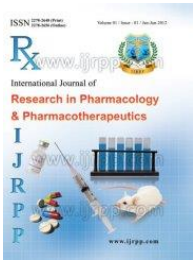
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 ***IJRPP |Vol.8 | Issue 1 | Jan - Mar - 2019***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Research article ***Open Access***

Study on the prevalence of asymptomatic bacterial vaginal infections in pre and post menopausal diabetic women

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ABSTRACT

Vaginal infections including candidal vaginitis and bacterial vaginosis collectively represent perhaps the most common affliction in pre menopausal women. Type 2 Diabetes is another very important cause for increase in the occurrence of vaginal infections in the pre menopausal age group. Objectives of the present study on the prevalence of bacterial vaginal infections in asymptomatic pre and post menopausal diabetic women. The samples from the 50 diabetic and non diabetic pre and post menopausal diabetic women were evaluated for the study from Dhanalakshmi Srinivasan Medical College Hospital, Siruvachur, Perambalur, Tamil Nadu from period of 2015 to 2016. Vaginal samples were taken from the patients during the period of surgical wound dressing before the wound was cleaned with antiseptic solution. The swab was examined by Gram staining, inoculated onto culture plates and incubated aerobically and anaerobically. Antibiotic susceptibility pattern was performed by Kirby-bauer method. The pathogenic bacteria like *E.coli*, *S. aureus* were found to be more in diabetic women than the non diabetic women. *C. albicans* was found to be significantly more in diabetic women. In addition *lactobacilli*, *Peptostreptococcus* and *Bacteroids* were anaerobes commonly reported. Methicillin resistant *S. aureus* strains were found to be sensistive to Vancomycin. Gram negative isolates were highly sensitive to nitrofurantoin, amikacin and gentamicin.

INTRODUCTION

Vaginitis is a very common disease for women of reproductive age all over the world. As vaginal infections and symptoms greatly impact women's quality of life and vaginitis has been associated with serious public health consequences, it is essential to diagnose and treat the conditions correctly. Hence, there is a great need of diagnosing these conditions (Hillier and Lau 1997). There are a number of factors which influence the growth of organisms in the vagina. These include pH, glycogen content, vascularity and hormonal status (Ling et al. 2010)

Urinary tract infections (UTI) and vaginal infections, infections including candidial vaginitis and bacterial vaginosis (BV), collectively represent perhaps the most common affliction in women. Such infections occur with greater frequency after menopause (Vitali et al. 2007). Post menopausal women have decreased estrogen production with thinning and in activity of vaginal epithelium, together with reduction in acidity and rise in pH. As estrogen–deficent vagina can result in abvious problems such as discomfort and dysparenunia and also can lead to an environment that promotes the growth of abnormal flora, which may lead to a variety of infections, including frequent urinary tract infections and potential for renal compromise (Sobel et al. 1998) [12-25].

Type 2 Diabetes is another very important cause for increase in the occurrence of vaginal infections in the pre menopausal age group. Poor glycemic control in diabetics is also thought to result in impaired action of polymorphouclear leucocytes resulting in decreased ability to resist infection from opportunistic organisms. Evaluating the prevalence of asymptomatic vaginal infections may throw light on the nature of the infections, the need for prompt investigations and management of vaginits in diabetic pre menopausal women and possibly any changes in the recommendations for treatment protocol in such patients (Larsen et al. 1982) Thus the objective of the present study was to analyse the prevalence of bacterial vaginal infections in asymptomatic pre and post menopausal type 2 diabetic women and compare the results with that of non diabetic women of similar category [11].

Materials and Methods

This study was conducted at Dhanalakshmi Srinivasan Medical College Hospital, Siruvachur, Perambalur, Tamil Nadu from period of 2015 to 2016. Our study includes randomly selected diabetic and non-diabetic women of age 40-70 yrs attending the Diabetic Out Patient Department and the Gynaecology OPD of Dhanalakshmi Srinivasan Medical College Hospital. After obtaining informed consent from all the subjects, their detailed clinical history was assessed with regard to age, sex distribution, lifestyle pattern and various risk factors. Women were categorized as Pre menopausal (with regular or irregular menustral periods) and post menopausal (absence of menses for atleast 12 months) according to their menustral status. They were free from the vaginal symptoms-vaginal dryness, vaginal itching, any abnormal discharge from vagina and painful sexual intercourse. Diabetic history, medical history, reproductive history and sexual history were assessed using the questionnaire.

Three high vaginal swabs were collected using sterile cotton swabs from lateral or posterior wall of the vagina. The swabs were transported immediately to the microbiology labortatory and processed. First swab was used for preparing direct smear and wet mount. Second swab was inoculated onto blood agar, Mac Conkey agar and Columbia agar and incubated aerobically. Third swab was inoculated in Robertson's cooked meat medium and incubated anaerobically. After incubation Identification of bacteria from positive cultures was done with standard microbiological technique which included Gram staining and biochemical reactions. Antibiotic susceptibility testing of the bacterial isolates were done by disc diffusion technique using Muller Hinton Agar and the susceptibility or resistance of the isolates were noted by Kirby Bauer method (Koneman et al., 2006) [1-10].

Observation and Results

A total of 50 diabetic subjects and 50 non diabetic group were enrolled in the present study. Age distribution of the cases is illustrated in table 1. Distribution of cases based on the menustral status is shown in able 2. Diabetic status of the subjects were shown in table 3 [14].

Table 1: Age Distribution of the cases

Age in yrs	Study group	Control group	Total
40-50	21	27	48
50-60	19	16	35
60-70	10	7	17

Table 2: Distribution of cases based on the menustral status

	Study group	Control group	Total
Pre menopause	19	27	46
Post menopause	31	23	54
Total	50	50	100

Table 3 Distribution of cases according to the duration of diabetes

Duration of Diabetes	Pre menopause	Postmenopause	Total
0-5 yrs	11 (57.9%)	10 (32.3%)	21 (42%)
5-10 yrs	8 (42.1%)	13(41.9%)	21(42%)
>10 yrs	-	8 (25.8%)	8(16%)

The microorganisms isolated were (Table 4) Bacteria, Candida sp. and Trichomonas sp. Majority of the bacteria in the vagina of both diabetic and nondiabetic women were found to be anaerobes. In addition to *Lactobacillus*, *Peptostreptococcus* and *Bacteroides* were the anaerobes reported. The antibiotic susceptibility of test organisms is illustrated in table 5a & b. Three of the S. Aureus isolates were found to be oxacillin resistant (MRSA). MRSA strains were found to be sensisitve to Vancomycin. The Gram negative isolates were found to be highly sensitive to Nitrofurantoin, Amikacin, Gentamycin [15].

Table.4 Microorganisms Isolated from culture

Organism	Diabetic women (n=50) No of organisms			Non diabetic women (n=50)No of organisms			Total (n=100)
	Pre menopause (n=19)	Post menopause (n=19)	Total (n=50)	Pre menopause (n=27)	Post menopause (n=23)	Total (n=50)	
E. coli	4 (21.1%)	5(16.1%)	9(18%)	3(11.1%)	3(13%)	6(12%)	15(15%)
Klebsiella	0	0	0	0	2(8.7%)	2(4%)	2(2%)
S. aureus	2(10.5%)	4(12.9%)	6(12%)	2(7.4%)	1(4.3%)	3(6%)	9(9%)
Coagulase negative *S. aureus*	3(15.8%)	8(25.8%)	11(22%)	7(25.9%)	4(17.4%)	11(22%)	22(22%)
Micrococci	2(10.5%)	1(3.2%)	3(6%)	2(7.4%)	2(8.7%)	4(8%)	7(7%)
Group B *Streptococcus*	1(5.3%)	2(6.5%)	3(6%)	3(11.1%)	3(13%)	6(12%)	9(9%)
Streptococcus viridians	2(10.5%)	0	2(4%)	0	0	0	2(2%)
Enterococci	1(5.3%)	2(6.5%)	3(6%)	2(7.4%)	0	2(4%)	5(5%)
Diphtheroids	1(5.3%)	7(22.6%)	8(16%)	6(22.2%)	5(21.7%)	11(22%)	19(19%)
Lactobacilli	5(26.3%)	8(25.8%)	13(26%)	11(40.7%)	7(30.4%)	18(36%)	31(31%)
Peptostreptococcus	7(36.8%)	9(29%)	16(32%0	7(25.9%)	7(21.7%)	14(28%)	30(30%)
Bacteroides	4(21.1%)	5(16.1%)	9(18%)	5(18.5%)	2(21.7%)	7(14%)	16(16%)
Candida	4(21.1%)	5(16.1%)	9(18%)	5(18.5%)	2(21.7%)	7(14%)	16(16%)
Trichomonas	1(5.3%)	0	1(2%)	1(3.7%)	0	1(2%0	2(%)
No growth	0	1(3.2%)	1(2%)	0	1(4.3%)	1(2%)	2(2%)

Table 5a: Antibiotic Sensitivity for Gram Positive organisms

	Penicillin	Amoxicillin	Amoxyclav	Cotrimoxazole	Cephalexin	Cephazolin	Cefuroxim	Erythromycin	Azithromycin	Ciprofloxacin	Ofloxacin	Tetracyclin	Piperacillin	Oxacilin	Vancomycin
S.aureus	9 (100%)	2 (22.2%)	6 (66.7%)	2 (22.2%)	8 (88.9%)	8 (88.9%)	8 (88.9%	6 (66.7%)	8 (88.9%)	2 (22.2%)	1 (11.1%)	7 (77.8%)	7 (77.8%)	6 (66.7%)	9 (100%)
CoNS (n=220	11 (50%)	15 (68.1%)	20 (90.9%)	19 (86.3%)	19 (86.3%)	19 (86.3%)	20 (90.9%)	22 (100%)	20 (90.9%)	14 (63.6%0	15 (68.1%)	18 (81.8%)	21 (95.4%)	22 (100)	
Micrococci (n=7)	2 (28%)	2 (28.5%)	4 (57.1%)	5 (71.4%)	3 (42.8%)	3 (42.8%)	4 (57.1%)	5 (71.4%)	5 (71.4%)	3 (42.8%)	2 (28.5%)	6 (85.7%)	7 (100)	7 (100)	
Group B Streptococcus (n=9)	5 (55%)	6 (66%)	8 (88%)	6 (66%)	4 (44%)	3 (33%)	2 (22%)	3 (33%)	3 (33%)	5 (55%)	6 (66%)	4 (44%)	6 (66%)	6 (66%)	
Streptococcus viridians (n=2)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)	1 (50%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)	
Enterococci (n=5)	3 (60%0	3 (60%)	4 (80%0	4 (80%)	4 (80%)	4 (80%)	4 (80%)	5 (100%)	5 (100%)	4 (80%)	4 (80%)	4 (80%)	5 (100%)	3 (60%)	
Diptheroids (n=19)	15 (78.9%)	16 (84.2%)	18 (94.7%)	17 (89.4%)	17 (89.4%0	18 (94.7%)	18 (94.7%)	19 (100)	18 (94.7%)	15 (78.9%)	16 (84.2%)	18 (94.7%)	19 (100)	16 (84.2%)	

Table 5b: Antibiotic Sensitivity for Gram Positive organisms

	Norfloxacin	Nalidixic acid	Nitrofurantoin	Gentamycin	Amikacin	Aztreonam	Cefotaxim	Ceftriaxone	Cefixme	Cefdimir	Ciprofloxacin	Ofloxacin	Cefuroxime
E.coli (n=5)	0	0	15 (100%)	10 (66.7%)	15 (100%)	10 (66.7%)	8 (53.3%)	8 (53.3%)	6 (40%)	4 (26.7%)	2 (13.3%)	2 (13.3%)	4 (26.7%)
CoNS (n=220	2 (100%)	0	2 (100%)	2 (100%)	2 (100%)	0	2 (100%)	2 (100%)	1 (50%)	0	2 (100%)	2 (100%)	0

DISCUSSION

The vaginal ecology plays a vital role in the pathogenesis and prevention of any vaginal infection in women, especially with diabetes. For this reason, the vaginal microbial flora has been extensively studied in younger women. However a little is known about the vaginal flora of community dwelling pre and post menopausal diabetic women.

Our study demonstrates the prevalence of vaginal commensals as well as the potential pathogens, in an essentially healthy, asymptomatic sample of pre and post menopausal women and compares it with diabetic women of the same category. The present study confirms other reports that the vaginal flora of postmenopausal women is often abnormal in terms of being colonized by potentially pathogenic organisms (Boskey et al. 1999; Bruce and Reid 1993).

The microorganisms isolated in this present study were predominantly bacteria with candida and trichomonas contributing to the remaining. The bacterial isolates included *E.coli, Klebsiella, S. aureus, Micrococci, Group B Streptococci, Streptococcus viridians, Enterococcus, Dipheroids, lactobacilli, Peptostreptococcus, bacteroids.* Hiller et al (1993) reported similar spectrum of vaginal microflora in pregnanat women. Our study showed diabetic women are significantly more prone to develop vaginitis (both bacterial and fungal) than non diabetic women in correlation with the study by Rahman et al (1991)

Like the earlier study Rahman et al (1991), we also found E. coli to be the most pathogenic bacteria (15%) isolated from culture. However the prevalence of *E.coli* among the pre and post menopausal women was found to be similar. Our study correlates well with the study of Burton and Reid (2002) which reported E.coli in 21% of post menopausal women. The rate of colonization of *E.coli* was inversely associated with the presence of Lactobacillus as noted in the present study in agreement with the existing literatures (Gupta et al.1998; Wendy et al.2003). This suggest that the presence of lactobacilli plays an important role in the defence mechanism in prevention of uropathogens invasion and subsequent UTI.

Diabetic women have been found to have higher prevalence of *E. coli* than non diabetic women in accordance with the study by Wendy et al (2003). Similar to the studies of Stamey and Sexton (1975); Hooton and Stamm (1996), we also found that diabetic women with recent history of UTI were at high risk of *E. coli* vaginal colonization. This may be because of the fact that type 1 fimbriated *E. coli* adhere in significantly high numbers to the uroepithelial cell of diabetic women than the non diabetic women as demonstrated by Geerlings et al (2002). *Klebsiella* were seen in only in only two of the non diabetic post menopausal women. *Klebsiella* was surprisingly absent in the diabetic women.

The present study reported *S. aureus* in 9.3% of post menopausal women. Burton and Reid (2002) also reported *S. aureus* in7.5% of asymptomataic women. The infection was also found to be more prevalent in diabetic women (12%) when compared to non diabetic (6%0 women and also more frequent in women with uncontrolled diabetes (21.1%) than the women whose diabetes was under control (6.5%). Nine percentage of the women carried Group B Streptococcus (GBS). A range of 5 to 40% of a vaginal carriers had been found in various studies due to difference in the sample sites and cultural methods employed (Zhu et al . 1996). GBS was isolated more frequently from diabetic women who are under control. This doesnot agree with the study by Williams et al (1975) where GBS was found to be more prevalent in pooly controlled diabetics.

The coagulase negative Staphylococcus aureus which is considered as the skin commensal were found in 22%. Its prevalence is similar in both the diabetic and nondiabetic women. Streptococcal viridians is found in 2% of our study. Itis found in the 4% diabetic women. Anaerobes isolated in the present study were *lactobacillus, Peptrostreptococcus, Bacteroides,* which is similar to the study by Aggarwal et al (2003). Our data demonstrate that the diabetic women have much lower prevalence of vaginal lactobacilli than healthy non diabetic women. Eschenbach et al (1989) demonstrated 96% of healthy premenopausal women had vaginal lactobacilli. Only 34.8% of women in our study had vaginal lactobacilli and fewer than half had heavy growth of lactobacilli. The prevalence of lactobacillus much lower in postmenopausal women than the premenopausal women. This correlates well with a study by Wendy et al (2003).

The *peptostreptococci* and *Bacteroides* were the most frequent anaerobes microorganisms recovered from the pre and post menopausal women next to lactobacilli. Similar anaerobes were recovered from

post menopausal women in a study by Hiller et al (1997). In a study by Larson et al (1982), *peptostreptococcus* and *Bacteroides* were reported at a higher frequency in women who did not receive estrogen replacement therapy.

Candida was isolated in 16% of the women in our study. Similar to the study of Grigoriu et al (2006). Peer et al (1993) isolated candida sp in 25% of asymptomatic diabetic women. Candida was found to be significantly more in diabetic women than non diabetic control group in agreement with study of Goswami et al (2000). In the present study candida has been reported more in premenopausal than postmenopausal women. Candida has been isolated in 13% of post menopausal women which correlates well with the studies of Spinillo et al (1997). But is higher than that reported in other studies. *Trichomnas vaginalis* was found about 2% in premenopausal women in coherence with the previous studies.

CONCLUSION

Bacterial vaginal infections are one of the least understood infections in pre and post menopausal age group. In the present study prevalence of asymptomatic bacterial vaginal infections in pre and postmenopausal diabetic and non diabetic women. The pathogenic bacteria like E.coli, S.aureus were found to be more in diabetic women than the non diabetic women. C. albians was found to be significantly more in diabetic women. In addition lactobacilli, peptostreptococcus and Bacteroids were isolated. Methicillin resistant *S.aureus* strains were found to be sensistive to Vancomycin. Gram negative isolates were highly sensitive to nitrofurantoin, amikacin and gentamicin.

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