Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i7.7091

AN EXPLORATION OF ASYMPTOMATIC BACTERIURIA IN TYPE 2 DIABETICS WITH EMPHASIS ON THE PRESENCE OF PYURIA.

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ABSTRACT

Introduction: Diabetes mellitus (DM) predisposes individuals to infections due to impaired immune function and metabolic abnormalities. ASB, defined as the presence of bacteria in urine without symptoms of urinary tract infection (UTI), poses significant health risks, including progression to symptomatic UTIs or other complications.

Materials and Methods: A cross-sectional study was conducted involving 97 diabetic participants (males=45, females=52) from a general medicine outpatient department. Urine samples were collected under sterile conditions and cultured to identify bacterial growth, with subsequent antibiotic susceptibility testing performed.

Results: The study found a 15.46% prevalence of ASB, with higher rates observed in females (60%) compared to males (40%). Among isolated organisms, Escherichia coli predominated, particularly in females. Associations were observed between ASB prevalence and factors such as HbA1c levels and duration of diabetes, highlighting longer duration and poorer glycemic control as potential risk factors. In the present study, Cotrimoxazole was effective across Gram-positive and Gram-negative isolates, including ESBL No MRSA observed. In the present study, the overall prevalence of pyuria was 27%. Predominantly pyuria was noted among females.

Conclusion: Understanding these dynamics is crucial for tailored management strategies in diabetic care to mitigate the risks associated with ASB and improve overall health outcomes in this vulnerable population.

Keywords: Diabetes, asymptomatic bacteriuria, Pyuria

1. Introduction:

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. It is associated with decrease in production and utilization of insulin, resulting in body's inability to utilize nutrients properly. 1

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 382 million in 2013. Based on the current trends, the International Diabetes Federation projects that 592 million individuals will have diabetes by the year 2035.] The rising incidence of DM and the sheer number of people with DM living in India have given this country the dubious distinction of being the "Diabetes Capital" of the world.2

Many investigators agree that clinical evidence points to a higher prevalence of infectious diseases in people with diabetes, despite some researchers' contention that differences in infection risk factors between patients with and without diabetes are the result of uncontrolled or biassed studies (e.g., the increased likelihood of other disease diagnoses due to frequent medical appointments in individuals with diabetes).3

ASB is defined as the existence of bacteria in the urine of a patient who has not shown any symptoms or signs of a UTI. It is detected via urine culture of a properly collected urine specimen as per certified medical guidelines.3

Neonates, preschool children, pregnant women, the elderly, diabetics, catheterized patients, and patients with aberrant urinary tracts or renal illness are all at risk for asymptomatic bacteriuria. Though there is presently no agreement on how to treat asymptomatic bacteriuria in diverse demographic groups, treating asymptomatic bacteriuria in diabetic patients is recommended because these individuals may proceed to symptomatic UTI or suffer UTI sequelae.4

Microscopic pyuria is widely accepted as a predictor of UTIs. It is a condition wherein one can observe, in a fresh unspun, unstained urine specimen, ≥5 leukocytes under a microscope at a high magnification. However, in these cases, often, no bacteria are detected via conventional urine culture.5 Accordingly, the bulk of available data suggests that the inflammatory response to a UTI is complex (dependent on the interaction between pathogen and host), and that the presence or absence of pyuria alone, albeit informative in a large number of cases, is not always an accurate indication of the presence or absence of UTI.

The present study aimed to determine the prevalence of asymptomatic bacteriuria in diabetic patients with emphasis on pyuria.

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The present study aimed to determine the prevalence of asymptomatic bacteriuria in diabetic patients with emphasis on pyuria.

Materials and Methods: This study adopted cross-sectional design to assess the prevalence of asymptomatic bacteriuria with pyuria among diabetic patients. Institutional ethical committee approval was obtained. Participants' medical history, diabetes duration, glycemic control (HbA1c levels), diabetic complications, and medication history collected through interviews. Sample collection procedure was explained to the patients clearly, a Mid-Stream Urine (MSU) specimen (after washing of the perineal area in females and penile area in males) collected in a well labelled screw capped universal container which was promptly transported to the laboratory. All urine samples were inoculated on Mac-Conkey and Blood agar plates aerobically at 37^0 for 18 hours. A second urine sample will be taken in males whose urine culture shows mixed growth or growth < 10^5 colony forming unit /milliliter (cfu/ ml). Identification of the isolates will be carried out using standard microbiological methods. Semiquantitative estimation of colony count will be used. 6 Antibiotic susceptibility testing (AST) of the isolates will be performed on Mueller Hinton Agar by Kirby-

Bauer disc diffusion method. Selection of antibiotic discs were as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines.7

Inclusion Criteria

A representative sample of diabetic patients (Type 2) recruited from outpatient department of general medicine, Government General Hospital, coming for regular medication (Antidiabetic)

Exclusion Criteria: Symptomatic UTIs, Renal failure, Obstructive uropathy, Indwelling catheters, Pregnant females, Immuno-compromised patients. Patients who had received antimicrobial drugs during the previous 2 weeks.

Results: In our study, a total of 97 diabetic individuals(males=45 and females= 52) participated. The age of participants ranged between 35-85 in years. Among these 15 patients (15.46%) were found to be positive for ASB. Out of 52 female participants, ASB was observed in 9 (60%) individuals. Among 45 male participants, ASB was observed in 6(40%) individuals. Majority of individuals yielded growth belongs to the age group of 50-70 years and accounted for 66.67%. (Table.1)

Table. 1Age and gender wise distribution of participants with ASB

Age	No of participants	ASB	Male	Female
<50-60	11	5	1	4
61-70	65	5	2	3
71-80	12	3	1	2
>80	9	2	2	0
Total	97	15 (15.46%)	6(40%)	9 (60%)

In our study, a total of 15 organisms were isolated from study participants. Majority were Gram negative bacilli 9 (60%) followed by Gram positive cocci 6(40%). Escherichia coli was the predominant organism isolated from participants (46.67%). Further, E.coli was the isolate commonly observed from the samples collected from females. (Table.2)

Table.2 Gender wise distribution of the isolate

Organism	Male	Female
Escherichia coli [7]	1 (14.29 %)	6(85.71%)
Klebsiella [2]	1(50%)	1(50%)
Proteus [1]	1(100%)	0(0%)
Staphylococcus aureus [2]	1(50%)	1(50%)
CONS [2]	1(50%)	1(50%)
occus [1]	1(100%)	0(0%)

Table .3 depicts the association of asymptomatic bacteriuria and HbA1c levels. A total of 57 diabetic patients HbA1c levels were found to be ≤7. Out of these, 4 (7%) individuals showed asymptomatic bacteriuria. Higher HbA1c levels (>7) showed 11(27%).

Table. 3 Association of HbA1c levels with ASB

HbA1C	No. of Diabetic participants	ASB[%]
≤7	57	4 [7%]
>7	40	11 [27%]

In our study, duration of diabetes is broadly categorized into three groups. Majority of participants (n=59) were from less than 10 years of duration with diabetes and ASB was found to be 3(5.09%). But, preponderance of asymtomtomatic bacteriuria was observed in individuals with prolonged duration of diabetes and accounted for 45.45%. (Table.4)

Table. 4 Association of diabetes duration with ASB

Duration of diabetics(YEARS)	Total participants	ASB
<10	59	3 (5.09)
10-19	27	7 (25.9)
>20	11	5 (45.45)

In our study, participants were classified into three group based on type of antidiabetics. The "Oral Only" group, 7 out of 77 participants (9%) hadASB. Similarly, in the "Insulin Only" group, 2 out of 7 participants (28%) showed ASB , and in the "Oral + Insulin" group, 6 out of 13 participants (46.15%) showed ASB.(Table.5)

Table. 5 Association of type of antidiabetics with ASB.

Type of antidiabetics	Number of participants	ASB%
Oral only	77	7 (9%)
Insulin only	7	2 (28%)
Oral +insulin	13	6 (46.15%)

The susceptibility of Cotrimoxazole across both Gram-positive and Gram-negative isolates, including those with ESBL, suggests its potential effectiveness in the studied population. The absence of MRSA is noteworthy for infection control practices and treatment decisions. The specific details in Table 6 would provide a more granular understanding of the antibiotic susceptibility patterns for different bacterial strains (Table.6)

Table. 6Antibiotic susceptibility of uropatogens

Antibiotic	E.coli (n=7)	Klebsiella (n=2)	Proteus (n=1)	S.aureus (n=2)	CONS (n=2)	Enterococcus (n=1)
Amikacin	6	2	1	2	2	1
Amoxyclav	5	1	1	2	2	1
Ciprofloxacin	3	1	1	1	2	0
Cefixime	4	2	1	1	1	0
Cotrimaxazole	7	2	1	2	2	1
Nitrofurantoin	5	2	0	2	2	1
Ceftazidime	5	2	0	1	2	1
Vancomycin	-	-	-	2	2	1

A relatively smaller number of participants belonged to the age group<50-60 , but a higher percentage (45.45%) had ASB. Pyuria was observed in 27.27% of cases. In our study, the largest participant group was 61-70 years.. While the absolute number of ASB cases is higher (5), the percentage is lower (7.69%) compared to the <50-60 group. Pyuria was observed in 4.62% of cases. A smaller group (71-80), but a higher percentage (25%) had ASB. Pyuria was observed in 8.33% of cases. Further, a relatively smaller group more than 80 years, and a moderate percentage (22.22%) had ASB. Pyuria was observed in 11.11% of cases. Predominantly pyuria was noted among females compared to males. Table.7.

Table. 7Age wise distribution of ASB with and without pyuria

Age	Participants	ASB	Pyuria	Non-pyuria
<50-60	11	5 (45.45%)	3(27.27%)	2(18.18%)
61-70	65	5(7.69%)	3(4.62%)	2(3.07%)
71-80	12	3(25%)	1(8.33%)	2(16.67%)
>80	9	2 (22.22%)	1 (11.11%)	1 (11.11%)

Longer diabetes duration (>20 years) is associated with a higher percentage of ASB. The presence of pyuria seems to vary across different duration groups, with the highest percentage in the 10-19 years group. The non-pyuria percentage is notable in the >20 years duration group, suggesting a significant proportion without pyuria despite ASB.(Table.8)

Table . 8 Distribution of participants based on diabetes duration with ASB and pyuria

Duration of diabetes	Participants	ASB	Pyuria	Non-pyuria
<10	59	3(5.08%)	3(5.08%)	0 (0%)
10-19	27	7(25.93%)	5 (18.52%)	2(7.40%)
>20	11	5(45.45%)	1(9.10%)	4(36.36%)

Discussion:

According to the findings of the present study, the prevalence of ASB among T2D patients was 15%. Preponderance of ASB was observed among females (9/15) compared to males (6/15). Similar to the current study,previous studies reported a 9–27% prevalence of ASB in women with DM and 0.7–11% in men with DM. Predilection of ASB is due to their short urethra located close to the warm, moist, vulvar, and perianal areas that are colonized with enteric bacteria.8 Another study reported, 27% female participants and 9.4% male participants with T2D developed ASB and an overall prevalence of 20.1% was noted.9 As per the meta analysis conducted by Renko et al, prevalence of ASB was 12.5%. 10 The highest percentage of patients with ASB in our study were found in the 50-70 years age range, with 10 out of 15 ASB positive samples falling in that group. This age range was comparable to that reported by Meiland et al, where 61.8% of the diabetic patients positive for ASB were in the age range of 56-75 years.11

The importance of glycemic control (HbA1c) as a risk factor for diabetic people developing ASB has been debated. Studies in the literature argue both for and against its relevance as a risk factor. Our results show a significant association between the HbA1c levels and the risk of diabetic patients developing ASB. Similarly, Turan et al. have confirmed in their study that poor glycemic control is a risk factor for ASB.12 Similar conclusions have been reached by Nicolle et al. 13 and He et al.14 But this not in comparison with the studies conducted by Renko *et al* 10 and Zhanel *et al*.15 As per their observations, glycosuria is not an important determinant for occurrence of ASB.

Ongoing abnormal blood sugar levels raise osmotic pressure in bodily fluids, disrupt immune cell metabolism, and reduce chemotaxis, phagocytosis, and bactericidal activity. Because to the buildup of AGEs in the urinary tract mucosa, there is an endogenous "invisible injury" in the mucosa without destruction, and the mucosal barrier and protection are reduced, resulting in ASB.However, the differences in the findings on the relationship between glycemic control and ASB are due to the wide range of populations and selection criteria utilized in these studies.

Our study found significant association between duration of diabetes and the prevalence of bacteriuria similar to studies conducted by various researchers.16,17,18,19,20,21,22,23. Vejlsgaard et al,24 observed a higher occurrence of bacteriuria in individuals with diabetes mellitus lasting over 20 years, in contrast to those with a shorter duration of the disease. Furthermore, Vejlsgaard identified a correlation between bacteriuria and diabetic complications such as retinopathy, neuropathy, ischemic heart disease (IHD), and peripheral vascular disease. Only one study found that a longer illness period was associated with ASB in T2DM patients.25

Our study found Esherichia coli as the most common isolate among patients with ASB. Multiple studies have reported a variety of pathogens in their results. A study that is very much in line with our findings was conducted in Sudan, which also found E. coli (56.4%), Klebsiella pneumonia (23.0%), and Enterococcus faecalis (12.8%) to be the most common organisms.26 Another study conducted by Kiranmala et al showed *E. coli* (67%) as common isolate followed by Klebsiella (14%) and Enterococcus (9%).27 Similarly, a study conducted by Danasegaran et al.,E. coli (35%) was the predominant isolate followed by Klebsiella species (29%).28 The results of our study are contradictory to the study conducted by Odetoyin *et al*. As per their study, Staphylococcal aureus (80.9%), Klebsiella (9.5%), Enterococcus faecalis (4.8%), and *E. coli* (4.8%). 29 Further, as per the

study conducted by Bissong et al, coagulase-negative staphylococci was the predominant isolate (36.3%) isolated, followed by Klebsiella (15.9%), Candida (13.7%), E. coli (10.8%), and Serratia (10.8%).30 This inconsistency would be probably due to study design or sample size difference.

The results of the study indicate both gram-positive and gram-negative bacteria are showing an alarming trend in antibiotic resistance. One or more antimicrobial drugs showed intermediate to low levels of resistance, indicating a widespread problem that affects several antibiotic classes. But the most notable finding was the high prevalence of resistance to ciprofloxacin in particular.

There might be a number of reasons behind the increased ciprofloxacin resistance. The antibiotic's ease of availability is one important element. Ciprofloxacin is widely available and often recommended by medical practitioners as well as, regrettably, through unapproved routes. This antibiotic's accessibility over-the-counter and affordable price may encourage its indiscriminate and broad usage. In our study, all the isolates including ESBL strain were found to be susceptible to Cotrimaxzole. This observation is contradictory to the study conducted by Bissong et al. 30

As per Lyamuya et al, all isolates exhibited modest levels of resistance to trimethoprim-sulfamethoxazole, ciprofloxacin, amoxicillin/clavulanic acid, ceftriaxone, gentamycin, and ciprofloxacin, while substantial levels of resistance to tetracyclines were found.31 In our study most common isolate Escherichia coli showed good susceptibility to Cotrimaxazole followed by Amikacin, Amoxicillin/clavulanic acid,Nitrofuratoin and Ceftazidime. Venkatesan *et al.* observed that *E. coli*, the most prevalent organism in their study, was most sensitive to, Nitrofuratoin , Amikacin, and Gentamicin.32

In our study asymptomatic pyuria (ASP) in type 2 diabetic individuals was 8%. The prevalence of ASP of is inconsistent with the previous study conducted by Nakano et al.33 As per Nakano et al 31, middle-aged（<65 years) diabetic women showed the prevalence of ASP 12.5%. In our study, inspite of asymptomatic bacteriuria confirmed by urine cultures , 7 urine samples showed no pyuria. Although urine cultures are gold standard for confirming ASB, urinary leukocyte counts are preferred diagnostic method for ASB. Probably due to its easier and faster to perform , more convenient and acceptable to patients, less expensive.

In a study conducted by Zhanel et al., where urinary leukocytes were assessed using the more precise hemocytometer method, it was found that absolute urinary leukocyte counts exceeded or were equal to 10 mm^3 in 77.6% of the urine samples collected from 85 patients with confirmed bacteriuria as determined by two consecutive cultures.34

Further, among 5 women participants, sterile pyuria was observed. All the women with sterile pyuria belonged to the age group between 50-60 years. Based on population-based research, sterile pyuria is prevalent in 2.6% of males and 13.9% of females, signifying its common occurrence. 35 The incidence of this condition varies among different demographic groups. For example, a study revealed that 23% of inpatients exhibited sterile pyuria, excluding those with urinary tract infections (UTIs). Additionally, women are more prone to experiencing sterile pyuria compared to men, particularly in cases related to pelvic infections.36

Strengths of the study

In the present study, the recruitment strategy involved targeting participants from the outpatient department who were actively seeking regular medication for diabetes. Unlike studies that exclusively involve diseased populations, this research design acknowledges the diversity and complexity of health conditions that individuals may face while still actively managing their medical needs.

Second, we explored the association between various demographic details with ASB and Pyuria.

Third, our data showed that association with a higher risk of ASB and pyuria in diabetic women compared men. Because diabetic women are more likely to experience urinary hurdles it is possible that gender-specific variables, such as hormonal or anatomical variations, increase susceptibility to these issues when diabetes is present.

Limitations:

One of the limitations of the study is sample size. A lower sample size might restrict how far the study's conclusions can be applied. It's critical to specify the precise population to which the findings apply and to identify any potential obstacles to generalising the results to other populations and statistical analysis was performed by using simple percentage method.

Second, the assumption that individuals in the health checkup population were asymptomatic is a crucial aspect of our study design. However, it's essential to acknowledge that if this assumption proves incorrect, it could introduce bias into our findings, potentially leading to an overestimation or exaggeration of the association between asymptomatic bacteriuria (ASB) and diabetes.

Future perspectives

In order to further our understanding of the link between these factors, future study should examine methods to evaluate the asymptomatic status and take into account the implications of symptomatic cases on the found correlation between ASB and diabetes.

Undoubtedly, observing for the emergence of symptomatic bacteriuria by undertaking a follow-up with individuals who have asymptomatic bacteriuria (ASB) might be an important component of longitudinal study. This follow-up can shed light on the symptoms' development as well as the normal course of ASB and its possible consequences. This understanding of the clinical implications of ASB and contribute valuable information to the existing literature on the progression of bacteriuria in different populations

Conclusion: The study emphasises how crucial it is to take diabetes duration, glycemic control, and gender into account when determining the prevalence of ASB in people with diabetes. The promise of Cotrimoxazole as a therapy option is suggested by its effectiveness against a variety of bacterial strains. The results further highlight the necessity of more investigation into the complexities of ASB presentation and therapy in diabetic populations with different illness durations.

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