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Full Length Research Paper

Asymptomatic bacteriuria in patients with type-2 diabetes mellitus

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This study was to investigate the prevalence of asymptomatic bacteriuria (ASB) in patients clinically diagnosed with type 2 diabetes mellitus and to determine the antibiotic sensitivity pattern of bacterial isolates. One hundred and thirty type 2 diabetics comprising 56 males and 74 females (aged between 30 - 59 years) attending the Central Hospital, Benin- City, Nigeria were studied. Mid-stream urines were collected from patients who gave informed consent aseptically into sterile McCartney bottles 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. White blood cells (WBC) and red blood cells (RBC) were detected in 87 and 6% of samples while ketones and proteins were 6% and 96% respectively present in the samples. Significant bacteriuria (≥10^5 cfu /ml) was observed in some samples. Bacteria isolated included *Escherichia coli* with a prevalence of 56.9%, followed by *Klebsiella pneumoniae* (12.7%), *Staphylococcus aureus* (8.5%) and *Proteus* sp. (6.3%). *E. coli, K. pneumoniae, S. aureus* and *Proteus* sp. were most sensitive to cotrimazaxole, amoxicillin, nalidixic acid and ciprofloxacin but a large number of bacteria were resistant to tetracycline, chloramphenicol and ampicillin. The misuse of some antibiotics is a major factor responsible for bacterial resistance. Therefore, treatment of ASB in diabetics must be by drugs prescribed by physicians after proper laboratory analysis.

Key words: Bacteriuria, type 2 diabetes mellitus, antibiotics, Nigeria, white blood cell, red blood cell.

INTRODUCTION

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 diabetes (Nicolle, 2003). Diabetes mellitus is a major health problem in Nigeria. 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 women (Meiland et al., 2006). 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 (Geerling et al., 2001; Stapleton, 2002; Nicolle, 2005).

Asymptomatic bacteriuria is a major risk factor for the development of UTI in pregnancy due to physiological changes (Assel et al., 2009). Other factors include low socio-economic status, sickle cell trait, and grand multi-parity. 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 patient (Alebiosu et al., 2003; Kaas, 1956).

Most bacterial aetiologic agents in asymptomatic bacteriuria have been reported to include *Klebsiella pneumoniae, Escherichia coli, Streptococcus agalactiae, Enterococcus faecalis*, coagulase negative

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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)

Staphylococcus and *Streptococcus pyogenes* (Alebiosu et al., 2003; Olaitan, 2006; Assel et al., 2009). 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 septrin (Alebiosu et al., 2003). 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. (Dytan and Chua, 1999).

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 infections (Baqai et al., 2008; Vazquez and Sobel, 1995). Increased UTI in pregnant women has led to high morbidity and mortality with the subsequent increase in nosocomial infections (Vazque and Sobel, 1995).

The objective of this study was to investigate the aetiologic agents of ASB in patients with type 2 diabetes mellitus and the antibiotic sensitivity pattern among isolates.

MATERIALS AND METHODS

A total of 130 urine samples were collected from patients with type 2 diabetes mellitus with UTI attending the Central Hospital, Benin - City, Nigeria. Urinary tract infection here is characterized by a urinalysis result showing more than ten pus cells per high power field and no previous history of antibiotic intake, non-steroidal anti-inflammatory drugs and immuno-suppressors in the preceeding 2 weeks. The study had the approval of the Ethics Committee of the Central Hospital Benin-City. All patients gave their informed consents.

Patients were aged 30 - 59 years (females were aged range 30 - 44 years and males 45 - 59 years). The females were all pregnant. 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 estimated as reported (Alebiosu et al., 2003).

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. 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 bacteriuria (Hajeri et al., 2008).

Midstream urine samples were collected from patients into sterile MacCarthey bottles 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 ≥ 10^5 cfu/ ml were considered as significant bacteriuria (Flower et al., 1991; Harding et al., 2002). The organisms were identified using standard cultural, morphological and biochemical techniques (Bucchana and Gibbon, 1974).

Urinalysis was carried out using a dipstick while antibiotic sensitivity test was done by the disc diffusion test (Kirby-Bauer method) using Mueller-Hinton agar (Lab.39)???? What is this?. The antibiotics used included tetracycline, chloramphenicol, amoxicillin, cotrimoxazole, ciprofloxacin, and gentamycin (Mast Diagnostics, Merseyside, and U.K). Resistance and sensitivity to antibiotics was measured by the method of Baker and Breach (1980). When the antibiotic agent was 16 mm or higher, it was recorded as sensitive and resistant when less than 16 mm. The sensitivity plates were incubated aerobically for 18 h, and the zone of inhibition was recorded.

RESULTS

There were 74 females and 56 males with diabetes mellitus. The mean fasting blood glucose and two hour post-prandial blood glucose levels were 6.2 ± 1.4 $mmol^1$/L (range 5.1 - 7.3 mmol/L) and 9.4 ± 1.0 mm^1/L (range 9.0 - 9.8 mmol/L), respectively. Also, urinalysis showed that ketone bodies were present in 6%, protein 96%, WBC 87% and RBC in 6% cases.

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.

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. 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 *K. pneumoniae* (12.7%) and

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

Bacteria	No. of occurrence	% occurrence
Escherichia coli	28	59.6
Klebsiella pneumoniae	6	12.7
Staphylococcus aureus	4	8.5
Proteus sp.	3	6.3
Streptococcus pyogenes	2	4.3
Enterococcus faecalis	2	4.3
Strept. saprophyticus	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						
	EC	Isolates KP	SA	P	SP	EF	SS
Tetracycline	4	1	2	0	0	1	0
Choramphenicol	6	2	0	0	1	2	0
Amoxycillin	8	6	3	3	1	2	1
Ampicillin	0	0	0	1	0	0	1
Cotrimoxazole	16	6	4	3	0	0	1
Nalidixic	10	6	2	2	0	1	1
Gentamycin	4	0	0	1	1	0	1
Ciprofloxacin	12	6	4	2	1	1	0
n = number of isolate	28	6	4	3	2	2	2

An isolate with a zone of inhibition ≥ 16 mm is sensitive while ≤ 15 mm is resistant to a particular antibiotic.
EC- *Escherichia coli,* KP- *Klebsiella pneumonia,*SA- *Staphylococcus aureus,* P- *Proteus sp.* EF- *Enterococcus faecalis,* SS- *Strept. Saprophyticus.*

S. aureus (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.

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 is higher than results of previous studies that showed 21% in Karachi (Baqai et al., 2003), 26% in Nigeria (Alebiosu et al., 2003), 9.3% in Ethiopia (Uncu et al., 2002), in Ghana (Turpun et al., 2007) and 19% in Bahrain (Hajeri, 2008). 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% (Alebiosu et al., 2003). The variations in percentages of ASB have been attributed to factors such as geographical variations, ethnicity of the subjects and variation in the screening test (Assel et al., 2009).

E. coli was the most common pathogen isolated in this study (59.6%). This is in contrast to the report of Alebiosu et al. (2003) 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 (Assel et al., 2009; Olaitan 2006; Hajeri, 2008; Baqai et al., 2008). This is why in general practice most work on pathogenesis of UTI focuses on *E. coli* because of its high prevalence in UTI (Johnson, 2003). In chronic UTI, a slow growing *E. coli* with atypical colony morphology and multi-drug resistance has been reported (Triizsch et al., 2003). 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 reported (Assel et al., 2009).

Other bacteria isolated include *K. pneumoniae* (12.7%), *S. aureus* (8.3%), *Proteus* (6.3%) and *S. pyogenes*, *S. faecelis* and *S. saprophyticus* (43% each). *Klebsiella* spp. and *E. coli* are known to be important causes of both nosocomial and community acquired UTI.

Patton (1991) reported that *Klebsiella, Proteus* and *Enterococcus* spp. are the next most frequent isolates after *E. coli* in ASB. This is in line with the results of the present study where *Klebsiella* and *Proteus* had occurrences of 12.7% and 6.3% respectively, next to *E. coli* of 59.6%.

Results of antibiotic sensitivity showed that cotrimoxazole and ciprofloxacin where 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 (Olusanya and Olutiola, 1984). 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 (2006) 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.

In conclusion, a high prevalence of ASB was established in both males and females. The high prevalence of ASB of 36.15 % in this study is of major public health importance. The predominant pathogen was *E. coli* and this organism is beginning to acquire resistance to some of the clinically used antibiotics. The level of resistance to antibiotics recorded in this study is of great concern. The authors recommend improved personnel hygiene which is likely to reduce ASB that may be complicated in UTI. The use of unprescribed antibiotics and their abuse is a problem and appropriate public health programmes would help resolve this issue.

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