# LEVELS OF VITAMIN B12 AND HOMOCYSTEINE IN PRE-ECLAMPTIC PATIENTS WITH AND WITHOUT PLACENTA ABRUPTION

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## ABSTRACT

### OBJECTIVES

*This study aimed to determine vitamin B12 and homocysteine levels in pre-eclamptic patients with and without placenta abruption.*

### METHODOLOGY

*A case-control study comprised 60 participants (cases n =30 and control n = 30) ages 17-28 years to evaluate the correlation between vitamin B12 and homocysteine. Each participant was interviewed for data collection regarding their age, education, income, gestation weeks, and family history of hypertension and their blood pressure, urinary albumin, height, weight, and body mass index in kg/m2 (BMI). Blood samples (5 ml) were collected from each subject through sterilized syringes; 3 ml of blood was transferred to the ETDA tube and 2 ml into the clot activator Gel tube. Each sample was centrifuged and stored at -20C. Vitamin B12 and homocysteine ELISA kits were used for measuring the vitamin B12 as well as homocysteine levels in each blood sample. Vitamin B12 was analyzed by Chemiluminescent Microparticle Intrinsic Factor assay. For both Vitamin B12 and homocysteine analysis, a commercially available ARCHITECH kit was used. SPSS version 26.0 was used.*

### RESULTS

*It was observed that vitamin B12 in both cases and control were the most frequent. In case twenty-three (77%) and control twenty-two (73%) are in the normal range. Five (17%) and seven (23%) are deficient in both case and control, respectively. Only two (7%) and one (3%) had a high vitamin b12 in the case and control, respectively. On the other hand, none of the cases was deficient with homocysteine, while only three (3%) in control were deficient with homocysteine. Three (10%) and six (20%) had high homocysteine levels than normal in the case and control, respectively.*

### CONCLUSION

*Our results highlight the importance of Vitamin B12 and homocysteine in pregnancy. Further large-scale studies should evaluate the relationship between Vitamin B12 and homocysteine in pre-eclampsia and abruption.*


**KEYWORDS:** *Preeclampsia, Vitamin B12, Homocysteine, Placenta Abruption*

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## INTRODUCTION

Pre-eclampsia is a hypertensive condition that typically happens after 20 gestational weeks and is characterized by a protein found in the mother's urine and raised blood pressure. If this condition is left untreated, it can be fatal to both the mother and fetus.[1] Preeclampsia (multifactorial condition) is caused by the mutual interaction of environmental factors such as diet, weight gain in the gestational

period, twin pregnancies, maternal age, previous pre-eclampsia history, pre-existing maternal illnesses (including infections, chronic hypertension, and diabetes) and genetic factors.[4,5] Both placental and maternal aspects perform a role in pre-eclampsia, as Inflammation and dysfunction of endothelial cells are the significant contributors. Pregnancy Induce Hypertension, along with pre-eclampsia, confounds 2-8% of total pregnancies and, internationally, is a primary reason for perinatal and maternal mortality. Moreover, pre-eclampsia complications may lead to maternal mortality, morbidity, and adverse perinatal outcomes.[6] Maternal death due to pre-eclampsia was reported less in developed countries than in developing countries. Still, there is a high maternal morbidity rate due to pre-eclampsia, as it is the main reason for ICU (intensive care unit) admissions at the time of pregnancy.[8] Developing countries have a high (7%) prevalence rate of this disease compared to developed countries.[9,10] Two irregular metabolisms of lipids might enlighten pre-eclampsia. During PIH (pregnancy-induced hypertension), lipid level in the serum significantly increases. Systemic vasospasm is one of the most remarkable physiologic alterations in pre-eclampsia and is responsible for reduced virtual perfusion of all organs. There is also evidence of cardiovascular diseases in females with pre-eclampsia in the later stage of their life, suggesting long-lasting effects of pre-eclampsia even after pregnancies. Modifications in human lifestyle, for example, dietary patterns and delayed childbirth, have increased the global commonness of placental-associated complaints in the previous decades.[7] Cobalamin (Vitamin B12, water-soluble vitamin, an essential growth factor) has a metabolic function, is associated closely with homocysteine and folate, and is found only in animal-derived foods. It is essential to perform a function in the cellular production of energy, red blood cell production, synthesis and methylation of DNA, and neurological function. It may affect the placentation and growth of the fetus.[12] The deficiency of vitamin B12 (Cobalamin) is common in various low-middle earnings countries and can affect the above three-quarters of pregnant populations.[13,14] It is stated that a continuing decline in vitamin B12 concentration occurs during pregnancy, though it does not signify body deficiency or stores and can be related to dietary intake. The association of hyperhomocysteinemia and pre-eclampsia was suggested initially by Larsen, S. and has not been confirmed by all authors, although the majority of evidence suggests a positive association. Damaging the epithelium cells because of oxidative stress activated through homocysteine has also been considered a mechanism in pre-eclampsia's pathophysiology.[18] Another hypothesis proposes that homocysteine indirectly acts by its oxidation and its affiliation with the creation of ROS (reactive oxygen species).[6] Apoptosis of placenta in females having pre-eclampsia seems to be based on distressed one-carbon metabolites, including vitamin B12, folate, and polyunsaturated fatty acids with long-chain by oxidative stress, elevated homocysteine, and altered angiogenesis.[15] Although much knowledge regarding the risk factors associated with pre-eclampsia has been reported so far, the exact physiology of this disease is still not fully understood. Therefore, the current study has been designed to investigate whether vitamin B12 level and homocysteine in pregnant females with 28 weeks of gestation is a risk factor for pre-eclampsia development.

## METHODOLOGY

A case-control study has been conducted to assess the association of vitamin B12 with homocysteine levels in cases and compare them to the control group after taking ethical approval from Graduate Study Committee (GSC). This current research was approved by Advanced Study and Research Board (ASRB) under (ASRB000774/AV/IBMS) and Ethical Board under DIR/KMUEB/AV/000556. Sixty subjects participated in the study from Lady Reading Hospital (LRH) in the gynaecology ward. Patients were selected with newly diagnosed placenta abruption, whereas, for the control group, patients with pre-eclampsia without placenta abruption. Patients diagnosed with placenta abruption, 28 weeks of gestation, and only primigravida patients were included. Patients with normal pregnancy, less than 28 weeks of gestation, and taking Vitamin B12 supplementation are not included in this study because the fetus has not developed properly yet. The study was conducted after taking written consent from the patients on pre-designed proforma. Each participant was interviewed for data collection regarding their age, education, income, gestation weeks, and family history of hypertension and their blood pressure, urinary albumin, height, weight, and body mass index in kg/m2 (BMI). Blood samples (5 ml) were collected from each subject through sterilized syringes; 3 ml of blood was transferred to the ETDA tube and 2 ml into the clot activator Gel tube. Each sample was centrifuged and stored at -

20C. Vitamin B12 and homocysteine ELISA kits were used for measuring the vitamin B12 as well as homocysteine levels in each blood sample. Vitamin B12 was analyzed by Chemiluminescent Microparticle Intrinsic Factor assay. For both Vitamin B12 and homocysteine analysis, a commercially available ARCHITECH kit was used according to manufacturer instructions, and the test principle is based on the quantitative determination of vitamin B12 in human plasma on the ARCHITECH system. On the other hand, test-principal for homocysteine was based on the quantitative determination of total L-homocysteine values in human serum on the ARCHITECH system. The study results were analyzed using SPSS.22. The data's normality was checked using the Shapiro Walk test of normality. For numerical data presentations, means and standard deviations were used, and categorical data representation was done using frequency and percentages. An independent sample t-test was used to evaluate the difference between the two groups. Co-relation was used to check the association between vitamin B12 and homocysteine.

## RESULTS

The overall age difference between the case and control groups at baseline was 0.96. There was an insignificant difference between both groups Body Mass Index (BMI). The patients showed headaches, dizziness, vomiting, and nausea. A Chi-square test was used to evaluate the relationship between Vitamin B12 and homocysteine with different categorical variables.

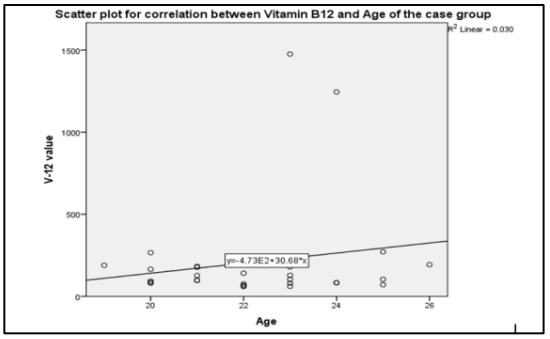


**Figure 1: Scatter Plot for the Correlation between the Age of the Case and Vitamin B12 Level (R=.172, R2=0.03, P=.364)**

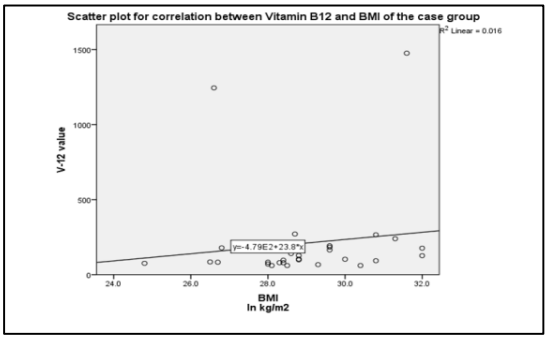


**Figure 2: Scatter Plot for Correlation between the Vitamin B12 and BMI of the Case Group (r=.128, $R^2$=0.016, P=.501)**

**Table 1: Demographic Characteristics of the Cases and Control**

| Demographic Characteristics | Cases N=30 | % | Control N=30 | % | P-Value |
|---|---|---|---|---|---|
| **Age Categories (Years)** | | | | | |
| (17-19) | 01 | 03% | 06 | 20% | .221 |
| (20-22) | 16 | 53% | 17 | 57% | |
| (23-25) | 12 | 40% | 05 | 17% | |
| (26-28) | 01 | 03% | 02 | 07% | |
| **BMI (Kg/m$^2$)** | | | | | |
| Healthy (18.5 – 24.9) | 01 | 03% | 07 | 23% | .220 |
| Overweight (25-29.9) | 21 | 70% | 19 | 63% | |
| Obese (30 and above) | 08 | 27% | 04 | 14% | |
| **Education** | | | | | |
| Illiterate | 09 | 30% | 11 | 37% | .881 |
| Under Primary | 07 | 23% | 06 | 20% | |
| Primary | 03 | 10% | 03 | 10% | |
| Middle | 01 | 03% | 06 | 20% | |
| Matric(SSC) | 10 | 33% | 02 | 07% | |
| Higher Secondary(HSSC) | 0 | 0% | 02 | 07% | |
| **Monthly Income** | | | | | |
| 2500-7499 | 03 | 10% | 08 | 27% | .548 |
| 7500-12499 | 17 | 57% | 16 | 53% | |
| 12500-17499 | 10 | 33% | 05 | 17% | |
| 17500-22499 | 0 | 01% | 03% | | |

**Table 2: Clinical Characteristics of Studied Population**

| Clinical Characteristics | Cases N=30 | Case Group Proportion | Control N=30 | Case Group Proportion |
|---|---|---|---|---|
| **Platelets Count** | | | | |
| 1. Normal (Greater than | 29 | 97% | 30 | 100% |
| 2. Low (100-150) | 01 | 03% | 0 | 0% |
| **HB level (g/dl)** | | | | |
| Normal HB (10-14) | 28 | 93% | 27 | 90% |
| Anaemia (Less than 10) | 02 | 07% | 03 | 10% |

**Table 3: Vitamin B12 Levels and Homocysteine in Both Groups**

| B-12 level Pmol/L | Case | Frequency | Control | Frequency |
|---|---|---|---|---|
| B-12 deficiency (<73) | 05 | 17% | 07 | 23% |
| B-12 Normal (73-388) | 23 | 77% | 22 | 73% |
| B-12 Surplus (>388) | 02 | 07% | 01 | 03% |
| **Mean and standard deviation of Vitamin B-12 level** | | | | |
| Mean | 209.47 | | | |
| Standard deviation | 320.19 | | | |
| **Homocysteine µmol/L** | | | | |
| Homocysteine deficiency (<4) | 0 | 0% | 03 | 10% |
| Homocysteine Normal (4-15) | 27 | 90% | 21 | 70% |
| Homocysteine surplus (>15) | 03 | 10% | 06 | 20% |
| **Mean and standard deviation of Homocysteine level** | | | | |
| Mean | 140.77 | | | |
| Standard deviation | 82.55 | | | |

**Table 4: Shows Chi-Square Test between Different Categorical Variables (N=60)**

| Demographic Characteristics | Cases N=30 | % | Control N=30 | % | P-Value |
|---|---|---|---|---|---|
| **Age Categories (years)** | | | | | |
| Age Group (17-19) | 01 | 03% | 06 | 20% | .162 |
| Age Group (20-22) | 16 | 53% | 17 | 57% | |
| Age Group (23-25) | 12 | 40% | 05 | 17% | |
| Age Group (26-28) | 01 | 03% | 01 | 07% | |
| **BMI category (Kg/m2)** | | | | | |
| Healthy (18.5 – 24.9) | 01 | 03% | 07 | 23% | .777 |
| Overweight (25-29.9) | 21 | 70% | 19 | 63% | |
| Obese (30 and above) | 08 | 27% | 04 | 14% | |
| **Height (cm)** | | | | | |
| 140-144 | 01 | 03% | 02 | 07% | .976 |
| 145-149 | 04 | 13% | 06 | 20% | |
| 150-154 | 12 | 40% | 12 | 40% | |
| 155-160 | 13 | 43% | 10 | 33% | |
| **Monthly Income (Rs)** | | | | | |
| 2500-7499 | 03 | 10% | 08 | 27% | .875 |
| 7500-12499 | 17 | 57% | 16 | 53% | |
| 12500-17499 | 10 | 33% | 05 | 17% | |
| 17500-22499 | 0% | 01 | 03% | | |
| 17500-22499 | 0% | 01 | 03% | | |

## DISCUSSION

Pre-eclampsia is considered one of the major causes of maternal and neonatal morbidity and mortality in low and middle-income countries. Homocysteine is formed during dietary methionine metabolism (largely present in animal protein). Folic acid and vitamin B12 (VitB12) are required for Homocysteine metabolism, and its deficiency can result in increased Homocysteine concentrations. Vitamin B12 is a water-soluble vitamin that is an essential growth factor. It is essential for properly forming red blood cells, DNA synthesis, and neurological function. It is important for the production and methylation of DNA and has a role in the cellular production of energy. Therefore, the main objective of the current study is to determine the levels of vitamin B12 and homocysteine in pre-eclamptic patients with placenta abruption and without placenta abruption. It was declared that the mean age of cases and controls was 22.23±1.79 in the case and 21.27±2.21 in the control group. Similarly, a study reported that the mean age was 24.00±4.14 and 25.84±5.43 in control, and the BMI of the cases were 24.3±1.2 while in control, the mean and standard deviation of BMI was 21.7±1.7.[2] whereas the current study declared that the mean BMI of the cases was 28.93±1.72 and 26.87±2.65 in the control group (3 and 4). Similar to current findings, a study reported that the age in the case and control were 24.40±3.85 and 24.19±3.67, respectively. In contrast, the 75 BMI was reported as 26.87±4.70 in cases and 24.79±3.94 in the control group.[3] Another study reported that the risk of the studied disease rises with increasing BMI. It was demonstrated that a quick increase is a threat across BMI distribution, showing pre-eclampsia risk is elevated within universal BMI categories. Because of this, women with a 28 BMI are 40% more at risk of pre-eclampsia onset than women with a 25 BMI (adjusted OR: 1.4; 95% confidence interval, 1.1, 1.6), although both have laid under the overweight category by conventional cut points. Secondly, by doubling and tripling BMI values defined as overweight or obese compared to a BMI of 21, the risk substantially increases at what are classically viewed as "normal" BMI values. Women with 24 BMI, usually classified as under the normal weight category, were 70% more at risk to increase pre-eclampsia than women with 21 BMI. Thirdly, it was suggested as a result of the risk curve that a strong and protective consequence of pregnancy is underweight. Women with a BMI of 21 were likely to have pre-eclampsia onset compared to women with a BMI of 17.[5] The underlying mechanisms of BMI–pre-eclampsia correlation have not yet been cleared. A decrease in placental perfusion, secondary to abnormal implantation and subsequent reduced placental vascularization, is the defining feature of pre-eclampsia.[6] The current study declared that in all the cases, 30 (100%) were primary and secondary educated and in control, 28 (93%) were primary

and secondary educated. Higher and above education was 38 (48.7%) in the control group, 39 (47.6%) in 76 cases, while in our study, only 2 (7%) were in the control group. Similarly, a study reported that the percentage of primary and secondary education was 40 (52.4%) in cases while 40 (51.3 %) in control.[4] The Pearson correlation test tested the association between homocysteine and vitamin B12. Our study found a non-significant relationship between Vitamin B12 and homocysteine (0.3% variation in the homocysteine level and Vitamin B12l; r= -0.55, P=0.679 in both cases and control). In contrast to current findings, a study by Zhao et al.[16] reported a significant correlation between homocysteine and vitamin B12 (r=0.34, p=0.001). The current study demonstrated no significant relationship between pre-eclampsia and homocysteine levels. In contrast, Acilmis et al.[17] have shown that the occurrence of hypertension disorder in pregnancy is related to an increase in homocysteine levels in pregnant women and that the level is related to the severity of the disease. Similarly, a study by Zhao et al. reported that the level of homocysteine in pre-eclampsia was significantly higher than in the control group. As a result of the univariate analysis, it was declared that the mean and standard deviation of systolic blood pressure in cases was 144.33 ± 12.51 mmHg and in control was 141.67 ± 11.01 mmHg whereas diastolic blood pressure was 99.50 ± 8.65 mmHg in cases and 100.17 ± 8.95 mmHg in controls. Similarly, a study reported slightly different systolic and diastolic blood pressure in cases and control i-e., 151.5±13.4 in cases, 111.0±8.7 in controls, and diastolic blood pressure was 100.1±10.0 and 68.6±7.4 in control respectively.[1] Another study reported that the systolic blood pressure was 145.57±17.46 in cases while 19.65±8.36 mmHg in controls, whereas diastolic blood pressure was 94.86±11.16 in cases and 77.19±5.26 in control.[3] The present study revealed that the homocysteine levels in cases and control were 9.02 ± 4.73 and 34.18 ± 133.43. Similarly, the study reported homocysteine levels in cases 8.4±4.9 and 8.0±4.8 in control. Another study reported that vitamin B12 was 287.2±158.7 in cases and 250.4±127.1 in control, whereas according to current findings, the mean and standard deviation of vitamin B12 in cases were 209.47 ± 320.19 SD greater than the mean value and 140.77 ± 82.55 in the control group. Another study reported that vitamin B12 were 336.30±43.70 in cases whereas 387.60±45.76 in the control group. Homocysteine was observed at 8.29±2.78 among cases and 7.25±1.37 in the control group.[4] In the pipeline of current findings, it was declared that Vitamin B12 ($p<0.05$) and homocysteine ($p<0.01$) were significantly higher in pre-eclampsia as compared to the normotensive control group. There was a positive association of homocysteine with systolic blood pressure (r=0.151, p=0.000, n=578) and diastolic blood pressure (r=0.213, p=0.000, n=578) in the whole cohort. Similarly, a positive association of homocysteine with diastolic blood pressure was observed in control (r=0.106, p=0.046, n=350) and pre-eclampsia (r=0.164, p=0.014, n=223) groups.

## LIMITATIONS

The sample size of our study was small and was taken from one hospital. Evaluating the relationship between Vitamin B12 and homocysteine in pre-eclampsia and abruption requires further studies. Insufficient nutrients during these phases can harm the function of vital tissues and down-regulate the growth of the fetus.

## CONCLUSION

Our results highlight the importance of Vitamin B12 and homocysteine in pregnant females with pre-eclampsia and placenta abruption. Insufficient nutrients during these phases can harm the function of vital tissues and down-regulate the growth of the fetus. Such changes can harm the fetus's survival but can show a long-lasting impact and increase the threat of type-2 diabetes, obesity, and cardiovascular diseases in children. Biological and epidemiological indications suggest maternal nutritional insufficiency can lead to pregnancy complications.

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