LEVELS OF INTERLEUKIN-1β, INTERLEUKIN-8 AND SIALIDASE VAGINAL SMEARS OF PREGNANT WOMEN WITH BACTERIAL VAGINOSIS AS PREDICTORS OF TREATMENT FAILURE WITH METRONIDAZOLE

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ABSTRACT:
Bacterial vaginosis is a health problem of pregnant women, due to its increased prevalence, and increased maternal and perinatal morbidity and mortality, especially its relationship with preterm labor. There is a decrease in recovery with standard treatment of metronidazole. To date there has been no map to predict treatment failure. Maternal immune response factors and germ virulence factors are expected to be used as a high-risk case of treatment failure. This study intends to determine whether the levels of interleukin-1β, interleukin-8 and sialidase in vaginal smears of pregnant women against the risk can be used as predictors of failure of treatment of pregnant women with BV with Metronidazol. This study used an observational case control study design. The case group was single pregnant women living with a gestational age of less than 20 weeks with BV infection who had failure treatment with metronidazole and the control group was pregnant women with BV infection who recovered with metronidazole treatment. The research was conducted at the Obstetrics Polyclinic of Sanjiwani Hospital. The study material was taken from the vaginal smear of pregnant women with BV and an elisa examination was carried out to determine the level of interleukin-1β. In this study, 26 pregnant women were found as cases of treatment failure and 26 pregnant women as controls. There were no differences in the average maternal age, gestational age and parity between cases and controls. There were significant differences in mean IL-1β levels (p = 0.004), IL-8 levels (p = 0.013) and sialidase (p = 0.008), in the case group compared to controls.

The cutoff of IL-1β, IL-8 and sialidase levels as predictors of treatment failure was 1401.17 ng/dl, 329.02 ng/dl and 2.38 ng/dl, respectively. Based on the results of the study above, cutoffs of IL-1β, IL-8 and sialidase levels as predictors of treatment failure were 1401.17 ng / dl, 329.02 ng / dl and 2.38 ng / dl, respectively. Further research is needed to determine the influence of high levels as a risk factor for treatment failure.

Keywords: Bacterial vaginosis, interleukin-1β, IL-8, sialidase, treatment failure

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INTRODUCTION

Bacterial vaginosis is still a health problem of pregnant women, since its prevalence increases, and the increase in maternal and perinatal morbidity and mortality. The prevalence of BV is higher in young pregnant women, especially in pregnancies less than 20 weeks. The standard treatment for BV infection to date is metronidazole. The cure rate for an acute episode of BV infection decreased compared to when metronidazole was initially used. The cause of treatment failure is not known for certain. In clinical treatment, it is important to know the cases that are at risk of treatment failure. To date there are no predictors for predicting treatment failure. Maternal immune response factors such as interleukin-1β (IL-1β), interleukin-8 (IL-8) and germ virulence factors such as sialidase levels, are expected to be used as a high-risk case of treatment failure.

Detection of the innate immune system to determine pathogenic virulence factors has emerged as a new paradigm for knowing pathogenic virulence (Diabate et al., 2015) (Bamogo et al., 2021). In BV infection, levels of IL-1β, IL-8, and sialidase are thought to be used as predictors to determine the risk of failure of BV treatment with metronidazole.

In vaginal smears pro-inflammatory cytokine interleukin-1β (IL-1β), IL-8 and sialidase, it is used as a specific point of care (POC) because it is directly related to the concentration of BV germs (Ballash et al., 2020) (Doyle et al., 2020). In the event of an excessive immune response, elevated levels of IL-1β, IL-8 and sialidase, can be dangerous by inducing excessive inflammatory reactions and oxidative stress, and this will cause damage to tissues and become a place to start pathogenic colonies (Dinarello, 2018) (Cavalli & Dinarello, 2018). This study is a basic study to determine the cutoff of IL-1β, IL-8 and sialidase levels, which can be used as a predictor of treatment failure in pregnant women with BV < 20 weeks gestational age with metronidazole.

RESEARCH METHODS

Observational research nested case control study at the Obstetrics Polyclinic of Sanjiwani Hospital from July to September 2022. The case group was pregnant women with BV infection who had failed treatment with metronidazole and the control group was pregnant women with BV infection who recovered with metronidazole treatment. The affordable population is pregnant women less than 20 weeks with BV infection who are given treatment with metronidazole who came to the Obstetric Polyclinic of Sanjiwani Gianyar Hospital within the research time period. The sample was a gestational mother of gestational age < 20 weeks single living with BV and the examination material was a vaginal smear.

Purposive consecutive sampling of the affordable population after meeting the inclusion and exclusion criteria. Vaginal smear examination for BV torture is carried out with Nugent Score, BV diagnosis is established if a fishy-smelling diluted
homogeneous discharge is found with a Nugent Score of 7-10. Examination of IL-1β, IL-8 and sialidase levels with the ELISA technique at the Integrated Biomedical Laboratory of the Faculty of Medicine, Udayana University, Bali. Patients were given treatment with Metronidazole twice 500 mg orally for 7 days, conducted clinical re-examination and Nugent scores of vaginal smear material after treatment. Treatment failure if clinical discharge with a Nugent score of 7-10, and treatment success if complaints are reduced or disappear with a Nugent score of 0-3.

Test normality with Kosmorgorov-Smirnov to determine whether IL-1β, IL-8 and sialidase levels in the case and control groups were normally distributed. The independent t test was used to determine the difference in the average levels of IL-1β, IL-8 and sialidase in the case and control groups. ROC curve analysis was performed to determine whether IL-1β, IL-8 and sialidase levels could be used as predictors of treatment success in BV. If an area below the r0.7 curve is obtained, then IL-1β, IL-8 and sialidase levels are considered to be used as predictors of treatment failure. Furthermore, cut-off-point values of IL-1β, IL-8 and sialidase levels will be determined which can be used as predictors of treatment failure. The data were analyzed with the help of IBM SPSS Statistics 21.

RESULTS AND DISCUSSION

During the period from July to September 2022, there were 504 visits by pregnant women with 125 patients with clinical vaginal discharge in pregnant women for less than 20 weeks, and 89 cases of vaginal discharge caused by BV. Of the 89 BV cases given treatment with metronidazole, 47 (53.9%) cases recovered with a Nugent score of 0-3 and 37 (41.6%) cases of treatment failure and the remaining 4 (4.5%) cases with a Nugent Intermediate score with a score of 4-6. The control case study was taken sequentially (consecutive) from patients who experienced treatment failure as a case, namely 26 pregnant women and patients who recovered as a control as many as 26 cases.

The pregnant adolescents with bacterial vaginosis had higher levels of IL-1 beta, IL-6 and IL-8 (P < 0.05). Sialidase was solely detected in 35 adolescents (67.2%) with bacterial vaginosis (Ferreira et al., 2015).

1. Distribution of maternal age characteristics, parity, gestational age, IL-1β, IL-8 and sialidase levels in the case group and control group

The distribution of maternal age, parity and gestational age characteristics in the case and control groups can be seen in table 1.

Table 1. Distribution of characteristics of maternal age, parity, gestational age in the case group and control group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case Group (n=26)</th>
<th>Control Group (n=26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Average±SD)</td>
<td>(Average±SD)</td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Mother's age (years)</th>
<th>Parity</th>
<th>Gestational Age (weeks)</th>
<th>Until IL-1β (ng/ml)</th>
<th>Until IL-8 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27,15 ± 3,41</td>
<td>0,46 ± 0,582</td>
<td>13,54 ± 4,42</td>
<td>1674,92 ± 413,87</td>
<td>424,63 ± 180,49</td>
</tr>
<tr>
<td>27,77 ± 4,08</td>
<td>0,8 ± 0,801</td>
<td>14,92 ± 3,09</td>
<td>1284,82 ± 514,15</td>
<td>324,02 ± 84,48</td>
</tr>
<tr>
<td>0,558</td>
<td>0,081</td>
<td>0,197</td>
<td>0,004</td>
<td>0,01</td>
</tr>
</tbody>
</table>

Normality tests obtained the distribution of data on maternal age, parity, gestational age, levels of interleukin-1β, interleukin-8 and sialidase in the case group and normal distributed controls. Based on the average comparison test, with the student t test, it was found that the average maternal age, parity and gestational age in the case and control groups did not differ in meaning.

In this study, significant differences were found in the average levels of IL-1β (p = 0.004), IL-8 levels (p = 0.013) and sialidase (p = 0.008), in the group of cases compared to controls.

2. ROC analysis of IL-1β levels as a predictor of treatment failure in pregnant women with BV

The results of the ROC analysis in Figure 1 show that the area under the ROC curve >0.7 is 0.840. This shows that IL-1β levels can be used as a predictor of treatment failure in pregnant women < 20 weeks with BV. The results of the ROC analysis also show a meaningful p-value (p = 0.001). The cutoff point value for IL-1β levels as a predictor of treatment failure is 1401.17 ng/dl.

![ROC Curve of IL-1β levels as a predictor of treatment failure in pregnant women < 20 mg with BV.](image)

3. ROC analysis of IL-8 levels as a predictor of treatment failure in pregnant women with BV

The results of the ROC analysis in Figure 2 show that the area under the ROC curve >0.7 is 0.826. This shows that IL-8 levels can be used as a predictor of treatment failure in pregnant women < 20 weeks with BV. The results of the ROC analysis also showed a meaningful p-value (p < 0.001). The cutoff point value for IL-8 levels as a predictor of treatment failure is 329.02 ng/dl.
Levels Of Interleukin-1β, Interleukin-8 And Sialidase Vaginal Smears Of Pregnant Women With Bacterial Vaginosis As Predictors Of Treatment Failure With Metronidazole

4. ROC analysis of sialidase levels as a predictor of treatment failure in pregnant women with BV

The results of the ROC analysis in Figure 3 show that the area under the ROC curve >0.7 is 0.842. This shows that sialidase levels can be used as a predictor of treatment failure in pregnant women < 20 weeks with BV. The results of the ROC analysis also showed a meaningful p-value (p < 0.001). The cutoff point value for IL-1β levels as a predictor of treatment failure is 2.38 ng/dl.

Discussion

The increase in the prevalence of BV in this study reached 71.2% and treatment failure occurred in 54% of pregnant women with BV. These results, according to a study in a tertiary hospital in Nigeria, found a prevalence rate of BV in pregnant women of 63.7% and treatment failure of 41.6%. Given this high prevalence they recommend regular screening and treatment during the antenatal period to prevent BV-related side effects in pregnancy and childbirth (Ajani, et al., 2012).

In this study, the average levels of IL-1β, IL-8 and sialidase were higher in the case group than in the control group. The cutoff point value for IL-1β levels as a predictor of treatment failure is 1401.17
Using a cutoff point value above, high interleukin-1β levels were obtained 70.0% in the case group.

BV status causes a dramatic increase in IL-1β concentrations (roughly 10 to 20 times), suggesting that the innate immune system reacts strongly and seeks to combat abnormal microbial colonization. (Cauci et al., 2003).

In vaginal smears, pro-inflammatory cytokine interleukin-1β (IL-1β) is used as a specific point of care (POC) because it is directly related to the concentration of BV germs with a sensitivity of 77% and a specificity of 72% (Mathys, 2020).

Concentrations of vaginal IL-1β had a strong positive correlation with levels of sialidase (P < .001) and prolidase (P < .001). Conversely, such enzymes were negatively correlated with the ratio of IL-8/IL-1β (both P < .001) and were not significantly associated with concentrations of IL-8. Notably, the number of vaginal neutrophils had a negative correlation with sialidase (P = .007). (Cauci et al., 2008)

Using a cutoff point value of IL-8 levels of 329.02 ng/dl, high IL-8 levels were obtained in 66.6% of the case group. In the case of BV, it is generally found that IL-8 levels are higher than mothers without BV, but an increase in excessive neutrophiles is not found in BV (Sakai, et al. 2004). The absence of an increased neutrophil response, resulting in reduced protection factors and affecting the inflammatory response and treatment failure (Cauci, et al. 2002).

The cutoff point value for sialidase levels as a predictor of treatment failure is 2.38 ng/dl. Using the cutoff point value above, a high sialidase level was obtained at 70.8% pin the case group.

Research shows a relationship between clinical, sialidase levels and the presence of G. vaginalis germs, and this proves the role of sialidase as a risk factor for germ virulence (Verstraelen, 2013: (Zhang et al., 2016).

With the cutoff value of IL-1β, IL-8 and sialidase levels, it is further expected to be able to conduct quantitative observational research to determine the magnitude of the role of high levels of IL-1β, IL-8 and sialidase on the incidence of treatment failure. If proven, clinical trial research with other treatment modalities is needed, in single form or in combination with metronidazole in cases of high risk of treatment failure.

CONCLUSION

Based on the results of the study above, cut off levels of IL-1β, IL-8 and sialidase as predictors of treatment failure were 1401.17 ng / dl, 329.02 ng / dl and 2.38 ng / dl, respectively. Further research is needed to determine the role of high levels of IL-1β, IL-8 and sialidase as risk factors for treatment failure.

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