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Role of Oral Lactoferrin in Prevention of Recurrent Bacterial Vaginosis in Third Trimester of Pregnancy

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ABSTRACT

Background: One of the most common conditions affecting women's health throughout the childbearing years is bacterial vaginosis (BV), which have a negative impact on pregnancy. Nutraceutical proteins and probiotics (such as lactoferrin) can help restore the equilibrium of the vaginal microbiome, which can be of significant use in avoiding unfavorable pregnancy outcomes. **Aim of Study:** This study aimed to evaluate the efficacy of lactoferrin in prevention of recurrent bacterial vaginosis in third trimester singleton pregnancy.

Patients and method: This was randomized controlled clinical trial conducted at Obstetrics and Gynecology Department, Zagazig University, Outpatient Clinic. The study included 66 cases with history of receurent bacterial vaginosis divided into two groups: Study group that received lactoferrin oral consumption of 2 capsules/day for 5 days, then 10 consecutive days at the dosage of 1 capsule/day (pravotinR). Control group, which did not receive lactoferrin. All of the ladies took 1 capsule of lactoferrin or a placebo daily for 10 days straight each month during the follow-up period.

Results: There was significant differences in microbiological cure rate as detected by Nugent scores in studied groups at T1 and T2 where the study group had microbiological cure rate than control one (57.6% and 9.1%) versus (72.7% and 18%) respectively. Low APGAR1, preterm, CS delivery type, LBW and PROM were significantly higher with control group.

Conclusion: Repeated sessions of orally delivered probiotics in conjunction with lactoferrin are beneficial in preventing BV recurrences, as evidenced by much greater clinical and microbiological cure rates, as well as a favourable safety profile.

Keywords: Bacterial vaginosis, Prevention, Lactoferrin.

INTRODUCTION

One of the most prevalent illnesses affecting women of reproductive age is bacterial vaginosis (BV). From 5% in Asia and Australia to 59% in Southern and Eastern Africa, BV is prevalent. Prevalence rates range from 8% to 51%, making pregnant women one of the populations most susceptible to the negative effects of BV [(1)]. There are BV-related consequences include pelvic inflammatory disease (PID), which can lead to miscarriage, premature birth, and preterm premature rupture of the membranes (PPROM). Moreover, regardless of symptoms, BV is linked to a number of sexually transmitted diseases (STIs), including Chlamydia trachomatis, Neisseria gonorrhoeae, HSV-2, and an increased risk of HIV-1 acquisition [(2)]. After 4 weeks of treatment, patients with BV treated with metronidazole or clindamycin can see an 80% reduction in symptoms. Nonetheless, recurrence has been seen in 40% to 50% of instances after stopping treatment for a year [(3)]. Known gastrointestinal side effects of clindamycin and metronidazole include nausea and vomiting, and stomach discomfort, despite the fact that both medicines have the potential to cause a number of side effects. Another issue with common BV treatment approaches is that they do not take into account the effects of disrupting vaginal ecosystems and the subsequent risk of disease recurrence [(4)]. Because BV treatment should put an emphasis on getting rid of patients' clinical symptoms and lowering the recurrence rate, and replenishing the vaginal microbiota, probiotics may reduce the recurrence rate of the infection [(5)].

A milk glycoprotein called lactoferrin (LF), which binds to iron and is involved in a wide range of biological processes, including an antibacterial impact, helps certain probiotic strains proliferate [(6)]. In ferrokinetics, lactoferrin is crucial. As a result of its strong affinity for binding free iron, LF restricts the amount of ions available for microbial metabolism [(7)]. Because of its bacteriostatic and bactericidal properties, LF plays a part in the host's defence mechanisms. Moreover, it prevents the growth of other bacteria including fungi and viruses. The immune system is also modulated by lactoferrin, and new research suggests that lactoferrin directly affects the generation and operation of neutrophils and monocytes [(8)].

The transporter of iron in the serum, serum transferrin, is physically and chemically identical to the iron-binding, non-haem protein known as LF. It is created by mucosal epithelial cells, widely present in bodily fluids, and abundantly secreted in milk [(9)]. Thus the aim of this study was to evaluate the effectiveness of LF as a new strategy for prevention of recurrent bacterial vaginosis in third trimester singleton pregnancies.

PATIENT AND METHODS

This randomized controlled clinical trial was conducted at Obstetrics and Gynecology Department, Zagazig University Outpatient Clinic through the period

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from July 2022 to January 2023 on 66 patients with history of receurent bacterial vaginosis.

Inclusion criteria: Pregnant ladies between the ages of 20 and 37. Primigravida. 28-40 week gestation. History of receurent bacterial vaginosis.

Exclusion criteria: Multiple gestations. Multiparity. Medical disorders as: Endocrine disorders, chronic hypertension, and diabetes mellitus are a few examples. Immune system disorders. Renal conditions. Blood disorders. Allergy to lactoferrin. Using vaginal douches. Patients who had antimicrobial treatment within four weeks of the sample's taking. No bacterial vaginosis infections. Patients unable to respond, mentally ill pregnant women. Patients refuse participation.

Methods:

All patients were subjected to the following: thorough physical examination and careful history taking with an emphasis on: obstetric history, history of the current condition, medical history, and surgical history.

Collection of samples for swab:

- Sterile vaginal speculum was inserted when patients were positioned in the dorsal lithiotomy posture. By using a sterile cotton swab, samples are taken from the vault of the vagina and the posterior vaginal fornix for culture.
- After the sawbs, the slides were air dried and labelled with the patient study number, then were smeared on clean glass slides and delivered to the lab the same day.
- Gram stain was used to stain the slides: Amsel criteria and the Nugent gramme stain grading system were used to identify bacterial vaginosis.
- Gram-negative, Gram-variable, and curved Gram-variable rods (such as Prevotella and Gardnerella vaginalis) are counted to get the Nugent score (i.e mobilincus). Bacterial vaginosis is consistent with a score of 7–10.

Treatment protocol

Among enrolled women those who had symptoms of bacterial vaginosis was women with a history of recurrent bacterial vaginosis who received standard antibiotic therapy (metronidazole 500 mg daily for 7 days) were randomly assigned to one of two research arms.

- **Study group:** That received lactoferrin via oral ingestion (2 sachets/day for 5 days, then 10 consecutive days at the dosage of 1 sachet/day) (pravotinR).
- **Control group :** Not reciving lactoferrin

All of the women took 1 sache of lactoferrin each day for 10 days straight each month during the follow-up period.

Samples collection and laboratory procedures

All of the women had the careful rotation of two vaginal swabs on the vaginal sidewall during each visit. The initial swab was right away placed in the medium for the microbiological analysis (culture for excluding yeasts, bacteria or trichomonas). The fresh smear on a glass slide was performed using the second swab, and the Nugent score was determined in accordance with the procedure outlined by **Nugent *et al***. [10] following fixation and Gram staining.

Follow up:

- Swabs were repeated after one month (T1) and two months (T2) at the gynaecological centres, where all of the participants' visits were three, from T0 to T2 (T2). Each clinical evaluation involved the collection of two cervical swabs (for vaginal culture and Nugent score).
- The patient's medical history was taken during the initial consultation (the baseline), the informed consent form was signed, and the women were separated into the two study arms.
- The purpose of visits T1 and T2 was to evaluate the investigational products' efficacy and safety. The patients self-reported whether the symptoms being assessed (vaginal discharge, itching) were present or absent during gynaecological appointments.
- The clinical cure rate for BV symptoms was used to evaluate efficacy (described as the lack of irritation or discharge from the vagina).
- A healthy Nugent score (ranging from 0 to 3) and remission of the vaginal microbiota are indicators of the microbiological cure rate, which is also known as the overall cure rate (lack of symptoms and a Nugent score of 7 or above).

Also, the percentage of women in each group who reported symptoms and a Nugent score of at least 3 during the follow-up period was used to compute the recurrence rate.

- Side effects are noted in order to evaluate safety.
- An adverse event is deemed severe if it resulted in death, serious bodily harm, the need for hospitalisation, or permanent harm.

Ethical approval: The Local Ethics Commission granted its clearance for our study after receiving the verbal and written consents of all participants.

Statistical analysis:

With the help of Epi-Info version 6 and SPP for Windows version 8, data were entered, verified, and analysed. Chi-squared test and student t test were employed. $P \leq 0.05$ was considered significant

RESULT

Both groups were compatible regarding age, occupation and gestational age with non-significant difference (table 1).

Table (1): Basic characteristics of studied groups

Variable	STUDY GROUP (*n*=33)		CONTROL GROUP (n=33)		t	P
Age : (year)						
Mean ± SD	26.58 ± 8.2		27.56 ± 9.1		0.73	0.46
Range	28 – 39		27 – 40			NS
Gestational Age:(weeks)						
Mean ± SD	32.58 ± 8.2		30.56 ± 9.1		0.73	0.46
Range	18 – 35		19 – 36			NS
Variable	**No**	**%**	**No**	**%**	χ^2	**P**
Occupation:						
Not working(House wife)	23	59.7	20	60.6	3.49	0.06
Working	10	30.3	13	29.4		NS

No significance differences were reported in methods of contraception, however IUCD were the most used methods in both groups (Table 2).

Table (2): Relation between bacterial vaginosis of the studied groups and methods of contraception

Variable	STUDY GROUP (*n*=33)		CONTROL GROUP (n=33)		χ^2	P
	No	**%**	**No**	**%**		
Previous Contraception:						
Not use	5	15.3	6	18.2		
Hormonal	9	27.2	10	30.3		
IUD	10	30.3	9	27.2	**1.572**	**0.321**
Barriers	9	27.2	10	30.3		

There was highly significant differences in clinical cure rate of bacterial vaginosis (BV) of the studied groups at T1 and T2 where study group had higher cure rate than control one (75.7% and 93.3%) versus (36.3% and 48.4%) respectively (Table 3).

Table (3): clinical cure rate of bacterial vaginosis (BV) of the studied groups

	STUDY GROUP (*n*=33)		CONTROL GROUP (n=33)		P-value
	No	**%**	**No**	**%**	
T0	0	0	0	0	
T1	25	75.7	12	36.3	<0.001 (HS)
T2	31	93.3	16	48.4	<0.001 (HS)

T0 = baseline; T1 = 1 month; T2 = 2 month . HS :highly significant

There was highly significant differences in recurrence rate of bacterial vaginosis (BV) of the studied groups at T1 and T2 , study group had lower recurrence rate than control one (57.6% and 9.1%) versus (72.7% and 18%) respectively (Table 4).

Table (4): Recurrence rate of bacterial vaginosis (BV) during the follow-up of the studied group

Variable	STUDY GROUP (*n*=33)		CONTROL GROUP (n=33)		P-value
	No	**%**	**No**	**%**	
T0	0	0	0	0	
T1	19	57.6	24	72.7	<0.001 (HS)
T2	3	9.1	6	18	<0.001 (HS)

T0 = baseline; T1 = 1 month; T2 = 2 month **,** HS **:**highly significant

There was increase microbiological cure rate as detected by Nugent scores in study group from T1 to T2 (Table 5).

Table (5): Microbiological cure rate as detected by Nugent scores in study group

Variable	STUDY GROUP					
	≤3		4-6		≥7	
	No	%	No	%	No	%
T0	0	0	0	0	33	100
T1	14	42.4	19	57.6	0	0
T2	27	82	6	18	0	0

T0 = baseline; T1 = 1 month; T2 = 2 month

There was increase of microbiological cure rate as detected by Nugent scores in control group from T1 to T2 (Table 6).

Table (6): Microbiological cure rate as detected by Nugent scores in control group

Variable	CONTROL GROUP					
	≤3		4-6		≥7	
	No	%	No	%	No	%
T0	0	0	0	0	33	100
T1	8	24.2	21	63.7	4	12.2
T2	5	15.1	26	78.8	2	6.1

T0 = baseline; T1 = 1 month; T2 = 2 month

There were significant differences in microbiological cure rate as detected by Nugent scores in studied groups at T1 and T2 where study group had microbiological cure rate than control one (57.6% and 9.1%) versus (72.7% and 18%) respectively (Table 7).

Table (7): Microbiological cure rate as detected by Nugent scores in studied groups

Variable	STUDY GROUP						CONTROL GROUP						P-value
	≤3		4-6		≥7		≤3		4-6		≥7		
	No	%	No	%	No	%	No	%	No	%	N0	%	
T0	0	0	0	0	33	100	0	0	0	0	33	100	P<0.05
T1	14	42.4	19	57.6	0	0	8	24.2	21	63.7	4	12.2	P<0.05
T2	27	82	6	18	0	0	5	15.1	26	78.8	2	6.1	P<0.05

T0 = baseline; T1 = 1 month; T2 = 2 month

DISCUSSION

The results of the current study showed that there were highly significant differences in the clinical and bacteriological cure rates of bacterial vaginosis (BV) of the studied groups at T1 and T2. The study group had a higher cure rate than the control group (75.7% & 93.3%) compared to 36.3% & 48.4% and 57.6% & 9.1% versus 72.7% & 18% respectively. This is in agreement with a prior clinical trial by **Alberti *et al.*** [11], which demonstrated that oral lactoferrin administration after two weeks of treatment showed a noticeable increase in the colonisation of the vagina by *L. acidophilus* GLA-14 and *L. rhamnosus* HN001 in healthy women, whereas the placebo had no effect on the lactobacilli in the treated women's vagina.

Russo *et al.* [12, 13] investigated the same medication on women participating in two independent randomised clinical trials (RCTs) with severe vaginal infections, such as recurrent bacterial vaginosis (RBV) and recurrent vaginal candidiasis (RVC). Respecta® complex efficacy was evaluated in the first RCT as a supplement in adult women with recurrent BV to metronidazole. In both the induction phase (oral metronidazole 500 mg twice daily for seven days plus Respecta® complex for 15 days) and the maintenance phase (oral Respecta® complex for 10 consecutive days beginning on the first day of menstruation, for six months). The results demonstrated a significant regression of the Nugent score and resolution of BV symptoms. The technology provides a viable and risk-free method of preventing recurrent BV in addition to being an alternative, nonantibiotic treatment, according to the significant findings after three and six months of follow-up [12]. The second RCT experiment conducted by **Russo *et al.*** [13] assessed Respecta® complex's potential to lessen vulvo-vaginal candidiasis (VVC) recurrence. Women with RVVC received a maintenance regimen of probiotics and lactoferrin for six months as part of a prospective,

randomised, double-blind clinical study. This was followed by typical therapeutic induction phase using vaginal clotrimazole and the Respecta® combination (for 15 days). The results after three and six months of follow-up revealed a significant decline in the RVVC recurrence rate.

There was a lot of data to back up Gardnerella vaginalis' pathogenicity and the notion that Gardnerella spp. biofilm may be to blame for the high recurrence rate and failure of conventional antibiotic therapies. This clearly implies that additional bacteria present in the biofilm are secondary invaders that exploit weak points in the host's defences. Antibiotic resistance is thought to be one of the causes of persistent and recurrent BV, and bacteria in biofilms react to antibiotic therapy differently than their planktonic counterparts [(14)].

Another study by **Laue *et al.* [(15)]** revealed that 17 women who ate yoghurt containing several lactobacilli strains for 4 weeks after receiving metronidazole (500 mg twice daily for 7 days) were all BV-free based on Amsel criteria, whereas only 11 out of 17 were BV-free. These strains included Lactobacillus delbrueckii ssp. Bulgaricus and Lactobacillus crispatus LbV. Furthermore, supplementation with lactobacilli throughout the intervention phase resulted in a significant improvement in both the Nugent score and symptoms (such as discharge and odour).

CONCLUSION

The current study's findings support the notion that probiotics taken orally in conjunction with lactoferrin in repeated courses are beneficial in preventing recurrences of BV, as shown by much improved clinical and microbiological cure rates as well as a favourable safety profile. After taking certain probiotics and lactoferrin orally, women with RBV reported a significant decrease in their Nugent score and a remission of symptoms such as vaginal discharge and itching.

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