

Asymptomatic Bacteriuria In Patients With Type-2 Diabetes Mellitus

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Abstract : Background: Diabetic subjects, especially women, show high prevalence of asymptomatic bacteriuria (ASB). The aim of the present study was to evaluate the prevalence of asymptomatic bacteriuria (ASB) in patients with type 2 diabetes mellitus and to find out the antibiotic sensitivity pattern of bacterial isolates. Methods: One hundred and thirty type 2 diabetics comprising 56 males and 74 females (aged between 30 - 59 years) attending the outpatient department of Chhattisgarh Institute of Medical sciences hospital, Chhattisgarh, India was recruited in the study. Mid-stream urines were collected from patients aseptically into sterile wide mouth container and examined microscopically, culturally using standard techniques and tested for glucose, post-prandial glucose, protein and ketone using a dipstick. Samples were cultured on blood agar, McConkey agar and Cysteine Lactose Electrolyte Deficient (CLED) media and incubated at 37°C aerobically for 24 h. Isolates were tested against antibiotics which included tetracycline, chloramphenicol, ciprofloxacin and cotrimoxazole by the disc diffusion method. Results: Significant bacteriuria was observed in Forty-seven (36.15%) of urine samples (34 females and 13 males). Bacteria isolated included Escherichia coli (56.9%), Enterobacter sp. (12.7%), Klebsiella pneumoniae (8.5%) and Proteus sp. (6.3%). E. coli, Enterobacter sp. K. pneumoniae, S. aureus and Proteus sp. were most sensitive to cotrimoxazole, amoxicillin, nalidixic acid and ciprofloxacin but a large number of bacteria were resistant to tetracycline, chloramphenicol and ampicillin. Conclusion: Screening for ASB is necessary in diabetic patients especially if pyuria is detected in urine analysis since ASB has been found to be a risk factor for developing symptomatic urinary tract infection [Singh L et al NJIRM 2013; 4(6) : 1-4]

Key Words: Bacteriuria, Type 2 diabetes mellitus, Escherichia coli

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Introduction: The term bacteriuria means the presence of bacteria in urine and it is taken to be significant if 10^5 organisms per millilitre of a fresh "clean catch" urine specimen are present in any patient¹. Asymptomatic bacteriuria is a major risk factor for the development of UTI in pregnancy due to physiological changes².

Diabetes mellitus is a major health problem in India. The prevalence of asymptomatic bacteriuria (ASB) in women has been reported as in school children (6 - 7%), during pregnancy (6% asymptomatic) and 10 - 12% among elderly women³. Diabetes mellitus has a number of long term effects on the genitourinary system. This effect predisposes to bacterial urinary tract infection (UTI) in the patient with diabetes⁴. Development of asymptomatic UTI in diabetic women has been reported to be much more common than in non-diabetic women, men, and from diabetic out-patients with urinary tract infections^{5,6}.

Most bacterial aetiologic agents in asymptomatic bacteriuria have been reported to include Klebsiella

pneumoniae, Escherichia coli, Enterobacter sp. Streptococcus agalactiae, Enterococcus faecalis, coagulase negative Staphylococcus and Streptococcus pyogenes^{1,2,7}. A number of these organisms such as K. pneumoniae, E. coli and E. faecalis have been reported to be very sensitive to nitrofurantoin, gentamycin, ciprofloxacin and ofloxacin but resistant to ampicillin, tetracycline and septrin¹. However, Olaitan (2006) reported that cotrimoxazole was the most effective antimicrobial agent against E. coli, S. aureus and E. faecalis, but resistant to ampicillin and tetracycline. Also, increased resistance against amoxicillin, cotrimoxazole and nalidixic acid has been shown against K. pneumoniae, E. coli, and Enterobacter sp.^{7, 8}. Untreated ASB predisposes the individual to recurrent UTI which can cause renal disease. Patients with diabetes mellitus have been reported to have increased rates of UTI infections⁹. Increased UTI in pregnant women has led to high morbidity and mortality with the subsequent increase in nosocomial infections¹⁰.

For women, two consecutive specimens with isolation of the same species in quantitative counts of at least 10,000 colony forming units/ml (cfu/ml) of urine while for men, a single specimen with one bacterial species isolated in a quantitative count of at least 10,000 cfu / ml is regarded as asymptomatic bacteriuria¹¹. Therefore, the aim of the present study was to evaluate the prevalence of ASB in type 2 diabetic patients and to determine antibiotic sensitivity pattern among isolates.

Materials And Methods: A one hundred and thirty type 2 diabetes mellitus patients with asymptomatic UTI, consecutively attending the outpatient department of Chhattisgarh Institute of Medical sciences' hospital, Bilaspur, Chhattisgarh, India were recruited in the study. The physical examination and the interview of the subjects were carried based on a study protocol by the researchers. Subjects with overt diabetic nephropathy (proteinuria) or nephropathy from other causes were excluded. In addition, subjects with symptoms of UTI (including dysuria, frequency, fever, urgency and abdominal discomfort) or use of antimicrobial drugs or non-steroidal anti-inflammatory drugs or immunosuppressors in the last 14 days were excluded by the study. Urinary tract infection was defined by a urinalysis result showing more than ten pus cells per high power field .

Patients were aged between 30 - 59 years (females were aged range 30 - 44 years and males 45 - 59 years). Clinical parameters including duration of diabetes mellitus, drug therapy, clinical symptomatology especially urinary complaints, and last menstrual period were recorded. Fasting blood sugar and two-hour post prandial blood sugar were estimated¹.

Midstream urine samples were collected from patients into sterile wide mouth container for urinalysis. Samples were inoculated on MacConkey agar, Blood agar and Cysteine Lactose Electrolyte Deficient (CLED) media using a calibrated loop to determine colony forming unit. The plates were incubated at 37°C aerobically for 24 h. Cultures

with colony counts $\geq 10^5$ cfu/ ml were considered as significant bacteriuria^{12,13}. The organisms were identified using standard cultural, morphological and biochemical techniques¹⁴. Antimicrobial sensitivity testing was carried out on Mueller Hinton Agar (MHA) plates with commercially available discs (Hi-Media, Mumbai) by the Kirby-Bauer disc diffusion method and interpreted according to NCCLS criteria¹⁵.

Asymptomatic bacteriuria in patients with diabetes mellitus is the presence of a significant quantity of bacteria in a urine specimen properly collected from a person without symptoms or signs of UTI. The study protocol was approved by the Institutional Ethical Committee and full informed written consent was obtained from all patients. Data are presented as percentages.

Results: A total of one hundred and third type 2 diabetic subjects were selected for the study. In this there were 74 females and 56 males. Table 1 shows ASB with age and sex of patients. ASB was highest in age groups 45 - 49 in males and 35 - 39 in females. . Forty-seven (36.15%) of urine samples had significant bacteriuria (34 females and 13 males). Their ages ranged from 30 - 59 years with a mean of 48 ± 3.0 years. The highest percentage from females was the age group of females with a high child bearing rate. Table 2 shows the bacteria isolated in ASB and the percentage prevalence of the organisms.

E. coli were found to be the most prevalent in ASB (59.6%), followed by *Enterobacter* sp. (12.7%) and *K. pneumoniae* (8.5%). The least prevalent organisms were *Streptococcus pyogenes*, *E. faecalis* and *S. saprophyticus* (4.3% each). Of the 28 *E. coli* isolated, 22 (78.5%) were from females. The antibiotic sensitivity test is shown in Table 3. The results showed that *E. coli*, *K. pneumoniae*, *S. aureus* and *Proteus* spp. were highly sensitive to cotrimoxazole, amoxycillin, nalidixic acid and Ciprofloxacin. Very few bacteria were sensitive to tetracycline, chloramphenicol and erythromycin. Only *Proteus* and *S. saprophyticus* were sensitive to ampicillin.

Table 1. The age and sex distribution of diabetics with asymptomatic bacteriuria (ASB).

Age (years)	Males number with ASB / %	Females number with ASB / %	Total number with ASB / %
30-34	2(15.4)	2(5.9)	4(8.5)
35-39	1 (7.7)	16(47.1)	17(36.2)
40-44	1 (7.7)	6(17.6)	7(14.9)
45-49	4 (30.7)	4(11.8)	8(17.0)
50-54	3(23.1)	4(11.8)	7(14.9)
55-59	2(15.4)	2(5.9)	4(8.5)
Total	13(27.66)	34(72.34)	47(100)

Table 2. Bacteria isolated from urine samples of patients with type -2 diabetes mellitus with ASB.

Bacteria	No. of occurrence	Occurrence (%)
<i>Escherichia coli</i>	28	59.6
<i>Enterobacter</i> sp.	6	12.7
<i>Klebsiella pneumoniae</i>	4	8.5
<i>Proteus</i> sp.	3	6.3
<i>Staphylococcus aureus</i>	2	4.3
<i>Enterococcus faecalis</i>	2	4.3
<i>Strept. saprophyticus</i>	2	4.3
n =	47	100

n = number of occurrence / percent occurrence.

Table 3. Bacterial isolates from diabetic patients that was sensitive to the different antibiotics.

Antibiotics	Number of each bacterial isolates sensitive to the different Antibiotics					
	<i>Escherichia coli</i>	<i>Enterobacter</i> sp.	<i>Klebsiella pneumoniae</i>	<i>Proteus</i> sp.	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>
Tetracycline	4	1	2	0	0	1
Choramphenicol	6	2	0	0	1	2
Amoxycillin	8	6	3	3	1	2
Ampicillin	0	0	0	1	0	0
Cotrimoxazole	16	6	4	3	0	0
Nalidixic	10	6	2	2	0	1
Gentamycin	4	0	0	1	1	0
Ciprofloxacin	12	6	4	2	1	1
n = number of isolate	28	6	4	3	2	2

Discussion: The present study showed that asymptomatic bacteriuria (ASB) was present in 47 (36.15%) out of 130 patients with type 2 diabetes mellitus. This result was higher when compared to previous studies which showed 21% in Karachi⁹, 26% in Nigeria¹, and 19% in Bahrain¹¹. The population studies in these reports are comparable to the number of patients in this study. Some studies have even reported much lower values of between 5.8 - 19%¹. The variations in percentages of ASB have been attributed to factors such as geographical variations, ethnicity of the subjects and variation in the screening test². *E. coli* was the most common pathogen isolated in this study (59.6%). This is in contrast to the report of Alebiosu et al. where *K. pneumoniae* was the most common isolates from

ASB. However, the result is consistent with the majority of reports where *E. coli* had been reported to be the major pathogen in ASB^{2,7,9,13}. This is why

in general practice most work on pathogenesis of UTI focuses on *E. coli* because of its high prevalence in UTI¹⁶. In chronic UTI, a slow growing *E. coli* with atypical colony morphology and multi-drug resistance has been reported¹³. Most of the *E. coli* isolated in this study was from the pregnant females, the highest prevalence being among the age group of 35 - 39 years. This is in agreement with previous reports where a high incidence of *E. coli* in ASB in diabetics women have been reported². Other bacteria isolated include *Enterobacter* sp. (12.7%), *K. pneumoniae* (8.3%), *Proteus* (6.3%) and *S. aureus*, *S. faecalis* and *S. saprophyticus* (4.3% each). *Klebsiella* spp. and *E. coli* are known to be important causes of both nosocomial and community acquired UTI.

Results of antibiotic sensitivity showed that cotrimoxazole and ciprofloxacin were very effective against most of the isolates. However, *S.*

saprophyticus was resistant to both antibiotics. The sensitivity of *E. coli* in this study is in agreement with previous reports ^{7, 17}. The high sensitivity to these two antibiotics may be due to their broad spectra on bacteria. Very few isolates were sensitive to tetracycline, ampicillin, chloramphenicol and erythromycin. Olaitan reported that the high prevalence of resistance to some of the commonly used antibiotics such as ampicillin and tetracycline may be due to their abuse and low cost of purchase. These factors are common in the study environment where some patients buy drugs without a physician's prescription.

Conclusion : A high prevalence of ASB was established in both males and females. The main pathogen was *E. coli* and this organism is beginning to acquire resistance to some of the clinically used antibiotics. The authors suggested that screening for ASB is warranted in diabetic patients particularly if pyuria is detected in urine analysis since ASB has been found to be a risk factor for developing symptomatic urinary tract infection.

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