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Asymptomatic Bacteriuria in Relation to Diabetic Women Attending Hawassa University Referral Hospital, Southern Ethiopia

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Abstract

The aim of the current study was to investigate the association between diabetes and ASB in women, identify the bacteria involved and determine their antimicrobial resistance. Diabetic (n=100) and non-diabetic (n=100) non-pregnant women, with no abnormalities of the urinary tract were involved in the study. Clean-catch midstream urine samples were collected for bacterial isolation and identification. Questionnaire was used to collect demographic and clinical information of the patient. ASB was diagnosed in 22(22%) diabetic and 15(15%) non-diabetic women. *Escherichia coli*, *Staphylococcus* aureus, coagulase negative *staphylococci*, *Klebsiella pneumoniae* and Proteus species were isolated from both groups of study subjects. There was no statistically significant difference in the relative frequency of isolated bacteria species among the two groups of patients (P>0.05). Most of the isolated bacteria were resistant to amoxicillin (96%), penicillin (92%) and vancomycin (70%), but susceptible to ceftriaxone (60%), norfloxacin (62%) and gentamicin (62%). Occurrence of ASB was not associated with diabetic status in women attending Hawassa University Referral Teaching Hospital.

Keywords: Antimicrobial resistance; Asymptomatic bacteriuria; Diabetes; *Escherichia coli*; Hawassa

Introduction

Diabetes mellitus (DM) is a common metabolic disorder resulting from defects in insulin action or production [1]. WHO estimated the number of diabetic cases in Ethiopia to be 800,000 by the year 2000, and the number is expected to increase to 1.8 million by 2030 [2]. A recent estimate suggested that diabetes mellitus was the 5th leading cause of death worldwide and is responsible for about 3 million deaths annually [3]. Diabetes causes several abnormalities of the host defense system that might result in a higher risk of certain infections, including urinary tract infection (UTI). A higher glucose concentration in the urine may create favorable culture medium for pathogenic microorganisms [4]. Urinary tract infections in patients with DM are reported mainly from developed countries [5]. Many UTIs are asymptomatic, especially in women with or without diabetes. Several recent reports have noted a higher prevalence of asymptomatic bacteriuria (ASB) among women with diabetes than those without diabetes [6]. Bacterial infection of the urinary tract is usually acquired by ascending route from the urethra to the bladder. The infection may then proceed to the kidney, occasionally bacteria infecting the urinary tract invade the blood stream to cause septicemia [7]. Both symptomatic and asymptomatic UTIs are reported to occur with increased frequency in women with diabetes [8]. Patients with diabetes have an increased risk of infections with the urinary tract being the most common site [9].

The aim of the present study, therefore, was to investigate whether diabetes was associated with ASB, identify risk factors associated with ASB in diabetic and non-diabetic women, to identify bacteria involved in ASB and to determine the antimicrobial resistance of isolated bacteria.

Materials and Methods

Study area

The study was conducted during May-August 2011 at Hawassa University Teaching Referral Hospital. Hawassa is located in the East African Great Rift Valley on the shores of Lake Hawassa, 275 km south of Addis Ababa. The city is the capital of the Southern Nations Nationalities and Peoples Region (SNNPR) and located within a latitude and longitude of 7°3'N 38°28'E coordinates at an altitude of about 1708 meters above sea level.

Patients

The study involved a total of 200 subjects. One hundred were diabetic women who attended the weekly diabetic clinic at

Hawassa University Teaching Referral Hospital for diagnosis and treatment; and 100 controls were women with cardiac disease who had their fasting blood sugar tested previously and found to be within the normal range.

Check list was used to collect risk factors (duration of diabetes, degree of diabetes control and diabetic complication) for ASB form patients' medical record. Information about demographic variables was collected through questionnaires administered to the study subjects.

Inclusion criteria

Cases: Women with DM (type 1 and 2) over 18 years old, without UTI, and controls: outpatient women without diabetes, receiving treatment for other medical conditions (Cardiac patients), had no abnormalities of the urinary tract and attending the hospital in the same period were included in the study.

Exclusion criteria

Subjects with pregnancy, known urinary tract abnormalities, recent hospitalization or surgery within the past 4 months, symptoms of urinary tract infection and subjects who used antimicrobial agents in the 14 days preceding the sampling were excluded from being involved in the study.

Ethical consideration

Participation in the study was on voluntary basis. Those willing to participate were requested to sign an informed consent prepared in English and translated into a local language-Amharic. Ethical clearance was obtained from ethical clearance committee of Hawassa University Referral Teaching Hospital. Moreover, permissions to obtain information from patients' documents were requested in writing to the medical director's office and implemented when allowed to do so.

Research design and sampling technique

The study was a case-control study, in which women with diabetes were defined as cases whereas controls were women attending outpatient clinic at the hospital during the same time who had their fasting blood sugar tested and found non-diabetic. All volunteer consecutive diabetic (n=100) and non-diabetic (n=100) women patients attending the clinics during the study were included.

Urine Specimen collections

Approximately 20 ml midstream clean catch urine specimens were collected in sterile screw capped bottles. The participants of the study were asked to clean their labia with water and soap before urine collection. The sample containers were labeled with patient's name, date and time of collection and the samples were processed in laboratory within 2 hours of collection.

Isolation of asymptomatic bacteriuria (ASB)

A 0.002 ml calibrated loop was vertically inserted into a well-mixed urine sample just below the surface and a loopful of the sample was removed and inoculated on blood, mannitol salt and MacConkey agar (all from OXOID, Basingstoke, England) simultaneously. The spreaded plates were then incubated in inverted position for 24 hours at 37°C. And the colonies on the agar plates were counted, the number of bacteria per milliliter of urine was determined and expressed as cfu/ml. A count of ≥ 105 cfu/ml was considered as significant to indicate ASB [10].

Isolation and identification of bacteria

The isolates in ASB cases were identified by colony morphology, characteristics of Gram stained smears, primary and secondary biochemical tests. Isolates were stored on nutrient agar (OXOID) slants for further characterization [10].

Antimicrobial susceptibility test

Antimicrobial susceptibility test was conducted using the Kirby Bauer disk diffusion method. Each isolate was taken from the storage medium (nutrient agar slant) and before antimicrobial testing was sub cultured on blood agar and incubated at 37°C for 24 hours. About 3-5 well isolated colonies of similar appearance were selected and the top of each selected colonies was touched with sterile loop. Then the inoculum was transferred into a tube containing 5 ml of sterile nutrient broth (Oxoid, Basingstoke, England) and mixed gently to form homogeneous suspension. The broth culture was incubated at 37°C for 2 to 6 hours. Finally, the turbidity was adjusted to 0.5 MacFarland standards [10].

A sterile cotton swab was dipped into the suspension and the excess broth was removed. The swab was then used to distribute the bacteria evenly over the entire surface of Muller Hinton agar (Oxoid, Basingstoke, England). The inoculated plates were left at room temperature to dry for 3-5 minutes and antibiotic disks were carefully placed with the aid of sterile forceps [10].

After placing the discs, the plates were incubated aerobically at 37°C for 16-18 hours. The diameter of the zone of complete inhibition, was measured to the nearest whole number using millimeter scale ruler on the under surface of the plate. The clear zone size around each disk was interpreted as susceptible (S), intermediate (I) or Resistant (R) according to NCCLS [10]. The following antibiotic discs were used for antimicrobial susceptibility testing: Amoxicillin (AML, 2 g), Penicillin (P, 10 g), Ceftriaxone (CRO, 30 g), Vancomycin (VA, 30 g), Gentamicin (CN, 10 g) and Norfloxacin (NOR, 10 g).

Information on risk factors and data analysis

In addition to DM status, information regarding other factors which could affect the occurrence of ASB was collected for each study subject. Data about duration (≤ 10, >10yrs) type (1, 2), degree of control (poor, fair, good) and complication of diabetes (present, absent), age and body mass index (BMI) were collected

from patient records. Income, marital status and frequency of sexual intercourse were collected using questionnaire.

Data were stored in a Microsoft Excel spread sheet and analyzed with Stata version 9 (Stata Corp. College Station, TX). Statistical associations between ASB and DM status of the study subjects and other risk factors considered in the study were analyzed using Chi Square test. A P value <0.05 was considered as statistically significant.

Results

Prevalence of asymptomatic bacteriuria (ASB)

Of the total 200 women included in the study 37 (18.5%) had asymptomatic bacteriuria (ASB). ASB in diabetic and non-diabetic women was 22.0% and 15.0%, respectively. There was no difference in the occurrence of ASB among diabetic and non-diabetic women (P>0.05).

In addition to assessing the association between ASB and DM we also analyzed the effects of age, income, marital status, frequency of sexual intercourse, BMI, on occurrence of ASB. ASB appears to be not affected by all of these factors (P>0.05) (**Table 1**).

Table 1. Association of risk factors with ASB occurrence in diabetic and non-diabetic women.

Risk Factors	Group	No. Examined	ASB Positive	Percent	Chi Square	P Value
Age	18-35	73	12	16.4	0.6888	0.88
	36-49	65	12	18.5		
	50-64	46	9	19.6		
	65-75	16	4	25		
Income	≤ 1000	152	28	18.4	0.0026	0.96
	>1000	48	9	18.8		
Marital Status	Unmarried	39	7	17.9	0.3688	0.95
	Married	136	26	19.1		
	Divorced	9	1	11.1		
	Widow	16	3	18.8		
Sexual Intercourse	At least Weekly	90	18	20	3.3591	0.19
	Monthly	85	12	14.1		
	Never	23	7	30.4		
Body Mass Index (Kg/m^2)	<19	15	3	20	0.6701	0.72
	19-25	38	10	26.3		
	>25	47	9	19.1		
Overall		200	37	18.5		

Association between ASB and other potential risk factors in diabetic women were also analyzed in the study. A statistically significant association (P<0.05) between ASB and duration and degree of diabetes control were observed. ASB was high in women who have been with diabetes for more than 10 years (40.0%) as compared to those who were diabetic for less than 10 years (17.7%). Women with poor diabetes control had the highest (35.6%) prevalence of ASB as compared to women with fair (17.4%) and good (6.3%) degree of control (**Table 2**). Frequency of sexual intercourse, body mass index, type of diabetes and presence of diabetic complications, however, were not significantly associated with ASB (P>0.05).

Table 2. Association of diabetes related risk factors with ASB in diabetic women.

Risk Factors	Group	No. Examined	ASB Positive	Percent	Chi Square	P Value
Diabetes type	1	27	5	18.5	0.26	0.609

	2	73	17	23.3		
Duration of diabetes	≤ 10 years	79	14	17.7	4.58	0.032
	>10 years	21	8	40		
Degree of diabetes control	Poor	45	16	35.6	9.73	0.008
	Fair	23	4	17.4		
	Good	32	2	6.3		
Diabetes complication	Absent	62	12	19.4	0.67	0.415
	Present	38	10	26.3		
Overall		100	22	22		

Bacteria isolated from cases of ASB

A total of 50 bacterial isolates of 5 different species/genera were recovered from urine specimen of cases of ASB. The bacteria isolated from urine samples were *E. coli*, *S. aureus*, coagulase negative *staphylococci*, *K. pneumoniae* and *Proteus* spp. in the order of their frequency of isolation (**Table 3**). There was no significant association (P>0.05) (**Table 3**) between diabetic status and frequency of isolation of any of the bacteria species identified in the study. Of the 50 isolates recovered 16(32.0%), 11(22.0%), 9(18.0%), 7(14.0%), and 7(14.0%) were *E. coli*, *S. aureus*, coagulase negative staphylococci (CoNS), *K. pneumoniae* and *Proteus* spp. respectively.

Table 3. Frequency and statistical association of bacteria with diabetic status of women (n=200).

Bacteria	Isolation frequency (%)		Statistical analysis	
	Diabetic (n=100)	Non diabetic (n=100)	Chi square	P value
Escherichia coli	10(35.7)	6(27.3)	1.08	0.29
Staphylococcus aureus	7(25.0)	4(18.2)	0.86	0.35
Coagulase negagtive stapylococci	4(14.3)	5(22.7)	0.14	0.7
Klebsiella pneumoniae	4(14.3)	3(13.6)	0.14	0.7
Proteus species	3(10.7)	4(18.2)	0.12	0.72
Total	28(100)	22(100)	P>0.05	

Antimicrobial susceptibility

Antimicrobial susceptibility testing of all isolated bacteria (n=50) was done by Kirby Bauer disk diffusion method. Of the 50 isolates of bacteria of different species 48(96.0%), 46(92.0%) and 35(70.0%) were resistant to amoxicillin, penicillin and vancomycin respectively. 49(98.0%) of the isolates were resistant to 1 or more antimicrobials, while 47(94.0%) of the isolates were resistant to 2 or more antimicrobials. On the other hand, most of the isolates were susceptible to norfloxacin (62.0%), ceftriaxone (60.0%) and gentamicin (62.0%). **Table 4** shows the susceptibility/resistance of all the isolates recovered from both study groups.

Table 4. Antimicrobial susceptibility of bacteria isolated from ASB in both diabetic and non-diabetic women at Hawassa University Referral Hospital.

Organism	Status	Antimicrobial Agent (%)					
		CRO	NOR	CN	P	VA	AML
Escherichia coli (n=16)	S	68.75	37.5	31.25	6.25	18.75	6.25
	I	31.25	6.25	12.5	0	12.5	0
	R	0	56.25	56.25	93.75	68.75	93.75
Staphylococcus aureus (n=11)	S	36.36	54.55	72.73	0	9.09	0
	I	18.18	18.18	18.18	9.09	18.18	9.09
	R	45.45	27.27	9.09	90.91	72.73	90.91
Klebsiella pneumoniae (n=7)	S	85.71	100	85.71	0	14.29	0
	I	14.29	0	14.29	0	14.29	0
	R	0	0	0	100	71.43	100

Proteus spp. (n=7)	S	57.14	71.43	71.43	14.29	0	0
	I	42.86	14.29	28.57	0	28.57	0
	R	0	14.29	0	85.71	71.43	100
CNS (n=9)	S	55.56	77.78	77.78	0	11.11	0
	I	33.33	0	22.22	11.11	22.22	0
	R	11.11	22.22	0	88.89	66.67	100

NOR, Norfloxacin; P, Penicillin; VA, Vancomycin; CN, Gentamicin; AML, Amoxicillin; CRO, Ceftriaxone. S, susceptible; I, intermediate; R, resistant; CNS, coagulase negative staphylococci

Discussion

The proportion of diabetic women with ASB in the present study (22.0%) lies within the range of prevalence of ASB in diabetic women [11]. In another study the prevalence of ASB in a group of 130 women with diabetes was reported as 36.15%, with a diagnostic criterion for ASB of 1–2 urine specimens with 105 -colony forming units per ml of urine [12]. However, in our study diabetes was not found associated with ASB.

Our result was in agreement with Bonadio et al. [13] who failed to demonstrated significant difference in the prevalence of ASB between women with and without diabetes. The result was also consistent with Ghenghesh et al. [5] where the prevalence of uropathogens was not significantly associated with diabetes. Results of the present study, however, contradict previous reports of high proportion of ASB in diabetic than non diabetic women [14]. This difference might in part be explained by differences in study protocols. As different from our study these studies, used observation of at least 10^5 cfu/ml of urine in 2 consecutive samples as the diagnostic criterion for ASB.

Although, age has been postulated as the most important risk factor for ASB in both diabetic and non-diabetic individuals [9], the current study failed to demonstrate an association between age and the presence of ASB. This observation was in agreement with Ghenghesh et al. [5] who reported that age was not a risk factor for the development of ASB in women with type 1 or type 2 DM and the control group.

There was no statistically significant association between BMI and presence of ASB among diabetic patients in the current study. Contrary to our observation Geerlings et al. [9] noted significant positive association of low BMI with ASB in women with type 2 diabetes. Frequent sexual intercourse has been reported to be a risk factor for development of ASB in diabetic and non-diabetic women [18]. However, we found no difference in proportion of ASB among groups with different frequency of sexual intercourse. On the other hand, this finding was in agreement with the report that the association between diabetes and ASB could not be due to frequency of sexual intercourse [9].

Some reports have shown that the duration of diabetes was associated with ASB in diabetic patients [14-17]. Similarly, in the present study, a significant association was observed between duration of diabetes and presence of ASB in diabetic women. Many studies involving women with diabetes have reported an association between ASB and long-term complications of diabetes, such as retinopathy, nephropathy, and neuropathy [18]. However, a significant association was not observed between the presence and absence of diabetic complications in relation to prevalence ASB in diabetic patients in the current study, this finding compares favorably with the report of [19]. Our study has demonstrated significant association between ASB and degree of diabetic control, in line with Janifer et al. [20] who also found that poor diabetic control was associated with UTI in both men and women diabetics.

E. coli were the predominant microorganism isolated from both diabetic and non diabetic women with ASB. Similar result was observed where E. coli were the most frequent bacteria isolated from women with diabetes and without diabetes [12]. Zamanzad et al. [14] also indicated that *Escherichia coli* were the most prevalent bacteria in patients with ASB.

In agreement with the current result [21] reported that antibiogram result of bacteria isolated from urine sample of diabetic patients showed that most of them were resistant to amoxicillin and penicillin. The current result showed (94.0%) of the isolates were resistant to 2 or more antimicrobials. A large number of the organisms isolated in this study were sensitive to gentamicin, norfloxacin and ceftriaxone. The result was also supported by Al- Dulaimi [22] that most of the isolated bacteria from diabetic women were sensitive to norfloxacin and ceftriaxone. Very few isolates were sensitive to amoxicillin, penicillin and vancomycin. The possible explanation for this is that most of these drugs were easily available and may be used indiscriminately, which may lead to an increased prevalence of resistant organisms.

Conclusion

The present study identified that longer duration of diabetes and poor glucose controls predispose diabetic patients for ASB. *Staphylococci* and *E. coli* were the most prevalent etiological agents in ASB in both diabetic and non-diabetic women. Most isolates were susceptible to gentamicin, norfloxacin and ceftriaxone but very few isolates were sensitive to amoxicillin, penicillin and vancomycin in both of the study groups. Routine urine culture and drug sensitivity is recommended for diabetic women who have duration of diabetes greater than 10 years and poor glucose control in the absence of urinary symptom. Gentamicin, Norfloxacin and Ceftriaxone may be preferred for treatment of ASB in diabetic patients.

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