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Prevalence of Asymptomatic Bacteriuria among Antenatal Women Attending a Tertiary Care Hospital, Davangere, India

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Abstract: ***Introduction: Asymptomatic Bacteriuria (ASB) is the commonest bacterial infection which requires medical treatment in pregnancy which can be diagnosed by urine culture and rapid screening tests. AIMS: To determine the prevalence of ASB in pregnant women, to isolate, identify the etiological agents that cause ASB and to determine its antimicrobial susceptibility pattern. Methodology: This study consists of 100 pregnant female patients in the age group of 18-45 years without any signs and symptoms of UTI. Clean catch midstream urine samples were collected and Urine culture was done using conventional microbiological techniques. Biochemical testing was used to identify the organisms and antibiotic sensitivity was done by the Kirby Bauer disc diffusion method according to standard CLSI guidelines. Rapid screening tests were done by wet mount, gram's staining, reagent strip test such as UROCOLOR 10 which detects both nitrite and leukocyte esterase (LE). Results: Out of 100 samples, 87 were sterile. Organisms in pure culture in significant number were obtained in 13 cases. ASB was more common in the low socioeconomic group, in the age group of 21-25years and the organism most commonly isolated was E.coli 5 (38%). The screening tests like wet mount showed 92% specificity, Gram's staining showed 76% of sensitivity, LE showed 91% of specificity whereas nitrite test showed 98% of specificity. Conclusion: Urinary tract infection (UTI) are more common in pregnant than non-pregnant women. UTI are associated with risk to both the foetus and the mother, including pyelonephritis, LBW and increased perinatal mortality. The earlier diagnosis and proper antimicrobial treatment in these women would prevent the obstetric complications.***

Keywords: Asymptomatic bacteriuria, Antenatal women, Rapid screening test, Antibiotic resistance

1. Introduction

Urinary Tract Infection (UTI) is the commonest of all bacterial infections affecting human beings throughout their life span especially in women[1].UTI during pregnancy is classified into symptomatic and asymptomatic[2.]Asymptomatic Bacteriuria (ASB) isdefined as persistently and actively multiplying bacteria in significant numbers i.e. 10^5 bacteria /milliliter within urinary tract without any obvious symptoms.[3]The term asymptomatic bacteriuria of pregnancy refer to the presence of a positive urine culture in an asymptomatic pregnant female.[3]

Pregnancy causes hormonal and mechanical changes and increases the risk of urinary stasis and vesicoureteral reflex.[4]The anatomical and physiological changes imposed on urinary tract by pregnancy, as well as pressure on the uterus by the gravid uterus and muscle relaxant effect of progesterone predisposes women with ABU to UTI.[2]Other conditions associated with increased incidence include low socioeconomic status, grand multiparity and advancing age[5]. The pregnant females are two times more commonly affected than age matched non pregnant females.[3]

Global prevalence of ASB varies widely and in pregnancy it is 1.9% to 9.5%.[6] While in India it is found to be on higher side i.e. between 5-12%.[3]Detection of ASB during pregnancy is important as women identified with ABU in early pregnancy have 20-30 fold increased risk of developing pyelonephritis during pregnancy, compared with women without bacteriuria.[3, 2] Quantitative urine culture is the gold standard for diagnosis of ASB[8]. Screening methods may be useful because full bacteriological analysis could be reserved for those patients who are symptomatic or have a positive screening test results. Because ASB is detectable and treatable, screening of urine during pregnancy may be justified.[7]

Comparative clinical trials have reported that antimicrobial treatment of ABU during pregnancy decreases the risk of subsequent pyelonephritis from 20-30% to 1-4% and decreases the frequency of low birth weight infants and preterm delivery.Therefore allantenatal women require screening for bacteriuria by urine culture at least once in early pregnancy so that they can be treated with appropriate antibiotics for 3-7 days[8].

Because the majority of urine specimens submitted to clinical laboratory for culture are negative or have bacterial colony counts below levels consideredbeing clinically significant, several non-culture screening tests have been devised for the rapid detection of bacteria. The screening test most commonly used are the Wet mount,Gram's staining along with the combination of chemical Nitrite and Leukocyte esterase dipstick test.[9]In Wet mount technique urine is examined microscopically as a wet prepration to detect significant pyuriaie WBC's in a excess of 10^7 WBC/ml of urine,red cells, casts, yeast cells,Trichomonasvaginalis,motile trophozites and bacteria. In Gram's staining technique the gram's stained smear is prepared and examined.[11]Urine from a healthy person does

not contain nitrite. The detection of nitrite in urine is a useful test in the investigation of urinary tract infections caused by nitrate reducing bacteria.[10]Urinary pathogens, e.g. *E.coli, Proteus species and Klebsiella species* are able to reduce the nitrate normally present in urine to nitrite.[11]

The presence of leukocytes in urine indicates inflammation of the urinary tract, the commonest cause of which is a urinary tract infection.[10]It detects the enzyme from both active and lyzed WBC. Leukocyte Esterase testing is an alternative method of detecting pyuria when it is not possible to examine fresh urine microscopically for white cells or when the urine is not fresh and likely to contain mostly lyzedWBCs.Leukocyte Esterase can be detected using a reagent strip test such as UROCOLOR 10 which detects both nitrite and leukocytes(Leukocyte esterase)[11]. One advantage of this test is that leukocytes need not be viable for leukocyte esterase activity to be detected.[9]

We screened pregnant women who attended the antenatal clinic for ASB with wet mount, Gram's staining, reagent strip test such as UROCOLOR 10 which detects both Nitrite and Leukocyte esterase and compared the results with urine culture.

2. Materials and Methods

This study consists of 100 pregnant female patients in the age group of 18-45 years without any signs and symptoms of UTI, attending the routine antenatal checkup in ChigateriGeneral Hospital andBapujiHospitalDavangere, Karnataka. Control group subjects will be 50 non-pregnant females of the age group of 18 to 45 years, without any symptoms and signs of UTI.

Institutional approval from the Institutional Ethics Committee was taken prior to the study. Informed consent was taken from all the subjects participated in the study after explaining the study details in the subject's mother tongue.

Inclusion criteria

1) All pregnant women without any signs and symptoms of UTI.

Exclusion criteria

1) History of UTI symptoms (dysuria, frequency, urgency and burning micturitionetc.).
2) Pregnancy with diabetes mellitus / hypertension.
3) History of antibiotic therapy in the previous 2 weeks.
4) Pyrexia.
5) Known congenital anomalies of the urinary tract.
6) Vaginal bleeding, suprapubic pain, multiple pregnancies, history of pre-term delivery, Preterm Premature Rupture of Membrane, intrauterine growth retardation.

Sample collection and processing:

About 30 ml of clean,catchmidstream urine samples were collected in 100 ml sterile, dry, leak – proof container with instruction on how to collect a clean catch mid-stream urine (MSU).[11] The MSU requires that the first 10-30 ml of the voided urine be discarded and the second midstream be sampled. In the female patient, adequate periurethral cleansing is necessary to reduce the probability of contamination. For cleaning, water and soap solution without anti -bacterial activity wereused.[12] Urine is an excellent culture media and bacteria will multiply if specimen is left at room temperature for any appreciable time. For this reason, urine specimen were transported to the laboratory immediately after obtaining and were processed within 1 hour or in case of delay they were refrigerated at 4°C (upto to 24 hours), until culture can be performed.[13]

The specimens were subjected to semi-quantitative culture method. The culture were done by surface streaking method on 5% sheep blood agar, McConkey's agar and CystineLactoseElectrolyte Deficient agar. For this, sterile standard nichrome loop of 28 SWG was used which had a internal diameter of 3.28 mm and volume holding capacity of 0.004 ml.The plates were incubated at 37° C for 24 hours. Prolonged incubation was done for further 24 hours if no growth obtained. It was reported as significant if the growth obtained is confluent or number of colonies corresponding to 10^5colony forming unit (CFU)/ml i.e. 40 colonies or more. Insignificant growth was reported in samples in which colony count obtained corresponding less than 10^5 CFU/ml of urine except in case of growth of *Staphylococcus aureus* where even 10^2 CFU/ml was taken as significant. The identification of isolates were done by gram's staining, motility test, catalase test, oxidase test, coagulase test and routine biochemical tests as per cowan and steels manual.[3,14] Antibiotic sensitivity testing was done by emulsifying selected colonies in normal saline at a turbidity compared to 0.5 MacFarland's standard. Using sterile swabs, suspensions were inoculated on Muller – Hinton agar in accordance with modified Kirby Bauer method as per recommendation of CLSI guidelines(2014) and incubated at 35-37°C for 18-24 hours.[5,15] Sensitivity testing were done using drugs safe in pregnancy namely Amoxicyllin(25µg), Ampicillin(3oµg), Cephalexin(30µg), Cefuroxime(30µg), Cefotaxim(30µg), Amikacin(30µg), Gentamycin(10µg), Nitrofurantoin(300µg).[16] Interpretation was done by comparing the diameter of zone of inhibition with those of a standard table in three grades of susceptibility which are sensitive, intermediate and resistant.[5] Standardization of antibiotic susceptibility testing was done by using standard strains of *E.coli* ATCC 25922 and *Staphylococcus aureus* ATCC25933. Significant bacteriuric pregnant females were advised to take treatment.[6, 16]

Because the majority of urine specimens collected for culture was negative or had bacterial colony counts below levels considered being clinically significant,and the complete bacteriological analysis was both time consuming and expensive, several non culture screening tests were used for rapid detection of bacteriuria.[9]

At first, urine samples were examined microscopically as a wet preparation to detect significant pyuria that is WBC's in excess of 10^7 WBC/ml of urine. Detecting bacteria in uncentrifuged urine indicates urinary infection, pyuria that can be quantified by counting WBC's on estimating numbers by examining a drop of urine on a slide (1 WBC per lower power field corresponds to 3 cells per µl).

The Gram's stain was another method used to estimate bacteriuria.A drop of uncentrifuged well mixed urine was taken on a clean grease free slide and stained by gram's method of staining and examined under the oil immersion microscope. Presence of >1 bacteria per oil immersion field correlates with the significant bacteriuria of $>10^5$ CFU/ml of urine.[7]

Urine from a healthy person does not contain nitrite. The detection of nitrite in urine is a useful test in the investigation of urinary tract infection caused by nitrate reducing bacteria. The presence of leukocytes in urine indicated inflammation of urinary tract.[10]A nitrite reagent strip which also detects leukocyte esterasesUROCOLOR 10 was used. The test detects nitrite in a concentration as low as 11micromol/l[10].

3. Results

100 pregnant women attending the antenatal clinics of ChigateriGeneralHospital and Bapuji Hospital, Davangere, during the two months period of June – July, 2014 were taken for study.Out of 100 samples, 87 (87%) were sterile. Organisms in pure culture in significant number were obtained in 13 cases (13%). In 13 samples pure growth of organism $>10^5$ CFU/ml were obtained. None of the samples showed growth contamination.

Table 1: Effect of age on asymptomatic bacteriuria

Age in years	Total no.of patients	asymptomatic bacteriuria	Percentage %
15 – 20	21	4	31
21 – 25	61	6	46
26 – 30	11	2	15
31 – 35	7	1	8

According to Table – 1, occurrence of ASB varies with age. It occurs more among women with age group of 21-25 years 6 (46%), in 15-20 years 4 (31%), 26-30 years 2 (15%), 31-35 years 1 (8%). No cases were found in age group 35 and above in our study.

Table 2: Effect of socio-economic status on ASB

Socioeconomic status	Total no.of patients	asymptomatic bacteriuria	Percentage %
Low	71	10	77
Middle	24	2	15
Upper	5	1	8
Total	100	13	100

According to table – 2, antenatal women from low socioeconomic status had highest (77%) of incidence followed by middle (15%) and upper (8%).

Table 3: Result of wet film preparation with respect to culture

Wet film	Culture		
	+	-	Total
+	5	7	12
-	8	80	88
Total	13	87	100

According to table – 3, the number of samples showing wet film positive – culture positive, wet film positive- culture negative, wet film negative – culture positive, wet film negative- culture negative are 5, 7, 8 and 80 respectively.

Table 4: Result of Gram stain with respect to culture

Gram stain	Culture		
	+	-	Total
+	10	12	22
-	3	75	78
Total	13	87	100

According to table – 4, the number of samples showing gram stain positive – culture positive, gram stain positive – culture negative, gram stain negative – culture positive, gram stain negative – culture negative are 10, 12, 3 and 75 respectively.

Table 5: Result of Leukocyte esterase test with respect to culture

Leukocyte esterase	Culture		
	+	-	Total
+	7	8	15
-	6	79	85
Total	13	87	100

According to table – 5, the number of samples showing Leukocyte esterase positive – culture positive, Leukocyte esterase positive – culture negative, Leukocyte esterase negative – culture positive, Leukocyte esterase negative – culture negative are 7, 6, 8 and 79 respectively.

Table 6: Result of Nitrite test with respect to culture

Nitrite	Culture		
	+	-	Total
+	6	2	8
-	7	85	92
Total	13	87	100

According to table – 6,the number of samples showing Nitrite positive – culture positive, Nitrite positive – culture negative, Nitrite negative – culture positive, Nitrite negative – culture negative are 6, 7, 2 and 85 respectively.

Table 7: Organisms present in ASB

Total no. of patients ASB	Organism present	No. of patients	Percentage %
13	*E.coli*	5	38
	Staphylococcus aureus	3	23
	Klebsiella pneumonia	2	15
	Acinetobacter	1	8
	Proteus mirabilis	1	8
	Pseudomonas	1	8

According to table 7, the most common bacteria found in ASB is *E.coli* (38%) followed by *Staphylococcusaureus(23%), Klebsiella pneumonia* (15%),*Acinetobacter*(8%), *Proteus – mirabilis(* 8%), *Pseudomonas(8%).*

Table 8: Antibiotic susceptibility of the isolates

Organisms	No.of isolates	Amikacin (30µg)	Amoxycillin (25µg)	Ampicillin (10µg)	Cefotoxime (30µg)	Cephalexin (30µg)	Gentamycin (10µg)	Cefuroxime (30µg)	Nitrofurantoinin (300µg)
E.coli	5	4	-	-	2	1	3	3	2
Staphylococcus aureus	3	2	-	-	1	-	2	1	1
Klebsiella pneumonia	2	1	-	-	-	-	2	1	1
Acinetobacter	1	1	-	-	1	-	1	1	1
Proteus mirabilis	1	1	-	-	-	1	1	1	1
Pseudomonas	1	1	-	-	-	-	1	1	1

According to table 8, all the strains were resistant to Ampicilin,Amoxycilin. There was varying susceptibility to other antibiotics.

Table 10: Comparison of screening tests at significant bacteriuria

Tests	*Sensitivity*	*Specificity*	*PPV*	*NPV*
Wet film	38%	92%	47%	90%
Gram stain	76%	86%	45%	96%
LE	46%	91%	47%	92%
Nitrite tests	54%	98%	75%	92%

According to table 10, PPV – positive predictive value, NPV – negative predictive value comparing the various screening tests, it was seen that gram stain had the maximum sensitivity 76% and highest negative predictive value 96% and low positive predictive value of 45%. Wet film has high specificity of 92% and least sensitivity of 38%. Leukocyte esterase has high specificity of 91% and low sensitivity of 46%. Nitrite test has highest specificity of 98% and highest positive predictive value of 75%.

4. Discussion

The urinary tract is second only to the respiratory tract in acquiring microbial infections, especially in females. The gold standard for the detection of bacteriuria is urine culture. However, the full bacteriological analysis is both time consuming and expensive and a vast majority of antenatal urine specimens will be negative. Thus a number of other screening methods have been proposed like Wet mount,Gram's staining and combination of Leukocyte esterase and Nitrite reduction test.[8]

In our study of 100 pregnant women, 13 (13%) were found to be suffering from asymptomatic bacteriuria. BalamuruganS et al(2014) reported that the prevalence of asymptomatic bacteriuria as 13% which is correlating with our study.

In our study incidence of urinary infection is more in the age group of 21-25 years i.e. 46%. Chandel et al (2012) also observed incidence of bacteriuria was more in the age group of 20-25 years.

In our study incidence of bacteriuria was more in low socioeconomic status group that is 77% compared to 15% in middle class group and 8% in upper class group. Muktikesh et al (2013) reported that the prevalence of bacteriuria is more in low socioeconomic group 62.4%.

In our study of 100 pregnant women, 13 (13%) were found to be suffering from asymptomatic bacteriuria and the organisms isolated were *E.coli 5* (38%), *Staphylococcus aureus* 3 (23%), *Klebsiella pneumonia* 2 (15%), *Acinetobacter*1(8%), *Proteus mirabilis* 1 (8%), *Pseudomonas* 1 (8%). In our study we found *E.coli* 5 (38%) whereas CA Turpin et al (2007) 37%, Muktikesh, et al (2013) (39%) which were similar to our study. In our study *Staphylococcus aureus* 3 (23%) whereas CA Turpin, et al (2007) had 31% which is closer to our studies.

In our study we got *Klebsiella pneumonia* 2 (15%) whereas ShanweelAhamad et al (2011) had 17% which were almost similar to our study.

Acinetobacter, Proteus mirabilis,Pseudomonas i.e. 8% each found from our study when compared to the other studies they are almost similar. In our present study 4 rapid tests were used to screen asymptomaticbacteriuriain pregnant women.

a) Wet film : of uncentrifuged urine

In our study, this test had poor sensitivity 38% and PPV 41% but good specificity 92%.

Previous study like Taneja N et al have shown this test to have sensitivity 40%, specificity of 90.9% and PPV 28.5% which were similar to our study.

b) Gram's stain :

Gram staining of uncentrifuged urine samples in our study had a sensitivity of 76%, specificity of 86% NPV 96%, but poor PPV 45%. Study of Tanejaet,al have shown this test to have sensitivity 70%, specificity 77.5%, NPV 96.6%, PPV 22% which showscentrifuged urine samples have high false positive result. Hence uncentrifuged urine is better.

c) Leukocyte esterase test :

Leukocyte esterase test in our study had moderate sensitivity of 46% but high specificity 91%. Other study like Balamurugan S et al has shown this test to have sensitivity of 85% and specificity of 71% which were almost similar to our study.

d) Nitrite test

Nitrite test in our study had the highest specificity 54% and sensitivity 94%. Other study like Balamurugan S et al, have shown this test to have sensitivity of 62% and specificity of 71% which were similar to our study.

5. Conclusion

From the present study it is observed that ASB is present in 13% of antenatal women in our hospital. The earlier

diagnosis and proper antimicrobial treatment in these women would prevent the obstetric complications. Culture is the gold standard but it is time consuming and expensive, though many screening tests are available, no one is 100% sensitive. Leukocyte esterase and Nitrite test is a rapid and inexpensive method to ruleout UTI in antenatal women. Alternatively Wet film and Gram's stain can be used as screening method.

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References

[1] ShamweelAhemd, ShehlaShakooh. Prevalence of asymptomatic bacteriuria among pregnant women in Kashmir. Srilanka Journal of Obstetrics and Gynecology 2011; 33:158-162.
[2] Muktikesh Dash, SusmitaSahu, IndraniMohanty, MoningiVenkat, Jyotimayee, Rani Sahu. Prevalence, risk factors and antimicrobial resistance of asymptomatic bacteriuria among antenatal women. Journal of Basic and Clinic Reproductive Sciences 2013; 2(Issue 2):92-96.
[3] ChandelLata R,Karga Anil, Thakur Kamlesh, MoktaKiran K, SoodAnuradha, ChauhanSmriti. Prevalence of pregnancy associated asymptomatic bacteriuria: A study done in a Tertiary care hospital. The Journal of Obstetrics and Gynaecology of India 2012; 62(5):511-514.
[4] Emilie Katherine Johnson. Urinary tract infections in pregnancy. Medscape reference, Drug Diseases and Procedures 2014.
[5] Akinola B. Ajayi, Charles Nwabuisi, AbiodunP,Nanji S,Nanji S Ajayi,Adeola F, Olurotimi O. Asymptomatic bacteriuria in antenatal patients in Ilorin, Nigeria. Oman Medical Journal 2012; 27(1).
[6] Gayathree L, Shetty S, Deshpande S R, Venkatesha D T. Screening for asymptomatic bacteriuria. In Pregnancy: An evaluation of various screening tests in Hassan District Hospital, India. Journal of Clinical and Diagnostic Research 2010; 4:2702-06.
[7] Jayalakshmi J. Evaluation of various screening tests to detect asymptomatic bacteriuria in pregnant women. Indian Journal of Pathol – Microbiol 2007; 51(3): 379-81.
[8] Balamurugan S, Chaitannya Shah, Jayapriya S, Priyadarshini S, Jeya M, Ramesh Rao K. Reagent strip testing (RST) for asymptomatic bacteriuria (ASB) in pregnant women: A cost-effective screening tool in under – resourced settings. Journal of Clinical and Diagnostic Research 2012; 6:671-673.
[9] KonemanColor Atlas and Text book of Diagnostic Microbiology. Part-1, pp.20-25.
[10] District laboratory practice in tropical countries. Part-1, Monica Cheesbourg, pp.380-382.
[11] District laboratory practice in tropical countries. Part – 2, Monica Cheesbourg pp.106-115.
[12] Topley and Wilson's microbiology and microbial infections. pp.677-680.
[13] Gradwohl's clinical laboratory methods and diagnosis. Volume 2, Part VII, page 1136-1139.
[14] Colle J.G, Marimon BP, Fraser AG, Simmons A, Mackie and McCartney practical Medical Microbiology, 14th ED, London, Elesvier 1996.
[15] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty third Informational supplements. Clinical and Laboratory Standards Institute Wayne PA 2014:M100-523
[16] Vaishali Jain, Vinita Das, AnjooAgarwal, AmithPandey. Asymptomatic bacteriuria and obstetric outcome following treatment in early versus late pregnancy in North Indian women. Indian Journal of Medical Research 2013; 137:753-758.
[17] CA Turpin, BridgeetMirkah, KA Danso, EH Frimpong. Asymptomatic bacteriuria in pregnant women attending clinic at KomfoAnokyeTeaching Hospital, Kumsai, Ghana. Ghana Medical Journal 2007; 41(1):26-29.
[18] Taneja N, Kavya M, Rangmei M, Deo N, Sharma M. Evaluation of three screening methods for detection of urinary tract infection in antenatal women. J ObstetGynocol India,2004; 54(3):267-270.