Research Article

Effectiveness of Oral Prebiotics as Adjuvant Theraphy in Reproductive Aged Women with Vaginal Discharge

Efektivitaas Probiotik Oral sebagai Terapi AjuvanKeputihan pada pasien usia reproduksi

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Abstract

Objective: To investigate the efficacy of oral probiotics and prove the high proportion of cure and satisfaction levels of post-treatment patients with a combination of antimicrobial-probiotic oral Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 compared to a combination of antimicrobial-placebo in the treatment of reproductive aged patients with vaginal discharge in the outpatient obstetrics and gynecologic clinic in Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia and Arifin Achmad Regional Hospital Pekanbaru, Riau, Indonesia.

Methods: This was a randomized, double-blind, placebo-controlled trial involving 50 subjects consisting of reproductive aged women. Data were collected using syndromic approach, probiotics were given as an adjuvant for standard antimicrobial therapy versus placebo as control, response was recorded 4 weeks later, for cure proportion and satisfaction level. Statistical analysis was performed to assess the variables. Interim analysis with conditional power assesment and futility testing were performed at midway due to insufficient sample size. Research was approved by Ethics Commitee for Health Researches Faculty of Medicine University of Indonesia Dr. Cipto Mangunkusumo Hospital in March 2016.

Results: A total of 50 subjects participated in this study and analyzed (25 subjects in each group), cure proportion 56% (14) of the treatment and 60% (15) on the control group, with relative risk of 1.1, Chi-square test p value 0.77 (95% CI; 0.57 to 2 , 11). High satisfaction level (score ≥67) was higher in the placebo (52.6%, 10 subjects) compared to probiotic group (47.4%, 9 subjects), p value 0.65 (≥0.05). Conditional power and futility testing curve, revealed Z = -0.2865, conditional power 0.11 to 0.13, and futility index of 0.87 to 0.88, equals to low possibility of statistical significance with full sample size (84).

Conclusion: There was no clinical and statistical difference in the proportion of cure rate and the level of satisfaction in patients of probiotics vs placebo groups after treatment for 4 weeks. The initial hypothesis of higher proportion of the cure rate in the treatment group still cannot be excluded, due to insufficient samples.

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Keywords: bacterial vaginosis, lactobacillus reuteri RC-14, lactobacillus rhamnosus GR-1, randomized double blind controlled trial, trichomoniasis, vaginal discharge, vulvovaginal candidiasis

Abstrak

Tujuan: Mengetahui efektivitas klinis dan dibuktikan tingginya proporsi kesembuhan dan tingkat kepuasan pascaterapi pasien kombinasi antimikroba-probiotik oral Lactobacillus rhamnosus GR-1 dan Lactobacillus reuteri RC-14 dibanding kombinasi antimikroba-placebo pada pengobatan pasien usia reproduksi dengan keputihan di poliklinik rawat jalan obstetrik dan ginekologi Rumah Sakit Dr. Cipto Mangnukusumo (RSCM) dan Rumah Sakit Umum Daerah (RSUD) Arifin Achmad Pekanbaru, Riau.

Metode: Penelitian ini merupakan penelitian uji klinis acak tersamar ganda dengan 50 subjek perempuan usia reproduksi, dengan 25 subjek pada setiap kelompok. Data dikumpulkan dengan pendekatan sindromik, pemberian probiotik sebagai ajuvan terapi antimikroba standar versus plasebo sebagai kontrol, dicatat respon terapi 4 minggu. Analisis statistik dilakukan untuk menguji variabel. Analisis interim, conditional power dan uji futilitas dilakukan di tengah penelitian karena jumlah sampel awal tidak tercapai. Penelitan ini sudah lolos kaji etik dan mendapat persetujuan pelaksanaan dari Komite Etik Penelitian Kesehatan FKUI-RSCM pada bulan Maret 2016.

Hasil: Sebanyak 50 subjek dapat terkumpul dan dianalisa (25 subjek pada tiap kelompok), proporsi kesembuhan 56% (14) pada kelompok perlakuan dan 60% (15) dari kelompok kontrol, dengan risiko relatif sebesar 1,1, nilai p 0,77 (95% CI; 0,57-2,11). Proporsi tingkat kepuasan tinggi (skor ≥67) lebih besar pada kelompok plasebo (52,6%, 10 subjek) dibanding kelompok probiotik sebesar 47,4% (9 subjek), dengan p 0,65 (≥0,05). Analisis conditional power dan uji futilitas, diperoleh nilai Z = -0.2865, conditional power 0,11-0,13 dan indeks futilitas 0,88-0,87, sehingga kemungkinan kecil penelitian akan bermakna bila dilanjutkan hingga tercapai sampel total (84).

Kesimpulan: Tidak ditemukan perbedaan proporsi yang bermakna secara klinis maupun statistik pada kesembuhan maupun kepuasan pada kelompok probiotik vs plasebo setelah terapi selama 4 minggu, namun hipotesis awal proporsi kesembuhan kelompok probiotik lebih tinggi belum bisa ditolak, karena jumlah sampel belum memadai.

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Kata kunci: bakterial vaginosis, keputihan, lactobacillus reuteri RC-14, lactobacillus rhamnosus GR-1, randomized double blind controlled trial, trichomoniasis, vulvovaginal candidiasis

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INTRODUCTION

Vaginal discharge is the most frequent gynecological complaint found in primary health care, with its annual visit amounted to 10 millions/year in the United States in 2004[1], which can be classified as normal or abnormal.[2] Abnormal vaginal discharge may cause discomfort, reduced productivity, and even serious reproductive complications in both pregnant and non pregnant women, if left untreated.[2] The most common cause of abnormal vaginal discharge is bacterial vaginosis (BV) in 22-50% cases, vulvovaginal candidiasis (VVC) in 17-39% cases, and trichomoniasis in 4-35% cases.[2]

The condition is caused by imbalance of normal vaginal flora (i.e. reduced lactobacilli and increased comensal/pathogenic microbes such as G. vaginalis, Mycoplasma hominis, Bacteroides species, Peptostreptococcus species, Fusobacterium species, Prevotella species, and Atopobium vaginae) in the vagina that caused increased vaginal pH, reduced protection of vaginal mucosa, and increased probability of invasion from other microbes that may result in abnormal vaginal discharge.[2,3]

Several risk factors that may contribute to the condition include age below 25 years of age or being older than 40 years old, uncircumsized male sexual partner, black ethnicity, sexual partner more than one, changing sexual partner within the last 30 days, female to female sexual encounter, immunodeficiency (e.g. HIV), diabetes mellitus, poor vaginal hygiene, sexual intercourse more than once a week, after vaginal delivery or postabortal patients, and vaginal douching.[4-8]

The diagnostic flowchart above is practical to be used in general everyday primary health outpatient service without the use of other diagnostic tools. This approach is known as the syndromic approach.[9]

Probiotics are defined as live microorganisms that may confer health benefit to the human host if given in sufficient amount.[10] Studies have already proved the effectiveness of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 either as adjuvant or main treatment of vaginal discharge caused by BV, VVC, notably by Anukam et al in 2006, where the treatment group of oral probiotics has increased rate of cure (88% vs 40%) compared to controls, in a randomized double
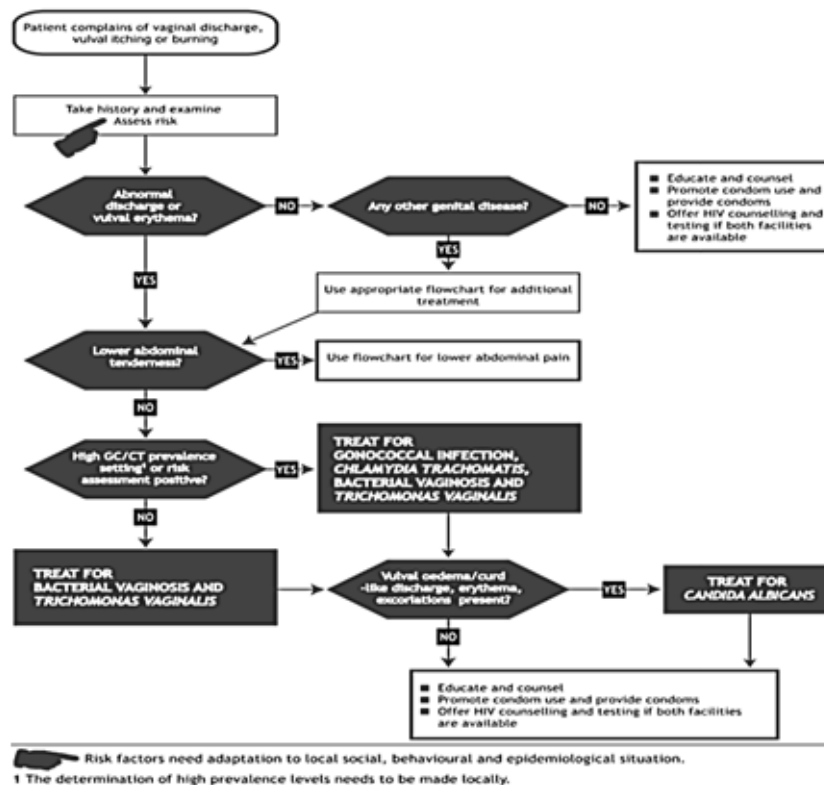

Figure 1. WHO Syndromic Approach Flowchart for Vaginal Discharge

blind controlled trial study[11] or other successful trials to prove the effectiveness of oral probiotics in skim milk form (Reid et al) or capsules (Martinez et al) to cure vaginal discharge caused by VVC.[10,12]

Trials involving the use of oral probiotics for vaginal discharge in Indonesia have never been conducted. This study is determined to see if the use of similar oral probiotic containing Lactobaci-llus rhamnosus GR-1 and Lactobacillus reuteri RC-14 will confer similar benefits for Indonesian women as other studies above.

OBJECTIVES

In this study, the proportion of patients of reproductive age cured clinically of vaginal discharged was significantly higher on combined antimicrobes- oral probiotics Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 compared to combined antimicrobes-placebo after 4 weeks treatment. Therefore the goal was to acknowledge the clinical effectiveness and prove the high proportion of cure and satisfaction levels of post-treatment patients with a combination of antimicrobial-probiotic oral Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 compared to a combination of antimicrobial-placebo in the treatment of patients of reproductive age with vaginal discharge in the clinic outpatient obstetrics and gynecology, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia, and Arifin Achmad Regional Hospital Pekanbaru, Riau, Indonesia.

METHODS

This was a randomized, double-blind, placebo-controlled trial with initial sample size of 84 subjects, and by consecutive sampling, only 50 subjects were able to be analyzed with target population of reproductive age women who visited with complain of vaginal discharge to outpatient clinic at Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia and Arifin Achmad Hospital Pekanbaru, Riau, Indonesia. Subjects age are between 15-49 years old. Inclusion criteria in this study were; reproductive age women, with vaginal discharge complain, married or had had sexual intercourse, and signed consent to be included in the study. Exclusion criteria were physiologic vaginal discharge, consuming antibiotics or corticosteroid, had HIV, diabetes mellitus, currently pregnant, postpartum/post-abortion in the past 40 days,

menstruation, sexual partner more than one, cervical polyp, malignancy, urogenital fistula, allergic reaction, menopausal women, allergy to the antimicrobes or probiotics used in this study.

After consenting to the study, the subjects then underwent history taking and physical examination by using a special case report form, by syndromic approach. The patient was then given standard antimicrobes according to the suspected findings (BV, VVC, Trichomoniasis, or combined) and randomized to have oral probiotics containing each $2,5 \times 10^9$ Lactobacillus rhambosus GR-1 and Lactobacillus reuteri RC-14, or placebo by contacting the pharmacy prior to administration. The adjuvan had already packaged in a way that is difficult to differentiate between treatment and placebo (blinding) by the central pharmacy. The patient was then instructed to return for visit after 4 weeks of daily probiotics.

All statistical analyses were performed using SPSS17 for Windows. The Chi Square test was used to analyze the difference of proportion of cure and the level of satisfaction between the groups. Interim analysis, conditional power and futility analysis was performed, since the sample size was lower to determine the probability of significancy had the study continued to full sample size.

RESULTS

A total of 50 subjects were recruited in this the study. Interim analysis was performed, with O'Brien-Flemming at sample size 24 and 50 women, with p=1 (p>0.05) on both analysis, corresponds to insignificance results We performed conditional power and futility analysis. The z score the time of 50 sample size is -0.2865, which equal to conditional power of 0.11-0.13 corresponding to futility index of 0.87-0.89, therefore the study will less likely to achieve statistical significance even if continued to full sample size (futility index > 0.8).

The subjects age are between 22-48 years old reproductive aged women. The age distribution followed the normal distribution curve on all respondent (Saphoro-Wilk; p = 0.29). Age of the subjects were 15-24 years old (10%), 25-34 years old (43%), 25-44 years old (44%). The mean age of treatment group was 35.7 years old (SD 6.17) and 34.6 years old (SD 7.17) on the treatment and control group respectively. Most of the subject age

were between 35-44 years old on treatment (48%) and control (40%) group.

Majority of the subjects on treatment group was employee (64%). No multiple sexual partners was noted on both groups. The frequency of sexual intercourse was more than 1 time per week on both group 84% and 88% on treatment and control group respectively, vaginal douching habit was found on 40% subject on both groups, no subject was found to have multiple sexual partner.

Proportion of Cure

In this study, the cure proportion of all subjects was 58%. The proportion of cure was 56% and 60% on treatment and control group respectively, with relative risk on the uncured subjects was 1.1 (p=0.77, 95% CI = 0.57-2.1) as seen on Table 1, also the following calculation below.

Table 1. Cure Distribution on Treatment and Control

Groups	Not cured		Cure		Total	
	n	%	n	%	n	%
Treatment	11	44	14	56	25	50
Expected count	10.5		14.5			
Control	10	40	15	60	25	50
Expected count	10.5		14.5			
Total	21		29		50	100

Experiment Event Rate (EER) = $\dfrac{a}{a + b}$ = 0.44

Control Event Rate (CER) = $\dfrac{c}{c + d}$ = 0.4

Relative Risk (RR)= EER / CER = 0.44/0.4 = 1.1

Satisfaction level was recorded using specific form based on treatment satisfaction Questionnaire for Medication (TSQM VERSION II). The most common satisfaction level in treatment group is on the medium level (score 34-66), that is 44% (CI 90%, 23.1-64.9), followed by high satisfaction level (36%) and lastly is low satisfaction level (20%). On the control group, however, the majority on satisfaction level is at high level (score ≥67) on 40 % of subjects (CI 95%, 19.4-60.6), followed by medium level (32%) and low level of satisfaction (28%).

On comparison (Table 2), low and high satisfaction level (score 0-33) is mostly seen on control (placebo) group (58.3 and 52.6% respectively) compared to that of treatment (probiotics) group (41.7 and 47.4% respectively), while on the medium satisfaction level mostly observed on treatment group (57.9%) compared to that of control (42.1%). Statistical test revealed p 0.65 (p ≥ 0.05), corresponds to no significant difference of treatment and control (placebo) group.

On the sexual intercourse analysis, the respondents that were cured mostly had sexual intercourse frequency of more than one time per week, as much as 86.2%. Similarly, that uncured subjects had similar frequency of sexual intercourse as much as 85.7%. The result was statustically analyzed and reveled p = 1.0 (p ≥ 0.05) and relative risk of 1, corresponds to insignificant difference between the groups.

Multivariate analysis for confounding variable was not performed in this study due to no ethnical difference of both treatment (probiotics) and control (placebo) group and also of no apparent significant difference on sexual intercourse frequency analysis, therefore was not fulfilling the requirement of (p < 0.25) significance level to be included in multivariate analysis.

Table 2. Comparative Proportion of Treatment Satisfaction Level of Treatment (Probiotics) and Control (Placebo) Group

Satisfaction Level	Treatment (Probiotics)		Control (Placebo)		Total		p value
	n	%	n	%	n	%	
Low	5	41.7	7	58.3	12	100	
Medium	11	57.9	8	42.1	19	100	0.65
High	9	47.4	10	52.6	19	100	
Total	25	50.0	25	50.0	50	100	

The sample size for treatment and control group was similar (25 subject each), this size was smaller to initial sample size of total 84 subjects, corresponds to lower power as have been discussed earlier on the result. The subjects were mostly at the age of 35-44 years old and normally distributed. Difference in demographic data was seen on the education level, whereas on the treatment group, half of the subjects had duration of education of more than or equal to 13 years, while on the control group, duration of education was equally distributed on two subgroups (duration of education of 10-12 years and more than or equal to 13 years) as much as 36% on each subgroups. On both groups, we did not find any subject with multiple sexual partner and frequency of sexual intercourse on both groups were more than once a week (probiotics 84%, placebo 88%). Subjects on the treatment group were mostly working (64%) reversely different with that of the placebo group. There was no side effect or adverse incident related to the study procedure in this study.

We found that the proportion of vaginal discharge patients that were cured was mostly on the control (placebo) group as much as 15 subjects (60%) compared to the treatment (probiotics) group as much as 14 subjects (56%). However this difference was only for 1 subject, with effect difference of 4%, and relative risk calculation of 1.1. Based on these result, (effect size 4% of 20% expected initially), these results were statistically and clinically insignificant. Moreover, Chi Square test revealed p 0.77 (>0.05), thus the difference between the groups was insignificant.

From the satisfactory level, we found that the placebo group had more satisfactory level according to subjects compared to the probiotics groups, and statistical test did not reveal significant difference of three satisfaction level between the groups (p 0.65).

On the analysis based on frequency of sexual intercourse, the cure rate was higher on group with more than one time sexual intercourse per week (86%) compared to 13.8% on the other group. Theoritically, frequency of sexual intercourse was considered to be connected to vaginal ecosystem and increased risk of BV, by reducing colonization of H_2O_2 forming lactobaccili or to increase BV related species such as Gardnerella vaginalis.[13] Contradictively similar result was reported by

Foxman on his case control study in 1990, including 85 women, that stated sexual intercourse frequency of more than one per week is related to increased risk of VVC with OR 1.73 to 2.98 increasing with frequency per week.[14] However, a cross sectional study in Indonesia by Dwiana Ocviyanti et al in 2010 on 492 women aged 15-50 years old, regarding risk factors for BV did not mention that sexual intercourse frequency per week to be considered a risk factor, instead that it was found that uncircumsized male (OR 6.25) and age of more than 40 years old (OR 3.15).[8] While other observational cohort study in 2011 revealed that digital insertion sexual act is related to increased colonization of Gardnerella vaginalis and the researchers also stressed on the notion that sexual intercourse frequency indeed did not relate with increased risk of BV.[13] So we can conclude that frequency of sexual intercourse per week did not relate with increased risk of vaginal discharge on both groups. In this study, there was also no ethnical difference between the subjects therefore was not included in analysis since all the subjects was of Asian origin.

Insufficient sample in this study was due to difficulty in fulfilling the initial 84 subjects during the study process, particularly on Arifin Achmad Hospital, resulting in subjects only collected from Dr. Cipto Mangunkusumo Hospital (50 subjects, 25 in each group). The difficulty was due to luckiness of patients that fulfill the inclusion criteria for the study and high drop out particularly on Arifin Achmad Hospital. However, we predicted that this luckiness of subjects, will not affect the outcome had the study had sufficient sample size was achieved, as with futility index testing of 0.87-0.89. Addition of sample up to initial size of 84 subjects will not change the result to be statistically significant, since it was not significant in 50 subjects.

Anukam et al and Martinez et al had conducted similar studies in 2006 and 2009, respectively, with the former was on bacterial vaginosis patients and the latter was on candidiasis patients, which both yield considerably different results from both studies, 88% (p<0.001) cure rate on the former and less discharge (10.3% vs 34.6%, p=0.014) on the latter, which most probably due to tighter inclusion criteria (using Nugent criteria, sialidase enzyme, culture) and outcome analysis which was compared using culture, therefore resulting in more accurate outcome from only depending on

clinical-syndromic approach as applied in our study.[11,12] Sialidase enzyme is produced by anaerobic bacteria such as Prevotella and Bacteroides spp, that promotes bacterial adhesion toward vaginal epithelium and musinase activity, helping vaginal mucose invasion by BV related bacterias.[15] This enzymatic assessment is superior compared to Nugent and Amsel criteria with sensitivity of 88% (95% CI, 81 to 93%) and spesitivity of 95% (95% CI, 91 to 98%).[15]

This study used probiotics as adjuvant of standard antimicrobial treatment, therefore it is difficult to assess difference of cure on both groups, considering that antimicrobial treatment alone in this study (placebo group) was capable of resulting in 60% cure rate compared to treatment group, with effect size of 4%, far below expected of 20%. One literature also mentioned that standard metronidazole treatment as applied in this study for BV treatment based on CDC guideline 2015 alone may result in cure rate as much as 70%.[16]

Another weakness of this study, besides the humble syndromic-clinical criteria used, is also due to difficulty in collecting samples at the second hospital (Arifin Achmad Hospital Pekanbaru), whereas after 10 months of study near the expired date of probiotics used in this study, subjects fulfilling inclusion and exclusion criteria were only 4 samples, and none of them return for re-assessment (drop out).

Moreover, in this study, one of the possibilities of insignificant result of the study was due to the difference of interpretation of physicians assessing each subjects that are changing (due to resident's rotation) every month, albeit already trained by us, may contribute to variation of cure assessment from one physician or another. Therefore, there is a possibility of enhancing the interpretation had we use other objective means, such as photography documentation.

Therefore we may that insignificant comparison in proportion of cure on both groups in this study that is not different from one another is due to lackness of sample size, inaccurate inclusion criteria due to only using syndromic-clinical approach, and response assessment that was not confirmed by culture result such as previously mentioned similar studies.[11,12]

The results are potential to be studied further by tightening the inclusion criteria with Nugent and Amsel criteria, sialidase enzyme detection, or culture, to ensure that the clinical results observed are in accordance to laboratory confirmation for more objective assessments. This study is the first study in Indonesia done with double blind Randomized Clinical Trial to prove the clincial effectiveness of combined antimicrobes-oral probiotics Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 compared to antimicrobes-placebo for treatment of reproductive age women with vaginal discharge.

CONCLUSION

There was no clinical and statistical difference in the proportion of cure and the level of satisfaction in patients of reproductive age with vaginal discharge in the treatment with combination of antimicrobial-oral probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 compared to combination of antimicrobial-placebo after treatment for 4 weeks. However, in this study, the initial hypothesis of higher proportion of cure at the treatment group (probiotic) compared to placebo still cannot be excluded, due to insufficient samples collected.

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