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Prevalence of asymptomatic bacteriuria among pregnant women in Nnewi, southeast Nigeria

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ABSTRACT

Asymptomatic bacteriuria (ASB) in pregnancy is associated with obstetric complications including pre-eclampsia, pyelonephritis, preterm labour, low birth weight and prematurity. Determining the prevalence of asymptomatic bacteriuria among pregnant women locally is needed to justify routine screening for ASB in pregnancy. This cross sectional, case controlled study examined 440 women comprising equal numbers of pregnant and non pregnant women attending various clinics at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Nigeria with the aim of determining the prevalence of ASB among them and the antibiotic sensitivity of the isolated organisms. Urine culture using MacConkay and blood agar were used to isolate bacteria organisms. Significant bacteriuria was defined as the finding of single pure isolate of $\geq 10^5$ in two consecutive, freshly voided, midstream urine. The prevalence of asymptomatic bacteriuria among the pregnant women was 19.5% (n=43) and 6.4% (n=14) for the non pregnant women. (x^2=16.9,0R=3.6, P=0.00). There was no significant influence of age (x^2=1.89,P=0.39), parity (x^2=3.92,P=0.14) or social class (x^2=5.7, p=0.22) on the prevalence of asymptomatic bacteriuria in pregnancy. *Escherichia coli (E. coli)* was the commonest isolated organism, 30(52.6%) followed by *Staphylococcus aureus*, 10 (17.5%) and *Klebsiella sp*, 9 (15.8%). The isolated *E. coli* was 100% sensitive to ofloxacin, nitrofurantoin and nalidixic acid and 83.3%, 73.3% and 66.7% sensitive to augmentin, gentamicin and cefuroxime, respectively. Sensitivity to ampicillin and cotrimoxazole were 43.3% and 30.0%, respectively. The prevalence of asymptomatic bacteriuria among pregnant women attending antenatal clinic in NAUTH is high and the isolated organisms were mainly susceptible to ofloxacin, nitrofurantoin and nalidixic acid. Routine screening for ASB in pregnancy in the hospital is recommended for all pregnant women.

Keywords: Prevalence, Asymptomatic bacteriuria, Nnewi, Nigeria.

INTRODUCTION

The changes that occur both in the structure and function of the urinary tract in pregnancy encourage upper urinary tract infections. These changes include dilatation of the renal calyces and the ureters and occur as early as the 14th week of pregnancy, thus implicating hormonal relaxation of the muscular layers of the renal tract (Cunningham et al., 2005). There is also

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further dilatation beginning at mid pregnancy because of the ureter compression by the enlarging gravid uterus. Increased vesicourethral reflux also increases this potential for multiplication of bacteria within the renal tract. As a result, urinary tract infections are the most common infections that complicate pregnancy.

True bacteriuria is defined as the presence of up to 100,000 colony forming units (CFUs) of a single pathogen per ml of two freshly voided mid-stream, clean catch urine specimens or a single catheterization specimen (Cunningham et al., 2005).

Asymptomatic bacteriuria (ASB) therefore, is the presence of true bacteriuria in an individual without symptoms of urinary tract infection. (Samuels and Colombo, 2002).The prevalence of ASB in pregnancy varies across the various regions of the world. In Nigeria the highest incidence of 86.6% was reported in Benin City, Niger Delta area (Akerele et al, 2001). In the southwestern part of the country, it ranges from 12.2% in Ado-Ekiti (Oyagade et al., 2004) to 21% reported in Ibadan (Ifemeje et al., 2006; Akiloye et al., 2006). In Enugu, south-east Nigeria, Ezeome et al. (2006), reported an incidence of 15.1% The significance of ASB in pregnancy is that more than 50% of untreated cases progress to symptomatic urinary tract infections with adverse obstetric outcomes such as pyelonephritis, preterm labour, low birth weight, hypertensive disease, chorioamnio-nitis and prematurity (Ezeome et al., 2006; Ullah et al., 2006).

In view of these identified complica-tions associated with ASB, many authors have proposed routine urine screening for ASB in all pregnant women at their first prenatal visit (Akerele et al., 2001; Oyagade et al., 2004; Ifemeje et al., 2006; Akiloye et al., 2006).

Prominent among these recommendations was the U.S. Preventive Services Task Force reaffirmation statement that strongly recommended routine screening for all pregnant women between 12^{th}-16^{th} of pregnancy (Calonge et al., 2008). This recommendation no doubt, carries with it some financial implications which may limit its applicability in resource constrained regions of the world like the sub-Saharan Africa. Therefore, in other to adjust local management protocols with respect to routine screening for asymptomatic bacteriuria in pregnant women, it is necessary to determine its prevalence within the locality.

This study therefore, aims at determining the prevalence of ASB among pregnant women in Nnewi as well as the influence of sociodemographic characteristics. The findings from this study will form the basis for making recommendations with respect to routine screening for ASB among pregnant women in our locality.

MATERIALS AND METHODS

Study site

The study was carried out in the Antenatal clinic, Family Planning clinic, Gynaecology and General Outpatient clinics and the Microbiology Department of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, which is a tertiary health institution in Nigeria with a catchment area of the five States of the South East geopolitical region. These states include Anambra, Enugu, Abia, Imo and Ebonyi States. It also receives referrals from some parts of Delta State of Nigeria.

Study population

The study population comprised consecutive pregnant women attending the antenatal clinic and an equal number of non pregnant women, who were attending the gynaecology, family planning and the general outpatient clinics of the hospital who met the inclusion criteria.

Inclusion criteria

- All pregnant women at gestational age ≤ 30 weeks;
- All women attending family planning, gynaecology and general outpatient clinics within the age of 15-49 years.

Exclusion criteria

- All patients with history of urinary tract infection symptoms, diabetes mellitus, hypertension, renal diseases or sickle cell disease;
- Women with previously treated urinary tract infections;
- Women who are HIV positive;
- Women who are currently on antibiotics or had taken antibiotics during the course of the index pregnancy;

Sample size

The minimum sampling size was determined using the statistical formula of Fischer (Hassan, 1990) for calculating sample size.

$N = Z^2pq/d^2$ Where; N = Minimum sample size for a statistically significant survey

Z = Normal deviant at the portion of 95% Confidence interval = 1.96

p = prevalence of bacteriuria in pregnancy in Enugu = 15.1% (Ezeome IV, 2006);

q = 1- p; d = Margin of error acceptable or measure o precision = 0.05

N = (1.96 X1.96)X 0.15 X 0.85

0.05 X 0.05; N = 195.92 = 196 pregnant women. We used a sample population of 220 pregnant women and 220 non pregnant women (a total of 440) to improve the power of the study.

Data collection

At the various clinics, women were first educated on the purpose and nature of the study as a group. Those who gave consent for the study were clinically evaluated for symptoms of urinary tract infections and the presence of the other exclusion criteria. The eligible women who met the inclusion criteria were recruited for the study and their sociodemographic data obtained through coded semi structured pre-tested questionnaires that were administered by the researchers.

The information obtained were age, gestational age, parity, level of education, occupation, religion and occupation of the husband. Attached to the questionnaire was a specimen proformal for documentation of the laboratory findings. Patients were assigned to social classes utilizing the method developed by Olusanya et al (1985) which considers the educational level of the woman and the occupation of the husband (Thirty women were not classified because they were single)

Laboratory methods

After completing the questionnaires, the eligible women were taught how to collect clean-catch midstream urine sample by thorough antero-posterior swabbing of the vulva with sterile water before urine collection. Thereafter, they were provided with two sterile universal containers. Two samples of 10–15 ml urine were obtained and immediately sent to the laboratory.

Urine microscopy

At the laboratory, 10 ml of the urine sample was poured into a test tube and centrifuged for 5 minutes. The supernatant was discarded while a drop of the deposit was placed on a grease free slide and covered with a cover slip. It was then examined under the microscope at 40 X magnification. The urine specimen was also examined for pus cells, bacteria, epithelial cells and red blood cells.

Urine culture

The urine samples were also immediately cultured on chocolate and MacConkey agar, and samples not cultured within two hours were stored at 4 °C.

Using a sterile standard wire loop that delivers 0.002 ml of urine, a loopful of urine sample was streaked evenly on dried plates of chocolate and MacConkey agar to obtain discrete colonies. The plates were incubated aerobically at 37 °C for 24 hours and were then counted for pure isolates. Identification of bacteria pathogens were made on the basis of Gram reaction, morphology and biochemical characteristics. Only colony counts yielding bacterial growth of 10^5 cfu/ml or more of pure isolates from the two samples

were deemed significant. Any urine specimen containing high colony counts with more than one species of bacteria was considered as being contaminated and hence, discarded.

Testing for antimicrobial sensitivities of the isolates

Using a sterile wire loop, small portion of the isolate on the plates were collected and streaked evenly onto a nutrient agar. Using a pair of sterile forceps, the sensitivity disc was placed on the surface. The plate was incubated at 37 °C for 24 hours and the plates were read for zones of inhibition.

All the women who were positive for asymptomatic bacteriuria were referred to their attending physicians for treatment in accordance with the antibiotic sensitivity pattern.

Data analysis

Statistical analysis of the data collected was done with Epi-info statistical package, version 3.5.2 (2008). Continuous variables are expressed as means and standard deviations, while statistical relationships between variables were explored using chi square test and a p-value of < 0.05 was considered statistically significant. Student's T-test was used to calculate the difference of means of the studied populations. The result is presented in tables.

Ethical considerations

Ethics approval for the study was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethics Committee. Questionnaires were administered only to those patients who gave their consent, after due group counseling. Confidentiality was maintained at all stages of the research work and all the participating women had the right to withdraw from the study at any time after counseling. The research posed no hazards to the respondents.

RESULTS

The prevalence of asymptomatic bacteriuria among the pregnant women was 19.5% (n=43) and 6.4% (n=14) for the non pregnant women. This difference was statistically significant (x2=16.9, 0R=3.6, P<0.01).

The mean age of the women was 28.67 ± 5.55 years while the mean parity was 2.01± 1.61. Table 1 shows the sociodemographic profile of the respondents. Most of women were married (91.1%, n=401), had achieved at least secondary education (93.4%, n=411) and were traders (38.2%, n=168). The modal social class was class III (40.2%, n=167). There was no significant difference (T-statistic=1.97, p=0.05) between the mean age of the pregnant women (28.2 ±5.3 years) and that of the non-pregnant women (29.2 ±.7 years)

Table 2 shows the prevalence rates of asymptomatic bacteriuria specific to various sociodemographic characteristics of the women. The prevalence was highest among women aged 30 years and above (22.8%, n=23) the grandmultiparae (34.8%, n=8), women with secondary education (22.5%, n=29), the widows (25.0%, n=1) and those who belong to social class IV (28.3%, n=15). However, there was no significant influence of age (x^2=1.89, P=0.39), parity (x^2=3.92, P=0.14) or social class (x^2=5.7, p=0.22).

Escherichia coli was the most common isolated organism, 30 (52.6%) followed by *Staphylococcus aureus*, 10 (17.5%) and *Klebsiella sp*., 9 (15.8%). This is shown in Table 3.

Table 4 shows that the isolated *E. coli* was 100% sensitive to nitrofurantoin and nalidixic acid. It was 83.3%, 73.3% and 66.7% sensitive to amoxicillin-clavulanic acid combination, gentamicin and cefuroxime respectively. Its sensitivity to ampicillin and cotrimoxazole were 43.3% and 30.0% respectively.

Table I: Distribution of Sociodemographic profile of the studied women.

Socio-demographic Characteristics	Pregnant women	Non-pregnant women	Total		T-Statistic	P-value
	No (%)	No (%)	No	%		
Age						
< 20	7(3.2)	5(2.3)	12	2.7		
20 – 24	46(20.9)	47(21.4)	93	21.1		
25-29	66(30.0)	56(25.5)	122	27.7	1.97	0.05
30-34	65(29.5)	68(30.9)	133	30.2		
35-39	35(15.9)	39(17.7)	74	16.9		
≥40	1(0.5)	5(2.3)	6	1.4		
Parity						
0-1	107(48.6)	74(33.6)	181	41.1	2.48	0.01
2-4	90(40.9)	130(59.1)	220	50.0		
≥5	23(10.5)	16(7.3)	39	8.9		
Marital status						
Married	205(93.2)	196(89.1)	401	91.1		
Single	10(4.5)	20(9.0)	30	6.8		
Widowed	4(1.8)	3(1.4)	7	1.6		
Separated/divorced	1(0.5)	1(0.5)	2	0.5		
Educational level						
Tertiary	77(35.0)	73(33.2)	150	34.1		
Secondary	129(58.9)	132(60.0)	261	59.3		
Primary	14(6.4)	14(6.4)	28	6.4		
No formal education	0(0.0)	1(0.5)	1	0.2		
Social class						
I	21	22	43	10.5		

II	63	47	110	26.8
III	77	90	167	40.7
IV	53	27	80	19.5
V	5	5	10	2.5
Occupation				
Trader	67(30.5)	101(45.9)	168	38.2
Public servant	67(30.5)	61(27.7)	128	29.1
Student	35(15.9)	26(11.8)	61	13.9
Housewife	37(16.8)	20(9.1)	57	13.0
Artisan	14(6.4)	12(5.5)	26	5.9
Religion				
Catholic	102(46.4)	99(45.0)	201	45.7
Pentecostal	59(26.8)	66(30.0)	125	28.4
Anglican	57(25.9)	55(25.0)	112	25.5
Jehovah Witness	1(0.4%)	0(0.0)	1	0.2
Sabbath	1(0.4%)	0(0.0)	1	0.2

Table 2: Influence of socio demographic characteristics on the prevalence of ASB among the pregnant women.

Characteristics	**Positive urine culture (%)**	**Negative urine culture (%)**	X^2	**df**	**P-value**
Age					
< 20	2(28.6)	5(71.4)			
20 – 30	18(16.1)	94(83.9)	1.89	2	0.39
≥30	23(22.8)	78(77.2)			
Parity					
0-1	18(16.8)	89(83.2)			
2-4	17(18.9)	73(81.1)	3.92	2	0.14
≥5	8(34.8)	15(65.2)			
Marital status					
Married	41(20.0)	164(80.0)			
Single	1(10.0)	9(90.0)			
Widowed	1(25.0)	3(75.0)	0.93	3	0.82
Separated/divorced	0(0.0)	1(100.0)			
Highest educational level					
Tertiary	11(14.3)	66(85.7)			
Secondary	29(22.5)	100(77.5)			
Primary	3(21.4)	11(78.6)	2.09	3	0.35
Social class					
I	3(14.3)	18(85.7)			
II	7(11.5)	54(88.5)	5.73	4	0.22
III	17(22.1)	60(77.9)			
IV	15(28.3)	38(71.7)			
V	1(20.0)	4(80.0)			

Religion					
Catholic	24(23.5)	78(76.5)	2.65	4	0.61
Pentecostal	11(18.6)	48(81.4)			
Anglican	8(14.0)	49(86.0)			
Jehovah Witness	0(0.0)	1(100.0)			
Sabbath	0(0.0)	1(100.0)			

Table 3: Bacterial isolates among pregnant women with significant bacteriuria.

Bacterial Isolates	**Number of women with isolates**	**Percentage**
Escherichia coli	30	52.6
Staphylococcus aureus	10	17.5
Klebsiella species	9	15.8
Other Coliforms	5	8.8
Other species	3	5.3
Total Isolates	**57**	**100.0**

Table 4: Distribution of the antibiotic sensitivity of the predominant organism (*E.coli*) isolated in women with ASB in pregnancy.

Antibiotics	**Number of sensitive isolates (*E. coli*)**	**Percentage**
Ofloxacin	30	100.0
Nalidixic acid	30	100.0
Nitrofurantoin	30	100.0
Augmentin	25	83.3
Gentamicin	22	73.3
Cefuroxime	20	67.7
Ampicillin	13	43.3
Cotrimoxazole	9	30.0

DISCUSSION

Urinary tract infections, especially upper tract infections in pregnancy are associated with adverse maternal and perinatal effects. Asymptomatic bacteriuria is a significant risk factor for symptomatic urinary tract infections. Hence it has become a standard practice in developed countries to screen for, and treat all cases of ASB in pregnancy.

The prevalence of ASB in pregnancy varies widely among regions and even within the same country. This study found a high prevalence rate of 19.5% among the pregnant women which was comparable to 21.0% reported in Ibadan, Nigeria (Ifemeje et al., 2006; Akinloye et al., 2006). However, it is lower than 86.6% reported in Benin City, Nigeria (Akerele et al., 2001).

In Enugu, southeast Nigeria, Ezeome et al. (2006) reported a prevalence of 15.1% and this is lower than 19.5% found in this study. In Ethiopia and Ghana both in tropical Africa, rates of 9.8% (Tadesse, 2007) and 7.3% (Turpin et al., 2007) had been reported respectively. The high prevalence rate of ASB in pregnancy found in this study indicates the need for routine screening for ASB among the antenatal mothers in our hospital.

As our antenatal women pay for their investigations from their pockets, it is possible therefore, that a policy of routine screening for ASB in pregnancy in our environment may suffer a setback on the basis of financial requirements for urine culture. This can be mitigated however, by exploring the use of simpler screening tests which have shown considerable efficacy in detecting bacteriuria such as the combination of nitrite test and the leucocyte esterase test (Kovavisarach et al., 2009). Although these tests may not be as sensitive and specific as urine culture, they can be used to select those pregnant women who would require further urine culture. In spite of the perceived high cost, it is considered beneficial to screen every pregnant woman at booking considering the high prevalence of ASB found in this study and its attendant obstetric and perinatal complications.

With respect to the influence of maternal age on ASB in pregnancy, this study did not establish any significant relationship of ASB in pregnancy with maternal age, although the rate was highest among those who were 30 years and above. This is similar to the report of Kovavisarach et al. (2009) in Thailand, Hazhir et al. (2007) in Iran and Akinloye (2006) in Nigeria. However, Omole – Ohonsi et al. (2008) in Kano, northern Nigeria and Tugrul et al. (2005) in Turkey reported increasing prevalence rate with increasing maternal age.

As regards the effect of parity, while increased prevalence of ASB in pregnancy was found among the multiparous women in

Ibadan (Akinloye, 2006), no significant association was found in this study between ASB in pregnancy and parity. However, the rate was highest among the grandmultiparous women, indicating a closer surveillance for asymptomatic bacteriuria among this obstetric population.

Though most pregnant women with positive urine culture in this study belong to low socioeconomic status (class III & IV), this was not statistically significant. This corroborates the report of Kovavisarach et al. (2009) in Thailand and Hazhir et al. (2007) in Iran. However, low socioeconomic status had been associated with an increased prevalence of ASB in pregnancy (Fatima and Ishrat, 2006).

It is implied from this study that routine screening for ASB in pregnancy should be recommended for all pregnant mothers irrespective of their parity and socioeconomic status in our environment.

Escherichia coli which is widely documented as the most common isolated organism in cases of ASB in pregnancy (Ullah et al., 2007; Hernández Blas et al., 2007; Abdullah and Al-Moslih, 2005) was similarly found in this study. The organism showed poor sensitivity to ampicillin and cotrimoxazole, the two commonly used antibiotics for treatment of urinary tract infections in pregnancy. This had also been noted in previous studies (Akinloye, 2006; Tadesse et al., 2007; Oyagade et al., 2004; Oyetunji et al., 2004; Aboderin et al., 2004). Therefore, treatment of ASB among the pregnant women should utilize the more effective antibiotics which, in this case include nitrofurantoin, amoxicillin-clavulanic acid combination, cefuroxime and gentamicin. All these antibiotics are safe in pregnancy. However, nitrofurantoin can cause haemolysis in G6PD deficient neonates and hence should be avoided in late trimester.

Conclusion

The prevalence of asymptomatic bacteriuria in pregnancy among women attending antenatal clinic in NAUTH Nnewi is high and significantly higher than that among the non pregnant women. There is no significant association between ASB in pregnancy and age, parity or social class. The predominant organism is *E. coli* with poor sensitivity to ampicillin and cotrimoxazole.

Routine screening for ASB in pregnancy in this hospital is thereby recommended for all pregnant women to prevent its adverse obstetric and perinatal outcome.

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