CYSTATIN C AS A SCREENING BIOMARKER OF GESTATIONAL DIABETES MELLITUS

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ABSTRACT

Objective: To evaluate cystatin C as a screening biomarker for early detection of gestational diabetes mellitus (GDM).

Study Design: Case control study.

Place and Duration of Study: Army Medical College and Pak Emirates Military Hospital, from Dec 2017 Jun 2018.

Methodology: This case control study was conducted at department of Chemical Pathology, Army Medical College and Pak Emirates Military Hospital Rawalpindi. A total of 30 women with gestational diabetes (cases) and 30 healthy pregnant women (control) were recruited in the study. HbA1c, cystatin C and insulin levels were performed on samples of all the participants. Seventy-five gram OGGT was performed on all subjects. Paired t-test and Odds ratio were calculated for cases and controls.

Results: The cystatin C levels were high with increasing parity. Paired sample t test showed strong association of cystatin C to HbA1c, fasting and post load glucose levels in patients of GDM (HbA1c t (60)=36.0, p<0.001, Fasting glucose t (60) = 34.3, p<0.001, One-hour glucose t (60)=27.6, p<0.001 and Two hours glucose t (60)=22.9, p<0.001). Adjusted odds ratios (OR) based on cut off value of cystatin C (>0.95) of maternal plasma showed positive association with one-hour (OR=4.7) and two-hour (OR=4.3) post load plasma glucose levels in OGTT.

Conclusion: This study strongly suggested that cystatin C may be used as preliminary screening biomarker for early detection of GDM. Patients having elevated levels of cystatin C then can be further evaluated using OGTT. This two-step approach has potential to rationalize the diagnostic work up of this category of patients and would also result in evidence-based patient management.

Keyword: Cystatin C, GDM, OGTT.

INTRODUCTION

Gestational diabetes mellitus (GDM) is the degree of glucose intolerance in pregnant women without prior diagnosis of diabetes[1-3]. In normal pregnancies, fasting and postprandial insulin levels are raised because of hyperplasia of pancreatic β-cell[4], and insulin resistance is increased due to diabetogenic hormones including growth hormone, corticotrophin releasing hormone, placental lactogen, and progesterone especially in third trimester[5]. GDM occurs when β-cell fail to overcome this increased insulin resistance.

The proportion of all pregnancies with GDM is high all over the globe, ranging 1%-14% in various countries[6]. Only in the United States prevalence is approximately 6% of all pregnancies. There is rapid increase in prevalence in Asian countries due to obesity and sedentary lifestyle[4]. Gestational diabetes is the major risk factor in development of type II diabetes, metabolic syndrome and cardiovascular diseases in future[7]. For diagnosing GDM in pregnant women, a traditional standard Oral Glucose Tolerance Test (OGTT) has been in use for many years but many variables affect the results of OGTT[2,3].

Cystatin C, a protease inhibitor, is produced in all nucleated cells in a constant amount[8], and its serum concentration does not depend on muscle mass and protein intake. It is mainly metabolized in the kidneys. The primary advantage of using cystatin C as biomarker is that its generation does not vary across populations[9], so can be used as sensitive marker in detection of

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disorders influenced by demographic features and health status. Cystatin C levels are only affected in certain clinical scenario, e.g. rapid cell turn over, thyroid disorders and use of corticosteroids[8].

Cystatin C has been used as a sensitive marker of the renal functions[10,11], but previous studies have also shown it as a predictive marker of prediabetes in subjects having normal values of fasting blood glucose[12]. It can be used as a screening test for prevention as well as early identification of the microvascular and macrovascular complication of diabetes i.e., diabetic retinopathy, peripheral neuropathy, lower limb involvement[13], and coronary artery disease.

Some studies suggest an association between Cystatin C and insulin resistance in type II DM patients[14], although the underlying causal mechanism between them is currently unknown[2], Different studies provided evidence for the positive association between Cystatin C and insulin resistance and development of GDM in Asian women[2,3]. Cystatin C can be used as a reliable, useful and uniform marker of GDM[2]. It is a potential marker of GDM which may replace the traditional OGTT[3]. Raised Cystatin C levels are associated with insulin resistance as well as central adiposity which are contributing factors in GDM[15]. Cystatin C is highly expressed in omental & subcutaneous adipose tissue, adipocytes, pre-adipocytes, endothelial cells and macrophages. Its association with leptin has also been proven which is a crude marker of body fat mass. Cystatin C secretion is increased in GDM due to increase in adipose tissue in pregnancy[2,3] and by placenta. No local study on evaluation of cystatin C as potential biomarker for GDM was found despite thorough literature search. Keeping in view the need for a simple and reliable marker for GDM this study was planned to assess and compare Cystatin C with OGTT.

METHODOLOGY

It was a case control study, carried out at department of Chemical Pathology, Army Medical College and Pak Emirates Military Hospital Rawalpindi, from Dec 2017 to Jun 2018. It was started after the approval of ethics review committee of the institute. A total number of 60 pregnant ladies were included in this study. Pregnant ladies having gestational diabetes mellitus diagnosed on basis of OGTT, using American diabetes association (ADA) 2013 criteria were included in case group[16,17].

Other pregnant ladies who had normal OGTT with same duration of pregnancy were taken as control. All those pregnant women who were advised OGTT by the obstetrician at 22-28 weeks of pregnancy were enrolled for this study. Diagnosed patients of overt diabetes mellitus, women having eclampsia and preeclampsia in previous pregnancies and chronic kidney disease were excluded from this study. All the participants of study were informed about the procedure and their consent was taken. They were advised to visit laboratory reception in medical fasting state. Their age, Weight, parity and blood pressure were recorded. A 3ml venous blood was taken in sodium fluoride tube, 4ml sample in plain tube and 2ml sample in EDTA tube. Sodium fluoride containing sample were processed immediately for analysis of fasting glucose level. The samples in plain tube were processed for analysis of cholesterol, triglycerides, fasting insulin and cystatin C levels. Glycosylated hemoglobin levels were performed on EDTA sample. All the participants were then given 75-g oral glucose load as per standard protocol. Blood samples for glucose analysis were drawn at one hour and two hours after glucose load. All the biochemical tests were performed on selectra XL analyzer, except insulin which was estimated by chemilumine-scent enzyme immunoassay (Immulite). The insulin resistance was evaluated by using the formula for the homeostasis model assessment of insulin resistance (HOMA-IR).

Mean and SD were calculated for quantitative data. Paired sample t-test was used to analyze the association of different parameters in cases and controls. Odds ratio was calculated for one hour and two hour post glucose load levels

of plasma glucose with Cystatin C, using the cut off level of >0.95 mg/L for Cystatin C[3].

RESULTS

The demographic data of all the study participants was divided intwo groups. Group 1 was having Cystatin C cut off levels >0.95 mg/L and group 2 having levels <0.95 mg/L. Levels of diabetic profile, cholesterol, triglycerides and lipid profile were again divided on basis of cystatin C value as shown in table-II. Association of Cystatin C levels (cut off 0.95 mg/L) and diabetic profile was determined using chi square test. The *p*-values are given in table-III. Paired sample t-test showed a statistically significant difference between the levels of cystatin C and different glucose values during OGTT (table-IV). Comparison of cystatin C and different value of OGTT and HbA1c using adjusted odd value with 95% CI shows positive association (significant at >1) withone-hour (OR=4.7) and two-hour (OR=4.3) post load plasma glucose levels in OGTT.

Table-I: Demographic data on basis of cystatin-C levels.

	Mean ± SD (>0.95)	Mean ± SD (<0.95)
Age	29.5 ± 5.4	28.8 ± 4.8
Weight	67.3 ± 4.9	66.7 ± 5.7
Parity	1.8 ± 1.2	1.4 ± 1.2
Gravida	3.4 ± 1.7	2.9 ± 1.6

Table-II: Biochemical profile on basis of cystatin C cutoff levels.

	Mean ± SD (>0.95)	Mean ± SD (<0.95)
Cystatin C	1.4 ± 0 .6	0.7 ± 0.2
HbA1c	5.6 ± 1.4	5.2 ± 0.5
Fasting	5.3 ± 1.3	4.9 ± .6
One hour	8.9 ± 2.9	8.3 ± 1.7
Two hours	7. 4 ± 2.6	6.6 ± 1.6
Insulin	6.4 ± 13.3	7.1 ± 14.5
HOMA IR	1.3 ± 2.8	1.5 ± 3
Cholesterol	6.2 ± 1.6	5.5 ± 1.6
Triglycerides	2.2 ± 0.8	1.8 ± 0.5

Table-III: Comparison of Glucose levels on basis of cystatin C levels.

	>0.95	<0.95	*p*-value
	Mean ± SD	Mean ± SD	
Fasting	5.3 ± 1.3	4.9 ± 0.5	0.051
One hour	8.9 ± 2.9	8.3 ± 1.7	0.025
Two hours	7.4 ± 2.6	6.6 ± 1.6	0.006
Insulin	6.4 ± 13.3	7.1 ± 14.5	0.878
HbA1c	5.6 ± 1.4	5.2 ± 0.5	0.370

Table-IV: Paired sample t test.

	t (60)	*p*-value
Fasting	34.3	<0.001
One hour	27.6	<0.001
Two hours	22.9	<0.001
HbA1C	36.0	<0.001

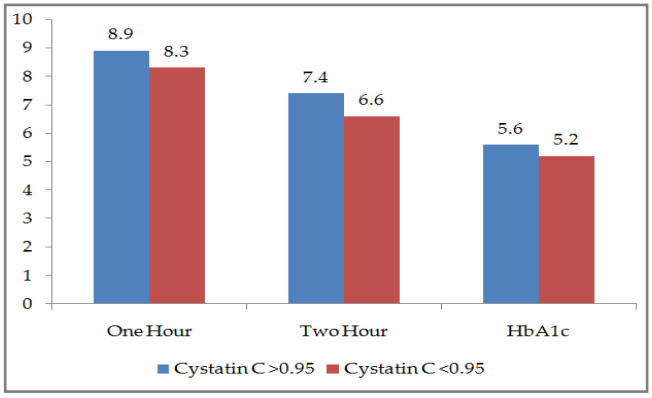

Figure: Comparison of diabetic profile.

DISCUSSION

Most pregnant women have increased weight due to sedentary life especially after conception leading to obesity which itself contribute towards insulin resistance. Early diagnosis and management of gestational diabetes prevents complications in mother such as polyhydramnios, hypertension and in neonates such as fetal death, shoulder dystocia and neonatal hypoglycemia[2,16,18]. Different criteria have been established for GDM but there is still need for international consensus to define this uncertainty in diagnosis of GDM[19,20]. Different cost-effective biomarkers are concern for care givers, healthcare managers and clinicians to give best clinical outcomes.

Cystatin C, non-glycosylated protein is produced by all nucleated cells at a constant rate[12], so it's not wrong to say it is a "house keeping gene agent". Its concentration is not dependent on physiological factors i.e. age, sex, changes in muscle mass, and nutrition[21]. Previous *et al*[21],

studies have shown markedly increased cystatin C levels in some pathological conditions and shown its association with insulin resistance. Use of serum cystatin C as screening biomarker has some advantages over glucose tolerance test as a single blood sample is required and it can be analyzed on any automated chemistry platform without any prerequisite like medical fasting state and special sampling techniques[9]. Comparison of parity and gravidity of study participants with cystatin C levels revealed that there was a positive correlation between increased parity and gravidity and cystatin C value. These finding are consistent with results of study by Zadeh *et al*[2].

Lipid parameter which included cholesterol and triglycerides also showed a significant increase with increasing levels of cystatin C. Surrender *et al*[22], had the same finding in their study and their results are comparable to our finding[2,22]. Dyslipidemia is an important component of metabolic syndrome, and most of the patients with diabetes mellitus have deranged lipid levels which further contribute to complications of the disease. Keeping in view the impact of financial burden on health care system due to these metabolic abnormalities, many studies have recommended utility of cystatin C as a predictive marker of metabolic syndrome[2,12,23,24].

In diabetes mellitus, HOMA-IR is a significant marker and its values are also corelated to GDM. Though many studies have documented a strong association between raised cystatin C levels and HOMA-IR but results of our study showed no statistically significant correlation between these two parameters. Similar results were also discussed in a study by Ilhan *et al*[25].

Study participants were divided in two groups based on cystatin C cut off levels (0.95 mg/L). Comparison of fasting, one hour and two-hourglucose values obtained during OGTT in two groups showed a strong association of glucose values with cystatin C levels of >0.95mg/L. All the subjects having cystatin C above the cutoff had significantly higher glucose values at all three levels as compared to those having cystatin C lower than cutoff. Intergroup variation between cystatin C groups were compared using chi square and paired sample t-test. These findings strongly support the association of cystatin C levels with diabetes mellitus. The same relationship was also established by Yousaf and Zao[1,2].

Although mean value of HBA1c was higher in cases as compared to control group but this difference was not significant statistically. This finding can be explained because the duration of development of GDM may be less than three months in many patients thus leading to near normal values of HBA1c. Cystatin C can be used as a preliminary screening test to identify patients as risk of developing GDM. All women having cystatin C levels >0.95 mg/L, can then be subjected to OGTT. By following this approach only selected patients can then be subjected to OGTT thus making it convenient for patient as well as decreasing the burden on diagnostic facilities.

As the number of participants was limited in our study, further subgroup analysis was not performed. It could not be documented due to small sample size that at which glucose cutoff values, the levels of cystatin C started to rise. However, a significant relation of cystatin C with one hour and two-hour OGTT glucose groups was established in our local population.

CONCLUSION

This study strongly suggested that cystatin C may be used as preliminary screening biomarker for early detection of GDM. Patients having elevated levels of cystatin C then can be further evaluated using OGTT. This two-step approach has potential to rationalize the diagnostic work up of this category of patients and would also result in evidence-based patient management.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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