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COMPARISON OF VITAMIN B12 LEVELS WITH PRE-ECLAMPSIA VERSUS NORMOTENSIVE PATIENTS

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Abstract

Objective: *To compare serum vitamin B12 levels in patients with pre-eclampsia and normotensive pregnant patients.*

Study Design: *A case-control study*

Place and Duration of Study: *Department of Gynae & Obs., National Hospital and Medical Centre, Lahore. 3 Months (20 February 2025 to 25 May 2025)*

Methodology: *This case-control study was conducted on 181 pregnant women, included 91 pre-eclamptic pregnant women as cases and 90 normotensive pregnant women as controls. Serum vitamin B12 levels were measured by chemiluminescence assay and compared between both groups using the independent sample t-test.*

Results

Mean serum vitamin B12 levels were significantly lower in the pre-eclampsia group than in the normotensive group (338.54 ± 96.27 pg/ml vs 421.83 ± 108.64 pg/ml; p<0.001). Blood pressure was significantly higher in pre-eclamptic patients, while other baseline variables were comparable between the groups.

Conclusion

Serum vitamin B12 levels were lower in patients with pre-eclampsia than in normotensive pregnant patients. Lower serum vitamin B12 levels were associated with pre-eclampsia and could have value in maternal risk assessment.

INTRODUCTION

Pre-eclampsia is a major hypertensive disorder of pregnancy linked to significant maternal and fetal complications. Vitamin B12 deficiency may contribute to its development through impaired homocysteine regulation and endothelial dysfunction. It continues to be a significant cause of maternal and perinatal morbidity and mortality especially in low and middle-income environments. One of the nutritional elements that are being studied is the vitamin B12 due to its role in the one-carbon metabolism, DNA synthesis, methylation reactions, and homocysteine regulation [1]. Vitamin B12 deficiency can also cause endothelial injury by disabling remethylation of homocysteine to hyperhomocysteinemia, oxidative stress, and vascular dysfunction, all of which are the causes of pre-eclampsia [2]. Some of the studies have used the vitamin B12 status of women with pre-eclampsia in comparison to normotensive pregnant women. Mahmoud et al. also found a reduction of serum

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vitamin B12 in preeclamptic women and indicated its correlation with unfavorable Doppler results and pregnancy outcome, which might have contributed to the severity of the disease and placental insufficiency [4]. Similarly, Yadav et al. have shown there is a substantial correlation between the variability in homocysteine, vitamin B12 and folate levels in women with pre-eclampsia, which supports the biological plausibility of the same [3]. Other sources have also attributed the pathogenesis of pre-eclampsia to oxidative stress and nutrient imbalance, among which is the vitamin B12 imbalance [3].

In the near past, larger studies have reinforced this correlation. Mardali et al. conducted a systematic review and meta-analysis and concluded that abnormal maternal serum vitamin B12 levels were related to pre-eclampsia, with some studies showing a stronger and more uniform association [1]. In a similar study, Farzaneh et al. measured several vitamins and reported that serum vitamin B12 status was one of the micronutrient data that was linked to pre-eclampsia and indicated that maternal vitamin status could contribute to the risk or progression of the disease [5]. Although these findings were made, evidence is still heterogeneous and population specific data are still required. Existing variance in nutrition, intake of supplements, socioeconomic status, and laboratory procedures might influence the reported levels of vitamin B12 in pregnancy. Thus, the current study was compared to the level of vitamin B12 in pre-eclampsia patients, and normotensive pregnant patients, to further elucidate the relationship between vitamin B12 status and pre-eclampsia and to add valuable information to the risk assessment and preventive actions of mothers [6].

Aim of the Study

This study aims to compare the levels of Vitamin B12 in pre-eclamptic pregnant women and normotensive pregnant women.

Hypothesis

Alternate Hypothesis: Serum vitamin B12 levels are lower among women with pre-eclampsia compared with normotensive pregnant women.

Methods and Procedures

Research Design

The study was a case-control study which was carried out at the Department of Gynae & Obs., National Hospital and Medical Centre, Lahore. The study duration was 3 months from (20 February 2025 to 25 May 2025 after approval of synopsis. The researchers compared the serum vitamin B12 concentration in patients with pre-eclampsia and the normotensive pregnant women.

Study Sampling

The sample size was calculated using the WHO sample size calculator for comparison of two means. The level of significance was kept at 5%, and the power of the test was 80%. The estimated mean vitamin B12 levels were 350.7 ± 283.1 pg/ml in pre-eclamptic pregnant women and 424.8 ± 364.7 pg/ml in normotensive pregnant women.

A total sample size of 181 participants was recruited using non-probability consecutive sampling. The participants included 91 pre-eclamptic pregnant women as cases and 90 normotensive pregnant women as controls.

Operational definition of pre-eclampsia:

Pre-eclampsia was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation, along with proteinuria of ≥1+ on urine dipstick or significant proteinuria on laboratory testing. Diagnosis was made according to ACOG criteria.

Inclusion criteria

- Women aged 20–35 years
- Singleton pregnancy at 34–40 weeks of gestation
- Non-obese women with BMI <30 kg/m²
- Cases: pregnant women diagnosed with pre-eclampsia according to the operational definition

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- Controls: BP <140/90 mmHg without history of hypertension or proteinuria.
- History of vitamin supplementation was recorded.

Exclusion criteria:

Multiple gestation
Gestational diabetes
Chronic hypertension
Type 1 or type 2 diabetes mellitus
Seizure disorder
Renal disease
Liver disease
These conditions were assessed from the patients' medical records..

Study Duration:

The research was conducted in 3 months following the synopsis approval.

Ethical Considerations

Prior to data collection, written approval was obtained from the hospital ethical committee. Written informed consent was taken from all participants. Confidentiality of participants' personal and clinical information was maintained throughout the study. All collected data were used only for research purposes.

Data Collection Procedure

A predefined proforma was used to record demographic and clinical data such as name, maternal age, gestational age, parity, height, weight to determine the BMI, systolic and diastolic pressure levels of blood.

1. Clinical assessment

All the participants were clinically assessed and appropriate obstetric and demographic information was recorded. The measurement of blood pressure was taken and the patients were classified according to the study definitions as cases and controls.

2. Laboratory analysis

Each participant was asked to give a blood sample of 5 ml by a competent phlebotomist whose experience in blood collection was not less than 2 years. The serum vitamin B12 level was assessed in the pathology laboratory, in which the blood samples were sent with the chemiluminescence assay (CLIA) technique.

Data Analysis

The SPSS version 27.0 was used to enter all data and analyze them. Numerical variables, such as maternal age, gestational age, BMI, systolic blood pressure, diastolic blood pressure, and vitamin B12 levels, had a mean given along with the standard deviation. The frequency and percentages of the categorical variable, parity were displayed. The independent sample t-test was used in comparison of vitamin B12 levels in cases and controls. The statistically significant p-value was considered as 0.05. Stratification of data was also done in terms of maternal age, gestational age, BMI and parity and same test was done after stratification.

Results

Maternal age, gestational age, BMI, and parity were similar in pre-eclampsia and normotensive groups because these differences were not significant. Nevertheless, there was a great difference in systolic and diastolic blood pressure in the pre-eclampsia group.

The comparison between the mean serum vitamin B12 level among cases and controls is expressed. The average vitamin B12 concentration was less in the pre-eclampsia group compared to the normotensive group and the difference was significant.

Women with pre-eclampsia had lower mean vitamin B12 levels compared to normotensive women in all age groups of women with pre-eclampsia. The difference was statistically significant among the age categories.

The serum vitamin B 12 levels comparison upon stratifying by the gestational age. The pre-eclampsia group had low levels of vitamin B12 compared to the normotensive group in both

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gestational age groups (table 4). The statistically significant difference shows that the association was constant in different gestational age categories.

The levels of serum vitamin B12 are stratified by BMI. Both groups of BMI patients had lower vitamin B12 levels among pre-eclamptic patients than normotensive patients (table 5). The large p-values show that BMI did not have a significant influence on the observed association.

The results of the comparison between the serum vitamin B12 levels based on the parity are presented. The mean vitamin B12 levels of both primigravida and multigravida women with pre-eclampsia were lower than their normotensive counterparts. These statistically significant differences between the two parity groups also indicate the correlation between low levels of vitamin B12 and pre-eclampsia.

Table 1: Baseline Characteristics of Study Participants

Variable	Pre-eclampsia Group (n=91) Mean ± SD / n (%)	Normotensive Group (n=90) Mean ± SD / n (%)	p-value
Maternal age (years)	28.64 ± 4.12	27.98 ± 3.89	0.271
BMI (kg/m²)	27.41 ± 1.89	26.98 ± 1.76	0.114
Gestational age (weeks)	36.48 ± 1.72	36.91 ± 1.65	0.089
Gravidity			0.268
Primigravida	49 (53.8%)	41 (45.6%)	
Multigravida	42 (46.2%)	49 (54.4%)	
Systolic blood pressure (mmHg)	154.32 ± 12.41	113.27 ± 6.84	0.001
Diastolic blood pressure (mmHg)	98.16 ± 8.73	74.92 ± 5.31	0.001

Table 2: Comparison of Serum Vitamin B12 Levels Between Cases and Controls

Variable	Pre-eclampsia Group (n=91) Mean ± SD	Normotensive Group (n=90) Mean ± SD	p-value
Serum Vitamin B12 (pg/ml)	338.54 ± 96.27	421.83 ± 108.64	0.001

Table 3: Stratification of Serum Vitamin B12 Levels by Maternal Age

Maternal Age Group (years)	Pre-eclampsia Group Mean ± SD	Normotensive Group Mean ± SD	p-value
20–25	329.41 ± 89.36	417.52 ± 101.47	0.002
26–30	341.85 ± 94.18	425.61 ± 109.83	0.001
31–35	345.92 ± 103.24	423.17 ± 114.55	0.010

Table 4: Stratification of Serum Vitamin B12 Levels by Gestational Age

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Gestational Age Group (weeks)	Pre-eclampsia Group Mean ± SD	Normotensive Group Mean ± SD	p-value
34–36	332.76 ± 92.11	414.29 ± 103.62	0.001
37–40	344.89 ± 99.34	428.76 ± 111.18	0.001

Table 5: Stratification of Serum Vitamin B12 Levels by BMI

BMI Group (kg/m^2)	Pre-eclampsia Group Mean ± SD	Normotensive Group Mean ± SD	p-value
<25	346.17 ± 91.84	432.54 ± 105.28	0.003
25–29.9	334.28 ± 98.46	416.39 ± 109.72	0.001

Table 6: Stratification of Serum Vitamin B12 Levels by Parity

Parity	Pre-eclampsia Group Mean ± SD	Normotensive Group Mean ± SD	p-value
Primigravida	331.92 ± 95.77	418.46 ± 104.91	0.001
Multigravida	346.27 ± 96.81	424.65 ± 112.37	0.002

Hypothesis Result

The study findings supported the research hypothesis that serum vitamin B12 levels differ between patients with pre-eclampsia and normotensive pregnant patients. Since the mean vitamin B12 level was significantly lower in the pre-eclampsia group (p 0.001), the null hypothesis was rejected and the alternative hypothesis was accepted.

Discussion

The present study demonstrated significantly lower serum vitamin B12 levels in women with pre-eclampsia than in normotensive pregnant women (338.54 ± 96.27 vs. 421.83 ± 108.64 pg/ml; p=0.001). Because age, BMI, gestational age and parity were comparable between groups, this difference suggests an independent association between reduced vitamin B12 status and pre-eclampsia.

These findings are comparable with national evidence from Pakistan. Bawar et al. reported vitamin B12 deficiency in primigravida patients with pre-eclampsia and found an inverse relationship between vitamin B12 and homocysteine, supporting the role of impaired one-carbon metabolism in the disease process[8]. The present study extends this local evidence by including a normotensive comparison group and showing consistently lower B12 levels across age, gestational-age, BMI and parity strata.

Regional studies from South Asia also support these findings. Mujawar et al. in India observed lower vitamin B12 and folate with higher homocysteine among pre-eclamptic women[15]. Similarly, Yadav et al. in Nepal reported an association of homocysteine with vitamin B12 and folate levels in women with pre-eclampsia[2]. These results are biologically plausible because vitamin B12 deficiency can elevate homocysteine, which may contribute to endothelial dysfunction, oxidative stress and placental vascular injury.

Internationally, the present results agree with Shahbazian et al., who found lower vitamin B12 in Iranian pre-eclamptic women than healthy pregnant controls[16]. A systematic review and meta-analysis by Mardali et al. also concluded that maternal vitamin B12 levels are significantly lower in pre-eclampsia, although heterogeneity was high[1]. However, not all studies agree. Sanchez et al. in Peru and Serrano et al. in Colombia found weaker or non-independent associations between vitamin B12 and pre-eclampsia[17,18]. Differences in diet, supplementation, ethnicity, assay methods

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and adjustment for folate or homocysteine may explain these inconsistencies.

Strengths

This study included a reasonable sample size with almost equal cases and controls. Serum vitamin B12 was measured, and both groups were comparable in baseline characteristics.

Limitations

This was a single-centre case-control study, so causality cannot be established. Vitamin B12 was measured only once, and factors such as diet, supplementation, folate, homocysteine, and socioeconomic status were not assessed.

Conclusion

Serum vitamin B12 levels were lower in pre-eclamptic women than in normotensive pregnant women. This suggests a possible association between vitamin B12 deficiency and pre-eclampsia, potentially through hyperhomocysteinemia, oxidative stress, and endothelial dysfunction.

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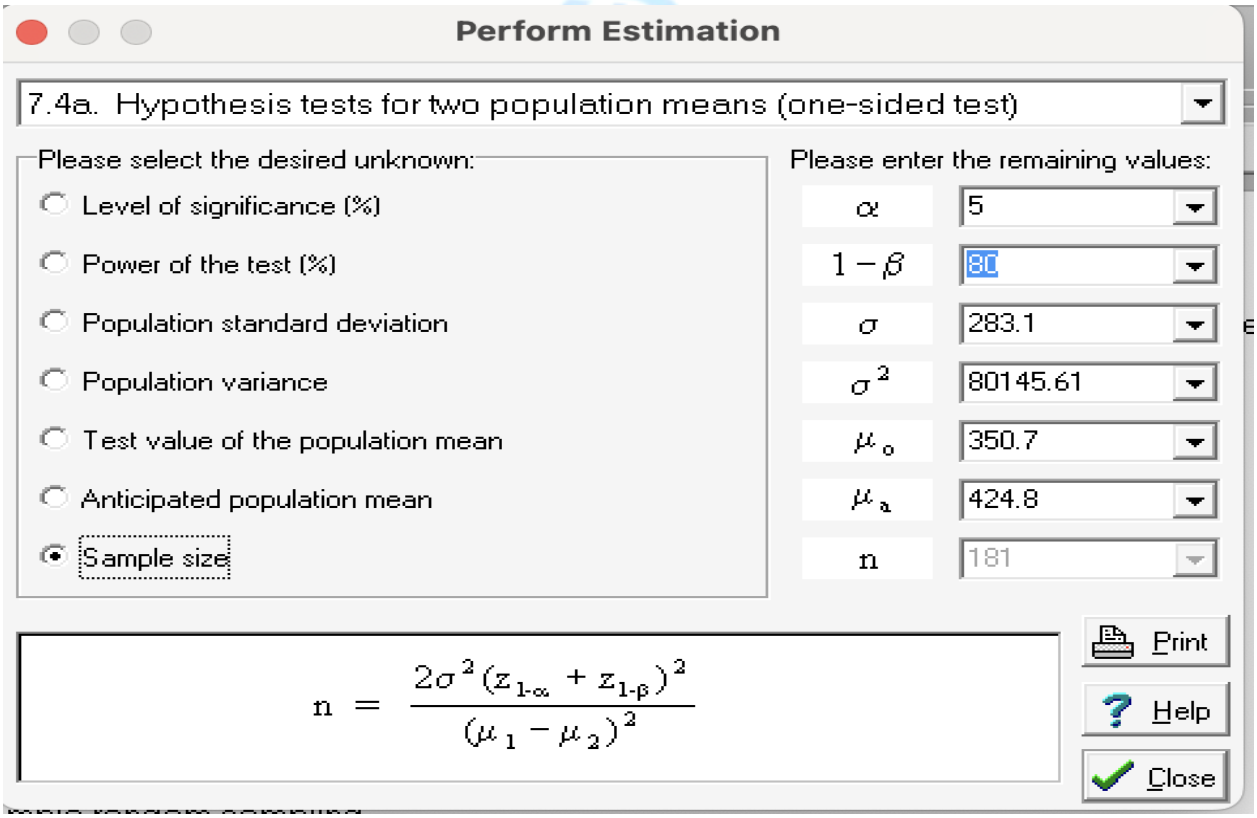
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Annexure:



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Declaration:
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