

Assessment of association of Folate and Homocysteine levels with development of Pre-eclampsia among pregnant women

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ABSTRACT

Objective: To determine the association of folate and homocysteine with pre-eclampsia and to ascertain the consequences of pre-eclampsia on foetal birth weight.

Study Design: A cross-sectional, case-control study.

Place and Duration: From 3th May 2019 to 3rd October 2019 at the Gynecology Department of Jinnah Medical & Dental College in Karachi.

Methodology: A total of 76 women with pre-eclampsia were randomly selected after establishing diagnostic criteria on blood pressure and proteinuria. Serum folate and Homocysteine levels were determined by collecting antecubital vein blood and running the assays. Based on the assay results, the study participants were divided into three groups: severe pre-eclampsia, mild pre-eclampsia, and normotensive women. Women were also evaluated for gestational age, maternal age, infant birth weight, and body mass index.

Results: Homocysteine was found substantially higher in maternal serum in severe pre-eclampsia than in the mild pre-eclampsia population and controls ($p=0.02$). However, homocysteine levels were not important in differentiating the control groups from moderate pre-eclampsia ($p>0.05$). No significant variation was noted in folic acid ($p>0.05$). Additionally, pre-eclampsia was significantly correlated with gestational age and the child's birth weight ($p>0.05$).

Conclusion: The study's findings indicated homocysteine is significantly associated with the development of pre-eclampsia and could act as a potential biomarker in its detection.

Keywords: Pre-eclampsia, Fetal outcome, Obstetric complications, Homocysteine level, Folic acid, Gestational age

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INTRODUCTION

Pre-eclampsia is characterized as the presentation of proteinuria and hypertension following the twentieth week of gestation in

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women with no history of hypertension¹. It is one of the major causes of fetal-maternal mortalities worldwide, affecting 5-8% pregnant women². To date, the underlying etiology couldn't be fully understood, yet some studies have developed a correlation between preeclampsia and folate and homocysteine profile³. Since the start of the 21st century, many researchers have evaluated the association of maternal concentration of homocysteine. They also analyzed folate and the potential risk of development of Pre-eclampsia³. Resultantly, growing evidence predicts that hyper-homocysteinemia could be an underlying cause of endothelial dysfunctioning. That is stimulated by oxidative stress in pre-eclampsia⁴. Physiologically, homocysteine acts as an intermediate amino acid converted into methionine or cysteine. During its metabolism, homocysteine necessitates folate as a co-substrate and vitamin B12 as a cofactor for the optimum functioning of the enzymes involved in the metabolic pathway, such as the methionine synthase enzyme⁵. Therefore, decreased folate levels disturb the regular metabolic pathway, consequently elevating the levels of homocysteine in the blood. Thus multiplying the risk of development pre-eclampsia⁶.

This association has also been validated through vitro studies. Through the conduction of experiments in vitro studies on human trophoblastic cells taken from the placenta of healthy

pregnant women, it has been observed that a higher concentration of homocysteine is capable of inducing apoptosis of these trophoblastic cells⁷. However, this apoptosis, along with other associated pathological, either suppressed or reverted upon treatment of trophoblastic cells with folic acid. Further, in vitro studies have concluded that culturing of human trophoblastic cells acquired from placental tissue during the initial months of pregnancy in the presence of folic acid triggers an elevation in extra villous trophoblastic invasion and vascular density (angiogenesis)⁸.

Although the association between the incidence of pre-eclampsia and folate and Hcy is widely under discussion worldwide, Pakistan still lags in this study area. A study concluded that around 25% of women from the studied population suffered from pre-eclampsia in Pakistan⁹, reflecting the severity of the situation and demanding the conduction of rational studies in this domain. Therefore, the study is designed to ascertain the association of two biomarkers with the incidence of pre-eclampsia in Pakistan. Additionally, the consequence of pre-eclampsia on fetal birth weight is also evaluated. So, we conducted this study with an objective to determine the association of folate and homocysteine with pre-eclampsia and to ascertain the consequences of pre-eclampsia on foetal birth weight.

METHODOLOGY

The cross-sectional, case-control study was conducted at the Gynaecology Department of Jinnah Medical & Dental College in Karachi for six months from 3rd May 2019 to 3rd October 2019. A sample of 78 pregnant women, who were in the third trimester of pregnancy, was randomly selected, through a simple random sampling technique. Women with any comorbidity or history of previous feto-maternal condition were excluded from the study. The study participants were informed of the study's objective, and their consent was acquired. Participants were initially inquired of maternal age and the body mass index (BMI) to assess if these variables correlate with the development of pre-eclampsia. To evaluate the two biomarkers, folate and homocysteine, maternal blood samples were taken by the antecubital veins after the consent of the ethical committee. Serum was separated, and chemical assays were run to ascertain

the concentration of the biomarkers in the maternal blood. After assessing the levels of biomolecules, the pre-eclamptic status of the participated women was assessed through the following criteria: women with blood pressure greater than 160/110 mmHg and proteinuria greater than 5 g/day were defined as pre-eclamptic¹⁰. In contrast, mild pre-eclamptic status was designated when neither blood pressure nor proteinuria matched the criterion mentioned above¹¹. Healthy normotensive women whose evaluation found no evidence of proteinuria were designated as controls. Resultantly, 3 study groups: pre-eclampsia, mild-eclampsia, and control groups were formed from our initially selected participants. Pre-eclamptic and control participants were then evaluated to correlate the disease status (pre-eclampsia) with their homocysteine and folate levels. The participants were also followed at the time of delivery of the baby, assessed for birth weight.

Statistical Analysis: Computer software SPSS. 20.0 was used for statistical analysis. All values were specified as standard and mean differences. A one-way variance analysis to compare group media was performed. The Tukey test was used for all contrast procedures on the pair. Furthermore, correlation analysis was done with the Pearson product-moment correlation coefficient.

RESULTS

Among the total of 78 participants, three groups, pre-eclampsia (P), mild pre-eclampsia (MP), and normotensive women (control), were formed with 26 participants in each group. On comparison of baseline variables and fetal health among the groups, it was found that no significant difference occurred between the three groups in terms of maternal age and body mass index (BMI). However, fetal birth weight and gestational age negatively correlated with the severity of pre-eclampsia. The inverse correlation was found to be significant (Table-I). Furthermore, evaluation of biomarkers dictated that folate was highest among severe pre-eclamptic women compared to the other two groups. However, the difference in the levels was not significant ($p=0.853$). Similarly, homocysteine levels were also found to significantly highest among the pre-eclamptic population ($p<0.05$); however, no significant difference was found between mild eclamptic and control groups (Table-II).

Table-I: Comparison of Multiple Variables among Three Study Groups (N=78)

	Pre-eclampsia (n=26)	Mild Pre-eclampsia (n=26)	Control (n=26)	P-value	P-MP	P-Control	MP-Control
Maternal Age (years)	30.03±5.21	29.61±4.73	29.6±3.59	>0.05	$p>0.05$	$p>0.05$	$p>0.05$
Fetal Birthweight(g)	1906.50 ±846	2731.7±452	3236.9±321	<0.05	$p<0.05$	$p<0.05$	$p<0.05$
Gestational Age(week)	32.50 ±3.93	35.2±2.09	37.6±1.24	<0.05	$p<0.05$	$p<0.05$	$p<0.05$
BMI	28±2.50	28.2±2.2	28.3±2.4	>0.05	$p>0.05$	$p>0.05$	$p>0.05$

Table-II: Comparison of Amount of Homocysteine and folic acid levels in among Three Studied Groups (N=78)

	Pre-eclampsia (n=26)	Mild Pre-eclampsia (n=26)	Control (n=26)	P-value	P-MP	P-Control	MP-Control
Homocysteine umol/l)	6.79±1.55	5.53±0.96	5.8±2.13	0.001	$p<0.05$	$p<0.05$	$p<0.05$
Folic Acid (ng/mL)	10.23(7.16-13.4)	10(8.1-11.6)	9.70(7.6-13.2)	0.852			

DISCUSSION

The findings revealed that the plasma concentrations of homocysteine were higher among pregnant women with extreme pre-eclampsia relative to moderate pre-eclampsia and control groups. However, this change in homocysteine levels was not correlated with folic acid in maternal plasma.

Against the established literature, our study recorded the highest folate levels in the pre-eclamptic group, which contrasts with previously held studies. Serrano et AL., while finding the correlation between three biomarkers, folate, homocysteine, and Vit B12, concluded that an adequate amount of folate is important for feto-maternal health and to keep the homocysteine in normal ranges and to mitigate the risk of pre-eclamptic development¹². Similarly, another study effect of folic acid supplementations was compared among users and non-users. It was found that the risk of pre-eclampsia was reduced among users than non-users. However, certain guidelines were set for the folate doses during the gestational period¹³. In 1997 in Colombia, given the importance of folic acid, it was decided to fortify wheat flour with the appropriate amount of folic acid¹⁴; unfortunately, no credible reports are officially available on the implications of this supplementation on general public. Additionally, since the start of the century, it is nationally recommended to begin folic acid intake (1 mg/day) from the start of pregnancy throughout the first trimester to mitigate the risks of the development of neural tube defects in children. A similar daily intake is also suggested to be continued during the rest of the conception until 6 months postnatal, along with an appropriate dose of ferrous sulfate, to decrease the prevalence of anemia.

Literature dictates that pre-eclampsia is developed in two stages. The first stage occurs during the first or second trimester characterized by abnormal implantation, while in the second stage, endothelial dysfunction occurs. Folic acid is used by the body to prevent this endothelial dysfunction¹⁵.

Similar to folate, homocysteine levels are also found to be significantly elevated in severe pre-eclamptic groups. A high level of Hcy has been experimentally proved to be a causative agent of vascular diseases and disruptor of endothelial cells, which is characteristic of pre-eclampsia. These results were similar to already established studies. A study conducted by Zao and his colleague concluded a significant difference of Hcy between the pre-eclamptic and control group¹⁶. Dodd's et al. concluded that elevated levels of Hcy in early pregnancy are the risk factor of pre-eclampsia¹⁷. Similarly, Acilmis et al., found that serum level of Hcy is a strong indicator of pre-eclampsia where an increase in concentration reflects the development of severity in disease¹⁸. Many other associated studies have proved the negative correlation of Hcy and folate against our study results^{19,12}.

Besides, severe pre-eclampsia is significantly associated with the birth weight of the infant and gestational age. It was found that women who developed pre-eclampsia delivered the babies early than the appropriate time. Moreover, babies were found to be unhealthy and low in weight, as already found in previous studies²⁰.

The results of this study don't completely comply with previous studies. It is recommended to conduct further studies with larger sample size and for a longer duration to ascertain the association between studied variables. Moreover, investigative studies should be conducted to determine the supplementation and dietary habits of normal healthy mothers.

CONCLUSION

The study's findings indicated homocysteine is significantly associated with the development of pre-eclampsia and could act as a potential biomarker in its detection.

AUTHOR'S CONTRIBUTION

Ghafoor S: Conceived idea, Designed research study, Data analysis, Manuscript writing, Final critical review of manuscript

Ali N: Conceived idea, Designed research study, Data analysis, Manuscript writing, Manuscript writing, Data analysis, Data collection, Literature review

Salah F: Manuscript writing, Data compilation, Data analysis

Hussain SF: Manuscript writing, Data compilation, Data analysis, Data collection, Literature review

Zareen S: Data collection, Literature review

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