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# VITAMIN B 12 AND HOMOCYSTEINE LEVELS IN PRIMI GRAVIDA PATIENTS WITH PRE-ECLAMPSIA: AN EXPERIENCE FROM A TERTIARY CARE HOSPITAL OF PAKISTAN

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## ABSTRACT

**Objective:** To determine the frequency of serum vitamin B12 and homocysteine levels in patients with Pre-eclampsia.

**Methodology:** This cross-sectional study was carried out in Department of Gynecology and Obstetrics, Lady Reading Hospital, Peshawar in collaboration with the Department of Biochemistry of Khyber Medical University (KMU), from May 2019 to December 2019 after obtaining ethical approval from Advanced Study and Research Board (ASRB). Using non-probability convenient sample technique, 60 patients who were Primi Gravida with Pre-eclampsia were admitted via OPD or labour room. Data was collected from hospital record, charts and documented and analyzed in SPSS version 22.

**Results:** The mean age of the patients in this study was  $21.75 \pm 2.05$  years. The mean vitamin B 12 levels recorded in our study was  $175 \pm 34.39$  pmol/l. Vitamin B 12 levels in most of the participants, 45(75%) were in normal ranges. 12(20%) patients were deficient in vitamin B 12 levels while 03(05%) patients had high levels of vitamin B12. The mean serum Homocysteine level in our study was  $9.63 \pm 3.46$  umol/l. Most of the participants 48 (80%) had normal levels while 09(15%) patients had high levels of serum Homocysteine while only 03 (05%) patients were deficient in serum Homocysteine levels.

**Conclusion:** Majority of the patients with Pre eclampsia had normal levels of Homocysteine and vitamin B12. There was an inverse correlation between serum Vitamin B12 and Homocysteine levels in Pre-eclamptic patients

**Keywords:** Pre-eclampsia; Placental Abruption; Vitamin B 12; Homocysteine.

## INTRODUCTION

Pre-eclampsia is a pregnancy-specific condition arising after 20 weeks of gestation with new-onset hypertension (over 140 mmHg systolic or over 90 mmHg diastolic) and the coexistence of one or more of the following new-onset conditions: proteinuria and other maternal organ dysfunction like renal, liver, neurological, and haematological complications along with uteroplacental dysfunction completely resolving after 6 weeks postpartum.<sup>1</sup> Pre-eclampsia presents with a vast range of abnormal symptoms, signs and biochemistry for example; right upper quadrant or epigastric abdominal pain, oliguria or acute kidney shutdown, headaches, blurring of vision, etc. Pre-eclampsia occurs in 2-8% of pregnancies ranging from mild to severe forms. In its extreme form, it can result in serious maternal and fetal morbidity as well as mortality, accounting for about 15% of direct maternal deaths.<sup>2</sup>

The basic pathophysiology of Pre-eclampsia is de-

fective placentation leading to endothelial dysfunction and hence oxidative stress. It starts with deficient spiral artery invasion by cytotrophoblasts, developing the spiral arteries into high resistance maternal conduits.<sup>3</sup> The resultant decrease in blood supply makes vessels more sensitive to vasoconstrictors resulting in chronic placental ischemia and oxidative stress. The chronic placental ischemia is responsible for fetal complications seen in Pre-eclampsia like intrauterine growth restriction and stillbirth while oxidative stress-mediated reaction owes to maternal endothelial dysfunction and hyperpermeability giving rise to hypertension and classic signs and symptoms of multi-organ involvement, including placental abruption.<sup>4</sup> Placental abruption is a rare but serious complication that may manifest itself in by separation of the placenta from the uterus due to uteroplacental insufficiency. Preterm placental abruption is also associated with Pre-eclampsia in the next pregnancy.<sup>5</sup> Due to high-risk condition, researchers have always been interested to devise screening methods, history base or investigations to diagnose Pre-eclampsia,

well in time. Low serum Folate and serum vitamin B12 levels with high serum concentrations of Homocysteine have been found in patients with hypertensive disorders of pregnancy which results in damage to endothelial cells, forming the basis of pathophysiology of Pre-eclampsia.<sup>6-8</sup> Homocysteine, a thiol-containing amino acid is formed by demethylation of Methionine and is metabolized in kidneys through two processes. Firstly; remethylation by folic acid and vitamin B 12, secondly; via transsulfuration by vitamin B6.<sup>9</sup> Hyperhomocysteinemia is considered responsible for multiple neurodegenerative, neuropsychiatric, autoimmune, renal and vascular diseases including Pre-eclampsia, placental abruption and Intrauterine growth retardation.<sup>8,10</sup> Defective metabolism of Homocysteine or already compromised kidneys in Pre-eclampsia, failing to excrete increase Homocysteine concentrations is responsible for its increased levels.<sup>11</sup> Vitamin B 12 is a water-soluble vitamin also called cobalamin and acts as a co-factor in metabolism at the cellular level of the body. The remethylation of Homocysteine to Methionine depends on the presence of folic acid and vitamin B 12 in our body. The deficiency of either of them results in the accumulation of Homocysteine levels in the body, which is pathogenic.<sup>12</sup> Pre-eclampsia and Placental abruption both originate from placental vascular bed abnormal function. It is well established that supplementation of vitamin B12, vitamin B6 and folic acid decreases the serum concentrations of Homocysteine, which can reduce morbidity in the pregnant population.<sup>13</sup>

This study was designed to find out the levels of serum vitamin B12 and Homocysteine in our Pre-eclamptic population because the group represents the severity of hypertensive disorders in pregnancy. This would mean the use of this investigation in future antenatal population to prevent the grave and moribund condition by supplementing our antenatal hypertensive population with vitamin B12.

## METHODOLOGY

This cross-sectional study was carried out in Department of Gynecology and Obstetrics, Lady Reading Hospital, Peshawar in collaboration with the Department of Biochemistry of Khyber Medical University (KMU), from May 2019 to December 2019 after obtaining ethical approval from Advanced Study and Research Board (ASRB) of KMU.

Using non-probability convenient sample technique, 60 patients who were Primi Gravida after 28 weeks of gestation with Pre eclampsia were admitted via OPD or Labour room. Patients with more than 1 pregnancy, who did not consent, less than 28 weeks of gestation and patients taking vitamin B 12 supplements were excluded from the study. Diagnosis of Pre-eclampsia was made after history, examination and investigations which included routine and specific investigations, for Pre eclampsia. Verbal and written consent was taken for examination and investigations. Participants were asked to give 5 ml of blood. Blood was taken by applying an aseptic technique through the sterilized syringes. 3 ml of blood was transferred to EDTA tube and 2 ml into clot activator Gel tube. Each sample was taken for centrifugation, within an hour. After centrifugation samples were stored at -20C. After the completion of the required sample size, all samples were transferred for analysis of vitamin B12 and Homocysteine with help of the biochemistry department, KMU. Vitamin B12 and homocysteine levels were analysed by Chemiluminescent Microparticle Intrinsic Factor and Chemiluminescent Microparticle Immunoassay (CMIA), respectively by using a less time consuming and uncomplicated kit of ARCHIT-TECH which is commercially available. Data was collected, documented and analyzed in SPSS version 22. Continuous variables were presented as Mean and Standard deviation while categorical variables were presented as frequency and percentage. Spearman's rank correlation was also applied on Homo-

cysteine with blood pressure, hemoglobin and Body mass index. P-value  $\leq 0.05$  was considered as significant.

## RESULTS

A total of 60 patients were registered in the study. The mean age of the patient was found to be  $21.75 \pm 2.05$  years. Most of the patients, 55% were found to be in the age group 20-22 years while only 05% of patients were found in the age group ranging 23-25 years. The mean height recorded was  $153 \pm 6.1$  cm while the mean weight of the participants was calculated to be  $65.18 \pm 6.58$  kg. The mean BMI was calculated as  $27.90 \pm 2.46$  (Kg/m<sup>2</sup>) as given in Table No: 1. Analysis of BMI categorization shows the number of healthy participants with normal BMI was 8 (13%), overweight patients were 40 (66%) and obese were 12 (20%). Presenting complaints and symptoms at the time of arrival of the patients are described in Table No: 2. These patients presented with complaints of high blood pressure and a variety of symptoms. The prevailing symptoms were found to be headache, dizziness, nausea and vomiting, followed by swollen body and abdominal pain. The mean blood pressure was recorded as 142/100 mmHg. The mean urine albumin was recorded as 2+. Thus most of the patients in our study presented with moderate Pre-eclampsia depending on a cocktail of sign and symptoms at an average gestational age of 36 weeks  $\pm 2$  weeks.

The mean vitamin B-12 and Homocysteine was recorded  $175.12 \pm 34.39$  pmol/l and  $9.63 \pm 3.46$  umol/l respectively. Vitamin B 12 levels in most of the participants, 45(75%) were in normal ranges. About 12 (20%) patients were deficient in vitamin B 12 levels while 03(05%) patients had high levels of vitamin B12. Serum and plasma samples obtained from newly diagnosed cases and were analyzed for Hemoglobin% (HB%), platelet count, vitamin B 12 and Ho-

Table No 1: Demographic characteristic of the patients

Age (years)	21.75 ± 2.05
Weight (Kg)	65.18 ± 6.58
Height (cm)	152.83 ± 4.41
Systolic Blood Pressure mmHg	143.1 ± 11.76
Diastolic Blood Pressure mmHg	99.83 ± 8.73
BMI (Kg/m <sup>2</sup> )	27.90 ± 2.46
VIT B12 Level (pmol/L)	175.12 ± 34.39
Haemoglobin g/dl	11.68 ± 0.98
Platelets / cmm.	304.52 ± 69.31
Homocysteine (umol/L)	9.63 ± 3.46
Urinary albumin	2.22 ± 0.64

Table 2: Presenting complaints and symptoms at the time of arrival (n = 60)

Variables		n (%)
Complaints	Headache	60 (100%)
	Dizziness	60 (100%)
	Nausea /vomiting	60 (100%)
	Swelling in face and feet	48 (80%)
	Abdominal pain	15 (25%)
	Rapid weight gain	3 (5%)

Table 3: Vitamin B12 and homocysteine in patients with pre-eclampsia

Variables		n (%)
B12 (pmol/l)	Deficiency (< 73)	12 (20%)
	Normal (73 - 388)	45 (75%)
	Surplus (> 388)	3 (5%)
Homocysteine (umol/l)	Deficiency (< 4)	3 (5%)
	Normal (4 -15)	48 (80%)
	Surplus (> 15)	9 (15%)

Table 4: Correlation among homocysteine with study parameters

Parameters		VIT B12 Level (pmol/L)	HB%	Systolic BP	Diastolic BP	BMI (Kg/m <sup>2</sup> )
Homocysteine (umol/L)	r <sub>s</sub>	-0.204	-0.163	-0.093	0.006	-0.327
	p-value	0.028	0.212	0.483	0.962	0.011

Homocysteine levels. Most of the participants 48 (80%) had normal levels while 09(15%) patients had high levels of serum Homocysteine while only 03 (05%) patients were deficient in serum Homocysteine levels as given in Table No: 3. Among the 09 patients with raised serum Homocysteine levels, 05(55%) had normal vitamin B12 levels, 03(33%) patients had vitamin B12 deficiency and only 01(11%) had increased vitamin B12 levels.

Inverse correlation between serum Vitamin B12 and Homocysteine levels was found in Pre-eclamptic patients (r<sub>s</sub> = -0.204, p = 0.028). The correlation of Homocysteine was also determined with the blood pressure, HB and BMI (Kg/m<sup>2</sup>) as given in Table No 4. The insignificant correlation of Homocysteine was also determined with the blood pressure (systolic: r<sub>s</sub> = -0.093, p = 0.483 & diastolic: r<sub>s</sub> = 0.006, p = 0.962). Though, analysis also showed that significantly inverse cor-

relation between Homocysteine and BMI (r<sub>s</sub> = -0.327, p = 0.011).

## DISCUSSION

In our study the mean serum vitamin B12 value was found to be 175.12 ± 34.39 pmol/l in our study. We found out that most of the patients had normal serum vitamin B12 levels. Similar findings were seen by a study done by Serrano NC et al who determined the mean vitamin B 12 levels of 287.2 ± 250.4 pmol/l. Although the average determined by them was high as compared to our study population mean but their study also could not ascertain any association between vitamin B12 levels and Pre-eclampsia.<sup>6,14</sup> The mean serum Homocysteine level in our study was recorded to be 09 umol/l. Normal Homocysteine levels (4-15umol/l) were seen in 48 (80%) women, 09 (15%) patients had a surplus, while 03 (5%) patients had a deficiency of serum Homocysteine. A recent subcontinent study by Miglani S et al also confirmed normal serum Homocysteine levels, mean value being 13.9 umol/l in the Pre-eclamptic population, though it was higher than the antenatal population without hypertensive disorders of pregnancy.<sup>15</sup> Miglani S et al in their study also discovered levels of serum Homocysteine not to be significantly different in terms of severity, from mild to severe Pre eclampsia.<sup>15</sup> On the contrary, an Indian study in its results showed that the increase in serum levels of Homocysteine was directly proportional to an increase in severity of Pre-eclampsia, with the highest Homocysteine levels seen in eclampsia.<sup>16</sup>

Another study demonstrated that detection of serum vitamin B 12 levels can often be misleading, in the early stages of vitamin B12 deficiency. The vitamin B12 levels may be normal or increased, so a deficiency of vitamin B12 is better exhibited by elevated serum Homocysteine levels.<sup>17</sup> Our study shows a casual relationship between in-

crease serum Homocysteine and vitamin B12 levels. A study by Robert OH further supports the finding of our study, emphasizing that 50% of patients may have normal levels of vitamin B12 levels in blood in the early deficient stage while having high serum Homocysteine levels. Therefore a more reliable method of determining vitamin B12 deficiency is to check for serum Homocysteine and methylmalonic acid.<sup>18,19</sup> Acilmis YG et al also showed that Homocysteine levels were higher in severe pre-eclampsia vs mild Pre-eclampsia but vitamin B 12 deficiencies were not significantly associated with high Homocysteine levels.<sup>20</sup>

A combined study between gynaecology and the biochemistry department strengthens the analysis of our study but at the same time the small sample size of our study limits the results of our study. The small sample size was due to self-sponsored investigations. This stresses the need for larger studies to potentiate the findings of our results. Studies with a larger sample size would mean funding or availability of free tests within the hospital to analyze the serum vitamin B12 and Homocysteine levels as these tests are expensive.

## CONCLUSION

Majority of the patients with Pre eclampsia had normal levels of Homocysteine and vitamin B12. There was an inverse correlation between serum Vitamin B12 and Homocysteine levels in Pre-eclamptic patients

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#### Author's Contribution

SB helped in data collection, data analysis, data interpretation, and writing the manuscript. F helped in the collection of data, and data analysis and contributed in writing the discussion. TS contributed in the analysis and interpretation of data and finalize the manuscript. RN conceived the idea, designed the study, and finalized the statistical portion of the manuscript. Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

#### Conflict of Interest

Authors declared no conflict of interest

#### Grant Support and Financial Disclosure

None

#### Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.