**Research Article**

# Levels of 25-Hydroxyvitamin D in Normotensive Pregnancy and Severe Preeclampsia

## *Kadar 25-Hydroxyvitamin D pada Kehamilan Normotensif dan Preeklamsia Berat*

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## Abstract

**Objective**: To determine the ratio of 25-hydroxyvitamin D levels in normotensive pregnancy and severe preeclampsia.

**Methods**: This study was an analytic cross-sectional study with t-test. The subject of this study consists of 17 samples normotensive pregnancy and 17 samples severe preeclampsia. This study was conducted and evaluated from August 2016 until December 2016 at Department of Obstetrics and Gynecology Faculty of Medicine Universitas Sam Ratulangi Prof. Dr. R.D. Kandou Hospital Manado and satellite hospital in Manado. Samples were taken from serum as much as 5 ccs and were analyzed using CLIA at Prodia clinical laboratory. Data were analyzed with SPSS version 20.0.

**Results**: By using the t- test, there were significant differences in 25-hydroxyvitamin D levels between normotensive pregnancy group (24.771 ± 6.9567ng/ml) and severe preeclamptic group (17.712 ± 3.7513ng/ml), p = 0.001.

**Conclusion**: Levels of 25-hydroxyvitamin D in normotensive pregnancy significantly higher compared to severe preeclampsia so it can be concluded that the levels of 25-hydroxyvitamin D were associated with preeclampsia.

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**Keywords**: 25-hydroxyvitamin D, normotensive, severe preeclampsia

## *Abstrak*

***Tujuan**: Mengetahui perbandingan kadar 25-hydroxyvitamin D pada kehamilan normotensif dan preeklamsia berat.*

***Metode**: Penelitian ini merupakan suatu penelitian potong lintang bersifat analitik dengan uji t terhadap subjek yang terdiri atas 17 sampel normotensi dan 17 sampel preeklamsia berat. Penelitian dilaksanakan dan dievaluasi sejak bulan Agustus 2016 sampai Desember 2016 di Departemen Obstetri dan Ginekologi Fakultas Kedokteran Universitas Sam Ratulangi / RSUP Prof. Dr. R.D. Kandou Manado dan rumah sakit jejaring di Manado. Sampel diambil dari serum sebanyak 5 cc dan dikirim ke Laboratorium klinik Prodia untuk diproses dengan metode CLIA. Data dianalisis dengan SPSS versi 20.0*

***Hasil**: Dengan menggunakan uji t, didapatkan perbedaan bermakna kadar 25-hydroxyvitamin D pada kelompok normotensif (24,771 ± 6,9567) dan kelompok preeklamsia (17,712 ± 3,7513), p= 0.001.*

***Kesimpulan**: Kadar 25-hydroxyvitamin D pada kehamilan dengan normotensif lebih tinggi secara bermakna dibandingkan dengan preeklamsia berat sehingga dapat disimpulkan bahwa kadar 25-hydroxyvitamin D berhubungan dengan kejadian preeklamsia.*

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***Kata kunci**: 25-hydroxyvitamin D, normotensif, preeklamsia*

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## INTRODUCTION

Preeclampsia is still one of the major complications during pregnancy, delivery and post-partum period. Preeclampsia, one of the causes of death other than bleeding and infection.[1] The effect can lead to the disruption of maternal and fetal well-being. Despite considerable progress in the field of obstetrics and perinatology services for antenatal and neonatal care, but nonetheless, preeclampsia remains one of the most common causes of maternal and perinatal morbidity and mortality.[2] Every year nearly 40.000 women, especially in developing countries die from preeclampsia or eclampsia. Preeclampsia or eclampsia are the second leading cause of maternal death in Pakistan.

The prevalence of preeclampsia ranges between 7%-10% of all pregnancies. In the United States, the incidence was 23.6 cases/1000 pregnancies. Whereas in the UK the incidence of severe preeclampsia ranges from 5/1000 pregnant women and 5/10,000 pregnant women in eclampsia. And the incidence of preeclampsia tends to increase from year to years.[3] In Indonesia, this disorder is still the top three highest contributors to maternal mortality after bleeding and infection, with incidence rates varying between 2.1 to 8.5%. The pathogenesis of preeclampsia until now remains unclear. Preeclampsia is a disorder characterised by hypertension and proteinuria occurred > 20 weeks of gestation. Around the world, about 2-3%

of all pregnant women can develop preeclampsia. This condition is a major cause of maternal and perinatal morbidity and mortality.[3,4]

Vitamin D plays an important role in calcium metabolism, immune system, proliferation and cell differentiation, the process of infection and cancer.[5,6] Within the last ten years, research on vitamin D prove that vitamin D effects on conception, pregnancy and health neonatal.[7] Vitamin D (cholecalciferol) formed by the skin during exposure to sunlight (ultraviolet radiation), and also absorbed from food. Absorption of cholecalciferol converted in the liver to 25(OH) cholecalciferol or 25- hydroxyvitamin D (25(OH)D), and then by 1α-hydroxylation in the kidney to 1,25 hydroxylase dihydroxycholecalciferol or 1,25(OH)2D3, most forms active group vitamin D.

Vitamin D receptors are also present in the placenta. Levels of 1,25(OH)2D3 increases during pregnancy through increased 1α-hydroxylase. The discovery of the levels of 25(OH)D in maternal and umbilical cord serum proved that the development of the placenta and fetus are directly related to vitamin D intake and sun exposure.[8] Levels of 25(OH)D were lower in the first trimester associated with the incidence of preeclampsia.[9] Intake of vitamin D during early life is required so that the risk of preeclampsia can be reduced up to 50% pregnancy.[10]

Magnus (2001) observed that the incidence of preeclampsia-related with seasons. The incidence of preeclampsia is reduced in summer with high sun exposure.[11] Vitamin D deficiency (levels of 25(OH)D <17.5 nmol/l) and insufficiency (levels of 25(OH)D <50nmol/l) was found in tropical countries, for example, India and Bangladesh with high sun exposure.[12] Green (2008) investigated the levels of 25(OH)D in reproductive age women in Jakarta and Kuala Lumpur, and it showed that 60% reproductive-aged women have vitamin D insufficiency.

Deficiency of vitamin D (25(OH)D) causes endothelial dysfunction through the molecular mechanism. Endothelial dysfunction that occurs in preeclampsia begins with exposure of the endothelial cell membrane by mediators released as a result of placental ischemia and hypoxia, among the products of lipid peroxidation, resulting in damage of the cell membrane. Disruption of cell membranes earlier can disturb endothelial function, and even cause damage to the entire structure of endothelial cells. As a result of damage to endothelial cells, the endothelial function as a mechanical barrier endothelial lost so that leakage that causes extravasation of fluid into the extravascular intra, in addition to producing endothelial function and NO PGI2 also decreased, resulting in vasoconstriction and increased blood pressure.[13-15] In addition to the endothelial damage also causes many other disorders, such as, decreased production of prostacyclin, platelet aggregation in areas of damaged endothelium which will also produce thromboxane A2. The typical changes in the glomerular capillaries form of glomerular endotheliosis, increased capillary permeability, increased coagulation factors.

There also are found vitamin D (25(OH)D) effect on trophoblast invasion and angiogenesis when implantation process, so this is an important factor in the pathophysiology preeclampsia.[5] Many other studies that try to prove the pathogenesis of preeclampsia associated with defence vitamin D, for example regarding the response, oxidative stress and angiogenesis.[13-15]

## OBJECTIVE

This study was performed to investigate the levels of 25-hydroxyvitamin D in normotensive pregnancy and severe preeclampsia.

## METHOD

This research is an analytical cross-sectional study with t-test to compare the levels of 25-hydroxyvitamin D in normotensive pregnant women and severe preeclampsia. This research was conducted and evaluated from August 2016 to December 2016 in the Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sam Ratulangi / Prof. Dr. R.D. Kandou Manado and hospital networks in Manado to research subjects who meet the inclusion and exclusion criteria. The inclusion criteria include pregnant women 20 weeks gestation to term with severe preeclampsia, superimposed preeclampsia and eclampsia and willing to participate in research. Exclusion criteria included pregnant women with chronic hypertension, heart disease, liver, kidney, diabetes mellitus, fetal growth retardation, and refuse to join the study. Subjects consisted of 17 samples and 17 samples of normotensive severe preeclampsia. After anamnesis, physical examination and have

informed consent, serum samples were taken from as many as 5 ccs and put into a sterile sample container, centrifuged and stored at -20°C and then processed in Prodia a clinical laboratory method chemiluminescent immunoassay (CLIA). This study has also been approved by the Integrated Health Research Unit (UPKT) Prof. Dr. R.D. Kandou Manado. Data were analyzed with SPSS version 20.0.

## RESULT

In Table 1 shows the subject of research in the normotensive group most aged 21 to 35 years is 13 people (76.47%), Minahasans many as 14 people (82.36%), job IRT 11 (64.7%), multigravida as many as 11 people (64.71%) and the wedding one time as many as 15 people (88.24%). While in severe preeclampsia group there are at most ages 21-35 years as many as 9 people (52.94%), Minahasans 10 (58.83%), job IRT 16 people (94.12%), multigravida 10 people (58.82%) and the wedding one time as many as 15 people (88.24%).

**Table 1.** Research Subject Characteristics

| Characteristics | Normotensive | | Preeclampsia | |
|---|---|---|---|---|
| | n | % | n | % |
| Age | | | | |
| < 20 years | - | 0 | 2 | 11.76 |
| 21-35 years | 13 | 76.47 | 9 | 52.94 |
| > 36 years | 4 | 23.53 | 6 | 35.30 |
| Ethnic | | | | |
| Minahasa | 14 | 82.36 | 10 | 58.83 |
| Sangihe | 1 | 5.88 | 4 | 23.53 |
| Gorontalo | 1 | 5.88 | 1 | 5.88 |
| Etc | 1 | 5.88 | 2 | 11.76 |
| Occupation | | | | |
| Housewife | 11 | 64.7 | 16 | 94.12 |
| Private employee | 1 | 5.88 | 1 | 5.88 |
| Government employee | 4 | 23.53 | - | - |
| Student | 1 | 5.88 | - | - |
| Parity | | | | |
| Primigravidity | 6 | 35.29 | 7 | 41.18 |
| Multigravidity | 11 | 64.71 | 10 | 58.82 |
| Marriage | | | | |
| 1 | 15 | 88.24 | 15 | 88.24 |
| > 1 | 2 | 11.76 | 2 | 11.76 |

**Table 2.** Variable Distribution of Age and Blood Pressure (BP) In normotensive Pregnancy and Severe Preeclampsia

| Variable | Normotensive Pregnancy (n = 17) | Severe Preeclampsia (n = 17) | p |
|---|---|---|---|
| Age | 31.41 ± 5.455 | 30.94 ± 9.692 | 0.863 |
| Systolic BP | 122.35 ± 10.326 | 168.24 ± 14.246 | 0.000 |
| Diastolic BP | 78.82 ± 9.275 | 108.82 ± 6.002 | 0.000 |

**Table 3.** 25-Hydroxyvitamin DLevels in Normotensive Pregnancy and Severe Preeclampsia

| Variable | Normotensive Pregnancy (n = 17) | Severe Preeclampsia (n = 17) | p |
|---|---|---|---|
| Kadar 25-hydroxyvitamin D (ng/ml) | | | |
| Mean ± SD | 24.771 ± 6.9567 | 17.712 ± 3.7513 | 0.001 |
| Median | 24.700 | 17.500 | |
| Highest value | 14.8 | 7.9 | |
| Lowest value | 40.7 | 22.4 | |

From the above table, for the age variable t-test (mean difference between two independent variables), it was found there was no significant difference between the age groups of normotensive and severe preeclampsia group, so it was concluded there was no correlation between the incidence of severe preeclampsia with maternal age. Whereas for the variable systolic blood pressure and diastolic blood pressure test and the Mann-Whitney there are significant differences between the systolic and diastolic blood pressure in normotensive and severe preeclampsia group, so it was concluded there is a correlation between the incidence of severe preeclampsia with systolic and diastolic blood pressure.

In Table 3, 25-hydroxyvitamin D levels in pregnancy with normotensive obtained the highest levels of 40.7 ng/ml and the lowest levels in normotensive 14.8 ng/ml. As for severe preeclampsia 25-hydroxyvitamin D levels high of 22.4 ng/ml while 25- hydroxyvitamin D levels low of 7.9 ng/ml.

By using the t-test (mean difference between two independent variable), it was concluded that there are significant differences in vitamin D levels in each of the normotensive group (24.771 ± 6.9567) and severe preeclampsia group (17.712 ± 3.7513), (p = 0.001), meaning that there is a relationship between the incidence of severe preeclampsia with blood levels of vitamin D (Table 3 and Figure1).

Shapiro-Wilk normality test showed that the distribution of data levels of vitamin D in the normotensive group and Severe Preeclampsia were normally distributed (p = 0.57 and p = 0.13).

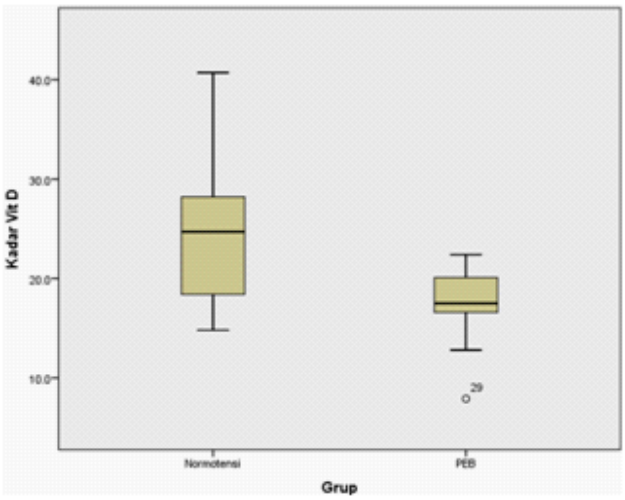


**Figure 1.** Graphs the levels of 25-hydroxyvitamin D in normotensive pregnancy and severe preeclampsia

## DISCUSSION

The basic characteristics of the study sample include age, ethnicity, occupation, parity, marriage with sample number 34 and is divided into two groups: group with normotensive pregnancy and severe preeclampsia group. Table 1 can be seen from the distribution of study subjects based on the age of the mother is 16 to 46 years. The highest percentage in the age group 21-35 years both in the normotensive group as many as 13 people (76.47%) and severe preeclampsia group were nine people (52.94%). Followed age > 36 yrs namely the normotensive group of 4 people (23.53%) and severe preeclampsia six people (35.3%). In this study, the Minahasa tribe is the tribe most studied as many as 14 people in the normotensive group (82.36%) and ten men in the severe preeclampsia group (58.83%), because the study was conducted in the city of Manado, North Sulawesi where Minahasans a majority interest. In this study, it was found that the incidence of severe preeclampsia increases in the productive age and IRT. This contrasts with research conducted by SQ Wei et al in June 2012 which reported that there was no significant difference between preeclampsia and nonpreeclampsia terms of maternal age, education and ethnicity.[5]

In this group, the highest percentage obtained parity characteristics are good multigravida in normotensive group (64.7%) and severe preeclampsia (58.82%). This is due to the distribution of samples high in this group. Preeclampsia often occurs at a young age and primiparous, it is suspected because of the existence of a mechanism immunology.[13,16] In Table 3 shows the relationship of vitamin D (25(OH)D) in pregnancy normotensive, and severe preeclampsia in which it was concluded there are significant differences in the levels of vitamin D (25(OH)D) in the normotensive and severe preeclampsia, with an average value levels vitamin D (25(OH)D) in normotensive 24.771 ± 6.9567 ng/ml while in severe preeclampsia group was 17.712 ± 3.7513 ng/ml (p = 0.001). This suggests there is a correlation between levels of vitamin D (25(OH)D) and the incidence of severe preeclampsia.

Research in Surabaya Hanifa et al. (2015) that compared 18 women of severe preeclampsia and 18 normotensive women found a significant difference between levels of vitamin D (25(OH)D) in the normotensive with severe preeclampsia. Levels of vitamin (25(OH)D) in normotensive

(11.7 ± 4.8) compared with severe preeclampsia (15.9 ± 4.5). The hypothesis about the levels of vitamin D (25(OH)D) maternal determine the risk of preeclampsia.[18]

From several studies reported that a deficiency of vitamin D (25(OH)D) related to an inflammatory process due to endothelial dysfunction. Is a layer of endothelial cells lining the vascular wall facing the lumen and attached to the subendothelial tissue consisting of collagen and various glycosaminoglycans including fibronectin.[17] Formerly considered that endothelial function is as a structural barrier between the circulation of the surrounding tissue, but it is now known that the endothelial function of regulating vascular tone, prevents thrombosis, regulate the activity of the fibrinolytic system, preventing the adhesion of leukocytes and regulates vascular growth. Issued endothelial vasoactive substances include nitric oxide (NO) which is also called endothelial-derived relaxing factor (EDRF), endothelial-derived hyperpolarising factor (EDHF), prostacyclin (PGI2), bradykinin, acetylcholine, serotonin and histamine. Among other vasoconstrictor substance endothelin, platelet activating factor (PAF), angiotensin II, prostaglandin H2, thrombin and nicotine. Deficiency of vitamin D (25(OH)D) causes endothelial dysfunction through the molecular mechanism. Where the result of a deficiency of vitamin D (25(OH)D) causes the release of most of the proinflammatory factors including the increase in NFkB (nuclear factor-kB), interleukin-6, a decrease in the vitamin D receptor, 1α-hydroxylase, and calcium levels. Wherein the calcium associated with vitamin absorption D.[5,20,21] If endothelial impaired in by many things such as oxidative stress, shear stress hemodynamic, hypercholesterolemia and exposure of inflammatory cytokines, the regulatory function becomes abnormal and is called endothelial dysfunction.[18-21] Deficiency of vitamin D (25(OH)D) in pregnancy predisposes proinflammatory response and oxidative stress resulting in endothelial dysfunction that is the hallmark of preeclampsia. There also are found vitamin D (25 (OH)D) effect on trophoblast invasion and angiogenesis when implantation process, so this is an important factor in the pathophysiology preeclampsia.[5] Several studies have also reported a deficiency of vitamin D (25(OH)D) and the risk of preeclampsia. Marya et al. suggest that the intake of calcium and vitamin D can lower blood pressure, thereby reducing the incidence of preeclampsia. Other researchers such as Haugen et al. (2009) Haryana et al. (2012), Abdulbari (2013), Dharma et al. (2005) also suggests the same result.[14,15,19-21]

## CONCLUSION

From this study, it can be concluded that the obtained 25-hydroxyvitamin D levels were lower in patients with severe preeclampsia. The results of the analysis of the relationship of 25-hydroxyvitamin D levels with the incidence of severe preeclampsia in getting that blood levels of 25-hydroxyvitamin D a pregnant woman is associated with severe preeclampsia.

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