ORIGINAL ARTICLE

USEFULNESS OF NITRITE TEST IN SCREENING FOR URINARY TRACT INFECTION IN CHILDREN WITH SICKLE CELL ANAEMIA

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ABSTRACT

Background: Screening for urinary tract infection (UTI) among children with SCA can lead to early detection of UTI in childhood. This may necessitate further evaluation and early effective interventions with reduction in the number of individuals who develop end-stage renal disease (ESRD). **Objectives:** To evaluate the usefulness of nitrite test in screening for UTI in children with SCA. **Methods**: Two hundred and seventy two children with SCA both in steady state and in crisis (anaemic and vaso-oclusive), aged 6 months to 15 years were screened for significant bacteriuria. The study was conducted over a period of 8 months (February to September, 2012). Urine samples were aseptically collected and incubated aerobically at 37°C for 24 hours. The study was conducted in the paediatric medical ward, emergency paediatric unit and institute of child health, Banzazzau all of the Department of Paediatrics, Ahmadu Bello University Teaching Hospital, Zaria. Children whose urine samples yielded $\geq 10^5$cfu/ ml of urine on two consecutive cultures were regarded as having significant bacteriuria. **Results:** Bacterial isolates were detected in 22 (8.1%) of the 272 subjects with SCA. Of the 272 children with SCA, 66 (24.3) had positive nitrite test. The nitrite test has the sensitivity, specificity and positive and negative predictive values of 68.2%, 79.6% and 22.7% and 96.6% respectively. **Conclusion: N**itrite test is not a useful screening tool for the diagnosis of UTI in SCA children.

KEYWORDS : Bacteriuria, Children, Nitrite test, Screening, Sickle Cell Anaemia.

INTRODUCTION

Urine screening tests are often used in children to guide early diagnosis and treatment of UTI and to establish whether these tests are sufficiently sensitive to avoid urine culture in children with negative results. These tests are not designed to replace urine culture, the reference standard, because they neither identify the causal organism nor establish antibiotic sensitivities that guide the choice of antibiotic.[1] Any urine screening test should be compared with the reference standard (urine culture) for diagnosis of UTI. But urine culture has practical problems: 18 – 48 hours are needed for detection of bacterial growth, it is expensive and needs a microbiological laboratory with skilled technicians [1].

Validity of a screening test depends on the test's sensitivity, specificity, positive and negative predictive values. Sensitivity of a test is the ability of a test to correctly classify an individual as 'diseased' while the ability of a test to correctly classify an individual as disease-free is called the test's specificity.[2] Positive predictive value is the percentage of patients with a positive test who actually have the disease while the specificity of a test is the percentage of patients with a negative test who do not have the disease.[2]

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Various urine screening tests are used in the detection of suspected UTI and nitrite test is one of the useful screening tools for UTI in children with SCA. Nitrite in the urine is produced by the action of bacteria on dietary nitrate through the action of the bacterial enzyme, nitrate reductase and this is indicated by the development of pink colour on the pad within 60 seconds.[3-7] This reduction is time dependent because positive test requires a prolonged bladder incubation time of about 4 hours of significant residual urine.[7] Gram negative organisms like E. coli, Klebsiella spp and Proteus spp are implicated in the conversion of nitrate to nitrite through an enzyme, nitrate reductase. However, certain organisms such as Staphylococcus species, Enterococcus species and Pseudomonas species do not produce nitrate reductase, so false negative results will be obtained when infection is due to these organisms.[3]

Other screening tests like urine dipstick for leukocyte esterase, proteinuria and haematuria as well as microscopic test for pyuria may detect UTI but with a lot of shortfalls as compared to nitrite test. In positive nitrite test,Gram-negative bacteria reduce dietary nitrate to nitrites and it is very easy, quick to perform and relatively cheap.[7] Leukocyte esterase test uses an enzyme leukocyte esterase that suggests the presence of leukocytes in urine.[2] The test is, however, not commercially available and not suitable for non-potty trained children. Proteinuria and haematuria are not very reliable because the tests are associated with false positive results. Pyuria is more time consuming, more expensive and involved urine centrifuging and microscopic examination.[2]

Urinary tract infection is one of the significant causes of morbidity and mortality in children, especially those with SCA who have been reported to be at increased risk of infection.[6,8] More so, UTI may lead to renal scarring with subsequent development of hypertension and end-stage renal failure. Urine culture remains the gold standard for the diagnosis of UTI but takes at least 48 hours to give a result.1[1,9] Because of the clinical importance of early diagnosis and delays with the reference standard, screening such children for UTI by simple means is very important with a view to instituting prompt treatment and prevent renal scarring. This study, therefore, aimed to evaluate the significance of nitrite test in detecting UTI in children with SCA.

MATERIALS AND METHODS

This cross-sectional study consisted of consecutively selected children with SCA (in steady state and in crisis) aged 6 months to 15 years. The study was conducted in the paediatric medical ward, emergency paediatric unit and institute of child health, Banzazzau all of the Department of Paediatrics, Ahmadu Bello University Teaching Hospital, Zaria. The study was conducted over a period of 8 months (February to September, 2012). Children with SCA who were excluded from the study included those who were on antibiotics one week preceding enrolment into the study, those with confirmed (or suspected) congenital urogenital anomalies and those who had recent (<1 week) manipulative urogenital procedure (like catheterization and cystoscopy). Also those whose parents or guardians did not consent and those with HbSC and other forms of sickle cell disease other than SCA were excluded. Ethical approval was obtained from the Ahmadu Bello University Teaching Hospital Research Committee and a written consent obtained from guardians of subjects.

Urine sample collection and urinalysis

For each patient that has achieved bladder control, midstream urine specimen (5mls) was aseptically collected at the time of presentation into two sterile universal bottles. For adolescent girls a trained female resident doctor assisted in collection of the specimens. For infants, moribund patients and children who were not toilet trained, suprapubic bladder aspiration (SPA) was used for obtaining the urine specimens. All urine specimens were obtained under aseptic procedures as described by Anochieetal 1.0 in order to minimize bacterial contamination of the urine. None of the subjects was given water to induce diuresis as this may reduce the bladder time and reduce the likelihood of the bacteria to convert nitrates to nitrites. Samples were analysed within an hour of collection, otherwise they were kept in a refrigerator and or preserved in boric acid and analysed within 3-5 hours. Five milliliters of the urine was immediately subjected to urinalysis using Multistix 10SG (Bayer corporation, USA, 1999) according to the manufacturer's instructions. An aliquot of non-centrifuged urine was tested by dipping the multistix strip into the urine for the presence of nitrate-reducing bacteria. Nitrite test utilizes an amine impregnated pad to detect the presence of urinary nitrite.4The test result was considered positive if the dipstick turned pink or red for nitrites within 1 - 2 minutes of contact with the urine.11

A portion of another aliquot was inoculated onto blood and MacConkey agar plates and incubated aerobically at 37oC for 48 to 72 hours. A pure colony count of ≥ 105 organisms/ ml of urine were considered a significant growth. Other sets of culture plates were incubated in carbon dioxide extinction jar at the same temperature for isolation of anaerobes. In case of significant bacteriuria, systematic bacteriology and biochemical tests using standard techniques; catalase, oxidase, sugar fermentation, motility, urease, citrate, indole, hydrogen sulfide and gas production were carried out based on bacterial gram reactions. Antimicrobial sensitivity test were carried out using modified Kirby-Bauer's diffusion methods where zones of inhibition were measured.[12] Those with positive culture results were treated accordingly.

Data analysis

Data was analyzed using Epi Info version 3.5.3 statistical software. Values were expressed as frequency, mean and standard deviation. Chi-square test was used to determine the level of significance. P-values less than 0.05 was considered significant.

RESULTS

Two hundred and seventy two subjects with SCA aged six months to 15 years were studied. Their mean age was 6.4 ±3.8 years. There were 156 (57.4%) males and 116 (42.6%) females with male to female ratio of 1.3: 1 (Table I). Of the 272 children with SCA, 87 (32%) were in crisis and 185 (68.0%) were in steady state. Of the 87 subjects with SCA in crisis, 55 (63.2%) had vaso-oclussive crisis while 32 (36.8%) had anaemic crisis. Of the 272 children with SCA, 66 (24.3%) had positive nitrite test out of which 36 (23.1%) were males and 30 (25.9%) were females and the difference was not statistically significance ($\chi 2=0.15$, $p=0.70$). Positive nitrite test was commonly found among the under-fives occurring in 29 (43.9%) children while 19 (28.9%) and 18 (27.2%) children with positive nitrite test were among the 5-9 years and 10-15 years respectively (table I).

Of the 272 children with SCA studied, 22 had significant bacteriuria giving an overall prevalence of 8.1%. A total of 22 bacteria were isolated from the urine samples, mainly gram negative organisms. The most frequently isolated organisms were Escherichia coli (11; 50.0%) and Klebsiella pneumonia (5; 22.7%). The least common isolates were Staphylococcus aureus(2; 9.1%) and Salmonella typhi(1; 4.6%; Table II). The organisms were mainly sensitive to ceftriazone and resistant to co-trimoxazole.

Among the study population, 66 (24.3%) had positive nitrite test while 206 (75.7%) had negative nitrite test. Of the 87 subjects with SCA in crisis, 34 (39.1%)had positive nitrite test while 32 (17.3%) out of the 185 subjects with SCA in steady state had positive nitrite test. Positive nitrite test was commonly associated with SCA in crisis than insteady state; $\chi 2=15.3$, $p=0.00$. This is shown in Table III. Among the 32 subjects with SCA with anaemic crisis, 20

(62.5%) had positive nitrite test while 14 (25.5%) of those with vaso-oclussive crisis had positive nitrite test. There were more subjects with anemic than with vaso-oclussive crisis; $\chi2=11.7$, p=0.001. Table III.

Of the 22 children with SCA and significant bacteriuria, 15 (68.2%) had positive nitrite test, while only 78 (31.2%) out of 250 with no bacteriuria had positive nitrite test. The screening test had a sensitivity and specificity of 68.2% and 79.6% respectively. The positive and negative predictive values were 22.7% and 96.6% respectively. Nitrituria was significantly associated with increased risk of bacteriuria ($p<0.002$) (Table IV).

Table 1: Age And Sex Distribution of SCA Subjects With And Without Positive Nitrite Test

	Positive nitrite test, n (%)		Negative nitrite test, n (%)		
Ages (Years)	Males	Females	Males	Females	Total n (%)
< 5	15 (41.7)	14 (46.7)	33 (27.5)	30 (34.9)	87 (32.0)
5-9	10 (27.8)	10 (27.8)	38 (31.7)	31 (36.0)	93 (34.2)
10-15	11 (30.5)	7 (23.3)	49 (40.8)	25 (29.1)	92 (33.8)
TOTAL	**36 (100)**	**30 (100)**	**120 (100)**	**86 (100)**	**272 (100)**

Table 2: Urinary Bacterial Isolates Among Subjects With SCA

Organisms	Frequency (%)
Escherichia coli	11 (50.0)
Klebsiella species	5 (22.7)
Proteus species	3 (13.6)
Staphylococcus aureus	2 (9.1)
Salmonella typhi	1 (4.6)
TOTAL	**22 (100.0)**

Table 3: Nitrite Test Among Children With Sca In Crisis And In Steady State

	SCA in crisis, n (%)		SCA in steady state, n (%)	Total
Nitrite test	Anaemic crisis	Vaso-oclusive crisis		
Positive nitrite	20 (62.5)	14 (25.5)	32 (17.3)	66 (24.3)
Negative nitrite	12 (37.5)	41 (74.5)	153 (82.7)	206 (75.7)
TOTAL	**32 (100.0)**	**55 (100)**	**185 (100.0)**	**272 (100.0)**

Table 4: Urine Microscopy (nitrituria) And Culture

	Urine culture		
Nitrituria	Significant growth (%)	Insignificant growth (%)	Total
Positive nitrite	15 (68.2)	51 (20.4)	66 (24.3)
Negative nitrite	7 (31.8)	199 (79.6)	206 (75.7)
TOTAL	**22 (100)**	**250 (100)**	**272 (100)**

($\chi2=22.59$, p=<0.00002)

Sensitivity = 68.2%
Specificity = 79.6%
Positive predictive value = 22.7%
Negative predictive value = 96.6%

DISCUSSION

This study revealed that there were more children with positive nitrite test among children with SCA in crisis as compared to those children with SCA in steady state. This finding may be explained by the fact that the SCA crisis may be precipitated by *E. coli or klebsiella spp* UTI as these were the commonest organisms isolated in this study. These organisms can actually reduce nitrate to nitrite. Nitrituria being commoner among children with SCA in anaemic crisis as compared to those with SCA in vaso-occlusive crisis in this study may be due to*E. coli* or *Klebsiella spp* urosepsis as a precipitating factor for the anaemic crisis due to haemolysis. The finding of 66 children with SCA with positive nitrite test in this study was lower than that reported by Powell *etal*[5] where they reported positive nitrite test in 83 and 104 children with asymptomatic and symptomatic UTI respectively. This could be due to the fact that there were more symptomatic than asymptomatic children in their study.

The 68.2% sensitivity obtained in this study is higher than that reported by Brown *etal*[6] and Wamanda*etal*[12] who reported the sensitivities of 18.9% and 28.9% respectively. Goldsmith and Campos[13] also reported a lower sensitivity of 29.0%. The higher sensitivity observed in this study as compared to the other studies[6,12,13] may be due to type of organisms isolated from the urine of these subjects. Gram negative organisms were the predominant pathogens isolated and these pathogens can reduce nitrate to nitrite.Some of these subjects may have increased urine bladder time precipitating the conversion of nitrate to nitrite as none of these subjects studied was given water to induce diuresis and reduce bladder urine time. Gram negative organisms need to be in the bladder for about 2 - 4 hours for them to convert dietary nitrate to nitrite.[7]

The high specificity (79.6%) of nitrite test reported in this study is in keeping with findings by other workers.[6,14,15] The high specificity of this test indicates that the test could correctly identify 79.6% of the subjects without the disease as true negatives. Since this study revealed a screening test with low sensitivity and high specificity, nearly all of the false positives may be correctly identified as disease negative. A previous study[16] revealed that a test with a high sensitivity but low specificity results in many patients who are disease free being told of the possibility that they have the disease and are then subjected to further investigations. The low positive predictive value of 22.7% observed in this study is in contrast with that observed by Wammanda*et al*[12] who reported value of 80.8%.

In conclusion, the low sensitivity, high specificity and very low positive predictive value obtained in this study do not support the usefulness of the nitrite test as a screening tool for UTI. There is a need for urine culture, the gold standard, for the diagnosis of UTI in children with SCA.

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