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Correlation of Nitrite, Leucocyte Esterase and Protein Detection to Diagnosis of Urinary Tract Infections

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Authors' contributions

This work was carried out in collaboration between both authors. Author KTW designed the study and wrote the protocol. While author ILO managed the literature searches, analyses of the study and draft of the manuscript. Both authors managed the laboratory processes, read and approved the final manuscript.

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ABSTRACT

Aim: To determine the relationship between detection of nitrite, Leucocyte esterase (LE) and protein in urine and significant bacteriuria.
Study Design: Cross-sectional descriptive study.
Place and Duration of Study: Department of Medical Microbiology and Parasitology, University of Port Harcourt Teaching Hospital, between March and September 2015.
Methodology: 240 urine samples were analyzed. Dipstick analysis using Combi-UriScreen 10SL reagent strips (Axiom Medical limited, UK) and culture for significant bacteriuria were performed according to manufacturer's instruction/ using standard protocols. Data was coded, entered into Microsoft Excel ® version 2010 and analysed using *Epi-Info version* 7.02. Categorical data were presented as frequencies and percentages using tables. Univariate analysis using logistic regression (Odds Ratio) was used to determine the association between the presence of nitrite, LE

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and protein and significant bacterial yield in urine. A *P*-value of ≤ 0.05 was considered statistically significant. Likelihood ratios were calculated.

Results: 23 (23.2%) out of 99 samples with significant bacteriuria were nitrite positive, while 42 (42.4%) and 45 (45.5%) were positive for leucocyte esterase and protein respectively. Nitrite (*P* = 0.001, OR = 5.03, *95% CI = 2.02-12.93*) and leucocyte esterase positivity (*P* = 0.001, OR = 3.59, *95% CI = 1.91-6.80*) were significantly associated with significant bacteriuria while proteinuria was not *(P* = 0.989, OR = 1.03, 95% CI = 0.60-1.79*).* Nitrite positivity alone had the best positive likelihood ratio (4.09, 95% CI: 1.91, 8.78) followed by the combination of nitrite and LE positivity (3.65, 95% CI: 1.90, 7.03).

Conclusion: The use of dipstick analysis of urine as a screening tool for samples to be cultured may be a very effective way of reducing laboratory costs and wastage of man hours, which both ultimately improve the effectiveness of clinical laboratories especially in resource-poor settings.

Keywords: Urinary tract infection; nitrite; leucocyte esterase; protein; dipstick analysis.

1. INTRODUCTION

Urinary tract infections (UTIs) are inflammatory diseases of the urinary tract due to the presence of micro-organisms. They are very commonly encountered in medical practice particularly in women, and cause significant morbidity [1,2].

UTIs have the potential for serious and life-threatening sequelae if left untreated or undertreated. Possible sequelae include pyelonephritis which can lead to renal scarring and sepsis and in pregnant women, intrauterine growth restriction and low birth-weight infants, increased risk of preterm labor, preterm birth, pregnancy-induced hypertension, preeclampsia, amnionitis and anemia [2].

The gold standard for the diagnosis of a urinary tract infection is the detection of the pathogen in the presence of clinical symptoms. Confirmatory diagnosis of UTI requires that the urine is cultured. The standard diagnostic criterion for UTI is the presence of significant bacteriuria which is generally defined as the presence of 10^5 colony forming units (CFU) of bacteria per milliliter (ml) of a properly collected mid-stream urine sample. However, occasionally in symptomatic patients, in children, men and pregnant women or in the presence of antibiotics, the minimum count may fall to 10^3 to 10^4 CFU of bacteria per ml or lower [3].

In most clinical laboratories, urine cultures are the most common type of culture requested for and the most frequently processed samples, accounting for 24%–40% of submitted cultures with as many as 80% of these submitted from the outpatient setting, with the processes undertaken being quite cumbersome, time consuming, expensive and requiring specialized human and material resources. However, a significant number of these turn out to be negative [1,4].

Screening methods are available that attempt to rapidly separate those specimens containing significant counts of bacteria from negative specimens. These methods have been said to compare well with culture on specimens containing 10^5 CFU of bacteria per ml or greater but perform poorly when colony counts are lower [3].

Gram staining for screening urine specimens is rapid and economical with respect to the stains required but is labor intensive and requires specialized training. On the other hand, Urine dip sticks, usually multi-sticks, which is one of the most frequently used instruments for diagnosis of suspected UTI are commercially available, they detect nitrite; the result of action of bacterial nitrate reductase on nitrate, leukocyte esterase (LE); an enzyme produced by neutrophils as well as protein and blood (as indicators of inflammation) among others. Dipstick tests are rapid, inexpensive, and simple to perform, but their sensitivity has been reported to be low in some patient populations [1,3].

Leukocyte esterase (LE) is used to test for the presence of white blood cells in the urine as an indication of an inflammatory process of the urinary tract. It is produced by neutrophils and indicates pyuria, which is associated with UTI. The principle of leukocyte esterase (LE) test is that the esterase released from activated neutrophils reacts with indoxil carbonic acid ester; indoxil is released by the esterase and reacts with diazoniun salt and is oxidized yielding a violet azo dye. The intensity of the color is correlated to leukocyte counts according to the fabricant [5,6]. Positive test results are clinically

significant. Organisms other than uropathogens can produce leukocyte esterase therefore; this is a highly sensitive (72% to 97%) but poorly specific (41% to 86%) test for UTI in women [7-9]. A positive Nitrite result usually indicates infection, with a specificity of 92% to100% and a sensitivity of 19% to 48% however, a negative result does not necessarily rule out infection if the patient is symptomatic because false negatives may occur if the bacterial load is low, if the implicated bacterium doesn't produce nitrate reductase e.g Enterococci or the urine has not been present in the bladder for a time sufficient for the reaction to occur (i.e recently voided or dilute urine [10,3].

The combination of the LE and urinary nitrite test is said to provide an excellent screening for establishing the presence or otherwise of a urinary tract infection [10]. It has thus been recommended by some authors that it is urine samples that test positive for both nitrite and leukocyte esterase that should be cultured for pathogenic bacteria [3]. Outpatient screening algorithms have been proposed that incorporate enzyme screening in a "reflexive" urine test; i.e. Urinalysis is performed, and if positive for leukocyte esterase or nitrate reductase, a culture is set up, and if negative, a culture will not be done [3].

Proteinuria which is the presence of increased quantities of protein in the urine is useful in the diagnosis of proteinuric renal disease, is an important predictor of progressive kidney damage in patients with suspected or proven chronic kidney disease including diabetic nephropathy, reflux nephropathy and early glomerulonephritis and is a potent independent cardiovascular risk marker and predictor [11].

UTI is often associated with proteinuria however; the relationship between proteinuria and UTI remains incompletely defined. Widely divergent views on the association between proteinuria and UTI have been reported but no causal relationship has been defined between these.

Because of the reported association between UTI and proteinuria and the high prevalence of asymptomatic UTI, many published guidelines and expert consensus opinions recommend the exclusion of a UTI if a test result for urinary total protein is positive or prior to the diagnosis of microalbuminuria in patients with diabetes [12-14].

This study was carried out to determine the relationship between presence of Leucocyte esterase, nitrite and protein and significant bacterial yield in urine.

2. METHODOLOGY

This study was carried out in the Medical Microbiology Laboratory of the University of Port Harcourt Teaching Hospital, one of the tertiary health facilities in Southern Nigeria. A total of two hundred and forty (240) samples sent in for urine microscopy, culture and sensitivity were analyzed accordingly. They were examined macroscopically and microscopically using standard protocols. Dipstick analysis using Combi-UriScreen 10SL reagent strips (Axiom Medical limited, UK) and culture for significant bacteriuria were performed using standard protocols/ according to manufacturer's instruction (Health Protection Agency (2009). Investigation of urine. National Standard Method BSOP 41 Issue 7. http://www.hpa.org.uk/srmd/div_esl_su/pdf_bacteriology.htm.)

Data was coded and entered into Microsoft Excel ® version 2010 and then imported into the statistical software *Epi-Info version* 7.02 for analysis. Categorical data were presented in the form of frequencies and percentages using tables. Univariate analysis using logistic regression (Odds Ratio) was used to determine the association between the independent variables (Presence of nitrite, leucocyte esterase and protein in urine) and the dependent variable (Significant bacterial yield). A *P* value of ≤ 0.05 was considered statistically significant. Likelihood ratios were calculated to tell us which test will best help us rule-in or rule-out UTI.

3. RESULTS

The prevalence of UTI in our study as defined by a significant bacterial yield of > 10^5 CFU/ml was (41%) (99 out of 240 samples) while 31(13%), 66(28%) and 108(45%) were positive for Nitrite, leucocyte esterase and protein respectively (Table 1).

Twenty-three out of ninety-nine (23.2%) samples with significant bacteriuria were nitrite positive, while forty-two (42.4%) and forty-five (45.5%) were positive for leucocyte esterase and protein respectively. On the other hand, eight (5.7%), twenty-four (17%) and sixty-three (44.7%) out of the one hundred and forty-one samples with

insignificant bacterial yield were nitrite, leucocyte esterase and protein positive respectively (Table 2).

The presence of Nitrite (*P* = 0.001, OR = 5.03, *95% CI = 2.02-12.93*) and leucocyte esterase (*P* = 0.001, OR = 3.59, *95% CI = 1.91-6.80*) on dipstick analysis were significantly associated with significant bacteriuria (Table 2).

Forty-five percent of samples with significant bacteriuria also had proteinuria; however, there was no statistical significant association *(P* = 0.989, OR = 1.03, 95% CI = 0.60-1.79*)*.

Likelihood ratios (LR) were calculated for nitrite, leucocyte esterase and protein alone as well as for the combination of nitrite and LE.

The positive (LR+) and negative (LR-) likelihood ratios for nitrite was 4.09 (4.09, 95% CI: 1.91, 8.78) and 0.81 (0.81, 95% CI: 0.72, 0.91) respectively while that for leucocyte esterase was 2.49 (2.49, 95% CI: 1.62, 3.83) and 0.69 (0.69, 95% CI: 0.58, 0.83) respectively. On the other hand the LR+ and LR- for both nitrite and LE was 3.65 (3.65, 95% CI: 1.90, 7.03) and 0.99 (0.99, 95% CI: 0.78, 1.24) respectively.

4. DISCUSSION

Dipstick analysis of urine is advantageous because it is easy to perform, rapid, cost-effective, requires the use of fewer materials and can be carried out in primary care settings giving an immediate result as compared to culture which requires at least 24 hours as well as multiple materials, power supply and manpower [3]. It has also been reported severally to be an effective screening tool for excluding bacteriuria [1,3,4].

Table 1. Distribution of the urine samples with regard to the presence of significant bacteriuria, leucocyte esterase, nitrite and protein

Variables	Frequency (n)	Percentage (%)
Significant bacterial yield		
Yes	99	41.25
No	141	58.75
Total	240	100.0
Presence of nitrite in urine		
Yes	31	12.92
No	209	87.08
Total	240	100.0
Presence of leucocyte esterase in urine		
Yes	66	27.50
No	174	72.50
Total	240	100.0
Presence of protein in urine		
Yes	108	45.0
No	132	55.0
Total	240	100.0

Table 2. Univariate analysis of the association between the presence of leucocyte esterase, nitrite, protein and significant bacterial yield

Characteristic	Significant bacterial yield		Odds ratio (OR)	*P*-value	95% confidence interval (CI)
	Yes	No			
Presence of nitrite in urine					
Yes	23	8	5.03	0.001	2.02-12.93
No	76	133			
Presence of leukocyte esterase in urine					
Yes	42	24	3.59	0.001	1.91-6.80
No	57	117			
Presence of protein in urine					
Yes	45	63	1.03	0.989	0.60-1.79
No	54	78			

Various other studies have shown a positive association between nitrite and leucocyte esterase positivity and significant bacteriuria as observed in our study [4,15,16].

Nitrite detection has been reported to increase the probability of a urinary tract infection, with likelihood ratio [LR] of 2.6 to 10.6 (though the sensitivity is relatively low) while the detection of leukocyte esterase increases the probability to a lesser degree (LR of 1.0 to 2.6) [4,17]. Researchers in another study reported that Nitrite alone had a relatively high pooled LR+ implying usefulness in ruling in disease but it may not be a useful test for ruling out disease due to its relatively poor LR- [15]. This was the case in our study with nitrite alone having the highest LR+ (4.09, 95% CI: 1.91, 8.78) and a relatively poor LR- (0.81, 95% CI: 0.72, 0.91).

LE alone on the other hand, is reportedly a relatively poor criterion both for ruling in and ruling out disease [15]. Similarly, in this study, LE alone had the poorest LR+ (2.49, 95% CI: 1.62, 3.83) and LR- (0.69, 95% CI: 0.58, 0.83) and so appears to be more useful in ruling in UTI.

However, they further stated that a strategy which combines the results of LE and Nitrite testing appears to offer the best performance both for ruling in and ruling out UTI [15]. This was based on the finding that a dipstick test positive for both nitrite and LE had the highest positive likelihood ratio (28.2, 95% CI: 17.3, 46.0) suggesting that this test combination may be used to rule in disease. Also, that a dipstick test negative for both LE and nitrite had the best LR- (0.20, 95% CI: 0.16, 0.26) suggesting that this combination may be used to rule out disease. It also states that on the other hand, dipstick positive for either and negative for the other is less informative for the diagnosis of UTI and could be regarded as indeterminate, requiring further investigation. This was attributed to the poor LR- associated with nitrite alone and the poor LR+ and LR- associated with LE positivity alone [15].

The limitation of this approach would be in cases of UTI caused by organisms that do not elaborate nitrate reductase. Our results indicate that the combination of nitrite and LE positivity is more suggestive of UTI (3.65, 95% CI: 1.90, 7.03) than the absence of both ruling out UTI.

Proteinuria in our study was associated with a poor LR+ (1.02, 95% CI: 0.77, 1.35) and LR- (0.99, 95% CI: 0.78, 1.24) suggesting that it is only very minimally useful in ruling in or ruling out UTI. Other reviews suggest between 63 and 83% of cases of culture-confirmed UTI having reagent-strip positive tests for protein [10]. Data from various studies are inconsistent about the value of proteinuria in confirming UTI [1,15]. The high prevalence of proteinuria in our urine samples may indicate the presence of renal or metabolic co-pathologies associated with proteinuria in these patients with UTI; which may even serve as predisposing factors for the development of UTI.

5. CONCLUSION AND RECOMMENDATION

The presence of leucocyte esterase and nitrite in urine seems to be a positive predictor for significant bacteriuria therefore we recommend the use of dipstick analysis of urine as a screening tool for samples to be cultured. It is recommended that urine samples positive for nitrite alone or nitrite plus LE should be cultured (except in patients with obvious clinical manifestations of UTI) as this may be a very effective way of reducing laboratory costs as well as reducing wastage of man hours, which both work towards ultimately improving the effectiveness of the clinical laboratories especially in resource-poor settings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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