

Low Maternal Serum Folic Acid as Risk Factor of Preterm Delivery

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ABSTRACT

Introduction: Preterm delivery increases the risk of infant mortality and morbidity higher than that of term born babies, so it is still a big problem in the field of obstetrics. Preterm delivery is not only influenced by anatomical factors, but nutritional status can also be a risk factor. Various studies have reported the role of folic acid on the risk of preterm delivery. Folic acid is thought to play a role in preterm labor through inflammatory and infectious pathways, this pathway is triggered by low serum folic acid levels interfering with Hcy metabolism which causes an increase in Hcy levels. High levels of Hcy in serum induce oxidative stress processes in vascular tissue and placental endothelium. The occurrence of oxidative stress and cell apoptosis will release proinflammatory mediators that cause preterm labor. This study aims to prove the role of maternal serum folic acid levels in preterm delivery.

Methods: This study used a case-control design that was carried out from January 2021 to June 2021. The target population of the case in this study was pregnant women in the process of preterm labor with control of preterm pregnant women. The sample in this study was taken by consecutive sampling (non random sampling). Examination of folic acid from venous blood samples was carried out using the ELISA method.

Results: This study involved 27 cases (preterm delivery) and 27 controls (preterm pregnancy). In this study, serum folic acid levels were found to be lower in the case group compared to the control group (cases vs. controls: 9.86 ± 1.93 ng/mL vs. 13.59 ± 1.24 ng/mL), all samples the case and control groups received folic acid supplementation. In this study, the maternal serum folic acid threshold was found at a concentration of 12.2 ng/mL, the researchers also obtained a risk value (OR) of 5.71 which indicated that pregnant women with serum folate levels below the value of 12.2 ng/mL had a risk of preterm delivery. 6 times greater when compared to pregnant women with serum folate levels above this value. Folic acid plays a role in the developmental phase of EVT, angiogenesis, and secretion of MMP which is important in placental development. Low serum folic acid levels also increase the process of angiogenesis, expression of MMP2, MMP3, and MMP9 thereby increasing the risk of preterm labor.

Conclusion: Low folic acid serum level in pregnancy is a risk factor for the occurrence of preterm labor by six times.

Keywords: Folic acid, labor, preterm.

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I. INTRODUCTION

Preterm labor is delivery that occurs before 37 weeks of gestation, calculated from the first day of the last menstrual period. Preterm delivery increases the risk of infant mortality and morbidity higher than that of babies born at term so that it is still a big problem in the field of obstetrics. Preterm labor is influenced by various micronutrient factors that are still controversial, one of which is folic acid. Further research is needed on the role of folic acid as a risk factor for preterm delivery.

Preterm delivery causes severe morbidity in infants such as neonatal infection, respiratory failure syndrome,

intraventricular hemorrhage, cerebral palsy, and enterocolitis [1], [2]. Preterm delivery also increases the cost of care in hospitals, that the cost of hospitalization per case for preterm delivery in government hospitals reached 8,465 US dollars, and in private hospitals it was recorded at 19,640 US dollars. dollars, from the recording of these costs include the cost of medical equipment maintenance, nutrition, and paramedical services.

The causes of preterm labor are multifactorial, where almost 70% of preterm births are caused by spontaneous birth (spontaneous labor) or rupture of membranes and iatrogenic factors [3]. In addition, preterm delivery is not only influenced by the anatomical structure of the cervix, but also

by a history of infectious diseases and habits of the mother during pregnancy, nutritional status can also be one of the risk factors for preterm delivery. During pregnancy the nutritional status of the mother is very important to note because it not only plays a role in fetal growth but also in the delivery process such as the incidence of preterm labor, one of which is folic acid [4].

Folic acid has a role in the mechanism of preterm labor through infection and inflammation. Low folic acid levels are associated with impaired T-cell and neutrophil function [5]. A study conducted by [5] in Nepal stated that adequate folic acid supplementation in pregnant women can reduce inflammatory mediators during pregnancy. A study conducted by [6] said that in preterm labor there was a decrease in folic acid levels and an increase in inflammatory mediators. Low levels of folic acid also interfere with the work of enzymes that metabolize serum Hcy in pregnant women, causing an increase in serum Hcy, an increase in serum Hcy causing an increase in oxidative stress, narrowing of the placental arterioles, damage to the placental endothelium until placental thrombosis occurs which in turn causes preterm labor [3].

II. MATERIALS AND METHODS

The sample in this study was taken by consecutive sampling (non random sampling) where every preterm birth that occurred at 24 weeks to 36 weeks 6 days of pregnancy that met the inclusion and exclusion criteria was used as a case sample of 27 people and the selection of control samples was taken from pregnancy. non-inpartum preterm with a total sample of 27 people who came to the ER and IRJ obstetrics and gynecology at the Sanglah Hospital Denpasar and the FK UNUD educational network hospital in the period January 2021 to June 2021.

The method for the examination of folic acid is using the ELISA method with the following examination principle: monoclonal antibodies specific for human folic acid are coated on a microplate. Standards and samples containing folic acid were put into wells containing immobilized antibodies. The color change was stopped with a stop solution and the color intensity was measured using a spectrophotometer (ELISA reader).

The data that has been collected is then processed using SPSS 26-version for Windows. Determination of the cutoff point of folic acid levels using the ROC curve, after the analysis, the cutoff point value of folic acid will be found based on the ROC curve. Folic acid levels that are less than the ROC cut off point are classified as low, while folic acid levels that are more than the ROC cut off points are said to be normal. Bivariate analysis with Chi-square test and calculation of odds ratio was performed to assess low folic acid level on the risk of preterm delivery. Statistical significance was obtained with $p < 0.05$.

III. RESULTS

This study involved 27 cases (preterm delivery) and 27 controls (preterm pregnancy) who underwent folic acid examination through venous blood. Characteristics in this

study include age, BMI, and parity. The characteristics of the sample in this study can be seen in Table I. In this study, there was no significant difference in maternal age, BMI, and parity between the case group and the control group ($p > 0.05$).

TABLE I: RESEARCH SAMPLE CHARACTERISTICS

Characteristics	Cases (n=27)	Controls (n=27)	p
Maternal age (median, min-max) (years)	26 (16-40)	24 (16-40)	0.054 [¶]
Body mass index (mean \pm SD) (kg/m ²)	22.11 \pm 1.36	22.41 \pm 1.16	0.345 [¶]
parity (median, min-max)	1(0-4)	0 (0-3)	0.328 [¶]

*Significance ($p < 0.05$); [¶]Mann Whitney U-test; [¶]T-test

A. Serum Folic Acid Levels in Case and Control Groups

In this study, the mean serum folic acid level in the case group was 9.86 ± 1.93 ng/mL and the mean serum folic acid level in the control group was 13.59 ± 1.24 ng/mL. There was a significant difference in serum levels of folic acid between the case group and the control group (mean difference 3.72; 95% CI 2.84-4.61; $p < 0.001$) (Table II). Distribution of folic acid serum levels by case and control group can be seen in Fig. 1.

TABLE II: DIFFERENCES IN SERUM FOLIC ACID LEVELS IN CASE AND CONTROL GROUPS

Variable	(Mean \pm SD) (ng/mL)	Mean Difference	CI 95%	p
Serum levels of folic acid in the case group	9.86 \pm 1.93	3.72	2.84-4.61	<0.001*
Serum levels of folic acid in the control group	13.59 \pm 1.24			

*Significance ($p < 0.05$)

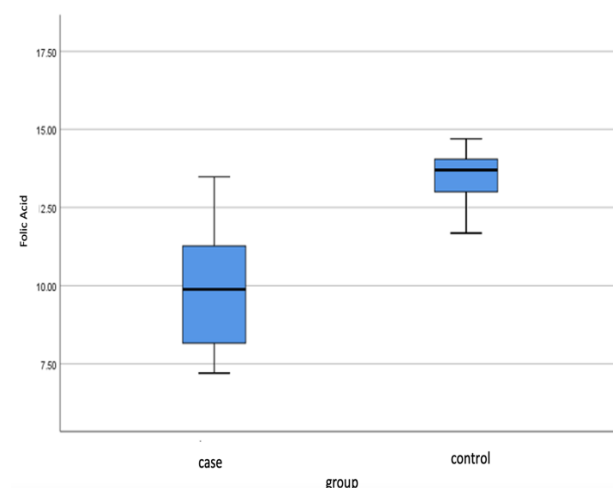


Fig. 1. Boxplot of folic acid serum levels by case and control group.

B. Analysis of Serum Folic Acid Levels in Preterm Labor

In the case group, there were 20 (74%) samples with low serum folic acid levels (≤ 12.2 ng/mL), compared to the control group with 9 (33%) samples with low serum folic acid levels. Mothers with low serum folic acid levels had a 6 times greater risk of having preterm delivery compared to mothers who had folic acid serum levels of more than 12.2 ng/mL (95% CI 1.76-18.50; $p = 0.003$). Analysis of low serum folic acid levels on preterm labor can be seen in Table III.

TABLE III: ANALYSIS OF SERUM FOLIC ACID LEVELS IN PRETERM LABOR

Variables	Case	Control	OR	CI 95%	p
Low serum folic acid ($\leq 12,2$ ng/mL)	20 (74%)	9 (33%)	5.71	1.76-18.50	0.003*
Normal serum folic acid ($>12,2$ ng/mL)	7 (26%)	18 (67%)			

*Significance ($p < 0,05$)

The cut off point for serum folic acid levels in this study was 12.2 ng/mL (AUC 0.952; sensitivity 88%, specificity 89%, $p < 0.001$) (Fig. 2 and Table IV).

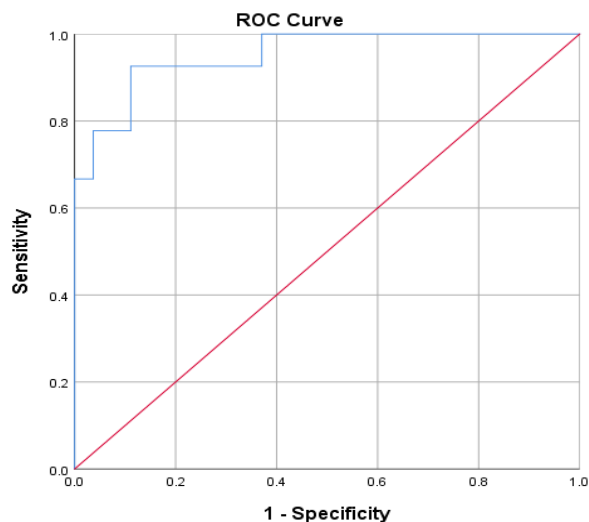


TABLE IV: CUTOFF OF FOLIC ACID SERUM LEVEL BASED ON THE ROC CURVE

AUC	CI 95%	Sensitivity	Specificity	Cutoff point	p
0,952	0.902-0.982	88%	89%	12.2	$< 0.001^*$

*Significance ($p < 0,05$)

IV. DISCUSSIONS

A. Sample Characteristics

In this study, the basic characteristics that were evaluated were maternal age, BMI, and parity. The median age of the mother was 26 (16-40) years in the case group and 24 (16-40) years in the control group, there was no significant difference in the age factor ($p: 0.054$). These results are in accordance with previous research, [5] reported that mothers aged 24-30 years had a low risk of preterm delivery, research by [7] showed that the age group 25- 29 years old were not at risk of having preterm delivery with an adjusted OR of 1.03 (0.89–1.19), and a study by [8] also reported that the age most at risk for preterm delivery was 28 years with adjusted OR was 1.10 (95% CI 1.02–1.19).

The increase in maternal age is directly proportional to the increased risk of preterm delivery, this is due to changes in the myometrium structure and vascular structure of the placenta as well as an increase in various medical complications that can affect the condition of pregnancy. Another mechanism that underlies the influence of older age on preterm labor is a deficiency of progesterone which is a hormone that is very important in preventing uterine contractility during pregnancy [9].

Another factor that affects preterm delivery is BMI. In this study, the median maternal BMI was 22.11 ± 1.36 kg/m² in the

case group and 22.41 ± 1.16 kg/m² in the control group. In this study, the BMI of the case and control group was included in the normal category, according to a study by [10] the risk of preterm delivery was reported at BMI 40 kg/m² (OR 2.99 95% CI 2.28-3.92). Another study conducted by [11] which examined the role of BMI on preterm delivery involving 33,740 research samples found that the overweight category (OR 1.43; 95% CI 1.16-1.77) and obesity (OR 1.76; 95% CI 1.37-2.26) is a risk factor for preterm labor.

B. Low Serum Folic Acid Levels in Preterm Labor

In this study, serum folic acid levels were found to be lower in the case group compared to the control group (cases vs. controls: 9.86 ± 1.93 ng/mL vs. 13.59 ± 1.24 ng/mL), all samples the case and control groups received folic acid supplementation, but the study did not record the level of adherence of pregnant women to taking folic acid during pregnancy.

The causes of low serum levels of folic acid in women with preterm delivery are multifactorial, supplementation and the level of compliance of pregnant women in consuming folic acid are the most important factors [12]. In this regard, the folate supplementation program since the preconception period has succeeded in reducing the rate of folate deficiency, other factors that influence differences in serum levels of folic acid in pregnant women such as differences in demographic location, social level, BMI (overweight and obesity), and education level [13].

Genetic abnormalities (polymorphisms) also affect serum levels of folic acid, the MTHFR 677C>T polymorphism has been known to cause a decrease in the activity of the MTHFR enzyme which causes a decrease in the efficiency of the transformation of folate to L-methylfolate which is more chemically active. Another genetic variant associated with decreased MTHFR enzyme activity is MTHFR 1298A->C, which is reported to reduce MTHFR enzyme efficiency up to 68% lower than the non-polymorphic variant (wild type). Low levels of the active metabolites of folic acid, such as 5-MTHF and 5-FTHF, can increase the risk of preterm labor [14].

C. Low Serum Folic Acid Level as a Risk Factor for Preterm Labor

In this study, from the analysis of the ROC curve, the maternal serum folic acid threshold was found at a concentration of 12.2 ng/mL. Chi-square analysis obtained a risk value (OR) of 5.71 which indicates that pregnant women with serum folate levels below the value of 12.2 ng/mL have a risk of preterm delivery 6 times greater than pregnant women with serum folate levels above this value.

The relationship of folic acid with preterm delivery can be explained as follows, folic acid is known to play a role in placental development and determines whether the formation of the placenta is stable. This is because folic acid plays a role in the developmental phase of EVT, angiogenesis, and MMP secretion which is important in placental development. Low

serum folic acid levels also increase the process of angiogenesis, the expression of MMP2, MMP3, and MMP9 thereby increasing the risk of preterm labor [15].

Viewed from the path of infection and inflammation, folic acid acts as an anti-inflammatory and plays a role in preventing preterm labor. Inflammation has been known as one of the factors causing preterm labor, normal serum folic acid levels can reduce levels of CXC chemokines such as GRO α , ENA-78, and IL-8 as well as CC chemokines such as monocyte chemoattractant peptide-1 and RANTES [16].

Folic acid plays a role in the remethylation of Hcy to methionine, where low serum folic acid levels are associated with increased Hcy levels. High serum Hcy levels can cause cytotoxic conditions and oxidative stress in vascular tissue and placental endothelium. The occurrence of oxidative stress can interfere with the DNA repair process that leads to cell apoptosis and results in preterm labor [17]-[20].

V. CONCLUSION

From this study, it can be concluded that low serum folic acid level (≤ 12.2 ng/mL) in pregnancy is a risk factor for the occurrence of preterm labor by six times (OR= 5.71; 95% CI 1.76-18.50); $p = 0.003$.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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