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Research Article

Effect of Synbiotic on Interleukin 10 and Tumor Necrosis Factor- α Serum Level in Pregnant Women with Bacterial Vaginosis

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Abstract

Background and Objective: Bacterial Vaginosis (BV) is the bacterial infection commonly found in women within reproductive age with 19-24(%) of availability. The aim of this study is to determine the effect of synbiotic on IL-10 and TNF- α serum level in pregnant women with bacterial vaginosis. **Materials and Methods:** This study was followed by 44 pregnant women with bacterial vaginosis diagnosed by Amsel criteria and the inclusion and exclusion criteria. Sample group consists of 22 women who receive synbiotic and standard treatment and the control group consists of 22 women who receive placebo and standard treatment. **Results:** All women are followed up for 2 weeks after treatment is started. The change of mean difference of IL-10 before and after treatment in sample group is -321.38 ± 625.71 and in control group is -182.65 ± 473.16 . The data are analyzed using Mann-Whitney test with p-value 0.302. This study shows significantly elevated of IL-10 serum level in both groups after treatment. Although in the sample group indicates more elevated of IL-10 serum level after treatment, this difference is not significant between both groups. The change of mean difference of TNF- α before and after treatment in sample group is 100.37 ± 119.57 and in control group is 80.16 ± 134.81 . The data are analyzed using Mann-Whitney test with p-value 0.189. **Conclusion:** This study shows the significant derivation of TNF- α serum level in both groups after treatment. Although in the sample group indicates more derivation of TNF- α serum level after treatment, this difference is not significant in both groups.

Key words: Bacterial vaginosis, synbiotic, interleukin 10, tumor necrosis factor- α , pregnancy

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bacterial Vaginosis (BV) is a modification of the vaginal flora that is characterized by a diminished or absent flora of lactobacilli, which increases the vaginal pH and leads to a significantly increased colonization by several anaerobic or facultative microorganisms, including *Gardenerella vaginalis*, *Prevotella* sp., *Bactericides* sp., *Mobiluncus* sp., Gram-positive cocci and genital mycoplasma.

Bacterial Vaginosis (BV) is the bacterial infection which commonly is found in women of reproductive age that is 19-24%. There is a range of 10-30% of pregnant women shows appropriate symptoms of bacterial vaginosis. However, half of them are asymptomatic¹.

As matter of fact, the harm of vaginal flora changes has played strong relationships to the risk of preterm birth as well as early membranes rupture. The previous study has shown the increase in preterm birth diagnosed with bacterial vaginosis. It is the change of endogen vaginosis microbial based on the decrease of the *Lactobacillus* level which functions to produce hydrogen peroxide².

The treatment of vaginal infections is currently less inadequate since it finds a high rate of post-therapy recurrence that because current treatments presumably do not aim to restore an acidic vaginal environment and do not trigger the proliferation of lactic acid-producing bacteria³.

A previous study by Bandyopadhyay and Mandal⁴ showed that synbiotics as a type of nutritional supplement in the form of probiotic and prebiotic combined can reduce the concentration of inflammatory markers in patients with type 2 of diabetes mellitus which ultimately can reduce the risk of heart disease. As well as other studies have shown the improvements in the gastrointestinal microbiome environment and increased immune system by giving synbiotics⁵.

Probiotics are living microorganisms (commonly bacteria). Those have similarities to beneficial microorganisms that are naturally lived in the gut. Probiotics are available in various forms as dietary supplements. Based on a literary study conducted by Yeganegi *et al.*⁶ against 11 randomized controlled trials, the administration of *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* spp. shows no incidences of malformation or miscarriage. The administration of *Lactobacillus acidophilus* in the form of suppository yogurt has been proven safe and effective for the treatment of bacterial vaginosis in pregnancy.

The interesting Immunosuppressive stokin within this study is TGF- β and IL-10. The TGF- β is a protein that inhibits the activation of lymphocytes as well as monocytes derived

from phagocytes. The IL-10 is an anti-inflammatory cytokine produced primarily by monocytes and is known to inhibit other pro-inflammatory cytokines such as IFN- γ , IL-12, IL-3, TNF- α and GM-CSF produced by macrophages and T cells⁷.

There is a growth of Tumor Necrosis Factor- α (TNF- α) within women with bacterial vaginosis then those have no bacterial vaginosis. The changes of TNF- α are either found in women with bacterial vaginosis before and after antibiotic therapy. However, the levels of TNF- α were lower after antibiotic therapy. In the study of BV combination and Lactobacilli appear to be more triggering IL-10 from exposure to a single lung as seen for gasseri L and A Vaginae and L Rhamnosus and G vaginalis. The balance between pro-inflammatory cytokines and anti-inflammatory cytokine production for BV may change in favor of an anti-inflammatory response when lactobacilli exists⁷.

The social, clinical and economic implications of adverse outcomes associated with BV have led to increase attention to diagnosis and treatment. The attempts to correct the imbalance in vaginal micro ecology only with the use of antibiotics have been substantially unsuccessful, especially in the management of recurrences, while recent advances in our understanding of microbiological, immune and metabolic alterations in the vaginal micro environment of women with BV have raised further interest in the therapeutic use of probiotics.

Based on the overall statement, this study proposed to compare the effect of giving symbiotic on microbial changes in the vagina of pregnant women with Bacterial Vaginosis by looking at changes in serum levels of IL-10 and TNF- α before and after the synbiotics gift.

MATERIALS AND METHODS

This research was the Randomized Double-Blind Clinical test to determine the effect of synbiotics on the levels of IL-10 and TNF- α before and after the given synbiotics. This study was conducted at some of Public Health Center (Puskesmas) in Makassar. This study was performed from January, 2017 until the sample was adequate.

The research sample was pregnant women from 20 weeks of gestational age or greater with complain white vaginal discharge based on Amsel criteria who visited the Maternal and Child Health room of some Public Health Center (Puskesmas) in Makassar. The subject that qualified with inclusion criteria underwent sampling, then the sample is divided into two group: group A and group B with total amount (A-B) 44 samples. The determination of the

order of groups based on the tables had been made according to the randomization of the block. Group A received a symbiotic that should be consumed 2 times a day (morning and afternoon) for 2 weeks and antibiotics (clindamycin 2×300 mg) orally for 7 days. The samples of Group B were given milk containing no probiotics or prebiotics and clindamycin 2×300 mg orally for 7 days. Grouping of samples in both groups were done in Double-Blind. After 2 weeks, both group sampling were taken to see changes in serum level of IL-10 and TNF-α.

Statistical analysis: The data analyzed and processed through SPSS tool. The statistical test was done through Wilcoxon's paired T-test hypothesis that aims to compare the means of serum level of IL-10 and TNF-α before and after each sample and control. Then, the result of the comparison was tested through Mann-Whitney's unpaired t-test hypothesis to compare the means difference of serum IL-10 and TNF-α level between the Sample group and the Control group.

RESULTS

The randomized Double-Blind Clinical test has been conducted to compare the effect of synbiotics on microbial changes in the vagina of pregnant women with bacterial vaginosis by looking at the changes in the levels of IL-10 and TNF-α before and after given synbiotics.

About 80 data before the research period. However, it produced a decrease of data as it was simply in the qualification of inclusion and exclusion criteria with 44 data as samples. From the sample, it divided the characteristics of the research sample based on age, parity, occupation, education and average gestational age as shown in Table 1.

The average maternal age of the research sample in group A and B were 20-35 years analyzed from 18 samples (81.8%). The average of parity in groups A and B were <2 with group A obtained from 20 samples (90.9%) and B obtained from 16 samples (72.7%). The majority of the research sample from group A and B were not working, from group A was 20 samples (90.9%) and group B was 19 samples (86.4%). The majority of the research sample in group A and B with education <12 years was 90% from 20 samples. The average of research sample pregnancy age in group A was 30.82 ± 5.68 and B was 28.77 ± 4.21 . There was no significant correlation between age, parity, occupation, education and gestational age with bacterial vaginosis before given synbiotic and after given synbiotic ($p > 0.05$).

Based on Table 2, there was an increase of the mean level of the IL-10 after treatment. In group A, the mean value of the IL-10 was higher than group B. In group A, the mean value before treatment was $222.13 \text{ pg mL}^{-1}$ and increased to $543.51 \text{ pg mL}^{-1}$ after treatment. The increase was $321.38 \text{ pg mL}^{-1}$. Meanwhile, in group B, the mean value before treatment is 96.24 pg mL^{-1} and increased to $278.89 \text{ pg mL}^{-1}$ after treatment. As shown in Table 2, the increase in group A was also greater than that of group B.

The result of the statistical test was the p-value of 0.000. The p-value less than 0.05 ($p\text{-value} < 0.05$) indicated the difference was statistically significant. It means that there was a correlation between IL-10 serum level in both groups before and after treatment.

As to reconsider, the mean level of TNF-α was decreased after treatment for both group. Before given treatment, the mean level serum of TNF-α in group A was $274.44 \pm 203.80 \text{ ng L}^{-1}$. After given clindamycin with synbiotics, the mean level of TNF-α decreased to

Table 1: Characteristic of the research sample

Variables	Group A		Group B		Total		p-value
	N	%	N	%	N	%	
Age (years old)							
<20	3	13.6	2	9.1	5	11.4	0.766
20-35	18	81.8	18	81.8	36	81.8	
>35	1	4.5	2	9.1	3	6.8	
Parity (amount)							
<2	20	90.9	16	72.7	36	81.8	0.081
2-5	1	4.5	6	27.3	7	15.9	
>5	1	4.5	0	0	1	2.3	
Occupation							
Not working	20	90.9	19	86.4	39	88.6	1.000
Working	2	9.1	3	13.6	5	11.4	
Education (year)							
<12	20	90.9	20	90.9	40	90.9	1.000
>12	2	9.1	2	9.1	4	9.1	
Gestational age (average)	30.82 ± 5.68		28.77 ± 4.21				0.182

Table 2: Comparison of the level of the IL-10 and TNF- α serum in pregnant women with bacterial vaginosis before and after treatment

Group treatments	Before treatment (Mean $\pm$ SD)	After treatment (Mean $\pm$ SD)	p-value
IL-10			
Group A (clindamycin+synbiotic)	222.13 $\pm$ 432.81	543.51 $\pm$ 984.96	0.000
Group B (clindamycin+placebo)	96.24 $\pm$ 170.51	278.89 $\pm$ 535.13	0.000
TNF-α serum (ng L⁻¹)			
Group A (Clindamycin+synbiotic)	274.44 $\pm$ 203.80	174.07 $\pm$ 174.78	0.000*
Group B (Clindamycin+placebo)	190.35 $\pm$ 149.70	110.19 $\pm$ 64.35	0.000*

174.07 $\pm$ 174.78 ng L⁻¹. While in the group B where clindamycin given with placebo, the mean serum level of TNF- α before treatment was 190.35 $\pm$ 149.70 ng L⁻¹ and it was decreased after treatment to 110.19 $\pm$ 64.35 ng L⁻¹. Based on Wilcoxon test shows that TNF- α serum level change was statistically significant because the $p < 0.05$.

There is the difference in the mean level of TNF- α in both groups. Based on the Mann-Whitney test, the difference of the mean level of TNF- α serum in both groups was statistically significant because $p > 0.05$. Thus, based on statistical results, the treatment in both groups was able to provide an advantage in improving TNF- α serum level.

DISCUSSION

In this study, researchers obtained 44 pregnant women with complain white vaginal discharge and were diagnosed with bacterial vaginosis based on Amsel criteria. About 22 pregnant women were treated with clindamycin and synbiotics and 22 of them were treated with clindamycin as standard treatment and placebo.

There was no difference in sample characteristics which is age, parity, occupation, education and gestational age. Authors obtained the $p > 0.05$ indicated that both sample groups were not significantly different in any equivalent and could be considered equivalent. In a study conducted by O'Sullivan *et al.*⁸, the elevated levels of both systemic and local cytokines in pregnant women with BV are not influenced by age of respondent, parity and gestational age.

This study demonstrated, there was a significant increase in IL-10 serum level in pregnant women with bacterial vaginosis after treatment in both groups.

There was a significant decrease in serum level TNF- α after therapy in both groups. Although the synbiotic group has elevated IL-10 serum level which is greater than the placebo group and the synbiotic group has decreased serum level TNF- α greater than the placebo group, the changes in both groups had no significant difference.

Based on research conducted by Lisangan *et al.*⁹, proven that on the effect of yogurt gift on anaerobic intestinal micro flora during clindamycin gift, the number of lactobacillus and

bacteroides remains stable in patients, whereas in the clindamycin group therapy, the amount of *Lactobacillus*, eubacteria, *Veillonella* and Bacteroides decreases during clindamycin gift and later increases after the end of the study. The number of Lactobacillus decreases on the 7th day and again increases on the 14th day. While in the group given the number of yogurts remains stable, although the improvement in the number of B lactis has been found.

The results of previous research are incompatible with this study. In this study, there is no significant difference between treatment with the gift of synbiotics and treatment by not giving symbiotic to pregnant women. The results of this study lead to the increased use of IL-10 as an indicator of preterm birth. This is consistent with a study conducted by Mastromarino *et al.*¹⁰ that the IL-10 level in women with preterm birth is higher as compared to women with term births. These results suggested that IL-10 level may be used as a predictor of preterm birth or other pathological conditions in pregnancy. As it is known that the IL-10 increases with bacterial vaginosis can cause preterm birth.

With the addition of synbiotics as an adjunctive therapy of bacterial vaginosis, it is able to provide better recovery and is expected to reduce the incidence of bacterial vaginosis recurrence when compared with only standard therapy. Various studies have demonstrated the manifestation of probiotic supplementation to restore the normal vaginal flora in women with bacterial vaginosis in both non-pregnant and pregnant women¹¹.

In this study, the sample group that has mean of level TNF- α before therapy is 274.44 $\pm$ 203.80 ng L⁻¹ and after clindamycin with given synbiotics has mean serum TNF- α dropped to 174.07 $\pm$ 174.78 ng L⁻¹ and that decreased serum TNF- α level found to be statistically significant. While in the control group, the serum level TNF- α before treatment is 190.35 $\pm$ 149.70 ng L⁻¹ and decreases after treatment is given to be 110.19 $\pm$ 64.35 ng L⁻¹, the decrease in TNF- α levels in this group is also found to be statistically significant.

The increased serum level TNF- α in both groups support the ongoing inflammatory process due to the infection related to bacterial vaginosis. Both the clindamycin group along with synbiotics and in the clindamycin group with placebo showed a marked decrease in serum level TNF- α .

The increased serum level TNF- α is certainly detrimental, especially if it is not controlled by reducing the incidence of infection that becomes a cause. As it is known that TNF- α is involved in the activation of prostaglandin synthesis where there is an increase of PGE2 production into the amniotic fluid during preterm labor-related infections, thus it stimulates the contraction of the uterus¹².

In this study, symbiotically with the probiotic contains of *Lactobacillus rhamnosus* (2×10^8 CFU) and *Lactobacillus acidophilus* (2×10^8 CFU) is used and given for 2 weeks.

The comparison of the mean difference of serum level TNF- α that decreases before and after therapy between groups are given clindamycin along with synbiotics and clindamycin group without synbiotics. The mean difference of serum TNF- α that decreases in clindamycin group along with symbiotic is 100.37 ± 119.57 ng dL⁻¹, while in clindamycin group without symbiotic is 80.16 ± 134.81 ng dL⁻¹. Statistically, no difference is found on the difference in the mean of serum level TNF- α in both groups. It suggested a decrease in serum level TNF- α as well in both therapy groups.

According to Bandyopadhyay and Mandal⁴, in their study, they find 8 weeks of symbiotic treatment for inflammatory markers TNF- α , a significant decrease in serum level TNF- α is found when compared to those given placebo in diabetes mellitus type 2.

In a study conducted by Larsson *et al.*¹³, where they address *Lactobacillus rhamnosus* and *Lactobacillus reuteri* at a dose of 2.5×10^9 CFU for 2 consecutive months after 7 days standard antibiotic therapy for bacterial vaginosis, they find no significant difference in the Nugent score on day 30 of post-therapy than in the placebo-treated group. But they find a significant difference in the probiotic group after 90 and 180 days of follow-up. It suggested that although probiotic treatment does not show any difference in the initial 30 days of monitoring, the probiotics continues to improve vaginal flora, so recurrence in the probiotic group is lower than in the placebo group.

In a study conducted by Larsson *et al.*¹³ proves that they use *Lactobacillus gasseri* and *Lactobacillus rhamnosus* as additional therapies after clindamycin on women with bacterial vaginosis. They find no difference to Nugent score after one month of therapy as compared to the placebo group. Both groups show similar improvement rates, but they find a significant difference after 2 and 6 months of therapy, where probiotic groups had a better effect on healing.

In this study, there was a change in the mean difference of decrease serum level TNF- α in both groups which is not statistically different. However, in a group with given synbiotics showed a greater mean difference in the decrease of serum level TNF- α than in the placebo group.

The absence of statistical differences in the two groups was due to the possibility of a short follow-up period of only 2 weeks, so there is not enough time needed by synbiotics to have a meaningful effect. If longer observation is conducted, in the group with synbiotics would probably showed a better decrease in TNF- α level after therapy than in the placebo group.

CONCLUSION AND SUGGESTION

It is concluded that there was a decrease in serum level TNF- α and elevated IL-10 serum level in pregnancy with bacterial vaginosis. Synbiotics may be considered as supplementation in bacterial vaginosis therapy during pregnancy, because it can lower the level of TNF- α larger and raise the level of IL-10 is greater than just given standard antibiotic therapy. We recommend that follow-up would be performed, so it is better to assess changes in the level of IL-10 and TNF- α in both studied groups. An assessment of clinical and microbiological changes are firstly required in each group before assessing the level of IL-10 and TNF- α . Further studies of recurrence and preterm delivery in each therapy group should be undertaken.

SIGNIFICANCE STATEMENT

This study discovers the new medical treatment that can be beneficial for pregnant women biological and medical problem. This study will help the researcher to uncover the critical areas of symbiotic effect on the levels of IL-10 and TNF- α that is conducted before and after the given symbiotic where many researchers were not able to explore. Thus a new comprehension on pregnant women medical handling may be arrived at.

ACKNOWLEDGMENT

The research on the effect of synbiotic on interleukin 10 and Tumor Necrosis Factor- α Serum Level in pregnant women with bacterial vaginosis has been empirically done. Reconsidering the result of the observation, it is proven after conducting a test that giving synbiotic to pregnant women can elevate IL-10 serum and decrease TNF- α level serum. For the best of help from the authors, this new comprehension of medical treatment for pregnant women expectedly be beneficial for the advances of pregnant women medical treatment. The giving of Synbiotics on pregnant women is seen as best solution to avoid any medical effect of pregnancy.

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