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.
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
Semi-quantitative culture method - semi-quantitative technique of inoculation in suitable culture media - by standard loop method was done to determine significant bacteriuria.

Culture media used
MacConkey agar, Blood agar, Lactose sugars bile salt, neutralized 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 10^5 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 (χ²=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/µl. Hence, the relation between CD4+ count and presence of intestinal parasites could not be established. The earlier study showed that intestinal opportunistic protozoal infection and diarrhea are more common in patients with low CD4+ count [5-7].

| 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    |

| 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     |

| 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 |

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

| Bacterial isolates | HIV-positive patients with UTI | Total (12) |
|--------------------|--------------------------------|------------|
|                    | Male | Female | Male | Female |
| Escherichia coli   | 04   | 04     | 08   | 08     | (66.7) |
| Klebsiella         | 02   | 00     | 02   | 02     | (16.7) |
| Pseudomonas aeruginosa | 01   | 00     | 01   | 01     | (08.3) |
| Staphylococcus aureus | 00   | 01     | 01   | 01     | (08.3) |
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 (χ²=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 in 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 (8.3%) 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 their 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 (>10⁵ 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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