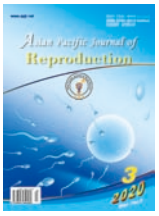

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Efficacy of standard therapy with synbiotic or without synbiotic to reduce *Gardnerella vaginalis*, *Atopobium vaginae* and *Megaesphaera* phylotype Ⅰ in pregnant women with bacterial vaginosis

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ABSTRACT

Objective: To evaluate whether addition of symbiotic to clindamycin could reduce *Gardnerella vaginalis*, *Atopobium vaginae*, and *Megasphaera* phylotype Ⅰ in pregnant women with bacterial vaginosis.

Methods: This randomized controlled trial (RSUP Dr. Wahidin Sudirohusodo Makassar clinical trial registry UH17010021) included 61 samples. The intervention group was given clindamycin and synbiotic while the control group was given clindamycin and placebo (without synbiotic). Wilcoxon test and hypothesis test of two independent samples were used to compare the treatment efficacy.

Results: This study showed a significant difference in Nugent score before and after treatment in each group. But there was no difference in Nugent score between the intervention group and the control group after treatment or in Nugent scores reduction in both groups. The most common type of bacteria found was *Megasphaera* phylotype Ⅰ. There were no significant differences in the three types (*Gardnerella vaginalis, Atopobium vaginae*, and *Megasphaera* phylotype Ⅰ) of bacteria after treatment between both groups. Additionally, there was no difference in therapeutic effect between the intervention group and the control group.

Conclusions: Clindamycin along with synbiotics is no more effective for treated bacterial vaginosis than clindamycin without synbiotics. *Megaesphaera* is the most commonly found bacteria, which cannot be eradicated with clindamycin.

KEYWORDS: Bacterial vaginosis; Synbiotic; Nugent score; Amsel criteria

1. Introduction

The imbalance of healthy vaginal flora caused by a decrease in the proportion of *Lactobacillus*, resulting in excessive growth of anaerobic bacteria, could characterize bacterial vaginosis[1–4]. This condition was associated with an increased risk of developing pregnancy complications such as preterm labor, low birth weight, and pelvic inflammatory disease and could increase the risk of sexually transmitted diseases[5]. Pregnant women who met the criteria for being diagnosed with bacterial vaginosis were about 10%-30%, but some of them were asymptomatic[6–8].

Gardnerella (G.) vaginae were not the only bacterial that was caused by bacterial vaginosis, although it exhibited high sensitivity (100%) and low specificity (49%)[9,10]. Presumably, there was a relationship between *G. vaginae* with *Atopobium (A.) vaginae*, because both bacteria set together and that *A. vaginae* were very rarely found alone[11]. Another bacteria was *Megasphaera* associated with biofilms in the vagina[12].

When it met the Amsel criteria or used a wet mount or Gram staining to calculate a Nugent score, the diagnosis of bacterial vaginosis performed. The method could not identify some of the bacterial morphotypes associated with bacterial vaginosis. By using a polymerase chain reaction (PCR) test, other suspected bacteria that were associated with bacterial vaginosis could be diagnosed[5,13].

Standard therapy for bacterial vaginosis was metronidazole or clindamycin in either orally or intra-vaginally, with a cure rate of about 80%[14–16]. A previous study reported that a cure rate

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had reached 48%-96% on the administration of both antibiotic with a recurrence rate of 49%-66% after seven days of treatment. Although the treatment was effective in preventing the proliferation of anaerobic bacteria, it did not automatically restore the typical vaginal ecosystems, so it was possible to be relapsed or recurrent[7]. The treatment with antibiotics was also associated with other side effects and disadvantages, such as the occurrence of super-infective of other pathogenic microorganisms[16,17].

Synbiotics were supplements containing probiotics and prebiotics that played a role in stimulating *Lactobacillus* growth, potentially optimizing, restoring, and maintaining the vaginal ecosystem[18]. Vaginal infections, especially bacterial vaginosis on pregnancy that would increase the risk of preterm labor and subsequently associated with perinatal mortality and morbidity, the inefficient current standard treatment, drug resistance, and high recurrence rates[19,20] were also the reasons for this study. This study has compared the effect between clindamycin with synbiotic and clindamycin without synbiotic against *G. vaginalis*, *A. vaginae*, and *Megasphaera* using PCR examination. Based on Amsel criteria and Nugent score, bacterial vaginosis was clinical. This study is expected to be significant in developing studies in women medical health. Principally, this research comes to offer any idea to prevent bacteria in women pregnancy.

2. Materials and methods

2.1. Design of study

This study was a double-blind clinical trial of pregnant women suffering from bacterial vaginosis that met the inclusion criteria as approved and registered at RSUP Dr. Wahidin Sudirohusodo Makassar clinical trial registry UH17010021. This study classified pregnant women into two groups. The intervention group was treated with clindamycin+synbiotic, while the control group was treated with clindamycin+placebo. The determination of the order of groups based on tables was according to the randomization of the block. There were 80 research samples, but only 61 samples were analyzed (Figure 1). The sample consisted of 33 pregnant women with bacterial vaginosis treated with clindamycin along with synbiotic and 28 people treated with clindamycin and placebo.

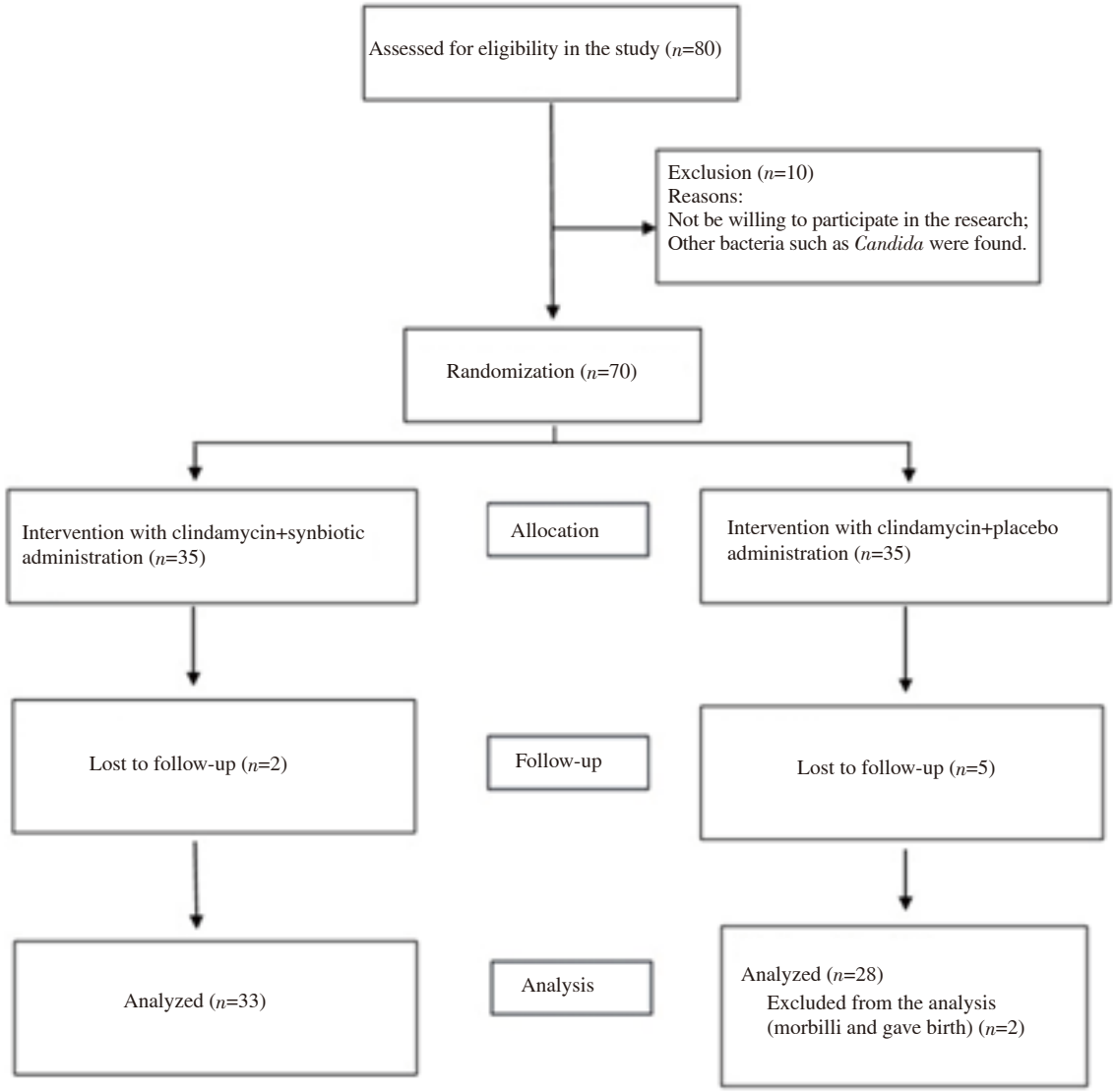

Figure 1. Flowchart of screening of participants.

2.2. Population of study

The inclusion criteria were pregnant women with single pregnancy, gestational age ≥20 weeks with bacterial vaginosis based on Amsel criteria [3 of 4 criteria that involved: a) Thin, white, yellow, homogeneous discharge; b) Clue cells on wet mount microscopy; c) a vaginal fluid pH of over 4.5 when placing the discharge on litmus paper, and d) Release of fishy odor when adding 10% potassium hydroxide solution to wet mount - also known as "whiff test"], no blood in the vaginal fluid, no suspicion of other vaginal infections (candidiasis, trichomoniasis) during clinical examination, factually did not use antibiotic and vaginal douching in the last two weeks. They had agreed to take part in this study by signing a consent letter. The exclusion criteria were neither taking drugs nor synbiotic regularly, and Gram staining results could not be read or damaged preparations, not willing to participate in research and examination.

The study was conducted at Hasanuddin University Education Hospital. This study period commenced in December 2016 until the sample size was sufficient.

2.3. Study interventions

The intervention group was given 300 mg oral clindamycin twice per day for 7 days and consumed synbiotic twice daily for 14 days. Oral synbiotic contained *Lactobacillus rhamnosus* (2.0×10^8) CFU, *Lactobacillus acidophilus* (2.0×10^8) CFU, *Bifidobacterium longum* (8.5×10^7) CFU, *Bifidobacterium bifidum* (8.5×10^7) CFU, streptococcus thermophiles (6.8×10^8) CFU (probiotics), and fructo-oligosaccharide 509.08 mg (prebiotics). The control group was given clindamycin (2.0×300) mg orally for 7 days and milk (as placebo) containing no probiotics and prebiotics twice daily for 14 days.

All participants that met the inclusion criteria were taken and had given informed consent to be a research sample. The research participants were examined by using a speculum and vaginal smears to confirm the diagnosis of bacterial vaginosis based on Amsel criteria. The vaginal smears of pregnant women diagnosed by bacterial vaginosis were followed by Gram stain examination to calculate the score based on the Nugent criteria and PCR test to assess *G. vaginae*, *A. vaginae*, *Megaesphaera* phylotype Ⅰ. This study had done Gram-staining and PCR tests before and after administration treatment.

A diagnosis of bacterial vaginosis was made if 3 of the 4 Amsel criteria were met, or a wet mount or Gram staining was used to calculate a Nugent score. The Amsel criteria were: 1) fishy-odor or amine-like when given potassium hydroxide (whiff test); 2) Gray-colored or white homogeneous vaginal discharge; 3) Increased vaginal acidity (4.7-5.7); 4) Clue cell found, squamous cell covered by bacteria on examination by using wet preparations. Nugent criteria were determined with quantification of bacterial morphotypes by using a microscope on vaginal smears that were given Gram staining. Scoring ranges were from 0-10. It was normal if the score was 0-3. It was intermediate if the score was 4-7. If the score was more than 7, it indicated a bacterial vaginosis.

2.4. Randomization

The grouping of samples into the intervention group and the control group was done in double-blind. The grouping of samples was regulated by using the randomization of blocks. After fulfilling the inclusion criteria, the sample was determined whether it belonged to the intervention group and the control group by the nurse, and the medication was given by the nurse. Drug packaging for the intervention group and the control group had the same packaging.

2.5. Study outcomes

Healing bacterial vaginosis based on Amsel criteria change and Nugent score, and identification of *G. vaginae*, *A. vaginae*, *Megaesphaera* phylotype Ⅰ and its changes in post-treatment.

2.6. Sample size

In this study, the sample size in each intervention and control group was calculated based on the following formula:

$$n = \frac{2}{d^2} \times C_{p,\,power}$$

n was the number of samples required in each intervention and control group, d was the standardized difference that measured the combined standard deviation between the two proportions of the intervention and control groups, while $C_{P,\,power}$ was a constant defined from the P-value (5%) and the test strength (power = 80%). The following formula was used to measure the standardized difference:

$$d = \frac{(p_1 - p_2)}{\sqrt{[\bar{p}(1-\bar{p})]}}$$

p_1 and p_2 were the proportions of recovery from bacterial vaginosis assessed based on the Nugent score obtained in previous research, as 80% in oral probiotics and 40% in the control, and p was the mean of both proportions (0.8+0.4)/2 = 0.6. The value of d in this study was:

$$d = \frac{(0.8\text{-}0.4)}{\sqrt{0.6(1\text{-}0.6)}} = \frac{0.4}{\sqrt{0.24}} = \frac{0.4}{0.49} = 0.82$$

Using the P-value of 5% and the strength of the test of 80%, the minimum sample size in each intervention and control group was 24 research samples.

2.7. Statistical analysis

These data were collected, analyzed, and processed with SPSS version 18 for Windows program. Shapiro-Wilk normality test was used to check data normality. Non-parameter was analyzed with Wilcoxon test (Mann Whitney test). *Chi*-square statistic test for analysis of characteristic of the samples. $P<0.05$ was considered as statistically significant.

2.8. Ethical approval

This study requested the ethical goodness of the Biomedical Research Ethics Commission on Human, Medical Faculty, Hasanuddin University, Makassar, Indonesia and obtained legal, ethical permission as 82/H4.8.4.5.31/PP36-KOMETIK/2017 by a leader of ethics Prof. Dr. dr. Suryani As'ad, M.Sc., Sp.GK and secretary Dr. Agussalim Bukhari, M.Med., Ph.D., Sp.GK.

3. Results

3.1. Samples characteristics

The result of the *Chi*-square statistic test showed that the samples between the two groups did not have significant differences in age, education, occupation, and gestational age as a homogenous sample group ($P>0.05$) (Table 1).

3.2. Results of sample identification after tests

Based on Shapiro-Wilk normality test, this study found that the difference between Nugent scores before and after the intervention had a normal distribution. Based on the Mann Whitney test, it was found that there was no significant differences in Nugent score before and after treatment either in clindamycin+synbiotic group or clindamycin+placebo group ($P>0.05$). After treatment, there was no difference in Nugent scores between clindamycin+synbiotic group (30.44) and clindamycin+placebo group (31.66) ($P=0.305$) with Student's *t*-test. More healing occurred in the clindamycin+synbiotic group (15 people) than the clindamycin+placebo group (13 people).

Finally, based on the PCR examination before the treatment, it appeared that *Megasphaera* phylotype Ⅰ was found in 95.1% (58/61) of bacterial vaginosis sufferers in pregnant women who were studied. For *A. vaginae* and *G. vaginae*, the corresponding data were 19.7% (12/61) and 49.2% (30/61), respectively.

3.3. Differences in PCR result of G. vaginalis, A. vaginae, Megasphaera phylotype I before and after treatment

Based on Mc Nemar test, there was no difference in PCR results of *G. vaginae*, *A. vaginae* and *Megasphaera* phylotype Ⅰ before and after treatment ($P>0.05$). It suggested that the administration of clindamycin+synbiotic or clindamycin+placebo show no difference to reduce *G. vaginae*, *A. vaginae* and *Megasphaera* phylotype Ⅰ (Table 2).

Table 1. Characteristics of the control and intervention groups [*n*(%)].

Variables	Control (*n*=28)	Intervention (*n*=33)	Total	*P*	*df* (Pearson *Chi* square)
Age (years)					2
15-24	9(32.1)	16(48.5)	25(41.0)		
25-35	17(60.7)	15(45.5)	32(52.5)	0.430	
>35	2(7.1)	2(6.1)	4(6.6)		
Education					2
Junior high school	1(3.6)	6(18.2)	7(11.5)		
Senior high school	26(92.9)	25(75.8)	51(83.6)	0.170	
College	1(3.6)	2(6.1)	3(4.9)		
Job					1
Working	26(92.9)	32(97.0)	58(95.1)	0.459	
Not working	2(7.1)	1(3.0)	3(4.9)		
Gestational age (weeks)					1
20-28	12(44.4)	13(39.4)	25(41.7)	0.693	
29-40	15(55.6)	20(60.6)	35(58.3)		

Note: *Chi*-square test is used.

Table 2. Different results of *G. vaginalis*, *A. vaginae* and *Megasphaera* phylotype Ⅰ before and after treatment using PCR test [*n*(%)].

Treatment		*Gardnerella vaginae*			*Atopobium vaginae*			*Megasphaera* phylotype Ⅰ		
		Before treatment	After treatment	*P*	Before treatment	After treatment	*P*	Before treatment	After treatment	*P*
Clindamycin+Synbiotic (*n*=33)	Negative	15(45.5)	22(66.7)	0.065	24(72.7)	30(90.9)	0.070	2(6.1)	5(15.2)	0.375
Clindamycin+Placebo (*n*=28)	Negative	16(48.5)	22(66.7)	0.109	25(75.8)	27(81.8)	0.500	1(3.0)	2(6.1)	1.000

Note: Mc Nemar test is used.

3.4. Difference in effectiveness of clindamycin+synbiotic compared to clindamycin+placebo

In this section, there were no significant differences in effectiveness between the clindamycin+synbiotic group and the clindamycin+placebo group. Therefore, it was concluded that clindamycin+synbiotic treatment was no more effective than clindamycin+placebo.

4. Discussion

Synbiotic was a combination of prebiotics to improve health. The purpose of giving prebiotics together with probiotics was to make good bacteria contained in probiotics could survive[21-23]. Prebiotics that contained oligosaccharide, fructose-oligosaccharide, or inulin in the soybean could increase the resistance of *Lactobacillus acidophilus* LAFTI L10, *Bifidobacterium lactic* LAFTI B94 (B94) or *Lactobacillus casei* LAFTI L26 LAFTI to survive *in vivo*. In the vaginal ecosystem, synbiotic allegedly optimized, maintained and repaired the natural microbes in the vagina[24,25].

The primary purpose of giving synbiotic was to increase the concentration of *Lactobacillus* in the vagina[26]. Based on research conducted by Reid *et al*[27], the oral administration of probiotics containing *Lactobacillus rhamnosus* and *Lactobacillus fermentum* (2 times daily for 14 days) in women with asymptomatic infection and having a history of recurrence of candida infections, bacterial vaginosis and urinary tract infection, showed improvement of bacterial colonization of bacterial vaginosis within 1 week after consumption. Giving *Lactobacillus* strain orally could improve vaginal flora.

The results of this study showed there is no significant difference in age, education, occupation, and gestational age between the clindamycin+synbiotic group and the clindamycin+placebo group. The absence of differences in sample characteristics reduced the risk of bias in terms of sample selection.

Rehman *et al*[28] reported that administering probiotics together with clindamycin might increase good bacteria such as *Lactobacillus* compared with probiotics given after antibiotic. In that study, it was concluded that giving probiotics along with clindamycin was useful to stabilize intestinal metabolic hemostatic by reducing toxic metabolism and preventing damage to endogenous microbiota. This was consistent with this study's data that there was an increase in the concentrations of large Gram-positive rods (*Lactobacillus* morphotype) in both intervention groups, and more increases were found in samples given clindamycin+synbiotic than clindamycin+placebo.

Based on a study conducted by Sullivan *et al*[29] on the effect of administration of yogurt on anaerobic intestinal microflora during clindamycin administration, the number of *Lactobacillus* and *Bacteroides* remained stable in patients given clindamycin along with yogurt, whereas in the treatment group, there was only clindamycin given. The amount of *Lactobacillus*, *Eubacteria*, *Veilonella*, and *Bacteroides* decreased during clindamycin administration and increased again after the end of the study. Sullivan *et al* report that the number of *Lactobacillus* decreased on the 7th day and increased again on the 14th day, while in the group given yogurt, the number remained stable and found its improvement in the number of type B lactic. There is a report that *Lactobacillus* is susceptible to clindamycin. However, in this study, we found that the amount of *Lactobacillus* not decreased after treatment in both groups[17].

In this study, we also obtained eleven samples in the clindamycin group with synbiotics and six samples in the clindamycin and placebo group who still showed an abnormal Nugent score (score 4-6, intermediate). Coste *et al*[7] reported that prebiotics administration might improve vaginal flora to return to normal, although from the results of the 8th and 24th days, some samples still indicated abnormal Nugent score (33% and 16%). Several published studies had reported that *G. vaginalis* could be detected in women who did not clinically meet the criteria for bacterial vaginosis. However, this study generally did not define optimal flora or normal flora and did not determine whether women with intermediate Nugent score (score 4-6) met criteria for diagnosis of clinical bacterial vaginosis or women with normal vaginal flora[6].

In this study, there was no difference in the effectiveness of clindamycin treatment along with synbiotic *versus* clindamycin and placebo. Similarly, the mean difference in Nugent score reduction in both groups did not show any significant difference.

In this study, most bacteria were found as *Megasphaera* phylotype Ⅰ (95.1%). It was similar to the results reported by Fredricks *et al*[30] that *Megasphaera* phylotype Ⅰ was detected in 95.0% of the 264 participants who suffered from bacterial vaginosis through PCR test. In addition to *Megasphaera*, they also found three types of bacteria, namely *Clostridiales* (BVAB 1-3), *Leptotrichia/Sneathia*, *A. vaginae*. Similar results were reported by Tamrakar *et al*[31] that bacterial vaginosis-related bacteria, including BVAB2, *Megasphaera*, *Leprotrichia*, and *Eggerthella*-like bacterium were associated with bacterial vaginosis in 163 pregnant women in Japan. Similarly, Hinchlifle *et al*[32] reported that *Megasphaera* phylotype Ⅰ was more common than *Megasphaera* phylotype Ⅱ (76% *vs.* 22%), and the number of bacterial colonies of *Megasphaera* phylotype Ⅰ was much higher (5×) in all patients with bacterial vaginosis than in women with healthy flora. It should be noted that although *Megasphaera* phylotype Ⅰ was strongly correlated with bacterial vaginosis, it could still be detected in some clinically normal women with Nugent scores as well as on PCR.

Nelson *et al*[33] and Ferris *et al*[34] reported that *Megasphaera* phylotype Ⅰ was associated with a risk of spontaneous preterm

labor (odds ratio 6.2, 95% *CI*: 1.9-20.6). An inadequate diagnosis, antibiotic resistance, and the presence of polymicrobial biofilms inherent in the vaginal epithelium, including *A. vaginae* and *G. vaginalis* were the main components suspected to be the cause of unsatisfactory treatment outcomes. *A. vaginae* were resistant to metronidazole but were responsive to clindamycin. Unfortunately, clindamycin also destroyed *Lactobacilli* that served to produce lactic acid to maintain the normal vaginal ecosystem[13]. On the contrary, in this study there was no significant decrease in the amount of large Gram-positive rods (*Lactobacillus* morphotype) in both groups.

In conclusion, this study was only to evaluate the efficacy of symbiotic addition to clindamycin, not to evaluate the recurrence rate of bacterial vaginosis. So, this study was limited only for curative aspects, not for prevention. On the other hand, this study excludes other bacteria that caused fluor albus such as *Trichomonas vaginalis* or *Candida* microscopically. Hence, after the treatment for bacterial vaginosis, the bacteria that might have previously existed still provided post-therapy complaints. During the research, the most common type of bacteria that was found was *Megasphaera* phylotype Ⅰ. No significant differences were found in the three types (*G. vaginalis*, *A. vaginae* and *Megasphaera* phylotype Ⅰ) of bacteria after therapy. Besides, there was no difference in therapeutic effect in the intervention group given clindamycin+synbiotic compared with the control group.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

Dr. Deviana Soraya Riu MD SpOG, as the corresponding author, led the research processes, mapped the framework of the research, decided the study population and methods of research, interpreted the data obtained, and wrote the research into a manuscript. Efendi Lukas MD SpOG, being an advisor and consultant for this research, certainly and fully contributed himself to supervising and assessing this manuscript scientific credibility, and giving any evaluative suggestion for improvement of the research implementation. Firdaus Kasim MD, M.Sc and Rizalinda Sjahril MD participated in the research and assisted the researcher (corresponding author) in implementing data statistics, doing a statistical treatment based on the materials in this research, and reporting the interpretation of statistical data to the corresponding author.

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