ABSTRACT

Background: Improved survival of sickle cell anaemia subjects has led to a growing population of adult sicklers. The pattern of urinary tract infections (UTI) and prevalence of nephropathy in adult Nigerians with sickle cell anaemia remains largely speculative as available data are mainly on paediatric population.

Methodology: Bio-data, information on symptoms of UTI/kidney damage and urine specimens were obtained from 100 consecutive stable Hemoglobin-SS subjects and 100 age and sex-matched healthy haemoglobin–AA controls aged ≥ 16 years were subjected to urinalysis, microscopy, culture and sensitivity as well as albumin and creatinine estimation.

Results: Significant bacteriuria was more frequent in subjects with HbSS than haemoglobin-A controls: 24(24%) vs 5 (5%), p=0.0002. Escherichia coli, Staphylococcus aureus and Klebsiella spp. accounted for 45.8%, 37.5% and 16.7% respectively of the isolates in HbSS subjects. Twenty-seven (27%) HbSS subjects had Albumin/ Creatinine ratio >300mg/g out of which 8 (29.6%) of these had UTI.

Antibiotics sensitivities in the HbSS subjects were: ceftazidime (79.1%), ciprofloxacin (58.6%), perfloxacin (58.3%), gentamycin (58.3%), nalidixic acid (37.5%) and nitrofurantoin (45.8%).

Conclusion: Urinary tract infection is commoner in HbSS than HbAA subjects. E.coli is the commonest cause. Empirical use of ceftazidime or ciprofloxacin, an oral preparation is suggested while awaiting sensitivity results.

Keywords: UTI, nephropathy, adults, sickle cell anaemia.

INTRODUCTION

Urinary tract infection (UTI) is common worldwide and occurs in all age groups [1]. It results from invasion of the genito-urinary tract by pathogenic micro-organism. Urinary tract infection is associated with clinical, histologic or immunologic evidence of host injury [1, 2].

Conditions predisposing to UTI include diabetes mellitus, pregnancy, hypo-proteinaemic states and sickle cell anaemia (HbSS) among others [3]. Sickle cell anaemia (HbSS) is an inherited disease in which normally disc-shaped red blood cells (RBCs) become crescent-shaped and function abnormally due to valine substituting glutamine at position six of the beta-globin chain. This causes the RBCs to clog up...
in blood vessels and vital organs. In Nigeria, 25-35% of its over 160 million population carry the haemoglobin S-gene; however the prevalence of HbSS is 2-3%, translating to a population of about 3.5 million Nigerians with HbSS [4].

Sickle cell anaemia patients are prone to infections due to asplenia which makes them susceptible especially to encapsulated organisms such as pneumococcus, salmonella and mycobacterium [5, 6]. Defective alternate complement pathways together with excessive iron deposition in tissues also provide a good milieu for bacteria growth [7]. Common sites of infection are the chest, bone, gall bladder and genito-urinary tract. However, UTI accounts for 75% of all infections in this population [8]. Chronic UTIs in sickle cell anaemia subjects is a cause of kidney damage which may have grave implications in terms of morbidity and mortality [9-12]. Increased survival to adulthood further increases the likelihood of HbSS individuals developing nephropathy [4].

Most studies on UTI in sickle cell anaemia have been conducted in the paediatric age group with only few data on adult subjects. Marked improvement in survival of HbSS individuals in Nigeria to adulthood has created a large pool of adults with increased potential for the development of UTI/nephropathy; these are largely uncharacterised. We studied the prevalence and pattern of UTI (significant bacteriuria) among adult HbSS subjects attending a tertiary care clinic in Lagos, Nigeria.

**METHODOLOGY**

**Patients selection:** A hundred sickle cell anaemia (HbSS) subjects (confirmed by low voltage electrophoresis using cellulose acetate paper) in steady state (defined as that period when the subject with sickle cell anaemia is free from infection, pain, or other acute disease processes for at least 3 months) [13], aged 16 – 65 years attending the sickle cell clinic of the Lagos University Teaching Hospital (LUTH) and who were not on immunosuppressive or antimicrobial therapy for ≥ 3 weeks prior to recruitment and sample collection were enrolled for the study. HbSS subjects with recent use of antimicrobial and or immunosuppressive therapy (< three weeks), not in steady state and who did not give their consent were excluded from the study.

One hundred age and sex matched HbAA (confirmed by low voltage electrophoresis using cellulose acetate paper) apparently healthy controls (defined as absence of history/symptoms suggestive of kidney disease or acute medical illness) not on immunosuppressive and or antimicrobial therapy for ≥ three weeks prior to recruitment and sample collection were enrolled. Controls subjects consisted of workers, patients’ relatives and students in Lagos University Teaching Hospital, Idi-Araba, Lagos.

**Sample collection:** Informed consent was obtained from all study subjects prior to commencement of study. All participants were properly educated and carefully instructed by the investigators on how to collect clean catch mid-stream urine into sterile universal bottles and correctly fill the self-administered questionnaire.

All urine samples so collected were sent to microbiology laboratory and processed.

**Laboratory methods:** Urinalysis was performed by dipstick method using the Bayer® diagnostics 9 point reagent strip (Bayer AG 51368 Leverkusen, Germany). The samples were screened for glucose, protein, nitrite, leucocytes, blood, pH, specific gravity and the strips were read by comparing with the colour charts on the reagent test bottle 30-60 seconds after insertion into the urine samples.

**Urine microscopy:** 5mls of each urine sample was centrifuged at 1500 revolutions per minute (RPM) for 5mins, the supernatant was discarded and the sediment re-suspended with 100ml of the patients urine sample using a micropipette. Fifty microlitre of re-suspended sediment was thereafter placed on a clean glass slide and examined under the microscope. The number of white blood cells and red blood cells were counted under high power field (HPF). Counts less than 5 per HPF were regarded as normal while counts of 5 and above per HPF were regarded as significant.

**Culture:** A calibrated standardized platinum wire loop was immersed vertically (after flaming and cooling to sterilize) into each thoroughly mixed urine sample. A loopful of urine was then carefully taken out vertically from the specimen bottle, discharged fully
and streaked on the entire surface of cystine lactose electrolyte deficient (CLED) medium and another loopful of urine into well dried blood agar plate. The two plates were incubated aerobically at 37°C for twenty four hours. The number of bacterial colonies was counted after 24 hours of incubation. Significant bacteriuria is defined as a minimum of 100,000 colony forming units (CFU) of a single strain per millilitre (ml) in any subject [14]. UTI was therefore defined in this study as presence of significant bacteriuria [15].

**Bacterial identification:** Identification of bacteria species isolated from urine sample of subject with significant growth of bacteria was performed using analytical profile index (API 20E, bio Merieux inc., USA) for gram-negative bacilli while the morphology on plates and biochemical reactions were used to identify gram-positive organisms.

**Sensitivity testing:** Three to five colonies of the organism to be tested were picked with a sterilized platinum wire-loop from pure culture plate of the organism and streaked evenly over a well-dried iso-sensed agar plate. A strip containing multiple antibiotics disc was carefully placed on the inoculated iso-sense agar plate, care being taken to ensure that all the antibiotics discs were firmly in contact with the culture medium. Culture plates were thereafter incubated aerobically at 37°C for twenty four hours. The zone diameters were measured and classified thereafter as sensitive or resistant respectively as follows: ceftazidime (sensitive ≥18 and resistant ≤14), gentamycin (sensitive ≥15 and resistant ≤12), cephalexin (sensitive ≥23 and resistant ≤14), co-amoxiclav (sensitive ≥20 and resistant ≤19), ciprofloxacin (sensitive ≥21 and resistant ≤15), perflaxin (sensitive ≥19 and resistant ≤15), nalidixic acid (sensitive ≥19 and resistant ≤13), nitrofurantoin (sensitive ≥17 and resistant ≤14), trimetoprim/sulfamethoxazole (sensitive ≥16 and resistant ≤10), amoxicillin (sensitive ≥29 and resistant ≤28). Only the organisms showing full sensitivity to the antibiotic tested were regarded as being sensitive. Random urine samples were collected from all subjects. Urine albumin was determined in first-morning void specimens by nephelometry (BN II, Dade Behring Diagnostic, Marburg, Germany). Urinary creatinine ratio (ACR, an index of renal damage) was calculated thereafter.

**Questionnaire:** Biodata of the study population were recorded. These included name, age, gender, level of education and religion. Information on symptoms suggestive of urinary tract infection and kidney damage including dysuria, nocturia, and increased frequency of micturition, loin pain, haematuria and frothiness of urine were elicited for. History of passage of stone in urine as well as sexual history were also elicited and documented.

**Data analysis:** Data were analyzed using EPI-info statistical package version 6.0 (CDC 1600 Clifton Road, Atlanta, GA30329-4027 USA). 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 <0.05 was considered to be statistically significant.

### RESULTS

A hundred HbSS subjects attending the Lagos University Teaching Hospital out-patient clinic as well as one hundred HbAA control subjects participated in the study. The HbSS group consisted of 55 females (55.0%) and 45 males (45.0%) whose ages ranged from 16-53 years. The HbAA (control) group consisted of 52 males and 48 females whose age ranged from 17-54 years. The mean age of the HbSS and HbAA subjects were 25.34 ± 7.88 years and 25.95 ± 8.45 years respectively (p=0.6). Table 1 shows the age and gender distribution of the study population.

The frequency of occurrence of symptoms of urinary tract infection in the study population is shown in table 2. Loin pain, the commonest symptom, occurred in 60% of HbSS subjects.

Thirteen HbSS and 5 HbAA subjects had significant pyuria i.e. ≥5 pus cell/hpf. Oxalate crystals were present in 4 HbSS subjects while 2 HbAA subjects had oxalate crystals. Yeast cells were found in two HbSS subjects but none of the HbAA subjects had yeast cells in their urine samples.

Out of 27 HbSS subjects with dysuria, 7 had significant bacteriuria, while only one of the 5 HbAA subjects with dysuria had significant bacteriuria (p=0.78) Out of the six HbSS subjects with haematuria, only one had significant bacteriuria while none of the
HbAA control participants with haematuria had significant bacteriuria (Fisher exact=1.0). Thirteen of the 60 HbSS subjects with loin pain had significant bacteriuria while none of the 8(8%) HbAA control subjects having loin pain had significant bacteriuria (Fisher exact=0.3).

Twelve (30%) of 40 sexually active HbSS subjects had UTI, while only 5 (11%) of 46 sexually active HbAA control subjects had UTI (χ²=4.94, p=0.026).

A total of 9 (9%) HbSS subjects had sexual contact 3 weeks prior to this study, 2 of which had UTI, while one of the 24 HbAA control subjects who had sexual contact 3 weeks prior to the study had UTI (Fisher exact=0.7).

Thirty HbSS subjects had leucocyturia out of which 13 (43%) had significant bacteriuria. One of the 2 HbAA subjects with leucocyturia had significant bacteriuria (p=0.0001). Nitrituria was present in 17 HbSS subjects while 2 HbAA subjects had nitrituria.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Response</th>
<th>HbSS subjects</th>
<th>Significant bacteriuria</th>
<th>HbAA subjects</th>
<th>Significant bacteriuria</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n)</td>
<td>(n)</td>
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<td>7</td>
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<td></td>
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<td>1</td>
<td>1.0*</td>
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<td></td>
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<td>94</td>
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<td>16</td>
<td></td>
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</tr>
</tbody>
</table>

UTI- urinary tract infection, *Fisher’s exact

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Thirty HbSS subjects had leucocyturia out of which 13 (43%) had significant bacteriuria. One of the 2 HbAA subjects with leucocyturia had significant bacteriuria (p=0.0001). Nitrituria was present in 17 HbSS subjects while 2 HbAA subjects had nitrituria.

Urine culture: A total of 24 and 5 cultures were positive in the HbSS and HbAA subjects respectively (p=0.001). E. coli was isolated in 11(45.8%), Staphylococcus aureus in 9(37.5%) and Klebsiella spp in 4(16.7%) urine samples of HbSS subjects. The isolates obtained in the HbAA controls were: E. coli 3(60%), Staphylococcus aureus 1 (20%) and Klebsiella specie 1 (20%).
Seventeen (71%) of the 24 positive cultures in the HbSS subjects and 2 (40%) of the 5 positive cultures in the HbAA subjects had nitrituria. Nineteen (79%) of the 24 culture-positive HbSS subjects had pH of 5 while five of the positive cultures had pH of 6. Among the HbAA subjects, two each of the positive cultures had pH of 5 and 6 respectively while one positive culture had pH of 8 ($\chi^2 = 13.5$, $p$-value = 0.0002). Table 3 shows the sensitivity pattern of the micro-organisms to antibiotics.

Twenty-seven (27%) of HbSS subjects had Albumin/Creatinine ratio >300mg/g out of which 8 (29.6%) of these had UTI but one HbAA subject had albumin/creatinine ratio of >300mg/g and none had UTI ($\chi^2 = 0.46$, $P$ value = 0.498).

**DISCUSSION**

This study evaluated the prevalence of UTI (significant bacteriuria) and nephropathy in adult HbSS subjects, highlighting the clinical and laboratory characteristics of UTI in the study population. It also identified the common aetiologic agents of UTI in the study population and their antimicrobial sensitivity.

The prevalence of UTI among adult sickle cell anaemia subjects from this study was 24% and 5% in the HbAA control subjects. Akinbami et al. reported a prevalence of 12.85% while Iwalokun et al documented a prevalence of 18.4% in their respectively studies on HbSS population [16, 17]. The higher prevalence in our own study may be due to the fact that Akinbami et al. had a younger age group (14-55 years) coupled with the fact that some of their subjects were on antibiotic at the time the study was commenced while Iwalokun et al included children in their own study population. However, there was a statistically significant relationship between recent sexual activity and UTI ($p = 0.003$). Those exposed to sexual activity prior to the survey had higher prevalence of UTI. This finding, like in the general population is expected as sexual activity increases the risk of UTI [18].

This is similar to the findings of other workers[19-21]. Past history of UTI in HbSS subjects was also found to be a risk factor for another UTI ($p = 0.008$). This finding is not unexpected because sickle cell anaemia subjects are prone to having recurrent UTI due to renal ischaemic damage [3].

There was a high occurrence of history of loin pain in the HbSS population, however this was independent of the presence of significant bacteriuria ($p = 0.5$). Although loin pain is a symptom of upper UTI, it is possible that bone pains from the back were reported as loin pains in HbSS subjects: this may explain the high frequency of loin pain in the HbSS subjects. Renal infarction may also be responsible for some of the loin pain [22].

Haematuria may be the first complaint in HbSS patients [23]. In this study, 6% of the HbSS subjects had hematuria but only one had UTI (Fishers exact = 1). This is possibly so because haematuria can
occur in HbSS subjects without UTI as a result of renal medullary vascular obstruction, papillary necrosis and neoplasms [24-26]. This is lower than 25% reported by Akinkugbe et al in an earlier study in Ibadan [27]. Dysuria was found in 27% of HbSS subjects out of which only 7% had UTI. New-onset vaso-occlusive crisis without UTI which may simulate dysuria in HbSS subjects [28].

The lower frequency of UTI in the HbAA subjects compared with the HbSS group may be due to the higher activity of urinary siderocalin which restricted bacterial growth in the HbAA subjects [29]. Also, HbSS subjects are known to have iron overload which encourages bacterial growth [30].

Thirteen (43%) of the thirty HbSS subjects with leucocyturia had significant bacteriuria while one of the two HbAA subjects with leucocyturia had significant bacteriuria (P=0.0001). Leucocyturia has also been documented as a marker of UTI in other studies [2,31-33]. Interestingly, significant bacteriuria was also found in 16% (n=11) of HbSS subjects without leucocyturia. This may be due to the poor leucocyte response that may occur in HbSS subjects[33].

Escherichia coli specie was the most common organism isolated from the urine sample of most HbSS subjects with UTI accounting for 45.8%. This was closely followed by Staphylococcus aureus (37.5%) and Klebsiella specie (16.7%). In the HbAA control group, Escherichia coli specie was also found in majority of subjects, constituting 60% of the positive cultures while Staphylococcus aureus and Klebsiella specie were isolated in 20% each of the HbAA subjects. This finding is similar to those of Akinbami et al. and Iwalokun et al. who in two separate studies isolated was E.coli as the most common organism followed by Klesiella specie and Staphylococcus specie [16, 17].

Two HbSS subjects and none of the HbAA controls had yeast cells cultured from their urine. Candiduria occurs in usually in the setting of immunosuppression. However, there has been a non-conclusive debate on whether or not there is immunosuppression in stable sickle cell anaemia subjects [34-37].

The anti-microbial agent to which E. coli and Klebsiella specie were most sensitive were Ceftazidine, ciprofloxacin and perfloxacin while Staphylococcus specie was sensitive to co-amoxiclav. Ciprofloxacin and Perfoxacin may be preferred in uncomplicated UTI due to the availability of oral preparations and minimal renal side effect profile. This finding is similar to that of Brown et al. who found Ciprofloxacin as useful first line drugs in the treatment of UTI [38].

This study shows that 27% of HbSS subjects had evidence of nephropathy (albumin/creatinine ratio >300mg/g) as opposed to 1% in HbAA subjects, although a sizeable number of both study populations had UTI. Sickle cell anaemia is a risk factor for nephropathy because of chronic ischaemic damage4. Our finding is similar to that of Abdu A et al. in northern Nigeria [39]. Other studies have shown varying values. For instance, Bolarinwa et al. [40] found evidence of nephropathy in 50% of their study population while Iwalokun BA, Aleem A et al. and Ephraim RK et al. reported evidence of nephropathy in 22.3%, 41% and 68.4% respectively [16, 40-42]. This variability might have resulted from different indices used to define nephropathy as well as environmental factors. These studies however show the high prevalence of nephropathy in HbSS study population.

CONCLUSION AND RECOMMENDATION
Urinary tract infection is common in HbSS subjects, with a prevalence of 24% in steady state. The presence of leucocytes and or nitrite on urinalysis is highly suggestive of UTI in adult HbSS subjects.

Ciprofloxacin and Perfoxacin are oral antimicrobial agents which may be used empirically while awaiting culture and sensitivity results. Nephropathy is common in HbSS subjects. Routine urinalysis is recommended in all HbSS subjects for early detection and treatment to delay the progression to end-stage kidney disease.

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