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ASYMPTOMATIC BACTERIURIA IN PATIENTS WITH DIABETES MELLITUS IN ILE-IFE, SOUTH-WEST, NIGERIA
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ASYMPTOMATIC BACTERIURIA IN PATIENTS WITH DIABETES MELLITUS IN ILE-IFE, SOUTH-WEST, NIGERIA

W. B. ODETOYIN, A. O. ABODERIN, R. T. IKEM, B. A. KOLAWOLE and A. O. OYELESE

ABSTRACT

Objectives: **To investigate the prevalence and associates of asymptomatic bacteriuria (ASB) in a sample of Nigerian diabetic patients.**
Design: **Cross-sectional descriptive and analytic study.**
Setting: **The Wesley Guild Hospital and Ife State Hospital, both units of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria.**
Subjects: **One hundred and thirty five diabetic patients and 57 non-diabetic patients as controls.**
Main outcome measures: **Demographic parameters of participants were recorded. Significant bacteriuria was determined for each of the mid-stream urine specimen obtained from all the subjects. Organisms isolated were identified and evaluated for antibiotic susceptibility patterns.**
Results: **There was a significant difference in the prevalence of ASB in the two groups. Prevalence of ASB was 16% and 3.5% in the diabetic patients and control respectively (p=0.03). Demographic parameters except age were not related to the presence of ASB. ASB was found in 54.4% of diabetic patients with poor glycaemia control compared with 2.9% in diabetics with good glycaemia control (p = 0.006). Organisms associated with ASB were *Staphylococcus aureus, Klebsiella sp, Escherichia coli* and *Enterococcus faecalis,* however the most predominant was *Staphylococcus aureus.* These organisms were largely resistant to the common antibiotics tested such as cotrimoxazole and gentamicin but susceptible to nitrofurantoin.**
Conclusions: **The prevalence of ASB is high in diabetic patients and poor glucose control can be considered a predisposing factor.**

INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disease. Apart from the vasculopathies (micro and macrovascular complications), diabetic patients are more prone to infections compared with non-diabetic patients (1). This is because diabetes causes several abnormalities in the host defence system. In a study of patients with bacteraemia, Carton *et al* (2) demonstrated that two thirds of the patients with infection were diabetic. They also showed that the most prevalent type of infection among diabetic patients was urinary tract infection (UTI). Apart from impaired host defence system, the high level of glucose concentration in urine may serve as culture medium for pathogenic microorganisms (3). Some serious complications of UTI such as emphysematous cystitis, pyelonephritis, renal or perinephric abcess, bacteraemia and renal papillary necrosis occur more commonly in diabetic patients (4).

Urinary tract infections may be symptomatic or asymptomatic; whether the symptomatic UTIs are preceded by asymptomatic bacteriuria (ASB) is not clearly understood (5). While some studies showed

no significant difference in the prevalence of ASB between diabetic and non- diabetic populations, majority of other studies reported a three fold increase in the prevalence of UTI in women with diabetes compared to non-diabetic women (6). This prevalence varies from 7.9% (7) to 26% (8). Associated risk factors for ASB include age, gender, marital status, duration of diabetes control and presence of complications of diabetes (4). It has been stated that though several researchers have looked at the problem of ASB among diabetics in different populations, there are still unanswered questions. The long-term consequences of ASB are not well established, and the exact role of ASB in diabetic women in a predisposition to sepsis or perinephric abscess is also unknown (9). Furthermore antimicrobial resistance is a large and growing problem in infections globally and particularly in most of Africa with a need to document resistance patterns as part of global efforts for containment of antimicrobial resistance. Because of the increased frequency of UTI and its complications observed among diabetic patients we investigated the prevalence and risk factors of ASB in diabetic patients attending the diabetes clinic of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, South-West Nigeria. We also identified the organisms responsible for ASB in this group and the antimicrobial sensitivity of such organisms.

MATERIALS AND METHODS

Study design and subjects: This was a cross sectional, descriptive and analytic study of consecutive diabetic patients who attended the diabetes clinic of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) between July 2004 and July 2005. A total of 135 patients were studied.

The participants included were type 2 diabetic patients who gave their consent. Patients whose clinical conditions were likely to influence the diagnosis such as pregnant women, recent hospitalisation or surgery (within the past four months), urinary tract abnormalities (including recent urinary tract instrumentation) or symptoms of UTI were excluded. Other exclusion criteria were recent use of antimicrobial drugs (within the preceeding 14 days), and history of renal disease. The following demographic parameters were recorded: age, gender, marital status, type of marriage, and duration of DM as well as presence of associated systemic arterial hypertension. Hypertension in the patients was defined (in all age groups) as a systolic blood pressure > 140mmHg, and a diastolic blood pressure > 90 mmHg, or previous use of antihypertensive drugs. Fifty-seven non-diabetic subjects matched for age and sex and screened for ASB served as controls.

Laboratory diagnosis: Mid-stream clean voiding urinary specimens were collected for determination of ASB and albuminuria. All urine samples were cultured within two hours of collection. Specimens were inoculated on Cysteine Lactose Electrolyte - Deficient (CLED) and chocolate agar plates using standard platinum wire loop (0.001ml). The plates were incubated aerobically at 37°C for 24 hours. After 24 hour incubation, the plates were examined. Cultures with colony counts $\geq 10^5$ml were considered as significant bacteriuria. The cultural and morphological characteristics of distinct and isolated colonies from each significant growth were determined. These isolates were gram –stained and characterised using the methods described by Cowan (10). ASB was defined as the presence of at least 10^5 colony forming units (cfu / ml) of one or two bacterial species in the clean-catch midstream urine samples of individuals without symptoms of UTI. Contaminated urine was defined as the presence of at least three different microorganisms in one urine specimen. One such specimen was excluded.

The antibiotic susceptibility testing of the isolates was performed by the disc diffusion technique as modified bythe National Committee for Clinical Laboratory Standards (NCCLS) (11). The following antibiotic discs gentamicin (GEN) 10μg, erythromycin (ERY) 5μg, nalidixic acid (NAL) 10μg, nitrofurantoin (NIT) 300μg, tetracycline (TET) 10μg, augmentin (AUG) 30μg, cefuroxime (CEF) 5μg, cotrimoxazole (COT) 25μg, chloramphenicol (CXM) 10μg, amoxicilin (AMX) 25μg, ofloxacin (OFL) 30μg and vancomycin (VAN) 30μg were included. *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (NCTC 10418) were used as control organisms.

Determination of albuminuria was carried out using Albustix (Bayer) strips coated with reagent (yellow). Each strip was dipped into a sample of urine to be tested for albumin for a few seconds. Then, the strips were inspected for colour change within 60 seconds. No colour change (yellow) indicated negative results, very light green indicated trace, and light green to deep green indicated positive results.

Statistical analysis: Simple proportions were used and categorical data were compared using chi-square. Subjects and non-diabetic controls were categorised into two depending on the presence or absence of ASB. Further comparisons were drawn between diabetic patients with good glycaemic control and those with poor control. Good glycaemic control was defined as fasting blood glucose $\geq 3 \leq 5$ mmol / l. The presence of isolates for both subjects and controls were also determined and expressed as percentages.

Differences between patients with and without ASB were tested with the students t -test for

continuous variable (age, duration of diabetes). P-value of < 0.05 was considered to be statistically significant.

RESULTS

A total of 192 persons participated in the study, 135 were diabetic (subjects) and 57 were non-diabetic (controls). Table 1 shows the demograghic parameters of subjects and controls.

Twenty one (16%) of the 135 diabetic patients had significant bacteriuria compared with two (3.5%) of the 57 non-diabetic patients. Bacteriuria was more prevalent in patients with DM (X^2= 4.55; p = 0.03, Fisher's exact) . The odds for diabetic patients to develop ASB was four times than for non-diabetic patients [OR 4.43 (95% CI= 1.0-28.3)].

Age was a significant risk factor for ASB among diabetic patients (p = 0.003) . The other risk factors (gender, marital status, type of marriage, duration of diabetes) were not related to the presence of ASB, so were the presence of hypertension and albuminuria.

Table 1
Variables of the study for DM patients with and without ASB

Variable	Diabetic		P-value	Non-diabetic		P-value
	ASB	No ASB		ASB	No ASB	
Age (years)	65.52±9.4	57.76±10.97	0.003	56±5.65	51.22±10.61	0.53
Gender						
Male	8(10.8)	66(89.2)	0.48	0(0)	27(100)	
Female	13(21.3)	48(78.7)		2(7)	28(93)	
Marital Status						
Married	18(15)	102(85)	0.7	1(2.1)	47(97.9)	0.29
Widowed	3 (20)	12(80)		1(11.1)	8(88.9)	
Type of Marriage						
Polygamous	6(19.4)	25(80.6)	0.36	1(5.3)	18(94.7)	
Monogamous	10(11.2)	79(88.8)		0(0)	29(100)	
DM duration (years)	6.81±5.18	6.34±5.17	0.7			
Blood pressure						
Hypertensive	15(19.7)	61(80.3)	0.1			
Normotensive	6(10.2)	53(89.8)				
Albuminuria						
Negative	12(17.6)	56(83.4)	0.7			
Trace	7(13.5)	45(86.5)				

Table 2 shows the prevalence of ASB in relation to glycaemic control. Eighteen (54.5%) of the thirty three subjects with poor glycaemia control had ASB compared to 3/102 (2.9%) of subjects with good glycaemic control. The difference was statistically significant, X^2= 12.57; p=0.006 (Fisher's exact). The odd of developing bacteriuria was almost forty times in subjects with poor glycaemic control compared with those with good control [OR 39.6 (95% CI= 10.395-150.851)].

TABLE 2
Prevalence of bacteriuria in relation to blood glucose control

Blood glucose control	Number	Presence of ASB (%)
Poor glucose control	33	18 54.4
Good glucose control	102	3 2.9

X^2= 12.57; p=0.006 (Fisher's exact). Odds ratio=39.6 (95% CI= 10.395-150.851)

Table 3 shows the prevalence of bacterial isolates in both subjects and controls. Nineteen isolates were gram-positive and three were gram-negative. Of the gram-positive isolates, the most common organism was *Staphylococcus aureus*, 17 (80.9%) and one (50%) in the subjects and controls respectively. *Enterococcus faecalis* one (4.8%) was isolated from one of the subjects. Of the gram-negative rods, *Klebsiella sp* was the predominant gram-negative rods seen, two (9.5%) and one (50%) in the subjects and controls respectively. The other gram-negative organism was *Escherichia coli* one (4.8%) from one of the subjects.

Table 3
Prevalence of bacterial isolates from both subjects and controls

Isolate	Subjects No. (%)	Controls No. (%)
Staphylococcus aureus	17 80.9	1 50
Enterococcus faecalis	1 4.8	-
Klebsiella sp	2 9.5	1 50
Escherichia coli	1 4.8	-
Total	21 100	2 100

The resistance profile of the isolates against different antimicrobial agents is shown in Table 4.

The susceptibility testing results of the isolates showed that the organisms were multi-resistant. A large number of *Staphylococcus aureus* isolates were resistant to cotrimoxazole (94.1%), nalidixic acid (94.1%), gentamicin (94.1%), erythromycin (88%), and cefuroxime (82.4%). Similarly, it was observed that the *S. aureus* isolates were resistant to vancomycin (60%), augmentin (Amoxicillin clavulanic acid) (59%) and chloramphenicol (53%). On the other hand, the *S. aureus* isolates were sensitive to nitrofurantoin (82%) and tetracycline (71%). Like *S. aureus*, *Escherichia coli* was also resistant to cotrimoxazole (100%) and *Enterococcus faecalis* to gentamicin (100%).

Of note is the general resistance of the isolates to cotrimoxazole (85.7%), gentamicin (81%), nalidixic acid (85.7%) and ofloxacin (71.4%). Contrary to this trend is the relatively low level of resistance to nitrofurantoin (23.8%) and tetracycline (28.6%).

Table 4
Urine isolates from diabetic patients and their antibiotic resistance pattern

Isolate	No. of Isolates	AMX (%)	COT (%)	NIT (%)	GEN (%)	TET (%)	NAL (%)	OFL (%)	CXM (%)	ERY (%)	VAN (%)	AUG (%)	CEF (%)
Staphylococcus aureus	17	10(58.8)	16(94.1)	3(18)	16(94.1)	5(29)	16(94.1)	13(76)	9(53)	15(88)	6/10(60)	10(58.8)	14(82.4)
Enterococcus faecalis	1	0(0)	0(0)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)
Klebsiella sp	2	1(50)	1(50)	1(50)	0(0)	1(50)	1(50)	1(50)	0(0)	ND	ND	1(50)	ND
Escherichia coli	1	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	ND	ND	0(0)	ND
Total	21	11 (52.4)	18 (85.7)	5 (23.8)	17 (81)	6 (28.6)	18 (85.7)	15 (71.4)	10 (47.6)	16/18 (88.9)	6/18 (33.3)	11 (52.4)	15/18 (83.3)

AMX= Amoxicillin, COT= Cotrimoxazole, NIT= Nitrofurantoin, GEN=Gentamicin, TET= Tetracycline, NAL= Nalidixic acid, OFL= Ofloxacin, CXM = Chloramphenicol, ERY= Erythromycin, VAN= Vancomycin, AUG= Augmentin (Amoxicillin clavulanic acid), CEF= Cefuroxime, ND= Not determined

DISCUSSION

The results of this study have shown that the prevalence of ASB among patients with diabetes is higher than in an apparently healthy control group. 16% of diabetic patients investigated had ASB compared with 3.5% of non diabetic control patients. This difference between the two groups is statistically significant (P = 0.03). The study also showed that the odds of diabetic patients having ASB is four times more than non diabetic individuals. This is comparable to other studies by Jaspan *et al.* (12) (18.7%), Ooi *et al.* (13) (15.8%) and Veljsgaard (14) (17%). In contrast, higher prevalence of ASB in patients with diabetes have been reported in other studies 26%; 26.6% and 31.7% by Geerlings *et al.* (15), Alebiosu *et al.* (16) Makuyana *et al.* (17) respectively.

The higher susceptibility of diabetic patients to bacterial infection is not clearly explained; however factors such as bladder dysfunction due to autonomic nerve dysfunction and defective polymorphonuclear leucocyte functions (opsonization, chemotaxis, phagocytosis and killing) are potential contributing factors (18). Alterations of bacterial adhesion to uroepithelial cells, partly explained by changes of the chemistry and concentration of Tamm-Horsfall protein also promote urinary-tract infection (19).

Various susceptibility factors for asymptomatic bacteriuria among diabetic patients some of which have been studied in many other works were investigated in our study. These include age, sex, marital status, type of marriage, duration of diabetes mellitus, hypertension, nephropathy, glucosuria, and albuminuria (7,13). Our study showed that age was a risk factor for ASB in DM patients. Geerlings *et al.* (8) had previously reported that age was the most important risk factor for ASB in type 2 diabetic patients in Netherlands. Other studies, however, have reported contradictory results with most not showing an increased incidence of ASB in diabetic patients (7,14,17). Furthermore, our study showed that significant bacteriuria was more likely to occur in patients with poor blood glucose control as demonstrated by blood glucose levels ($p = 0.006$) . Hyperglycaemia is responsible for causing endothelial dysfunction, oxidative stress, and increased formation of advanced glycosylation end products, which may play a role in the development of diabetic complications including asymptomatic bacteriuria (17).

This study also showed that both gram-positive and gram-negative organisms were cultured from the urine of the diabetic patients. That *Staphylococcus aureus* is the predominant organism isolated is comparable to other studies by Olusanya *et al.* (20) and Ozumba (21) where coagulase positive *Staphylococcus* was the most common isolate. A longer term follow up of pathogenic microorganisms responsible for UTI also showed an increase in the prevalence of *S. aureus* over time, corresponding to an increase in the occurrence of methicillin-resistant *S. aureus* (MRSA) (22). In another study on the isolation of *Staphylococcus aureus* from urinary tract and its association with subsequent bacteremia, Muder *et al.* (23) demonstrated that *Staphylococcus aureus* is a primary urinary pathogen among long term care patients and often secondary to staphylococcal bacteremia arising elsewhere. *Staphylococcus aureus* has been known to exhibit its pathogenic properties due to its ability to colonise, multiply and spread widely in tissues through the production of substances such as exotoxins, leucocidin, exofoliative toxins, toxic shock syndrome toxin, enterotoxin as well as components of its cell wall. The isolation of *Klebsiella sp* is also of importance since *Klebsiella sp* is an important cause of both nosocomial and community acquired urinary tract infection (24).

Our results showed that the isolates were highly resistant to cotrimoxazole, gentamicin and nalidixic acid. Cotrimoxazole is a drug commonly prescribed for various ailments ranging from common cold through cough to diarrhoea. Gentamicin, though an injectable drug is also readily available off-counter. Various workers have reported high rates of resistance to cotrimoxazole (3,16,24). These drugs cannot therefore be used as a first line treatment without susceptibility testing.

Interestingly, almost all the isolates tested were susceptible to nitrofurantoin and tetracycline. This is similar to the findings of Kayima *et al.* (25) in Kenya where 93% of all isolates (both gram-negative and gram-positive) were sensitive to nitrofurantoin. This is also comparable to reports on antimicrobial sensitivity pattern of isolates from various other centers (24, 26). The implication of this finding is that nitrofurantoin, though an old drug, it is still effective in the treatment of urinary tract infection.

Strikingly, though the gram-negative organisms isolated were susceptible to inhibition by the fluoroquinolone, ofloxacin, the gram-positive organisms were resistant. This pattern of high-level resistance is as described for the parent quinolone, nalidixic acid. This finding questions the usefulness of fluoroquinolone in the empiric treatment of UTI.

ASB could be considered a complication in diabetic populations that are seen in clinical practice in Ile-Ife, south-west Nigeria, and age and poor glycaemic control were significant associated factors. There is a preponderance of *Staphylococcus aureus* demonstrating the important role of this organism in diabetes related UTI. There is a need to follow-up patients with ASB with a view to determining what proportion will eventually develop symptomatic bacteriuria. Such prospective studies are also needed to show how impaired glycaemia influence outcome of ASB and whether patients with poor control should be screened routinely especially in resource limited settings.

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TYPE 2 DIABETES MELLITUS: CLINICAL AND AETIOLOGIC TYPES, THERAPY AND QUALITY OF GLYCAEMIC CONTROL OF AMBULATORY PATIENTS
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TYPE 2 DIABETES MELLITUS: CLINICAL AND AETIOLOGIC TYPES, THERAPY AND QUALITY OF GLYCAEMIC CONTROL OF AMBULATORY PATIENTS

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ABSTRACT

Background: **Type 2 diabetes is a heterogeneous disease with multiple causes revolving around beta cell dysfunction, insulin resistance and enhanced hepatic glucose output. Clinical judgement based on obesity status, age of onset and the clinical perception of residual beta cell insulin secretory function (hence insulin-requiring or not), has been used to determine therapeutic choices for each patient. Further laboratory testing of the clinically defined type 2 diabetes unmasks the various aetiologic types within the single clinical group.**

Objective: **To determine the aetiological types of the clinically defined type 2 diabetic patients, their chosen therapies at recruitment and the quality of glycaemic control achieved.**

Design: **Descriptive cross-sectional study.**

Setting: **Diabetes out-patient clinic of Kenyatta National Hospital, Nairobi, Kenya.**

Results: **A total of 124 patients with clinical type 2 diabetes were included, 49.2% were males. The mean duration of diabetes in males was 26.09 (20.95) months and that of females was 28.68 (20.54) months. The aetiological grouping revealed the following proportions: Type 1A-3.2%, Type 1B-12.1%, LADA-5.7%, and "true" type 2 diabetes 79.0%. All the patients with Type 1A were apparently, and rightly so, on "insulin-only" treatment even though they did not achieve optimal glycaemic control with HbA_1c % = 9.06. However the study patients who were type 1B and LADA were distributed all over the treatment groups where most of them did not achieve optimal glycaemic control, range of HbA_1c of 8.46 -10.6%. The patients with "true" type 2 were also distributed all over the treatment groups where only subjects on 'diet only' treatment had good HbA_1c of 6.72% but those in other treatment groups did not achieve optimal glycaemic control of HbA_1c, 8.07 - 9.32%.**

Conclusion: **Type 2 diabetes is a heterogeneous disease where clinical judgement alone does not adequately tell the various aetiological types apart without additional laboratory testing of C-peptide levels and GAD antibody status. This may partly explain the inappropriate treatment choices for the various aetiological types with consequent sub-optimal glycaemic control of those patients.**

INTRODUCTION

The National Diabetes Data Group (1979) classification (1) subdivided diabetes according to perceived need for insulin therapy. The WHO (1997) classification (2) sought to replace the previous categories with a functional measure based on insulin deficiency.

Type 2 diabetes is a heterogeneous disease with multiple causes revolving around beta cell