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Reliability of Rapid Urine Test for the Laboratory Diagnosis of Urinary Tract Infections among the Out-patients

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Abstract

Objectives: UTIs could be wrongly diagnosed by chromogenic dipsticks especially in the case of early infection. Hence, the need to evaluate the sensitivity of the dipstick in comparisms to conventional method, microscopy.

Methods: Sample size was obtained using standard normal distribution within 95% CI. Three hundred and seventy urine samples were collected after obtaining consent and ethical approval. WBCs and RBCs were counted after staining the slide and view at high-power microscope. All the positive samples were subjected to urinalysis using chromogenic dipsticks with ten biomarkers. The data was processed using descriptive statistics and represented with Graph Pad Prism version 6.01.

Results: The gender distribution 33.5% and 66.5% for male and female respectively while age ranges from less than 1 year to greater than 60 years. Only two hundred (200) had WBCs by microscopy with 0-4 cells (73.5%), 5-9 (9.5%) and 10+(17%). These positive samples were subjected to urinalysis. Among other biomarkers, protein and nitrite are 49.5% and 36% respectively. High alkalinity (pH 9) was observed in the 18% of the urine sample investigated. Out of the positive samples to microscopy, only 27.5% were confirmed positive by chromogenic dipsticks.

Conclusion: The sensitivity of the dipstick cannot be absolutely relying upon because of undetectable level of some enzymes required for the colour change. This can result into false negative in the diagnosis of UTIs.

Keywords: UTIs; Biomarkers; Microscopy; Undetectable level; Leucocytes

Background

Urinalysis is laboratory diagnosis that provides the health professional with valuable information about the patient's health indices which includes indications of renal, urological and liver disease, diabetes mellitus, Urinary Tract Infection (UTI) and general hydration. Urinalysis can be used to screen for substances that would not usually be expected to be present in urine, for example, glucose, leucocytes, nitrite and blood [1]. Although urinalysis is an effective screening tool it should not be used in isolation to guide treatment because false positives, for example, nitrites, and false negatives, for example, glucose, can occur if the sample is contaminated or allowed to stand for too long [2].

Urinary Tract Infections (UTIs) are among the most common bacterial infections. It has been estimated that symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually [3]. UTIs have become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and they are the second most common cause of bacteremia in hospitalized patients [4,5].

Asymptomatic bacteriuria is common in about 2% to 10% of individuals especially pregnant women and therefore associated with risks of premature birth and possible pyelonephritis if left untreated [6,7]. The diagnosis is preferably based on urine culture although culture results are typical not available until 24 to 48 hours after specimen collection [8]. Enhanced urinalysis for the diagnosis of UTIs has earlier been proposed with gram staining uncentrifuged urine for bacteria detection coupled with leucocyte counts using haemocyctometer [8].

Material and Methods

A cross sectional study was conducted on out-patients from

selected hospitals which cut across different age groups. Three locations in Oyo State were randomly selected which include Ibadan, Shaki and Ogbomoso. All the out-patients enrolled in the study were duly informed about the study and verbal consents were obtained before collecting the urine sample along with their age and gender. Urine samples were collected by standard mid-stream clean-catch method from all the consented subjects using sterile universal bottles and transported to the laboratory for urinalysis and culturing. The study was conducted from 2017 to 2020.

Sample size determination

The consent of the subjects will be sought by filling the Informed Consent Form. A total of three hundred and seventy (370) midstream urine samples will be collected with sample size determination according to Mulualem, et al. [9].

$$\frac{Z^2_{1-\alpha/2} \times P\,(1-P)}{d^2}$$

Z is standard Normal Distribution where region within 95% confidence interval is accepted.

P is proportion of the target population expected with the desired characteristics, 60% (0.6) according to previous study of [9].

Microscopy urinalysis

A 10-mL aliquot of urine was centrifuged at 1500 rpm for 5 minutes. The supernatant was removed and the sediment was resuspended into solution with 1 mL of supernatant. One drop (0.4 mL) of the resuspended sediment was placed onto a microscope slide and stained with methylene blue stain, covered, and examined under 100X and 400X magnifications. The results as the number of WBCs and RBCs per High-Power Field (HPF) and bacteriuria (400X magnification) were interpreted according to the method Wanjian, et al. [10].

Rapid test strips urinalysis

Urine samples were investigated for macroscopic analysis using standard dip sticks which is contains ten markers and result is read based on the manufacturer's recommendation and the method of Wanjian, et al. [10]. The markers on the dip sticks were blood (Erythrocytes and haemoglobins), Specific Gravity (SG), Leucocytes (LEU), pH, Ketones (KET), Glucose (GLU), Nitrites (NIT), Proteins (PRO), Urobilinogen (URO), Bilirubin (BIL).

Statistical analysis

Data obtained were entered into excel 2013 version and analyzed using SPSS version 20 to generate descriptive statistics. Descriptive statistics such as frequency and percentage were employed to analyze the data. Frequency tables were used for the macroscopic data, GraphPad Prism version 6.01 was used for all the bar chart while Excel version 2013 was used for all the pie charts.

Results

The sex and age distribution of the participants that gave consent about sample collection (Urine) using the sample universal bottle was observed to 33.5% male and 66.5% female. The age distribution was observed to range from less than 1 to greater than 60 years. The highest occurring age group is 30-39 years (**Table 1**).

Chromogenic combo test strips with ten biomarkers used in the macroscopic study revealed that only 22% and 16.5% were positive to haemoglobin and erythrocyte which is an indication of red blood cells in the urine. The percentage occurrence of bilirubin, urogen, ketone, glucose, protein, and nitrite were 5.5%, 15%, 2%, 12%, 49.5% and 36% respectively as shown in **Table 1**.

The pH of the urine samples was found to range from 5 to 9 however it was observed that pH 6 was the highest occurring (41%) while pH 8 was the lowest occurring (8%) in the study (**Figure 1**). The specific gravity of the urine samples investigated ranges from 1.000 to 1.030. The minimum occurring specific gravity is 1.020 which is 3% while 1.010, 1.000 and 1.005 are having higher occurrence with 20%, 23% and 26% respectively (**Figure 2**).

Table 1: Descriptive and macroscopic analysis of urine samples using Comb 10 strips.

Variables	Variables	Frequency	Percentage
Sex	Male	67	33.5
	Female	133	66.5
Age	0-9	20	10
	10-19	19	9.5
	20-29	24	12
	30-39	46	23
	40-49	36	18
	50-59	10	5
	60+	45	22.5
Hb	Positive	44	22
	Negative	156	78
Ery	Positive	33	16.5
	Negative	167	83.5
Bil	Positive	11	5.5
	Negative	189	94.5
Uro	Positive	30	15
	Negative	170	85
Ket	Positive	4	2
	Negative	196	98
Glu	Positive	24	12
	Negative	176	88
Pro	Positive	99	49.5
	Negative	101	50.5
Nit	Positive	72	36
	Negative	128	64

Note: The results of the macroscopic parameters of the urine samples evaluated with percentage occurrence of the parameters considered as positive while those that do not contain the properties were considered negative.

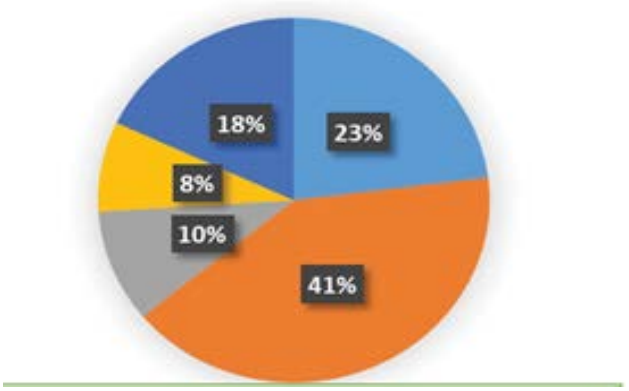

Figure 1 pH value of the urine samples investigated. The degree of the acidity and alkalinity of the urine samples were expressed with percentages based on the evaluation using chromogenic dipsticks technique.

Note: 5 6 7 8 9

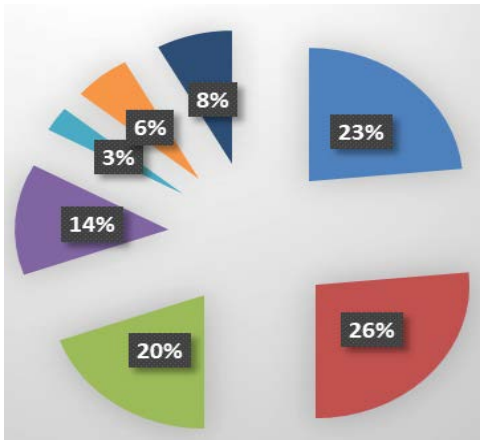

Figure 2 Specific gravity of the urine samples investigated. The specific gravity of the urine samples which indicates amount of the dissolved substances in the urine were expressed in percentages based on the evaluation using chromogenic dipsticks technique.

Note: 1 1.005 1.01 1.015 1.02

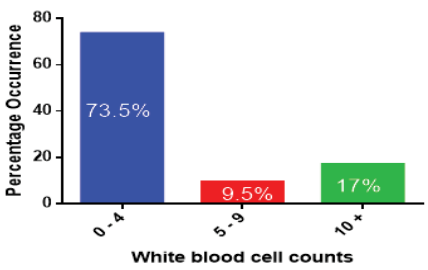

Figure 3 White Blood Cells (WBC) counts using wet prep. The WBC in the urine samples were evaluated using microscope under high power objectives and the percentages were calculated based on the cell numbers.

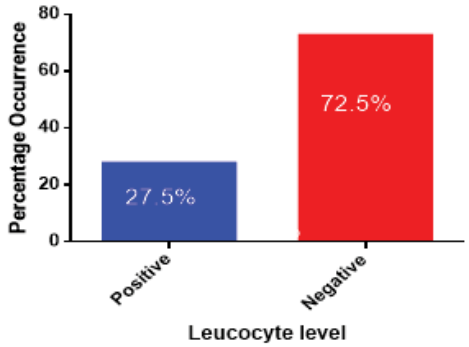

Figure 4 Leucocyte level of the urine samples investigated with combo 10 strip. The percentage of the leucocytes in the urine samples confirmed with the presence of WBC by microscopy as detected by the chromogenic dipsticks.

The microscopic count of the white blood cells (Leucocytes) using convectional wet prep method revealed that 147 (73.5%) of the urine samples were having leucocyte counts of less than 4 cells. The White Blood Cells level of 5-9 cells was found to be 19 (9.5%) while that of leucocyte counts greater than 10 cells was observed to be 34 (17.0%) as shown in **Figure 3**.

The Leucocyte occurrence in the urine sample was observed to be 55 (27.5%) in the test performed with Combo 10 test strips. However, the remaining sample tested negative were 145 (72.5%) leucocytes as shown in **Figure 4**.

Discussion

There were more female from whom the urine samples were collected and the age was from less than one year to 60 years. Some of the samples obtained from the out-patients contain red blood cells because of the presence of haemoglobin and erythrocytes. Other chemical substances found in the urine samples are bilirubin, urogen, ketone, glucose, protein and nitrites. The use of rapid kit for the diagnosis of UTIs could be important because of the speed in obtaining result which is similar to report of Hagay [11] that rapid test was good predictor of bacteriuria especially in pregnancy. It was found in this study that hemoglobin and erythrocyte in the urine samples were 22% and 16.5% respectively. The proportion of Urobilinogen in the freshly voided urine samples was observed to be 15% while ketone was 4% in all the samples investigated. The occurrence of the glucose in all the samples confirmed positive by microscopy was 12% however, protein level was 49.5%. The proportion of nitrite in the samples was 36%. It has earlier reported by Birgul, et al. [12] that NIT test has high specificity for detecting asymptomatic bacteriuria however it has about low specificity which similar to our finding where sensitivity was found to be 36%. The positive NIT test indicates that nitrites has been produced from the reduction of nitrite by enteric bacteria especially genera of *Enterobacteriaceae* [13]. The sensitivity level of the NIT test has also been reported to be low (37%-67%) by several studies [6,7,12]. False negative

may occur when the UTIs have been caused by organisms that do not contain nitrate reductase or when urine has not stayed for sufficient long period in the bladder for the reduction of nitrate to occur or lack of nitrate-related diet [12]. The pH that most common in all the patients is pH 6 while few of the patients had void urine that has pH of 8 in the investigation. The pH of normal urine is normally acidic however, pH ranges from 5.0-8.0 is considered normal according to Higgins [14] which falls within the pH ranges obtained in our work. It was found that 18% of the out-patients had pH that tends towards high alkality (pH 9) which is an indication of certain bacteria like Proteus, Klebsiella or Pseudomonas based on the report of Higgins, [14]. The specific gravity which is an indication of the level of dissolved substances in the urine. The prominent specific gravity in the urine sample investigated is 1.005 however the normal SG range of urine is 1.001-1.035 [15], depending on the solutes contained in the urine which is range obtained in this study. Low specific gravity of 1.000 and 1.005 were observed to have higher proportion in this work which indicate high fluid intake [16]. The presence of leucocyte in the urine was an indication of infection in the patients enrolled in this study. It was found that 27.5% of the samples investigated were positive leucocytes by chromogenic combo test strips however; all the samples were found with white blood cells although very low counts (0-4 cells) was found to be 73.5%. The weakness of the chromogenic combo test strip could be inability to detect low level of white blood cells (leucocytes) in the urine samples. The occurrence of leucocytes is associated with UTI however may indicate more severe renal problems [14].

Conclusion

There are more female than male associated with urinary tract infection. Some of the disease parameters like urogen, ketone, bilirubin, glucose, protein and nitrite were found in the urine sample which implies occurrence of disease in the patient. Detection and enumeration of white blood cells (Leucocytes) by chromogenic combo strip and microscopy observed in this work indicates that there is an infection in the out-patients. The specificity of chromogenic combo strip was observed not to detect white blood cells (leucocyte) ≤ 4 cells. So, specificity of the chromogenic combo strip is based on the increased leucocyte counts that is the leucocyte level must be at detectable level.

Conflict of Interest

The authors declared that there is no conflict of interest.

Research Funding

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Authors Contribution

All the authors contributed to the research and writing of the manuscript and approves the publication.

Informed Consent

The out-patients were duly informed verbally before samples were collected for the analysis.

Ethical Approval

The research was approved by The Research Ethics Committee, Faculty of Natural Sciences, Ajayi Crowther University, Oyo, The approval number (FNS/ERC/2017/021) was given for the conduct of the research. s.

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