloxacin, ceftriaxone, ceftazidime and gentamycin. Haemoglobin electrophoresis was done for all the controls, only children without SCA were enrolled as controls.

Statistical analysis

Data collected were entered into computer database and analyzed using STATA version 16.0 software package. Quantitative variables were summarized using measures of central tendency (mean and median) and measures of dispersion (standard deviation). Categorical variables were summarized using frequency and percentages Fisher's exact test for significant difference between SCA and non-SCA patients with UTI. Odds ratio was used to determine the odds of asymptomatic bacteriuria in children with SCA and those without SCA. A *P*-value of < 0.05 was considered statistically significant.

Results

Of the 139 children with SCA in steady state and 139 age and sex matched controls without SCA, there were 81 (58.3%) males and 58 (41.7%) females, giving a male:female ratio of 1.4:1 in each of the study groups. The mean age was 4.25 ± 3.04 years.

One hundred and twenty-three (88.5%) of the first urine samples examined in children with SCA, had insignificant growth, 5 (3.6%) had doubtful growth whereas the remaining 11 (7.9%) had significant growth. All the 11 with significant growth and the 5 with doubtful growths were cultured again, and only 8 (5.8%) of the samples had significant growth on second culture. Amongst the control subjects, 131 (94.2%) of the first urine sample had insignificant growth, 3 (2.2%) had doubtful growth whereas 5 (3.6%) had significant growth. Only 2 (1.4%) of the sample in the control had significant bacteriuria on second urine culture. Thus, prevalence of asymptomatic bacteriuria in children with SCA was 5.8% and 1.4% in the children without SCA. Children with SCA were 4 times likely to have asymptomatic

matic bacteriuria compared to those without SCA (OR=4.1832, 95% CI=0.87-20.07, p=0.07). Six (75.0%) of the SCA subjects who had asymptomatic bacteriuria were females while two (25.0%) were males giving a female to male ratio of 3:1. On the other hand, the two children without SCA who had significant bacteriuria were both females. There was no statistically significant difference in the prevalence of asymptomatic bacteriuria between the subjects and the controls (Fischer's exact, p = 0.053).

The most common isolates were Gram negative organisms that constituted 87.5% of isolates with *Escherichia coli* (*E. coli*) accounting for 62.5% of the cases in children with SCA and 50.0% in children without SCA

Table 1.

Aetiologic agent	SCA		Controls	
	N	%	N	%
E.coli	5	62.5	1	50
Klebsiella	2	25.0	1	50
S aureus	1	12.5	-	-
Total	8	100%	2	100%

Majority of the bacterial isolates were sensitive to Ofloxacin, ciprofloxacin, Gentamycin, ceftazidime, Ceftriaxone and Nitrofurantoin both the study groups. However, many of the isolates showed poor sensitivity to amoxicillin, cotrimoxazole, nalidixic acid, and chloramphenicol. Tables 2 and 3.

Only four (50.0%) of the children with SCA and one (50.0%) of the children without SCA with positive urine cultures had significant leucocyturia(10 WBC/mm³).Of the 8 children with SCA who had culture proven UTI, only 3 tested positive for leucocyte esterase, 1 for nitrite and 5 for mild proteinuria as shown in Table 4. While in the children without SCA, leucocyte esterase and nitrite were positive in only one of the patients with positive urine culture.

Organisms	Antibiotic sensitivity pattern													
	N	GM	AM	AU	CO	ND	NF	CEF	CFT	CFD	CIP	OFL	CPC	
<i>E.coli</i>	5	4 (80%)	3 (60%)	4 (80%)	2 (40%)	2 (40%)	4 (80%)	3 (60%)	4 (80%)	4 (80%)	5 (100%)	5 (100%)	2 (40%)	
<i>Klebsiella spp</i>	2	2 (100%)	-	1 (50%)	1 (50%)	1 (50%)	2 (100%)	1 (50%)	1 (50%)	2 (100%)	1 (50%)	2 (100%)	-	
<i>S.aureus</i>	1	1 (100%)	-	-	-	-	-	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	-	
Total sensitivity	8	7 (88%)	3 (38%)	5 (63%)	3 (38%)	3 (38%)	6 (76%)	5 (63%)	6 (76%)	7 (88%)	7 (88%)	8 (100%)	2 (25%)	

N= Number of isolates, GM=gentamicin, AM=amoxicillin, AU=augmentin, CO=cotrimoxazole, ND=Nalidixic acid, NF=nitrofurantoin, CEF=Cefuroxime, CFT=ceftriaxone, CFD=Ceftazidime, CIP=ciprofloxacin, OFL=Ofloxacin CPC=chloramphenicol

Organisms	Antibiotic sensitivity pattern													
	N	GM	AM	AU	CO	ND	NF	CEF	CFT	CFD	CIP	OFL	CPC	
<i>E.coli</i>	1	1	1	1	-	1	1	1	1	1	1	1	-	
<i>Klebsiella spp</i>	1	1	-	-	-	-	1	-	1	1	1	1	-	
Total	2	2	1	1	-	-	2	1	2	2	2	2	-	

N= Number of isolates, GM=gentamicin, AM=amoxicillin, AU=augmentin, CO=cotrimoxazole, ND=Nalidixic acid, NF=nitrofurantoin, CEF=Cefuroxime, CFT=ceftriaxone, CFD=Ceftazidime, CIP=ciprofloxacin, OFL=Ofloxacin CPC=chloramphenicol

Urinalysis	Culture result	
	Positive	Negative
<i>Proteinuria</i>		
Yes	5	1
No	3	130
<i>Leucocyte esterase</i>		
Yes	3	0
No	5	131
<i>Nitrite</i>		
Yes	1	0
No	7	131

Discussion

The findings from our study revealed that asymptomatic bacteriuria was more common in children with SCA when compared to their counterparts without SCA. The findings are in keeping with reports from previous studies and further emphasize the increased risk of UTIs in children with SCA.^{11,12,15} Although not statistically significant, our study also demonstrated that children with SCA are four times more likely to have asymptomatic bacteriuria compared to children without SCA. The

higher prevalence of ASB in children with SCA, may partly be explained by the compromised kidney and repeated vaso-occlusive episodes which leads to altered blood flow in the renal vasculature, causing papillary necrosis and loss of urinary concentrating and acidifying ability of the nephrons.¹² This results in the formation of abnormally dilute and alkaline urine, which favours bacterial proliferation, and increases susceptibility to UTI.

Our study reported a higher prevalence of asymptomatic bacteriuria in girls in both children with SCA and without SCA. Outside infancy, UTI is more common in girls than in boys.¹⁶ Several reasons including anatomical differences have been linked to the increased risk.¹⁷ This may explain why the incidence of asymptomatic bacteriuria was higher in females than males.^{18,19}

Similar to symptomatic UTI, the most common pathogen implicated in asymptomatic bacteriuria in both children with SCA and without SCA is gram negative bacteria especially *E. coli*.^{11, 15, 18, 20} Our study reports a similar finding with *E. coli* being the most common isolate followed by *Klebsiella spp.* In spite of the similarity in the pathogenic organisms implicated in UTI and asymptomatic bacteriuria, there are concerns that bacterial strains related to asymptomatic bacteriuria express fewer virulence factors than those bacterial strains involved in symptomatic UTI.²¹ This may be the reason for the varying opinion in terms of the medical importance of asymptomatic bacteriuria.^{22,23} These opinions may not be readily applicable to children with SCA as they are a special group with impaired immunologic function.⁹

Previous studies in children with SCA reported that the majority of the bacterial isolates in asymptomatic bacteriuria and symptomatic UTI showed low sensitivity or resistance to ampicillin, cotrimoxazole and nalidixic acid, and that sensitivity was more to cephalosporins and quinolones.^{11,15,20,24} Similarly, our study also detected that the isolated pathogens had poor sensitivity to amoxicillin and cotrimoxazole. This implies that the use of ampicillin, amoxicillin, cotrimoxazole and nalidixic acid for empirical or first line treatment for UTI in children with SCA should be avoided. It is possible that frequent use of antibiotics due to recurrent febrile illnesses and the routine use of penicillin as chemoprophylaxis in children with SCA may have contributed to the

low susceptibility recorded in these studies.

Urinalysis although a very useful tool for screening for urinary abnormalities, was not very helpful in our patients. Leukocyte esterase and nitrites were present in less than half of the patients with positive culture. However, mild proteinuria was seen in most of the patients with positive isolates. The low sensitivity of nitrite test has been reported in previous studies.^{11, 25} The sensitivity of the nitrite test in the present study may have been affected by the time of urine specimen collection, as spot urine samples were used. The use of first morning urine sample yields a higher sensitivity of the nitrite test compared to the use of randomly collected urine sample.²⁶

Conclusion

Asymptomatic bacteriuria is more common in children with SCA when compared to their counterparts without SCA. Similar to symptomatic UTI, asymptomatic bacteriuria is more common in girls. Most isolated pathogens showed low susceptibility to ampicillin, amoxicillin, cotrimoxazole and nalidixic acid and implies that the use of these drugs for empirical or first line treatment for UTI in children with SCA should be avoided.

Area for future research should include prospective studies to explore the risk factors and medical importance of asymptomatic bacteriuria in children with SCA.

Author contributions

Ibrahim: Concept, study design, data collection, data analysis, manuscript writing, critical review

Mudi: Concept, data analysis, manuscript writing, critical review

Asani: Concept, study design, manuscript writing, critical review

Aliu-Isah: Study design, data collection, data analysis, manuscript writing

Garba: Data collection, data analysis, manuscript writing

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