PREVALENCE OF URINE GLUCOSE-ASSOCIATED BACTERIURIA IN DIABETICS AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF THE ISOLATED BACTERIA

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Abstract

Objectives: This study aimed at determining the influence of urine glucose on prevalence of bacteriuria in diabetic patients and to carry out antibiotic susceptibility patterns testing on the isolated bacteria.

Methods: This is cross-sectional study conducted at Diabetes Clinic-Muhimbili National Hospital, by collecting 133 midstream urine (MSU) specimens from 133 patients who were attending the clinic during the study period. The MSU specimens were then cultured on cystine lactose electrolyte deficient (CLED) agar medium using the standard wire loop technique. The isolated microorganisms were identified and microbial counts determined by using conventional methods. Urine glucose levels (UGLs) were determined using urinalysis reagent strips (Acon Laboratory Inc., USA). Results were interpreted and classified as negative, trace, positive (+), positive (++) or positive (+++). Antibiotic susceptibility patterns of the isolated bacteria were conducted using disk-diffusion method in accordance with National Committee on Clinical Laboratory Standards (NCCLS) guidelines. Checklists of clinical histories were also used for collection of complementary data on the patient weight and age. All the obtained data were coded and entered in computer and then analyzed using computer software SPSS version 10.

Results: Majority of the patients 118(88.7%) manifested no significant bacterial growth (NSBG). Out of the patients who manifested NSBG, majority (73%) had no/negative urine glucose levels (UGLs). *Escherichia coli* were the most frequently isolated microorganism (18.8%), which were also the most predominantly isolated microorganisms from patients with negative urine glucose levels. *Klebsiella* spp and *Pseudomonas aeruginosa* were found in all patients with glycosuria. More females (12%) manifested higher UGLs in comparison to males' counterpart (7.5%), which coincided with the higher bacterial counts in female diabetics. All the isolated microorganisms were found to be susceptible to nalidixic acid (30μg). Nevertheless, all the microorganisms were resistant to ampicillins (10μg), flucloxacillin (10μg) and tetracycline (30μg) with exception of *E. coli* which was susceptible to the latter drug.

Conclusion: The study revealed that *E. coli* was the most frequently isolated microorganisms (18.8%) and bacteriuria was relatively more prevalent in female patients (25%) compared to males (16%). Notwithstanding, majority of the patients (88.7%) manifested NSBG. Direct correlation between bacteriuria and UGLs was observed though its relation is still unclear. The antibiotic resistances identified make it necessary for antibiotic susceptibility testing to be carried out prior antibiotic prescription.

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Introduction

Recent data show that about 200 million people suffer from diabetes mellitus worldwide, and it shows that this number may well double by the year 2025. [1] In Tanzania it is estimated that approximately 350,000 individuals are diabetics (2-4). Diabetes mellitus is a chronic disease, a metabolic disorder characterized by hyperglycemia (fasting plasma glucose 7.7 mmol/L), or acquired deficiency in production of insulin by the pancreatic ß-cells, or by ineffectiveness of the insulin produced.[3]

Such a deficiency leads to increased concentration of glucose in blood which in turn leads to damage of many of the body's systems especially the blood vessels and nerves (5-6). Glucose will also overflow into the urine once the blood glucose level is high, that is above the renal threshold, which in most people is 10.0mmol/L.[1, 3, 7]

To date there are two principal forms of diabetes, which are designated as Type 1 diabetes (Insulin-dependent diabetes mellitus-IDDM) and Type 2 diabetes (Non-insulin dependent diabetes mellitus-NIDDM). In type 1 diabetes, patients usually require daily administration of insulin, which is a life-saving medication. In type 2 diabetes, treatment is usually dietary although supplementary oral hypoglycemic drugs or insulin often becomes necessary due to the body's inability to respond to the action of insulin produced by the pancrease.[8] Symptoms of diabetes may be pronounced or subdued. In type 1 diabetes, the classic symptoms are increased frequency of urination (polyuria), thirst (polydipsia), weight loss and feeling of lassitude. Those symptoms may be less marked in type 2 diabetes, which may also happen without early symptoms and the disease is diagnosed only several years after its onset, when complications are already present and probably have become more severe.[9]

Patients with diabetes are more predisposed to infections. This predisposition is due to a combination of angiopathy, neuropathy and hyperglycemia.[3, 10] Impaired host defense mechanisms such as impaired granulocyte function, decreased cellular immunity, impaired complement function and decreased lymphokine response that may be influenced by glycemic control are also frequent.[11, 12] The urinary tract is also common site of infection in many diabetics[13], apart from the fact that diabetes is a significant risk factor in nosocomial urinary tract infections (UTIs)[14] Complications from UTIs usually manifested in diabetics include acute lobular nephronia, intrarenal abscess, perinephric abscess, emphysematous cystitis, emphysematous pyelonephritis, papillary neurosis and metastatic infections.[1, 3] This study aims to determine the influence of urine glucose (glycosuria) on prevalence of bacteriuria in diabetic patients and to carry out antimicrobial susceptibility tests on the isolated microorganisms.

Methodology

Study design

This was a cross-sectional study that involved a total of 133 patients attending the diabetes clinic at MNH, from which 133 midstream urine (MSU) specimens were collected. Clean catch MSU, were collected after clear instructions to the patient to avoid contamination of the urine with microflora present in lower urethra, vagina, rectum and skin. This was followed by enquiries on the patient's age and weight through checklist of clinical histories.

Specimens were cultured on the same day (within an hour) of collection in cystein lactose electrolyte deficient agar (CLED) onto Petri dishes using a calibrated 1 microlitre bacteriologic loop. The inoculated plates were aerobically incubated overnight at 37°C. Significant bacterial growth (SBG) was defined by a confluent growth or when 1ml revealed $\geq 10^5$ colonies expressed as colony forming units (Cfu/ml) of a pure culture; otherwise the growth was referred to be non-significant bacterial growth (NSBG). Preliminary microbiological assays (Gram staining technique, growth onto non-and-selective culture media) and subsequent biochemical tests on the isolated microorganisms were performed in accordance to standard microbiological methods.[15, 16].

Determination of urine glucose levels

Un-centrifuged aliquots of MSU samples from the above shortly described step were employed for determination of urine glucose levels (UGLs) using urinalysis reagent strips (Acon Laboratory Inc., USA) as previously described (14). Results were interpreted and classified as negative, trace, positive (+), positive (++) or positive (+++) according to manufacturer's recommendations.

Susceptibility tests

After microbial isolation and identification; each of obtained pure culture of the microorganisms (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus* spp and *Klebsiella* spp) from patients who manifested SBG was separately suspended in about 5ml of Peptone water (BDH, UK). Each microbial suspension was aerobically incubated at 35°C overnight and then vortexed thoroughly to break all clumps of colonies. The resultant bacterial suspensions were visually compared to the 0.5 McFarland turbidity standard (15, 16) prior conducting the sensitivity testing using the Stokes technique of comparative disk diffusion method. Approximately 1ml from each of the shortly prepared suspensions was overlaid onto Nutrient agar (Pronadisa, Spain) to the periphery of test sensitivity agar plate, then spread evenly to ensure confluent bacterial growth. The control microorganisms viz. *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC27853) and *Escherichia coli* (ATCC 25922) for the strain being tested was similarly treated and was inoculated to the centre of the plate. The plates were left to dry for 15 minutes allowing the medium to absorb the moisture from the inoculum. Disks of nalidixic acid (30ug), tetracycline (30ug), flucloxacillin (10ug)-(Sigma-Aldrich, USA), and ampicillin (10ug)-(DIBCO, USA) were placed onto the agar surface using flamed sterilized forceps and pressed lightly to ensure complete contact with the agar. The inoculated plates were inverted and aerobically incubated at 37°C overnight. Isolates which showed evidence of resistance to the assayed antibiotics were retested using the standardized method as described by the NCCL.[17] Subsequently, results were also interpreted by referring to standard zone diameter for antimicrobial susceptibility testing according to NCCLS guidelines.[17]

Data management

All data obtained from laboratory as well as from the patients checklists were coded, entered in computer and analyzed using the Statistical Package for the Social Sciences (SPSS+ 10.0, 1999) software (SPSS Inc., Chicago, IL). Differences in prevalence and levels of bacteriuria (cfu/ml) among various groups (categorized into the patient age, sex, body weight and urine glucose level) were analyzed by Paired Samples T-Test; and level of significance was set at $p<0.05$.

Ethical aspects

These were addressed by seeking both informed verbal and written consents from the patient. Prior to that, the College ethical committee was consulted and approved the study. The objectives of the study were succinctly explained and sufficient time was allocated for the patient to decide whether to participate in the study or not. The specimens were coded, such that no names were required for patients'

confidentiality. However, for those who were eager to know the study findings were given access to the results.

Results

Socio-demographic characteristics of the study population and prevalence of bacteriuria

A total of 133 MSU specimens from 133 patients of which 69(52%) were females and 64(48%) males were analyzed. There were significant age and weight differences among the patients; their ages ranged from 6-85 years with a median of 49 years-old and the weight from 20-93 kg with a median of 72.4 kg of body weight ($p<0.05$). *Escherichia coli* were the most frequently isolated microorganisms from 25(18.8%) patients, which were seconded by *P. aeruginosa (7%), Klebsiella* spp (6%) and *Staphylococcus* spp (6%) as depicted on Table 1. A few patients manifested mixed growth of both *E. coli* and *Ps. aeruginosa* (3%).

Table 1: Isolation frequency of bacteria among the studied diabetic patients

Isolated microorganisms	Frequency per patients' sex		Total (%) (n = 133)
	Females	Males	
None	36	43	79 (59.4)
Escherichia coli	15	10	25 (18.8)
Klebsiella species	6	3	9 (6.8)
Pseudomonas aeruginosa	9	1	10 (7.5)
Staphylococcus species	3	7	10 (7.5)

Levels of bacteriuria and urine glucose among diabetics

The presence of bacteria in the patients' urine specimens was categorized as SBG when the observed bacterial growth was $\geq 10^5$cfu/ml and NSBG when $\leq 10^5$cfu/ml when there was clinically insignificant bacterial growth. Study finding shows that among the patients with bacteriuria, only 11 (8.3%) females and 4 (3%) were males had SBG. Majority of the patients (88.7%) manifested NSBG whilst only 11.3% had SBG and the incidence of bacteriuria was high in the age range of 48-85 years, though statistically the difference is not significant ($p<0.05$). The most frequently isolated microorganism from patients with SBG/high levels of bacteriuria were *Klebsiella* spp, *E. coli* and *Ps. Aeruginosa.*.

The study also revealed that majority of patients with less than 60 kg of body weight had NSBG. Patients with body weight higher than 61 kg (39 patients) had also higher levels of bacteriuria compared to the rest ($p< 0.05$). However no significant differences were observed on levels of bacteriuria between males and females of this body weight-group. The study revealed an association between the patient's UGLs and levels of bacteriuria. Most of the patients 86(73%) who manifested NSBG also had negative UGLs. Results show that *E. coli* was the most isolated microorganism in patients with negative UGL while *Klebsiella* spp and *Ps. aeruginosa* were found in patients with all UGLs. For patients with SBG, more females were found to be having higher UGLs in comparison to males' counterpart (data not shown).

Susceptibility patterns of bacteria isolated from patients with SBG

All the isolated bacteria were found to be susceptible to nalidixic acid (30μg). On the other hand, all the bacteria were resistant to tetracycline (30μg) with exception of *E. coli.* But all the isolated bacteria were resistant to ampicillin (10μg) and flucloxacillin (10μg).

Discussion

Diabetes mellitus complications are the consequence of the metabolic derangements, which usually develop, often over many years. Some of the most common complications in diabetics are renal failure and microbial infections namely UTIs, skin infections, and respiratory infections.[18, 19] Often bacteria and yeasts are the major pathogens that attack diabetic patients.[11, 14] Of those, *E. coli* and *Klebsiella* spp are some of the very common enteric bacteria that are found in diabetics.[12] The latest WHO report on Global Burden of Disease estimates that about two thirds of diabetics live in developing countries.[1, 2] This implies that diabetes is no longer a condition of developed, 'industrialized' or 'Western' countries. This study finding has shown that patients over 61 kg of body weight (regardless their heights), had high levels of bacteriuria. This could be because of the altered fat metabolism that usually is attributable to low cell mediated immunity, as consequence of change in natural killer cells activity, cytotoxicity and phagocytosis.[20] Nevertheless, due to lack of records on patients' heights, the body mass index was not determined; this could have enabled investigation on how many patients were obese or underweight and relate these parameters with levels of bacteriuria. On the other hand, female patients manifested relatively higher frequency of SBG compared to males coinciding with previous finding[11, 19, 22], which could partially be explained by the female urinary tract anatomy. Presumably, the observed sexual-related difference in prevalence of bacteriuria might be due to the shortness of the female urethra compared to that of male.[7, 23] Hence enteric bacteria from the faecal matter can easily get access to the vagina ultimately to the urethra because of its closeness to the anus. Also females over 48 years of age manifested high SBG than other female patients. This probably is attributed to hormonal changes that occur after the onset of menopause, when older women become prone to minor infections, thus being at higher risk of developing UTIs.[20, 21] Usually because of to estrogen loss, there is a reduction of certain immune factors in the vagina that creates favorable environment for *E. coli* and other pathogens, which tends to adhere to the vaginal epithelial cells. In parallel to that there is also decline in *Lactobacilli* levels and the walls of urinary tract thin out reducing ability to resist infections.[11]

Results also show that majority (88.7%) of the patients manifested NSBG, which might be a good indicator of adequate patient monitoring and proper patient personal hygiene. Furthermore, majority of the patients (73%) with NSBG had negative UGLs; the relationship between the two is not fully understood, though high levels of blood sugars often increase susceptibility to infections.[7, 11] Our results are in consistence with that of Patterson & Andriole.[14], who also found that *E. coli* was the most predominant microorganism in diabetics. *Escherichia coli* usually reside in the intestines as normal flora though sometimes invades the urinary tract, and cause UTIs.[21] Result on susceptibility patterns testing revealed bacterial resistance to ampicillin and flucloxacillin. This finding is of great concern. Firstly, because of the observed drug resistance that to a greater extent may be attributable to the prophylactic use of the antimicrobial agents among the patients whether on prescription or auto-medication.[11, 14] Secondly, due to mutation in bacteria and hence emergence of resistant strains[23] Our study findings call for further investigations on the extent of antibiotic resistance country-wide. Moreover, in the meantime rational use of antimicrobial agents basing on antibiotic susceptibility profiling should certainly be implemented.

Conclusion

The study revealed that female patients had relatively higher levels of bacteriuria compared to males. Most of the patients (88.7%) manifested NSBG, and this implies that there was low incidence of UTIs among the patients. There is direct correlation between bacteriuria and UGLs though its relation is still unclear. The antibiotic resistances identified make it necessary for antibiotic susceptibility testing to be carried out prior antibiotic prescription. Moreover, this study recommends that in order to reduce the risk of bacterial infections, patients should wash genitalia using clean water with soaps or disinfectants after every defecation. We also recommend that female patients should dry themselves by wiping off backwards; this can prevent transfer of bacteria to urethra. Further studies using larger samples of diabetic patients should be conducted for better understanding the relationship between glucosuria, bacteriuria and other metabolic disorders associated with diabetes for betterment of the diabetic patient health care.

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