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Original Research Article

Investigating Serum Ferritin Levels and Gestational Diabetes Mellitus, in two Healthcare Institutions in Delta State, Nigeria

Amos Ekoh[1], Peter V. Orugbo[2]*, Patrick Okonta[1]

[1]*Department of Obstetrics and Gaenecology, Delta State University, Abraka, Nigeria*
[2]*Department of Chemical Pathology, Delta State University, Abraka, Nigeria*

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ABSTRACT

Gestational diabetes mellitus (GDM) is the most common metabolic disorder during pregnancy, which causes morbidity and mortality with long-term complications. Some studies have found correlation between increased serum ferritin levels and insulin resistance but the relationship between serum ferritin and risk of GDM has been conflicting. The study aims to correlate GDM risks and increased blood ferritin levels. It was a case-controlled study involving 126 pregnant women attending antenatal care clinic after 24 weeks gestation. A fasting blood sugar level of >92mg/dl and a 2-hour postprandial after a 75g glucose load, >153mg/dl, were included as cases (n=63). For each selected case, the next presenting normal non-diabetic patient matched for body mass index (BMI) was selected as control (n=63). Mean maternal age, gestational age, parity, BMI, family history of DM, previous history of congenital anomalies and stillbirths were similar between the two groups. However, differences in previous macrosomic babies in women with GDM compared to those without GDM ($p = 0.042$) was reported. The mean serum ferritin levels in women with GDM was 92.66 ng/ml compared to 39.89 ng/ml in women without GDM ($p<0.01$). Elevated serum ferritin levels was moderately and positively correlated with the risk of developing GDM ($r= 0.635, p<0.01$). With binary regression analysis, elevated serum ferritin level was found to be an independent risk factor in the development of GDM. There was a significant correlation between elevated serum ferritin levels and GDM. Therefore, elevated serum ferritin level is an independent risk factor for the development of GDM.

***Keywords*:** Body iron stores, Ferritin, Diabetes mellitus, Gestational diabetes mellitus

Introduction

The explosive increase in the diabetic population worldwide is a major public health concern both in the developed and developing countries.[1] Diabetes mellitus (DM) describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defect in insulin secretion, action or both. [2] Gestational diabetes mellitus (GDM) is a class of DM and is the commonest metabolic disorder in pregnancy. [3] GDM is carbohydrate intolerance of varied severity with onset or first recognition in pregnancy.[4] It usually develops between 24th and 28th weeks and resolves after pregnancy.[5] It accounts for 90% of diabetes in pregnancy.[6] GDM incidence is increasing globally, occurring in 7-14% of all pregnancies[7], while in Nigeria it is about 4%[8]. GDM-related risk factors have not been completely identified. However, family history of type 2 diabetes in first-degree relatives, maternal age (>35 years), previous pregnancy complicated by GDM or impaired glucose tolerance, fetal macrosomia, congenital malformation or unexplained IUFD, obesity, hypertension, elevated platelet count, increased haemoglobinand ferritin levels have been enumerated as potential risk factors.[9-16]

*Corresponding author. E mail: petervoke.orugbo@gmail.com
Tel: +2348036899761

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Maternal insulin resistance begins in the second trimester and peaks in the third trimester.[17] This is the result of increasing secretion of diabetogenic hormones such as human placental lactogen, growth hormone, progesterone, cortisol and prolactin. These hormones cause a decrease in insulin sensitivity in the peripheral tissues such as adipocytes and skeletal muscles by interfering with insulin receptor signaling. As a result, maternal glucose use declines and gluconeogenesis increases maximizing the availability of glucose to the fetus.[18] The mechanisms involved in the development of GDM are not completely understood.[19, 20, 21]

Iron is an essential element for both pregnant women and the developing fetus, its being regarded as a double-edged sword in living systems, as both deficiency and excess can be harmful.[22] Iron deficiency anaemia was shown to be associated with greater risk of neonatal morbidity such as preterm birth. On the other hand, it was argued that a higher maternal hemoglobin level from iron supplementation would decrease placental perfusion due to increase in blood viscosity and cause adverse pregnancy outcomes such as low birth weight, preeclampsia and still births.[23] Emerging evidence have pointed to a possible link between higher iron stores and abnormal glucose control (including type 2 diabetes) in pregnant individual. Thus, the need for prophylactic iron supplementation during pregnancy has become controversial.[24]

Serum ferritin, a major iron store protein plays a role in iron metabolism and its concentration provides an indirect estimation of body iron stores as it highly correlates with bone marrow iron[25]. Elevated serum ferritin concentrations have been found in many chronic inflammation-related diseases. Studies have shown a possible link between elevated serum ferritin levels and GDM and has also been related to the occurrence of long-term complications of diabetes both microvascular and macrovascular.[26]

Although the mechanism is not clear, elevated iron stores may induce diabetes through a variety of hypotheses including oxidative damage to pancreatic beta-cells, impairment of hepatic insulin extraction by the

liver and interference with insulin's ability to suppress hepatic glucose production leading to peripheral hyperinsulinaemia.[27] In fact, the initial and most common abnormality seen in iron overload conditions is liver insulin resistance. There is some evidence that iron overload also affects skeletal muscle, the main effector of insulin action. Iron plays a direct and causal role in diabetes pathogenesis, mediated cell failure and insulin resistance. Iron also regulates metabolism in most tissues involved in fuel homeostasis, with the adipocytes in particular, serving as an iron sensing role.[28]

Recent studies among healthy individuals and non-pregnant women have shown a positive associations of moderately elevated serum ferritin levels with risk factors for cardiovascular diseases (CVDs). Also, studies showed a significant relation between higher serum ferritin levels and insulin resistance syndrome.[29] In humans,Serum ferritin has its special characteristics in the buffering action between iron overload and iron deficiency.[30-33]

The effects of reduced serum ferritin levels on metabolic syndromes have been studied.[34,35] Serum ferritin can be used as a proxy for body iron stores as it is highly correlated with bone marrow iron.[37] However, the health policy about the amount of iron supplementation during pregnancy varies between countries. Iron deficiency is common among pregnant women and remains a global public health concern. Adequate iron is critical for β-cell function and glucose homeostasis but excess iron beyond the need increases systemic oxidative stress and majority of pregnant women routinely receive iron supplementation despite the fact that such prophylaxis is still a matter of controversy.[38-41]

There is a potential concern that some women who are not anaemic may be taking unneeded dose of supplemental iron during pregnancy. It has been suggested that such use may build up the mother's iron stores and increase blood viscosity, so that the utero-placental blood flow is impaired, or that the excess iron intake could cause toxic reactions.[42] Ferritin production also is increased with infection and inflammation, as part of the acute phase response. In the presence of infection, macrophages produce inflammatory cytokines that generate reactive oxygen species releasing free iron from ferritin.[43,44,45] Pregnant women with raised serum ferritin levels in the third trimester of pregnancy have a greatly increased risk of preeclampsia, intrauterine growth restriction and stillbirth.[46] Furthermore, a high serum ferritin levels have been linked with the development of GDM.[47] However, data on whether or not elevated serum ferritin level is an independent risk factor for GDM is conflicting. GDM is due to abnormality in the pancreatic beta-islet cell function or at receptor level causing insulin resistance. [48.50]

Free radical causes decrease in receptor number, post receptor defect in insulin action and alteration in glucose transport systems which mediate the peripheral insulin resistance in GDM.[51] Oxidative stress induced from excess iron accumulation can cause damage and death of the insulin producing pancreatic beta cells. This effect contributes to impairment of insulin synthesis and secretion.[52] Under normal circumstances, the pool of intracellular non transferrin-bound iron (NTBI) is maintained at a lower level by rapid incorporation of iron into ferritin to reduce the risk of oxidative stress. Furthermore, the action of superoxide dismutase and catalase mediate effective disposal of free radicals formed in the body. However, excess iron stores disrupt this mechanism.[53] Free radical mediated tissue damage is generally accepted as a major mechanism underlying the occurrence of GDM.[54]

Pregnancy exhibits increased susceptibility to oxidative stress, on the other hand, the placental environment induces protective mechanisms against free radicals as pregnancy progresses. This placental anti-oxidant potentials are able to counteract oxidative stress in normal pregnancy.[55] Pregnancy is a state whereby adaptation and equilibrium are easily disrupted as evidenced by the propensity towards the development of insulin resistance which lead to GDM in some cases, while free radical mediated tissue injury worsen the situation.[56] Reduction in free iron results in upregulation of transferrin receptors, enhancing iron absorption from GIT. Iron absorption is minimal when body iron stores are normal.[57,58]

The relationship between elevated serum ferritin levels and risk of developing GDM has been controversial. Several studies have shown association between serum ferritin levels and risk of GDM while others showed no association between serum ferritin levels and GDM. A number of other studies have associated increased ferritin levels with GDM.[59-65] However, a number of other studies found no association between elevated serum ferritin levels and GDM.[66-68] There therefore appears to be a conflict on the relationship between serum ferritin and GDM. Some studies found an association between serum ferritin and GDM while others found no association.

Gestational diabetes mellitus is associated with maternal morbidity and high perinatal morbidity and mortality. The increasing incidence of GDM is thought to reflect the global rise in the incidence of type 2 diabetes mellitus, related to energy intake in excess of energy expendicture.[69] Therefore, strategies addressing effective prevention, by identification of all possible risk factor are warranted. Emerging studies, though, still controversial, indicate that high serum ferritin during pregnancy and high total iron intake during pregnancy was significantly related to high GDM risk. However, there is paucity of data on the relationship between serum ferritin and the GDM risk in Nigerian women. Furthermore, considering the fact that supplemental iron is routinely prescribed in Nigeria, it is imperative that local studies should be conducted to determine if any association exists between serum iron and GDM. Therefore, this study will add to the body of literature on the subject matter and provide some evidence that may influence clinical practice.

Methodology

Study design

This was a case control study of pregnant women with GDM and those without GDM at Delta State Teaching Hospital (DELSUTH) Oghara, and Central Hospital (CH) Warri, both in Delta State. This study was conducted in the Department of Obstetrics and Gynaecology in DELSUTH, Oghara and its affiliate, Central Hospital, Warri, both in Delta State in collaboration with the Department of Chemical Pathology, where analyses too place.

The study population included 63 eligible pregnant women attending antenatal care at DELSUTH, Oghara and Central Hospital, Warri, after 24 weeks gestation. Cases were pregnant women with GDM while controls were normal pregnant women without GDM confirmed by normal oral glucose tolerance test (OGTT).The researcher recruited pregnant women who develop GDM and was aided by residents from both institutes. After all eligible participants provided informed written consent, the next presenting normal non-diabetic patient matched for BMI was chosen as a control for each selected GDM patient. Blood sample was collected from all consenting participants for serum ferritin estimation.

Criteria for making a diagnosis of GDM

Assessment of GDM was done by a one-step approach according to WHO diagnostic criteria using the 75g oral glucose tolerance test (OGTT) after 24 weeks gestation. OGTT was performed after an overnight fast of 8-14 hours, while the subject was on an unrestricted carbohydrate diet with unlimited physical activity for at least 3 days. Plasma glucose was measured at fasting, 1 hour and 2 hours after 75g glucose load. Women were diagnosed with GDM, if one or more of the 3 abnormal glucose levels were obtained; 1. Fasting plasma glucose levels of ≥92mg/dl, 2;

One-hour plasma glucose levels of ≥180mg/dl, 3. 2-hour plasma glucose level ≥153mg/dl.[40] Glucose was measured by the photometric method, using a blood glucose meter. (Accu-Chek, Roche Diabetes Care, Mannheim, Germany)

Inclusion criteria

- Singleton pregnancy
- Gestational age above 24weeks, relying upon the last menstrual period (LMP) or early ultrasound

Exclusion criteria

- Women with preexisting diabetes
- Urinary tract infection
- Respiratory tract infection
- Preeclampsia or pregnancy induced hypertension
- Multiple gestation
- Cardiac, hepatic or renal disease

- Drug or alcohol abuse
- Iron deficiency anaemia (Hb<10mg/dl) or iron overload (Hb>14mg/dl)
- Polyhydramnios

Sample size calculation
The desired sample size for each group was obtained using the sample size formula[70] for comparative study.

$$n = \left(\frac{r+1}{r}\right)\frac{SD^2\,(Z_\beta + Z_\alpha)^2}{d^2}$$

Where: n = desired sample size for each group
r = ratio of cases to control = 1 for equal number of case and control
SD = Standard deviation (obtained from previously published study =8.2).[62]
Zβ = Standard normal variate for power = for 80% power it is 0.8.
Zα= Standard normal variate (at 5% type 1 error (P<0.05) it is 1.96
d = Effect size (one half of standard deviation= 4.1)
Therefore

$$n = \frac{1+1}{1}\frac{8.2^2\,(0.84+1.96)^2}{4.1^2} = 2 \times \frac{67.24 \times 7.84}{16.81} = 62.72$$

Minimum sample size for each group is approximately 63

Procedure and sample collection
Eligible women were recruited after an informed written consent has been obtained. Participants' medical history, past and present obstetric history, gynaecological history and family history were obtained and a physical and obstetric examination performed by the attending registrar. Pulse, temperature (taken with digital thermometer [Ultramed®, Malaysia]), blood pressure (taken with digital sphygmomanometer [Dynarex®, China]) and BMI were calculated by dividing the prepregnancy weights (in kilograms) at booking and heights (in metres) measured using adult analogue weight-measuring scale with attached height-measuring stadio-meters (Techmel ZT-160®, USA) accurate to 0.1kg and 0.1cm respectively. All investigations results including full blood count (hematocrit, hemoglobin, mean corpuscular volume, platelet count and white blood cell), urinalysis, retroviral screening, hepatitis B surface antigen, hepatitis C virus, venereal disease research laboratory and obstetric ultrasound scan were obtained from their folders.
Venous blood sample was collected from consenting eligible patients and analyzed for serum ferritin levels.

Serum ferritin assay
Five millilitres of blood sample was obtained through venipuncture after application of tourniquet under aseptic measures, preferably in the morning, following an overnight fast for at least 12 hours and stored in a plain bottle. The blood sample collected was allowed to clot and retract to prevent haemolysis while centrifuging.The sample was centrifuged for 15 minutes at 3,000 rpm, and the serum decanted and stored at a temperature of -20^{0}C, until the laboratory assays are done using the Eurogenetics ferritin quantitative kit. It was an indirect enzyme-linked immunosorbent assay (ELISA) technique, based on two highly affinity monoclonal antibodies in an immune metric assay system (Delaware Biotech Kit, USA), to ensure an optimal sensitivity and specificity. The ELISA technique (Delaware Biotech Kit, USA) applicable in this study was an indirect method of detecting ferritin levels and was non-invasive with relatively little patient discomfort. The Ferritin Quantitative Test was therefore based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the ferritin molecule being sandwiched between the solid phase and enzyme-linked antibodies. After a 45-minute incubation at room temperature, the wells were washed with water to remove unbound- labelled antibodies. A solution of TMB Reagent was added and incubated at room temperature for 20 minutes, resulting in the development of a blue colour. The colour development was stopped with the addition of Stop Solution, and the colour changed to yellow and measured spectrophotometrically at 45 nm. The concentration of ferritin is directly proportional to the colour intensity of the test sample, to be read on microplate. In the calculation of feeritin concentrations a typical standard curve with optical density readings at 450 nm shown in the Y-axis against Ferritin concentrations on the X-axis was adopted (Figure 1).
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Ethical considerations
Ethical approval from the Delta State Hospitals Management Board dated January 20, 2020 (CHW/ECC VOL. 1/202) (Appendix I). Ethical Approval was also obtained from the Delta State University Teaching Hospital Health Research Ethics Committee, dated May 5, 2020 (DELSUTH/HREC/PAN/2020/006/0331) (Appendix II). Informed consent was also obtained from all participants before recruitment into the study. They were assured of confidentiality of information provided as the proforma to be used was coded and these codes linked to the names and hospital numbers domiciled with the primary researcher only. Patients who did not give consent or opt out of the study had their rights to treatment fully respected and did not in any way interfere with the patients management. The study was clearly explained in the language that the client understood, when they did not communicate in English. The counselling involved clear description of the method, the risks and benefits of the study before obtaining informed consent. Participation was voluntary, free and participants were given the option to end their participation at any stage of the study. Continuing in the study or refusal to participate did not change the standard recommended care protocol of the hospital.

Limitation of study
The weight at booking was utilized in calculating BMI as pre-pregnancy weight could not be determined accurately. This was a limitation as weight during pregnancy can vary considerably from prepregnancy weight and is contributed by materanal and fetal component. However, we believed that the weight taken at booking at early gestational age would not vary considerably from pre-pregnant weight. Moreover, both group (GDM and non GDM) involved pregnant women. Also, at the beginning of the study, it was anticipated that, there may be presence of inter and intra-observer variability in serum ferritin assay. This was mitigated by assaying all samples in one laboratory under the supervision of a chemical pathologist.

Data analysis
All collected data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 21. The results were displayed in tables and graphs. The differences in mean serum ferritin level of the two groups were statistically tested using the student T-test. Test of statistical significance for categorical variables was done using the Chi-squared test. Level of significance was set at $p<0.05$. Multivariate logistic regression was applied to determine the effect of possible confounders.

Ferritin Conc. (ng/ml)	Absorbance (450 nm)
0	0.074
15	0.150
80	0.362
250	1.017
500	1.699
1000	2.728

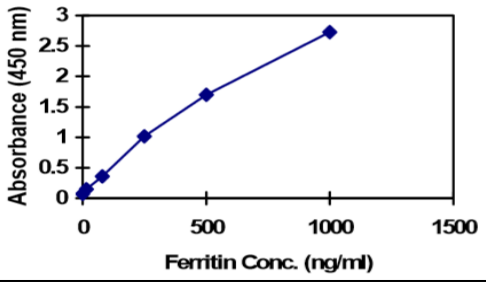

Figure 1: A typical standard curve with optical density readings at 450 nm utilized in the study to estimate ferritin levels.

Results and Discussion

This was a case-control study involving 126 consenting pregnant women (63 with GDM and 63 without GDM), aimed at determining the serum ferritin levels in women with GDM and without GDM, comparing the serum ferritin levels between the two groups and making recommendations based on the findings of the study, at Delta State University Teaching, Oghara and Central Hospital, Warri, both in Delta State.

Table 1 shows that there was no statistically significant difference in the mean maternal age, parity, gestational age and BMI between the two groups. However, majority of women with GDM and without GDM were aged 30-39 years (76.2% and 74.6%), with a mean age of 33.81 and 32.47 respectively (p=0.069). Most of the study participants were multiparous, with 42.9% of cases and 36.5% of controls. Highest proportion of patients with GDM were mildly obese (46.0%) while majority of participants without GDM were overweight (52.2%), with a mean BMI of 31.01 and 30.05 respectively (p=0.10). Gestational age did not also differ between the two groups. Results also showed that 20.0% of participants with GDM had previous history of macrosomic babies compared to 7.9% of patients without GDM, and the difference was statistically significant (p = 0.042) (Table 2). Though, more women in the GDM group had more stillbirths, congenital anomalies and family history of DM, the difference was not statistically significant between the two groups.

In this investigation, there was no significant difference between the two groups in terms of mean maternal age, gestational age, parity, BMI, family history of diabetes mellitus, prior history of congenital abnormalities, or stillbirths. This demonstrated that the two groups were appropriately matched to account for any potential confounders, particularly BMI, that might have affected the outcome of the result. Serum ferritin is an acute phase reactant and inflammation is usually associated with obesity, because adipocytes from adipose tissue can secrete proinflammatory cytokine, thus, increasing serum level of ferritin.

Table I: Baseline socio-demographic characteristics of study participants

Socio-demographic parameters		Controls	Cases	Total	Test statistic	P Value
		Frequency (%)	Frequency (%)	Frequency (%)		
EGA	Mean	31.66 ± 2.76	31.97 ± 3.12	31.80 ± 2.92	- 0.581 $^{\Delta}$	0.562
Booking Status	Booked	36 (57.1)	42 (66.7)	78 (61.9)	1.212*	0.271
	Unbooked	27 (42.9)	21 (33.3)	48 (38.1)		
Age Group	20 – 29 years	14 (22.2)	9 (14.3)	23 |(18.3)	3.097$^{\Delta}$	0.213
	30 – 39 years	47 (74.6)	48 (76.2)	95 (75.4)		
	40 years and above	2 (3.2)	6 (9.5)	8 (6.3		
	Mean	32.47 ± 4.12	33.81 ± 4.07	33.21 ± 4.34	- 1.832 $^{\Delta}$	0.069
Parity	Nulliparous	17 (27.0)	12 (19.0)	29 (23.0)	4.511*	0.211
	Primiparous	19 (30.2)	14 (22.2)	33 (26.2)		
	Multiparous	23 (36.5)	27 (42.9)	50 (39.7)		
	Grand Multiparous	4 (6.3)	10 (15.9)	14 (11.1)		
Marital Status	Single	9 (14.3)	4 (6.3)	13 (10.3)	2.144*	0.143
	Married	54 (85.7)	59 (93.7)	113 (89.7)		
Level of Education	No Formal Education	4 (6.3)	5 (7.9)	9 (7.1)	5.384*	0.146
	Primary	35 (55.6)	25 (39.7)	60 (47.6)		
	Secondary	15 (23.8)	14 (22.2)	29 (23.0)		
	Tertiary	9 (14.3)	19 (30.2)	28 (22.2)		
Religion	Christian	55 (87.3)	60 (95.2)	115 (91.3)	2.490*	0.115
	Muslim	8 (12.7)	3 (4.8)	11 (8.7)		
Occupation	Professional	10 (15.9)	16 (25.4)	26 (20.6)	1.753*	0.416
	Skilled	41 (65.1)	36 (57.1)	77 (61.1)		
	Unskilled	12 (19.0)	11 (17.5)	23 (18.3)		
BMI	20 - 24.9	0 (0.0)	3 (4.8)	3 (2.4	8.544*	0.074
	25 – 29.9	33 (52.4)	22 (34.9)	55 (43.7)		
	30 – 34.9	27 (42.9)	29 (46.0)	56 (44.4)		
	35 – 39.9	3 (4.8)	8 (12.7)	11 (8.7)		
	40 and above	0 (0.0)	1 (1.6)	1 (0.8)		
	Mean	30.05 ± 2.57	31.01 ± 3.82	30.53 ± 3.28	- 1.648 $^{\Delta}$	0.10

Test statistic = * chi squared. $^{\Delta}$ T-test

Also, both BMI and elevated serum ferritin levels have been shown to be independent predictor of insulin sensitivity, therefore controlling BMI, which is a significant confounding variable, increased the validity of the result. But, there was a statistically significant difference in previous macrosomic babies in women with GDM compared to those without GDM (p = 0.042). However, there is no causal relationship between previous macrosomic babies and GDM, and when subjected to logistic regression, it became insignificant.

The mean serum ferritin levels in participants with GDM was 92.66 ng/ml compared to 39.89 ng/ml without GDM, and the difference in the serum ferritin levels between the 2 groups were statistically significant (p-<0.01) (Table 3). The parameters used in the diagnosis of GDM including fasting blood sugar, 1-hour OGTT and 2-hour OGTT were significantly higher in patients with GDM than in patients without GDM. However, the difference in the mean packed cell volume between the two groups was not statistically significant (p = 0.603).Elevated serum ferritin levels was moderately and positively correlated with the risk of developing GDM (r=.635,p < .01). With binary regression analysis, elevated serum ferritin level was found to be an independent risk factor and a strong predictor for the development of GDM. This finding agrees with the report of Galal *et al* [62] on the association between elevated serum levels in early gestation and the risk of developing GDM. They concluded that high serum ferritin levels can be regarded as a significant good predictor for the development of GDM, with 100% sensitivity and high specificity. Similarly, Mohammed and colleagues[31] also showed a highly significant positive correlation between elevated serum ferritin and the risk of developing GDM (r= .680, p= .001) and they concluded that elevated serum ferritin was a significant variable to predict GDM.

Table 4 shows that elevated serum ferritin level is moderately and positively correlated to the risk of developing GDM and the relationship was statistically significant (r= 0.635;p<0.01). In the socio-demographic and clinical characteristics previous macrosomia was weakly and negatively correlated with GDM (r= -0.181, p=0.042). More so, Islam *et al*,[64] also found that the mean serum ferritin level was significantly higher in GDM than the non-GDM women. They also observed a significant linear correlation between elevated serum ferritin levels and 2-hour postprandial glucose (r=0.392, p < .001), and the likelihood of having GDM was 5 times higher in pregnant women with high serum ferritin levels than those with normal or low serum ferritin levels. Comparatively, findings by Amiri *et al*,[65] showed a statistically significant difference in the serum ferritin levels between GDM and non-GDM women. Figure 2 shows that there is significantly linear correlation between serum ferritin levels and 2-hour post 75g OGTT which was moderate and positive (r-0.1913, p = 0.01)

Table 5 shows that, elevated serum ferritin is an independent risk factor for the development of GDM, with a strong predictive value (7.307). Meanwhile, there was no significant association in the socio-demographic and clinical characteristics of the study participants with the development of GDM.

Serum ferritin is a proxy of total body iron stores and the association between elevated serum ferritin levels and the development of GDM as shown in these study has been attributable to the free radical mediated decrease in receptor number, post-receptor defect in insulin action and alteration in glucose transport, which mediate the peripheral insulin resistance. This free radical tissue damage is generally accepted as the major mechanism underlying the occurrence of GDM.[48,50] Hence, administration of iron supplements along with vitamin c in women with sufficient levels of iron stores, contribute to this free radical overproduction.

Contrary to the findings in this study, Zein *et al* [67] found no significant difference in the mean serum ferritin levels between women with GDM and without GDM. Also, Chan *et al*, [66] found no significant difference in the incidence of GDM among pregnant women in the iron supplement and placebo groups, at 28 weeks and 36 weeks gestation (OR 1.4, 95%CI 0.7-1.53 at 90% power). They concluded that, iron supplement from early pregnancy does not increase the risk of GDM.

Table 2: Baseline clinical characteristics of study participants

Clinical Characteristics		**Controls**	**Cases**	**Total**	**X^2**	**P Value**
		Frequency (%)	**Frequency (%)**	**Frequency (%)**		
Family Hx of DM	***Yes***	8 (12.7)	11 (17.5)	19 (15.1)	0.558	0.455
	No	55 (87.3)	52 (82.5)	107 (84.9)		
Previous GDM	***Yes***	3 (4.8)	9 (14.3)	12 (9.5)	3.316	0.069
	No	60 (95.2)	54 (85.7)	114 (90.5)		
Previous Macrosomia	***Yes***	5 (7.9)	13 (20.6)	18 (14.3)	4.148	0.042*
	No	58 (92.1)	50 (79.4)	108 (85.7)		
Previous Still birth	***Yes***	4 (6.3)	10 (15.9)	14 (11.1)	2.893	0.089
	No	59 (93.7)	53 (84.1)	112 (88.9)		
Previous Congenital Anomaly	***Yes***	4 (6.3)	10 (15.9)	14 (11.1)	2.893	0.089
	No	59 (93.7)	53 (84.1)	112 (88.9)		

Table 3: Study outcome measures

Outcome Measures	**Controls**	**Cases**	**Total**	**T**	**P Value**	**95% Confidence Interval**	
	Mean ± SD	***Mean ± SD***	***Mean ± SD***			**Lower**	**Upper**
Packed Cell Volume	31.81 ± 1.47	31.93 ± 1.25	31.88 ± 1.36	- 0.521	0.603	-0.610	0.356
Fasting Blood Sugar	77.98 ± 7.13	94.67 ± 8.56	86.33 ± 11.48	- 11.886	<0.01*	-19.461	-13.904
1H Post 75g OGTT	146.49 ± 10.31	167.32 ± 15.99	156.90 ± 16.99	- 8.689	<0.01*	- 25.569	- 16.082
2H Post 75g OGTT	108.87 ± 19.86	140.08 ± 15.01	124.48 ± 23.51	- 9.952	<0.01*	-37.413	-25.000
Serum Ferritin Levels	39.89 ± 14.08	92.66 ± 43.58	66.28 ± 41.74	- 9.143	<0.01*	-64.184	-41.340

Table 4: The correlation between test variables and GDM

Test Variables	Pearsons Correlation	P value
Sociodermographic Characteristics		
EGA	0.068	0.448
Age	0.098	0.273
Height	0.039	0.663
Weight	0.173	0.053
BMI	0.146	0.102
Clinical Characteristics		
Family Hx of DM	-0.067	0.459
Previous GDM	-0.162	0.070
Previous Macrosomia	-0.181	0.042*
Previous Still birth	-0.152	0.090
Previous Congenital Anomaly	-0.152	0.090
Outcome Measures		
Packed Cell Volume	0.047	0.603
Fasting Blood Sugar	0.730	<0.01*
1H Post 75g OGTT	0.615	<0.01*
2H Post 75g OGTT	0.666	<0.01*
Serum Ferritin Levels	0.635	<0.01*

This may be ascribed to the dose of iron supplement during the study period, as they adhered to the WHO recommended dose of 60mg elemental iron in pregnancy, also, there was no indiscriminate use of iron supplement. It may be argued that increase in iron store in this study was not sufficient enough, to cause any metabolic change in glucose metabolism. Other possibilities could be due to the fact that, the damaging effect of iron overload to the various component of glucose metabolism require a long-term exposure to high iron environment. Therefore, a relative sudden increase in body iron store with iron supplementation within a few months may not have an immediate adverse effect.

This study showed no statistically significant difference in the packed cell volume between the two groups. Studies have shown that the levels of hemoglobin is a reflection of iron stores and Lao *et al*, [10] found that high maternal haemoglobin reflecting a high serum ferritin, in early pregnancy is an independent risk factor for subsequent development of GDM. Women in the highest haemoglobin quintile (>13g/dl), had significantly higher incidence of GDM (18.7% VS 10.9%, p = 0.007). They also found that women with iron deficiency anaemia (Hb<10g/dl) were less likely to develop GDM than their non-anaemic counterparts (OR=0.52, 95% CI 0,27-0.98) and the prevalence of GDM was observed to be significantly reduced with increase in the duration of anaemia (p = 0.004). After adjusting for the effects of multiparity and BMI using multiple regression analysis, anaemia still emerged as significant predictor for decreased prevalence of GDM (OR=0.46, 95% CL 0.23-0.90). Also, studies have shown that, reduced iron stores from frequent blood donation have been demonstrated to reduce postprandial insulinaemia in healthy volunteers and improve insulin sensitivity, thus, constituting a protective factor for the development of GDM.[34,35]

Conclusion

This study discovered a link between increased blood ferritin levels and the likelihood of developing GDM. As a result, the Null hypothesis, which claims that there is no statistically significant difference in serum ferritin levels between GDM and non-GDM women, is rejected. Based on the current findings, it is recommended that routine iron supplementation in women at risk of diabetes be used with caution, unless they have iron deficiency anaemia.

Table 5: Logistic regression of test variables of study participants with GDM

Test Parameters	B	Wald	P Value	Odds Ratio	95% Confidence Interval	
					Lower	Upper
Previous Macrosomia	-1.242	0.769	0.381	0.289	0.018	4.643
Fasting Blood Sugar	0.160	6.726	0.009*	1.173	1.040	1.324
1H Post 75g OGTT	0.089	6.349	0.012*	1.093	1.020	1.172
2H Post 75g OGTT	0.033	3.998	0.046*	1.033	1.001	1.067
Serum Ferritin Levels	0.097	7.307	0.007*	1.102	1.027	1.182

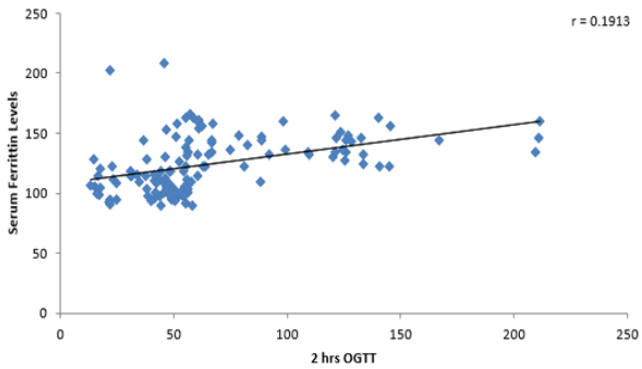

Figure 2: The correlation between serum ferritin levels and 2-hour post 75g OGTT

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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