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Original Article

Role of Serum Vitamin B12 and Glutathione Peroxidase during Third Trimester of Pregnancy with Gestational Diabetes Mellitus

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ABSTRACT

Objective: To investigate the role and relationship of serum Vitamin B12 and Glutathione peroxidase in pregnancy with gestational diabetes mellitus (GDM).

***Study Design*:** Comparative cross-sectional study.

Place and Duration of Study: Physiology Department, University of Health Sciences, Lahore Pakistan, from Feb 2019 to Mar 2020.

Methodology: Ninty pregnant women were recruited, fulfilling the inclusion and exclusion criteria. Women with GDM were the cases, and those without GDM were the controls. Serum Vitamin B12 and Glutathione peroxidase were measured by ELISA.

Results: Serum Vitamin B12 was significantly lower in cases compared to controls (158.98±7.38 and 357.48±42.07mg/dL, respectively, at $p<0.001$. Glutathione peroxidase was also significantly lower in cases compared to controls (2.68±1.13U/mL and 5.53±1.05U/mL, respectively, at $p<0.001$. Correlation analysis showed that Vitamin B12 and Glutathione peroxidase had a significant positive correlation in cases ($p<0.001$ and rho=0.76) and a non-significant correlation in controls ($p=0.87$ and rho=-0.024). Linear regression analysis showed that Vitamin B12 positively affects Glutathione peroxidase ($\beta=0.13$, $p<0.001$). Vitamin B12 and Glutathione peroxidase have a significant, negative effect on fasting blood glucose ($\beta=-0.06$, $p<0.001$ and $\beta=-3.66$, $p<0.001$, respectively).

Conclusion: Deficient Vitamin B12 leads to reduced Glutathione levels in GDM pregnancy. A deficiency of these two predicts elevated blood sugar levels.

Keywords: Gestational diabetes mellitus, Glutathione peroxidase, Oxidative stress, Vitamin B12.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a state of intolerance for glucose that is identified in pregnancy and is not present previously.[1] GDM is linked with maternal and fetal complications. Maternal complications comprise preeclampsia and type-2 diabetes mellitus (T2DM), while in the fetus, it causes shoulder dystocia and a long-term risk of obesity.[2,3] Recently, deficient Vitamin B12 (Vit B12) and Glutathione peroxidase (GPX) levels have been linked with oxidative stress, and GDM.[4,5] Vit B12 is an essential Vitamin, important for developing blood cells and the nervous system.[6] Vit B12 has antioxidant properties and helps destroy reactive oxygen species (ROS), along with the preservation of Glutathione.[6,7] Deficiency of Vit-B12 may occur due to poor dietary intake or absorption and increased cell turnover. Deficient Vit-B12 levels lead to elevated levels of ROS and homocysteine.[8] The antioxidant enzymes come into play to remove these free radicals. Glutathione peroxidase (GPX) is an antioxidant enzyme that protects against ROS and oxidative injury. When the level of free radicals increases, the combating antioxidants might exhaust, causing the free radical levels to surpass the antioxidant GPX levels. A deficiency of antioxidants may also occur due to inadequate nutritional intake of antioxidant enzymes and Vitamins.[9,10] As both Vit B12 and GPX are important factors in GDM pathogenesis and progression, the present study has been carried out with the objectives of comparing the serum levels of Vitamin B12 and Glutathione peroxidase in GDM and non-GDM group and determining the relationship between Vit B12 with GPX in GDM. To the best of our knowledge, no studies have been conducted in our population to determine the relationship between Vitamin B12 and Glutathione peroxidase in the third trimester of pregnancy complicated by gestational diabetes mellitus.

METHODOLOGY

The comparative cross-sectional study was conducted at various Government sector hospitals of

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rural and urban areas of Lahore from 2019 to 2020. The study was approved by the Ethical Committee of the University of Health Sciences Lahore (UHS/REG-19/ERC/401).

Inclusion Criteria: Pregnant women aged 19 to 40 years and at 28-32 weeks of gestation presenting at various Government sector hospitals of Lahore were included in this study.

Exclusion Criteria: Pregnant women with parity >5, pre-gestational diabetes, hypertension, chronic kidney and liver disease and taking Vit B12 and folate supplementation were excluded from the study.

A written, informed consent and detailed history were taken from the study participants.The sample size was calculated to be 30 in each group, but 90 pregnant women were sampled to increase the statistical power of the study. Among 90 pregnant women, 45 were normal pregnant women (Controls), and the other 45 had GDM (Cases).

5ml of venous blood was drawn from the study subjects after an overnight fast of 8 to 12 hours following the aseptic technique. Blood was centrifuged to extract the serum for Vitamin B12 and Glutathione peroxidase analysis. The serum was secured in labelled Eppendorf tubes, stored at -80^{o}C within 4 hours of collection, and thawed just before analysis. Serum levels of Vitamin B12 and Glutathione peroxidase were determined by ELISA (Bioassay Technology Laboratory). At the same time, blood glucose levels were determined by the glucose oxidase method.

The data were entered into Microsoft Excel sheets and then analyzed using Statistical Package for the social sciences (SPSS) version 23.00. The normality of distribution was analyzed utilizing the Shapiro-Wilk test. The group differences between cases and controls were analyzed using an independent sample t-test since the data was observed to have a normal distribution. Pearson correlation test was used to analyze the correlation between Vitamin B12 and Glutathione peroxidase. Linear regression analysis was done to see the causal association of Vitamin B12 with Glutathione peroxidase and their combined effect on gestational diabetes mellitus. At the same time, the significance level was taken at $p \leq 0.05$.

RESULTS

Among 90 pregnant women, 45(50%) were cases, and 45(50%) were controls. The mean age of the pregnant women was 28.95±4.31 years. Their mean gestational age was 29.71±1.50 weeks (Table-I). The two groups were age (p=0.45) and gestational age (p=0.29) matched. There was a statistically significant difference in blood sugar fasting levels between the two groups.

Table-I: Baseline and Biochemical Parameters of Study Population (n=90)

Baseline and Biochemical Parameters	Mean± SD
Age (years)	28.95±4.31
Parity	2.25±0.97
Gestational age (weeks)	29.71±1.50
BMI (Kg/m2)	25.54±3.06
Blood sugar fasting (mg/dL)	92.50±24.75
Vitamin B12 (mg/dL)	274.82±92.67
Glutathione peroxidase (U/mL)	4.12±1.36

It was observed that mean fasting blood sugar levels were significantly elevated in the GDM group, 103.74±5.88g/dL, compared to the non-GDM group, 79.36±6.20g/dL at p<0.001. In addition, Vitamin B12 levels were lower in the GDM group, 158.98±7.38mg/dL, compared to the non-GDM group, 357.48±42.07mg/dL at p<0.001, which was statistically highly significant. In addition, there were significantly lower levels of GPX in the GDM group, 2.68±1.13U/mL, compared to the non-GDM group, 5.53±1.05U/mL at p<0.001 (Table-II).

Table-II: Comparison between Gestational Diabetes Mellitus (GDM) and non-GDM Groups (n=90)

Variables	Gestational Diabetes Mellitus (n=45)	Non-GDM (n=45)	*p*-value
	Mean±SD	Mean±SD	
Gestational age	29.64±1.39	29.92±1.46	0.299
BSF	103.74±5.86	79.36±6.20	<0.001
Vit B12	158.98±7.38	357.48±42.07	<0.001
GPX	2.68±1.13	5.53±1.05	<0.001

There was significant negative correlation of Vit B12 with blood sugar fasting (p<0.001, r=-0.86**) in the GDM-group. Glutathione peroxidase also corre-lates significantly negatively with blood sugar fasting (p<0.001, r=-0.08**). Vitamin B12 has a statistically significant and positive correlation with GPX (p<0.001, r= 0.76**). At the same time, no significant correlation was observed between Vitamin B12 and Glutathione peroxidase in the non-GDM group (p=0.87 and r=-0.02) (Table-III). The linear regression analysis showed that Vitamin B12 positively affects Glutathione peroxi-dase levels, and the relationship was statistically signi-ficant (β=0.13, p<0.001). One unit change in B12 can bring about a 0.13-unit change in Glutathione peroxi-dase levels. Conversely, Vitamin B12 has a statistically

significant negative effect on fasting blood sugar levels (β=-0.06, p<0.001). Glutathione peroxidase also significantly negatively impacts blood sugar fasting levels (β=-3.66, p<0.001) (Table-IV).

Table-III: Correlation Analysis in GDM and Non- GDM Groups (n=90)

		Gestational Diabetes Mellitus			Non-GDM	
		GPX	BSF	Vit B12	GPX	Vit B12
GPX	Correlation	-	-.0861**	0.765**	-	-0.02
	Significance	-	<0.001	<0.001	-	0.87
BSF	Correlation	-0.86		-0.864**	-	-
	Significance	<0.001	-	<0.001	-	-
Vit B12	Correlation	0.76**	-.864**		-0.02	-
	Significance	<0.001	<0.001		0.87	-

GPX; Glutathione Peroxidase, BSF; Blood Sugar Fasting, Vit B12; Vitamin B12

Table-IV: Linear Regression Analysis (n=90)

Variables	Unstandardized Co-Efficient		t	p-value
	B	STD.Error		
Vit B12→GPX				
(Constant)	-13.19	3.521	3.746	<0.001
Vitamin B12	0.138	0.022	6.273	<0.001
GPX+Vit B12→BSF				
(Constant)	123.205	1.877	65.639	<0.001
Vitamin B12	0.065	0.01	6.50	<0.001
GPX	3.669	0.567	6.471	<0.001

DISCUSSION

The most striking findings of our study were the significantly lower levels (p<0.001) and significant positive correlation of Vitamin B12 and Glutathione peroxidase (p<0.001, rho=0.76**) in the GDM group as compared to the non-GDM group.

Our study duplicated the findings of Sukumar *et al*. (2016), who also reported significantly lower levels of Vit B12 in the GDM group.[10]

Our study also reproduced the findings of a local study conducted on GDM pregnancy. Deficient B12 levels in our study population can be due to lower socioeconomic status, a vegetarian diet, and poor consumption of B12 due to the high costs of animal-derived food in our study groups.[11,12]

Glutathione peroxidase levels were also significantly lower (p=0.0001) in pregnancy with GDM, just like the findings reported by another study.[13] Our study reproduced the findings of other international studies which reported lower levels of Glutathione peroxidase in diabetic pregnancy.[14] The lower levels of Glutathione peroxidase in our GDM group may be due to inadequate intake of antioxidant Vitamins or enzymes or exhaustion of endogenous GPX defence mechanism.[15]

Results of the Pearson correlation showed that lower Vitamin B12 levels reduced the Glutathione peroxidase levels (p=0.00, rho=0.76**) in GDM pregnancy. In addition, in the GDM group, there were lower Vitamin B12 (p=0.00, rho=-0.86) and Glutathione peroxidase (p=0.000, rho=-0.864) inversely correlated to blood sugar fasting. Al-Maskari *et al*. (2012) also stated lower Glutathione peroxidase levels in diabetic patients with lower Vitamin B12 levels.[16]

Our study observed that 1 unit decrease in B12 levels causes a 0.13-unit decrease in Glutathione peroxidase levels. A similar effect was seen in another study which observed that B12 also enhances the synthesis of GPX, deficiency of Vit B12 may also lead to inadequate Glutathione peroxidase levels causing oxidative stress.[17,18] As GDM is of multifactorial aetiology, oxidative stress is one of them. GDM is diagnosed in the second trimester of pregnancy when the foetus is exposed to the damaging effects of elevated blood glucose and oxidative stress. Therefore, timely detection and handling of GDM are very important to avoid the psychological and economic burden. Vitamin B12 is an antioxidant enzyme obtained easily from meat and dairy products. In addition to Vitamin B12, the exogenous antioxidant GPX enzyme may help reduce oxidative stress-related damage in GDM pregnancy. Though many studies have been conducted that discuss the role of Glutathione peroxidase,[19] and Vitamin B12 in GDM, studies correlating Glutathione peroxidase with Vitamin B12 in the last trimester of pregnancy with gestational diabetes mellitus have not been conducted yet, especially in the local population. As the current study reports statistically significant results, Vitamin B12 and antioxidant enzymes may be considered while managing pregnancy to help prevent oxidative damage-related pregnancy complications.

STUDY LIMITATIONS

Our study has certain limitations. Firstly, our sample size was moderate though the study population belonged to the same socioeconomic class and gestational age-matched, to reduce the confounders and provide ample statistical power of the study. Secondly, it is cross-sectional and comparative, which cannot establish a causal association.

CONCLUSION

Decreased levels of Vitamin B12 and Glutathione peroxidase were significantly associated with gestational diabetes mellitus. So, pregnant women should be urged to take the pregnancy-required amount of Vitamin B12, antioxidant enzymes andVitamins.

Conflict of Interest: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AK & AS: Conception, data acquisition, data analysis, drafting the manuscript, approval of the final version to be published.

AB & NA: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

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.

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