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High maternal homocysteine (Hcy) levels as a risk factor of preterm labor: a case-control study

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ABSTRACT

Background: Preterm labor is a serious perinatology problem with the consequences of increasing morbidity and mortality in neonates. Increased levels of homocysteine (Hcy) in serum have been reported to play a role in the pathogenesis of various pregnancy complications, including preterm labor. This study aimed to determine whether serum. Hcy levels are a risk factor for preterm labor.

Methods: This case-control study was conducted from January to June 2021. The case and control group included a consecutive woman with preterm and term labor. Homocysteine level was measured from maternal blood samples during labor. Maternal age, parity, and body mass index (BMI) data were also collected and compared between those two groups.

Results: A total of 30 preterm deliveries and 30 term deliveries were included in the study. The mean Hcy level in the preterm group was 17.26±6.814 mmol/L and in the term group was 9.788±4.532 mmol/L. Serum Hcy levels were classified as high (≥12.85 mmol/L) and low (<12.85 mmol/L) level. High serum Hcy level increased the risk of preterm labor by 9.118 times compared to low Hcy (p=0.001; 95% CI=2.581 – 32.211). There was no significant association between age, parity, and BMI with preterm labor (p>0.05).

Conclusion: High maternal serum Hcy level is a risk factor for preterm labor. Prospective studies with larger samples are required to confirm these results.

Keywords: Homocysteine, preterm labor, risk factors.
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INTRODUCTION

Preterm labor is still a burden in the obstetric field. The World Health Organization (WHO) has estimated about 15 million babies were born with preterm conditions. Globally, as many as one million infant deaths occur yearly due to preterm birth complications.[1] More than 60% of preterm births occurred in Africa and Southeast Asia.[2] A study at Prof IGNG Ngoerah Hospital in Bali reported that preterm birth was 9.4% in 2012 and increased to 23.7% in 2015.[3] The risk factors associated with preterm labor can be broadly divided into three main sources, namely maternal, fetal, and umbilical cord factors. Infection and inflammation through the apoptotic pathway are the main mechanisms of preterm labor. Infections and endotoxins may stimulate many proapoptotic factors during preterm labor. It was supported by the detection of those factors and also elements of programmed cell death (apoptosis) on the amniotic membrane of preterm labor women.[4]

Homocysteine (Hcy) is an intermediate metabolite with a very important role in the methionine cycle. Hcy will remain low in early pregnancy, reach 60% maximum concentration in the third trimester of pregnancy, and return to normal in 2-4 days after labor. Hcy is also an amino acid that has been shown to have a significant effect on vascular endothelial pathology.[5] Hyperhomocysteinemia (HHcy) was associated with changes in vascular morphology, increased oxidative stress leading to cellular apoptosis, loss of endothelial antithrombotic function, and induction of procoagulant.[6] Continuous oxidative stress due to HHcy will predispose inhibition to the conversion of methionine to S-adenosylmethionine (SAM). It is the main methyl group donor in the methyl transferase reaction in the lipid methylation process (conversion of phosphatidyl ethanolamine to phosphatidylcholine DHA), the strand formation process of DNA and histone proteins. Decreased SAM leads to reduced DNA methylation processes, which increase gene transcription and DNA strand destruction and trigger apoptosis.[7]

There are histopathological ischemic changes in the placental villi of women with a history of preterm labor accompanied by damage to vascular endothelial cells (VECs) due to peroxidase and free radicals formed from Hcy oxidation. Reduced nitric oxide (NO) production by VECs occurs when exposed to Hhcy chronically. It increases aggregation, activation, and adhesion of platelets impairs coagulation function and facilitates thrombus formation in the vascular pathway. A

previous study reported a significant negative correlation between infant birth weight and serum Hcy concentrations. Hcy serum concentration was higher in preterm mothers than in controls. The presence of VECs damage leads to many pathophysiological consequences, such as reduced NO and increased coagulation factors. This process will lead to placental and villi infarction, decreased placental perfusion, and fibrinoid vascular necrosis, which triggers fetal oxygenation and nutrition barriers.[8] HHcy shows convincing evidence of the risk of vascular damage, but the study of its effect on preterm labor, especially in pregnant women in Bali, is still limited. Therefore, this study aimed at high maternal serum Hcy levels as a risk factor for preterm labor.

METHODS

This case-control study was conducted in the Obstetric and Gynecology Department at Prof IGNG Ngoerah Hospital Denpasar and the affiliated hospital of Udayana University from January until June 2021. Single and live pregnancy women were included consecutively in the study. Patients with a gestational age of 24 weeks to less than 37 weeks were included in the case group, while those with a gestational age of 37 weeks or more were included in the control group. Patients with premature rupture of membranes, antepartum bleeding, congenital abnormalities in the fetus, medical disorders that accompany pregnant women such as asthma, diabetes mellitus, hypertension, heart disease, history of cervical surgery such as curettage, conization and others, have been treated with preterm labor. Imminence in this pregnancy and samples are taken, polyhydramnios, history of treatment with antibiotics, tocolytics and anti-inflammatory drugs in the last 1 week, and history of intercourse in the last 24 hours was excluded from the study. Serum Hcy levels were collected by taking 5 ccs of maternal venous blood. The samples were then measured using high-performance liquid chromatography (HPLC) and analyzed using enzyme-linked immunosorbent assay (ELISA). Maternal age, parity, and body mass index (BMI) data were also recorded.

Receiver operating characteristics (ROC) curves of 54 study samples were produced to investigate how well the Hcy level could predict preterm labor and determine the cut-off level of Hcy to preterm labor. The Chi-Square test was derived from creating a high-risk serum Hcy-risk model for preterm labor. A multivariate binary logistic regression test adjusted the confounding variables. All analyses were conducted with Statistical Program for Social Science (SPSS) version 25.0. for Windows. Results are presented as median (minimum-maximum) and number (%). Adjusted odds ratio (AOR) with a 95% confidence interval is presented to estimate the risk of high Hcy level in the preterm group after controlling other variables.

RESULTS

This study included 30 preterm labor women with a median gestational age of 33 weeks (26 - 35 weeks) and 30 term labor women with a median gestational age of 39 weeks (37 - 39 weeks), as seen in Table 1. The preterm labor group had a significantly higher median maternal age than the term labor group (p=0.023). There was no significant difference in BMI and parity between the preterm and term labor groups (p>0.05).

The mean Hcy level in the preterm group was higher than the term group (17.260 vs. 9.787 mmol/L). The receiver operating characteristic curve was constructed with the area under the curve (AUC) value was 0.818 (95% CI 0.712 – 0.924; p=0.000), as shown in Figure 1. The cut-off value of Hcy was 12.85 mmol/L, with a sensitivity of 76.7% and a specificity of 70%. Hcy levels were then classified based on the cut-off value into high (≥12.85 mmol/L) and low (<12.85 mmol/L) Hcy levels. More patients in the preterm labor group had high Hcy levels (76.7%), and only 30% of aterm labor group with high Hcy levels. This difference was statistically significant (p=0.001). The risk for preterm labor in the high Hcy level group was 7.667 times greater than the low Hcy level group (OR=7,667; 95% CI=2,424 – 24,245), as shown in Table 2.

Multivariate analysis was performed using the enter method binary logistic regression test to control for the confounding variable of maternal age. Age was classified into two groups, 20-35 years and <20 years or >35 years. High Hcy has an increased potential risk factor for preterm labor (adjusted OR: 9.118; 95% CI = 2.581 – 32.211; p = 0.001) after controlling the age variable.

DISCUSSION

Preterm labor is still a global problem because of the high incidence and increased morbidity and mortality due to complications of preterm labor in infants.[9] This study showed that the median maternal age was significantly higher in preterm labor. However, the multivariate test results showed that maternal age was not a risk of preterm labor. These results

Table 1. Subject characteristics.

Variables	Preterm (N=30)	Aterm (N=30)	p-value
Age (years), median (minimum-maximum)	26.50 (20-40)	24 (19-40)	0.023[a]
Body mass index (kg/m^2), median (minimum-maximum)	22.5 (20-24)	22.5 (20.5-24.5)	0.507[a]
Parity, median (minimum-maximum)	1 (0-4)	1.5 (0-4)	0.482[a]
Mann-Whitney test			

Table 2. Hcy level based on labor group.

Category of Hcy	Labor Group		p-value	OR (95% CI)
	Preterm (N=30)	Aterm (N=30)		
High (≥12,85 mmol/L)	23 (76.7%)	9 (30%)	0.001[a]	7.667 (2.424 – 24.245)
Low (<12,85 mmol/L)	7 (23.3%)	21 (70%)		

[a]Chi square test; OR: odds ratio

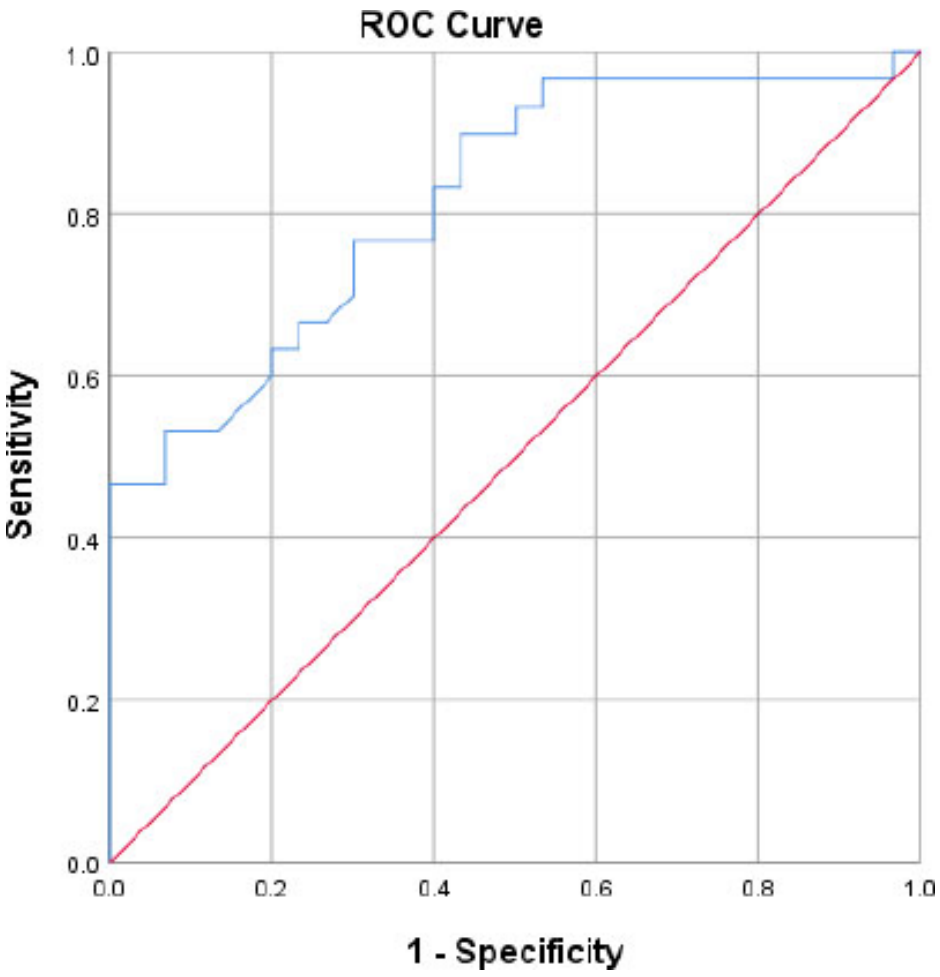

Figure 1. Receiver operating characteristics (ROC) curve.

follow previous studies, which showed no difference in maternal age between the preterm labor groups for indications of spontaneous and term or preterm labor.[10,11] In addition, there was no difference in the mean maternal age between groups with term labor, threatened preterm labor which reaching term, and preterm labor.[12] Different results were obtained from several previous studies showing the minimal effect of maternal age on preterm labor.[13,14] Maternal aged>40 years have a higher risk of preterm labor than those aged <40 years, with an increased risk of 1.13-1.61 times higher.[13-15] About 1.6-2 times increased risk of preterm labor was observed at maternal age <20 years compared to maternal age >20 years.[15,16]

Research on body mass index (BMI) as a risk factor for preterm labor still reported varying results. This study had no association between BMI and the incidence of preterm labor. Previous studies also reported no difference in the risk of preterm labor at 28-36 gestation weeks based on BMI.[17] Meanwhile, other studies reported that being underweight and overweight increased the risk of preterm labor.[18] Overweight and obese women increase the risk of preterm labor by 8.12 times and 15 times, respectively.[17,19] The risk of spontaneous preterm labor was found to increase almost 3 times in nulliparous obese women and 2.2 times in multiparous underweight women.[20] Abnormal response to oxytocin in obese women affects myometrial function. Oxytocin receptors are decreased in obese women at term. These changes are also associated with higher rates of post-term pregnancy and slower labor progress in obese women. Meanwhile, obesity is also characterized by an increased inflammatory response associated with proinflammatory cytokines and adipokines and changes in the hypothalamic-pituitary-adrenal axis which is responsible for releasing corticotrophin-releasing hormone, one of the risk factors for preterm labor.[19] Obesity also affects placental insufficiency, inflammatory conditions, insulin sensitivity and cellular oxidative stress that triggers preterm labor.[17]

There was no difference in parity between the preterm and term labor groups in this study. This result is supported by several previous studies, although some conflicting studies exist.[12,16] Nulliparous women have a 1.13-1.8 times higher risk of preterm labor than women.[15,21] Women with their fifth pregnancy also had the highest risk of having preterm labor, which was 1.26 times compared to other parities. Preterm labor is contributed by cervical damage, which plays a role in maintaining pregnancy. Damage to the cervix due to dilatation, curettage or excision of the premalignant lesion may increase the risk of preterm labor. This procedure is usually experienced by older women with more parity, producing an indirect effect of parity on preterm labor.[22]

Homocysteine (Hcy) is an important metabolite in the methionine cycle. It can be removed from the cycle when homocysteine is converted to cystathionine by a transsulfuration pathway that requires vitamin B6 as a cofactor.[23,24] Hcy levels are lower in normal pregnancy than in non-pregnant women. Hemodilution, due to increased blood volume and the glomerular filtration rate and the fetus may absorb some Hcy during pregnancy. Hcy levels decrease in early pregnancy, reach their lowest values in the second trimester, then increase slowly during the third trimester and reach levels in early pregnancy. Normal Hcy levels during pregnancy are 3.9-7.3 mmol/L before 16 gestation weeks (gw), 3.5-5.3 mmol/L at 20-24 gw, and 3.3-7.5 mmol/L after 36 gw.[23]

This study showed that the group with serum Hcy levels ≥12.85 mmol/L had a 9.118 times greater risk of preterm labor than those with serum Hcy levels <12.85 mmol/L. Previous studies also found that the group of mothers with preterm labor had higher serum Hcy than the control group.[7,25,26] Cut-off for Hcy level also varied in several studies. The group with serum Hcy levels >12.4 mmol/L had a 3.6 times greater risk of having preterm labor,[26] while research in China showed

the highest incidence of preterm labor was 7.39% in serum Hcy > 12.6 mmol/L.[25,27]

The role of inflammation and infection during pregnancy causes an increase in Hcy levels during pregnancy. Hyperhomocysteinemia (HHcy) promotes the production of hydrogen peroxide and superoxide free radicals, leading to oxidative injury of endothelial cells, damage to villi blood vessels and maternal-fetal blood circulation.[23] This metabolic disorder is characterized by changes in the metabolic pattern of long-chain polyunsaturated fatty acids (LCPUFAs) which contribute to higher platelet aggregation through the decreased synthesis of endothelium-derived relaxing factor and nitric oxide (NO), as well as tissue factor induction and stimulate smooth muscle cell proliferation.[5] Long-lasting HHcy conditions also increase procoagulants and endothelial dysfunction resulting in vascular sclerosis and microthrombi that interfere with uteroplacental circulation.[8] Protein peroxidase can cause loss of sulfhydryl groups and carbonyl group bonds with other amino acid rings. Proteolytic activity is inhibited in high ROS conditions, but accumulation occurs with long hydrophobic bonds that affect cell membrane function. Cell membrane phospholipids that undergo peroxidation will become rigid, and lose their selective permeability and integrity, thereby triggering the process of membrane apoptosis. This will facilitate tearing of the amniotic membrane, which predisposes contractions and preterm labor.[28]

The continuous oxidative stress due to HHcy conditions may also become an obstacle to the conversion of methionine to S-adenosylmethionine (SAM), which is the main methyl group donor in the methyl transferase reaction (conversion of phosphatidyl ethanolamine to phosphatidylcholine DHA), the process of DNA strands and histone proteins formation. Consequently, it causes a decrease in DNA methylation, destroying DNA strands, disrupting the DNA repair process, causing genetic mutations and triggering apoptosis.[7] DNA damage can lead to telomere reduction, a marker of cell aging. The loss of telomere function may induce cell cycle arrest and senescence (marked by irreversible cessation of cell growth). Amniochorionic cells divide rapidly during gestation to meet the increased demand for the fetus. This proliferative activity is a normal process expected to continue at term. However, senescent cells produce a unique inflammatory marker, the senescence-associated secretory phenotype (SASP). SASP will send a signal for contractions and labor. DNA damage may also induce independent telomere pathways when it is minimal. In this pathway, 8-OxoG is generated due to oxidative damage in telomere segments or G bases in DNA that are repaired by the specific enzyme 8-oxoguanine glycosylase (OGG1). The 8-OxoG: OGG1 complex activates Ras-GTPase and promotes p53-mediated apoptosis or NF-kB-mediated inflammation. The exact mechanism of this change remains unclear. The NF-kB-mediated inflammatory pathway produces an inflammatory response without apoptosis, mostly associated with preterm labor without amniotic membrane tearing.[28,29]

This study has several limitations. First, this study uses a retrospective approach so that the effect of increased Hcy levels on the incidence of preterm labor cannot be ascertained. Second, this study cannot rule out the involvement of genetic mutation factors as a cause of Hcy metabolic disorders. Third, this study did not include the possibility of cervical incompetence, only based on an anamnesis of a history of previous abortions.

CONCLUSION

In conclusion, high maternal serum Hcy levels in pregnancy increase the risk of preterm labor. The results of this study are expected to become basic epidemiological data regarding Hcy levels in preterm and term labor. It may assist clinical considerations in preventing cases of preterm labor. Further studies are required with a prospective approach and considering gestational age when assessing Hcy level as a risk factor for preterm delivery. Additional examinations to assess genetic and metabolic factors and cervical competence need to be carried out to see the possible effect on Hyc level and preterm delivery.

ETHICAL CLEARANCE

Ethics approval was obtained by the Ethics Research Committee of the Faculty of Medicine, Udayana University/Prof. Dr. IGNG. Ngoerah General Hospital under Study Number 2249/UN14.2.2.VII.14/LT/2020. A research permit was also obtained from the Education and Research Division of Prof. Dr. IGNG. Ngoerah General Hospital under Number LB.02.01/XIV.2.2.1/2888/2021.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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AUTHOR CONTRIBUTIONS

IPPK was the main author who carried out the study, including data collection and data analysis, and drafted the manuscript. All authors participated in the study's concept and design and read and approved the final manuscript.

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