Prevalence of Asymptomatic Bacteriuria during Pregnancy at a Tertiary Care Hospital of Province No. 2, Nepal

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ABSTRACT

Objectives: The objective of this study was to determine the prevalence of asymptomatic bacteriuria in pregnant women, identify the causative agent responsible for urinary tract infection (UTI) and its antibiotic susceptibility.

Methods: The mid-stream urine sample was streaked on the MacConkey agar (MA) and Blood agar (BA) medium by the semi-quantitative culture technique. Identification of significant isolates was done by standard microbiological techniques. Antibiotic susceptibility test of the isolated organisms was done by modified Kirby Bauer disc diffusion method.

Results: The prevalence rate of asymptomatic urinary tract infection (AUTI) among pregnant women was found to be 42%. The highest number of UTI cases found during pregnancy was in between age 21-25 years (52.22%), in second gravida (51.59%), during 3rd trimester of pregnancy (49.68%) and in winter with 52.22%. *E. coli* was principal organism to cause AUTI (35.48%) during pregnancy. Amikacin, imipenem and nalidixic acid were effective towards Gram negative bacilli whereas vancomycin, tetracycline and amoxyclav were effective towards Gram positive cocci.

Conclusion: All pregnant women visited for antenatal checkups should be advised for the culture and sensitivity test of their urine specimens which will reduce the maternal and child health complications. Different screening test and awareness programme should be conducted at regular interval of time for prevention of AUTI during pregnancy.

Key words: Asymptomatic bacteriuria, *E. coli*, Pregnancy, Pyelonephritis

INTRODUCTION

Asymptomatic bacteriuria (ASB) is the presence of bacteria more than 10^5 per ml in a midstream of urine sample (Yadav et al. 2014) in significant number of a person without symptoms of urinary tract infection (UTI) or bacteriuria. It is an important global health problem prevailing in all age groups. UTI can be classified based on association with complications as, complicated or uncomplicated UTI (Yadav and Prakash, 2016b) and also based on the site involved as upper urinary tract leads to symptomatic bacteriuria or lower urinary tract leads to asymptomatic bacteriuria (Emamghorashi et al. 2012).

The normal physiological changes during pregnancy with reduction in immunity, increased plasma, decreased urine volume and gestational induced glycosuria, pregnant women are more prone to UTI (Lucas and Cunningham, 1993). In addition, increased age, number of childbirths, number of intercourses per week, diabetes, recessive sickle cell anemia, previous history of UTI, immunodeficiency and urinary tract abnormalities can increase the risk of UTI in pregnant women (Giraldo et al. 2012; Raza et al. 2011). As per recent reports, around 25% to 30% women develop symptomatic UTI whereas 2% to 10% women develop ASB during pregnancy (Schnarr and Smaill 2008).

UTI aetiology are diverse showing the geographical variability of causative agents of Gram-negative bacteria

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such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Citrobacter* species, *Pseudomonas* species, *Proteus* species and Gram-positive bacteria like *Enterococcus* species, Streptococci, *Candida albicans* and *Staphylococcus saprophyticus* (Yadav et al. 2014). *E. coli* is the most common organism causing both community as well as hospital acquired UTI. Considering the importance of UTI in pregnant women which is responsible for several complications, its diagnosis and treatment are essential to maintain the health of mother and baby.

In Nepal, most of health care centers do not carry out routine urine culture test for every pregnant woman during her antenatal checkups presumably due to poor health education, high cost and time duration of culture result usually 2-3 days. Although, the clinicians and healthcare personnel look for the presence of glucose and protein analysis in urine by strip urinalysis method to diagnose UTI that poorly quantify the extent and grade of infection in pregnant women which do not give a clear picture of AUTI or UTI. Very few or negligible studies have been implicated in the prevalence and therapeutic consideration of AUTI at Southern Terai of Nepal. So, it is troublesome to know how frequent this disease is common in this reason among pregnant women. During pregnancy, the development of the AUTI to the symptomatic UTI can be prevented based on proper diagnosis. Therefore, this study was focused to identify the prime importance of causative agent of asymptomatic UTI and prevalence of this disease among at a tertiary care hospital of Dhanusha District at Province No. 2, Nepal which may be helpful to disseminate knowledge regarding asymptomatic UTI as many are unaware of it in this region.

MATERIALS AND METHODS

Study design: A cross-sectional descriptive study was conducted among the pregnant women attending for antenatal care check up at Department of Obstetrics and Gynecology and all the laboratory procedures were carried out in Microbiology Department of Clinical Pathology and Laboratory Medicine at Ram Janaki Hospital, Janakpurdham, Nepal from March 2017 to August 2018. This study included 378 women of reproductive age group who were pregnant.

Ethical consideration: Ethical approval was taken from Ram Janaki Technical Institute and Ram Janaki Hospital, Janakpurdham, Nepal. Informed written verbal consent was also obtained from the participants prior to the study

Inclusion and exclusion criteria of participants: Pregnant women on attending for antenatal check up at the hospital were included while members of staff of the hospital, patients with previous history of preterm labor or spontaneous abortion and those who did not give their consent were excluded.

Specimen collection and processing: The Mid Stream Urine (MSU) samples were collected in the clean, sterile, dry, wide-necked leak-proof container. The distinctive instruction was followed by the patient for the sample collection. When immediate processing was not possible, the specimen was refrigerated at 4-6°C, and when a delay of more than 2 hours, boric acid (1.8 % w/v) was added as preservative to the urine.

Culture of specimen: Media were prepared as instructed by the manufacturer company (Himedia). The urine sample was streaked on the MacConkey agar (MA) and Blood agar (BA) medium by the semi-quantitative culture technique using a standard loop. After mixing the urine sample in the container thoroughly, a loopful of sample was touched to the centre of the plate, from which the inoculum was spread in a line across the diameter of the plate. Without flaming the loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies. The plates were incubated aerobically at 37°C overnight.

The number of colonies was counted and the number of bacteria i.e. Colony Forming Unit (CFU) per ml urine estimated in accordance to the volume of urine inoculated and reported as:- Less than 10^4 /ml organisms - Not significant, 10^4 -10^5 /ml organisms - Doubtful significance (suggest repeat specimen) and More than 10^5 /ml organisms - Significant bacteriuria

Identification of the isolates: Identification of significant isolates was done by microbiological techniques as described in the Bergey's Manual of Systematic Bacteriology (2nd Edition) which involves morphological appearance of the colonies, staining reactions and biochemical properties. Gram positive organisms were tested by catalase test, oxidase test, coagulase test and their specific biochemical tests. Gram negative isolates were identified on the basis of various biochemical tests such as catalase test, oxidase test, O/F test, MR/VP test, SIM test, citrate test, urease test, TSI test.

Antibiotic susceptibility testing: Antibiotic susceptibility test of the isolated organisms was done

by modified Kirby Bauer disc diffusion method. Bacterial inoculum was prepared by suspending the freshly grown bacteria in 2 ml of sterile nutrient broth and incubated at 37 °C for 3-4 hours. The turbidity of tube was matched with 0.5 Mc Farland turbidity standards. The inoculum was then streaked on entire Muller-Hinton agar (MHA) plate. Antibiotic discs were placed around the outer edge of the plate and incubated overnight at 37 °C. Diameter of zone of inhibition was measured and zone diameter criterion was used to interpret the level of susceptibility to each antibiotic (CLSI, 2013).

Statistical analysis: The data were analyzed using SPSS 18.0 version and Microsoft excels 2007. The Chi-square test was used to test for the positive cases of UTI during pregnancy in relation to age, gravidity, seasons and trimester. The p-value ($p<0.05$) was considered as statistically significant.

RESULTS

Prevalence rate of AUTI among pregnant women: Out of total 378 pregnant women, the prevalence rate of AUTI among pregnant women was found to be 42% (Figure 1).

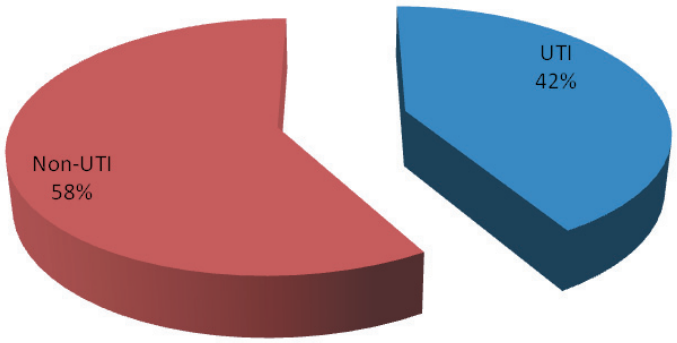

Figure 1: Prevalence rate of AUTI among pregnant women

Age-wise distribution of prevalence of AUTI in pregnant women: The highest number of AUTI cases found during pregnancy was in between age 21 to 25 years (52.22%) followed by age 26 to 30 years (19.74) (Table 1).

Table 1: Age-wise distribution of prevalence of AUTI in pregnant women

Age groups (years)	No. of pregnant women (%)	No. of positive cases (%)	p-value
≤ 20	78 (20.63)	26 (16.56)	
21-25	149 (39.41)	82 (52.22)	
26-30	105 (27.77)	31 (19.74)	0.09
>30	46 (12.16)	18 (11.46)	
Total	**378**	**157 (41.53)**	

Prevalence of AUTI in pregnant women in relation to gravidity: The more number of positive cases of AUTI during pregnancy was found in second gravida (51.59%) followed by prime gravida (29.93%). (Table 2).

Table 2: Prevalence of AUTI in pregnant women in relation to gravidity

Gravidity	No. of pregnant women (%)	No. of positive cases (%)	p-value
Prime gravida	133 (35.18)	47 (29.93)	
Second gravida	168 (44.44)	81 (51.59)	0.50
Multi gravid	77 (20.37)	29 (18.47)	
Total	**378**	**157**	

Trimester pattern of prevalence of AUTI in pregnant women: Most of the study participant were attacked by uropathogens during 3rd trimester of pregnancy (55.31%) and was statistically significant (p=0.0001) (Table 3).

Table 3: Prevalence of AUTI in pregnant women in relation to trimester

Trimester	No. of positive cases		Total	p-value
	Present (%)	Absent (%)		
1st trimester (1st 12 weeks)	26 (26.53)	72 (73.46)	98	
2nd trimester (13-28 weeks)	53 (38.12)	86 (61.87)	139	0.0001
3rd trimester (29-40 weeks)	78 (55.31)	63 (44.68)	141	

Bacteriological profile of uropathogens causing AUTI in pregnancy: *E. coli* was found to be predominant organism to cause urinary tract infection (35.48%) followed by *K. pneumoniae* (18.27%) during pregnancy (Figure 2).

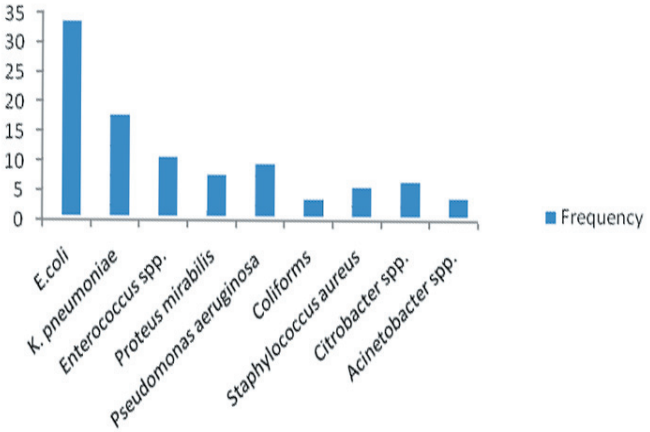

Figure 2: Profile of uropathogens causing AUTI in pregnancy

Antibiogram of Gram negative bacilli: More Gram negative bacilli were sensitive towards amikacin (69.89%) followed by imipenem (65.59%) and nalidixic acid (61.29%) while least sensitive towards cotrimoxazole (31.18%) (Table 4).

Table 4: Antibiotic susceptibility pattern of Gram negative bacilli

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	65 (69.89)	4 (4.30)	24 (25.80)
Gentamycin	41 (44.08)	15 (16.12)	37 (39.78)
Ciprofloxacin	49 (52.68)	10 (10.75)	34 (36.55)
Cotrimoxazole	29 (31.18)	2 (2.15)	62 (66.660
Norfloxacin	36 (38.70)	8 (8.60)	49 (52.68)
Nitrofurantoin	52 (55.91)	14 (15.05)	27 (29.03)
Nalidixic acid	57 (61.29)	3 (3.22)	33 (35.48)
Imipenem	61 (65.59)	7 (7.52)	25 (26.88)

Antibiogram of Gram positive cocci

More Gram positive cocci were sensitive towards vancomycin (77.41%) followed by tetracycline (73.11%) and amoxyclav (70.96%) while least sensitive towards erythromycin (49.46%) (Table 5).

Table 5: Antibiotic susceptibility pattern of Gram positive cocci

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxyclav	66 (70.96)	7 (7.52)	20 (21.50)
Ciprofloxacin	53 (56.98)	11 (11.82)	29 (31.18)
Erythromycin	46 (49.46)	9 (9.67)	38 (40.86)
Linezoid	60 (64.51)	2 (2.15)	31 (33.33)
Tetracycline	68 (73.11)	5 (5.37)	20 (21.50)
Teicoplanin	54 (58.06)	3 (3.22)	36 (38.70)
Vancomycin	72 (77.41)	3 (3.22)	18 (19.35)

DISCUSSION

The prevalence of significant asymptomatic bacteriuria (bacterial count >1x10^{5}cfu/ml) called the asymptomatic UTI in pregnancies. This study reveals the prevalence rate of AUTI among pregnant women was 42% which is almost in accordance with a study conducted by Valentina and Srirangaraj in 2016, the prevalence of UTI in pregnant women was 45%. Yadav and Yadav in 2018 from Chitwan Medical College reported AUTI of 51.83%. Similarly, Adabara et al. in 2012, Little et al. in 2016, Mokube et al. in 2013 and August et al. in 2012, accounted the prevalence of UTI as 75%, 66%, 23.5% and 21.15% respectively. Whereas, Mobasheri et al. in 2002, Hernandez et al. in 2007, Tadesse in 2007, Obirikorang et al. in 2012, Turpin et al. in 2007, Demilie et al. in 2012 and Thakre et al. in 2012, reported the low prevalence of UTI as 7- 10%.

The reason behind for higher prevalence could be attributed to the low income status of the patients, anemia, sexual activity during pregnancy, lack of proper personal, environmental hygiene, population susceptibility, poor housing, ventilation, sanitation and drainage systems (Dutta, 2008; Kolawole et al. 2009). The other established fact is that the urethra in females is shorter, wider and close to the anus contributes to the higher prevalence of UTI in women. Being in close proximity, the bacteria from the rectum can easily go up the urethra increasing the rate of infections (Kolawole et al. 2009; Ebie et al. 2009). Moreover, biochemical, hormonal and immunological normal physiological changes in pregnancy to reduce ureteric muscular tone in ureter and urethra, and increase in mechanical pressure from the gravid uterus, leading to urinary stasis, which act as good culture media, favoring the bacterial growth and multiplication in urine (Obiogbolu et al. 2001)

Age group in pregnancies revealed a significant difference in the prevalence of asymptomatic bacteriuria ($p<0.05$). The highest number of UTI cases found during pregnancy was in between 21 and 25 years. Similar finding was also obtained by Adeyeba et al. in 2002, Amadi et al. in 2007, Akinleye et al. in 2014, Valentina and Srirangaraj in 2016 and Yadav and Yadav in 2018. This could be recognized that the subjects with this age group are sexually more active which could favor the incidence of UTIs (Dutta 2008; Adeyeba et al. 2002).

Majority of the studies show a higher prevalence of UTI in multigravida and stress on the fact that the prevalence of UTI in pregnancy increases with parity (Emamghorashi et al. 2011). The result of this study demonstrates 29.93%, 51.59% and 18.47% of pregnant women suffered from asymptomatic UTI with respect to prime, second and multi gravida respectively. More number of positive cases of UTI during pregnancy was found in second gravida of 51.59% followed by prime gravida of pregnancy with 29.93% and was found to be statistically insignificant ($p=0.50$). This may be due to glycosuria, which is present in 70% of pregnant women, increases the urinary level of estrogen and progesterone, and decreases the patient's ability to fight invasive bacteria (Rizvi et al. 2011). In contrast to this study, Lavanya et al. in 2002, Marahatta et al. in 2011 and Valentina and Srirangaraj in 2016 found the higher prevalence of UTI associated with pregnancy among the primigravida.

Increased parity, age and gestational age increases the risk of UTI in pregnant women. Most of the study participants were infected by uropathogens during 3rd trimester of pregnancy. An article published by researcher of Chitwan Medical College, Nepal accounted the highest prevalence (45.33%) of asymptomatic bacteriuria was observed in the second trimester of pregnancy (Yadav and Yadav, 2018). A similar finding was also reported by Paty in 2018 with high percentage of asymptomatic bacteriuria in the second trimester of pregnancy which is in accordance with this study.

E. coli was found to be predominant organism to cause urinary tract infection. A similar result was also found by Valentina and Srirangaraj, 2016; Paty, 2018. This could be the reason for Gram negative bacteria being the dominant etiologic agent of UTI due to poor or unhygienic genital practices by pregnant women who may find it difficult to clean their anus properly after defecating or clean their genital after passing urine leading to infection by faecal contamination. Another reason behind for *E. coli* proliferation may be due to the increased levels of amino acids and lactose in pregnancy (Obiogbolu et al. 2009).

More Gram negative bacilli were sensitive towards amikacin followed by imipenem and nalidixic acid while least sensitive towards cotrimoxazole. More Gram positive cocci were sensitive towards vancomycin

followed by tetracycline and amoxyclav while least sensitive towards erythromycin. Valentina and Srirangaraj in 2016 reported among the Gram negative bacilli, higher degree of sensitivity was observed with Nitrofurantoin, Amikacin and Imipenem and among the Gram positive cocci, higher degree of sensitivity was observed with Vancomycin, Amoxyclav and Tetracycline. These findings are in line with the findings by Yadav and Yadav in 2018.

The diverse pattern of antimicrobial sensitivity and resistance among different communities and hospitals is due to indiscriminate use of antibiotics causing resistant strains (Yadav and Prakash, 2016a). The increase in antibiotic resistant pattern could be due to antibiotic abuse due to lack of knowledge to health practitioner and consumer. Also, the low cost and availability of antibiotics without prescriptions in this area could be another contributing factor for antibiotic abuse, and thus the resistance (Yadav and Yadav, in 2018)

CONCLUSION

This study highlights the moderate prevalence of UTI among pregnant women regardless to women's age, parity and gestational age. Routine screening and urine culture at least once in each trimester of pregnancy could be useful to investigate asymptomatic or symptomatic UTI to minimize maternal and neonatal morbidity and mortality.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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