

A STUDY OF URINE FROM HIV-POSITIVE AND HIV-NEGATIVE INDIVIDUALS AT ANDHRA PRADESH

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ABSTRACT

Objective: The aim of this study is to investigate the variations, if any, in urinary bacterial pathogens in HIV-positive and HIV-negative individuals.

Methods: Urine sample, macroscopic and microscopic examination for identification of bacteria use by method of Standard loop method, using identification methods, Grams-staining, Biochemical tests.

Results: Out of 75 HIV-positive males, 52 males were married, 23 males were unmarried; risk factors are sexually 28, occupationally majority were labor, 31, age group more in 19-25 age, only 9 HIV-positive patients presented with symptoms of urinary tract infection, *Escherichia coli* was the most frequently occurring bacterial isolate from positive urine culture followed by *Klebsiella* spp.

Conclusion: The study period of 1 year and 8 months revealed that it is difficult to locate/identify an HIV-positive person in a place like Kadapa on account of confidentiality/lack of awareness among the population. Having identified the HIV-positive cases, collection of urine samples from the patients also needed counseling and intervention by the respective clinicians, especially when patients were not having any symptoms. Out of 50 HIV-negative individuals, only 1 (2%) urine sample is showing significant bacteriuria.

Keywords: Urinary tract infection, HIV, AIDS, Bacteria, Antiretroviral therapy.

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INTRODUCTION

While the genitourinary system is both a primary site of HIV infection as well as a site for its complications, yet the genitourinary tract is generally protected. According to miles and associates, urinary tract infections (UTIs) occur in 17% of patients while there was a 16% incidence of urology-related symptoms. Symptoms of UTIs include dysuria, frequency, urgency, fever, and hematuria, but many patients are relatively asymptomatic. Although, infection may be present cultures are often negative. Most AIDS patients take prophylactic antimicrobials for opportunistic infections causing pneumonia or diarrhea and this renders culture negative [1].

The reported incidence of bacterial UTI in patients with AIDS is 7-50%. Bacterial infections are more common in AIDS patients with CD₄⁺ count of <200/ μ l. In patients with low CD₄⁺ counts, neurological symptoms may also occur. Bladder reflexia or hyporeflexia is a common neurological complication, which leads to urinary stasis and ultimately UTI. The most common bacterial pathogens in HIV-infected patients are *Escherichia coli*, *Enterobacter* (Enterococci), *Pseudomonas*, Group D *Streptococcus*, *Proteus* spp., *Klebsiella*, *Acinetobacter*, and *Staphylococcus aureus* [2].

METHODS

The present study was conducted from July 2014 to December 2016 in Fathima Institute of Medical Sciences (FIMS), Kadapa.

Study group

A total of 75 HIV reactive (positive) patients (age 15-59 years) were identified with the help of clinicians of Regional Institute of Medical Sciences (RIMS) and FIMS, Kadapa. These patients were being treated by the clinicians for recurring ailments. The study group comprised 55 male and 20 female HIV-positive patients.

Control group

A total of 50 HIV non-reactive individuals (34 male and 16 female), aged 18-60 years, admitted in RIMS and FIMS, Kadapa for various ailments in surgical wards were included in the study as control group. Urine samples were collected from these patients after collecting relevant information such as age, sex, occupation, present complaint, whether on ART (antiretroviral therapy), and treatment given. Urine samples were also collected with or without the presence of symptoms of UTI.

Total number of urine samples examined = 125

Laboratory diagnosis of HIV infection

The HIV-positive cases included in this study were identified through the clinicians who were treating them for their recurrent ailments. They were investigated and identified as HIV reactive by serological tests was done elsewhere. All of the patients were reported ELISA positive (as per WHO strategy-II 1993) for HIV-1/HIV-2 with at least two different kits [1].

The individuals included under the control group were screened for their HIV status in our laboratory by ELISA and TRIDOT (J. Mitra and co. Ltd, New Delhi) [3]. Patient admitted in RIMS and FIMS period 2014 to 2016.

Sample

A clean-catch midstream urine sample was collected in a wide mouth sterile container and examined within 2-3 hrs of collection.

Urine sample

Naked eye examination of urine sample was done and any relevant finding was noted.

Microscopic examination of urine

A wet film was prepared with a drop of urine and examined under the microscope for pus cells, RBC, organisms, etc.

Culture

Semiquantitative culture method - semiquantitative technique of inoculation in suitable culture media - by standard loop method was done to determine significant bacteriuria.

Culture media used

Maconcy agar, Blood agar, Lactose sugars bilesalt, neutralred all related biochemical reactions used.

Standard loop method

An inoculating loop of standard dimension was used to take up fixed and known volume of uncentrifuged urine and spread it over a plate of culture medium. A nichrome wire of 28 SWG was used to make a circular loop of 1 mm of internal diameter. It can hold 0.002 ml urine.

Total viable bacterial count/ml of sample = No of colonies × 500

Culture medium plate was touched with the loop containing urine from where inoculum was spread in a straight line across the diameter of plate. Without flaming, loop was drawn across entire plate, crossing the first inoculum streak numerous times to produce isolated colonies.

Inoculated plates were incubated overnight at 37°C in an incubator and colony count was noted.

Kass concept

In the presence of active UTI, the urine will contain 10⁵ bacteria or more per milliliter. This level is therefore considered to represent significant bacteriuria.

Identification of the organism

A single well-isolated colony was picked up with a sterile wire to prepare a pure subculture and the identification of the organism was done on the basis of:

- Gram staining,
- Motility test by hanging drop,
- Biochemical tests (catalase, oxidase, IMViC, urease and sugar fermentation tests, etc.) following the procedures mentioned in [4].

RESULTS

Out of 75 HIV-positive patients, 12 patients showed significant bacteriuria and was diagnosed to have UTI. The remaining cultures were sterile. No lesser counts were noted. Only one case of UTI was found in the group of HIV-negative individuals. Chi-square test revealed a statistically significant relationship between HIV status and UTI ($\chi^2=6.310$, $df=1$, $0.01 < p < 0.02$) (Tables 1-3).

Symptoms of UTI present in HIV-infected patients are shown in Table 4. Only 9 HIV-positive patients presented with symptoms of UTI.

Escherichia coli was the most frequently occurring bacterial isolate from positive urine culture followed by *Klebsiella* spp. (Table 5).

DISCUSSION

It shows the presence of risk factors, marital status, and occupation of 75 HIV-positive patients. In our study group, maximum patients were laborer (41%). Promiscuity appeared to be the highest risk factor among the study group. Among the 55 male patients, only 6 patients had circumcision, whereas 49 patients were uncircumcised. As the socioeconomic status of our study group was very poor, so we were unable to get information on the CD₄⁺ count of the patients. For four patients, the CD₄⁺ T-lymphocyte count done elsewhere were recorded and all of them were above 200/ μ l. Hence, the relation between CD₄⁺ count and presence of intestinal parasites could not be established. The

Table 1: Risk factors, marital status, and occupation of 75 HIV-positive patients

Variable	n (%)
Marital status	
Married	52
Unmarried	23
Risk factors	
Promiscuity	21
Blood transfusion	08
Intravenous drug users	01
Others (previous operation, spouse of HIV-positive person)	28
Occupation	
Driver	06
Laborer	31
Vendor	15
Student	04
Others	19
Circumcision in male	
Circumcised	06
Uncircumcised	49

Table 2: Age- and sex-wise distribution of HIV-positive patients with intestinal parasites

Age (years)	Total number of patients		Parasites detected	
	Male	Female	Male	Female
15-25	19	04	02	01
26-35	16	03	02	03
36-45	11	08	02	02
46-55	08	05	01	00
>55	01	00	00	00
Total	55	20	07	06

Table 3: Symptoms of UTI and HIV status

HIV status	HIV-positive	HIV-negative
Symptoms of UTI present	09	00
No symptoms of UTI	66	50
Culture positive >10 ⁵ colony/ml of urine	12	01

Table 4: Symptoms of UTI present in HIV-positive patients

Urinary symptoms	Male	Female
Fever	02	01
Dysuria	03	01
Frequency	02	02
Suprapubic pain	00	01
No symptoms of UTI	50	16

Table 5: Bacterial isolates from positive urine culture

Bacterial isolates	HIV-positive patients with UTI		Total (12)
	Male	Female	
<i>Escherichia coli</i>	04	04	08 (66.7)
<i>Klebsiella</i>	02	00	02 (16.7)
<i>Pseudomonas aeruginosa</i>	01	00	01 (8.3)
<i>Staphylococcus aureus</i>	00	01	01 (8.3)

earlier study showed that intestinal opportunistic protozoal infection and diarrhea are more common in patients with low CD₄⁺ count [5-7].

In our study of urine sample from 75 HIV-positive patients, 12 samples showed significant bacteriuria and diagnosed to have UTI. Among the 75 patients, 9 patients had symptoms of UTI which is given in Table 4. Fever, frequency, and dysuria were the most common symptoms in our study. Among the 66 asymptomatic patients, 3 were diagnosed to have UTI. Hence, asymptomatic bacteriuria is present in patients with HIV infection (3/75; 4%) than control group (1/50; 2%) [8]. Gugino *et al.* also documented the presence of asymptomatic bacteriuria in HIV-infected patients. In our study, the total number of UTI diagnosed in HIV-positive patients was 12 (16%). Chi-square test reveals a statistically significant relationship between HIV status and UTI ($\chi^2=6.310$, $df=1$, $0.01 < p < 0.02$). A similar study was conducted by Schonwald *et al.* [9] where the frequency of UTI in HIV-positive individuals was 16%. In the study of De Pinho *et al.*, UTI in AIDS patients versus asymptomatic HIV-positive individuals was 13.3% versus 3.2% [10]. According to Santos *et al.*, bacteriuria was significantly more frequent among AIDS patients (7.6%) than control group of HIV-negative individuals (0.91%) [11]. The study by Marques *et al.* showed that UTI was more common in HIV-positive patients (6.3%) [12]. A similar study by Evans *et al.* also showed the higher incidence of UTI (5.7%) [13] among HIV-positive patients than control group.

In our study, prevalence of UTI in HIV-positive patients was more in female (5/20; 25%) than male (7/55, 12.7%). In the study by Marques *et al.*, UTI in female and male HIV-positive patients was 8.7% and 6.3%, respectively. In our study, prevalence of UTI among HIV-positive patients is high (16%), but it is within the range as described by Heyns and Fisher (7-50%) [2]. It may be due to the fact that the rate of use of prophylactic antimicrobials for opportunistic infections is very low in this study group because of poor socioeconomic status [14,15].

Among the 12 culture-positive UTI cases, 8 (66.7%) were *E. coli*, followed by *Klebsiella 2* (16.7%) and *Pseudomonas/S. aureus 1* (8.33%) each (Table 5). In both symptomatic and asymptomatic groups, *E. coli* was the most predominant organism. The single isolate from HIV-negative group was also *E. coli*. In a similar study by De Pinho *et al.*, *E. coli* was the predominant pathogen (47.6%) among HIV-positive patients [11]. Marques *et al.* reported 63.1% of isolates to be *E. coli* and 5.3% *Acinetobacter* in his study but found in their study that Enterococci were the most frequent isolates in patients with HIV, whereas *E. coli* was most frequently isolated in controls [9]. The inference drawn in the present study is limited to a small number of patients. These findings may be useful for further exploration in a larger number of samples.

SUMMARY AND CONCLUSION

1. The study period of 1 year and 8 months revealed that it is difficult to locate/identify an HIV-positive person in a place like Kadapa on account of confidentiality/lack of awareness among the population.
2. Having identified the HIV-positive cases, collection of urine samples

from the patients also needed counseling and intervention by the respective clinicians, especially when patients were not having any symptoms.

3. Follow-up of the HIV-positive cases is difficult as they lose contact with the clinicians and not traceable after being discharged from the hospital.
4. As there was difficulty in identifying the HIV-positive cases, having located a case we collected urine samples from the patients.
5. Out of 75 HIV-positive cases, 12 (16%) are showing significant bacteriuria ($>10^5$ organisms/ml of urine). Among them, 9 patients have the symptoms of UTI.
6. *E. coli* (67%), *Klebsiella* (17%), *Pseudomonas aeruginosa* (8%), and *S. aureus* (8%) are the isolates from significant bacteriuria cases.
7. Out of 50 HIV-negative individuals, only 1 (2%) urine sample is showing significant bacteriuria.

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