Assessment of Serum Ferritin, CRP and Insulin Levels in First Trimester of Pregnancy as a Predictive Biomarker of Gestational Diabetes Mellitus: A Longitudinal Study

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ABSTRACT

Introduction: Gestational Diabetes mellitus (GDM) increases the risk of foetal morbidity and mortality heralding the future risk of development of type 2 diabetes in mother. The GDM develops mainly due to insulin resistance along with interplay of risk factors like advancing maternal age, family history of diabetes, obesity, ethnicity, history of macrosomia.

Aim: To detect whether estimation of serum ferritin, C-reactive protein and insulin in first trimester can predict the subsequent occurrence of GDM and whether susceptible mothers can be managed cautiously to prevent foeto-maternal complications.

Materials and Methods: This hospital-based longitudinal study was done in Department of Gynaecology and obstetrics in collaboration with the Department of Biochemistry, Medical College and Hospital, Kolkata, West Bengal, India, from January 2018 to June 2019. The study included 80 antenatal mothers attending the Antenatal Outpatient Department in first trimester. The blood samples were collected from them during first trimester and serum ferritin, C-reactive Protein (CRP) and insulin were estimated using different methods as specified later. The anthropometric measurements of the mother {Body Mass Index (BMI), skin fold thickness and waist hip ratio} were measured. These enrolled study participants have undergone Oral Glucose Tolerance Test (OGTT) with 75 gm of anhydrous glucose at 24-28 weeks of gestation. The performance parameters like sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio of ferritin and CRP was calculated.

Results: The mean age of participants who developed diabetes (n=12) 25.75±2.92 and who did not develop diabetes (n=68) 23.91±3.74 years. There was 15% prevalence of GDM in study population. The median concentration of serum ferritin and CRP was significantly higher in patients who developed GDM (n=12) among the study population (N=80). The sensitivity of serum ferritin {83.33% (95% confidence interval=51.59-97.91%)} was higher in comparison to CRP {31.82% (95% CI=13.86-54.87%)}.

Conclusion: There was a remarkable decline of insulin sensitivity with the advancement of pregnancy. This implies that latent insulin resistance may start from first trimester and screening in high risk ethnicity group like India should be a routine protocol.

Keywords: C-reactive protein, Insulin resistance, Macrosomia, Type 2 diabetes mellitus

INTRODUCTION

Gestational Diabetes Mellitus (GDM) is described by American College of Obstetrics and Gynaecology (2013) as "any degree of glucose intolerance that either has onset during pregnancy or first detected during pregnancy" [1]. In India, the toll of GDM has increased to 5 million with a random prevalence of 11.69% in West Bengal [2]. The aetiopathogenesis of gestational diabetes is still innocuous, though insulin resistance is main contributing factor due to pregnancy hormones like human placental lactogen (somatomamotropin), progesterone, cortisol [3]. However, literature reviews also suggest that gestational diabetes mellitus may simulate type 2 diabetes mellitus identified by the metabolic defects from β cell dysfunction [4]. There is about 20% risk of subsequent development of type 2 diabetes in mothers [5]. The occurrence of GDM has been attributed to interplay of multiple risk factors like advancing maternal age (>35 years), family history of type 2 diabetes in first degree relatives, obesity, macrosomia in previous pregnancy.

Gestational diabetes mellitus is associated with array of deadly maternal and foetal complications [6]. Thus, monitoring the longitudinal changes that takes place in carbohydrate metabolism during pregnancy plays is of immense importance. However, the screening and diagnostic guidelines varies in Indian clinical setting [7]. In low-resource, highly populated and high-risk setting of India the oral glucose tolerance test with 75 gm of anhydrous glucose at 24-28 weeks provides a cost effective and logistical screening test [8]. Despite advances in diagnostic tools, gestational diabetes is detected in late second and third trimester till date. This keeps a lacunae that some markers in first trimester may help in identifying or detecting the risk of the disease and arresting the complications.

Recent research has suggested the role of inflammatory markers like C-Reactive Protein (CRP), ferritin related to insulin resistance, an already established aetiology of GDM. But, there are very few studies like that of Feroz A et al., in this regard in Indian scenario especially in this part of the sub-continent [9]. In this instance, the rationale or justification behind this work is early assessment of the inflammatory markers like ferritin, CRP and insulin levels in first trimester may help to assess the risk of development of GDM. The study was designed to determine whether there is a relationship between serum ferritin, CRP and insulin in early pregnancy and risk of development of GDM and to differentiate GDM and non GDM.

MATERIALS AND METHODS

This hospital-based longitudinal study was done in Department of Gynaecology and Obstetrics in collaboration with the Department of Biochemistry at Medical College and Hospital, Kolkata, West Bengal, India, from January 2018 to June 2019. Ethical Clearance from the Institutional Ethics Committee (MC/Kol/Non-Spons/IEC/678/11-2017 dated 18/11/2017) was obtained. Informed consent was obtained from the subjects. Random sampling was used.

Sample size calculation: The sample size was calculated based on a formula used for cohort study:

$N=(Z/e)^2$, where,

Z=1.96 (two tailed) at 95% Confidence Interval (CI),

e=Allowable error around the expected/reported incidence of event of interest (here, it is prevalence of GDM=20%).

Considering 20%=0.2 error the sample size was 96 (approximately). The sample size was rounded about to 100 considering the non responder rate, however, during final data calculation the sample size approximated to 80 (due to non responders/dropouts).

Inclusion criteria: Antenatal mothers attending the antenatal Outpatient Department during first trimester with singleton pregnancy without any past history of GDM or previous history of DM were enrolled in the study.

Exclusion criteria: Antenatal mothers with previous history of GDM or any type of Diabetes Mellitus, iron deficiency anaemia, haemoglobinopathy or haematological disorder, Polycystic Ovary Syndrome (PCOS) or cardiovascular diseases, multiple pregnancies, infection, fever were excluded from the study.

Study Procedure

The participants were interviewed in person, during their routine antenatal visit to ensure their comfort and to have their fullest co-operation. Required venous blood sample were collected with a standard aseptic procedure after obtaining informed consent, processed and the desired parameters were estimated to derive the results.

Anthropometric measurements like Body Mass Index (BMI), waist hip ratio and skin fold thickness were done in each of the antenatal mothers during first trimester. Blood parameters like plasma glucose, CRP, insulin, ferritin, haemoglobin and haematocrit were estimated. The estimation of plasma glucose and CRP was done by hexokinase and immunoturbidimetry [10]. The estimation of serum ferritin and insulin were done using electrochemiluminiscence in Roche Cobas e411 [11]. All patients enrolled in the study have undergone oral glucose tolerance test between 24-28 weeks. The American Diabetes Association criteria were used as cut-off values [7]. The cut-off for fasting plasma glucose, 1 hour post prandial sample and 2 hour post prandial sample was <92 mg/dL, <180 mg/dL and <153 mg/dL respectively. The deviation of any one of the values was used as criteria for GDM.

STATISTICAL ANALYSIS

Results of the study were compiled; tabulated and analysed using appropriate statistical methods using Microsoft excel 2010 and IBM Statistical Package for Social Sciences (SPSS) version 21.0. The biological data of the individual were checked for Gaussian distribution using Kolmogorov Smirnov test and was considered in Gaussian (normal) distribution if p-value >0.05. Continuous data was expressed as Mean±SD. Man-whitney test was used for comparison median for non parametric distributed data. The binary logistic regression was done and odd's ratio was calculated to assess the risk with the predictive biomarkers. The performance parameters like sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio of ferritin and CRP was calculated.

RESULTS

It was observed that 12 pregnant patients among the 80 enrolled participants had developed GDM in the third trimester. The median and Inter-quartile Range (IQR) haemoglobin, haematocrit concentration of the participants were 11.35±1.37 gm/dL and 34.95±3.68%, respectively. The age range of the study population was 18-31 years. The mean age of participants who developed diabetes (n=12) 25.75±2.92 and who did not developed diabetes (n=68) 23.91±3.74 years [Table/Fig-1]. The baseline anthropometric measurements like BMI, waist-hip ratio and skin fold thickness of the study participants (n=80) was 22.81±1.80 kg/m², 0.78±0.05 and 24.18±3.68 mm respectively. The mean BMI, waist-hip ratio and skin fold thickness of the study participants who developed diabetes (n=12) was 22.63±1.95 kg/m², 0.79±0.06 and 25.08±5.07 mm, respectively.

Age group (years)	Developed GDM	Did not developed GDM	Total (N=80)
18-24	41	6	47
25-31	27	6	33
Mean±SD	23.91±3.74	25.75±2.92	24.2±3.68

[Table/Fig-1]: Distribution of cases according to ages.

The highest quartile or 75th percentile of the distribution of ferritin was considered for cut-off. The highest quartile of ferritin was >31.35 ng/mL. Among the study participants, 20 antenatal mothers had ferritin above 31.36 ng/mL whereas 60 antenatal mothers had ferritin below 31.35 ng/mL. The Fisher's-exact was employed to analyse the data and it was found that the p-value <0.00001, thus a significantly higher number of study participants developed GDM who had ferritin concentration >31.35 ng/mL (the third quartile or 75th percentile of the study population) in comparison to the lower quartile [Table/Fig-2].

Ferritin concentration	Developed GDM	Did not develop GDM	p-value
Level ≥31.35 ng/mL	10	10	
Level <31.35 ng/mL	2	58	<0.00001#
Total	12	68	

[Table/Fig-2]: Distribution of cases according to cut-off ferritin concentration (75th percentile).
#p-value <0.05 considered significant difference, calculated by Fischer's-exact test

Moreover, the subjects who developed GDM (n=12) had significantly higher concentration of ferritin (121.64 ng/mL) in comparison to the patient who did not develop GDM (n=68, 19.05 ng/mL) [Table/Fig-3].
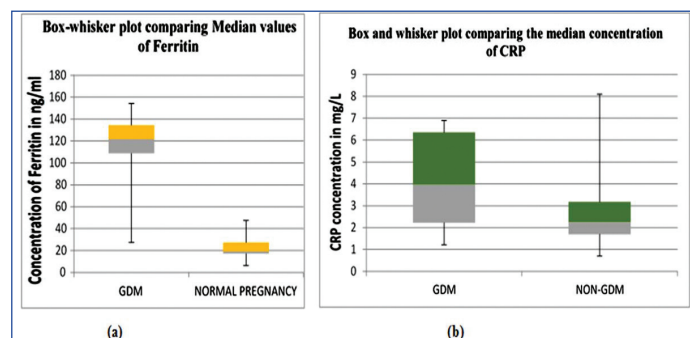
[Table/Fig-3]: The comparison of median concentration of (a)Ferritin and (b) CRP among patients who developed GDM (n=12) and who did not develop GDM (n=68).

The Mann-Whitney U-test was used to compare the median concentration (z=-5.060, p-value <0.05). Similarly, the 75th percentile for C-reactive protein was found to be 3.6 mg/L. It was found that seven out of 22 pregnant subjects who had CRP above this cut-off, developed GDM. This was significantly higher than the patients who did not develop diabetes as shown in [Table/Fig-4].

C-reactive protein	Developed GDM	Did not develop GDM	p-value
Level ≥3.6 mg/L	7	15	
Level <3.6 mg/L	5	53	0.0155#
Total	12	68	

[Table/Fig-4]: Distribution of cases according to cut-off C-reactive protein concentration (75th percentile).
#p<0.05 considered significant difference, calculated by Fischer's-exact test

The median concentration of CRP was higher in the subjects who developed GDM was significantly higher than subjects who did not develop GDM [Tab/Fig-3].

The unpaired student's t-test detailed that there was no significant statistical difference (p-value=0.36) among the subjects who developed GDM (n=12, mean insulin concentration=13.14±3.91 µU/mL) in comparison to subjects who did not develop GDM (n=68, mean insulin concentration=13.12±4.78. µU/mL) in first trimester. The homeostasis Model of Insulin Resistance (HOMA-IR) was estimated using Oxford's software considering Insulin resistance as the pathophysiology of GDM. However, there was no significant statistical difference (p-value=0.49) between the subjects who developed GDM (n=12) than who did not develop GDM (n=68).

Antenatal mothers having higher concentration of CRP {OR=1.56 (95% CI=0.73-3.34)} and waist-hip ratio {OR=2.84 (95% CI=(0.74-10.98)} in first trimester are at more risk of development of GDM [Table/Fig-5]. The pearson correlation coefficient suggested that there was a weak yet significant correlation between CRP and ferritin (r-value=0.274, p-value=0.014).

Definition of risk	Estimate measure	Point estimate 95% CI
Ferritin (ng/mL)	Odds ratio	1.2 (0.95-1.37)
C-reactive protein (mg/L)	Odds ratio	1.56 (0.73-3.34)
Body mass index (kg/m²)	Odds ratio	1.19 (0.48-2.95)
Insulin resistance (HOMA-IR)	Odds ratio	2.65 (0.13-54.5)
Waist-hip ratio	Odds ratio	2.84 (0.74-10.98)

[Table/Fig-5]: Binary logistic regression of biomarkers related to risk for development GDM.

The sensitivity of serum ferritin was 83.33% (95% CI=51.59-97.91%) was higher in comparison to C-reactive protein 31.82% (95% CI=13.86-54.87%) [Table/Fig-6].

Diagnostic performance	Ferritin	C-reactive protein
Sensitivity (%)	83.33% (51.59-97.91%)	31.82% (13.86-54.87%)
Specificity (%)	85.29% (74.61-92.72%)	91.38% (81.02-97.14%)
Positive predictive value	50% (34.85%-65.15%)	58.33% (33.16-79.80%)
Negative predictive value	96.67% (89.07-99.04%)	77.94% (72.43-82.61%)
Positive likelihood ratio	5.67 (3.03-10.6)	3.69 (1.31-10.42)
Negative likelihood ratio	0.20 (0.05-0.7)	0.75 (0.55-1)

[Table/Fig-6]: Diagnostic performance parameters of Ferritin and CRP (95% confidence interval).

DISCUSSION

The prevalence of diabetes in the present study population was 15% (12 out of 80). This was in concordance with the findings of Sain S et al., whose studies illustrated the prevalence of GDM in rural district of West Bengal was 11.69% [6]. The findings of the current study corroborate with the findings of Seshiah V et al., whose study demonstrated prevalence of 18.9% among urban population [2]. Among the 12 patients who developed GDM in the current study, 50% were more than 25 years and 50% less than 25 years. This data is contrary to the report of Savvidou M et al., which reported that women, who developed gestational diabetes, were older and mandates the recommendation of screening of GDM irrespective of the demographic parameters and risk stratification of antenatal mothers as per International Association of Diabetes and Pregnancy Study Group (IADPSG) protocol [8]. It is evident from [Table/Fig-2], that raised serum ferritin concentration is associated with GDM. This study findings are in accordance with the findings of historic Camden study (n=1456), a prospective study on Iran (n=1384) and case-control study conducted Vali-e-sar Hospital of Tehran [12-14]. In the Camden study, cases had a significant higher concentration of ferritin (62.8 ng/mL) as compared to the control (38.3 ng/mL). However, the values of ferritin were segmented in quintiles. The comparison of the highest segment of distribution of ferritin values

with the present study is shown in [Table/Fig-7] [12-14]. However, for the Camden study the values of ferritin were segmented in quintiles. The fact about iron reserve and related oxidative stress causing insulin resistance was uncertain. However, the aforementioned Iran study clearly detailed that iron within normal limits may decrease insulin production (oxidative damage of β cells) and increase insulin resistance (causes colocalisation of insulin dependent GLUT4 and IGF II receptor in adipocyte). It also causes glycation of the transferrin receptors and the glycated transferrin receptors cannot bind iron. This causes a spurious elevation of ferritin, as elevated free iron need to be stored as ferritin [15,16]. Thus, catalytic iron may affect glucose metabolism even in normal concentration. Thus, ferritin in absence of anaemia can be used as a surrogate marker of risk assessment of GDM. The raised CRP concentration among subjects who developed GDM are in concordance with the findings of nested case control study the famous Massachusetts General Hospital Obstetric Maternal Study (MOMS) where they elucidated that first trimester CRP levels were significantly raised in GDM compared with control subjects (3.1 mg/L vs 2.1 mg/L, p-value <0.01) [17]. The CRP increases in the physiological course of gestation [18]. Literature survey has reported that physical activity and insulin sensitivity increasing drugs like thiazolidinedione and metformin may be lead to lowering of CRP and in turn reduction of Insulin resistance [19,20]. A weak yet significant correlation of CRP with ferritin (r-value=0.274, p-value=0.014) suggested that inflammation, insulin resistance and GDM might share an interrelated molecular mechanism of causation. However, the current study had HOMA-IR to predict GDM an odds ratio 2.65. Yet there was no significant difference between the Insulin concentrations. This is in agreement with the fact that exaggerated insulin sensitivity in first trimester and like the findings of Ozgu-Erdinc AS et al. The present study similarly could not show first trimester insulin sensitivity indices test to have any diagnostic value for subsequent GDM [21,22]. In [Table/Fig-6], the CRP test shows a low sensitivity (31.82%) and high specificity (91.36%). However, the test has a negative predictive value of 77.94%, thus CRP has weaker potential than ferritin to predict the risk of GDM alone.

Authors	Place of study	Highest quartile of ferritin (ng/mL)
Chen X et al., [12]	Camden	58.3**
Amiri F et al., [13]	Iran	45
Sharifi F et al., [14]	Tehran	38.3
Present study	India	31.3

[Table/Fig-7]: Comparison of Ferritin concentration among various studies [12-14].
**The Quartile for the distribution was 62.8 ng/mL

Limitation(s)

The sample size of the study population was small. Further studies with large number of population is warranted. Moreover, there are strong genetic, racial and ethnic differences among the study participants, so whether the genetic association of this study population was also a confounding factor in statistical analysis cannot be ruled out. The dietary pattern of the study participants were not included in the study. Foetal outcome in cases that developed GDM has not been studied in this prospective study. Lastly, the serum insulin estimation was done in first trimester only and not re-estimated in third trimester, where the insulin resistance develops mostly. Further studies are needed for biochemical characterisation of inflammation biomarkers in order to work out whether any therapeutic intervention can affect the prognostic impact of them.

CONCLUSION(S)

In the present study, authors were able to detect higher first trimester CRP, ferritin and HOMA-IR levels among women diagnosed with GDM at 24-28 weeks of gestation. Thus, estimation of CRP, ferritin and insulin in the first trimester is easy and can categorise patients

who have a substantial risk of developing GDM. Though there was a no significant difference in the insulin concentration estimated during first trimester but HOMA-IR was higher in the GDM group well established by the odds ratio of 2.65. It is easier to stop something happening in the first place than to repair the damage after it has happened. Thus, early intervention for these patients may lead to a good perinatal outcome.

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REFERENCES

[1] Brussels, Belgium: International Diabetes Federation; 2013. International Diabetes Federation. IDF Diabetes Atlas. 6th ed.

[2] Seshiah V, Balaji V, Balaji MS, Sanjeevi CB, Green A. Gestational diabetes mellitus in India. J Assoc Physicians India. 2004;52:707-11.

[3] Clark CM, Qiu C, Amerman B, Porter B, Fineberg N, Aldasouqi S, et al. Gestational diabetes: Should it be added to the syndrome of insulin resistance? Diabetes Care. 1997;20:867-71.

[4] Babu GR, Tejaswi B, Kalavathi M, Vatsala GM, Murthy GV, Kinra S, et al. Assessment of screening practices for gestational hyperglycaemia in public health facilities: A descriptive study in bangalore, India. J Public Health Res. 2015;9;4(1):448.

[5] American Diabetes Association. Standards of medical care in diabetes- 2015. Diabetes Care. 2015;38:S1-S93.

[6] Sain S, Mukhopadhyay P, Saha TK, Ghosh R. Gestational diabetes: How risky are the mothers of rural Bengal, India. Global Journal Of Medicine And Public Health. 2012;1(06):01-09.

[7] American Diabetes Association: Gestational diabetes mellitus. Diabetes Care. 2018;42(Supp1):S165-S172.

[8] Savvidou M, Nelson SM, Makgoba M, Messow CM, Sattar N, Nicolaides K. First-Trimester Prediction of Gestational Diabetes Mellitus: Examining the Potential of Combining Maternal Characteristics and Laboratory Measures. Diabetes. 2010;59:3017-22.

[9] Feroz A, Surabhi G, Nasreen N, Shagufta M. Role of Maternal Serum Ferritin in Gestational Diabetes Mellitus. Int J Cur Res Rev. 2021;13(01):161-64.

[10] Price CP, Trull AK, Berry D, Gorman EG. Development and validation of a particleenhanced turbidimetric immunoassay for C-reactive protein. J Immunol Methods. 1987;99:205-11.

[11] Lotz J, Hafner G, Prellwitz W. Reference Study for Ferritin Assays. Kurzmitteilung Clin Lab. 1997;43(11):993-94.

[12] Chen X, Scholl TO, Stein TP. Association of elevated serum ferritin levels and the risk of gestational diabetes mellitus in pregnant women: The Camden study. Diabetes Care. 2006;29(5):1077-82.

[13] Amiri FN, Basirat Z, Omidvar S, Sharbatdaran M, Tilaki KH, Pouramir M. Comparison of the serum iron, ferritin levels and total iron-binding capacity between pregnant women with and without gestational diabetes. J Nat Sci Biol Med. 2013;4:302-05.

[14] Sharifi F, Ziaee A, Feizi A, Mousavinasab N, Anjomshoaa A, Mokhtari P. Serum ferritin concentration in gestational diabetes mellitus and risk of subsequent development of early postpartum diabetes mellitus. Diabetes Metab Syndr Obes. 2010;3:413-19.

[15] Jiang R, Manson JE, Meigs JB, Ma J, RifaiN , Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA. 2004;291:711-17.

[16] De Frozo RA. The triumvirate: β-cells, muscle, liver: A collusion responsible for NIDDM. Diabetes. 1988;37:667-87.

[17] Wolf M, Sandler L, Hsu K, Vossen-Smirnakis K, Ecker JL, Thadhani R. First-trimester C-reactive protein and subsequent gestational diabetes. Diabetes Care. 2003;26(3):819-24.

[18] Romem Y, Artal R. C-reactive protein in pregnancy and in the postpartum period. Am J Obstet Gynecol. 1985;151:380-83.

[19] López Caudana AE, López Ridaura R, González Villalpando C, Lazcano Ponce EC, Casanuevay López EM, Hernández Avila M, et al. Prediction of alterations in glucose metabolism by glucose and insulin measurements in early pregnancy. Arch Med Res. 2011;42(1):70-76.

[20] Saisho Y, Miyakoshi K, Tanaka M, Shimada A, Ikenoue S, Kadohira I, et al. Beta cell dysfunction and its clinical significance in gestational diabetes. Endocr J 2010:57(11):973-80.

[21] Grewal E, Kansara S, Kachhawa G, Ammini AC, Kriplani A, Aggarwal N, et al. Prediction of gestational diabetes mellitus at 24 to 28 weeks of gestation by using first-trimesterinsulin sensitivity indices in Asian Indian subjects. Metabolism 2012;61(5):715-20.

[22] Ozgu-Erdinc AS, Yilmaz S, Yeral MI, Seckin KD, Erkaya S, Danisman AN. Prediction of gestational diabetes mellitus in the first trimester: Comparison of C-reactive protein, fasting plasma glucose, insulin and insulin sensitivity indices. J Matern Fetal Neonatal Med. 2015:28(16):1957-62.

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