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Comparitive Study of Bactrial Flora among Diabetic and Nondiabetic Perimenopausal and Postmenopausal Women

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Bacterial vaginal infections are one of the least understood infections in perimenopausal and postmenopausal age group. This is the cross sectional study undertaken done to determine the prevalence of bacterial flora perimenopuasal and postmenopausal diabetic and non diabetic women attending Sree Balaji medical college and Hospital. *Escherichia coli* and *Staphylococcus aureus* were significantly more in poorly controlled diabetics than those who were under control. Our present study provided important information regarding the vaginal ecology of perimenopausal and postmenopausal women with and without diabetes. The relative depletion of lactobacilli among the women with increased prevalence of pathogens like E.coli, staph aureus supports the importance of lactobacilli as a potential barrier against pathogens.

Keywords: Diabetes type-2; postmenopausal women; recurrent urinary tract infection; estradiol hormone; risk factors for UTI.

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1. INTRODUCTION

The vaginal mucous membrane has normal physiological mechanism to prevent invasion by pathogenic microbes. Lactobacilli, a normal flora of vagina, protect the vagina from the invasion of various pathogens. Despite the defence mechanism, vaginal microflora is often disturbed due to various reasons. The vaginal microbiota also changes during the lifecycle of the female. Finer details of the composition and role of vaginal flora are still a matter of debate. Any inflammation or infection of the vagina is called vaginitis [1] Vaginitis is very common disease for women of reproductive agall over the world but children and postmenopausal women could also be affected. As vaginal infections and symptoms greatly impact women's quality of life and vaginitis have been associated with serious public health sequences, it is essential to diagnose and treat the ondition correctly [2]. Hence, there is a great need of diagnosing these conditions. There are number of factors which influence the growth of organisms in the vagina. These include pH, glycogen content, vascularity andhormonal status.

Urinary tract infections (UTis) and vaginal infections, including candidal vaginitis and bacterial vaginitis (BV), collectively represent perhaps the most common affliction in women such infections occur with greater frequency after menopause. Although there have been some bacteriological studies of vaginal flora [1] these studies have not led to new therapeutic options. Postmenopausal women have decreased estrogen production with thinning and inactivity of vaginal epithelium, together with reduction in acidity and rise of pH [3]. An estrogen deficient vagma can result in obvious problem, such as discomfort and dyspareunia, and also can lead to an environment that promotes the growth of abnormal flora, which may lead to variety of infections, including frequent urinary tract infections and potential for renal compromise. Type 2 diabetes is another very important cause for increase in the occurrence of vaginal infections in the perimenopausal age group. Poor glycemic control in diabetics is also thought to result in impaired action of polymorphonuclear leucocytes resulting in decreased ability to resist infection from opportunistic organisms [4].

The two most common cause of vaginitis are bacterial vaginosis and candida vaginitis. Bacterial vaginosis is caused by decrease of lactobacilli concurrent with overgrowth of several fastidious bacterial species which normally could be present in low concentration in the vagina [5] Candida vaginitis is a vaginal yeast infection where candida albicans is commonly the cause for the disorder [4] In diabetic women, vulvovaginitis is more common and it is often treated with antifungal agents on the assumption that the causative organism is only candida albicans. But it is not only candida, some bacteria and other organisms may cause infection. No prospective data on risk of microbiologically confirmed bacterial vaginal infection in relation to diabetes and its characteristics exist.

Recent advances molecular bacterial diagnostics have demonstrated many novel bacterial species that interact with other organisms and the menopausal vagina. A study [6] used vaginal cytology to document menopausal status and compared vaginal bacterial isolates obtained from postmenopausal women receiving Estrogen replacement therapy (ERT) with the isolates obtained from a separate postmenopausal population not receiving exogenous estrogen. Very few studies have evaluated the prevalence of bacterial vaginosis and vaginal microbial diversity in postmenopausal women and the prevalence of bacterial vaginal infections in perimenopausal women has not been studied. To our knowledge, there has been no study which demonstrates an association between diabetes and the risk of bacterial vaginal infections in peri and post menopausal women. Therefore it was determined to analyse the prevalence of bacterial vaginal infections in peri and post menopausal type 2 diabetic women and compare the results with that of non diabetic women of similar category [7]

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Menopause is defined as permanent cessation of menstruation at the end of reproductive life due to loss of ovarian follicular activity. It is the point of time when last and final menstruation occurs. Postmenopause is the phase of life that comes after the menopause [8]. Perimenopause is the part of the climacteric when the menstrual cycle is likely to be irregular. Climacteric is the period of time during which a woman passes from the reproductive to the non-reproductive state. This phase covers 5-10 years on either side of menopause [9-10]. Evaluating the prevalence of vaginal infection may throw light on the nature of infections; need for prompt investigations and management of vaginitis m

diabetic perimenopausal women and possibly any changes in the recommendations for treatment protocol in such patients would be very useful in determining the microbial sensitivity of such patients.

2. MATERIALS AND METHODS

Our Study was Single Centre, Cross sectional and analytical study included randomly selected Diabetic and Non- Diabetic women of age 45-60 years attending the Gynecology out patients department and Diabetology OPD of Sree Balaji Medical College Hospital, Chennai. The exclusion criteria were Age less than 45 years & more than 60 years, Pregnancy, Recent Hospitalization or Surgery (4 months), Use of Antimicrobials & Antifungals in the last 14 days and having any Malignancies. High vaginal swab samples from lateral or posterior wall of the vagina using sterile cotton swabs. Women with type 2 diabetes who were 45-60 years of age attending the outpatient department of Gynaecology and Diabetology of Sree Balaji medical college and Hospital were taken for this study. They were treated with oral hypoglycaemic agents or Insulin or Both. All were reported with symptoms like abnormal vaginal discharge, vaginal itching, vaginal dryness and painful sexual intercourse (Dyspareunia). The following laboratory data were analysed at the time of study Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS). Women without Diabetes were selected randomly from the patients who attend the Gynaecology OPD for various reasons. Their non diabetic status was confirmed by laboratory tests such as Fasting Blood Sugar and Postprandial Blood Sugar values. First swab was used for preparing direct smear and wet mount. Second swab was inoculated into culture media. Nutrient Agar, Blood Agar, Mac conkey Agar and Columbia Agar plates were incubated aerobically. Third swab was inoculated in Robertson's cooked meat medium and incubated anerobically. The first swab was used for direct microscopic examination by Gram stain. Gram stained smear was examined under oil immersion to look for the presence of gram positive cocci, clue cells of Bacterial vaginosis, candidal hyphae, spores, epithelial cells and Leucocytes.

3. RESULTS

This cross sectional study was carried out in Sree Balaji Medical College and Hospital, Chennai. 100 participants in the study group (Diabetic) and 100 participants in the control group (Non Diabetic) were enrolled in the study. In this study 94 women were Perimenopausal and 106 Postmenopausal (categorized according to the menstrual status). Vaginal discharge was collected by using sterile cottons high vaginal swabs and sent for culture and sensitivity. The discharge was cultured by using different culture media. The women who are all diabetic (study group)were in the mean age group of 51.0 with standard deviation 12.26.In control group, the mean age group is 51.2 with standard deviation with11.27 Table. 1 and 1a, Fig. 1. In the study group 45 women were in perimenopausal age group and 55 in the postmenopausal age group. In the control group 55 women were in perimenopausal age group and 51 in the post menopausal age group Table. 2 and Fig. 2).

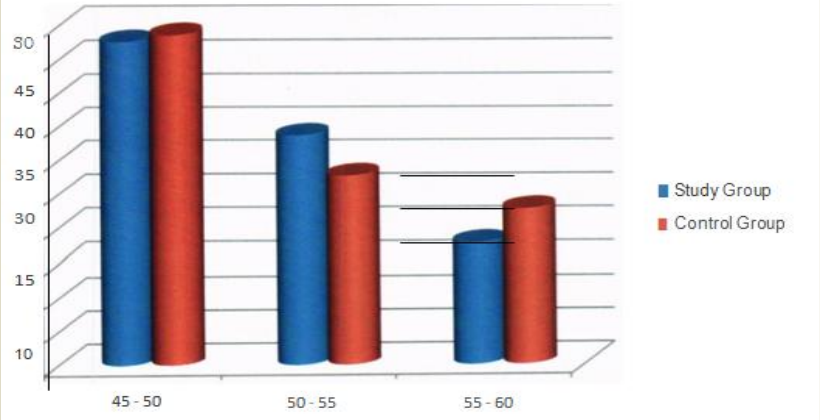

Fig. 1. Age wise Distribution of the studied patients

In the study group 45 women were in perimenopausal age group and 55 in the postmenopausal age group. In the control group 55 women were in perimenopausal age group and 51 in the post menopausal age group Table. 2 and Fig. 2.

This table shows distribution of menstrual status of diabetic women according to duration of diabetes Table. 3 and Fig. 3. Perimenopausal women, 0 to 5 years-28 women, 5 to 10 years-16 women, >10 years- I woman, Postmenopausal women, 0 to 5 years-23 women, 5 to 10 years-23 women, >10 years-9 women.

In study group *Staphylococcus aureus, E.coli, Diptheroids* and Beta haemolytic *Streptococci* were found to be more prevalent and no growth was observed in 27 subjects. In control group, micrococci, Enterococci, klebsiella, pseudomonas were found to bemore prevalent and no growth was observed in 41 subjects.

Table 1. Agewise distribution of the studied patients

Age (years)	Study Group (Diabetic)	Control Group (Non Diabetic)
45-50	48	49
50-55	34	28
55-60	18	23
Total	100	100

Table 1A. Mean age distribution of the studied patients

Group	Mean (years)	S.D.	p value
Study Group	51.0	12.26	0.08
Control Group	51.2	11.27	

Table 2. Distribution of cases based on menstrual Status

Menstrual status	Study Group (Diabetic)	Control Group(Non Diabetic)
Peri Menopause	45	49
Post Menopause	55	51
Total	100	100

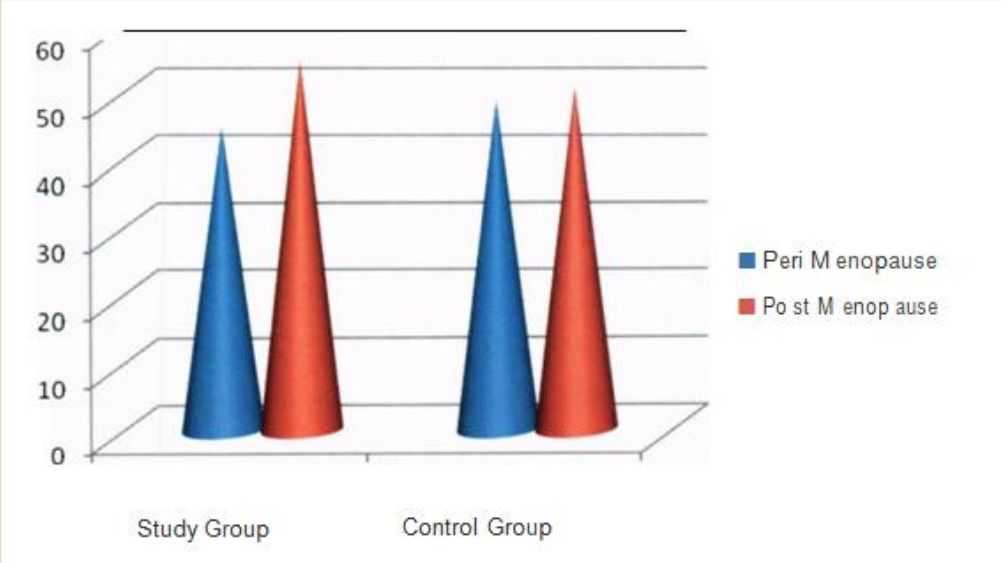

Fig. 2. Distribution of cases based on menstrual status

Table 3. Distribution of menstrual status of diabetic women according to theduration of diabetes

Duration of Diabetes	Peri menopause	Post menopause	Total	p value
0-5years	28	23	51	
5-10 years	16	23	39	
> 10 years	1	9	10	0.0716
Total	45	55	100	

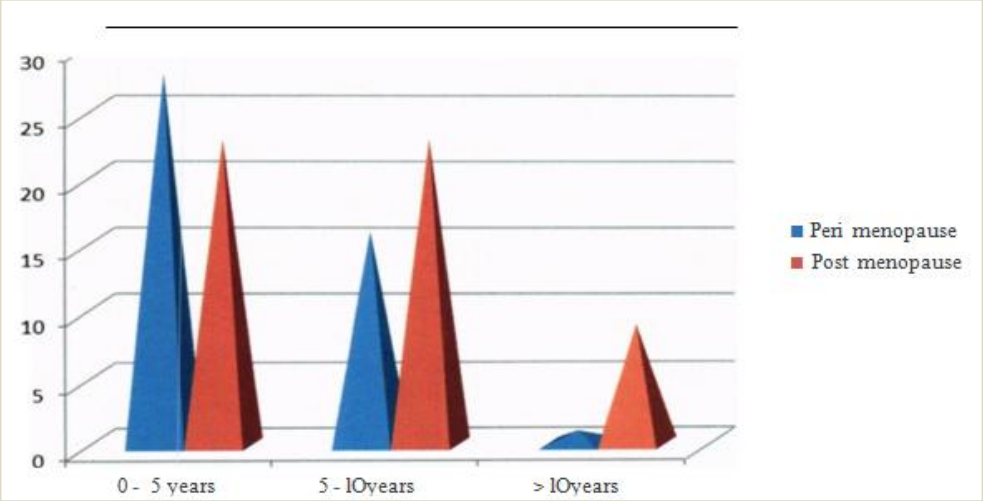

Fig. 3. Distribution of menstrual status of diabetic women

The prevalance of *Staphylococcus aureus* and *E. coli* was found to be similar in both perimenopausal and postmenopausal women and mixed growth was found only in postmenopausal women Table. 5 -6 a, Figs. 5-7.

Staphylococcus aureus, *Pseudomonas*, *Diptherods* were found to be more prevalent if diabetes is under control. The prevalence of mixed growth like *Diptheroids* and *candida albicans*, *E.coli* and *Candida albicans* were found to be same both in women whose diabetes is under control or not under control.

4. 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 studied in younger women. However a little is known about the vaginal flora of community dwelling perimenopausal and postmenopausal diabetic women. Our study demonstates the prevalence of vaginal commensals as well as the potential pathogens in the perimenopausal and postmenopausal women and compares it with diabetic women of same ategory.

The micro orgamsms isolated in the present study were predominantly bacteria with candida and trichomonas contributing to the rummage. The bacterial isolates included *E.coli, Klebsiella, Staphylococcus aureus, Microco ccus Enterococcus, streptococcus viridians, Diptheroids*, Beta hemolytic streptococci. Ross [11] reported similar spectrum of vaginal microflora in pregnant women. The common pathogenic bacteria isolated were *Staphylococcus aureus, E.coli and Diptheroids.* Our study showed diabetic women are significantly more prone to develop vaginitis than the non diabetic women in correlation with the study by Rahman et al. [12].

Like the earlier study 9 we also found Eschericia coli to be the most pathogenic bacteria isolated from the culture. However the prevalence of E.coli among the perimenopausal and postmenopausal women was found to be similar. Our study correlates well with the study of Jeremy P Barton and Gregor Reid [13] which reported E.coli in 21% of postmenopausal

women. Diabetic Women have higher prevalence of E.coli than the non diabetic in non diabetic in accordance with the study by wendy similar to the previous studies [14]. We also found that the diabetic women with the recent history of UTI were at the high risk of vaginal colonization. This may be because of the fact that the typel fimbiated E.coli adhere in significantly higher numbers to the uroepithelial cell of the diabetic women than the non diabetic women, as demonstrated by several studies [15- 19].

Table 4. Comparison of the prevalence of micro organisms among thediabetic and the non-diabetic women

Micro Organisms	Study Group	Control Group	p value
Staphylococcus aureus	20%	8%	
Pseudomonas sp.	8%	7%	
E.coli	12%	5%	
Streptococcus viridans	3%	10%	
Diptheroids	11%	1%	
Beta Haemolytic Streptococci	8%	9%	
No growth	27%	2%	
Micrococci	4%	41%	0.07
Enterococci	2%	6%	
Diptheroids and candida albicans	2%	1%	
Staphylococcus aureus & *Enterococci*	1%	1%	
E coli & *Candida albicans*	2%	1%	
E coli & Beta Haemolytic *Streptococci*	0	3%	
Klebsiella sp.	0	5%	
Total	**100**	**100**	

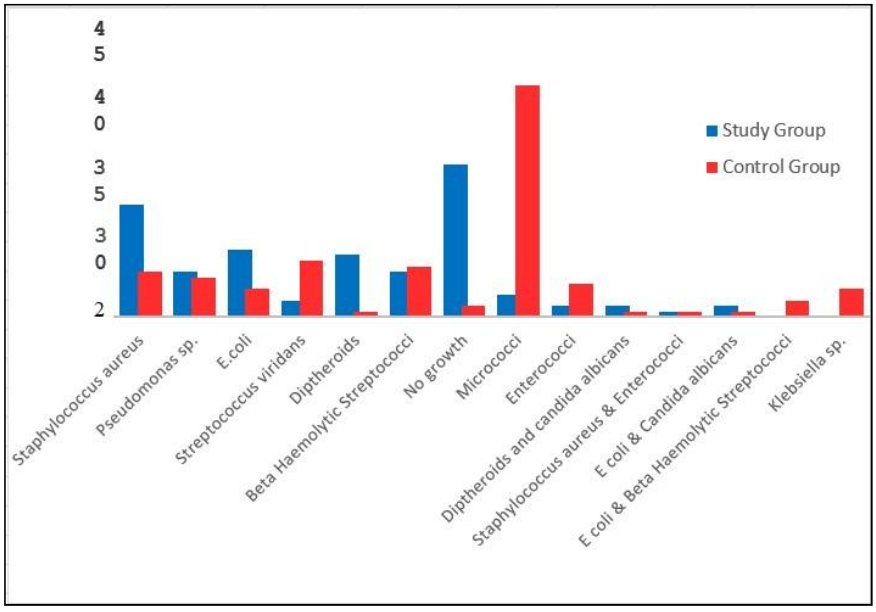

Fig. 4. Comparison of the prevalence of micro organisms

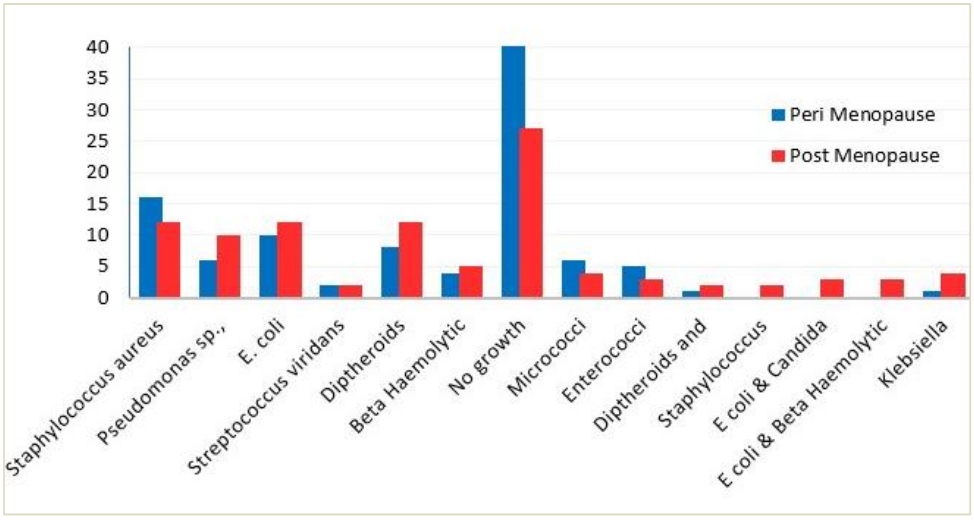

Fig. 5. Variation of micro organisms with respect to menopausal status

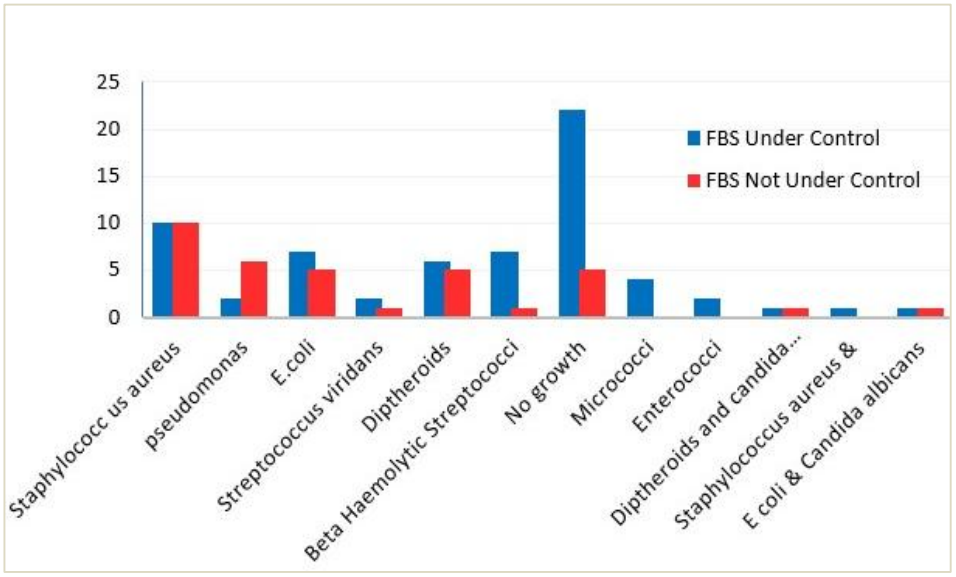

Fig. 6. Comparison of Prevalence of microorganism

Staphylococcus aureus was found to be more prevalent in diabetic women (20%) compared to the non diabetic women (8%) and also more frequent in women with uncontrolled diabetes (29%) than the women whose diabetes was not under control (15%). *Klebsiella* was seen only in 5 of the non diabetic women. klebsiella was surprisingly absent in the diabetic women. 10% of the women carried Beta haemolytic streptococci. A range of 5 to 40% of the vaginal carriers had been found in various studies due to difference in in the sample sites and cultural methods employed [19] It is the organism of most concern during pregnancy and in neonatal infection. It pathogenicity in menopause is not clearly known, which require further extensive studies in future. Although not statistically significant, Beta haemolytic S*treptococci* was isolated more frequently from diabetic women who are under control. This doesnot agree with the study by Williams DN et al. [9] where Beta haemolytic streptococci was found to be more prevalent in poorly controlled diabetes. The prevalence was found to be same in both perimenopausal and postmenopausal women.

Table 5. Variation of micro organisms with respect to menopausal status

Micro Organisms	Peri Menopause	Post Menopause	p value
Staphylococcus aureus	16%	12%	
Pseudomonas sp.,	6%	10%	
E. coli	10%	12%	
Streptococcus viridans	2%	2%	
Diptheroids	8%	12%	
Beta Haemolytic Streptococci	4%	5%	
No growth	41%	27%	
Micrococci	6%	4%	
Enterococci	5%	3%	
Diptheroids and candida albicans	1%	2%	
Staphylococcus aureus & Enterococci	0%	2%	0.064
E coli & Candida albicans	0%	3%	
E coli & BetaHaemolytic Streptococci	0%	3%	
Klebsiella	1%	4%	
Total	**100**	**100**	

Table 6. Comparison of Prevalence of micro organism based on FastingBlood Glucose control

Micro Organisms	FBS Under Control	FBS NotUnder Control	Total	p value
Staphylococc us aureus	10	10	20	
pseudomonas	2	6	8	
E.coli	7	5	12	
Streptococcus viridans	2	1	3	
Diptheroids	6	5	11	
Beta Haemolytic Streptococci	7	1	8	
No growth	22	5	27	
Micrococci	4	0	4	
Enterococci	2	0	2	
Diptheroids and candida albicans	1	1	2	0.03
Staphylococcus aureus & Enterococci	1	0	1	
E coli & Candida albicans	1	1	2	
Total	**65**	**35**	**100**	

Candida, an opportunistic pathogen, was isolated along with bacterial flora. The fungus was found more in diabetic women (4%). Candida was found to be significantly more in diabetic women (4%) than the non diabetic women (2%) in agreement. In the present study, candida has been reported more in the postmenopausal women than the perimenopausal women. Candida has been isolated in 5% of postmenopausal women which correlates with the other studies 6%were found by C. ROSS, and 3% by Fischer et al. [20] Cauci et al. [21] have recently suggested that the Nugent scoring system may not be adequate for evaluating normal vaginal flora and immediate grade colonization in women more than 40 years old, because in many cases no lactobacilli or BV associated micro organisms are detected. The Nugent scoring system is based on presence or absence of lactobacilli. However, the presence of lactobacilli m postmenopausal women traditionally has been thought to be reduced or absent. In these cases, the Nugent scores would indicate that BV is present, and

Table 6A. Comparison of Prevalence of micro organism based on PostPrandial Blood Glucose control

Micro Organisms	PPBS Under Control	PPBS Not Under Control	Total	p value
Staphylococcus aureus	6	14	20	
Pseudomonas	2	6	8	
E.coli	6	6	12	
Streptococcus viridans	1	2	3	
Diptheroids	5	6	11	
Beta Haemolytic *Streptococci*	6	2	8	
No growth	18	9	27	
Micrococci	1	3	4	0.03
Enterococci	2	0	2	
Diptheroids and *candida albicans*	1	1	2	
Staphylococcus *aureus* &*Enterococci*	0	1	1	
E coli & *Candida albicans*	1	1	2	
Total	**65**	**35**	**100**	

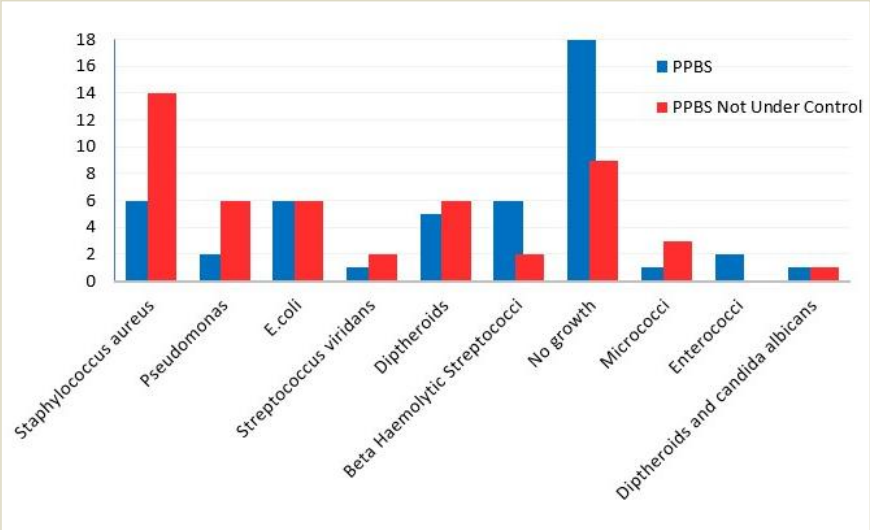

Fig. 7. Comparison of Prevalence of microorganism based on PostPrandial Blood Glucose control

thus, the correlation between Nugent score and the presence of "abnormal" vaginal microflora is more difficult to substantiate. Hence the usual scoring methods of bacterial vaginosis are not followed in our study.

5. CONCLUSION

In conclusion, pathogenic bacteria are also found as frequently as the candida in diabetic women. So, in diabetic women with genital symptoms, an attempt at diagnosis should be made prior to the commencement of therapy. The practice of initiating antifungal treatment for any vaginal infection in diabetic women without taking high vaginal swabs should be reviewed. However, in busy clinics, and where investigations cannot be performed, the use of empirical antifungal therapy alone may not be appropriate for recurrent infections and consideration should be given for the use of an antibiotics along with the antifungal drugs.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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