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ORIGINAL ARTICLE

VITAMIN B12, HOMOCYSTEINE AND GLUTATHIONE PEROXIDASE LEVELS IN WOMEN WITH GESTATIONAL DIABETES MELLITUS

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ABSTRACT:

Background: Vitamin B12, homocysteine and glutathione peroxidase are the key components of one carbon metabolism, essential for healthy pregnancy. Inconsistency exists as to whether imbalance of these components can contribute to gestational diabetes mellitus (GDM). The current study intended to determine the levels of vitamin B12, homocysteine and glutathione peroxidase in women with gestational diabetes mellitus.

Materials and Methods: This cross-sectional study was conducted at the Department of Physiology, University of Health Sciences, Lahore, from 1st January 2019 to 31st December 2020. The study involved 90 pregnant women, who were divided into two groups: pregnant women with GDM (n=45) and pregnant women without GDM (n=45). Serum glutathione peroxidase (GPX), homocysteine (Hcy), vitamin B12 (Vit B12), and fasting blood glucose (FBG) levels were measured in both groups at 28-32 weeks of gestation using ELISA and glucose oxidase method, respectively. Data analysis was done by using SPSS version 23. $p<0.05$ was set statistically significant.

Results: We recorded statistically significant differences between the two groups (GDM vs healthy group) regarding Vit B12 (149.00 pg/mL vs 357.00 pg/mL), Hcy (16.95 µmol/L vs 10.05 µmol/L) and GPX levels (3.15 U/ml vs 5.09 U/ml).

Conclusions: Vitamin B12 insufficiency is associated with elevated homocysteine and lower glutathione peroxidase levels in GDM. This is the first study that reports the interaction of one carbon metabolism and antioxidants in GDM in a local population from Pakistan.

KEY WORDS: estational Diabetes Mellitus; Vitamin B12; Homocysteine; Glutathione Peroxidase; One Carbon Metabolism.

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1. INTRODUCTION

Gestational diabetes mellitus (GDM) is a condition of elevated blood glucose levels, diagnosed for the first-time during pregnancy without prior diabetes mellitus.[1] This hyperglycaemic state proves to be fatal for mother and growing foetus and leads to complications in both. Foetal complications include respiratory distress syndrome, overgrown foetus, hyperbilirubinemia, electrolyte imbalance and poor immunity. These babies are prone to develop childhood obesity and type 2 diabetes mellitus (T2DM) later on. GDM mothers are also prone to develop metabolic and cardiovascular complications including hypertension and T2DM later on.[2] The short-term complications of GDM occur before it is even diagnosed.[3] So, it is essential to timely identify and modify the possible risk factors for GDM to help prevent occurrence of GDM and its related complications. Healthy pregnancy requires a balanced consumption of nutrients to fulfil the foetal and maternal needs.

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Vitamin B12 (Vit B12), homocysteine (Hcy) and glutathione peroxidase (GPx) are the key elements in one carbon metabolism, which is crucial for healthy pregnancy.[4] Vit B12 serves as a cofactor in one carbon metabolic pathway to sustain normal levels of homocysteine and GPX is an important antioxidant enzyme of this pathway.[5] Vit B12 deficiency is associated with dreadful pregnancy outcomes including GDM.[6] Additionally, Hcy and glutathione peroxidase (GPX) are also linked to complicated pregnancy.[7,8]

Our research problem (RP) was unawareness of the levels and relationships of Vit B12, Hcy and GPX in GDM population of Lahore, Pakistan. Also, the likely underlying path remains unclear. Thus, the inconsistency in the levels and relationship of vitamin B12, GPX and Hcy in GDM pregnancy and unavailability of relevant data relating to these RPs were our three KGs. Our three research questions (RQs) were "What would be the levels of vitamin B12, GPX and Hcy in GDM population of Lahore, Pakistan". Getting answers to these questions is the rationale of our study. The objective of our study was to determine the levels of Vitamin B12, homocysteine and glutathione peroxidase in women with GDM.

2. MATERIALS AND METHODS

This cross-sectional study design was conducted at the Department of Physiology, University of Health Sciences Lahore, from 1st January 2019 to 31st December 2020. The data was collected from Obstetrics and Gynaecology Department of Lady Willingdon Hospital Lahore. The study approval was granted by the Institutional Review Board of University of Health Sciences, Lahore. The sample size was calculated to be 90 keeping the prevalence of GDM and 95%CI. We included 90 pregnant women aged 18-40 years in our study in their last trimester (28-32 weeks) of pregnancy. The study participants were further categorized into two subgroups on the basis of blood glucose levels: pregnant women who had gestational diabetes and pregnant women with no gestational diabetes. GDM was diagnosed using the 75 OGTT criteria defined by American Diabetes Association (ADA).[9] Inclusion criteria for healthy control group was Pregnant women aged 18-40 years at 28-32 weeks of gestation with no gestational diabetes mellitus. The study participants who had past history of diabetes or gestational diabetes, hypertension, grand multiparity, renal or liver disease and were taking folate or B12 supplementation were excluded from the study.

Written informed consent was taken from the pregnant women who were willing to take part in the study after detailed exploration of history. The study groups were matched for age and gestational age. Gestational age was calculated from the last menstrual period. Weight, height, and body mass index (BMI) were recorded. Systolic and diastolic blood pressures were recorded with mercury sphygmomanometer in relaxed sitting position. 5ml of intravenous blood was drawn from the study participants following aseptic measures. Blood was centrifuged at 3000 rpm in temperature-controlled centrifuge machine for 10 minutes to extract the serum for measurement of biochemical parameters Vit B12, Hcy and GPx. Glucose oxidase method was used to measure fasting blood glucose levels. While serum Vit B12, Hcy and GPx levels were assessed by Enzyme Linked Immunosorbent Assay (ELISA) with standard kits (Bioassay Technology Laboratory, Shanghai, China).

All the demographic and biochemical variables were recorded and entered in Excel. SPSS software version 23.0 (IBM Corporation) was used to analyse the statistical data. We ran the Shapiro Wilk test to assess the normality distribution of biochemical parameters (Hcy, Vit B12, FBG, and GPx) and demographic characteristics of the two groups. Because of normal distributions of all parameters, independent sample T tests were used for the comparison of these parameters between two groups. The quantitative data/numerical variables are presented as mean and the categorical variables are represented as number of observations (n) and percentages (%). The results were considered statistically significant when the p-values were ≤ 0.05. Correlation among study variables was established by using Pearson Correlation test.

3. RESULTS

The demographic profile of the GDM patients and healthy control group is presented in Table 1. The mean age of GDM patients and healthy controls was 30±6.00 vs 29±6.00 years respectively. There was statistically significant difference ($p<0.001$) in mean BMI between GDM and non GDM group (33.00 vs 24.00 kg/m2) respectively. There was also statistically significant difference in number of miscarriages between GDM patients and healthy controls ($p=0.03$) as shown in Table 1.

The mean concentrations of Vit B12, Hcy, and GPx in GDM patients and healthy controls are presented in Table 2.

There was significant difference ($p<0.001$) in mean Vit B12 levels between GDM patients and control group (149.00 pg/mL vs 357.00 pg/mL respectively) as shown in Table-2. Hcy was found to be significantly increased ($p < 0.001$) in GDM patients compared to healthy controls (16.95 µmol/L vs 10.05 µmol/L respectively). A statistically significant decrease in the levels of GPx ($p < 0.001$) was found in GDM patients compared with the healthy controls (3.15 U/ml vs 5.09 U/ml respectively) (Table-2).

Table 1: Demographic Profile of the GDM Patients and Control Group

Variable	GDM Patients (n=45)			Controls (n=45)			p-value
	Mean ±SD	CI Upper Bound	CI Lower Bound	Mean ±SD	CI Upper Bound	CI Lower Bound	
Age (years)	30.00 ±6.00	36	24	29.00±6.00	35	23	NS
Gestational Age (weeks)	29.75 ±1.40	31.15	28.35	29.92 ±1.39	31.31	28.53	NS
Weight (kg)	80.03 ±8.32	88.35	71.71	60.05 ±7.50	67.55	52.55	0.001*
Height (cm)	161.00 ±3.80	164.8	157.2	157.70±4.72	162.42	152.98	NS
BMI (kg/m2)	33.00± 2.10	35.1	30.9	24.00 ± 1.83	25.83	22.17	0.001*
Systolic BP (mmHg)	108.00±12.01	120.01	95.99	106.00±14.1	120.1	91.9	NS
Diastolic BP (mmHg)	68.00 ±11.00	79	57	68.27 ± 8.25	76.52	60.02	NS
Level of education, literate	15			20			NS
Level of education, illiterate	15			10			NS
Consanguineous marriage	20			13			0.04*
Family history of diabetes	27			25			NS
History of miscarriage	28			22			0.03*

* Indicates significant $p < 0.05$.

Abbreviations: BMI=Body Mass Index, BP= Blood Pressure, NS: not significant.

Table 2: Chemical Biomarkers in GDM Patients and Control Group

Variable	GDM Patients (n=45)			Controls (n=45)			p-value
	Mean ± SD	CI Upper Bound	CI Lower Bound	Mean ±SD	CI Upper Bound	CI Lower Bound	
Hcy (µmol /L)	16.95 ±5.70	22.65	11.25	10.05 ± 3.35	13.4	6.7	0.034*
Vit B12 (pg/ml)	149.00 ±14.00	163	135	357.00 ±42.00	399	315	<0.001**
GPx(U/mL)	2.68±1.13	3.81	1.55	5.53±1.05	6.58	4.48	<0.001**
FBG (mg/dL)	139.00 ±12.66	151.66	126.34	85.00 ±6.22	91.22	78.78	<0.001**

* Indicates significant $p < 0.05$.

Abbreviations: Hcy=Homocysteine, Vit B12=Vitamin B12, GPx=Glutathione peroxidase, BMI=Body Mass Index, BP=Blood Pressure.

4. DISCUSSION

Our study reported Vit B12 deficiency (<150 pg/ml) in GDM group. It was noted that 96% of our GDM population was either B12 deficient or having insufficient levels of Vit B12 compared to 3.3% of control group of matching age and gestational age. Our study is in concordance with an Indian study that also reported Vit B12 deficiency in pregnant women with GDM.[10] Our study is also supported by the findings of previous studies that report significantly lower levels of B12 in diabetic patients.[11,12] But these studies link this B12 deficiency secondary to metformin intake. Deficient B12 levels in our study population may be due to poor socioeconomic living, vegetarian diet, and insufficient dietary consumption of B12 due to high costs of animal derived food in our population.[13]

Serum homocysteine was also markedly higher in GDM group compared with control group. Our findings are in concordance with various other studies which also link elevated Hcy with GDM.[14-17] Few of these studies link elevated Hcy levels with vitamin B12 deficiency and liver or kidney disease.[18] Vit B12 deficiency may be the likely cause of raised Hcy levels in our population as we excluded the GDM patients with liver and kidney disease and B12 levels were also deficient in our GDM group. Deficiency of B12 leads to higher Hcy levels which in turn elevates the reactive oxygen species (ROS) levels and causes oxidative damage.[19]

Glutathione peroxidase was also found to be low in both study groups. GDM patients had markedly lower levels of GPX compared with matched healthy controls. Our findings are also in concordance with multiple international studies, which reported GPX to be lower in GDM group.[20,21] There are few contradictory studies that report elevated levels of GPX in GDM patients.[22,23] The main dissimilarity between this and our study was the different gestational age of study population. Elevated levels of GPX reported by these studies may be attributed to an effective adaptive increase in this antioxidant enzyme to protect against damaging effects of free radicles. Lower GPX levels in our GDM patients may be due to inadequate intake of antioxidant vitamins and enzymes, fatigue of GPX to scavenge reactive species or combat Hcy induced oxidative stress.[24]

Deficiency of Vit B12 may lead to elevated Hcy levels as B12 is a key regulator of Hcy metabolism by boosting the activity of methionine synthase. This is in concordance with other studies that reported significant negative correlation between Vit B12 and Hcy in women with adverse pregnancy outcome including gestational diabetes and preeclampsia. Our study replicated the findings of studies who also reported lower levels of antioxidant enzyme GPX in diabetic patients with deficient B12 levels.[25] Vit B12 can also act as an antioxidant enzyme, by hunting the ROS, its deficiency can increase the levels of free radicles and consumption of antioxidant enzymes including GPX, enhancing the oxidative stress. Additionally, B12 may increase synthesis of GPX, a selenium enzyme and its deficiency may lead to lower levels of GPX directly enhancing oxidative damage. Our study explored the role of B12 in relation to Hcy and antioxidant enzyme GPX in GDM group who had deficient Vit B12 levels. Our study reported that deficiency of Vit B12 may also contribute to GDM by disturbing the antioxidant enzyme GPX, other than disturbed Hcy metabolism. In conclusion our study reports some potential link between one carbon metabolism and antioxidant enzyme GPX. This important relationship should be explored in all three trimesters of pregnancy so that newer pathways can be unveiled for the management of GDM.

5. CONCLUSION

The present study reveals a significant reduction in GPX and Vit B12 levels in GDM patients compared with a matched control group. There was significant elevation of Hcy levels in GDM patients compared with control group. Vit B12 deficiency was associated with significantly elevated levels of Hcy and decreased levels of GPx in GDM group compared with the healthy control group. The pregnant women should be closely observed for Vit B12 and antioxidant levels as they are prone to oxidative stress and Vit B12 and antioxidants deficiency.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	AK, AK, AS
Acquisition, Analysis or Interpretation of Data:	AK, AK, AS, HHP, MR, MSS
Manuscript Writing & Approval:	AK, AK, AS, HHP, MR, MSS

All the 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.