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A STUDY OF VARIATIONS IN THE IRON PROFILE AND VITAMIN – B_{12} LEVELS AS PREDICTIVE BIO-CHEMICAL MARKERS FOR GESTATIONAL DIABETES MELLITUS (GDM) IN PREGNANT WOMEN

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ABSTRACT

The prevalence of Gestational Diabetes Mellitus (GDM) is increasing globally and considerably more in developing countries like India.Pertaining to GDM there were some studies suggesting a relationship between iron profiles with impaired Oral Glucose Tolerance Test (OGTT) which is not yet clear. Very few studies are available regarding the relationship of Vitamin-B_{12} with GDM. The present study was designed to determine the relationship between iron profile and impaired OGTT in pregnant women with GDM and compare this relationship with that of normal pregnant women, along with comparison of Vitamin-B_{12} levels and also to establish the significance of iron profile and vitamin-B_{12} levels in the plasma as potent bio-chemical markers for impending GDM.The present study was conducted on 60 pregnant women visiting the OPD of Obstetrics in ASRAMS in 2015. 30 GDM women were included in Test group and 30 normal pregnant women in control group. Serum iron, serum ferritin, Total Iron Binding Capacity-TIBC and vitamin-B_{12} were estimated and statistical tools like Unpaired Student t-Test and Pearson Correlation Test were employed. Significant increase in levels of serum Iron and serum ferritin was seen in test when compared to controls $P < 0.0001$. No significant difference was found in TIBC between test and controls $P < 0.3$. Serum Vitamin-B_{12} in the test decreased compared to controls $P < 0.0001$. Increased serum iron and increased serum ferritin correlated positively with increased blood glucose in OGTT, while serum Vitamin-B_{12} is negatively correlated, inferring their significance as predictive biochemical markers for GDM. Larger comparative studies should be performed to establish this rationale.

KEY WORDS: ***Gestational Diabetes Mellitus, Iron Profile, Vitamin-B_{12}, Biochemical Markers.***

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INTRODUCTION

Gestational Diabetes Mellitus (GDM) is the most common of all metabolic disorders during pregnancy that complicates about 3 -14 % of all pregnancies annually [1]. The prevalence of GDM is increasing globally and considerably more in the developing countries like India [2-4]. GDM refers to the carbohydrate intolerance resulting in hyperglycaemia of variable sensitivity with the onset or first recognition during pregnancy. GDM as a disease entity adversely effects the maternal and foetal outcomes. GDM is known to cause several antenatal complications such as abortions, preeclampsia, and hydramnios and other perinatal complications like preterm labour, increased perineal lacerations during labour and increased necessity for caesarean sections in pregnant women; and it may also increase the risk of foetal demise, stillbirth, birth trauma, macrosomia, seizures and certain metabolic diseases in the baby [6-7]. So it is very much essential to diagnose GDM in an early stage in order to prevent the adverse maternal and foetal complications. However GDM related risk factors have not been completely identified yet. There were some studies suggesting a relationship between the iron profiles with the impaired Oral Glucose Tolerance Test (OGTT)[8]. But this relationship has been announced controversially in cross-sectional and case-control studies, which requires further investigations for confirmation [9]. Also there are only few studies available regarding the relationship of Vitamin-B_{12} with GDM in pregnant women, which needs further studies for the confirmation of its reliability [10]. So, the present study was designed to determine the relationship between the iron profile(serum iron, serum ferritin & Total Iron Binding Capacity-TIBC) and impaired OGTT in pregnant women with GDM and compare this relationship with that of normal pregnant women. This study also includes the comparison of Vitamin-B12 Levels in pregnant women with and without GDM. The results obtained from the study can be used to establish the efficiency of iron profile and vit-B12 levels in the plasma as potent bio-chemical markers for impending GDM.

MATERIALS AND METHODS

The present prospective study was carried out in the Department of Biochemistry attached to the central laboratory in the Teaching Hospital of Alluri Sitarama Raju Academy of Medical Sciences (ASRAMS)located in Eluru of West Godavari District, Andhra Pradesh; from May 2015 to July 2015. The study was conducted on the pregnant women with gestational age more than 13 weeks, visiting the Out Patient Department of Obstetrics in the Teaching Hospital of Alluri Sitarama Raju Academy of Medical Sciences (ASRAMS) for routine antenatal checkups between May 2015 to July 2015. After obtaining the clearance from the Institutional Ethical Committee to carry out the research study in the institution, a Fully Informed Consent was taken from all the subjects participating in the study. The blood samples required for the study were collected from the participating subjects following all aseptic conditions needed. The initial screening for GDM among the pregnant women was done by “One Step Glucose Challenge Test”. Subjects with a blood sugar level below 130 mg/dl in OGCT were considered normal with a good glycaemic status .Of them 30 healthy pregnant women were selected for the study and were included in the CONTROL GROUP. Subjects with a blood sugar level more than or equal to 130 mg/dl in OGCT were confirmed for GDM by “Oral Glucose Tolerance Test”. The diagnosis of GDM was made according to ADA guidelines, if anyone of the blood glucose values exceeded.

- Fasting > 92 mg/dl
- 1st Hour > 180 mg/dl
- 2nd Hour > 153 mg/dl

A total of 30 pregnant women with diagnosed GDM were selected for the study and were included in the TEST GROUP.The exclusion criteria of the study includes the Pregnant women with Overt Diabetes Mellitus (either type-1 or type-2); Iron overload prior to conceivement; Alcoholics; Major chronic diseases like any malignancies, tuberculosis, congestive cardiac failure, advanced liver failure, renal failures etc. Various parameters were estimated as follows

Parameter	Estimation
Blood glucose	Glucose oxidase and peroxidase method by using beckman- coulter au480 fully automated analyser
Serum iron	Bathophenanthroline method using colorimeter
Tibc	Bathophenanthroline method using colorimeter
Serum ferritin	Two-site sandwich immunoassay by fully automated electro chemi-luminescence immunoassay (e.c.l.i.a) on elecsys immunoassay analyzers.
Vitamin B_{12}	Competitive test principle using intrinsic factor specific for Vit B-12 by fully automated Electro Chemi-Luminescence Immunoassay (E.C.L.I.A) Elecsys immunoassay analyzers.

STATISTICAL ANALYSIS

The statistical methods used in the study were done in the software Microsoft Excel 2007 version. The quantitative data was expressed in terms of (Mean + Standard Deviation) and the significance of the difference in the mean values between the test and control groups was calculated by “Unpaired Student t-Test “at the significance of P value 0.05. The correlation between different analytes with blood glucose values in the test group was done by “Pearson Correlation Test “at the significance of P value 0.05.

OBSERVATIONS AND RESULTS

The results of the Blood Glucose levels in OGTT, Iron

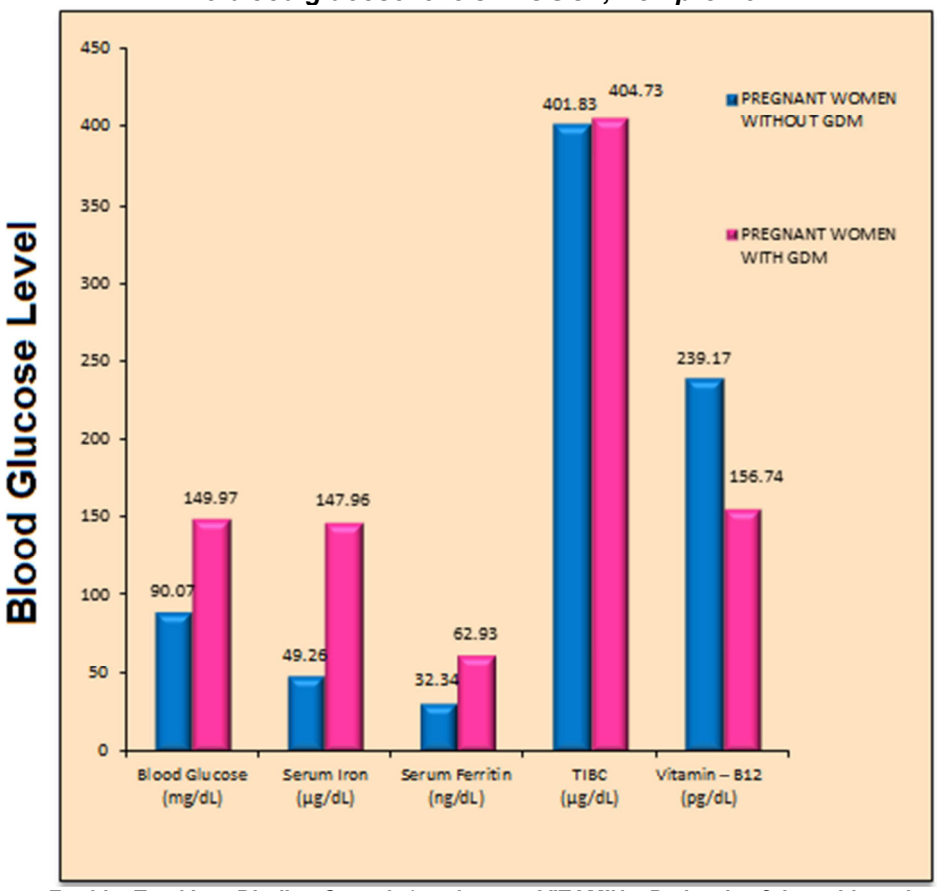
Profile (Serum Iron, Serum Ferritin, Total Iron Binding Capacity) and serum VITAMIN – B $_{12}$ levels of the subjects in the test and control group expressed in terms of (Mean ± SD) and the significance of the difference in the mean values between the two groups which was calculated by " Unpaired Student t- Test " at the significance of P value 0.05, were shown in Table : 1and the same in a graphical representation in Graph:1. The results of correlation of Blood glucose levels in OGTT with the levels of Serum Iron, Serum Ferritin and Vitamin – B_{12} among the pregnant women with GDM done by " Pearson Correlation Test " at the significance of P value 0.05, were shown in Table : 2 and in a graphical representation in Graph: 2.

Table 1
The blood glucose levels in OGCT, iron profile

Variables	Healthy Pregnant women Without gdm (n = 30), (mean±SD)	Pregnant women with diagnosed gdm (n = 30), (mean ± SD)	T – value Two Tailed	P – value
Blood Glucose levels in OGCT (mg/dl)	90.07 ± 6.75	149.97 ± 8.67	29.85	P < .0001
Serum Iron (µg/dl)	49.26 ± 5.92	147.96 ± 12.40	38.17	P < .0001
Serum Ferritin (ng/dl)	32.34 ± 5.18	62.93 ± 7.38	18.24	P < .0001
Total iron binding capacity (tibc), (µg/dl)	401.83 ± 9.15	404.73 ± 12.48	1.02	P < 0.3
Vitamin – B12 (pg/dl).	239.17 ± 21.64	156.74 ± 25.58	13.47	P < .0001

(Serum Iron, Serum Ferritin, Total Iron Binding Capacity) and serum VITAMIN – B_{12} levels of the subjects in the two groups in terms of (Mean ± SD), with t-value and P-value.

Graph 1
The blood glucose levels in OGCT, iron profile



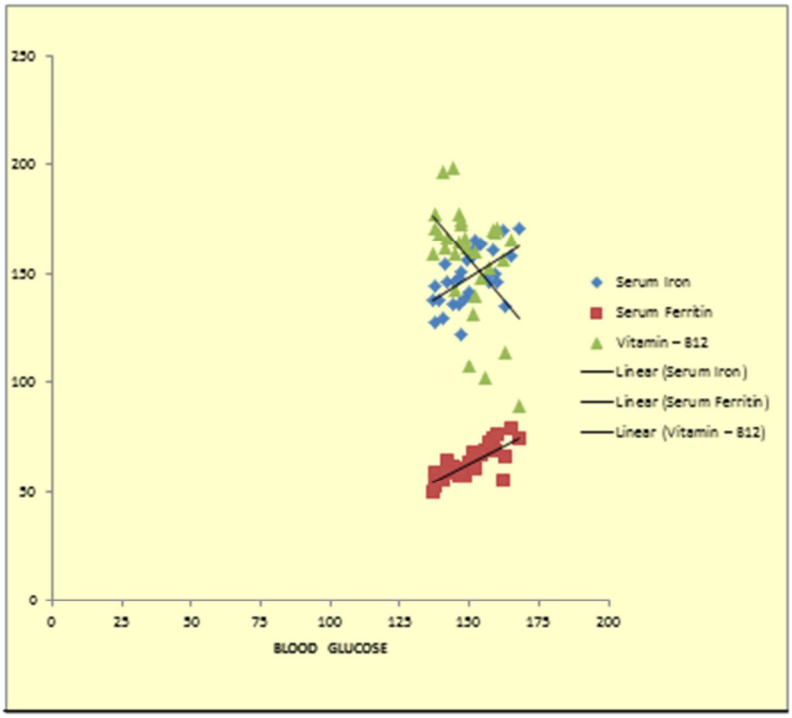
(Serum Iron, Serum Ferritin, Total Iron Binding Capacity) and serum VITAMIN – B_{12} levels of the subjects in the two groups.

Table 2
Correlation of Blood Glucose levels in OGCT

Variables	Serum iron (µg/dl)	Serum ferritin (ng/dl)	Vitamin – b_{12} (pg/dl)
Blood Glucose levels in OGCT (mg/dl) R– VALUE	0.568	0.77	- 0.504
P – value	<0.001	<0.001	<0.001

Correlation of Blood glucose levels in OGTT with the levels of Serum Iron, Serum Ferritin and Vitamin – B_{12} among the pregnant women with GDM.

Graph 2
Correlation of Blood Glucose levels in OGCT



Correlation of Blood glucose levels in OGTT with the levels of Serum Iron, Serum Ferritin and Vitamin – B_{12} among the pregnant women with GDM.

DISCUSSION

In the present study, the levels of various entities in the IRON PROFILE like 'serum iron, serum ferritin & Total Iron Binding Capacity (TIBC)' and serum VITAMIN – B_{12} levels were comparatively studied between pregnant women with GDM and normal pregnant women without GDM taken as controls. And the obtained values of the IRON PROFILE and serum VITAMIN-B_{12} levels in pregnant women with GDM were correlated with their increased blood glucose levels in OGCT. The study had shown a highly statistically significant increase in the levels of blood glucose (mg/dl) in OGCT in the pregnant women with GDM (Mean $\pm$ SD = 149.97 +8.6) when compared to the control group (Mean $\pm$ SD = 90.07 + 6.75) as P value is < 0.0001. Table: 1. A statistically significant increase in the levels of serum Iron (µg/dl) in the pregnant women with GDM (Mean $\pm$ SD = 147.96 +12.40) was seen, when compared to the control group (Mean $\pm$ SD = 49.26 $\pm$ 5.92) as P value is < 0.0001. Serum ferritin (ng/dl) was significantly increased in the pregnant women with GDM (Mean $\pm$ SD = 62.93+7.38) when compared to the controls (Mean $\pm$ SD = 32.34 + 5.18) as P value is < 0.0001. Table: 1. No statistically significant difference was found in TIBC (µg/dl), between pregnant women with GDM (Mean $\pm$ SD = 404.73 +12.48) and the controls (Mean $\pm$ SD = 401.83 + 9.15) as P value is < 0.3. Table: 1. the levels of serum Vitamin-B_{12} (pg/dl) in the pregnant women with GDM (Mean $\pm$ SD = 156.74 +25.58) were significantly low when compared to the controls (Mean $\pm$ SD = 239.17 + 21.64), as the P value is < 0.0001. Table: 1. A statistically significant positive correlation of increased blood glucose levels in OGCT with increased levels of serum iron was found with AR-value of 0.563, as the P value is < 0.001. And a similar positive correlation of increased blood glucose levels in OGCT with increased levels of serum ferritin was noted with ar-value of 0.77, as the P value is < 0.001.Table: 2. Also a statistically significant negative correlation of increased blood glucose levels in OGCT was observed with decreased levels of serum Vitamin-B_{12}, wither-value of -0.504, as the P value is < 0.001.Table: 2. In the present study, there is an increase in the levels of serum iron and serum ferritin with no significant change in levels of TIBC in pregnant women with GDM when compared with the pregnant women without GDM acting as controls. There is also a decrease in the Vitamin-B_{12} levels in the test group when compared to the controls. Graph: 1. The increased levels of serum iron in the pregnant women with GDM in the present study are almost on par with a study in Andhra Pradesh, where a similar statistical significance was noticed [12]. Likewise, some other investigations also reported that high iron levels in the body can be considered as a risk factor for developing GDM[13-15]. In another study there was a significant increase in the levels of serum ferritin in the pregnant women with GDM and statistically insignificant variation in the TIBC and serum iron levels between the pregnant women with and without GDM[8]. Similarly another study reported that elevated serum ferritin concentrations in early gestation are associated with an increased risk of GDM[11]. A recent study in India reported that a low level of plasma vitamin-B_{12} in pregnancy is associated with GDM probably by the relation of vitamin-B_{12} deficiency with adiposity there by inducing insulin resistance[10]. The underlying pathogenic mechanisms mediating the impaired glucose tolerance with increased serum iron and serum ferritin are numerous and incompletely understood. But a recent study hypothesized that the reactive oxygen species when combines with a transition metal like iron with

variable valencies, a highly reactive Hydroxyl radical is formed via fen ton reaction which is capable of causing oxidative damage to tissues[16]. Some surveys have displayed that increased body iron stores are involved in impaired glucose tolerance and gestational diabetes, due to the capability of iron compounds affecting insulin. Synthesis and secretion, increased lipid oxidation and subsequent reduction in glucose transport into the muscle; resulting in insulin resistance [17-18]. According to a recent study, in cases of high serum iron and high serum ferritin in the body which when accompanied with oxidative stress, there would be pancreatic inflammation leading to beta-cell failure causing decreased insulin production and also imparting insulin resistance[19]. As the pregnancy is a state of oxidative stress, when there is an accompanying increase in the levels of serum iron and ferritin along with other predisposing factors for GDM, there can be a high risk of developing GDM.

CONCLUSIONS

In our present study, significantly higher values of blood glucose levels were observed in the pregnant women with GDM when compared with that of normal pregnant women without GDM acting as controls. The study also shows that there is a significant increase in the serum iron and serum ferritin levels with an accompanying decrease in the serum vitamin-B_{12} level in the pregnant women with GDM when compared with that of normal pregnant women without GDM. The study showed that the increased blood glucose levels correlates significantly with the increased levels of the serum iron, serum ferritin and decreased levels of vitamin-B_{12} In the pregnant women with GDM.Thus it can be stated that the increased serum iron and serum ferritin and also decreased serum vitamin-B_{12} levelscan be considered as potent risk factors for GDM in pregnant women. Thereby the pregnant women with a significant increase in the serum iron and serum ferritin with an accompanying decrease in the serum vitamin-B_{12} levels are associated with an increased risk for developing Gestational Diabetes Mellitus. The results of the present study are strongly suggestive that, there is significance for the serum iron and serum ferritin in the iron profile and also the serum vitamin-B_{12} levels as predictive biochemical markers for GDM in pregnant women. Thus the estimation of serum iron, serum ferritin and serum vitamin-B_{12}in the early trimester of antenatal period can provide the effective screening and management of GDM. The emphasis should also be laid upon the cautious iron supplementation in the pregnant women with GDM, and the Vitamin-B_{12} supplementation should also be added to the list of routine supplementations given in the antenatal period to reduce the risk of GDM. Further comparative studies considering larger samples are necessary to establish this rationale.

AUTHORS CONTRIBUTION STATEMENT

Dr.Sunkarapalli Shiva Gautham Teja and Suchitakavuri conceived the present idea andshaped the research design and methodology. Dr. Sunkarapalli Shiva Gautham Teja carried out the research and took the lead in writing the manuscript. Suchitakavuri performed the analytic calculations and supported to the interpretation of the results. Both the authors discussed the results and contributed to the final manuscript.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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