

COMPARATIVE STUDY IN THE MANAGEMENT OF BACTERIAL VAGINOSIS METRONIDAZOLE VS. LACTOBACILLUS

Narmadha Selvaraj¹, Kalaivani Subramaniam²

¹Senior Assistant Professor, Department of Dermatology Venereology and Leprosy, KAPV Medical College, Trichy.

²Director and Professor, Institute of Venereology, Madras Medical College, Chennai.

ABSTRACT

BACKGROUND

The incidence and morbidity of bacterial vaginosis is high. But most of the patients are asymptomatic and recurrences are common even after effective treatment. Studies comparing the therapeutic efficacy of the drugs indicated for Bacterial vaginosis are also very minimal.

Aim of this study was to analyse the epidemiological pattern of bacterial vaginosis and to compare the efficacy of Metronidazole and Lactobacillus in the management of bacterial vaginosis.

MATERIALS AND METHODS

100 newly diagnosed Bacterial vaginosis patients were enrolled and randomly allotted into two study groups of Metronidazole for one week and Lactobacillus for two months respectively. The patients are followed up based on Amsel's criteria and Nugent's scoring system at the time of initiation of treatment, one week, one month, two months and six months after treatment. Ultra-sonogram was done at the end of two months. Statistical Analysis- chi square test with yate's correction and Fisher's exact test (2 tailed) to calculate the P value.

Settings and Design- Prospective, open labeled, comparative clinical trial.

RESULTS

Lactobacillus group was better than Metronidazole based on Amsel's criteria at six months follow up period and based on Nugent's scoring system at two months and six months follow up period.

CONCLUSION

Epidemiological interpretation in our study shows Bacterial vaginosis is common in the 24-40 years of age; marital status, earlier age at marriage, number of deliveries is directly proportionate to bacterial vaginosis incidence. Irregular menstrual cycle, number of sexual partners, and mode of delivery has no association with the incidence of Bacterial vaginosis. Moderate to profuse genital discharge is the commonest symptom observed. Whiff test (80%), Clue cells (90%) and alkaline vaginal pH > 5 has high degree of sensitivity.

KEYWORDS

Bacterial Vaginosis – Therapeutic Trial- Metronidazole Vs Lactobacillus.

HOW TO CITE THIS ARTICLE: Selvaraj N, Subramaniam K. Comparative study in the management of bacterial vaginosis metronidazole vs. lactobacillus. J. Evid. Based Med. Healthc. 2017; 4(9), 456-463. DOI: 10.18410/jebmh/2017/87

BACKGROUND

Bacterial vaginosis is due to the imbalance in the normal bacterial flora of vagina. Bacterial vaginosis is a polymicrobial syndrome involving the replacement of the normal vaginal lactobacillus by variety of anaerobic bacteria like Gardnerella vaginalis, Mycoplasma hominis, Prevotella biviadisens, Mobilincus and anaerobic gram negative rods. Bacterial vaginosis is the commonest cause of vaginal discharge (48%) in women with significant morbidity. If untreated patients are more prone for ascending

endometrial infections, preterm delivery, and increased risk of acquisition of Herpes genitalis, Gonorrhoea and HIV infections. But most of the cases are asymptomatic and recurrences are common even in treated cases. In this background we planned to conduct this study to compare the efficacy of Metronidazole, the commonly used drug and Lactobacillus, a probiotic alternative drug.

AIMS AND OBJECTIVES

1. To analyse the epidemiological pattern of the disease among the study population.
2. To compare the efficacy of Metronidazole and Lactobacillus in the management of Bacterial vaginosis.
3. To evaluate safety, adverse effects, compliance of Metronidazole and Lactobacillus.
4. To monitor the recurrence of Bacterial vaginosis following each therapeutic modality.

Financial or Other, Competing Interest: None.

Submission 29-12-2016, Peer Review 04-01-2017,

Acceptance 12-01-2017, Published 27-01-2017.

Corresponding Author:

*Dr. Narmadha Selvaraj,
#D49, 10th Cross, West,*

Thillainagar, Trichy-620018, Tamil Nadu.

E-mail: narmadha18@yahoo.com

DOI: 10.18410/jebmh/2017/87



MATERIALS AND METHODS

Trial Design- Prospective, open labelled, comparative clinical trial with the approval of ethical committee.

Inclusion Criteria

1. Patients aged above 18 years
2. Those who fulfilled Amsel's criteria and Nugent's criteria for Bacterial vaginosis.
3. Those who were willing to come for the follow up.

Exclusion Criteria

1. Patients aged less than 18 years
2. Pregnant individuals
3. Lactating mothers
4. Treatment with any antibiotics 6 weeks prior to study period
5. Patients with other causes of vaginal discharge
6. Hypersensitivity to any drugs
7. Cardiac valve replacement surgery patients
8. Underlying neurological disorder
9. Alcoholic
10. Immunosuppressed individuals.

100 patients with bacterial vaginosis those who attend the Venereology outpatient clinic for two years were included in this study. They were randomly allotted into two groups of 50 each to receive either metronidazole 400 mg bd for seven days or capsule lactobacillus 1 bd for first one month followed by 1 OD for the next month orally. All patients signed a written informed consent prior to the initiation of treatment.

A thorough history was taken. General examination, systemic examination, Genital examination under Cusco's bivalve self-retaining vaginal speculum was done. For those who denied sexual contact among unmarried category speculum examination was not done. Genital discharge smears were taken from the posterior fourchette for the unmarried patients and from the posterior blade of the vaginal speculum in married patients. Vaginal discharge was subjected for sniff test, whiff test, vaginal pH examination using litmus papers. Vaginal discharge was investigated under saline mount, KOH examination, Gram staining of vaginal smear. Cervical discharge taken from the cervical os of married patients was investigated under Gram staining. Endocervical swab was sent for gonococci culture in Amie's transporting media. Apart from routine urine examination VDRL, TPHA and HIV testing by ICTC was done to all patients.

The patients were followed up at one week, one month, two months and six months after starting treatment. At each visit vaginal pH, sniff test, Whiff test, saline mount and gram staining of vaginal secretion were repeated. USG was done at the end of two months of initiation of treatment.

OBSERVATION

In this trial the mean age of patients was 32.4±8.4 years. The highest age was 51 years and the lowest age was 20 years. The average duration of symptoms was 2 months.

Menstrual period was regular in 86 individuals, irregular in 10 patients and four attained menopause.

Out of 100 individuals 92 were married and the remaining 8 were unmarried. Among the married individuals 57 got married before the age of 20 years, 26 got married

in between 21-25 years of age and 9 got married above the age of 25 years.

Among the 92 married individuals 58 patients delivered normally, LSCS was done in 13 patients, abortion history present in 3 patients, Normal delivery and spontaneous abortion history among 11 patients and infertility noticed in 7 patients.

The number of sexual partners were calculated and analysed. 77 patients had mono partners which included two unmarried, multiple sex partners in 17 patients and 6 unmarried patients denied having sexual contacts.

According to symptoms 67 patients came with genital discharge, 10 with genital itching, 7 individuals with genital malodor and 16 patients were asymptomatic. On examination vaginal discharge was profuse among 53 patients, moderate in 37 patients. This indicates 16% were unaware about the vaginal discharge.

Cervical examination showed nil abnormality among 71 patients, erosion in the cervical os in 21 patients and neobothian follicle in 2 patients. Saline mount examination & Gram staining of vaginal secretion smear showed clue cells among 92% of smears. On adding a drop of 10 % KOH to the vaginal discharge 80% of patients showed rotten fishy odour (Positive Whiff test). Vaginal pH was < 5 in 11 patients, 5-5.5 in 72 patients and > 5.5 in 17 patients.

Urine examination showed positive urine sugar in six patients. Four of them were known diabetic. Two newly diagnosed diabetic patients were referred to diabetology op. VDRL was positive in two patients, showing 1:1 dilution and 1:2 dilutions respectively. But the TPHA reading was negative in both of them. Testing for HIV was reactive in 4 patients. They were referred to ART centre for obtaining anti retro viral therapy while simultaneously treating for bacterial vaginosis according to their study group.

On partner screening of the study group, non-specific urethritis was noted in 10 patients' partners, balanitis among 6 partners, genital wart among two partners, 52 were asymptomatic. While 30 patient's partners did not come for evaluation.

Patients were assessed on the basis of improvement of Amsel's criteria during their follow up period. Amsel's criteria 1& 2 was considered as good response to treatment, while Amsel's criteria 3-5 was considered as no response to treatment. At the end of one week metronidazole group showed good response in 92% of patients and Lactobacillus group showed 90% good response. At the end of one month both the groups showed 100% good response. Two patients lost from follow up at this time from metronidazole group. At the end of two months 97.7% from metronidazole group and 100 % from lactobacillus group showed good response. Recurrence seen in one patient in metronidazole group and six patients lost from follow up from metronidazole group at the end of two months. At the end of six months 76.2% in metronidazole group and 94% in lactobacillus group showed good response. Recurrence seen in ten patients (23.8%) of metronidazole group and in 3 patients (6%) of Lactobacillus group. Previously lost 8 patients from metronidazole group still missed at the end of six month period too. The following

graph 1 shows the pictorial complex bar chart expression for the treatment response to Metronidazole (Group 1) and

lactobacillus (Group 2) based on Amsel's criteria in the follow up period.

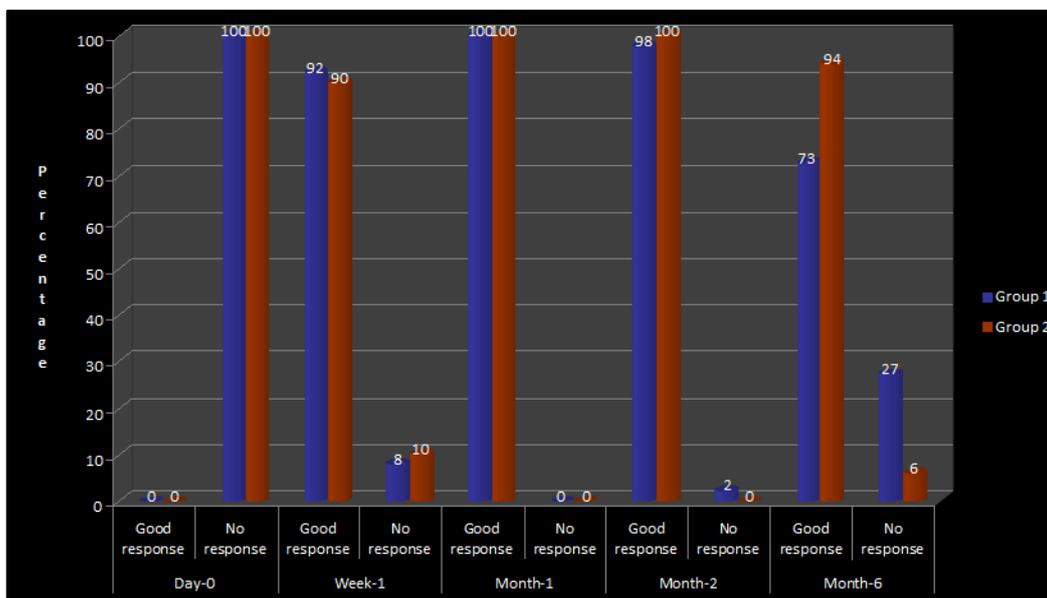


Figure 1. Treatment Response to Metronidazole (Group 1) and Lactobacillus (Group 2) in the follow up Periods of Day 0, At the End of One Week, At the End of One Month, Two Months and Six Months based on Amsel's Criteria

On assessment based on Nugent's scoring system at the follow up period the following grading response was taken into account. Grade 1 (Good response) – 0 to 3 score, Grade 2 (Moderate response) – Four to six score and Grade 3 (poor response)–seven to 10 score. At the end of one week metronidazole group showed good response among 40%, moderate response among 48% and poor response in 12% of study group. Lactobacillus group showed good response among 56%, moderate response in 40% and poor response in 4% of study group. At the end of one month Metronidazole group showed 81.3%, 18.4%, and 12% good, moderate and poor responses respectively. While

Lactobacillus group showed 56%, 40%, 4% good, moderate and poor responses. At the end of two months metronidazole group showed 61.4% good, 38.6% moderate response and 6 patients lost from follow up. In lactobacillus group 98% good and 2% moderate response seen at the end of two months. After six months 30.9% good, 59.5% moderate and 9.5% poor response noted in metronidazole group and 78% good response and 22% moderate response noted in Lactobacillus group. The following graph 2 represents the response following Metronidazole (Group 1) and Lactobacillus (Group 2) in the follow up period based on Nugent's scoring system.

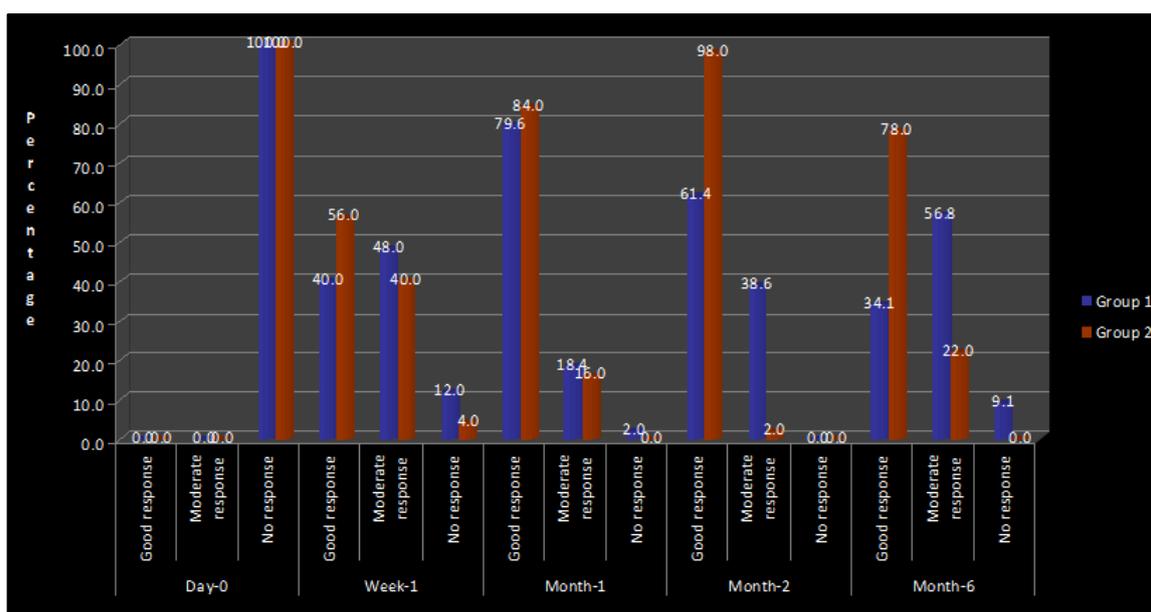


Figure 2. Treatment Response to Metronidazole (Group 1) and Lactobacillus (Group 2) in the follow up Periods of Day 0, At the End of One Week, At the End of One Month, Two Months and Six Months based on Nugent's Scoring System

DISCUSSION

The mean age of the patients in our study was 32.4±8.4 years. This correlated with the previous Morris study which stated that bacterial vaginosis was more common in the sexually active reproductive age group.¹

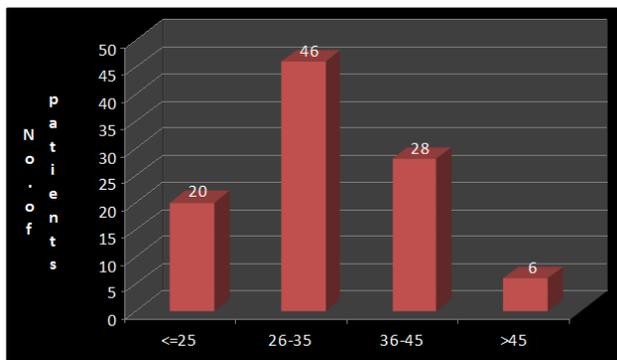


Figure 3. Incidence of Bacterial Vaginosis According to Age Wise Distribution in the Study Group

The mean duration of symptoms was 2 months, while the range was one week to 10 years. This depends upon the individual’s sensory adaptation. Genital discharge was the predominant symptom followed by genital itching and malodor.

Majority of the study group 86% had regular menstrual cycles. This shows the passive nature of the disease. Our study found out 92% of the patients was married or had history of sexual exposure. Those who had mono sexual partners were 77; multiple partners were 17 and 6 denied sexual contact. This differs from the previous Patiala study which concluded prevalence of Bacterial vaginosis in 37.5% married individuals.² This is depicted in the underlying simple pie chart and horizontal simple bar chart. Our study confirms the alteration of vaginal ecoflora after commencement of sexual activity. Our study coincides with the previous south Indian study by P.S. Rao et al which concluded the high incidence of bacterial vaginosis among married, sexually active individuals.³ In our study 57 out of 100 patients got married before the age of 20 years. This confirms that younger age at marriage has association with bacterial vaginosis which was already quoted in the Australian study by Larrison et al⁴ Amsel R et al study also showed the prevalence of bacterial vaginosis was lower with those who had no sexual exposure than those with previous sexual exposure.⁵

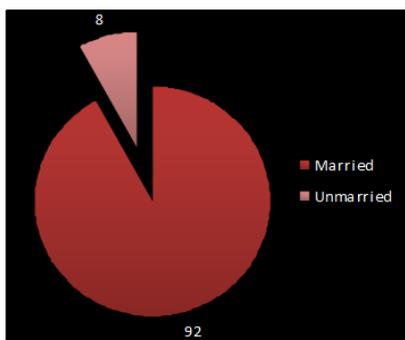


Figure 4. Incidence of Bacterial Vaginosis According to Marital Status

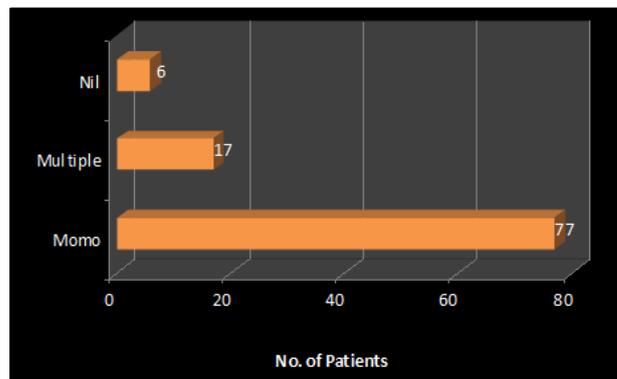


Figure 5. Incidence of Bacterial Vaginosis According to Number of Partners

In our study 15 patients had no children which included 8 unmarried, 21 had one child, 34 had two children, and 30 had 3 or more than three children. This study outlines the direct correlation of the number of pregnancies and the incidence of bacterial vaginosis. A study done by Bradshaw et al also confirmed the same above mentioned point.⁶

On analysing the sexual partners of the study group, ten of them had nonspecific urethritis, six partners had balanitis and 3 were HIV positive. Still we were not able to assess 30 patient’s partners. No study has shown the treatment of partner to decrease the risk of recurrence convincingly.⁷ But genital hygiene practices in the male partners is one of the prevention modality.⁸ we treated our patient’s partners with symptoms syndromically. Partner screening and syndromic diagnosis was explained in the pie chart below.

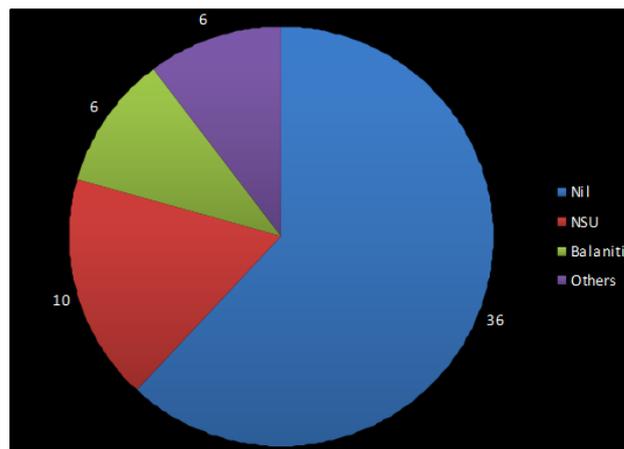


Figure 6. Incidence of Sexually Transmitted Infections in the Partners of the Study Groups

Cervical examination showed erosion in 21 patients and nebothian cyst in 2 patients. Lower abdomen tenderness and cervical motion tenderness & lower abdominal tenderness were negative in our study group. Ultra-sonogram abdomen also showed no free fluid which is the fore teller of ascending upper genital tract infection. As per the reference spontaneous PID that occurs without instrumentation may also be related to bacterial vaginosis.

Saline, KOH mount examination and gram smear ruled out other pathological causes for vaginal discharge like Trichomonas vaginitis and candidal vaginitis. But the leading hypothesis explains the absence of lactobacilli facilitates the

acquisition of other STIs. ⁹ Putrescine and cadaverine, the amines present in the vaginal secretion of bacterial vaginosis are responsible for the rotten fishy odor and they inhibit the germ tube formation of *Candida albicans*.¹⁰ In our study also we were unable to find out candidiasis in KOH mount. Vaginal pH was alkaline 5-5.5 in 72 patients and more than 5.5 in 17 patients. In bacterial vaginosis on pH determination if it is more than 4.5 then it is considered to be abnormal.¹¹ On saline wet mount examinations clue cells were found in 90 patients and clue cells along with pus cells reported among two patients. The presence of > 20 % clue cells is the best single diagnostic criteria of bacterial vaginosis.¹²

Though VDRL or syphilis was positive in 1:1 & 1:2 dilutions in two patients, TPHA which is the specific test for syphilis was negative for them, stating it was a false positive one. HIV was found out to be positive in two new patients; they were treated for bacterial vaginosis and referred to ICTC centre for ART simultaneously. The following Gram stain smear from vaginal secretion shows the Gram positive rods of lactobacillus species.

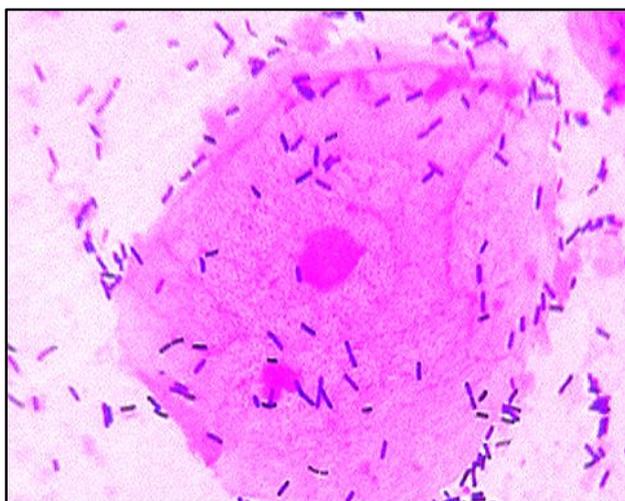


Figure 7. Showing Gram Positive Rods of Lactobacillus in the Gram Stain Smear of Vaginal Secretion

There were no symptoms and signs pertaining to UTI in our study group. On urine examination none of them showed abnormality in urine cell count, pH analysis except two were diagnosed as new diabetics. This contradicts the reference showing increased incidence of urethritis, cystitis among bacterial vaginosis patients.¹³

In our study, regarding complications following Bacterial vaginosis metronidazole group showed recurrence in 10 patients and lactobacillus group showed nil recurrences at the end of six months follow up. Up to 70% women developed recurrence of bacterial vaginosis within three months after completion of treatment according to Sobel JD

study.¹⁴ In a study by Boris J et al long term recurrence rates were approximately 50%.^{15,16} There was no significant association of pre term delivery and abortion with bacterial vaginosis in our study. Only 6 patients elicited the previous history of preterm birth and spontaneous abortion. Ascending genital infections due to bacterial vaginosis associated organisms and the altered intra uterine milieu cause infection of deciduas, placenta and amniotic fluid. This leads to bad obstetric history.¹⁷

On follow up, patients of both the groups, the cure or improvement was usually defined as resolution of two or more of Amsel's criteria for diagnosis.¹⁸

Amsel's criteria:

1. Excessive, homogenous, uniformly adherent vaginal discharge
2. Elevated vaginal pH >4.5
3. Positive amine test (whiff and sniff tests)
4. Clue cells constituting 20% or more of total vaginal epithelial cells.

The patients should fulfill 3 out of 4 criteria for bacterial vaginosis.¹⁹

Nugent's Scoring System

This is based on the bacterial morphology in Gram staining. Vaginal Gram staining has comparable sensitivity and greater specificity than vaginal culture which shows false positive results than Gram staining.²⁰

Bacteria	0	1+	2+	3+	4+
Lactobacilli	>30	6-30	1-5	<1	0
Gardnerella vaginalis	0	<1	1-5	6-30	>30
Mobiluncus	0	<1	1-5	6-30	>30

Score: 0-3 Normal
 4-6 Intermediate (test to be repeated later)
 7-10 Bacterial vaginosis.

The comparison of the two therapeutic modalities at the end of one week, one month, two months and six months of treatment was explained in the Table below. There were no similar studies with these two drugs exactly to compare our results.

Based on Amsel's criteria at 1 week, 1 month and 2 months period both the drugs had comparable therapeutic benefits. But after 6 months metronidazole group showed good response in 76.2% of study group and Lactobacillus group showed good response in 94% of study population. (P= 0.01)

Comparison of Amsels Criteria Between Two Study Groups at Different Time Points

Time point	Amsel's criteria	Group 1	Group 2	P-value
		No (%)	No (%)	
Day - 0	Good response	0 (0.0)	0 (0.0)	-
	No response	50 (100.0)	50 (100.0)	

Week - 1	Good response	46 (92.0)	45 (90.0)	1.00 (N.S.)#
	No response	4 (8.0)	5 (10.0)	
Month - 1	Good response	48 (100.0)	50 (100.0)	-
	No response	0 (0.0)	0 (0.0)	
Month - 2	Good response	43 (97.7)	50 (100.0)	0.47 (N.S.)#
	No response	1 (2.3)	0 (0.0)	
Month - 6	Good response	32 (72.7)	47 (94.0)	0.01 (Sig.)@
	No response	12 (27.3)	3 (6.0)	

Table 1. Based on Amsel's Criteria at 1 Week, 1 Month and 2 Months Period both the Drugs had Comparable Therapeutic benefits. But after 6 Months Metronidazole Group Showed Good Response in 76.2% of Study Group and Lactobacillus Group Showed Good Response in 94% of Study Population. (P= 0.01)

#Fishers Exact Test (2-tailed) was used to calculate the P-Value.

@ Chi-square test with Yates' correction was used to calculate the P-Value.

Inference

At 6th month, the proportion of 'good response' in Group 2 (94%) is significantly higher than Group 1 (72.7%) (P=0.01). However, there is no significant difference in the proportion of 'Good response' between Group 1 and Group 2 at other time points viz., Day-0, Week-1, Month-1 and Month-2 (P>0.05).

On comparing the efficacy based on Nugent's scoring system at 1 week and 1 month both groups were comparable. At 2 months and 6 months follow up period lactobacillus group showed good therapeutic response compared to Metronidazole. (p = < 0.0001 in both time period) The comparison of therapeutic response based on Nugent's scoring system is explained in the Table below.

At the end of study period Metronidazole group showed 30.9% good and 59.5% moderate therapeutic response and Lactobacillus group showed 78% good and 22% moderate therapeutic response.

Comparison of Nugent Criteria between Two Study Groups at Different Time Points

Time point	Nugent's criteria	Group 1	Group 2	P-value*
		No (%)	No (%)	
Day - 0	Good response	0 (0.0)	0 (0.0)	-
	Moderate response	0 (0.0)	0 (0.0)	
	No response	50 (100.0)	50 (100.0)	
Week - 1	Good response	20 (40.0)	28 (56.0)	0.16 (N.S.)
	Moderate response	24 (48.0)	20 (40.0)	
	No response	6 (12.0)	2 (4.0)	
Month - 1	Good response	39 (79.6)	42 (84.0)	0.56 (N.S.)
	Moderate response	9 (18.4)	8 (16.0)	
	No response	1 (2.0)	0 (0.0)	
Month - 2	Good response	27 (61.4)	49 (98.0)	<0.0001 (Sig.)@
	Moderate response	17 (38.6)	1 (2.0)	
	No response	0 (0.0)	0 (0.0)	
Month - 6	Good response	15 (34.1)	39 (78.0)	<0.0001 (Sig.)
	Moderate response	25 (56.8)	11 (22.0)	
	No response	4 (9.1)	0 (0.0)	

Table 2. At the End of Study Period Metronidazole Group Showed 30.9% Good and 59.5% Moderate Therapeutic Response and Lactobacillus Group Showed 78% Good and 22% Moderate Therapeutic Response

*Chi-square test was used to calculate the P-Value.

@ Chi-square test with Yates' correction was used to calculate the P-Value.

INFERENCE

- At 2nd month, the proportion of 'good response' in Group 2 (98%) is significantly higher than Group 1 (61.4%) (P<0.0001).
- At 6th month, there is significant difference in the distribution of Nugent's criteria between Group 1 and Group 2 (P<0.0001). Further analysis showed that the

- proportion of 'Good response' in Group 2 (78%) is significantly higher than Group 1(34.1%) (P<0.0001).
- However, there is no significant difference in the distribution of Nugent's criteria between Group 1 and Group 2 at other time points viz., Day-0, Week-1and Month-1 (P>0.05).

In a study by Reid G et al, oral lactobacillus for two months was compared with placebo. Microscopy revealed restoration of normal lactobacillus colonized micro flora in 37% women in lactobacillus group compared to placebo group 13%.²¹ Another Nigerian study which compared the oral lactobacillus with 0.75% Metronidazole gel showed 90% improvement in lactobacillus group and 55% improvement in the Metronidazole group.²² Kingsley Anukkam et al study initiated Metronidazole for one week in the study group followed by treating the patients in two groups. One group with probiotics and the other with placebo for one month. 88% in the probiotic group and 40% in the placebo group showed complete cure.²³ All the above mentioned studies though the methodology differs strongly correlated with our study response. But the draw backs in probiotic treatment are treatment period is long and the cost of treatment is also too high.

Our study also has certain draw backs. In the Metronidazole group 8 patients lost from follow up. The follow up period was very long. The treatment course also differed in the two comparable groups. For the metronidazole group the treatment period was one week and for Lactobacillus group the treatment period was two months. The cost of treatment was high for lactobacillus.

After two months of treatment with lactobacillus group ultra-sonogram showed fatty liver changes in eight patients and in one patient in metronidazole group. In literature few cases of liver abscess reported after lactobacillus ingestion. Our finding of fatty changes in liver after lactobacillus is questionable as pre-treatment ultra-sonogram was not done prior to the initiation of treatment. It would have been a valuable finding if USG was done at the initial visit and at follow up period.

CONCLUSION

Epidemiological interpretation in our study shows Bacterial vaginosis is common in the 24-40 years of age; marital status, earlier age at marriage, number of deliveries is directly proportionate to bacterial vaginosis incidence. Irregular menstrual cycle, number of sexual partners, and mode of delivery has no association with the incidence of Bacterial vaginosis. Moderate to profuse genital discharge is the commonest symptom observed. Whiff test (80%), Clue cells (90%) and alkaline vaginal pH > 5 has high degree of sensitivity.

On comparing the efficacy of Metronidazole 400 mg B.D for one week and C. Lactobacillus B.D for one month followed by od for the next one month, based on Amsel's criteria both groups are comparable in the 1 week, 1 month, 2 months period while at 6 months follow up Lactobacillus group was better than Metronidazole. On Nugent's criteria both groups were comparable in the 1 week and 1 month period. But at 2 months and 6 months follow up period lactobacillus group showed better response.

REFERENCES

[1] Morris MC, Rogers PA, Kinghorn GR. Is bacterial vaginosis a sexually transmitted infection? *Sex Transm Infect* 2001;77(1):63-68.

- [2] Chopra A, Mittal RR, Kanta S, et al. Vaginitis and vaginal flora-study of 100 cases. *Indian J Sex Transm Disease* 1993;14:52-54.
- [3] Rao PS, Devi S, Shriyan A, et al. Diagnosis of bacterial vaginosis in a rural set up: comparison of clinical algorithm, smear scoring and culture (smauc) by semi quantitative technique. *Indian J Med Microbiology* 2004;22(1):47-50.
- [4] Larrson P, Platz-Christensen J, Sundstorm E. Is bacterial vaginosis a sexually transmitted disease? *Int J STDs, AIDS* 1991;2:362-364.
- [5] De Seta F, Sartore A, Piccoli M, et al. Gynaecological infections in adolescence. *Minerva Gynaecol* 2000;52(9):327-332.
- [6] Bradshaw CS, Morton AN, Garland SM, et al. Higher-risk behavioral practices associated with bacterial vaginosis compared with vaginal candidiasis. *Obstet Gynaecol* 2005;106(1):105-114.
- [7] Holmes KK, Sparling PF, Stamm WE, et al. Sexually transmitted diseases. 4th edn. New York: The McGraw-Hill 2007: p. 580.
- [8] Hilliers S. Bacterial vaginosis. In: Holmes KK, Mardh PA, Sparling PF, eds. et al. Sexually transmitted diseases. 3rd edn. New York: McGraw-Hill 1999:563-586.
- [9] Schwebke JR. Abnormal vaginal flora as a biologic risk factor for acquisition of HIV infection and sexually transmitted diseases. *J Infect Dis* 2005;192(8):1315-1317.
- [10] Rodrigues AG, Mardh PA, Pina-Vaz C, et al. Is the lack of concurrence of bacterial vaginosis and vaginal candidosis explained by the presence of bacterial amines? *Am J Obstet Gynaecol* 1999;181(2):367-370.
- [11] Pheifer TA, Forsyth PS, Durfee MA, et al. Nonspecific vaginitis: role of haemophilus vaginalis and treatment with metronidazole. *N Eng J Med* 1978;298:1429-1434.
- [12] Garner HL, Dukes CD. Haemophilus vaginalis vaginitis. A newly defined specific infection previous classified non-specific vaginitis. *Am J Obstet Gynecol* 1955;69(5):962-976.
- [13] Reid G. Probiotic lactobacilli for urogenital health in women. *J Dis Gastroenterol* 2008;42(Suppl 3 PT 2):S234-236.
- [14] Sobel JD, Schmitt C, Meriwether C, et al. Long-term follow-up of patients with bacterial vaginosis treated with oral metronidazole and topical clindamycin. *J Infect Dis* 1993;167(3):783-784.
- [15] Agnew KJ, Hillier SL. The effect of treatment regimens for vaginitis and cervicitis on vaginal colonisation by lactobacilli. *Sex Transm Dis* 1995;22(5):269-273.
- [16] Boris J, Pålson C, Larsson PG, et al. Six years observation after successful treatment of bacterial vaginosis. *Infect Dis Obstet Gynaecol* 1997;5(4):297-302.

- [17] Platz-Chhristensen JJ, Brandberg A, Wiqvist N, et al. Increased prostaglandin concentrations in the cervical mucus of pregnant women with bacterial vaginosis. *Prostaglandins* 1992;43(2):133-134.
- [18] Amsel R, Totten PA, Spiegel CA, et al. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;74(1):14-22.
- [19] Chandeying V, Sutthijumroon S, Tungphaisal S. Comparison of three different nimorazole regimens in the treatment of bacterial vaginosis. *Asia-Oceania J Obstet Gynaecol* 1990;17:131-134.
- [20] Eschenbach DA, Hillier S, Critchlow C, et al. Diagnosis and clinical manifestation of bacterial vaginosis. *Am J Obstet Gynaecol* 1988;158(4):819-824.
- [21] Reid G, Charbonneau D, Erb J, et al. Oral use of lactobacillus rhamnosus GR-1 and L.fermentum RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immunol Med Microbiol* 2003;35(2):131-134.
- [22] Anukam KC, Osazuwa E, Osemene GI, et al. Clinical study comparing probiotic lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microbes and Infections* 2006;8(12-13):2772-2776.
- [23] Anukam K, Osazuwa E, Ahonkhai I, et al. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic lactobacillus rhamnosus GR-1 and lactobacillus reuteri RC-14: randomized, double-blind, placebo controlled trial. *Microbes and Infection* 2006;8(6):1450-1454.