Original Article

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Maternal and Foetal Blood Analysis in Term Pregnancies with and without Gestational Diabetes Mellitus: A Prospective Cohort Study

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: Gestational Diabetes Mellitus (GDM) is the development of carbohydrate intolerance of variable severity with onset or first recognition during pregnancy. Iron is essential for the beta cell functioning of the pancreas and glucose homeostasis in adequate quantities. However, excess iron levels can lead to the generation of an increased amount of free radicals, which can cause toxicity to the pancreatic beta cells, leading to insulin resistance by impairing glucose metabolism.

Aim: To compare maternal and foetal blood analysis in term pregnancies with and without GDM.

Materials and Methods: This prospective cohort study was conducted at the Department of Obstetrics and Gynaecology, JSS Medical College and Hospital Research Centre, Mysuru, Karnataka, India, on 120 term pregnant women, with 60 cases of GDM and 60 non GDM controls. Maternal blood and cord blood samples were used to measure Haemoglobin (Hb), Packed Cell Volume (PCV), serum iron, and serum ferritin in the mother and newborn at the time of delivery. Foetal blood analysis was performed in terms of foetal haemoglobin, iron, and ferritin. Birth weight was also measured. Statistical analysis was performed using the Chi-square test and Independent t-test, with a p-value <0.05 considered significant.

Results: The serum ferritin level of the mother was higher in GDM cases (mean value 89.47 ng/mL) than in non GDM controls (mean value 47.62 ng/mL), and this difference was statistically significant. Serum ferritin levels in newborns were significantly lower in the GDM group (85.43) compared to the non GDM group (102.71). Mean values of haemoglobin, PCV, and iron levels were not significantly higher in newborns of GDM mothers compared to non GDM mothers.

Conclusion: In GDM, serum ferritin was increased, indicating a marker of inflammation or iron overload, which increases oxidative stress that might affect placental iron transfer and haemoglobin synthesis in the foetus.

Keywords: Cord blood, Haemoglobin, Iron overload, Packed cell volume

INTRODUCTION

GDM is defined as carbohydrate intolerance of variable severity with its onset or first recognition during pregnancy. This definition is applicable whether insulin medication is required for the disease and considers the possibility that previously undetected glucose intolerance existed before the pregnancy [1]. The prevalence of GDM is estimated to be approximately 15% worldwide [2]. Indian women are at an increased risk of developing GDM compared to Caucasian women by 11-fold [3]. Many risk factors are responsible for the development of GDM, like increased Body Mass Index (BMI), increased maternal age, and a family history of diabetes mellitus [4]. Based on previous studies in India, it was found that approximately 3.8-21% of pregnant mothers had GDM, with variation across the country [5-8]. With advances in the understanding of the pathophysiology of GDM, several studies have reported a significant positive correlation between serum ferritin levels in pregnant mothers and the occurrence of GDM [9,10]. However, clear data regarding the role of serum ferritin as an independent factor in developing diabetes or whether the increase in serum ferritin levels is due to inflammation or increased iron pools among pregnant mothers is still lacking [11].

Ferritin plays a significant role in iron metabolism as one of the major iron storage proteins. It also serves as a marker of inflammation and an acute-phase protein. Serum ferritin levels have been found to be increased in many acute and chronic inflammatory disorders, like diabetes and cardiovascular disorders. Insulin resistance is caused by oxidative stress induced by increased iron stores. Iron, being a transition metal, fluctuates between Fe+2 and Fe+3 and, through the Fenton reaction, can form hydroxyl free radicals from oxygen, which causes cell injury [12,13]. Toxic and dysfunctional effects of iron may eventually result in aberrant metabolism, potentially impacting the risk of GDM. Studies in animals and epidemiology have demonstrated a strong correlation between increased serum ferritin storage and abnormalities of glucose metabolism, as well as a favourable correlation between ferritin and type 2 diabetes mellitus [14,15].

Iron deficiency is more common among pregnant women. In most developing nations like India, all pregnant women are prescribed iron tablets and consume iron supplements irrespective of their iron status [10]. Iron is essential for the beta cell functioning of the pancreas and glucose homeostasis in adequate quantities. However, excess iron levels can lead to the generation of an increased amount of free radicals, which can cause toxicity to the pancreatic beta cells, leading to insulin resistance by impairing glucose metabolism [16]. Approximately 90% of all complicated pregnancies are caused by GDM. GDM is linked to adverse outcomes among newborns, including macrosomia, birth injuries, shoulder dystocia, respiratory distress syndrome, hypoglycaemia, hyperbilirubinaemia, and childhood obesity. Women with GDM are also more likely to experience operative delivery, preeclampsia, gestational hypertension, and other potential morbidities [17]. Poorly regulated pregnancy-related diabetes mellitus is associated with increased foetal metabolic rate and oxygen consumption, as well as maternal and foetal hyperglycaemia and hyperinsulinaemia. As a result, the intrauterine environment becomes hypoxic,

stimulating erythropoiesis and increasing the foetal red cell mass, which raises the newborn's haemoglobin and PCV levels. If GDM is not properly treated, it can cause problems in the neonate, such as hypoglycaemia, hypocalcaemia, hyperbilirubinaemia, hypomagnesemia, and respiratory distress syndrome [17].

Most studies have focused on serum ferritin levels in early and mid-pregnancy and their association with GDM [18-21]. This study was conducted in term pregnancies with the aim of comparing maternal and foetal blood analysis in term pregnancies with and without GDM.

MATERIALS AND METHODS

This prospective cohort study was conducted at the Department of Obstetrics and Gynaecology, JSS Medical College and Hospital Research Centre, Mysuru, Karnataka, India from December 2020 to December 2022. The study received approval from the Institutional Ethics Committee (IEC) (JSS/MC/PG/5156/2020-21). Written informed consent was obtained from all enrolled patients.

Inclusion criteria: Participants with term gestation singleton pregnancies with and without GDM, irrespective of maternal age and parity were included in the study.

Exclusion criteria: Term pregnancies with anaemia (Hb <11 gm%-WHO criteria), Type-1 and Type-2 diabetes mellitus, hypertension, seizure disorder, acute or chronic liver disease, COVID-19 infection, history of drug abuse, and multifoetal gestation were excluded from the studys.

Sample size: Based on the incidence of GDM in the pilot study conducted at JSS Hospital, it was found to be 9%. Using this information, the sample size was estimated to be 120, with a 5% allowable error and 95% confidence interval. Purposive sampling technique was used to divide the sample into 60 study subjects in the GDM group and 60 study subjects in the control group. The diagnosis of GDM was made according to the Diabetes in Pregnancy Study group India (DIPSI) criteria [22].

A general physical, systemic, and obstetrics examination was performed, and eligible patients were enrolled in the study. At the time of admission, 3 mL of venous blood sample was drawn from the mother with aseptic precautions, and 3 mL of cord blood was collected from the maternal end of the umbilical cord in a plain vacutainer immediately after delivery. The sample was allowed to stand for 30 minutes to allow the blood to clot, and then it was centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum sample was processed using a fully automated integrated analyser COBAS 6000 Roche.

Maternal blood samples were used to measure Hb%, serum iron, and ferritin levels. Cord blood samples were used to estimate Hb%, PCV, iron levels, and serum ferritin in the newborn. Serum ferritin levels were estimated using the electrochemiluminescence method, and iron levels were measured using the ferrozine method [7,23].

STATISTICAL ANALYSIS

The data were entered into a Microsoft Excel datasheet and analysed using SPSS version 22.0 software. Categorical data were represented in the form of frequencies and proportions. The significance of the data was tested using the Chi-square test. Continuous data were represented as mean and standard deviation. The significance of the data was tested using an independent t-test. A p-value <0.005 was considered significant.

RESULTS

The mean age in the GDM group was 27.38±5.32 years, and in the non GDM group, it was 27.18±4.76 years. In the GDM group, 35% were primigravida, and 65% were multigravida. In the non GDM group, 43.3% were primigravida, and 56.7% were multigravida [Table/Fig-1].

Parameter		GDM cases (60) n (%)	Non GDM controls (60) n (%)	p-value
Age group (years)	<25	15 (25.0)	24 (40.0)	0.058
	26-30	35 (58.3)	22 (36.7)	
	>31	10 (16.7)	14 (23.3)	
Parity	Primi	21 (35.0)	26 (43.3)	0.350
	Multi	39 (65.0)	34 (56.7)	
Family history of DM	Absent	49 (81.7)	56 (93.3)	0.053
	Present	11 (18.3)	4 (6.7)	

[Table/Fig-1]: Distribution of the study subjects based on the social profile in both the groups.
Chi-square test

The mean BMI in the GDM group was 26.56±3.8 kg/m^2, and in the non GDM group, it was 24.25±3.3 kg/m^2 (p=0.001).

The mean maternal ferritin in the GDM group was 89.47±44.23, and in the non GDM group, it was 47.62±22.45 [Table/Fig-2].

Parameter	GDM cases (60) Mean±Standard deviation	Non GDM controls (60) Mean±Standard deviation	p-value
Maternal Hb (in g%)	12.01±0.87	12.30±1.07	0.105
Maternal ferritin (in ng/mL)	89.47±44.23	47.62±22.45	0.001*
Maternal iron (in mcg/mL)	72.85±26.03	82.46±37.14	0.103

[Table/Fig-2]: Maternal blood analysis in GDM cases and non GDM pregnant. Controls in study group.
Independent t-test; p-value <0.05 statistically significant

The mean foetal ferritin in the GDM group was 85.43±24.34 ng/mL, and in the non GDM group, it was 102.71±60.2 ng/mL. The mean birth weight in the GDM group was 3.16±0.49 kg, and in the non GDM group, it was 2.94±0.39 kg [Table/Fig-3].

Parameter	GDM cases (60) Mean±Standard deviation	Non GDM controls (60) Mean±Standard deviation	p-value
Foetal Hb (in gm%)	16.88±6.06	15.55±2.08	0.110
Foetal ferritin (in ng/mL)	85.43±24.34	102.71±60.20	0.040*
Foetal iron (in mcg/mL)	150.65±47.58	136.20±42.96	0.083
Birth weight (in kg)	3.16±0.49	2.94±0.39	0.008*
PCV (%)	48.34±11.92	47.17±8.04	0.529

[Table/Fig-3]: Foetal blood analysis in GDM cases and non GDM controls.
Independent t-test; p-value <0.05 statistically significant

DISCUSSION

In the present study, mean age of study subjects was found to be statistically insignificant between both the groups. In the study done by Sharifi F et al., the mean age of GDM subjects was 30±4.7 years of age and non GDM it was 30±4.9 years of age, in another study done by Inaniya P et al., most of the study subjects in GDM group (46.7%) as well as control group (48%) were aged 26-30 years, followed by 20-25 years, with a statistically insignificant p-value, similar to the findings of present study [19,20]. Soheilykhah S et al., reported a mean age of 29.4±5.4 years among GDM subjects [10]. Chen X et al., found a mean age of 22.14±0.13 years among study participants, which was much lower than in present study group [24].

In the present study, parity was found to be statistically insignificant between the GDM and non GDM groups, with multiparous women being more common in both groups (65% in GDM and 56.7% in non GDM subjects). Rajput R et al., Gopalan SK et al., and Kalyani KR et al., reported that nearly 76%, 58.5%, and 76% of subjects with GDM were multiparous, respectively, same as present study findings [21,25,26]. In this study, the history of diabetes mellitus among family members was found to be much lower in both the GDM and non GDM groups, with an insignificant p-value between the groups.

Rajput R et al., and Gopalan SK and Kalimuthu K, reported a family history of diabetes mellitus in 8.2% and 15.19% of those who developed GDM, respectively [21,25]. Sharifi F et al., found a family history of diabetes in 47% of GDM subjects and 4.6% among the non GDM group, with a statistically significant association, which contrasts with the findings of present study [19].

The mean maternal Hb in the GDM Group was 12.01±0.87, and in the non GDM Group, it was 12.3±1.07. There was no significant difference in the mean maternal Hb between the two groups. Sharifi F et al., reported a mean Hb of 12.8±0.8 in the GDM group and 12.5±0.58 in the non GDM group, with a statistically insignificant p-value [19]. Another study by Das A et al., showed that the incidence rate of GDM was significantly greater (44.12%) in the high haemoglobin group (Hb >13 gm/dL) compared to the normal haemoglobin group (10%) (Hb ≤13 gm/dL). Elevated ferritin levels have been suggested to contribute to the development of GDM [6].

In the present study, the mean maternal ferritin in the GDM group was 89.47±44.23, and in the non GDM group, it was 47.62±22.45. There was a significant difference in mean maternal ferritin between the two groups. Soheilykhah S et al., found a 1.4-fold greater risk of developing GDM in women with high ferritin levels compared to those with lower ferritin levels, with a cut-off value of 45 ng/mL [10]. Fu S et al., demonstrated a strong relationship between high ferritin levels and heme iron and the risk of GDM in their study [27]. Sharifi F et al., identified high blood ferritin concentrations as a separate risk factor for GDM [Table/Fig-4] [6,9,19,20,28,29].

Studies	GDM group	Non GDM group
Inaniya P et al., [20]	33.51 ng/mL	24.97 ng/mL
Jiang R et al., [28]	109 ng/mL	71.5 ng/mL
Sharifi F et al., [19]	112±28.4 pmol/L	65±16.9 pmol/L
Poonguzhalai S and Kalyanikutty KP [9]	33.02 ng/mL	27.98 ng/mL
Soubasi V et al., [29]	30.83±20.61 ng/mL	23.48±10.91 ng/mL
Das A et al., [6]	102.9 ng/mL	72.7 ng/mL
Present study	89.47±44.23 ng/mL	47.62±22.45 ng/mL

[Table/Fig-4]: Showing serum ferritin levels in GDM and non GDM groups in different studies [6,9,19,20,28,29].
Values are given as mean values and±standard deviation

The difference in mean ferritin levels between the two groups was found to be statistically significant (p-value <0.001). This indicates that higher ferritin levels greatly increase the risk of GDM. As ferritin is a protein that stores iron, excess iron-induced oxidative stress may lead to insulin resistance. The transition of iron between Fe2+ and Fe3+ and the subsequent Fenton reaction can generate hydroxyl radicals from oxygen, which can damage cells. The accumulation of excess iron in various tissues may result in faulty glucose absorption by muscle, adipocytes, liver, and other cell types, as well as poor insulin signaling in the liver. Therefore, if pregnant women are recommended to take iron supplements regardless of their iron status, it may lead to iron overload and subsequent production of free radicals, which can cause various issues, including GDM.

The mean foetal Hb in the GDM Group was 16.88±6.06 gm%, and in the non GDM Group, it was 15.55±2.08 gm%. The comparison of mean foetal Hb between the two groups was found to be statistically insignificant. Similar results were obtained in the study by Chauhan P et al., where cord blood Hb was 14.4±0.76 in the GDM group and 13.4±0.63 in the non GDM group [7]. Baki MA et al., found that foetal Hb (g/dL) was 19.00±1.39 in the GDM group and 17.47±1.6 in the non-GDM group [8]. El Raggal NM et al., indicated in their study that both the mother and foetus are vulnerable to developing iron deficiency anaemia among GDM mothers [30]. In the same study, foetal serum ferritin was found to be higher in newborns born to mothers with GDM compared to the control group, but the association was statistically insignificant, which contrasts with the findings in this study [30]. Hashim JM and Ameer S stated in their study that there was no significant difference between infants of diabetic mothers and the controls regarding PCV, MCV, and RDW (p-value >0.05) [31].

Birth weight was positively correlated with GDM in the study by Yang Y et al., (p-value=0.0002) [32]. Gillman MW et al., found that infants whose mothers had GDM had mean birth weights of 3.4 kg for females and 3.6 kg for males on average [33]. Jain R et al., found that LBW infants ranged from 9.9-21.9%, and >3.5 kg infants (macrosomia) ranged from 7.2-16.5%, and they also had elevated blood sugar levels, indicating that GDM is linked to higher birth weight in newborns [34]. The mean birth weight of infants of diabetic mothers in the study by Baki MA et al., was 3296±62 gm, compared to 2714±32 gm for children of non diabetic mothers (p-value <0.05) [8].

Birth weights were increased in newborns of GDM cases because in diabetes, a higher amount of blood glucose passes through the placenta into foetal circulation, leading to increased birth weight. Serum iron and Hb levels were increased in newborns of the GDM group, which could be due to increased transfer of iron from the mother caused by elevated demands in the foetus of diabetic mothers. Overall, it can be concluded that elevated ferritin levels are associated with an increased risk of developing GDM and can cause increased oxidative stress, which might affect placental iron transfer and foetal Hb synthesis.

Limitation(s)

In this study, measurement of serum ferritin levels was performed at term gestation. However, conducting earlier measurements during the antenatal period would have strengthened the study. Long-term follow-up of neonates was not conducted in this study.

CONCLUSION(S)

Serum indicators that can predict the probability of developing GDM easily can be routinely utilised to facilitate early diagnosis. In GDM, serum ferritin is elevated, indicating inflammation or iron overload, which increases oxidative stress and may, in turn, affect placental iron transfer and haemoglobin synthesis.

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