Original Research Article

Association of Uropathogens with Asymptomatic Urinary Tract Infection in Diabetes Mellitus Patients

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Abstract

Asymptomatic Bacteriuria has been maximally associated with Diabetes Mellitus. In the present study, a total of 125 midstream urine samples were processed, 40% showed significant bacteriuria. Macroscopically predominance was of pale color (88%) and cloudy in nature while microscopically was of pyuria (82%). Gram Negative (46%) isolates were in predominance followed by Gram Positive (36%) and Candida species (9%). In Gram negative isolates, E.coli (56.52%) was the prevailing organism while in Gram Positive isolates Staphylococcus aureus (36.8%). Nitrofurantoin was the most sensitive antibiotics against gram positive however Ciprofloxacin for Gram negative bacteria.

Keywords
ASB- Asymptomatic Bacteriuria, CLSI- Clinical Laboratory Standards Institute.

Introduction

Bacteriuria sans symptoms is termed as asymptomatic Bacteriuria. It is not well understood why patients with ASB do not develop symptoms. One possible mechanism is that bacteria with decreased virulence may colonize the urine rather than causing a symptomatic infection (Raz, 2003). Asymptomatic Bacteriuria has been maximally associated with Diabetes Mellitus in individuals with low immunity which can even lead to grave complications (Smith, 1972; Geerlings et al., 2000; Patterson, 1997). First and foremost is its conversion in symptomatic bacteriuria due to the involvement of the lower urinary tract followed by the infection of the upper urinary tract. Additionaly, ASB, if not treated can lead to occurrence of recurrent urinary tract infections which in turn leads to complications viz emphysematous cystitis, pyelonephritis, perinephric and renal abscess, neurogenic bladder and
vesicoureteral reflex, bacteremia and eventually renal failure. Pregnant women being at higher risk of mortality and morbidity with the subsequent increase in the nosocomial infections (Hu et al., 2004). An important aspect attached to it is, despite of the importance of ASB in diabetic patients, not much studies have been carried out in the developing countries which includes the ever progressing India as well. The present study was aimed to evaluate the causative agents which were causing ASB in the patients already diagnosed with Diabetes Mellitus.

**Materials and Methods**

A cross sectional prospective study was conducted on 125 midstream urine samples collected by clean catch technique from diagnosed cases of DM patients, admitted in various wards of MMIMSR, Mullana from August 2012 to March 2013. After collection, the urine samples were immediately transported to the department of Microbiology and were subjected to Direct Microscopy and Culture on Cysteine Lactose Electrolyte Deficient (CLED) agar. After overnight incubation of culture plates at 37°C a colony count of 10^5 CFU/mL or greater isolates were identified by Gram staining, battery of biochemical reactions and subjected for antibiotic susceptibility testing as per Clinical Laboratory Standards Institute (CLSI) guidelines. The Ethical clearance was taken from MMIMSR ethical committee.

**Results and Discussion**

The rate of uropathogens from diabetes mellitus patients with ASB was 40% (Table1). The macroscopic examination of Urine specimens is essential part of diagnosis, out of 50 culture positive, 88% were pale in color, 8% were having red and 4% were having red brown color. Appearance include 42% cloudy specimen, 40% milky and clear 18% (Table2). The microscopic examination of urine showing pyuria in 82% while only 4% cases showed hematuria. (Table3). The Gram Negative (46%) isolates were in predominance followed by Gram Positive (36%) and Candida species (18%). In Gram negative isolates, *E.coli* (56.52%) was the prevailing organism while in Gram Positive isolates *Staphylococcus aureus* (38.8%) (Table4). As per Antibiotic susceptibility pattern is concerned Nitrofurantoin and Gentamycin were the most sensitive antibiotics against *S.aureus*. On the other hand Ciprofloxacin and Nitrofurantoin were the effective antibiotics (100% each) against *S.saprophyticus*. However, in case of *Enterococcus species* Nitrofurantoin was found to be more sensitive. In case of Gram negative bacteria, the most sensitive drug was found to be Ciprofloxacin followed by Tetracycline (Table 5).

In present study asymptomatic bacteriuria was in 40% cases of diabetes mellitus (Table 1). It is comparable with Makuyana et al., (2002) who reported 32% while Yeshitela et al., (2012) reported 10.4%. Sibi et al., (2011) 68% and Vishal Sharma et al., (2012) 50.84%. (Table1) The variations in percentages of ASB have been attributed to factors such as geographical background, traditional variations of the subjects and difference in the screening test. Infection and host immunity have always been inversely proportional to each other. Whenever there is decrease in the immunity of the individual, the body gets predisposed to the infections which may be due to many causes and vice versa. One of the potential causes is the association of some secondary disease with the primary one. On the similar grounds, association of diseases viz hypertension, prostatic syndrome, chronic renal and pulmonary diseases with DM
patients, further lowers the immunity thereby making the patient more susceptible to asymptomatic urinary tract infection.

In present study 88% of patients were having pale color of urine (Table 2). According to Henry et al., (2012) pale urine of high specific gravity may be found in diabetes mellitus. In present study 8% were having red color of urine and 4% were having red-brown (Table 2) which may be due to chronic renal disease patients has been included in the study. Appearance of urine sample is another parameter in gross examination. In present study 42% of patients were having cloudy appearance of urine sample which was in concurrence with the presence of leukocytes, bacteria and yeast. Urine samples showed 40% milky appearance in the current study which may be due to neutrophils which is in concurrence with the microscopic finding.

Microscopic examination of specimens in diabetes mellitus patients with ASB is associated with the color of urine sample which in order is associated with different complications. In current study 88% of patient were having pyuria (more than five leukocytes/ high power field) (Table 3). This result was closely associated with the positive urine cultures and development of ASB. This was in concordance with the Mohammad et al., (2008) and Hale Turan et al., (2008) who stated a significant association with bacteriuria. In contrast with that Richard Colgan et al., (2006) stated that pyuria is not specific for UTI and may occur with other inflammatory disorders of the genitourinary tract. The prevalence of asymptomatic pyuria was significantly increased in patients with duration of diabetes leading to increase degree of nephropathy.

The presence of red blood cells in urine denotes hematuria. Ophori et al., (2010) in his study detected in 6% of cases. In present study 4% of patients were having hematuria who showed positive results for ASB (Table 3) which may be due to patients of chronic renal disease.

For the accurate identification of the causative organism culture is necessary. In the current study, predominant causative agents were gram negative bacteria (46%) while gram positive was (36%), isolate of yeast were least (18%) (Table 4) similar results were of Alebiosu et al., (2003) 69.7% and Gizachew et al., (2012) 57.3% where gram negative organisms showed predominance.

E.coli (56.52%) was the most frequent uropathogen isolated in present study (Table 4) concordance with, Makuyana et al., (1995) (26%), jha BK et al., (2009) (41.66%) Vishal Sharma et al., (2011) (41.5%), Ophori et al., (2006) (56.9%), Mohammad et al., (2010) (59.1%). E.coli have adhesions such as P and S fimbria responsible for binding uroepithelial cells subsequently increasing virulence. The result is consistent with the majority of reports where E.coli had been reported to be the major pathogen in ASB. This is in contrast to the report of Alebiosu et al., where K.pnemoniae was the most common isolates from ASB.

The most common isolates among gram positive were Enterococcus and S.aureus (38.8%) similar to Gizachew et al., Enterococcus species (11%) and Staphylococcus (25%) were the common species causing infection among gram positive bacteria. The fecal flora Enterococci which contaminates perineum may have been caused ASB.

Diabetic patients are prone to infection with Candida in the Urinary tract.
In the current study *Candida* (66.6%) isolates were present (Table 4), in accordance with this Kauffman *et al.*, states that *C.albicans* has been the yeast most commonly isolated from urine, accounting for 50%-70% of isolates. *Candida glabrata* (22.2%) and *Candida tropicalis* (11.1%) were also reported in present study. High blood sugars of mucous membranes makes a perfect environment for yeasts to grow.

Antibiotic Sensitivity Testing necessary for appropriate treatment thereby potentiating the prognosis of the disease.

Anibiotic Sensitivity Test (Table 5) of *E.coli, Klebsiella* and *Pseudomonas* showed Ciprofloxacin was the most sensitive drug and Ampicillin was the resistant drug which is in concordance with the Ophori *et al.*, Gizachew *et al.*, (2012).

### Table.1 Rate of Uropathogens from diabetes mellitus patients with ASB.

| TOTAL Samples processed | Total no. of bacterial isolates | PERCENTAGE | P value* |
|-------------------------|---------------------------------|------------|----------|
| 125                     | 50                              | 40%        | <0.0001  |

Table1- Illustrates the rate of uropathogens from diabetes mellitus patients with ASB. 40% of urine samples were positive.

### Table.2 Gross examination findings of specimen in Diabetes Mellitus patients with ASB

| Macroscopic examination of Urine | Number (N=50) | Percentage (%) |
|----------------------------------|---------------|----------------|
| Color                            |               |                |
| Pale                             | 44            | 88%            |
| Red                              | 4             | 8%             |
| Red-brown                        | 2             | 4%             |
| Appearance                       |               |                |
| Cloudy                           | 21            | 42%            |
| Milky                            | 20            | 40%            |
| Clear                            | 9             | 18%            |

Table2: Depicts the macroscopic examination of Culture positive Urine specimens. Of the 50 culture positive urine specimens, 44 (88%) specimens were pale in color, indicating definite relation with asymptomatic bacteriuria and culture positivity. 4(8%) were having red and 2(4%) were having red brown color. Appearance include 21(42%) cloudy specimen, 20(40%) milky and clear 9(18%).

### Table.3 Microscopic examination findings of specimens in Diabetes mellitus patients with ASB

| Microscopic examination | Number | Percentage | P value* |
|-------------------------|--------|------------|----------|
| Pyuria                  | 41     | 82%        |          |
| Hematuria               | 2      | 4%         | 0.0089   |

Table3 illustrates the microscopic examination of urine showing pyuria in 82% of diabetic cases. It suggests that presence of pus cells in microscopy indicate the presumptive diagnosis of urinary tract infection while only 4% cases showed hematuria.
Table 4: Distribution of Gram positive and Gram negative uropathogens in Diabetes mellitus patients with ASB

| Total no. of Isolates | Gram Positive isolates | Gram Negative Isolates |
|-----------------------|-------------------------|------------------------|
|                       | Bacteria                | Candida Species        |                        |
|                       | 18(36%)                 | 9(18%)                 | 23(46%)                |
| Rate of Isolates      |                         |                        |                        |
| S. aureus             | 7 (38.8%)               |                        |                        |
| S. saprophyticus      | 2 (11.1%)               |                        |                        |
| S. Epidermidis        | 2 (11.1%)               |                        |                        |
| Enterococcus faecalis | 7 (38.8%)               |                        |                        |
| E. coli               |                         |                        |                        |
| C. albicans           | 6 (66.6%)               |                        |                        |
| C. tropicalis         | 1 (11.1%)               |                        |                        |
| C. glabrata           | 2 (22.2%)               |                        |                        |
| E. coli               | 13 (56.52%)             |                        |                        |
| Klebsiella spp.       | 9 (39.1%)               |                        |                        |
| Pseudomonas           | 1 (4.34%)               |                        |                        |

Table 4: Depicts the distribution of Gram Negative and Gram positive uropathogens isolated. Gram Negative (46%) isolates were in predominance followed by Gram Positive (36%) and Candida species (9%). In Gram negative isolates, E.coli (56.52%) was the prevailing organism while in Gram Positive isolates Staphylococcus aureus (36.8%).

Table 5: Antimicrobial susceptibility patterns of Gram Positive and Gram Negative uropathogens of diabetic patient with ASB.

| Nature of Antibiotics | Gram Positive Bacteria | Gram Negative Bacteria |
|-----------------------|------------------------|------------------------|
| Staphylococcus aureus | N=7                    |                        |
| S. saprophyticus      | 4(57.1%)               | E. coli N=13           |
| S. epidermidis        | 2(100%)                | Klebsiella spp. N=9    |
| E. coli               | N=7                    |                        |
| Gentamycin            | 4(57.1%)               | 5(38.4%)               |
| Nitrofurantoin        | 4(57.1%)               | 3(33.3%)               |
| Ciprofloxacin         | 1(14.2%)               | 0%                     |
| Tetracycline          | -                      |                        |
| Ampicillin            | 3(42.8%)               |                        |

Table 5 depicts that Nitrofurantoin was the most sensitive antibiotics against Gram positive isolates: Staphylococcus aureus (57.1%), Staphylococcus saprophyticus (100%), Enterococcus faecalis (57%) and Ciprofloxacin for Gram negative isolates: E.coli (84.6%), Klebsiella spp (66.6%) and Pseudomonas (100%).

In case of Gram positive organisms, Nitrofurantoin and Gentamycin were the most sensitive antibiotics against S.aureus. On the other hand Ciprofloxacin and Nitrofurantoin were the effective antibiotics (100% each) against S.saprophyticus. However, in case of Enterococcus species Nitrofurantoin was found to be more sensitive.

In Patients of Diabetes Mellitus, Asymptomatic Bacteriuria was statistically significant. Macroscopic and microscopic examination is the essential part in diagnosis of ASB. The gram negative organisms, E.coli was prevailing due to presence of P and S fimbria responsible for binding uroepithelial cells. Staphylococcus aureus was predominant in Gram positive organisms. As early treatment can prevent further complications periodic scrutiny of diabetic patients for UTI should be done.
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