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Prevalence of Bacterial Urinary Tract Infection among HIV-Positive Patients on ART Attending Government Tertiary Hospital in Enugu

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Abstract

People living with Human Immunodeficiency Virus (HIV) are more likely to develop urinary tract infection (UTI) due to the suppression of their immunity. In order to determine the prevalence of UTI in HIV-positive patients attending Government Tertiary Hospital in Enugu, a total of 280 candidates were investigated between November 2018 and January 2019 using standard microbiological techniques. Out of the 63 subjects with UTI, 46 subjects had single bacterial infection while 17 subjects had double bacterial infections. Those with pyuria were 33, among which 17 subjects had UTI. Out of 247 subjects without pyuria, 46 had UTI. Those with symptoms of UTI were 49 while those without symptoms were 231. Out of the 231 subjects without symptoms, 47 had UTI (asymptomatic). However, there were statistically significant differences ($P<0.05$) for positive and negative cases in terms of gender, marital status, having flank pains and pyuria.

***Keywords**: prevalence, urinary tract infection, HIV, ART*

Introduction

One of the most common types of bacterial infections that affect humans both in the community and the health care settings is the urinary tract infection. The urinary tract includes the organs that collect and store urine and release it from the body and these organs include the kidneys, ureters, bladder, urethra and accessory structures. Urinary tract infection (UTI) is the infection of the urinary tract.[1] Urinary tract infection is a process whereby pathogenic microorganisms invade and

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multiply in the organs of the urinary tract system and this is manifested in symptomatic and asymptomatic patients as at least 100,000 organisms per milliliter of urine.[2] The bladder and urethra are most commonly infected but any part of the UTI can be infected. The infection of the bladder is known as cystitis while that of the kidneys is known as pyelonephritis.[3]

Due to their impaired immune systems, persons living with HIV are more likely to get a urinary tract infection (UTI), specifically those who are not doing well with antiretroviral drugs.[4] Due to the obvious structure of their sex organs, HIV-positive women are more likely to contract the virus. In HIV-positive people, pathogenic bacteria are a leading source of illness and death.[4] Urinary tract infection affects roughly 150 million individuals worldwide each year, impacting the financial system more than $6 billion.1 The most frequent bacterial infectious disease is urinary tract infection (UTI), which accounts for a large portion of the labor in clinical diagnostic laboratories. In many healthcare settings, it is also the most frequent illness in HIV-infected individuals.[1]

The study was done to determine the prevalence of bacterial urinary tract infection in HIV-positive patients on ART attending Government tertiary hospital in Enugu.

Materials and Methods

Study Area

The study was conducted at the ART clinic of a government tertiary hospital in Enugu, Enugu State.

Study Design and Period

A cross-sectional hospital-based study in the ART clinic of UNTH Ituku Ozalla was conducted from November 2018 to January 2019.

Ethical Clearance

Ethical clearance and permission were obtained from the Ethical Committee of UNTH Ituku Ozalla. All study records that identify subjects were kept confidential. All information that was collected in this study was given code numbers and no names was recorded. The keys to these code numbers and paper files were kept in a locked cabinet and computerized files was password-protected and all was only accessible to authorized persons. All the investigations done for participants of this study was free of charge but hospital care and treatments were paid for by the patients according to the rule of the hospital. Study participants were not compensated for their participation in this study but were given the results done so as to aid the clinician in the right choice of antimicrobial for their treatment.

Study Population

All HIV-positive patients on ART aged ≥18 years attending the ART Clinic of UNTH.

Inclusion and Exclusion Criteria of Study Participants

Inclusion criteria

- Males and females that are ≥18 years old and are HIV-positive. These are adults and are old enough to give consent for the study.
- UTI symptomatic and asymptomatic HIV-infected patients.
- HIV-positive patients who are on ART drugs.
- HIV-positive patients who gave their consent to participate in the study.

Exclusion criteria

- HIV-positive patients who are on antibiotics other than septrin within one month prior to the time of enrolment to the study.
- HIV-positive patients less than 18 years of age.
- HIV-positive pregnant women.

Sample Size Determination

The sample size was calculated using the formula for population less than 10,000:

$$nf = \frac{n}{1 + (\frac{n}{N})}$$

where,

nf = desired sample size when population <10,000

N = estimated size of the population = 1,680 (from the clinic records, UNTH sees an average of 560 ART patients per month giving 1680 per 3 months).

$$n = \frac{(Z)^2 pq}{d^2}$$

n = desired sample size when population >10,000

Z= standard normal deviate; corresponds to 95% confidence level (z=1.96)

p= proportion of target population with the characteristics (prevalence) = 26% = 0.26 (Samuel *et al.*, 2012).

q= 1- p = 1-0.26 = 0.74

d = precision = 5% = 0.05

n will be

$$n = \frac{(1.96)^2(0.26)(0.74)}{(0.05)^2}$$

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n = 295.65

Substituting for n,

$$nf = \frac{295.65}{1 + (\frac{295.65}{1680})}$$

nf = 250.55

Correcting for an attrition rate of 10% = 250.55 + 25.055

=275.605 = 276

Therefore, the minimum sample size for this study is 276.

Sampling Technique

Study participants was selected by simple random sampling technique using a table of random numbers. The list of HIV-positive patients on ART attending the ART clinic of UNTH on each clinic day was used as the sampling frame. Data collection was done two times per week. About 12 HIV-positive patients were sampled in each clinic day to ensure that data was properly collected.

Methods of Data Collection

Participants in the study gave their informed permission. An interviewer-administered structured questionnaire was used to obtain socio demographic data and relevant information. The medical histories of the participants, as well as their current CD4+ test results, were documented from their folders.

Sample collection

After a thorough discussion to the patients, urine samples were obtained. A clean catch mid-stream urine (MSU) sample was collected in a sterile, wide-mouthed, screw-capped container after they gave their consent. In the laboratory, they were processed right away. The urine samples were separated into two sterile test tubes, one for microscopic analysis and the other for culture inoculation.

Laboratory Investigation

Microscopy

The samples were thoroughly mixed in a container, and ten mL of each well–mixed urine sample was centrifuged for five minutes at 2000g. The supernatant was removed after centrifugation, and a drop of the sediment was deposited on a grease-free glass slide, which was then covered with a clean grease-free coverslip. It was studied under the microscope with X10 objective lens, then

confirmed using X40 objective lens. For pus cells, red blood cells, epithelial cells, casts, and crystals, their reporting system for identification was at high magnification.

Culture

On the surface of freshly prepared well dried blood agar and MacConkey agar media, 0.001 milliliter of well mixed un-centrifuged urine was inoculated with a calibrated wire loop. The media were made according to the manufacturer's instructions and incubated between 35^0C and 37^0C. The plates were checked for the presence of colonies after a 24 - 48-hour incubation period.[5]

Bacterial identification

It was regarded significant if there were more than 100 colonies per 0.001ml (10^5cfu/ml) of urine. The number of colonies forming units (CFUs) in the original urine sample was multiplied by 1000 to get the number of bacteria per milliliter.[5] Gram staining techniques and other biochemical assays such as indole, catalase and coagulase productions, methyl red, oxidase, and Voges-Proskauer reactions, as well as citrate utilization, were used to confirm the bacterial isolates.

Gram staining procedure

This test is used to determine if bacteria are Gram positive or Gram negative.

Procedure

Some of the distinct colonies were picked with a sterile wire loop, and a smear was produced on a clean grease-free glass slide by emulsifying the colonies with normal saline. The smear was allowed to air dry before being fixed with mild heat via the bunsen burner twice. The crystal violet was poured over the slide and left to stain for 60 seconds. The stain was removed using clean water. The slide was soaked with Lugol's iodine solution once more and left to stain for an additional 60 seconds. The iodine solution was washed away with clean water, and the smear was treated for a few seconds with acetone before being swiftly washed away with clean water. The smear was counter stained for two minutes with neutral red. Finally, the smear was rinsed away, and the back of the slide was wiped clean with absorbent cotton wool before being placed in a draining rack to air dry. The smear was examined using an oil immersion lens. The organisms' shape, color, and arrangement were noted. Gram positive organisms were purple/bluish in color, and Gram-negative organisms were pink/reddish.

Biochemical tests

Catalase test

Procedure: Few colonies of the organism were emulsified in distilled water on a clean glass slide and placed in a petri dish using the slide method. The dish was covered after two drops of three percent (3%) hydrogen peroxide were introduced. A positive reaction was shown by the presence of gas bubbles, whereas a negative reaction was indicated by the absence of gas bubbles.

Coagulase test

Procedure: Two different drops of normal saline were placed on a clean, grease-free slide to conduct the test. To generate thick suspensions, two colonies of the suspect organism were emulsified in each saline drop. The tip of a straight wire loop was dipped into the undiluted plasma, and any remaining plasma was blended with one of the bacterial solutions. A positive coagulase test was indicated by immediate coarse clumping of the mixture within ten seconds, whereas a negative test was indicated by no coarse clumping. The other suspension, which served as a negative control for the test and was used to distinguish non-specific granular appearance from actual coagulase clumping, had no plasma added to it.

Indole test

Procedure: A small amount of the test organism was cultured in peptone water overnight at 37^0C. The overnight peptone water culture was treated with a few drops of Kovac's reagent. A positive indole production was indicated by a red ring above the peptone water, whereas a negative indole production was indicated by no red ring above the peptone water.

Methyl red test

Procedure: A little amount of the test organism was placed in sterile glucose phosphate peptone water medium and cultured for 48 hours at 37°C. After incubation, five drops of the methyl red indicator were added to the culture, mixed, and read right away. A positive test resulted in a red colouration, whilst a negative test resulted in no change in colour.

Voges-Proskauer test

Procedure: The test organism was mixed with two mL of glucose phosphate peptone water medium. It was incubated for 48 hours at 37°C. 1 mL of 10% potassium hydroxide was introduced and allowed to stand at room temperature for one hour. A positive test was shown by a pink colouration, while a negative test was indicated by no colour change.

Citrate utilization test

Procedure: In bijou bottles, Simmon's citrate agar slants were prepared. They were allowed to solidify before the test organism was introduced into the medium using a sterile wire loop to stab the butt and then streak over the medium's surface. After that, the medium was incubated for 48 hours at 37°C. A positive citrate test is indicated by a blue colour, whereas a negative test is indicated by the original green colour.

Oxidase test

Procedure: A few drops of oxidase reagent were introduced to a few colonies on the culture plate. It was observed to see if it changed colour. A positive oxidase test indicated colonies that changed colour from blue to deep purple within 10 seconds, whereas a negative test indicated colonies that did not change colour to purple.

Data Analyses

Each research subject's clinical and laboratory data was entered into a standard registration format. After the data was validated for completeness, IBM SPSS Statistics version 20 was used to analyze it. Descriptive statistics were used to describe categorical variables (frequencies and percentages). At a 95% confidence interval, the paired student t-test and conventional One-way Analysis of Variance (ANOVA) were employed to compare mean differences between and among groups. The Chi-square (X2) test (at 95% confidence intervals) was conducted to see if there were any significant relationships between UTI and the subjects' baseline characteristics.

Results

Table 1 shows the prevalence of UTI among HIV-positive subjects on ART drugs according to signs and symptoms of UTI. Subjects with fever had the highest prevalence of UTI with frequency of 6 (33.3%). There was no statistically significant association between fever and UTI ($X^2 = 1.295$, P = 0.255). Study participants with dysuria had the highest prevalence of UTI with frequency of 6(42.9%). However, there is no significant association between dysuria and UTI ($X^2= 3.502$, P= 0.061). Those with frequent urination had the highest prevalence of UTI with frequency of 3(27.3%). Chi-square test at 95% C.I showed no statistically significant association between frequent urination and UTI ($X^2=0.150$, P= 0.699). Subjects with urgency had the highest prevalence of 8 (26.7%). There was no statistically significant association between urgency and UTI ($X^2= 0.335$, P= 0.563). Those with flank pain had the highest prevalence of UTI with frequency of 9 (42.9%). There was no statistically significant association between flank pain and UTI ($X^2= 5.395$, P= 0.020).

Table 1: Prevalence of UTI among HIV-positive subjects on ART drugs according to signs and symptoms of UTI

Variables	Number tested	Number positive for UTI (%)	Number negative for UTI (%)	X^2	P-value
Fever				**1.295**	**0.255**
Those with fever	18	6(33.3%)	12(66.7%)		
Those without fever	262	57(21.8%)	205(78.2%)		
Dysuria				**3.502**	**0.061**
Those with dysuria	14	6(42.9%)	8(57.1%)		

Those without dysuria	266	57(21.4%)	209(78.6%)		
Frequent urination				**0.150**	**0.699**
Those with frequent urination	11	3(27.3%)	8(72.7%)		
Those without frequent urination	269	60(22.3%)	209(77.7%)		
Urgency				**0.335**	**0.563**
Those with urgency	30	8(26.7%)	22(7.3%)		
Those without urgency	250	55(22.0%)	195(78.0%)		
Flank pain				**5.395**	**0.020**
Those with plank pain	21	9(42.9%)	12(57.1%)		
Those without flank pain	259	54(20.8%)	205(79.2%)		

Table 2 shows the prevalence of UTI among HIV-positive subjects on ART drugs according to subjects' medical history. Study participants without previous history of UTI had the highest prevalence of UTI with frequency of 51 (23.0%). There was no statistically significant association between history of UTI and UTI. Subjects who had been previously catheterized had the highest prevalence of UTI with frequency of 13 (34.2%). However, there was no statistically significant association between catheterization and UTI ($X^2 = 3.458$, $P = 0.063$). Subjects on 2nd line drugs had the highest prevalence of UTI with a frequency of 5 (23.8%). There was no statistically significant association between line of ART drugs and UTI ($X^2 = 0.022$, $P = 0.881$). Study subjects

exposed through mother-to-child transmission had the highest prevalence of UTI with frequency of 1 (33.3%). There was no statistically significant association between source of exposure and UTI ($X^2 = 0.565$, P = 0.754).

Table 2: Prevalence of UTI among HIV-positive subjects on ART drugs according to subjects' medical history

Variables	Number tested	Number positive for UTI (%)	Number negative for UTI (%)	X^2	P-value
History of UTI				**0.137**	**0.711**
Those with history of UTI	58	12(20.7%)	46(79.3%)		
Those without history of UTI	222	51(23.0%)	171(77.0%)		
Catheterization				**3.458**	**0.063**
Those previously catheterized	38	13(34.2%)	25(65.8%)		
Those not previously catheterized	242	50(20.7%)	192(79.3%)		
Line of ART drugs				**0.022**	**0.881**
Those on first line drugs	259	58(22.4%)	201(77.6%)		
Those on second line drugs	21	5(23.8%)	16(76.2%)		
Source of exposure				**0.565**	**0.754**
Homosexual contact	18	3(16.7%)	15(83.3%)		
Heterosexual contact	259	59(22.8%)	200(77.2%)		

Mother-to-child	3	1(33.3%)	2(66.7%)

Discussion

Subjects aged 42-49 years had the highest prevalence of UTI (18.6%), followed by those aged 26-33 years with a prevalence of 16 (22.9%), and those aged 58-65 years had the lowest prevalence of 1 (14.3%). This demonstrates that UTI affects people of all ages, and there is no statistically significant link between age and UTI. In a study conducted in Addis Ababa, Ethiopia, the age group 18-27 had the highest prevalence of 3 (21.4%), while the age group 48-57 had the lowest prevalence of 1 (7.7%). Urinary tract infection was found in all age categories, with no significant relationship between age and UTI.[6] In contrast, research performed in Jos among HIV positive persons found that the age group ≥ 46 years had the greatest prevalence of 8 (38.1%), while the age group 5-15 years had the lowest prevalence of 4 (15.4%). According to the findings, UTI was found in people of all ages, and there was a significant relationship between age and UTI.[4] HIV exposure, as well as other factors such as multiple sexual partners, injectable drug users, and unprotected sexual intercourse, could be responsible for the infection in all age groups.[8] In another study conducted in Osogbo, Nigeria and India among HIV-positive individuals, age group < 40 years had the highest prevalence of 39 (25.8%) and 8 (66.7%) respectively while age group > 40 years had a prevalence of 12 (13.2%) and 4 (33.7%) respectively. Increased sexual activity and unprotected sexual contact among the younger age group (under 40 years) may be risk factors for bacteria in urine and urinary tract infections.[8] However, in a study conducted in Osogbo among HIV-infected persons, there was a statistically significant relationship between age and UTI, whereas in a study conducted in India among HIV-infected persons, there was no statistically significant relationship between age and UTI, despite the fact that UTI occurred in all age groups.[9] People of all ages were found to have urinary tract infection, with HIV/AIDS exposure being the most common risk factor. Poor hygiene and sanitary circumstances, having very high blood sugar levels, increased sexual activity, and contamination of the vagina area from anal region after defecation are all risk factors, especially in women (Sheffield and Cunningham, 2005).

A total of 280 HIV-positive individuals on antiretroviral therapy (ART) were tested for bacterial UTI, with 70 males and 210 females. Bacterial UTI affected 10 percent of the total male population (7) and 26.7 percent (56) of the total female population. A total of 63 male subjects (90.0%) and 154 female subjects (73.3%) tested negative for urinary tract infection. Gender and UTI were found to have a significant relationship. This finding is consistent with several other research performed among HIV-positive individuals in Addis Ababa, Ethiopia, Plateau, and Ebonyi States in Nigeria, which found that UTI occurred more frequently in HIV-positive females than HIV-positive males.[6] This could be owing to anatomical differences between males and females, such as the female urethra being shorter and closer to the anus, and some females having poor vaginal hygiene.[4] These findings, however, contradict those of research conducted among HIV-positive people in Calabar, Nigeria, which found that females have a lower prevalence rate.[10] This could be as a result of a greater percentage of males having obstructive diseases that enhanced their risk of UTI, such as benign prostatic hyperplasia, stones, traumatic strictures, and urogenital malignancy.[11]

With a frequency of 63, Christians had the greatest rate of UTI (22.7 %). Religion, on the other hand, has no statistically significant link to UTI. The research area is predominantly Christian; therefore, more Christians were recruited for the study, resulting in them having the highest prevalence.

Conclusion

One of the problems that HIV/AIDS patients face is urinary tract infection. A total of 63 (22.5%) patients out of 280 HIV-positive patients on ART tested for UTI had urinary tract infection. Females had more UTI (both single and double bacterial infections) as compared to males.

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