Original Article

Association of Bacteriuria with the Presence of Nitrite and Pus Cells

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Background: This study attempts to investigate the level of association of bacteriuria with the presence of nitrite and pus cells in urine. Methodology: Over 220 subjects were examined; 20mL of mid-stream urine was collected and cultured on CLED and Blood agar using a calibrated wire loop of 0.001mL, then analyzed using combi 9 rapid test kits. About 5mL was centrifuge at 2000rpm for 5 minutes and the sediment was examined with a microscope. Results: Of the 220 subjects examined, 143(65%) yielded bacterial growth, 35(24%) with a count of $\leq 10^3$ CFU/mL, 46(32%) with 10^4 CFU/mL, and 62(43%) had $\geq 10^5$ CFU/mL. Nitrite was present in 54/220(26%) of the samples, and 43(80%) were culture positive; it has a sensitivity of 30% and specificity of 80. Pyuria was detected in 102/220(46%) samples, with 61(60%) yielding positive culture, the sensitivity and specificity were 43% and 60% respectively. Thirty-four (24%) of 143 subjects with positive culture, had both pyuria and nitrite in their urine, and 27(44%) and 7(21%) were positive for only pyuria and nitrite respectively. Statistical analysis showed a lack significant association between; bacteriuria and the presence of nitrite (P=0.13); between pyuria and bacteriuria (P=0.064). The occurrence of both pyuria and nitrite, was strongly linked to bacteriuria (P=0.0003). Conclusion: The presence of both pus cells and nitrite in urine signifies UTIs, but the occurrence of only one of the parameters are not reliable, and the absence of anyone or both parameters does not rule out UTIs.

Key words: Urinary Tract Infection (UTIs), Pyuria, Nitrite, Bacteriuria, mid-stream urine, Colony forming unit (CFU/mL)

Background

Urinary tract infection (UTI) is the invasion of the urinary system by microorganisms. With the exception of the urethra, the urinary system is considered sterile; but because of the normal floral colonizing the urethral, it is difficult to avoid contamination during micturition; hence a threshold of $\geq 10^5$ CFU/mL is set as a standard for defining UTIs. Urinalysis test detect the following parameters: ascorbic acid, bilirubin, blood, glucose, ketone, protein, nitrite, pH, and urobilinogen. The presence of each of these parameters in urine has its clinical significance. Studies have linked the presence of nitrite and pyuria (pus cells) to bacteriuria. (Ratna & Sharan 2017., Charles, 2011., Jayalakshmi & Jayaram, 2008., Prescott *et al*., 2008., Cheesbrough, 2000)

This study is interested in the presence of nitrite and pyuria or pus cells (presence of 5 and above white blood cells per microscopic field) which are indicators of bacterial infection. Knowledge of the relationship between the presence of nitrites and pus cells with bacteriuria can help in a proper diagnosis of bacterial UTI. Nitrites normally are not found in urine but result when bacteria reduce urinary nitrates to nitrites. Most uropathogens are well known for their ability to convert nitrates to nitrites, especially gram-negative rods; members of the enterobacteriaceae. It is believed that a positive dipstick nitrite test indicates UTIs, and these organisms are present in a significant number ($\geq 10^5$ CFU/mL). (Urquhart & Gould 2012., Guido *et al*., 2010. & Jeff *et al*, 2005). This study investigates the level of reliability of pyuria and Nitrite with bacteriuria.

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Methodology

Study Area

This study was conducted in Jalingo area of Taraba State Nigeria between May to September 2017. Subjects comprised of patients coming for urine microscopy, culture and sensitivity (MCS), all samples were collected and processed at the Government House Clinic Jalingo.

Ethical Clearance

The ethical approval was obtained prior the beginning of the study from the Taraba State Ministry of Health. An Informed consent was obtained from each participant and information gathered from this study and from the study participants at any point were treated confidentially in accordance with the ethics governing medical research. The results were made available to the attending physician for the benefit of those diagnosed with UTIs.

Sample Collection

About 10 to 20mL of an early morning mid-stream clean catch urine sample was collected from 220 patients into a sterile universal container. Since the subjects collect their urine samples, all necessary instructions to ensure aseptic sample collection or to prevent the contamination of the urine samples by normal vaginal, perineal and anterior urethral normal flora, the subjects were provided with sterile container, and instructed to

- Wash hands before and after sample collections.
- Push the fore skin of the penis (for males), females should spread their legs, clean the vulva and labia thoroughly using water.
- Pass urine, discarding the first part of the stream and collecting mid-stream urine in the provided sterile container.

Collected samples were labelled appropriately and processed immediately without delay.

Clinical Investigation

Using a calibrated wire loop, 0.001mL of urine sample was plated onto Cysteine Lactose Electrolyte Deficient (CLED) agar and blood agar. Plates were incubated at 37^0C for 48 hours. After incubation, colonies were counted and converted to a count per mL. The calibrated wire loop was a product of pro-lab diagnostics made from nickel and chromium in Canada. Both CLED and Nutrient agar used were manufactured by Antech Laboratories, USA. The blood agar was prepared from the nutrient agar by adding 5mL of blood to 100mL of molten agar at 45-50 °C.

Identification of isolates were through colonies appearance on the culture plate and their gram reaction. Gram positive bacteria were subjected to catalase test to differentiate *Staphylococci* from *Streptococci,* and coagulase test to differentiate *Staphylococcus aureus* from other coagulase negative *Staphylococci.* Analytical Profile Index (API) 20E test were performed on gram negative oxidase negative bacteria for species differentiation.

Urinalysis

Using combi 9 dipsticks manufactured by Macherey-Angel Germany, different urine parameters including nitrite, pH, bilirubin, urobilinogen, ascorbic acid, protein, ketones, and red blood cells were determined. In this study however, only nitrite was of interest.

Procedure

- The combi 9 rapid test strip was dipped in to the urine specimen in the universal container, ensuring all pads were soaked.
- It was withdrawn and allowed to stay for 1 to 2 minutes before reading.
- Then read the colour change by matching the colour on the dipped strip with the colour chart on the container of the urinalysis strips. The collected urine samples were analysed immediately so as to avoid autolysis.

Microscopic Examination

Microscopic examination of spun urine was carried out to enumerate the number of pus cells

Procedure

- About 5mL of each urine sample was dispensed into a

test tube, spun at 2,000 rpm for 5 minutes,

- Discarding the supernatant, the sediment was placed on a glass slide, and a cover slip placed over it and examined with x10 objective lens first, then x40 with the condenser iris closed sufficiently to give a good contrast.

Report: The presence of up to 5 white blood cells per high power field and above in 3 microscopic fields was considered positive.

Statistical Analysis

The data obtained were analyzed using statistical package for social sciences (SPSS) version 18. Correlation analysis was performed at 95% confidence level. A p-value of less than 0.05 was considered statistically significant, and null hypothesis was rejected. The lesser the p-value, the stronger the relationship.

Results

Urine Culture

One hundred and forty-three (65%) of the 220 study subjects yielded positive bacterial culture. *Escherichia coli* was the most prevalent organism; it was isolated in 73(51%) of the 143 culture positive subjects. Other isolates include *Staphylococcus aureus* 21(14.7%), *Klebsiella* species 8(5.6%), CoN *Staphylococci* 14(9.8%), *Proteus species* 7(4.9%), *Pseudomonas species* 10(7%), *Streptococcus species* 6(4.2%) and *Enterococcus species* 4(2.8%).

Microscopic Examination

Following microscopic examination of the 220 subjects, pyuria was detected in 102(46%) samples, and 54(25%) were positive for nitrite. Of the 102 positive for pyuria, 61(60%) yielded positive culture. Out of this positive culture, 15(25%) had a bacterial count of $\leq 10^3$ CFU/mL, 18(29%) with a count of 10^4 CFU/mL, and 28(46%) with a significant bacterial count (10^5 CFU/mL). From the 54 positive for nitrite, 43(80%) had positive culture; 10(23%) had bacterial count of $\leq 10^3$ CFU/mL, 10(23%) and 23(54%) with a count of 10^4 CFU/mL and $\leq 10^5$ CFU/mL respectively (Table 1). Forty-one (40%) of those positive for pyuria had sterile culture, and 11(21%) nitrite positive subjects, showed no bacterial growth. Of the 143 culture positive subjects, 82(57%) had no pus cells; 20 of the 82 had a bacterial count of 10^3 CFU/mL, 28 and 34 with a count of 10^4 CFU/mL and 10^5 CFU/mL respectively. A total of 100(70%) of the 143 subjects with positive culture were nitrite negative; details are provided in table 1 below.

Figure 1 below shows the relationship between the parameters; bacteriuria, pyuria, and nitrite at different bacterial count; 10^3 CFU/mL, 10^4 CFU/mL, and 10^5 CFU/mL, represented as a, c, and b respectively.

A total of 34(24%) of 143 subjects with positive culture, had both nitrite and pyuria, 27(19%) of the 143 were positive for pus cells only, 9(6%) of the 143 were positive for nitrite only, while 73(51%) of 143 culture positive had neither nitrite nor pus cells.

Eighteen (53%) of the 34 subjects positive for both pyuria and nitrite, yielded bacterial count of $\geq 10^5$ CFU/mL, 8(24%) yielded 10^4 CFU/mL, and the remaining 8(24%) had a growth $\leq 10^3$ CFU/mL.

In figure 1a, 18(51%) of the total 35 subjects with a bacterial count of $\leq 10^3$ CFU/mL were negative for pyuria and nitrite, 7(20%) with the same bacterial count had pyuria only, 2(6%) had only nitrite while 8(23%) had both pyuria and nitrite.

Similarly, of the total 46 with bacterial count of 10^4 CFU/mL, 26(57%) were negative for pyuria and nitrite, 10(22%) with the same count were positive for pyuria only, 2(4%) had nitrite only while 8(17%) had both the parameters; pyuria and nitrite (Figure 1c).

For bacterial count of 10^5 CFU/mL, over 29(47%) of the 62 recorded cases had neither pyuria nor nitrite, 10(16%) had only pus cells, 5(8%) had only nitrite while 18(29%) had both parameters (Figure 1b).

Seventy-seven (35%) of the total 220 urine specimen from the study subjects yielded no bacterial growth, despite the presence of pus cells in 36, nitrite in 6, both nitrite and pus cells in 5.

Statistical Analysis.

N bacteriuria and pus cells (P= 0.64); N bacteriuria and nitrite, (P= 0.13). Both of these p-values were greater than 0.05; therefore, the null hypothesis was accepted. But comparing samples with both parameters (pyuria and nitrite) with bacteriuria, (p=0.0003), and null hypothesis was rejected.

Table 1 Occurrence of Nitrite and Pyuria at Different Bacterial Count Categories

Urine Parameters	*No Positive*	*Culture Positive*	*≤10^3 CFU/mL*	*10^4 CFU/mL*	*≥10^5 CFU/mL*	*False Positive*	*False Negative*	*≤10^3 CFU/mL*	*10^4 CFU/mL*	*≥10^5 CFU/mL*
Pyuria	*102*	*61*	*15*	*18*	*28*	*41*	*82*	*20*	*28*	*34*
Nitrite	*54*	*43*	*10*	*10*	*23*	*11*	*100*	*25*	*36*	*39*

False positive: presence of urine parameter with no bacterial growth, False Negative: absence of urine parameter with a positive culture. CFU/mL: Colony Forming Unit per Milliliter.

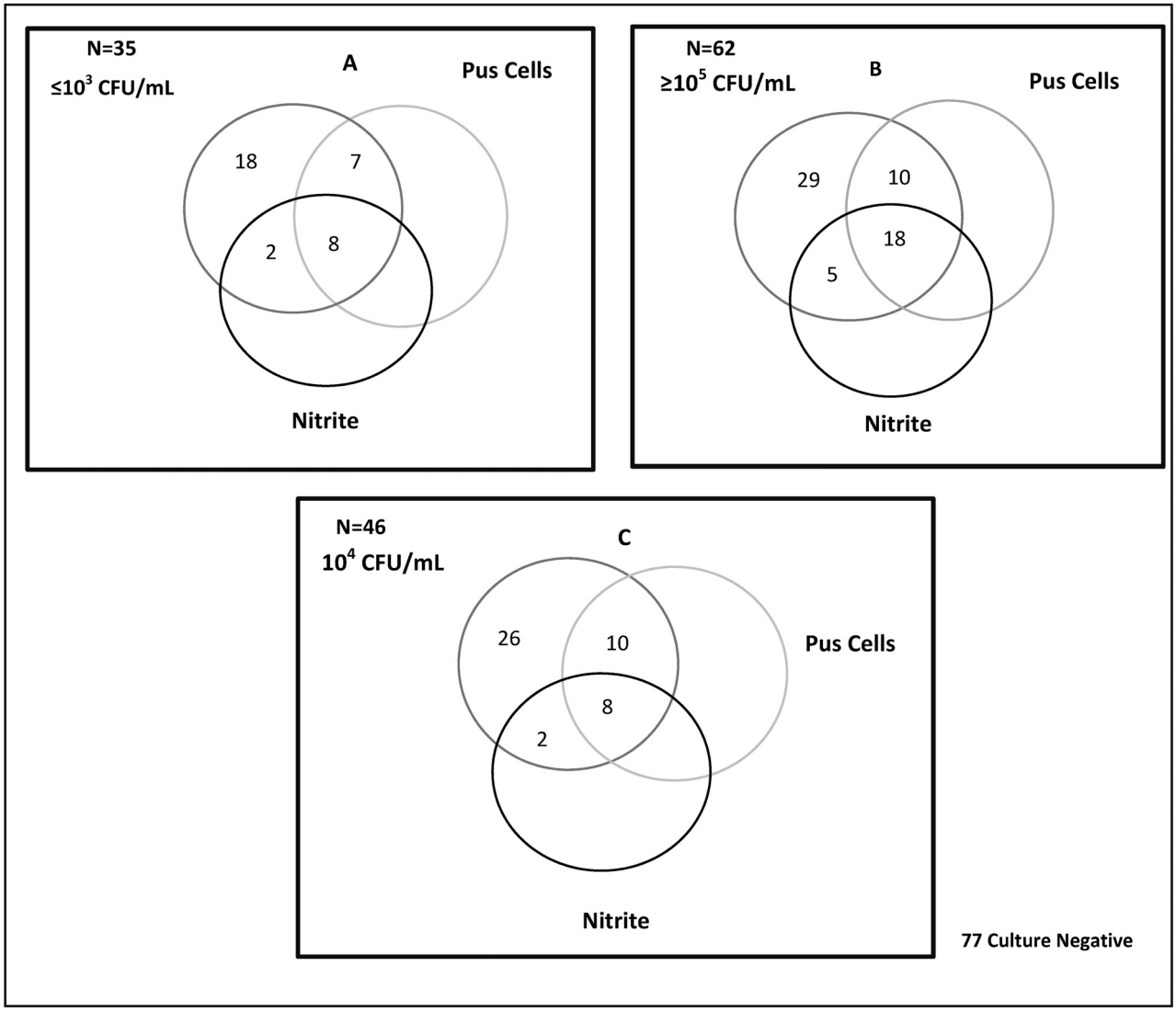

Fig.1 Venn Diagram Showing the Relationship Between Pyuria, Nitrite, and Bacteriuria at Different Bacterial Count

Discussion

It is a common knowledge that the presence of nitrites and pyuria are strong indicators of UTIs. In an attempt to investigate the association between these two parameters and UTIs, 220 subjects were examined. The results showed a lack of association between pyuria and bacteriuria. (P=0.064). This comes from the fact that only 61(60%) of 102 with pyuria had positive cultures, while the remaining 41(40%) of the 102 had negative culture results, and 41(29%) of the 143 with bacterial growth had no pus cells. This implies that pyuria has sensitivity of 43 percent (61/143*100); sensitivity is the ability to test positive when bacteriuria is significantly present. It has specificity of 60 percent (61/102*100). Specificity measures ability to test positive as the result

of bacteriuria not any other cause. A parallel can be drawn from the work of Ahmad, et al., (1997); in their study of pyuria and UTIs among HIV positive persons, they observed that asymptomatic bacteriuria without pyuria occurred in 1.4 percent of the patients. They also observed that one in every two cases with bacteriuria showed no pyuria. Akimbami *et al*., (2013) report a pyuria case of up to 8.97 percent without bacterial growth. Ratna and Sharan (2017) reported over 15(36%) subjects with positive culture without pyuria, and 3(10%) with pyuria but negative culture.

Although the cause of sterile pyuria is not fully understood, studies have shown that pyuria is not restricted to bacterial UTIs. Other conditions such as colonization with genitourinary *Mycobacterium tuberculosis*, gonorrhea, endocarditis, as well as other genital bacterial infections can also cause pyuria. (Medical Disabilities Guidelines, 2014).

In this study, 54(25%) of the study subjects were positive for nitrite. Of this number, urine specimen from 43(80%) yielded positive culture, with 11(20%) showing a sterile culture. One hundred (70%) of culture positive were negative for nitrite. The result showed that nitrite test has sensitivity and specificity of 30 percent and 80 percent respectively. Statistical analysis shows non-significant association between bacteriuria and the presence of nitrite ($P>0.05$). Although nitrite test is very vital in the diagnosis of UTI, and is highly specific for the presence of bacteriuria, several studies demonstrate significant bacteriuria in the absence of nitrite. (Grabe *et al*., 2008., & Cheesbrough, 2000). For instance, Heather and Deirdre (1999), reported over 91(18.9%) of the 479 women suspected with uncomplicated UTIs who had significant bacteriuria but were negative for nitrite. Raheela *et al*., (1996) had sensitivity of 69% and specificity of 84% which agree with the value of this study. Juliana et al., (2007) on the other hand had a sensitivity and specificity of 38.9% and 99.5% for bacteriuria respectively. Recent study on dipstick vs urine culture by Ratna and Sharan (2017); reported 17(37%) of nitrite positive urine samples with sterile culture, and 13(28%) positive culture with negative nitrite. The sensitivity of nitrite in their study was 69% and specificity was 89%. Urinary tract infection may not define by the presence of nitrite, because several factors can contribute to a false positive or negative result. For example, in a situation where bladder incubation time is reduced, a false negative results may be obtained and when dietary nitrate is less, or in a case where infection is caused by organisms that do not reduce nitrates such as Pseudomonas and gram positive bacteria, Mycobacteria etc. It can also occur when urine pH falls below 6.0, and in the presence of urobilinogen and urinary vitamin C. Recently voided urine, and diluted urine or urine with low bacteria counts ($\leq 10^3$ CFU/mL) can also lead to a false negative result. A false positive result can be due to the ingestion of substances that cause red urine e.g. beats etc. (Charles, 2011).

The presence of both parameters was strongly linked to bacteriuria. In this study, the sensitivity was 24%, the specificity however was (97%). The work of Raheela *et al*., (1996) and Juliana et al., (2007) corroborate this finding, though having a higher sensitivity, the specificity was 99% and 100% respectively. Ninama and Shah (2015) had a specificity of 97% for the occurrence of both parameters.

Conclusion

The presence of both nitrite and pyuria in urine is a strong and reliable evidence of bacteriuria, but if only one of the parameter is positive, it is not reliable. Due to the low sensitivity and specificity of nitrite or pyuria in the diagnosis of UTIs; urine culture should always be the ideal diagnostic method for UTIs. Though a positive result for either nitrite or pyuria is helpful, a negative result however, does not rule out UTI.

Referees

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