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Original Article

Obstetrics and Gynaecology Section

Association of High Serum Ferritin Level in Early Pregnancy with Development of Gestational Diabetes Mellitus- A Prospective Observational Study

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ABSTRACT

Introduction: Gestational Diabetes Mellitus (GDM) has a negative impact on maternal and perinatal outcome and several long-term complications. The evidence from different experimental studies have shown that high serum ferritin concentration can lead to pancreatic β-cell dysfunction and impaired glucose metabolism leading to GDM.

Aim: To determine the association of increased serum ferritin level in first trimester and GDM in course of pregnancy.

Materials and Methods: A prospective observational study was conducted in 204 women in Institute of Post Graduate Medical Education and Research and SSKM Hospital, West Bengal, India, during the period from January 2015 to December 2015. The blood samples were collected and screened for GDM by Oral Glucose Tolerance Test (OGTT) at the beginning of the study and then assayed for serum ferritin level who were screened negative. The women were divided into four groups by quartiles of serum ferritin levels (Q1 to Q4). Then they were followed-up with OGTT at 24-28 weeks and again at 32-34 weeks. Statistical analysis was done by using paired t-test, Chi-square test and Fisher's-exact test.

Results: The participants had an average serum ferritin concentration of 77.44 ng/mL. GDM prevalence within each serum ferritin quartile was 7.84%, 11.76%, 19.61% and 23.53% respectively (p-value=0.016). The odds ratio for GDM in the ferritin Q2-Q4 was 1.57 (CI=0.41-5.92), 2.87 (CI=0.84-9.83) and 3.62 (CI=1.08-12.11) compared with Q1, respectively. In addition, primigravida and women with high Haemoglobin (Hb) level (>13 gm%) have an increased risk of developing GDM.

Conclusion: Elevated serum ferritin level is associated with increased incidence of GDM irrespective of other risk factors. Iron supplementation should therefore be individualised based on serum ferritin in early pregnancy to minimise the risk of GDM.

Keywords: Haemoglobin, Impaired glucose tolerance, Iron supplementation

INTRODUCTION

The Gestational Diabetes Mellitus (GDM) is any degree of glucose intolerance resulting in hyperglycaemia with onset or first recognition during pregnancy [1]. It is associated with the high risk of foetal macrosomia and perinatal morbidity and mortality for the baby and a long-term risk of development of type-2 diabetes for the mother [2,3]. Most available evidences suggest that defects in the pathogenesis of GDM include decreased insulin secretion and insulin resistance. Overweight, obesity and age at pregnancy are some important modifiable risk factors of GDM but, it is important to identify other modifiable factors that might help to lower GDM risk. Depending on the population and the diagnostic tests applied, prevalence of GDM varies from 2.4-18% of all pregnancies [4,5]. In India it is difficult to estimate any uniform prevalence rate because of widespread differences in living conditions, socio-economic status and dietary habits. It is estimated that at any given point of time in India, about 4 million pregnant females are suffering from GDM [6].

The total iron requirement of a 55 kg woman is 1000 mg in pregnancy. The iron absorption capacity of body keeps on changing throughout pregnancy. Initially, the iron absorption is less. This is followed by a progressive rise in iron absorption in rest of the pregnancy. However, the amount of iron that can be absorbed from even an adequate diet is less than its requirements in later half of pregnancy. A woman must have atleast 300 mg of iron stores to meet the requirements fully in pregnancy [7,8]. But some studies suggest that iron stores are higher in GDM [9-11]. On the other hand, in one study iron-deficiency anaemia is reported to reduce the risk of GDM [12].

Iron is as many others trace elements essential for cellular functions. In pregnant women, an adequate iron store is important in preventing iron deficiency anaemia as well as insuring an uncomplicated pregnancy, normal development of the foetus and maturity of the newborn child. Apart from its important role in oxygen transport and exchange, iron is a potent catalyst for the production of hydroxyl radicals from hydrogen peroxide by the fenton reaction, which can result in oxidative damage of cells and apoptosis [13].

Although the precise mechanism of iron induced diabetes is unclear till now, it is presumed to be caused by three key mechanisms: 1) insulin deficiency; 2) insulin resistance; and 3) hepatic dysfunction [14]. In a mouse model of haemochromatosis, iron overload and oxidative stress cause apoptosis of pancreatic islets with a resultant reduced in insulin secretory capacity [15]. Pancreatic islets have a high susceptibility to oxidative damage, probably due to exclusive dependence on mitochondrial metabolism of glucose for glucose-induced insulin secretion and low expression of the antioxidant defence system [16]. In addition, a high expression of divalent metal transporter additionally predisposes them for more accumulation of iron than other cells and potentiates the risk of iron catalysed oxidative stress [17].

Therefore, increased iron concentration causing reduced insulin secretion coupled with pregnancy induced insulin resistance predisposes a woman to GDM. At present, 60 mg elemental iron is supplemented to all pregnant women as per national guideline-Intensified National Iron Plus Initiative [18].

The focus of the present study was to examine the relationship between iron stores evaluated by ferritin in early pregnancy as a

possible predictive factor of glucose intolerance and the association of risk for development of GDM in non anaemic, non obese Indian women. If this association is established, it will be possible to prevent GDM in some women by judicious supplementation of iron in future.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of Obstetrics and Gynaecology in Institute of Post Graduate Medical Education and Research and SSKM Hospital, Kolkata, West Bengal, India, from January 2015 to December 2015 after obtaining Ethical Committee Approval (inst/IEC/2015/246).

Inclusion criteria:

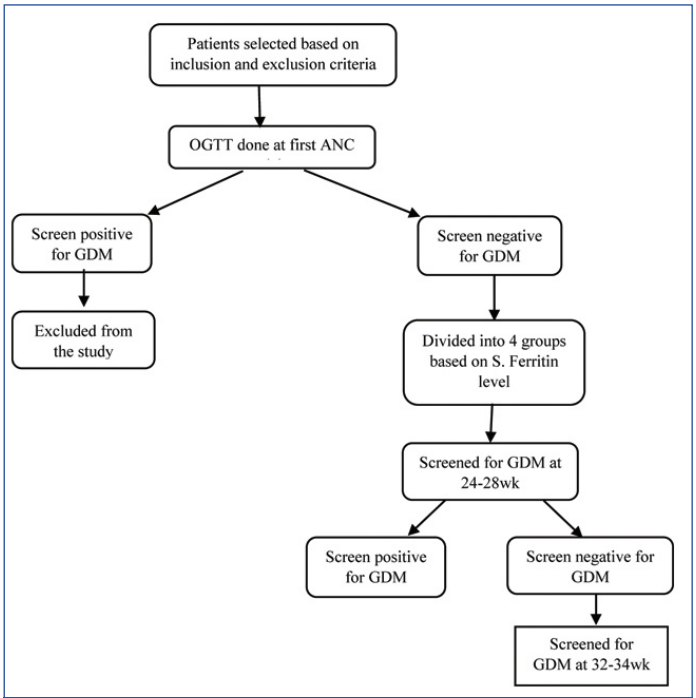
1. Mothers attending antenatal Outpatient Department (OPD) before 12 weeks of gestation (both primi and multigravida).
2. Singleton pregnancy.
3. Body Mass Index (BMI) within range of 18.5 to <25 kg/m^2.
4. Patients who were willing for regular follow-up.
5. Normal thyroid, liver, kidney function test.

Exclusion criteria:

1. Known hypertensive or diabetic.
2. Multiple pregnancies.
3. Mothers whose BMI <18.5 or ≥25 kg/m^2.
4. Any medical or surgical illness.
5. Past history of renal, hepatic or any other chronic illness.
6. History of GDM in previous pregnancy.

Study Procedure

As per the inclusion criteria, 204 eligible women were enrolled for the study [Table/Fig-1]. After taking proper consent the participants were screened for diabetes by 75-g OGTT using a 1-step approach as indicated by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) 2010 [19] and World Health Organisatiion (WHO) (2013) [20] screening criteria [Table/Fig-2].



[Table/Fig-1]: Flowchart of study procedure.
OGTT: Oral glucose tolerance test; ANC: Antenatal care; GDM: Gestational diabetes mellitus

IADPSG/WHO criteria [19,20]	Glucose threshold level (mg/dL)
Fasting	92
1 hour (after 75 gm glucose)	180
2 hours (after 75 gm glucose)	153
Any one of three values exceeding glucose threshold was needed for GDM diagnosis	

[Table/Fig-2]: IADPSG/WHO recommendation of diagnosing GDM.

Mothers attending antenatal OPD before 12 weeks of gestation (both primi and multigravida) during the stated study duration and who fit in the inclusion and exclusion criteria formed the sample population. Thus, total 204 patients were selected for the study. Serum ferritin level was estimated by Chemiluminescence Immunoassay (CLIA) technique in all the screened negative patients.

Then the patients were divided into four quartiles (Q) based on serum ferritin level. To get quartile values authors arranged the ferritin levels from lowest to highest values. Then median value was calculated. This median value was the Q2. At Q2 authors again split the data into 2 halves. The lower quartile (Q1) was the median of the lower half of the data and the upper quartile (Q3) was the median of the upper half of the data. Therefore, in a nutshell Q1 was the 25th percentile, Q2 was 50th percentile and Q3 is the 75th percentile. All participants were then screened for GDM again between 24-28 weeks, if negative then 32-34 weeks of gestation using the same test as mentioned before.

STATISTICAL ANALYSIS

Descriptive statistics were performed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Software Version 23.0. Categorical variables were expressed as percentages (%) and normally distributed variables as mean and standard deviation (SD). For paired comparisons the independent t-test was used. The Chi-square test was used for trend or Fischer's-exact test was used for intergroup comparison of categorical variables. All analysis was two tailed and p<0.05 was considered statistically significant. The frequency distribution of GDM across quartile of serum ferritin level was assessed for statistical significance by Chi-square test.

RESULTS

In present study, 32 (15.69%) women were found to have GDM and rest 172 (84.31%) are non GDM by OGTT. The average age of study population was 23.17 years with median value 22.5 (Range 18-31). The average Hb value in 1st quartile being ~11.2 which was lower than average value in 4th quartile ~12.6. The average value of ferritin in study population was 77.44 ng/mL [Table/Fig-3].

The mean serum ferritin level in GDM was 102.9 ng/mL that was significantly higher compared to that of non GDM which was 72.7 ng/mL [Table/Fig-4].

In [Table/Fig-5], 204 women were divided into four groups based on serum ferritin level (ng/mL)- 1st quartile <40.3, 2nd quartile 40.3-68.8, 3rd quartile 68.9-120.1, 4th quartile >120.1. The samples were then divided in quartile manner based on ferritin values in GDM and non GDM group. There were 51 patients in each quartile. GDM prevalence within each serum ferritin quartile was 7.84%, 11.76%, 19.61% and 23.53%, respectively (p=0.016).

Variables	Mean	Median	Minimum	Maximum	Average value in lower quartile (Q1)	Average value in upper quartile (Q4)	Standard deviation	Standard error
Age (years)	23.17	22.50	18	31	21	25	2.872	0.201
Hb (gm/dL)	11.93	11.75	10.20	14.6	11.20	12.6	1.004	0.070
Ferritin (ng/mL)	77.44	68.85	6.50	180.4	40.30	120.9	46.776	3.275

[Table/Fig-3]: General characteristics of maternal variable.

Test group	Mean±SD (ng/mL)	p-value
GDM	102.9±49.8	0.0007
Non GDM	72.7±44.8	
95% CI= -47.51 to -12.88, Standard error=8.78		

[Table/Fig-4]: Mean ferritin values in the groups (Independent t-test). GDM: Gestational diabetes mellitus, p-value <0.05 considered significant

Quartile based on serum ferritin level	GDM N=32	Non-GDM N=172	Total N=204	p-value
1st Quartile	4 (7.84%)	47 (92.16%)	51	0.016
2nd Quartile	6 (11.76%)	45 (88.24%)	51	
3rd Quartile	10 (19.61%)	41 (80.39%)	51	
4th Quartile	12 (23.53%)	39 (76.47%)	51	

[Table/Fig-5]: Distribution of GDM patients in study population in different quartile of serum Ferritin level (Chi-square test).

[Table/Fig-6] shows the association between GDM and serum ferritin level. There was significant increase in the risk of developing GDM as the ferritin value increases.

	Odds ratio	95% CI
Q2 vs Q1	1.57	0.41-5.92
Q3 vs Q1	2.87	0.84-9.83
Q4 vs Q1	3.62	1.08-12.11

[Table/Fig-6]: Association of GDM in different quartile of serum ferritin level (Fisher's-exact test).

Total 34 patients were present with Hb >13 gm/dL. The rate of occurrence of GDM was seen to be much higher in high haemoglobin group (44.12%) than that of normal haemoglobin group (10%). By applying Fisher's-exact test, the p-value was found to be statistically significant.

To find the association of GDM with dietary habit the study population was divided into vegetarian and nonvegetarian groups and occurrence of GDM was calculated. It was found that this association was not significant (p-value=0.89).

The total number of primigravida in study population was 104 of which 25 had GDM in contrast to only 7 GDM in 100 multigravida. This difference was statistically significant (p-value=0.001) [Table/Fig-7].

Variables	GDM (N=32)	Non GDM (N=172)	Total	p-value	Odds ratio	95% CI
High Hb (>13 gm/dL)	15 (44.12%)	19 (55.88%)	34	<0.0001	7.1	3.06 to 16.49
Normal Hb (≤13 gm/dL)	17 (10%)	153 (90%)	170			
Vegetarian	13 (15.29%)	72 (84.71%)	85	0.89	0.95	0.44 to 2.04
Non-vegetarian	19 (15.97%)	100 (84.03%)	119			
Primigravida	25 (24.04%)	79 (75.96%)	104	0.001	4.2	1.72 to 10.23
Multigravida	7 (7%)	93 (93%)	100			

[Table/Fig-7]: Showing association of different variable with GDM (Fisher's-exact test) (p<0.05 is statistically significant)

DISCUSSION

GDM is a growing problem. Its incidence is increasing worldwide due to urbanisation, sedentary lifestyle and change in dietary habits. GDM mothers are at increased risk of adverse pregnancy outcome as well as developing Type 2 DM in the future [21,22]. An attempt to identify modifiable risk factors for GDM is ongoing and iron is emerging one of the most consistent risk factors associated.

In present study, the association between GDM and serum ferritin level (best indicator of body iron stores) is assessed. After dividing mothers in four groups by ascending value of ferritin the distribution of GDM is noted among the groups. It was seen that there was significant increase in incidence of GDM with increasing value of ferritin, 12 in 4th quartile compared to only 4 in 1st quartile. The highest quartile had 3.6 times increased risk of developing GDM than lowest quartile. This result was consistent with previous research works as discussed below [10,11,23].

Soubasi V et al., reported that high maternal ferritin levels (>60 µg/L) were significantly associated with a higher rate of GDM and intra-uterine growth restriction [10]. Cheng Y et al., have shown in their study that elevated serum ferritin concentration in early pregnancy is associated with GDM [11]. They have seen that odds ratio for GDM was 2.31 in 4th quartile of serum ferritin as compared to 1st quartile. In present study, it was 3.62. The prevalence of GDM was also higher with increasing serum ferritin quartile. Chen X et al., examined the relationship between elevated serum ferritin levels and the risk of GDM and they found that elevated serum ferritin level (highest quartile >131.8 pmol/L) was significantly and positively correlated with pre-pregnancy BMI [22]. Women with the highest levels of serum ferritin had a 2-fold increased risk of developing GDM, when adjusted for confounding factors. In present study authors have excluded obesity as a confounding factor while selecting the cases. Tuomainen TP et al., reported 21.6% higher serum insulin levels in the 5th quartile of serum ferritin values (>216 µg/l) when compared with the 1st quartile (<57 µg/L) [23]. Bozzini C et al., reported an increased prevalence of body iron excess in patients with Metabolic Syndrome [24]. Wrede CE et al., investigated the association between insulin resistance syndrome and serum ferritin [25]. They found a significant association between a BMI value >25 kg/m^2 and hypertension, increased serum ferritin, and diabetes regardless of gender. Women with insulin resistance syndrome had significantly higher ferritin levels. [Table/Fig-8] summarises some recent similar type of studies in India, all are showing same trend of results [26-29].

The high Hb value was significantly associated with occurrence of GDM in present study (p-value <0.0001 and OR=7.1). Mehrabian F and Hosseini SM, in their prospective longitudinal study have found that women with high Hb levels (>12.5 gm/dL) had significantly higher rates of preeclampsia and GDM than those with normal Hb levels; the risks were 5.4 (95% CI=2.8 to10.5) and 3.7 times

Study	Place	Study design	Number of participants	Ferritin level in GDM	Ferritin level in Non GDM	Association	
				Mean±SD (ng/mL)		p-value	95% CI
Rasquinha SD et al., 2021 [26]	India	Case-control study	248	79.19±15.63	30.18±06.02	0.0001	10.019-9.691
Alam F et al., 2021 [27]	India	Prospective observational study	50	79.5±35.3	32.9±4.53	<0.05	_
Mahmood S et al., 2021 [28]	Bangladesh	Cross-sectional study	120	121.1±17.7	86.4±19.9	0.001	1.6 to 7.6
Chauhan P et al., 2020 [29]	India	Case-control study	100	38.1±4.6	33.5±2.7	<0.001	_
Present study (2021)	India	Prospective observational study	204	102.9 ± 49.8	72.7 ± 44.8	0.0007	-47.51 to -12.88

[Table/Fig-8]: Comparison of present study with previous study [26-29].

(95% CI=2.2 to 6.4), respectively [30]. Lao TT and Ho LF had has confirmed in their study, that women with iron deficiency anaemia was significantly associated with decreased prevalence of GDM (adjusted OR 0.46%, 95% CI 0.23 to 0.90) [12].

In this study, there was no significant association between dietary habit and GDM. The prevalence of GDM was almost similar in vegetarian and non-vegetarian groups (p-value=0.89, OR=0.95). However, Deepa R et al., showed that a pre-pregnancy low-carbohydrate dietary pattern with high protein and fat from animal-food sources (>3 times per week) was positively associated with GDM risk (p-value was <0.001, adjusted RR 2.1), whereas a pre-pregnancy low-carbohydrate dietary pattern with high protein and fat from vegetable food sources is not associated with the risk [31]. Tamrakar P showed in her study 3 fold increased risk of GDM among non-vegetarian but obesity was the main confounding factor [32].

It is proved that positive history of GDM in previous pregnancy is an independent risk factor for development of GDM in subsequent pregnancy [21]. In present study, the women with previous history of GDM were excluded during case selection. So, the association between previous history with subsequent GDM could not be assessed. But strikingly, it is seen that women who have previous pregnancy without GDM are much less likely to develop GDM than primigravida with odds ratio being 4.2. In previous study it is seen that high-parity is a predictor of GDM but age was a confounding factor [21]. A study conducted in Riyadh, Saudi Arabia has shown that multiparous women have 8.29 times more chance to develop GDM than nulliparous women. However, after adjustment of maternal age, it was seen that nulliparous women were 2.95 times more likely to develop GDM than parous women. For a parous woman the probability of developing GDM increased from 2-21% when age increased from 20 to 40 years [33].

Limitation(s)

First limitation of the study was small sample size. Due to limited resources in our setting, authors could not estimate serum ferritin level in all the possible subjects in first trimester. So, many patients could not be included in present study. Secondly, authors have not included the obstetric and perinatal outcome of the study subjects which could have given a more detailed information about the whole thing. Thirdly, authors have not followed-up subjects in postpartum period to know whether excess iron induced hyperglycaemia is a reversible condition or not. However, the findings of current study may serve as a platform for further longitudinal study in future.

CONCLUSION(S)

Iron is an essential mineral for the cells in the body. Either deficiency or overload will contribute certain pathogenic outcomes. Iron overload increases the prevalence of insulin resistance and even type 2 diabetes mellitus. In this study, increased incidence of GDM is seen to be associated with increased serum level of ferritin which represent total body iron store. As GDM is associated with increased iron load, all pregnant mother should be screened in the early pregnancy for Hb and ferritin and iron supplementation should only be provided to those with lower iron store to minimise the risk.

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