ORIGINAL ARTICLE

Serum Homocysteine as Determinant of Gestational Diabetes Mellitus

ASIA KANWAL[1], ASIMA KARIM[2], AISHA BASHIR[3], ASMA SALAM[4], AWAIS UR REHMAN[5]
[1,3]University of Health Sciences, Shalamar Medical & Dental College, Lahore
[2,4]University of Health Sciences, Lahore
[5]University Malaysia Sarawak, Malaysia
Correspondence to Dr. Asia Kanwal, Email: dr.aasia.kanwal@gmail.com, Cell: 03200477127

ABSTRACT

Research reports that serum Homocysteine levels are raised in diabetes as well as gestational diabetes mellitus. We wanted to compare serum homocysteine levels between pregnant women with gestational diabetes mellitus (GDM) and healthy pregnant controls and to find the association of homocysteine with GDM. Study design was Cross Sectional Comparative. The study was conducted at Department of Physiology & Cell Biology, University of Health Sciences, Lahore 2018 to 2019. Serum homocysteine levels were measured in 60 pregnant women in their third trimester of pregnancy. Among 60 pregnant women, 30 had GDM and 30 were healthy controls with no GDM. The diagnosis of gestational diabetes was made on fasting blood sugar levels ≥ 95 mg/dL. Mann Whitney U test was used to compare the groups. Correlation was established using spearman rho correlation. Regression analysis was done to find the association of serum homocysteine with blood sugar fasting. There was significant elevation of serum homocysteine levels in cases (16.95±5.70µmol/L) as compared to controls (10.05±3.35 µmol/L) at *p*=0.000. Regression analysis showed statistically significant and positive relationship of homocysteine with GDM (r=0.66, p=0.000). It was also depicted by regression analysis that there is significant positive relationship between homocysteine and gestational age (r=0.73, p=0.000).

Keywords: Gestational Diabetes Mellitus, Homocysteine, Reactive Oxygen Species

INTRODUCTION

Gestational diabetes mellitus (GDM) is an altered glucose tolerance detected for the first-time during pregnancy[1]. Asian countries have a high prevalence of GDM. A local study stated a frequency of 17.2%[4]. GDM is associated with maternal and fetal complications including shoulder dystocia, hypertension, type 2 diabetes mellitus, hypoglycemia, and respiratory distress syndrome[3]. The pathogenesis of GDM is multifactorial including genetic, metabolic, endocrine, and oxidative factors[3]. The main culprit in GDM is tissue resistance to insulin and raised blood glucose levels. This elevated blood glucose leads to oxidative stress that impairs glucose uptake, further enhancing hyperglycemia. Oxidative stress also increases homocysteine levels[6].

Homocysteine (Hcy) is an essential amino acid, produced in body during breakdown of methionine[9]. Liver and kidneys are involved in Hcy metabolism. Elevated Hcy levels increase reactive oxygen species (ROS) production and modify lipids and proteins[6]. Elevated homocysteine levels also damage the cells including beta cells of pancreas and affect the secretion of insulin[5,10]. Homocysteine levels drop during normal pregnancy[6,12].

Various international studies have hinted towards an association of homocysteine with GDM, but results are inconclusive[3,5,7], we sought to investigate this association in local women with GDM. To best of our knowledge, no studies are done to determine the relationship of homocysteine and GDM in our population.

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PATIENTS AND METHODS

Study design and Subjects: A cross sectional comparative study was conducted at Department of Physiology & Cell Biology, University of Health Sciences (UHS), Lahore. The study was approved by IRB, UHS, Lahore. Sample size was calculated to be 60, with 30 participants in each group. Convenient sampling technique was used. The study subjects were recruited from various hospitals of Lahore from 2019-2020. Cases were the pregnant women at 28-32 weeks of gestation, diagnosed with GDM. Controls were the pregnant women at 28-32 weeks of gestation, with normal blood glucose levels. The subjects with liver and kidney disease, past history of GDM, age>40 years and hypertension were excluded from the study. The subjects taking folate and multivitamins especially B12, were also excluded. A written informed consent was taken from the study subjects. A structured study questionnaire was used to extract information from the subjects.

Biochemical Parameters & Analysis: Blood samples of the subjects were taken to determine blood sugar fasting and homocysteine levels. Blood was centrifuged for 10 minutes at 3000 rpm to extract serum for homocysteine detection. The serum was stored at -80^{0}C. Serum was thawed prior to analysis. Serum homocysteine levels were determined by ELISA.

Statistical Analysis: Data was analyzed using SPSS version 23. Group comparisons were done by Mann-Whitney U test. The *p*-value <0.05 was considered as statistically significant. Spearman rho correlation was used to find the correlation between fasting blood sugar and homocysteine levels. To explore the association of homocysteine with fasting blood sugar, a multiple regression model was used.

RESULTS

The recorded values of age, gestational age, fasting blood sugar and homocysteine of study subjects are mentioned in Table-I. There were statistically significant differences of blood sugar fasting and serum homocysteine levels between two groups. Blood sugar fasting was significantly higher in GDM group (105.001±10.00) as compared to non GDM group (80.50±8.50) at p=0.000. Serum homocysteine levels were also significantly higher in GDM group (16.95±5.70) as compared to non GDM group (10.05±3.35) at p=0.000 (Table-II).

Correlation analysis depicted that homocysteine had significant positive correlation with blood sugar fasting (r=0.66, p=0.000. Gestational age also had significant positive correlation with homocysteine (r=0.73, p=0.000 (Table-III). So, increase in homocysteine levels cause increased fasting blood sugar levels and serum homocysteine levels increase as the gestational age advances.

Regression analysis showed that homocysteine was a highly significant determinant of blood sugar fasting (**β=**2.15, p=0.000). It was also observed that gestational age was also a significant predictor of homocysteine (**β =** 0.29, p=0.000) (Table-IV).

These findings were further analyzed through scatterplots, as given in Figure-1&II. Scatterplots showed that the homocysteine is a significant determinant of BSF with most of the observations lying in the 95% of confidence interval. Gestational age is also a significant predictor of homocysteine in GDM group and most of observations again lying in 95% CI.

Figure I: Scatter plot representing regression relationship between Homocysteine and blood sugar fasting of GDM group

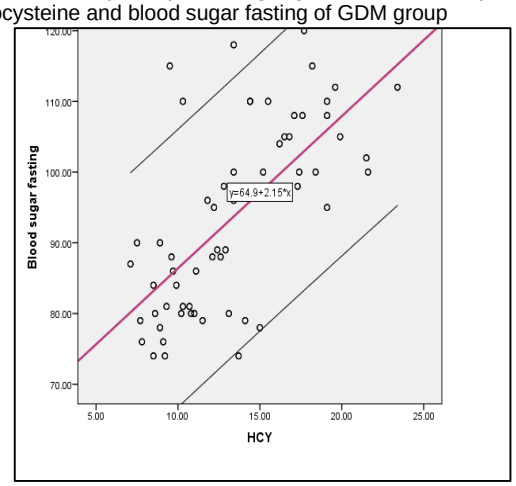

Figure-II: Scatter plot representing regression relationship between Homocysteine and gestational age of GDM group

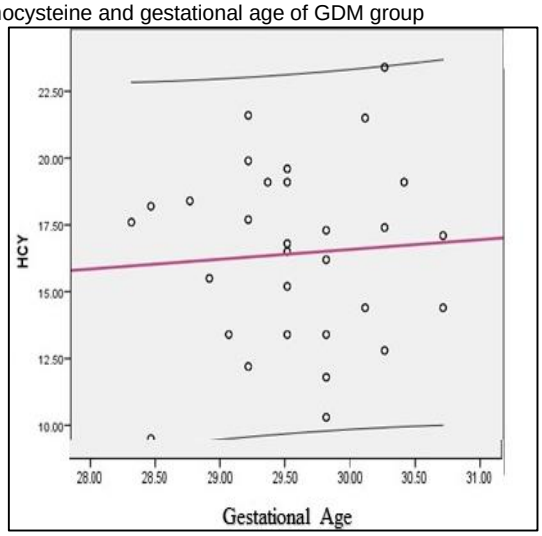

Table-I. Baseline characteristics of all study subjects

Variables	Median	IQR
Age (years)	29.00	6.00
Gestational age (weeks)	29.71	1.50
Fasting blood sugar (mg/dL)	92.50	24.75
Serum homocysteine (µmol/L)	12.85	7.28

Table-II. Comparison between GDM group and non GDM group

Variables	Cases		Controls		Significance
	Median	IQR	Median	IQR	
Age	30.40	4.21	27.50	2.98	0.008
Gestational age	29.64	1.39	29.92	1.46	0.299
Homocysteine	16.951	5.701	10.050	3.351	0.000
BSF	105.001	10.000	80.500	8.500	0.000

Table-III. Correlation of variables in GDM group

		Gestational Age	Blood sugar fasting	Homocysteine
Gestational Age	Correlation	1	.893**	.735**
	Significance		.000	.000
Blood sugar fasting	Correlation	.893**	1	.666**
	Significance	.000		.000
Homocysteine	Correlation	.735**	.666**	1
	Significance	.000	.000	

Table-I:. Multilinear regression analysis in GDM group

Variables	Homocysteine → Blood sugar fasting	Gestational age → Homocysteine
Constant	64.902	4.897
Significance	0.000	0.015
Standard error	2.503	1.996
Homocysteine (β)	2.150	
Standardised coefficient	0.666	
Significance	0.000	
Standard error	0.180	
Gestational age (β)		0.294
Standardised coefficient		0.208
Significance		0.000
Standard error		0.068
R^2	0.444	0.308
Adjusted R^2	0.441	0.089
F-significance	0.000	0.000

DISCUSSION

The most remarkable finding in our study is the highly significant association of elevated homocysteine levels with high fasting blood sugar levels (standardized **β** =0.666, p=0.000) in GDM group. There are multiple international studies available hinting towards the association of homocysteine with GDM. But many of these studies link elevated homocysteine levels with hepatic or renal impairment[8,11]. But our study excluded the GDM group with hepatic and renal impairment. Also, in our population no studies are available depicting the association of elevated homocysteine with GDM.

Gestational diabetes mellitus is a prevailing health problem complicating pregnancies. It has multifactorial etiology, and a lot of risk factors are associated with it. One of the risk factors for GDM is Homocysteine. Elevated homocysteine levels lead to tissue resistance to insulin and impairment of pancreatic beta cells[5,10].

An Indian study reported the same findings as ours that serum homocysteine levels increase as the third trimester advances[6]. Our finding is in line with a study conducted in China that also depicted the significant correlation of elevated homocysteine levels with GDM[3]. However, this Chinese study was conducted on different population with different dietary habits, living and nutritional status. But the main similarity between this study and our study was the GDM group without taking oral antidiabetic treatment or insulin therapy and the same trend of significant positive correlation of homocysteine with blood sugar fasting.

Similar to an Indian study, we also found significantly elevated levels of homocysteine in GDM group in their third trimester of pregnancy as compared to healthy pregnant controls[6]. A metanalysis by Gong et al., also revealed significantly higher homocysteine levels in women with GDM as compared to healthy controls[5]. A study by Begum et al., reported elevated homocysteine levels in GDM group[2]. A study conducted in Iran by Davari et al., also reported higher homocysteine levels in gestational diabetes mellitus as compared to healthy pregnant controls[12]. All of the above-mentioned studies support our findings. So, it is practical to consider that elevated serum homocysteine levels can be a factor causing elevated blood glucose levels. The present study had small sample size. So, it is advised to conduct future studies with larger sample size and interventional nature to find the causal association of homocysteine with GDM.

CONCLUSION

Elevated serum homocysteine levels have significant correlation with fasting blood sugar levels and can predict GDM. Serum homocysteine levels increase as the third trimester of pregnancy advances.

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