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Comparism of CD4 count of patients with UTI and those without UTI in HIV Positive Patients Attending Government Tertiary Hospital in Enugu, Nigeria

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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 comparison of CD4 Count of patients with UTI and those without 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 Flow Cytometry Automation and standard microbiological techniques. There were no statistically significant differences (P>0.05) for those with and without UTI in term of mean CD4 count and duration of ART treatment. 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. The need for regular monitoring of HIV positive individuals is emphasized due to the fact that the majority are asymptomatic for UTI knowing their underlying debility.

***Keywords**: CD4 Count, HIV, UTI, Bacteria*

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 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.1 Urinary tract infection is not just a hospital acquired infection but, in the community, is one of the significant illnesses that increased disease burden and is also an important source of morbidity. Main cause of UTI is the obstruction of the urinary tract including stone disease, pelvic-ureteric junction obstruction, benign prostate hyperplasia, urethral strictures and neuropathic bladder.[3]

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Asymptomatic UTI, also known as bacteria in urine with no clinical manifestation, is characterized as the presence of considerable bacteria in an individual's urine (10^5 colony forming unit/milliliter [cfu/mL]) without any clinical manifestations of UTI.[4] Bacteria in urine with no clinical manifestation is frequent, and its frequency varies according to maturity, gender, sex acts, and the existence of urogenital disorders. Women who do not have bacteria in urine with no clinical manifestation are less likely to get a clinical UTI than those who do.[5] Urinary tract infections can be either complicated or uncomplicated. It is complicated when an underlying aberration is thought to have aided the infection's occurrence, whereas it is uncomplicated when the infection occurs in a healthy urinary system with no architectural, operational, or preexisting human sickness to explain for the disease.[6] 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.[7] Bacterial organisms such as *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Morganella morganii, Proteus vulgaris, Proteus mirabilis,* and *Klebsiella pneumoniae* produce the bulk of UTIs.[8] Several microbes have indeed been detected in HIV/AIDS individuals' illnesses spread by infectious microorganisms.[8] 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]

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. A copy of the ethical clearance is shown in Appendix I. 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.[9]

q= 1- p = 1-0.26 = 0.74

d = precision = 5% = 0.05

n will be

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

n = 295.65

Substituting for n,

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

nf = 250.55

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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.[6]

Bacterial identification

It was regarded significant if there were more than 100 colonies per 0.001ml (10^5cfu/ml) of urine. The number of colony forming units (CFUs) in the original urine sample was multiplied by 1000 to get the number of bacteria per milliliter.[6] 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

The test is used to distinguish between bacteria that produce the catalase enzyme, such as Staphylococcus species, and bacteria that do not produce the enzyme, such as Streptococcus species.

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

Staphylococcus aureus is distinguished from other Staphylococci using this 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

This test is used to help distinguish Gram-negative bacilli.

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

This test is used to distinguish between different types of enterobacteria. It detects the formation adequate acid during glucose fermentation.

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

This test is used to distinguish between different types of enterobacteria.

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

Enterobacteria are identified using this method.

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

Pseudomonas aeruginosa is identified with this 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.

Antimicrobial susceptibility testing (AST)

The disk diffusion technique, as modified by the Clinical and Laboratory Standard Institute, was used to test the isolate's antibiotic susceptibility. Three to five chosen colonies of bacteria were picked from a pure culture and transferred to a tube containing 5 mL sterile normal saline. The suspension was gently mixed to form a homogeneous solution, and the turbidity was adjusted to a McFarland 0.5 standard. The plates were streaked with a sterile cotton swab, and the excess suspension was removed by gently pushing and rotating the swab against the tube's interior wall surface. The bacteria were then distributed equally throughout the Mueller Hinton agar surface using the swab. The seeded plates were let too dry for 5 minutes at room temperature before being impregnated with 10 antibiotic discs. The antibiotics disks used were from Oxoid Ltd, Basingstoke, and Hampshire, United Kingdom, and they are as follows: Erythromycin 10µg, Ceftriaxone 30µg, Ampicillin 30µg, Cloxacillin 10µg, Cephalexin 30µg, Levofloxacin 5µg, Ciprofloxacin 10µg, Gentamicin 10µg, Ofloxacin 10µg, Clindamycin 10µg, Nitrofurantoin 100µg, Chloramphenicol 10µg, Cefuroxime 10µg, Pefloxacin 10µg, Amoxicillin 30µg. For 18-24 hours, the plates were incubated at 37°C in an aerobic environment. Using a graduated caliper in millimeters, the diameters of the zone of inhibition surrounding the disc were measured to the nearest millimeter, and the isolates were categorized as sensitive, intermediate, or resistant according to CLSI.

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 comparison between the CD4 count of patients with UTI and those without UTI. The mean CD4 count of subjects with UTI was 539.98 ± 311.656 while that of subjects without UTI was 477.98 ± 281.669. Statistically, Independent student's t-test showed no significant difference between the mean CD4 count of HIV subjects with UTI and those without UTI (t = -1.420, P = 0.159).

Table 1: Comparing the CD4 count of patients with UTI and those without UTI

Variable	Mean CD4 count	± SD
UTI infection		
Positive for UTI	539.98	311.656
Negative for UTI	477.98	281.669
p-value	**0.134**	

Discussion

Urinary tract infection is one of the two diseases coexisting at the same time that HIV-infected patients face. Urinary tract infections, many of which are caused by dangerous bacteria, account for a remarkable number of patients who visit the hospital on a regular basis. They are one of the unscrupulous illnesses that HIV patients face.[1] Bacterial infections which are opportunistic in nature are a major cause of AIDS illness and death.[10]

Subjects with UTI had a mean CD4 count of 539.98 ± 311.656, while those without UTI had a mean CD4 count of 477.98 ± 281.669. The mean CD4 count of HIV patients with UTI and those without UTI did not differ statistically significantly. This finding matched that of an Indian study that found no statistically significant link between CD4 levels and UTI, but found considerable bacteriuria in patients with CD4 counts between 300 and 1000, with a mean CD4 of 581.[11] In contrast, other studies in Calabar and Ibadan, both in Nigeria and South Africa, among HIV-positive people, all found a statistically significant link between CD4 levels and UTI.[12] These data reveal that UTI was found in HIV-positive people regardless of their CD4 count, since UTI was found in people with both low and high CD4 counts.[13-14]

Conclusion

These data reveal that UTI was found in HIV-positive people regardless of their CD4 count, since UTI was found in people with both low and high CD4 counts.

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