IS THERE A LINK BETWEEN IRON-RELATED PARAMETERS AND INSULIN RESISTANCE IN GESTATIONAL DIABETES MELLITUS?

DISSERTATION

Submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

In partial fulfillment for the degree

DOCTOR OF MEDICINE IN BIOCHEMISTRY - BRANCH XIII

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DEPARTMENT OF BIOCHEMISTRY, CHRISTIAN MEDICAL COLLEGE VELLORE-632002, INDIA

CERTIFICATE

This is to certify that the study titled **"IS THERE A LINK BETWEEN IRON-RELATED PARAMETERS AND INSULIN RESISTANCE IN GESTATIONAL DIABETES MELLITUS?"** is the bona fide work of Dr. Lois Sara James, (Registration number 201823202) who conducted it under the guidance and supervision of Prof. Dr. Molly Jacob, Professor of Biochemistry, Christian Medical College, Vellore. The work in this dissertation has not been submitted to any other university for the award of a degree.

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DECLARATION

I hereby declare that the investigations, which form the subject matter of this study, were conducted by me under the supervision of Prof. Dr. Molly Jacob, Professor of Biochemistry, Christian Medical College, Vellore.

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TABLE OF CONTENTS

ABSTRACT

INTRODUCTION

Gestational diabetes mellitus (GDM) is a major health problem worldwide. Insulin resistance is a major feature of diabetes mellitus as well as a cause of GDM. Associations have been reported between increased body iron stores and insulin resistance. Serum levels of ferritin (a marker of body iron stores) and hepcidin (the central regulator of systemic iron homeostasis) have been reported to be elevated in women with GDM. Such elevations have been reported to be a significant risk factor for GDM. However, it is not clear how exactly these are linked to development of GDM.

AIM

The aim of this study was to estimate iron-related parameters in blood and insulin resistance in women with GDM, and to determine if they are associated.

MATERIAL AND METHODS

The study was carried out in the antenatal clinic of the Community Health and Development (CHAD) hospital of Christian Medical College, Vellore, Tamil Nadu, India. Primigravidae who were referred for an oral glucose tolerance test (OGTT) were recruited, after obtaining informed consent. A fasting blood sample was obtained from each woman and used to estimate levels of glucose, insulin, ferritin, iron, total iron-binding capacity (TIBC) and C- reactive protein (CRP).

RESULTS

One hundred and fifty-five primigravidae were screened. Twenty-five were found to have GDM. From among those screened, 25 age-matched (chronological and gestational ages) control subjects were chosen from among those who did not have GDM. Maternal age, gestational age at the time of recruitment, gestational age at delivery and birth weight of babies were similar in both groups. Levels of glucose in blood and insulin resistance (as assessed by HOMA-IR) were higher in those with GDM. Levels of haemoglobin and iron-related parameters were similar in the 2 groups. Iron-related parameters correlated with one another, in keeping with known biological relationships. Parameters linked to glucose and insulin were also significantly associated with one another, in keeping with known relationships. Iron-related parameters and indices of insulin resistance did not show any significant association with one another.

CONCLUSIONS

In this study, no significant association was found between iron-related parameters in blood and insulin resistance. However, the sample size in this study was small. An adequate number of subjects would need to be studied to confirm these observations.

Key words:

Gestational diabetes mellitus, insulin resistance, iron, ferritin, pregnancy

REVIEW OF LITERATURE

Diabetes mellitus (DM) is a disorder of metabolism characterized by hyperglycemia. It may be a consequence of decreased insulin production or insensitivity of cells to the action of insulin or both (Petersmann et al., 2018). India has been estimated to have about 62 million diabetics, which is the second highest in the world (Anjana et al., 2017). On the global front, there are around 451 million diabetics which is expected to rise to 693 million by the year 2045 (Cho et al., 2018).

According to the American Diabetic Association (ADA) diabetes mellitus is classified into (American Diabetic Association, 2020):

1. Type 1 diabetes mellitus: Absolute insulin deficiency due to destruction of pancreatic β-cells
2. Type 2 diabetes mellitus: Insulin resistance with insufficient insulin secretion to compensate
3. Gestational diabetes mellitus: Seen in pregnant women
4. Other types:
    a. Neonatal diabetes
    b. Maturity-onset diabetes of the young
    c. Drug induced diabetes
    d. Diseases of exocrine pancreas such as cystic fibrosis and pancreatitis

GESTATIONAL DIABETES MELLITUS

Introduction

Gestational diabetes mellitus (GDM) is glucose intolerance that is first diagnosed during pregnancy (Kaaja and Rönnemaa, 2008). Its prevalence has been estimated to be highly variable, with estimates ranging from 10.5% (lowest) to 24.2% (highest) among pregnant women (Plows et al., 2018; Zhu and Zhang, 2016), making it one of the most common metabolic disorders in pregnancy (Amiri et al., 2013). Its prevalence in India has been estimated to vary between 3.8% to 21% (Reddy et al., 2017).. Such variations in prevalence have been attributed to socio-economic status, dietary habits, geographical locations, and diagnostic criteria used

The most commonly used criteria for diagnosing GDM are those of the International Association of Diabetes and Pregnancy Study Group (IADPSG) which was based on the findings of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study (Chiefari et al., 2017; HAPO Study Cooperative Research Group et al., 2008). As per IADPSG, a fasting plasma glucose (FPG) estimation should be done for all women at their first antenatal visit, according to which a value $\geq$ 92mg/dL confirms GDM. Women with FPG $\leq$ 92mg/dL are again screened for GDM between 24 and 28 weeks where fasting plasma glucose levels $\geq$ 92 mg/dL or a 1-hour plasma glucose level (post

75g oral glucose tolerance test [OGTT]) ≥ 180 mg/dL or 2-hour plasma glucose level (post OGTT) ≥153 mg/dL is indicative of GDM (Plows et al., 2018).

Other criteria used in the diagnosis of GDM are given below. (Table 1)

Table 1: Criteria used in diagnosis of GDM

Criteria	**Pregnancies**	**Timing of OGTT**	**Steps**	**Glucose load (gm)**	**Glucose Threshold (mg/dL)**			
					Fasting	1 hour	2 hours	3 hours
O' Sullivan, 1964	All	24-28 weeks	2	100	90	166	146	124
WHO, 1999	All	24-28 weeks	1	75	126		140	
American Diabetic Association (ADA), 2004	High and medium risk	14-18 weeks for high risk, 28-32 weeks for medium risk	2	100	95	180	155	140
National institute for health and care excellence (NICE), 2015	High risk	As early as possible	1	75	101		140	
IADPSG, 2010 WHO, 2013 ADA, 2016	All	24-28 weeks	1	75	92	180	153	

Source: Reproduced from (Plows et al., 2018)

Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study was done to evaluate whether glucose intolerance in mother less than the diabetic range is associated with adverse pregnancy outcomes. In the study, hyperglycemia was compared with primary pregnancy outcomes like birth weight >90th centile for gestational age, primary cesarean delivery, clinical neonatal hypoglycemia, cord serum C-peptide >90th centile and with secondary outcomes like delivery before 37 weeks of gestation, shoulder dystocia or birth injury, need for intensive neonatal care, hyperbilirubinemia, and preeclampsia. A strong correlation was observed between increased glucose levels in mother and birth weight >90th centile and cord serum C-peptide >90th centile whereas weak correlation was observed between increased glucose levels in mother and preterm delivery, hypoglycemia in newborn, delivery by caesarean section, shoulder dystocia, need for intensive care for baby, hyperbilirubinemia (COUSTAN et al., 2010; HAPO Study Cooperative Research Group et al., 2008).

Risk factors for GDM include increased maternal age, obesity, increased maternal weight gain during pregnancy, previous history of GDM, and a family history of diabetes mellitus (Durnwald, 2015; Lao et al., 2006; Levy et al., 2010; Okosun et al., 2004). Polycystic ovarian syndrome (PCOS), a disease of insulin resistance, is also a risk factor for GDM (Ben-Haroush et al., 2004). There is direct or indirect involvement of these risk factors with beta cell dysfunction (Plows et al., 2018).

GDM is also associated with other post-partum maternal as well as fetal complications (HAPO Study Cooperative Research Group et al., 2008). Sixty percent of women with past history of GDM developed type 2 DM (T2DM) and 63% develop cardiovascular disease (CVD) later on in life (Peters et al., 1996; Shostrom et al., 2017).

Fetal complication includes macrosomia (which can lead to shoulder dystocia) and stillbirth. Macrosomia is due to fetal overgrowth which is because of increased production of insulin and insulin like growth factor 1(IGF-1) (Langer et al., 2005; Schwartz et al., 1994). Other complications seen in infants born to GDM mothers include neonatal hypoglycemia and childhood obesity (Esakoff et al., 2009; Vohr and Boney, 2008).

Pathophysiology of GDM

During the course of a normal pregnancy, insulin sensitivity changes over time. It increases early in the first trimester, but by third trimester, a state of insulin resistance develops (Buchanan and Xiang, 2005). Insulin resistance in late pregnancy is thought to be due to the rise in levels of placental hormones, such as estrogen, progesterone, leptin, cortisol, placental lactogen, and placental growth hormone (Catalano et al., 1991). The role of these hormones in the pathogenesis of GDM is supported by the fact that, post-delivery, maternal insulin sensitivity returns to pre- pregnancy levels

(Ryan et al., 1985). To maintain glucose homeostasis during pregnancy, the pancreatic beta cells undergo certain adaptive changes. These help to overcome insulin resistance during pregnancy. These include increased glucose-stimulated insulin secretion, beta cell hyperplasia and hypertrophy, increased insulin synthesis, increased glucose oxidation and cAMP metabolism and increased gap-junctional coupling among beta cells (Parsons et al., 1992; Plows et al., 2018; Sorenson and Parsons, 1985).

More than 80% of those with GDM manifest with dysfunction of beta cells and chronic insulin resistance (Buchanan and Xiang, 2005). These are, therefore, considered the major pathological mechanisms that result in GDM.

- **Pancreatic beta cell dysfunction**

Pancreatic beta cell dysfunction is a characteristic finding in GDM (Buchanan and Xiang, 2005; Plows et al., 2018). It is defined as the inability of beta cells to sense the blood glucose concentrations and subsequently cause the release of insulin in response (Weir et al., 2001). Women with GDM were found to have decreased expression of genes related to regulation of insulin secretion, such as glucokinase (Gck) and potassium voltage-gated channel KQT-like 1 (Kcnq1) (Prentki and Nolan, 2006) (Prentki and Nolan, 2006).

Insulin resistance causes worsening of the beta cell dysfunction. Beta cells secrete more insulin in response to the hyperglycemia caused by the reduced insulin-stimulated glucose uptake. Glucotoxicity occurs due to the contribution of glucose to β-cell failure (Ashcroft et al., 2017). Animal studies have shown that, not only beta cell dysfunction, but also a reduction in beta cell mass or beta cell number can contribute to GDM (Delghingaro-Augusto et al., 2009; Simmons et al., 2001). Beta cell dysfunction can occur before and after pregnancy and in many cases can progress to overt diabetes, so GDM is considered as early stage of T2DM (Buchanan et al., 2012).

- **Chronic insulin resistance**

Insulin is a polypeptide hormone made up of 51 amino acids, with two chains - A chain (21 amino acid) and B chain (30 amino acids) - held together by disulphide bridges. It is synthesized by the pancreatic beta cells (islets of Langerhans)(Dodson and Steiner, 1998; Wilcox, 2005). Plasma glucose levels are regulated mainly by insulin, along with glucagon. Insulin promotes glycogenesis and lipogenesis but suppresses gluconeogenesis and glycogenolysis (Wilcox, 2005).

Chronic insulin resistance has been reported to be one of the main causes of GDM (Catalano et al., 2003). Insulin resistance is a condition in which target tissues (mainly

liver, muscle, and adipose tissue) show impaired response to insulin. This often results in increased production of insulin from beta cells and hyperinsulinemia (Freeman and Pennings, 2019). Insulin resistance can result in impaired translocation of glucose transporter 4 (GLUT 4) which is the transporter involved in the entry of glucose into the cells (Figure 1). As compared to a normal pregnancy, insulin stimulated uptake of glucose is reduced by 54% in GDM (Catalano, 2014). Abnormalities of insulin signaling, such as decreased or increased expression of insulin receptor substrate (IRS)-1, phosphatidylinositol 3-kinase (PI3K) or an altered phosphorylation of insulin receptor, can result in insulin resistance (Barbour et al., 2007; Catalano, 2014).Glucose and free fatty acid concentrations in blood are increased due to the insulin resistance (Phelps et al., 1981).

Multiple studies have shown that when the acquired insulin resistance of pregnancy abates post-partum, women who had GDM end up with a greater insulin resistance than normal women (Buchanan and Xiang, 2005; Homko et al., 2001; Kautzky-Willer et al., 1997). This increase in insulin resistance in GDM as compared to normal pregnancy is due to alteration in insulin signaling pathway, abnormal subcellular localization of GLUT4 transporters, increased expression of the membrane glycoprotein PC-1 or reduced insulin-mediated glucose transport (Buchanan and Xiang, 2005).

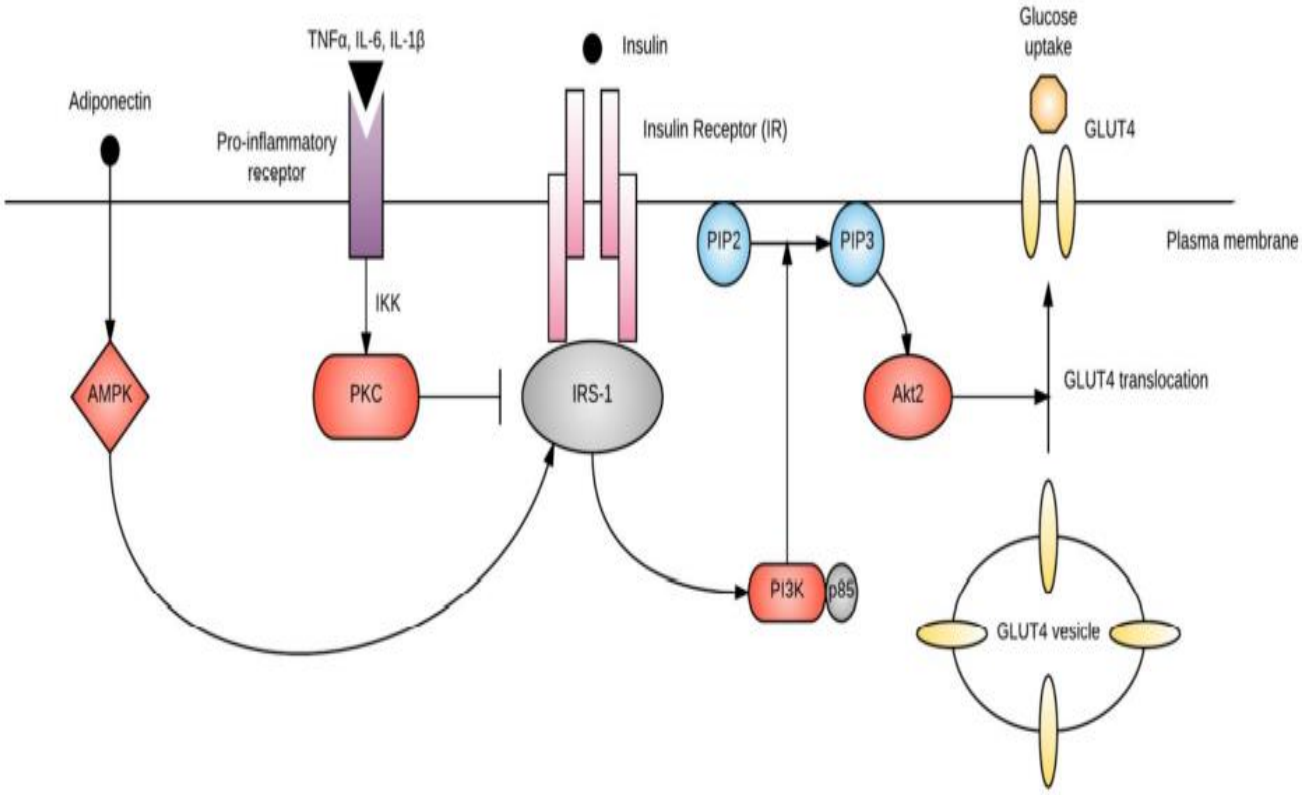

Figure 1: Insulin signal transduction pathway

Insulin binds to the insulin receptor (IR) which in turn activates IRS-1. Activated IRS-1 activates phosphatidylinositol-3-kinase (PI3K), which catalyzes the conversion of phosphatidylinositol-4, 5-bisphosphate (PIP_2) to phosphatidylinositol-3, 4, 5-phosphate (PIP_3). PIP_3 activates Akt, which induces the translocation of GLUT4 to the plasma membrane. GLUT4 increases uptake of glucose into the cell. IRS 1 is can also be activated by adiponectin promotes IRS-1 through AMP-activated protein kinase (AMPK). TNF α, IL 6 and IL 1β (proinflammatory cytokines) bind to their respective receptors and activate protein kinase C (PKC) via the IkB kinase (IKK). PKC inhibits IRS-1. **Source: Reproduced from (Plows et al., 2018)**

As mentioned earlier placental hormones like progesterone, estrogen, placental lactogen, leptin and placental growth hormone can cause insulin resistance in pregnancy. Progesterone decreases the expression of IRS 1 (Fig 1) thereby suppressing the phosphoinositol 3-kinase-mediated pathway thereby inhibiting GLUT4 translocation and glucose uptake into the cell (Wada et al., 2010). Estrogen can also decrease insulin sensitivity (González et al., 2000). Human placental growth hormone can increase in insulin levels and decrease insulin stimulated uptake of glucose. Both insulin-like and anti-insulin effects are associated with human placental lactogen (Barbour et al., 2002).

Leptin is a hormone synthesized by adipocytes and, in pregnancy, by the placenta as well (Masuzaki et al., 1997). It plays a role in satiety by stimulating anorexigenics, such as pro-opiomelanocortin (POMC), and inhibiting orexigenics, such as agouti-related peptide (AgRP) and neuropeptide Y (NPY) (Farr et al., 2015). Leptin resistance is normally seen in pregnancy, but it increases in GDM causing a state of hyperleptinemia (Honnorat et al., 2015). This contributes to fetal macrosomia by facilitating amino acid transport across the placenta (Pérez-Pérez et al., 2013).

Adiponectin is synthesized mainly by the adipocytes and decreased levels are seen in GDM (Williams et al., 2004). Studies have reported strong association between adiponectin and insulin resistance(Retnakaran et al., 2004). Adiponectin facilitate IRS 1 (Fig 1) action by activating AMP-activated protein kinase (AMPK), enhancing

insulin signaling and fatty acid oxidation (Yamauchi et al., 2002). It also causes exocytosis of insulin granules and upregulation of insulin gene expression, stimulating insulin secretion (Kishida et al., 2012).

GDM is characterized by increased circulating concentration of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β (Atègbo et al., 2006; Fasshauer et al., 2014). They act by decreasing release of insulin from beta cells and altering insulin signaling. They degrade IRS 1 via the STAT3-SOCS3 pathway, decrease tyrosine kinase activity of insulin receptor, enhance phosphorylation of IRS 1, thereby activating insulin resistance (Barbour et al., 2007; Kim et al., 2009).

Figure 2, shown below, depicts the relationship between β-cell dysfunction, insulin resistance, and GDM.

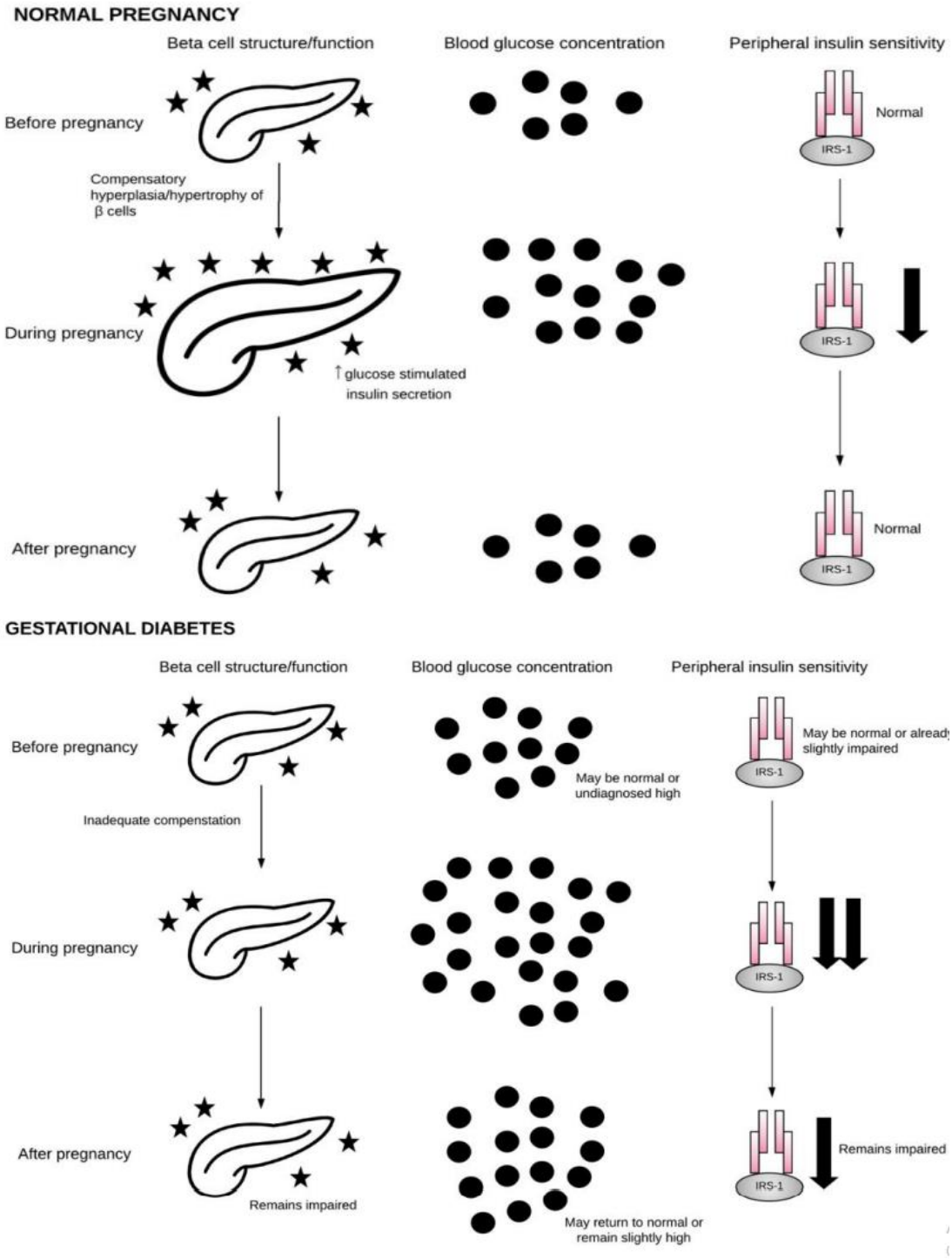

Figure 2: Relationship between β-cell dysfunction, insulin resistance, and GDM

There is hyperplasia and hypertrophy of beta cells in normal pregnancy. As insulin sensitivity fall with advancement of pregnancy, blood glucose levels rise. In GDM, beta cells fail to compensate for increasing insulin resistance resulting in hyperglycemia. Insulin sensitivity returns to normal after delivery. However, β-cell function may remain impaired following delivery. This may lead to GDM in subsequent pregnancy or T2DM in future. **Source: Reproduced from (Plows et al., 2018)**

IRON

Iron is an important trace mineral required for various metabolic reactions. It is a cofactor for a large number of enzymes in the body(Fu et al., 2016). Iron plays a role in transport of oxygen, production of neurotransmitters, functioning of immune system etc(Edison and Bajel, 2008; Gulec et al., 2014). The ability of iron to exist in two forms (ferric and ferrous) enables it to be part of electron transfer reactions. Iron levels have to be maintained in normal limits as excess iron can cause damage to membrane lipids, proteins and DNA due to production of oxygen free radicals (Fenton Reaction) (Gulec et al., 2014).

Absorption of iron

Iron absorption is highly regulated because there are no physiologically regulated mechanisms for iron excretion. To compensate for small amounts of iron loss (1-2 mg/day) caused by sloughing of intestinal epithelial cells, desquamation of skin and urinary cells, blood loss, or sweat, an equal amount of dietary iron is absorbed in the duodenum. When the needs are higher (like in pregnancy or increased erythropoiesis) iron absorption is enhanced whereas it is suppressed in iron overload (Hentze et al., 2010).

Dietary iron is of two types- heme and non heme, which are maximally absorbed in the duodenum and upper part of jejunum (Carpenter and Mahoney, 1992; Edison and Bajel, 2008).

(a) Absorption of non-heme iron (Figure 3)

Foods of plant origin are rich sources of non-heme iron(Anderson and Frazer, 2017). In the duodenum, iron, which is present in ferric (Fe^{3+}) form, is first reduced to ferrous form. This is achieved with the help of duodenal cytochrome b (DCYTB) which is a ferrireductase present on the brush border epithelium of the intestinal cells (McKie et al., 2001). Multiple studies have shown an upregulation of DCYTB during conditions of iron deficiency and acute hypoxia, clearly indicating importance of DCYTB in intestinal transport of iron (Collins et al., 2005; Latunde-Dada et al., 2011, 2002).

Ferrous iron is imported into the enterocyte through Divalent Metal ion Transporter 1 (DMT 1) present on the apical surface (Mackenzie and Garrick, 2005). DMT 1 can transport, in addition to iron, other divalent ions like manganese, cobalt and copper (Arredondo et al., 2014; Shawki et al., 2012). Importance of DMT 1 as an intestinal iron transporter (importer) is established by the fact that mutation of *SLC11A2* (gene encoding DMT 1) result in severe iron deficiency anemia (Blanco et al., 2009; Fleming et al., 1998, 1999; Mims et al., 2005).

Once inside the cell, iron is either stored as ferritin(Abboud and Haile, 2000) or released into circulation. It is transported out of the cell with help of ferroportin (FPN)(Donovan et al., 2005). This fate of whether iron will be stored or transported out depends on the body's requirement for iron i.e. when body iron demands are high, it will be transported out by FPN and when the demands are low iron, is stored as ferritin (Gulec et al., 2014). FPN is the only known iron export protein in mammals and is highly expressed in enterocytes, macrophages and hepatocytes (McKie et al., 2000). Hephaestin is a ferroxidase present on the basolateral surface, which works alongside FPN in the export of iron into the circulation (Abboud and Haile, 2000; Donovan et al., 2000). Ferrous iron is oxidized to ferric form with help of hephaestin.

Iron absorption (mainly of non heme iron) is affected by certain dietary factors which can either inhibit or enhance the process. Inhibitors of iron absorption include (a) Polyphenols which are present in tea, coffee, cocoa, red wine, vegetables like spinach, grains like red sorghum, spices like oregano, cinnamon etc. (b) Phytates- seen in cereals like bran, oats, seeds, nuts, vegetables, and fruit. (c) Calcium- can inhibit absorption of both non heme and heme iron. Enhancers include ascorbic acid, gastric hydrochloric acid and other reducing substances present in diet (Zijp et al., 2000).

(b) Absorption of heme iron

Heme iron in the diet is mainly of animal origin (from hemoglobin or myoglobin), and its absorption is not influenced by other dietary constituents (Anderson and Frazer, 2017). Uptake of heme into the duodenal enterocytes is mediated by a membrane protein called heme carrier protein 1 (HCP) (Shayeghi et al., 2005). Once inside the enterocyte, heme is acted upon by heme oxygenase to release the ferrous (Fe^{2+}) form of iron. Hereafter fate of heme iron is same as non heme-iron (Edison and Bajel, 2008).

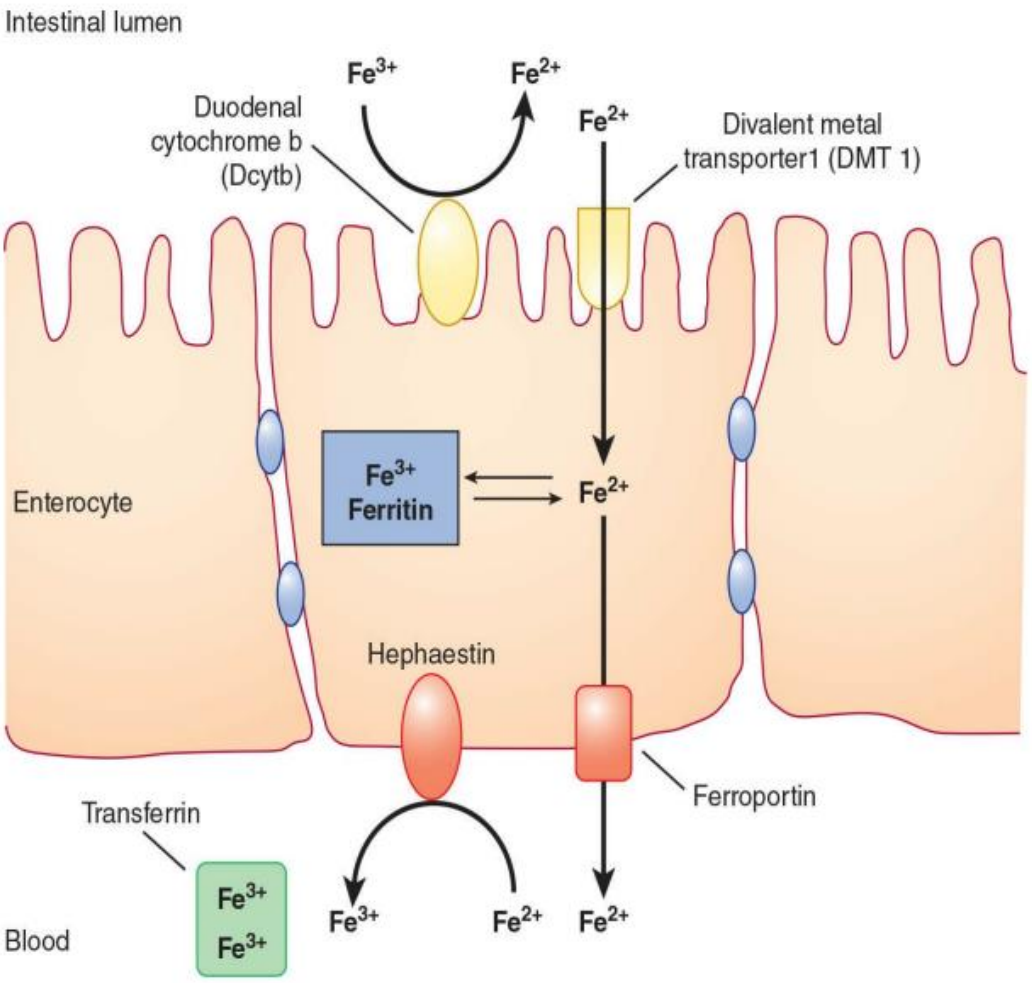

Figure 3: Absorption of non heme iron

Duodenal cytochrome b (Dcytb) has a ferrireductase activity, reduces ferric iron to ferrous iron. Divalent metal transporter1 (DMT1) transports this ferrous iron into enterocyte. Iron undergoes two fates in the enterocytes: it is either stored as ferritin or transported out by ferroportin. Hephaestin oxidizes the ferrous to its ferric form which binds to transferrin in the circulation. **Source: Reproduced from (Rodwell et al., 2018)**

Iron in circulation

In the circulation, iron is transported bound to transferrin (Tf) (Frazer and Anderson, 2014). Apotransferrin, a bilobed protein, binds two atom of Fe^{3+} to become holo-transferrin (Edison and Bajel, 2008). In normal conditions, the transferrin saturation is about 30%, which means only ~30% of the iron-binding sites on transferrin are occupied (Anderson and Frazer, 2017). Transferrin saturation is an indicator of the body iron status – low levels indicate iron deficiency and high levels indicate iron overload (Luck and Mason, 2012).

Cellular uptake of iron (Figure 4)

Uptake of iron by cells is initiated by the binding of transferrin to transferrin receptor (TfR) present on the membrane (Frazer and Anderson, 2014). Two types of transferrin receptors are present- transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2). The function of TfR1 is to mediate the uptake of transferrin by cells and it is present in all cells of the body, whereas TfR2 is present mainly in hepatocytes (Edison and Bajel, 2008; Kawabata et al., 1999). Binding of transferrin to TfR1 initiates receptor-mediated endocytosis and formation of intracellular endosomes containing Tf-TfR1 complex. The endosome formed is acidified by pumping H^+ ions into the endosome via proton pumps. Acidification induces a conformational change

in transferrin resulting in the release of iron. STEAP3, a metalloreductase, converts the ferric iron into its ferrous form (Ohgami et al., 2006, 2005). DMT 1 then transports the Fe^{2+} out into the cytosol from the endosomes (Fleming et al., 1998).

Apotransferrin remain bound to TfR1 and the complex is returned to the plasma where transferrin is released into blood. (Ponka et al., 1998).

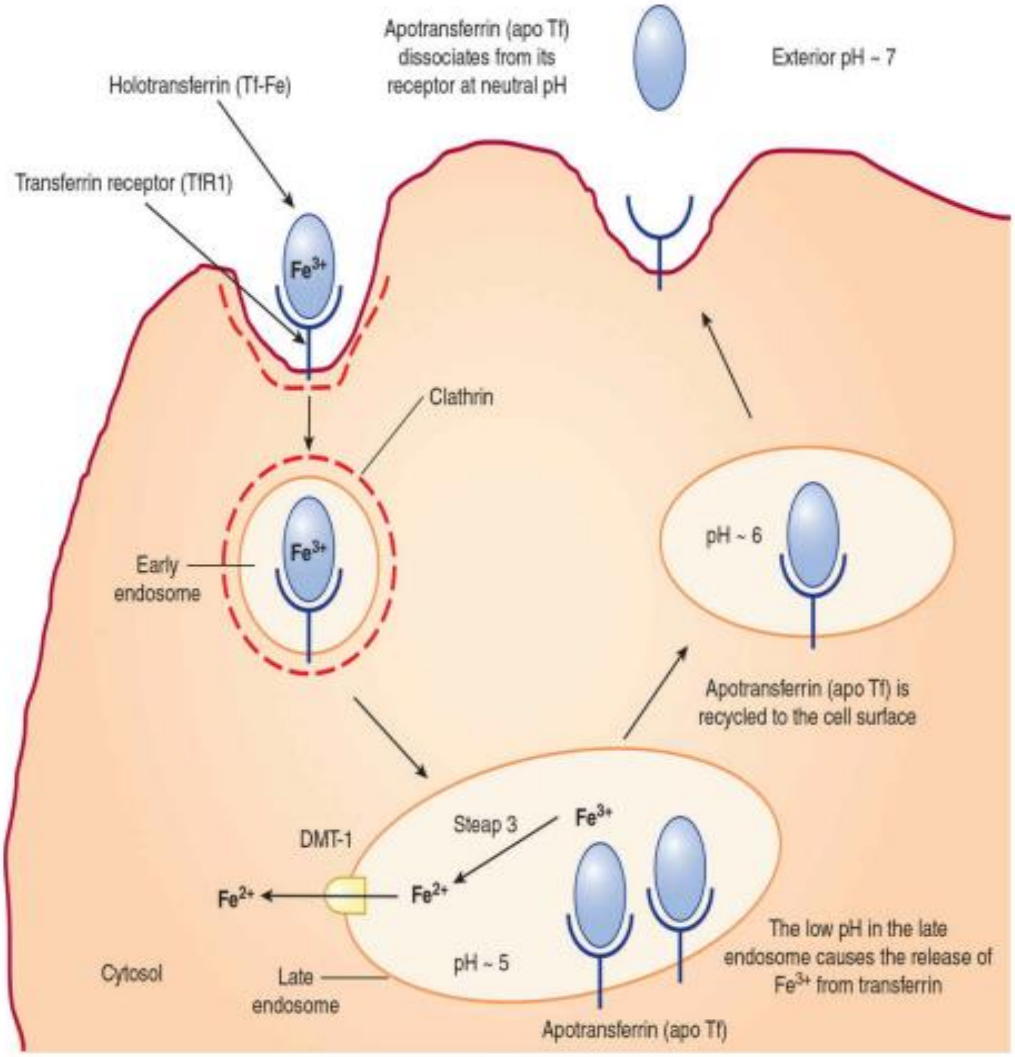

Figure 4: Cellular uptake of iron: Transferrin cycle

Holotransferrin (Tf-Fe^{3+}) binds to transferrin receptor 1 (TfR1) present on the surface of cells. They undergo invagination to form early endosomes. Early endosomes mature into late endosomes. Pumping of H^+ ions into the late endosomes resulting in a decrease in pH which cause dissociation of iron from transferrin. Ferric iron is converted to its ferrous form by Steap 3 which is then transported out into the cytosol via DMT1. Apotransferrin remain bound to TfR1, and this complex is returned to surface where transferrin gets released into circulation and TfR1 is reutilized. **Source: Reproduced from (Rodwell et al., 2018)**

Iron storage

The major storage form of iron is ferritin (Theil, 2013). It consists of 24 subunits that are arranged to form a spherical shell with a large central cavity which can hold 3000-4500 atoms of ferric iron. It consists of 2 types of subunits - heavy (H) and light (L) chain (Anderson and Frazer, 2017; Gulec et al., 2014). As the cellular iron concentrations rises, expression of ferritin increases leading to the accumulation of ferritin which fuses with lysosomes causing its degradation and the resulting mixture of Fe^{3+} cores and peptides is known as hemosiderin (Theil, 2013). Increased levels of hemosiderin are seen in disorders of iron overload.

Regulation of iron homeostasis

Iron homeostasis regulation can occur both at systemic and cellular levels.

(a) Systemic iron homeostasis

Hepcidin is the central regulator of systemic iron homeostasis (Ganz and Nemeth, 2012). It is produced by the liver as a prohormone (84 amino acid prepropeptide) which is cleaved by furin to give 25 amino acid-containing mature hepcidin (Hentze et al., 2010). Hepcidin can also be synthesized in small amounts by a variety of other cells including inflammatory monocytes, macrophages (Peyssonnaux et al., 2006)

and adipocytes (Bekri et al., 2006). It binds to α2- macroglobulin in the plasma (Peslova et al., 2009) and is excreted in the urine .(Hentze et al., 2010).

Hepcidin binds to ferroportin and causes it to be internalized and degraded , thereby reducing iron release from cells (Nemeth et al., 2004). It binds to the extracellular loop of ferroportin containing the amino acid cysteine (C) at 326th position. So, a mutation in C326S will result in impaired hepcidin binding to ferroportin, leading to a hepcidin-resistant state (Fernandes et al., 2009; Sham et al., 2005).

Iron recycling by macrophages

Macrophages play a major role in maintaining adequate levels of plasma iron by recycling iron from senescent erythrocytes. Consequently, less than 10% of the daily iron needs are met by intestinal absorption (Hentze et al., 2010). Macrophages of the reticuloendothelial system (RES) like spleen, liver phagocytose damaged or senescent erythrocytes to form phagocytic vesicles(Beaumont and Delaby, 2009). Heme, present in hemoglobin, is broken down to iron by action of the enzyme, heme oxygenase 1(Ganz, 2016). Iron, that is released, is transported out into the cytoplasm by divalent metal transporter, DMT1 (also callednatural resistance-associated macrophages protein 1; NRAMP1) (Soe-Lin et al., 2009). Ferroportin helps the in the iron export from macrophages, thus playing a role both in iron absorption in the intestine and iron recycling from macrophages (Rodwell et al., 2018).

Regulation of hepcidin synthesis

Hepcidin production is dependent on iron. When iron levels are high, more hepcidin is produced by the liver to limit duodenal iron absorption and release from macrophages. When iron levels are low, less hepcidin is produced, thus allowing increased duodenal iron absorption and recycling (Ganz and Nemeth, 2012). Other factors which influence hepcidin expression includes hypoxia, erythroid demand, and inflammation , (Edison and Bajel, 2008).

Regulation of hepcidin occurs at the level of transcription (Hentze et al., 2010). It is induced by inflammation, high iron levels and repressed by increased erythropoiesis and decreased iron levels (Edison and Bajel, 2008; Ganz and Nemeth, 2012). Iron dependent regulation of hepcidin occurs through the bone morphogenetic protein-SMAD regulatory pathway(Ganz, 2013). Interleukin 6 (IL 6), a key pro-inflammatory cytokine, enhances the hepcidin gene expression by activating the JAnus Kinase Signal Transducer and Activator of Transcription (JAK/STAT) pathway (Fleming, 2007). Erythroid regulators of hepcidin (e.g., erythroferrone) are produced by erythroid precursors in the bone marrow in response to increased iron demands during iron deficiency. It inhibit hepcidin activity independently of the BMP – SMAD pathway (Kautz et al., 2014).

(b) Cellular iron homeostasis

Iron-responsive elements (IRE)/Iron regulatory protein (IRP) system helps in the regulating the cellular iron homeostasis (Rouault, 2006). IRP interact with hairpin structures present in the 5' and 3' untranslated regions of mRNA known as IRE. Two types of IRPs are present- IRP 1 and IRP 2. Depending on whether the IRE is in 5' or 3' UTRs, IRE- IRP interaction can result in an increase or decrease in protein translation (Muckenthaler et al., 2008). Ferritin, FPN1, 5-aminolevulinic acid synthase 2, and hypoxia inducible factor 2α have IRE in 5' UTR and TfR1, and DMT1 have IRE in 3' UTR (Harms and Kaiser, 2015).

When cellular concentrations of iron are low, IRPs binds to IRE in 5' UTR of the ferritin mRNA inhibiting translation, so that little ferritin is produced. Binding of IRP to IREs in the 3' UTR of the TfR1 mRNA stabilizes it and increases its half-life, thus enabling more TfR1 to be translated and expressed on the cell membrane to facilitate increased iron intake. In condition of high cellular iron concentration, there is no IRP-IRE binding, causing translation of the ferritin mRNA and TfR1 mRNA degradation. This limits cellular iron uptake and promotes storage as ferritin (Anderson and Frazer, 2017).

IRON AND PREGNANCY

Iron requirements during pregnancy are high due to increased demands (Fu et al., 2016). As shown in Figure 5, there is 10-fold increase in the iron requirements during pregnancy (from 0.8 mg/day in the first trimester to 7.5 mg/day in the third trimester) (Bothwell, 2000). The excess iron is required for placental and fetal growth, increase in maternal red blood cell mass and to compensate for blood losses during delivery (Allen, 2001; Scott, 1972; Tapiero et al., 2001). Many major health organizations recommend iron supplementation throughout pregnancy as the dietary iron intake among pregnant women (approximately 15 mg/day), is insufficient to meet the increased demand (Institute of Medicine (US) Panel on Micronutrients, 2001).

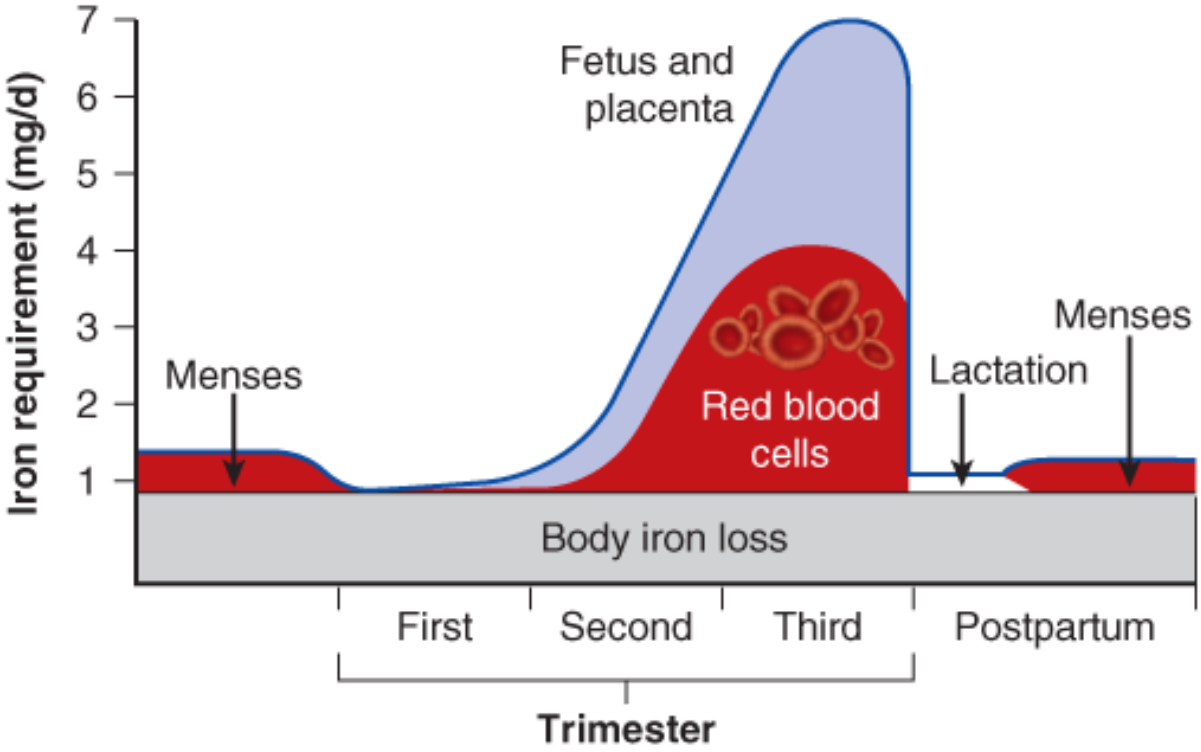

Figure 5: Iron requirements during pregnancy

This graph shows the variation in iron requirements during various phases of a normal pregnancy. In a nongravid female, iron requirement per day is approximately 1.5mg. During the first trimester, iron requirement is very minimal (0.8mg/day). As pregnancy progresses, iron requirement increases to a maximum of about 7mg/day during the third trimester to provide for the increased demand for growing fetus and placenta. In the postpartum period iron requirement again fall to pre-pregnancy levels **Source: Reproduced from (Cunningham et al., 2018)**

Iron deficiency in the mother can lead to complications, such as preterm delivery, low birth weight of baby, etc (Bothwell, 2000; Chen et al., 2006; Klebanoff et al., 1991). Studies have shown an association between severe anemia and increased risk of maternal mortality which ranges from 7 to 194 deaths per 100 000 live births in developing countries (Allen, 2000). An U-shaped association was observed between maternal hemoglobin concentrations and birth weight meaning that both abnormally high hemoglobin concentrations as well as low hemoglobin concentration are associated with lower birth weight babies (Garn et al., 1981; Singla et al., 1997; Steer, 2000) . Studies done on Welsh women with anemia showed a 1.18–1.75-fold higher relative risk of preterm birth and low birth weight (Murphy et al., 1986).

Iron transport across placenta (Figure 6)

In the maternal circulation, iron is bound to transferrin. Holo-transferrin binds to TfR1 on the maternal side of the syncytiotrophoblasts in the placenta and undergoes endocytosis. Acidification of vesicle results in dissociation of iron from transferrin and iron is transported into the cytosol by the divalent metal transporter-1 (DMT-1) (Rao and Georgieff, 2007). Iron enters the fetal circulation via ferroportin which is located on the fetal side of the syncytiotrophoblasts (Sangkhae and Nemeth, 2019).

Ferrous iron is then oxidized to ferric iron by Zyklopen, a ferroxidase, before it binds to transferrin in fetal circulation (McArdle et al., 2011).

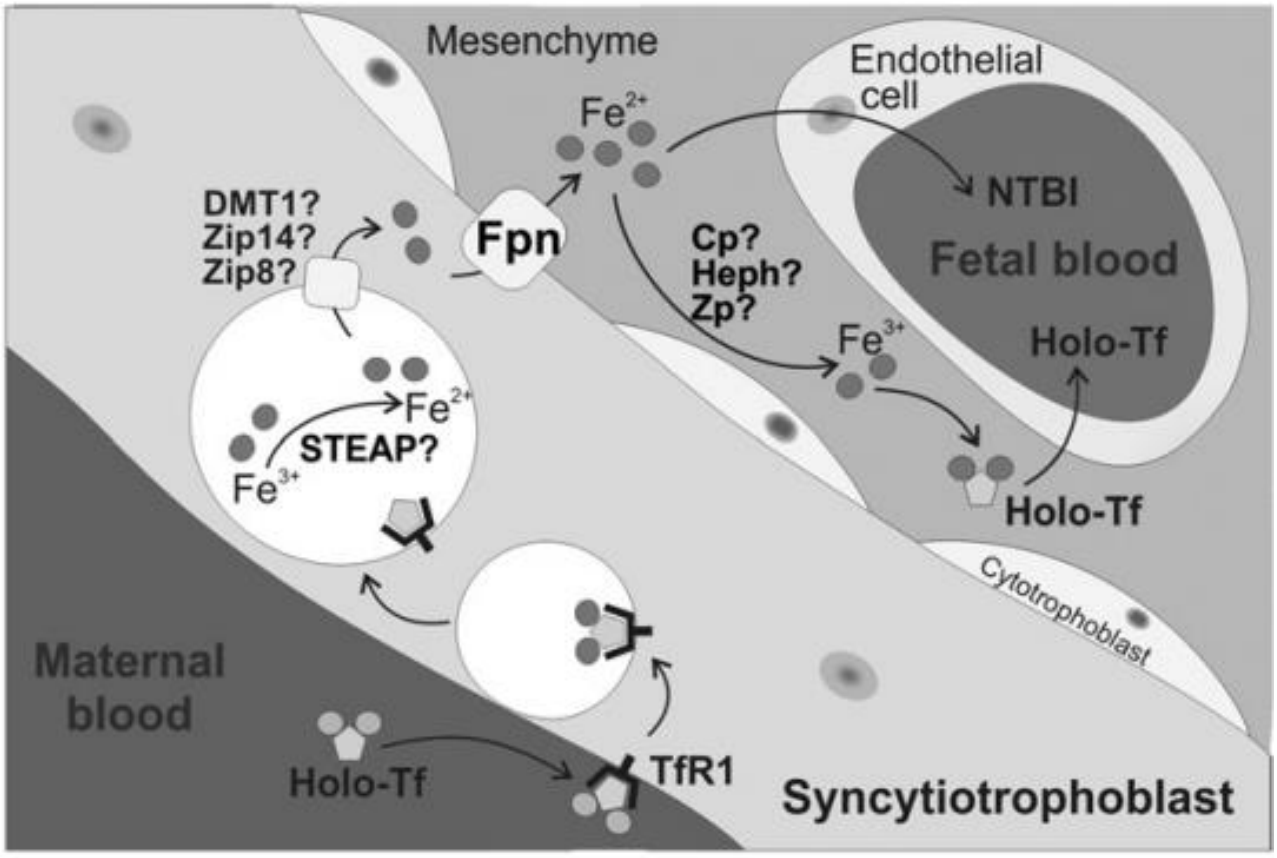

Figure 6: Iron transport across placenta

Iron transferrin bind to transferrin receptors on the maternal side of syncytiotrophoblast. This complex is endocytosed, and it forms an endosome. Acidification of the endosome cause the dissociation of the iron transferrin complex. Ferric iron is converted to its ferrous form by STEAP. Divalent metal transporter 1 (DMT 1) transports iron into the cytosol. Iron is exported out into the fetal circulation by Ferroportin. Zyklopen oxidizes ferrous iron to its ferric form which binds to transferrin in fetal circulation **Source: Reproduced from (Fisher and Nemeth, 2017).**

Regulation of placental iron transport

Maternal hepcidin is thought to regulate iron transport across the placenta to some extent (Fisher and Nemeth, 2017). It decreases to undetectable levels in second and third trimesters of pregnancy to maximize iron bioavailability and enhance transport across the placenta (Koenig et al., 2014; van Santen et al., 2013). Hepcidin is a negative regulator of placental transport of iron to the fetus. This fact was demonstrated in transgenic mice which over-expressed hepcidin; pups died shortly after birth due to severe iron deficiency (Nicolas et al., 2002).

IRON METABOLISM AND GDM

Iron excess has been implicated in the pathogenesis of diabetes mellitus (Beutler et al., 2003; Wilson et al., 2003). Studies have shown that increased serum ferritin levels (a marker of body iron stores) is associated with higher risk of developing diabetes mellitus (Forouhi et al., 2007; Rajpathak et al., 2006; Wrede et al., 2006). This relationship was first observed in patients with hereditary hemochromatosis (Pietrangelo, 2006). There are mainly three ways by which iron plays a role in the development of diabetes: by decreased insulin production, increased resistance to insulin and by causing liver dysfunction (Swaminathan et al., 2007).

It has been reported that iron overload in pregnancy may be involved in the development of GDM (Lao et al., 2001). *In vitro* studies have shown that iron overload is toxic to beta-cells of the pancreas, resulting in decreased insulin secretion and impaired glucose metabolism (Bowers et al., 2016; Liu et al., 2009). Studies done on Chinese and Malaysian subjects have shown an association between raised hemoglobin levels and increased risk of GDM (Lao et al., 2001; Tan et al., 2011). Lao et al showed that initial hemoglobin, hemoglobin levels in third trimester was similar in both GDM as well as controls whereas post-partum hemoglobin (day 3) was significantly higher in the GDM group (Lao et al., 2001). Other studies have shown that higher maternal hemoglobin levels in first trimester (Tan et al., 2011) and prepregnancy Hb levels higher than 13 g/dL (Hy et al., 2021) were independently associated with the risk of developing GDM. Women with prepregnancy Hb levels higher than 13 g/dL were also associated with GDM that required insulin therapy (Hy et al., 2021).

GDM women tend to have high iron and low insulin levels (Kaygusuz et al., 2013). Insulin synthesis in the pancreas can be affected by iron due to its pro-oxidant property (Rajpathak et al., 2009).

High levels of serum ferritin have been reported in women with GDM, as compared to those without (Amiri et al., 2013; Bowers et al., 2016; Chen et al., 2006; Derbent

et al., 2013; Fu et al., 2016; Khambalia et al., 2016). Amiri et al showed that concentration of serum iron and total iron binding capacity were similar in both GDM as well as controls whereas serum ferritin level was found to be significantly higher in women with GDM. In addition, the risk of developing GDM was increased 2.4-fold in pregnant women who had higher ferritin levels (> 80 ng/mL) as compared to pregnant women with lower ferritin levels. These findings were similar to other studies (Chen et al., 2006). This study, however, did not adjust for inflammation since ferritin is an acute phase reactant and can be elevated in conditions of inflammation. Both Chen et al and Bowers et al showed that women with higher levels of ferritin had a twofold increased risk of developing GDM. Even after adjusting for inflammatory markers like CRP and oxidized LDL, the association between ferritin and GDM remained significant (Bowers et al., 2016; Chen et al., 2006).

High levels of hepcidin in blood result in reduced absorption of dietary iron, recycling of iron from macrophages and release of iron from stores in the liver (Ganz and Nemeth, 2012). Women with GDM were found to have elevated levels of serum hepcidin (Rawal et al., 2017). Derbent et al (2013) showed that women with GDM had higher levels of hepcidin and ferritin as compared with controls. Hepcidin showed significant positive correlation with fasting glucose and fasting insulin levels. To determine whether the elevations in hepcidin and ferritin were related to

inflammation, CRP was analyzed, and this showed no correlation of CRP with ferritin and hepcidin levels.

A study done in Indian population showed GDM cases had significantly higher levels of ferritin as compared to controls and a negative correlation was established between ferritin and blood glucose (Yadav et al., 2017).

To summarize, there is evidence to suggest that elevations in iron parameters may be a significant risk factor for GDM. However, there is very little data from India. In addition, the precise mechanistic links between the two remains unclear.

SCOPE OF STUDY

Studies have shown an association between insulin resistance (IR) and increased serum ferritin levels. Serum levels of hepcidin (the central regulator of systemic iron homeostasis) and ferritin have been reported to be elevated in women with GDM. Such elevations may be a significant risk factor for GDM. However, it is not clear how exactly they are linked.

The available literature shows that elevated body iron stores (as indicated by serum levels of ferritin and/or hepcidin) are associated with development of GDM. The present study aims to look for links that may exist between iron parameters and insulin resistance in patients with GDM. This may help to ascertain if iron parameters (such as ferritin or hepcidin) may be useful in prediction of GDM. This assumes significance in the light of the fact that iron supplementation is routinely given to all pregnant women, irrespective of their iron status.

THE STUDY

HYPOTHESIS

Iron-related parameters in blood correlate with insulin resistance, in women with gestational diabetes mellitus (GDM).

AIM

The aim of this study was to estimate iron-related parameters in blood and indices of insulin resistance in women with GDM, and to determine if they correlate with one another.

OBJECTIVES

Primary objectives:

- To estimate iron-related parameters in blood from pregnant women, with and without GDM
- To estimate fasting blood glucose and insulin levels in pregnant women, with and without GDM
- To calculate indices of insulin resistance in pregnant women, with and without GDM

- To determine if any correlation exists between iron-related parameters in blood and indices of insulin resistance in pregnant women, with and without GDM

Secondary objective:

- To determine the outcomes of pregnancy in subjects, with and without GDM, and to correlate these with the parameters of interest in this study

MATERIALS

Equipment used

1. Table-top refrigerated centrifuge (MPW R 350, MPW, Poland)
2. -70^{O}C freezer (Cryo Scientific, Chennai, India)

Other materials used

1. For blood collection - red vacutainer tubes (BD Biosciences, Plymouth, UK)
2. Micro-tubes (Tarson Products Private Limited, Kolkata, India)
3. Micro-tips (Tarson Products Private Limited, Kolkata, India

METHODS

This study was approved by the Institutional Review Board (IRB) of Christian Medical College (CMC), Vellore, India (IRB approval no: 13458, dated 05.10.2020). The letter of approval for the study from the IRB is included as Appendix 1.

SUBJECTS

The study was carried out on subjects who attended the antenatal clinic of the Community Health and Development (CHAD) hospital, CMC, Vellore. Primigravidae who were referred for an oral glucose tolerance test (OGTT), at the time of their antenatal check-up, were recruited, after obtaining informed consent. The recruitment period was from March 2021 to September 2021.

The inclusion and exclusion criteria used were as follows:

Inclusion criteria

- Hb $\geq$ 11 g/dL
- Willing to participate in the study

Exclusion criteria:

- Hb < 11g/dL
- Already known to be diabetic
- Any complication of pregnancy

CALCULATION OF SAMPLE SIZE

Data from the publication by Derbent et al (2013) was used to calculate sample size as shown below, using nMaster 2.0. The data in this publication for serum ferritin was used for the purpose of calculation of sample size.

Two means - hypothesis testing for two means		
	Based on the publication by Derbent et al (2013)	
Standard deviation in group I (Cases-GDM)	13.37	13.37
Standard deviation in group II (controls)	9.66	9.66
Difference between means in the 2 groups	11.45	11.45
Effect size	0.994355	0.994355
Alpha error (%)	5	5
Power (1- beta) %	80	90
1 or 2 sided	2	2
Required sample size per group	16	22

Ref: *Derbent et al (2013). Serum hepcidin is associated with parameters of glucose metabolism in women with gestational diabetes mellitus. J. Matern. Fetal Neonatal Med 26, 1112–1115. doi:10.3109/14767058.2013.770462*

The formula that was used to calculate the sample size, using nMaster 2.0, was:

$$n = \frac{(Z_\alpha + Z_\beta)^2 * S^2 * 2}{d^2}$$

where,

- Z_α = Z value for α error
- Z_β = Z value for β error
- S = Common standard deviation for the two groups calculated as shown:

$$S^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

- Where n_1 = number of subjects in group 1 (cases- GDM) in the publication by Derbent et al (2013)
- n_2 = number of subjects in group 2 (controls) in the publication by Derbent et al (2013)
- s_1 = SD of mean in group 1(cases- GDM) in the publication
- s_2 = SD of mean in group 2(controls) in the publication

- d = Difference between the means in the 2 groups in the publication
- The calculated sample size, with a power of 90% was 22 in each group. Hence, it was proposed to study 25 subjects with GDM and 25 without GDM, a total of 50 subjects

INFORMED CONSENT

After identification of eligible subjects, based on the inclusion and exclusion criteria listed above, details of the study were explained to each subject. They were each provided with an information sheet about the study, which were made available in Tamil, English or Hindi, as per their preference. Written informed consent was obtained from each participant. The information sheet and the informed consent form used are included as Appendix 2.

DATA COLLECTION

Relevant socio-demographic and clinical information was collected from each participant. The proforma used for this is included as Appendix 3.

The weight and height of each subject was obtained from the hospital records. This information was used to calculate body mass index (BMI) for each subject which is given by the formula

Body mass index (BMI)= Weight (Kg)/(Height (m))^2

Information on supplementation with iron and folic acid tablets was noted from the hospital records. They were asked about how regular they were in taking these tablets.

The hemoglobin value for each subject was obtained from the hospital records. Information regarding the outcomes of pregnancy was obtained either from hospital records or by contacting the subjects by phone.

BLOOD SAMPLE COLLECTION

A fasting blood sample (6ml) was collected from each participant, at the time of the OGTT. Blood was collected in a plain red vacutainer tube (for clotted blood), from a peripheral vein in either arm, using aseptic precautions

PROCESSING AND STORAGE OF BLOOD SAMPLES

Serum was separated from the clotted blood samples obtained, by subjecting them to centrifugation at 2500*g* for 10 min. The serum obtained was divided into multiple aliquots and stored at -70^{o}C, until laboratory analyses were carried out. Serum samples were thawed on the day of analyses. They were used for estimation of serum iron, ferritin, total iron-binding capacity (TIBC), insulin and C-reactive protein.

DIAGNOSIS OF GDM

The criteria used to diagnose gestational diabetes mellitus were as follows: Fasting plasma glucose levels ≥ 92 mg/dL or a 1-hour plasma glucose level (post 75g oral glucose tolerance test [OGTT]) ≥ 180 mg/dL or 2-hour plasma glucose level (post OGTT) ≥153 mg/dL (American Diabetic Association, 2020).

ESTIMATION OF SERUM IRON

Estimation of serum iron was done in the diagnostic laboratory of the Department of Clinical Biochemistry, CMC, Vellore.

Analyzer used:

Roche Cobas c720 modular analyzer from Roche Diagnostics, Gmbh, Mannheim

Principle: Guanidine/ferrozine spectrophotometric method

Guanidine hydrochloride caused release of ferric ions from transferrin. This ferric ion was reduced to ferrous state by hydroxylamine. Ferrous ions reacted with the dye ferrozine, forming a purple-coloured complex. The intensity of color formed was directly proportional to the concentration of iron and was determined by measuring the absorbance at 546 nm.

ESTIMATION OF SERUM FERRITIN

Estimation of serum ferritin was done in the diagnostic laboratory of the Department of Clinical Biochemistry, CMC, and Vellore.

Analyzer used:

Siemens, Atellica IM

Principle of the method: Two-site sandwich immunoassay, using direct chemiluminescence technology

This method utilized two anti-ferritin antibodies. The first antibody was an acridinium-labelled polyclonal goat anti-ferritin antibody. The second antibody was a monoclonal mouse anti-ferritin antibody, which was covalently bound to paramagnetic particles. Solid phase for the immunoassay was provided by the paramagnetic particles. These antibodies were then added sequentially to the reaction chamber and formed a complex with ferritin present in the sample. A substrate was added which caused excitation of the acridinium, causing release of photons. The intensity of photons released was directly proportional to the concentration of ferritin in the sample.

ESTIMATION OF TOTAL IRON-BINDING CAPACITY (TIBC)

Estimation of TIBC was done in the diagnostic laboratory of the Department of Clinical Biochemistry, CMC, Vellore.

Analyzer used:

Roche Cobas c720 modular analyzer from Roche Diagnostics, Gmbh, Mannheim

Principle: Colorimetric method

Serum sample was added to a reagent solution consisting of a buffer and known concentrations of iron and ferrozine. The buffer provided the alkaline pH needed for binding of iron in the reagent to iron-binding sites on transferrin. The unbound iron reacted with ferrozine to give a purple complex. The intensity of color formed was directly proportional to the concentration of unbound iron and was determined by measuring the absorbance at 546 nm. Unbound iron binding capacity (UIBC) was calculated as the difference between the amount of iron added and the measured unbound iron. Total iron binding capacity (TIBC) was obtained by the sum of serum iron and UIBC.

CALCULATION OF TRANSFERRIN SATURATION

Transferrin saturation was calculated as the percentage of the ratio of serum iron to TIBC, as shown in the formula below:

Transferrin saturation (%) = (Serum iron/TIBC) x 100

ESTIMATION OF SERUM HIGH SENSITIVITY C-REACTIVE PROTEIN (hs-CRP)

Estimation of hs-CRP was carried out in the diagnostic laboratory of the Department of Clinical Biochemistry, CMC, Vellore.

Analyzer used:

Immulite 2000 xpi from Siemens, UK.

Principle: Solid-phase chemiluminescent immunometric assay

Anti-ligand-coated beads constituted the solid phase. The liquid phase contained an anti-CRP murine monoclonal antibody and rabbit polyclonal anti-CRP antibody in buffer. Anti-CRP murine monoclonal antibody was attached to the ligand, and rabbit polyclonal anti-CRP atibody was conjugated to alkaline phosphatase. The sample and the reagent were incubated together, with the anti-ligand-coated bead, for 30 minutes. The CRP in the sample formed a sandwich with the antibodies, anti-CRP murine monoclonal antibody and rabbit polyclonal anti-CRP antibody. A chemiluminescent substrate that emitted a light signal was added after washing away unbound antibodies. The intensity of light emitted was proportional to the concentration of hs-CRP in the sample.

ESTIMATION OF SERUM INSULIN

Estimation of serum insulin was carried out in the diagnostic laboratory of the Department of Clinical Biochemistry, CMC, Vellore.

Analyzer used:

Immulite 2000 xpi from Siemens, UK.

Principle Solid-phase chemiluminescent immunometric assay

Solid phase beads were coated with monoclonal murine anti-insulin antibody. The liquid phase comprised of two antibodies, which were conjugated to alkaline phosphatase i.e., polyclonal sheep anti-insulin antibody and monoclonal murine anti-insulin antibody. Sample and reagents were added to the reaction chamber. Insulin present in the sample formed a sandwich with monoclonal murine antibody on the solid phase, polyclonal sheep anti-insulin antibody and monoclonal murine antibody in the liquid phase. A chemiluminescent substrate that emitted a light signal was added after washing away unbound sample and reagent. The intensity of light emitted was proportional to the insulin concentration of the sample.

CALCULATION OF HOMEOSTASIS MODEL ASSESSMENT OF INSULIN RESISTANCE (HOMA-IR)

This was calculated by the formula:

HOMA-IR =Fasting plasma insulin (µIU/ mL) x fasting plasma glucose (mg/dL)/405

ESTIMATION OF PLASMA GLUCOSE

Estimation of plasma glucose was carried out in the diagnostic laboratory of the Community Health and Development Hospital (CHAD), CMC, Vellore.

Analyzer used:

Beckman Coulter

Principle: Hexokinase method

Glucose was converted to glucose-6-phosphate by the enzyme hexokinase, in the presence of ATP.

Glucose-6-phosphate dehydrogenase oxidized the glucose-6-phosphate formed to 6-phosphogluconate and NADPH. The amount of NADPH produced was directly proportional to the concentration of glucose in the sample and was determined by measuring the absorbance at 340 nm.

STATISTICAL TESTS

Statistical analysis was done using Statistical Package for Social Sciences version 21.0 (SPSS 21.0). Normality of data was assessed by Kolmogorov–Smirnov (KS) test. Normally distributed data were expressed as mean and standard deviation and skewed data were expressed as median and interquartile range. Independent t-test was used for comparison of data in two groups that were normally distributed and Mann-Whitney U test was used for comparisons of data that were not normally distributed. Bivariate correlation analyses were done using Pearson correlation for variables with normal distribution and Spearman's rank correlation for variables that are not normally distributed. A P-value less than 0.05 was considered statistically significant in all cases.

RESULTS

One hundred and fifty-five primigravidae were screened. Of these, 25 were diagnosed to have GDM, based on results of the OGTT. . They were matched, by chronological and gestational age, with 25 control participants, who had normal results for their OGTT.

Distribution of the data was determined by Kolmogorov-Smirnov (KS) test. Data on maternal age, serum iron, serum ferritin, transferrin saturation, serum insulin, HOMA-IR and C-reactive protein were found to be normally distributed. Gestational age at the time of recruitment, weight, height, body mass index, hemoglobin, plasma glucose values (fasting values and values at 1 and 2 hours after the glucose load), TIBC, gestational age at delivery and birth weight of infant were found to have skewed distributions.

Table 1: Characteristics of participants

	Controls (N=25)	**Cases (GDM) (N=25)**	**P value**
Maternal age (years)	26.68 ± 4.26	26.48 ± 3.87	0.863
Gestational age at the time of recruitment (weeks)	25.29 (24.29 - 27.14)	25.71 (24 – 27.58)	0.771
Weight (kg)	60 (54.50 – 65.35)	65 (54.80 – 71.50)	0.346
Height (cm)	153 (149.50 – 159)	155 (149.50 – 160)	0.648
Body mass index (BMI) (kg/m^2)	25.68 (23.57 – 26.96)	26.37 (22.77 – 28.75)	0.332
Haemoglobin (g/dL)	12 (11.45 – 12.5)	12.1 (11.55 – 12.5)	0.734

Data were expressed as means (± SD) or as medians with interquartile ranges (IQR), depending on the distribution of the data.

All the above characteristics were similar in both groups. Both women with GDM and without GDM were on iron and folic acid supplementation from 13 weeks of gestation. When enquired about the regularity of taking the supplements all the women said they were regular.

Table 2: Results of glucose tolerance test

	Controls (N=25)	**Cases (GDM) (N=25)**	**P value**
Fasting plasma glucose (mg/dL)	82 (74 – 85.50)	92 (80 – 98.50)	**0.003**
Plasma glucose 1 hr after glucose load (mg/dL)	131 (117.5 – 157.50)	181 (144.5 – 207)	**0.000**
Plasma glucose 2 hr after glucose load (mg/dL)	112 (98 – 128)	144 (119.5 – 172.50)	**0.000**

Data were expressed as medians with interquartile ranges (IQR).

Values of fasting plasma glucose. and at 1 and 2 hours after the glucose load were significantly higher in women with GDM than in the control subjects.

Table 3

	Controls	**Cases (GDM)**	**P value**
Serum insulin (µIU/mL)	5.65 ± 3.07 (N=16)	10.99 ± 10.63 (N=21)	0.060
HOMA-IR	1.15 ± 0.68 (N=16)	2.55 ± 2.49 (N=21)	0.036
Serum C-reactive protein (mg/L)	10.36 ± 9.71 (N=25)	17.49 ± 28.96 (N=25)	0.248

Data were expressed as means (± SD)

Nine of the 25 control subjects and 4 of the cases had insulin levels too low to be detected by the assay used (<2 µIU/mL). These subjects were excluded from the analysis involving insulin and HOMA-IR.

Serum insulin levels were higher in those with GDM (p=0.06). Values for HOMA-IR were significantly higher in the GDM group than in the control group. Serum CRP levels were similar in both groups.

Table 4: Iron-related parameters

	Controls (N=25)	**Cases (GDM) (N=25)**	**P value**
Serum iron (µg/dL)	94.32 ± 44.27	101 ± 74.09	0.700
Serum ferritin (µg/L)	21.49 ± 19.54	20.23 ± 19.77	0.821
Total iron-binding capacity (TIBC) (µg/dL)	451 (424.5 – 514.5)	440 (394.5 – 529)	0.516
Transferrin saturation (%)	20.84 ± 10.93	22.64 ± 16.66	0.654

Data were expressed as means (± SD) or as medians with interquartile ranges (IQR), depending on the distribution of the data.

All iron-related parameters in blood were similar in those with and without GDM.

Table 5: Outcomes of deliveries

	Controls (N=24)	**Cases (GDM) (N=22)**	**P Value**
Gestational age at delivery (weeks)	39.64 (39.29 – 40.11)	39.5 (38.97 – 40.14)	0.774
Birth weight (kg)	3.01 (2.62 – 3.32)	2.74 (2.40 – 3.03)	0.069

Data were expressed as medians with interquartile ranges (IQR).

Mean gestational age at delivery and birth weights of the babies in the 2 groups were similar.

Table 6: Bivariate analysis of iron-related parameters (N=50)

			Serum iron (µg/dL)	Serum ferritin (µg/L)	Total iron binding capacity (µg/dL)	Transferrin saturation (%)
	Serum iron (µg/dL)	Correlation Coefficient				.968
		P Value				0.000
	Serum ferritin (ng/mL)	Correlation Coefficient			-.590	
		P Value			0.000	
	Total iron binding capacity (TIBC) (µg/dL)	Correlation Coefficient		-.590		-.292
		P Value		0.000		0.039
	Transferrin saturation (%)	Correlation Coefficient	.968		-.292	
		P Value	0.000		0.039	

Correlation analysis was done using Spearman's correlation.

Serum iron showed significant positive correlation with transferrin saturation. TIBC correlated significantly and negatively with serum ferritin and transferrin saturation

Table 7: Bivariate analysis of metabolic parameters (N=37-50)

		Fasting plasma glucose (mg/dL)	Plasma glucose 1hr after glucose load (mg/dL)	Plasma glucose 2hr after glucose load (mg/dL)	Insulin (µIU/mL)	HOMA-IR
Fasting plasma glucose (mg/dL)	Correlation Coefficient			.311	.364	.562
	Sig. (2-tailed)			.028	.027	.000
Plasma glucose 1hr after glucose load (mg/dL)	Correlation Coefficient			.547		.323
	Sig. (2-tailed)			.000		.051
Plasma glucose 2hr after glucose load (mg/dL)	Correlation Coefficient	.311	.547			
	Sig. (2-tailed)	.028	.000			

Spearman's correlation coefficient was used for correlation analysis.

Fasting plasma glucose showed significant positive correlation with plasma glucose values 2hrs after glucose load, insulin levels and HOMA-IR. Plasma glucose 1hr after glucose load showed significant positive correlation with plasma glucose 2hr after glucose load and HOMA-IR.

Table 8: Bivariate analysis of metabolic and iron-related parameters (N= 37-50)

		Serum iron (µg/dL)	Serum ferritin (ng/mL)	Total iron-binding capacity (µg/dL)	Transferrin saturation (%)	Hemoglobin (g/dL)	CRP (mg/L)
Fasting plasma glucose (mg/dL)	Correlation Coefficient	-.398			-.323		.553
	Sig. (2-tailed)	.004			.022		.000
Plasma glucose 1 hr after glucose load (mg/dL)	Correlation Coefficient			-.279			.271
	Sig. (2-tailed)			.050			.057
Plasm glucose 2hr after glucose load (mg/dL)	Correlation Coefficient					.322	
	Sig. (2-tailed)					.023	
Insulin (µIU/mL)	Correlation Coefficient						
	Sig. (2-tailed)						
HOMA IR	Correlation Coefficient						.344
	Sig. (2-tailed)						.037

Spearman's correlation coefficient was used for correlation analysis. Fasting plasma glucose levels showed significant negative correlation with serum iron and transferrin saturation, and positive correlation with serum CRP levels. Plasma glucose levels at 1 hour after the glucose load showed significant negative correlation with TIBC and positive correlation with serum CRP levels. Plasma glucose levels at 2 hours after the glucose load showed significant positive correlation with hemoglobin. HOMA-IR values showed a significant positive correlation with serum CRP.

SUMMARY OF RESULTS

1. There were no significant differences between women with and without GDM, with regard to maternal age, gestational age at recruitment, weight, height, BMI and values of hemoglobin, serum iron, serum ferritin, TIBC, transferrin saturation and serum CRP.

2. Outcomes of deliveries, gestational age at delivery and birth weight of the babies were similar in both groups.

3. The iron-related parameters measured in blood correlated with one another.

4. Fasting plasma glucose levels showed significant positive correlation with insulin levels and HOMA-IR.

5. Fasting plasma glucose levels showed significant negative correlation with serum iron and transferrin saturation. Plasma glucose 1 hour after glucose load showed significant negative correlation with TIBC. Plasma glucose 2 hour after glucose load showed significant positive correlation with hemoglobin.

DISCUSSION

In this prospective case control study, primigravidae who were referred for an OGTT at 24-28 weeks of gestation were recruited. Based on the results of the OGTT, they were categorized into those with GDM and those without (control subjects). Twenty-five subjects were studied in each group.

The hypothesis postulated by the present study was that iron-related parameters in blood would correlate with insulin resistance in women with gestational diabetes mellitus. This was based on studies that have suggested that iron may be implicated in the pathogenesis of diabetes mellitus (Beutler et al., 2003; Wilson et al., 2003). Studies have shown that increased body iron stores (as indicated by elevated serum ferritin concentrations) were associated with increased risk of developing diabetes mellitus (Forouhi et al., 2007; Rajpathak et al., 2006; Wrede et al., 2006). Iron overload has been shown to be toxic to beta-cells of the pancreas, resulting in decreased insulin secretion and impaired glucose metabolism (Bowers et al., 2016; Liu et al., 2009). In pregnancy, iron excess has been suggested to be involved in the development of GDM (Lao et al., 2001). Studies have also shown an association between raised haemoglobin levels and increased risk of GDM (Lao et al., 2001; Tan et al., 2011). Patients with GDM were reported to have increased iron and decreased insulin levels (Kaygusuz et al., 2013). Iron has also been shown to affect insulin

synthesis in the pancreas, due to its pro-oxidant property (Rajpathak et al., 2009; Fernández-Real et al., 2002; Lenzen, 2008). An increase in insulin resistance in pregnancy has been reported to be associated with increased risk of GDM (Zhang et al., 2014).

Ferritin is the most studied biomarker of iron in relation to GDM (Rawal et al., 2017). It is the major storage form of iron, and its serum levels are an indicator of body iron stores because it correlates with the bone marrow iron (Kaneshige, 1981; Lipschitz et al., 1974). Increased levels of ferritin have been reported in chronic inflammatory conditions, as it is also a positive acute-phase reactant (Harrison and Arosio, 1996; Kalantar-Zadeh et al., 2004). GDM is accompanied by inflammation, as assessed by an increase in markers of inflammation such as C-reactive protein (CRP) (Qiu et al., 2004; Wolf et al., 2003). In normal pregnancy, serum ferritin levels have been reported to highest in the second trimester (12- 16 weeks); it falls by the third trimester (Fenton et al., 1977; Kaneshige, 1981; Romslo et al., 1983). In those with GDM, maternal serum ferritin levels have been reported to higher (even in the third trimester) than in control subjects (Lao et al., 2001; Lao and Tam, 1997).

In the present study, no differences were found in serum ferritin levels in those with GDM and in control subjects. Similar results have been reported before (Maitland et

al., 2014; Zein et al., 2015). Maitland et al (2014) conducted a randomized control trial to study whether obesity was a risk factor in the development of GDM. They recruited pregnant women between 15 and17 weeks of gestation (n=106). Of these, 27.4% developed GDM, as diagnosed by the criteria of the International Association of Diabetes and Pregnancy Study Groups Consensus Panel (IADPSG, 2010). Most parameters they studied (lipid profile, hsCRP, AST, ALT, ferritin, fructosamine, insulin, tissue plasminogen activator, interleukin 6, visfatin and leptin) were similar in women with and without GDM except for adiponectin, which was found to be lower in those with GDM. Zein et al (2015) recruited women at gestational ages less than 12 weeks (n =104). They were divided into 4 groups based on the subjects' serum ferritin levels at recruitment (<13μg/L, 13 to 21.55μg/L, 21.55 to 38.5μg/L and $\geq$ 38.5μg/L). Subjects in the 4 groups did not differ in terms of age, BMI, gestational age Hb, CRP and fasting plasma glucose (FPG) at time of recruitment. Dietary iron intake was found to be higher in the group with highest serum ferritin values, as assessed by a food frequency questionnaire. Of the subjects in that study, 15.4% developed GDM, as per IADPSG criteria (2010). Mean values for BMI, hemoglobin, FPG, and glucose levels post 1-h OGTT were similar in the 4 groups. The 2-h OGTT values were significantly higher in the group with the highest serum ferritin concentrations (at recruitment) than in the other 3 groups. At 24- 28 weeks (at the time of the OGTT) the subjects in the study were categorized into 3 groups, based on

ferritin values at this point as follows: <8μg/L, 8 to15μg/L, and ≥ 15μg/L. Values for FPG and 1-h OGTT values were found to be similar in the 3 groups. The 2-h OGTT values were significantly higher in the group with the highest serum ferritin concentrations. However, no association was found between high ferritin concentrations and incidence of GDM.

Other studies have reported associations between serum ferritin and increased risk of GDM (Amiri et al., 2013; Bowers et al., 2016; Chen et al., 2006; Yadav et al., 2017). In 2 of these papers, statistical analyses had adjusted for serum CRP concentrations to confirm that the elevated ferritin levels were due to increased iron stores and not due to inflammation (Bowers et al., 2016; Chen et al., 2006). Hence, these 2 studies showed that the increased serum ferritin levels observed were indicative of increased body iron stores, whereas in the other 2 studies (Amiri et al., 2013; Yadav et al., 2017) the possible effect of inflammation on serum ferritin levels was not ruled out.

The following table compares the findings of the present study with those by Chen et al (2006), Amiri et al (2013), Bowers et al (2016), Yadav et al (2017), Derbent et al (2013), Lao et al (2001), Soheilykhah et al (2017) and Tan et al (2011).

	Gestational age at which blood sample was collected for estimation of iron-related parameters	**Sample size**	**Population studied**	**Diagnosis of GDM based on**
Present study	24 - 28 weeks	50 (25 with GDM and 25 without)	Indian	One abnormal value of either fasting plasma glucose or 1 hour or 2 hours plasma glucose, post 75 gm glucose load (American Diabetic Association, 2020)
Study by Chen et al (2006)	15.6 $\pm$ 0.1 weeks	1456 subjects (45 with GDM and 1411 without)	American	Glucose challenge test (GCT) with 50 gm of glucose load. Those who tested positive on the GCT underwent an OGTT. Two abnormal values of the OGTT - fasting plasma glucose or 1 hour, 2 hours and 3 hours glucose levels, after a 100gm glucose

				load confirmed the diagnosis of GDM (Carpenter and Coustan, 1982)
Study by Amiri et al (2013)	24 - 28 weeks	200 subjects (100 with GDM and 100 without)	Iranian	Glucose challenge test (GCT) with 50 gm of glucose load. Those who tested positive on the GCT underwent an OGTT. Two abnormal values of the OGTT - fasting plasma glucose or 1 hour, 2 hours and 3 hours glucose levels, after a 100gm glucose load confirmed the diagnosis of GDM (Carpenter and Coustan, 1982)
Study by Bowers et al (2016)	9.4 $\pm$ 3.2 weeks	350 with GDM and 349 without	Danish	One abnormal value of either fasting plasma glucose or 2 hours plasma glucose, post 75 gm glucose load (World Health Organization, 1999)

Study by Yadav et al (2017)	28 weeks	90 subjects –(30 with GDM and 60 without)	Indian	Glucose challenge test (GCT) with 50 gm of glucose load. Those who tested positive on the GCT underwent an OGTT. Two abnormal values of the OGTT - fasting plasma glucose or 1 hour, 2 hours and 3 hours glucose levels, after a 100gm glucose load confirmed the diagnosis of GDM (Carpenter and Coustan, 1982)
Study by Derbent et al (2013)	24 - 28 weeks	30 with GDM, 72 without GDM	Turkish	Glucose challenge test (GCT) with 50 gm of glucose load. Those who tested positive on the GCT underwent an OGTT. Two abnormal values of the OGTT - fasting plasma glucose or 1 hour, 2 hours and 3 hours glucose levels, after a 100gm glucose load confirmed the diagnosis of

				GDM (Carpenter and Coustan, 1982)
Study by Lao et al (2001)	28 - 31 weeks	97 with GDM, 194 controls	Chinese	One abnormal value of either fasting plasma glucose or 2 hours plasma glucose post 75 gm glucose load ("WHO Expert Committee on Diabetes Mellitus," 1980).
Study by Soheilykhah et al (2017)	12-16 weeks	1384 subjects (306 with GDM, 1078 without GDM)	Iranian	One abnormal value of either fasting plasma glucose or 1 hour or 2 hours plasma glucose, post 75 gm glucose load (American Diabetic Association, 2020)
Study by Tan et al (2011)	$\leq$ 26 weeks	1538 subjects (182 with GDM, 1356	Malaysian	Glucose challenge test (GCT) with 50 gm of glucose load. Those who tested positive on the GCT underwent an OGTT. One abnormal value of either fasting

		without GDM)		plasma glucose or 2 hours plasma glucose, post 75 gm glucose load (World Health Organization, 1999)

As can be seen from the information summarized in the table above, the various studies shown differed in many methodological details. These include the gestational ages at which the blood samples were taken for estimation of iron-related parameters, the ethnicities of the subjects studied and the criteria used for diagnosis of GDM. Some of the methodological features are similar to one another. For example, the study by Derbent et al (2013) employed methodology and diagnostic criteria similar to those used by Chen at al (2016), Amiri et al (2013) and Yadav et al (2017), while Lao et al (2001) used methodology similar to the present study. The OGTTs in all the studies above were carried out between 24 to 28 weeks of gestation. Most of the above studies differed from the present one in the diagnostic criteria to diagnose GDM and in the gestational ages at which the estimations for iron-related parameters were carried out. These differences make it difficult to directly compare the results of these parameters in these studies with the present one.

Studies have suggested the involvement of high body iron stores in the pathogenesis of glucose intolerance (Cheng et al., 2020; Zein et al., 2015). A significant positive correlation was observed between serum ferritin concentrations in the first trimester and plasma glucose values 1-h after the glucose load in an OGTT ($r=0.208$, $p= 0.045$) and with 2-h OGTT values ($r=0.428$, $p <0.001$), and also between ferritin levels at mid-pregnancy with 2-h OGTT values ($r=0.245$, $p= 0.018$) (Zein et al, 2015). Cheng et al (2020) have reported similar findings of a significant positive association between serum ferritin and 1-h OGTT values ($r= 0.087$, $P = 0.011$).

The present study did not find any correlation between serum ferritin and glucose levels. However, it showed that serum iron and transferrin saturation correlated negatively with fasting plasma glucose and that TIBC correlated negatively with plasma glucose 1 hour after the glucose load. It also showed a significant positive correlation between hemoglobin and plasma glucose levels 2 hour after the glucose load. Other studies have reported an association between increased hemoglobin levels and risk of development of GDM (Lao et al., 2001; Tan et al., 2011). In these studies, iron-related parameters (such as serum iron, total iron-binding capacity and transferrin saturation) did not differ between the 2 groups, as observed in the present study. Similar findings have been reported in other studies as well (Amiri et al., 2013; Soheilykhah et al., 2017). It has been reported in a study done on individuals with

impaired glucose tolerance that insulin secretion and resistance was associated with plasma glucose levels; the association was stronger with the 1 h glucose levels post-OGTT than with fasting plasma glucose concentrations and 2-h glucose levels post OGTT (Abdul-Ghani et al., 2008).

Another important biomarker of iron is hepcidin. It is the central regulator of systemic iron homeostasis (Nemeth et al., 2003). It regulates iron homeostasis by binding to ferroportin, and causing it to undergo degradation (Nemeth et al., 2004). Studies have shown strong associations between serum hepcidin levels and risk of GDM (Derbent et al., 2013; Rawal et al., 2017). It was not possible to estimate hepcidin levels in blood in the present study, due to financial and logistical constraints. Hence, data is not available to make comparisons with the above published studies.

The mean ages of the subjects in the present study were similar in both the groups. Other studies (Derbent et al., 2013; Lao et al., 2001) have reported that women with GDM were older than those without. All the subjects in the present study were on iron and folic acid supplements. This was similar to the study by Lao et al (2001), whereas subjects in the study by Derbent et al (2013) were specifically stated as not being on iron or folic acid supplements.

Women who were obese have been reported to have a higher incidence of GDM (6–12%) than those who were not (2–4%); the risk of developing GDM was found to directly proportional to maternal body mass index (Ehrenberg et al., 2002; Gross et al., 1980). Studies have shown that women with BMI $\geq$ 30 have a 2.9-fold increased risk of developing GDM as compared to women with BMI $\leq$ 20 (Solomon et al., 1997). There was no difference in the BMI between the two groups in the present study. This finding was similar to Lao et al (2001), while Derbent et al (2013) showed that BMI values of women with GDM were significantly higher than those of women without GDM.

In the present study, parameters related to outcomes of pregnancy, such as mean gestational age at delivery and birth weights of the newborns, were similar in 2 groups. This is in contrast with studies which have shown women with GDM had adverse outcomes of pregnancies, such as preterm birth, abnormal fetal development (macrosomia) (Mayor, 2017; Yang et al., 2002). The fact that the number of subjects in the present study was small may account for the differences in observations.

CONCLUSION

In the present study, no significant differences in iron-related parameters were found in women with and without GDM. Serum iron and transferrin saturation correlated negatively with fasting plasma glucose and TIBC correlated negatively with plasma glucose 1 hour after glucose load. No correlation was found between ferritin and glucose levels.

LIMITATIONS OF THIS STUDY

The sample size in the present study was small. It was not possible to study more subjects due to financial and logistical constraints (due to the pandemic).

This study was unable to reliably document details of iron supplementation in the 2 groups. This is a factor likely to affect the iron status in the subjects.

Estimation of serum hepcidin may have provided additional information on the iron status of the subjects. However, it was not possible to carry out this estimation because of financial constraints and delays in procuring reagents (due to the pandemic).

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APPENDIX 1- IRB APPROVAL LETTER

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., Dr. Min (clinical)
Director, Christian Counselling Center
Chairperson, Ethics Committee

Dr. Anna Benjamin Pulimood, MD., Ph.D.,
Chairperson, Research Committee, Principal

Dr. Suceena Alexander, MD., DM., FASN.,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

February 09, 2021.

Dr. Lois Sara James,
PG Registrar,
Department of Biochemistry,
Christian Medical College,
Vellore – 632 004.

Sub: Fluid Research Grant: New Proposal:
Is there a link between iron-related parameters and insulin resistance in gestational diabetes mellitus?
Dr. Lois Sara James (Emp. No. 81702), PG Registrar, Biochemistry, Dr. Molly Jacob (Emp. No. 14509), Biochemistry, Dr. Joe Varghese (Emp. No. 20405), Dr. Padmanaban Venkatesan (Emp. No. 33341), Biochemistry, Dr. Jasmine Prasad (Emp. No. 20080), Community Health, DR. Anne George, Community Health, Dr. Thenmozhi Mani, Biostatistics.

Ref: IRB Min. No. 13458 [OBSERVE] dated 05.10.2020.

Dear Dr. Lois Sara James,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Suceena Alexander, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Suceena Alexander
Secretary (Ethics Committee)
Institutional Review Board

Dr. Suceena Alexander, MD.,DM.,FASN.
Secretary - (Ethics Committee)
Institutional Review Board
Christian Medical College,
Vellore - 632 002, Tamil Nadu, India.

CC: Dr. Molly Jacob, Department of Biochemistry, CMC

Ethics Committee Blue, Office of Research, I Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284508 Fax: 0416 – 2262788 E-mail: research@cmcvellore.ac.in

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

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Ref: IRB Min. No. 13458 [OBSERVE] dated 05.10.2020.

Dear Dr. Lois Sara James,

The Institutional Review Board **(Blue,** Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Is there a link between iron-related parameters and insulin resistance in gestational diabetes mellitus?" on October 05, 2020.

The Committee review the Following Documents:

1) IRB Application Format
2) Proforma
3) Patient information sheet and Consent Form (Tamil, English, Hini)
4) Cvs. Of Drs. Jasmin, Joe, Lois, Molly, Padmanabhan, Thenmozhi, Anne George.
5) No. of Documents 1 -4.

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on October 05, 2020 in the New IRB Room, Christian Medical College, Vellore 632 004.

Ethics Committee Blue, Office of Research, I Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
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Dr. B.J. Prashantham, M.A., Dr. Min (clinical)
Director, Christian Counselling Center
Chairperson, Ethics Committee

Dr. Anna Benjamin Pulimood, MD., Ph.D.,
Chairperson, Research Committee, Principal

Dr. Suceena Alexander, MD., DM., FASN.,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. B. J. Prashantham	MA (Counseling Psychology), MA(Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Suceena Alexander	MD., DM., FASN	Secretary – (Ethics Committee), IRB, Addl. Vice Principal (Research), Professor of Nephrology, CMC, Vellore	Internal Clinician
Dr. Jayaprakash Muliyil	BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, Vellore	External, Scientist & Epidemiologist
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil,BL.	Sr. Legal Officer, Vellore	External Legal Expert
Rev. Rainard Pearson	BA., B. Th., M. Div.,	Sr. Chaplin, CMC, Vellore.	Internal, Social Scientist
Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician
Dr. Barney Isaac	DNB (Respiratory Diseases)	Associate Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person

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Dr. Suceena Alexander, MD., DM., FASN.,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Joe Varghese	MBBS, MD Biochemistry	Professor, Department of Biochemistry	Internal Clinician
Dr. Balu Krishna	MBBS MD DNB DMRT	Professor, Department of Radiotherapy, CMC Vellore	Internal Clinician
Dr. Rohin Mittal	MS , DNB	Professor, Department of General Surgery, CMC Vellore	Internal Clinician
Dr. Shyam Kumar NK	DMRD, DNB, FRCR, FRANZCR	Professor, Radiology, CMC, Vellore	Internal, Clinician
Dr. Santosh Varughese	MBBS, MD	Professor, Nephrology, CMC, Vellore	Internal, Clinician
Dr. HS. Asha	MBBS, DNB	Professor, Department of Endocrinology, CMC Vellore	Internal Clinician
Dr. Ekta Rai	MD, MRCA	Professor, Head of the Unit 5, Department of Anaesthesia, CMC, Vellore	Internal, Clinician
Dr. Rekha Pai	MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal, Basic Medical Scientist
Dr. Winsely Rose	MD (Paed)	Professor, Paediatrics, CMC Vellore	Internal, Clinician
Dr. Premila Abraham	M.Sc. Ph.D	Professor, Department of Biochemistry, CMC, Vellore	Internal Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of Withdrawals for the study entitled: "Is there a link between iron-related parameters and insulin resistance in gestational diabetes mellitus?" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

The Institutional Ethics Committee expects to be informed about the progress of the project, Any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are

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Additional Vice-Principal (Research)

expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Fluid Grant Allocation:
A sum of 3,00,000/- INR (Rupees Three Lakh Only) will be granted for 2 years. 1,50,000/- INR (Rupees One Lakh fifty Thousand only) will be granted for 12 months as an 1st Installment. The rest of the 1,50,000/- INR (Rupees One Lakh fifty Thousand only) each will be released at the end of the first year as 2 nd Installment.

Yours sincerely,

Dr. Suceena Alexander
Secretary (Ethics Committee)
Institutional Review Board

Dr. Suceena Alexander, MD.,DM.,FASN.
Secretary - (Ethics Committee)
Institutional Review Board
Christian Medical College,
Vellore - 632 002, Tamil Nadu, India.

IRB Min. No. 13458 [OBSERVE] dated 05.10.2020

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APPENDIX 2- INFORMATION SHEET AND CONSENT FORM

INFORMATION SHEET FOR STUDY PARTICIPANTS

Departments of Biochemistry and Community Medicine, CMC, Vellore

Title of project: Is there a link between iron-related parameters and insulin resistance in gestational diabetes mellitus?

Date:

This is a study to be carried out by the Departments of Biochemistry and Community Medicine in CMC, Vellore. The aim of the study is to see if there is a connection between iron levels in blood and insulin resistance, in women who become diabetic in pregnancy (gestational diabetes mellitus).

This study will help provide doctors with a better understanding of the link between iron and insulin resistance in gestational diabetes mellitus.

For the purpose of this study, we need 6 ml of blood sample from each subject, which will be collected at the same time when blood is drawn for your glucose tolerance test. We would like to know if you are willing to participate in the study and provide a blood sample for it.

The collection of blood will not cause any harm to you or your baby. Blood samples collected will be used only for research purposes. If there is any sample remaining after this study is complete, we would like your permission for the samples to be stored and used for related studies in the future.

All information provided by you will be kept confidential.

You may not benefit directly by participation in this study. Participation in the study does not entitle you to concession or any other special treatment. You have to pay for all the routine tests that the doctor might ask you to do as part of your antenatal health check-up. However, you need not pay for the tests done as part of this study.

If you are not willing to participate in the study, you are free to do so. It will not, in any way, affect the treatment you will receive in the hospital.

For your further queries, please contact us on the numbers given below.

Dr. Lois Sara James, PG Registrar, Department of Biochemistry, CMC, Vellore, Contact number: 8860164833
Dr. Molly Jacob, Professor, Department of Biochemistry, CMC, Vellore, Contact number: 0416 – 2284267
Dr. Joe Varghese, Professor, Department of Biochemistry, CMC, Vellore, Contact number: 0416 – 2284267

Departments of Biochemistry & Community Medicine, Christian Medical College, Vellore.

Informed consent form for subjects

Study number:

Study title: Is there a link between iron-related parameters and insulin resistance in gestational diabetes mellitus?

Subject's full name:

Date of birth/age:

I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

I understand that when I participate in the study, a blood sample (6 mL) will be collected from me. [ ]

I understand that the blood sample collected will be used only for research purposes. If there is any sample remaining after this study is completed, I give permission for the sample to be stored and used for related studies in the future. [ ]

I understand that the ethics committee and the regulatory authorities will not need my permission to look at my health records, both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

I agree not to restrict the use of any data or results that arise from this study provided that such a use is only for scientific purpose(s). [ ]

I agree to take part in the above study. [ ]

Signature (or thumb impression) of the subject/legally acceptable

Date: _____/_____/_____

Signatory's name: _____

Signature of the investigator: _____

Date: _____/_____/_____

Study investigator's Name: _____

Signature or thumb impression of the witness: _____

Date: _____/_____/_____

Name & address of the witness: _____

ஆய்வு பங்கேற்பாளர்களுக்கான தகவல் தாள்

உயிர் வேதியியல் மற்றும் சமூக மருத்துவத் துறை, சி.எம்.சி, வேலூர்

ஆய்வு தலைப்பு : கர்ப்பகால நீரிழிவு நோயில் இரும்பு தொடர்பான அளவுகளுக்கும் இன்சுலின் எதிர்ப்புக்கும் தொடர்பு இருக்கிறதா?

தேதி :

வேலூரில் உள்ள சி.எம்.சி.யில் உள்ள உயிர் வேதியியல் மற்றும் சமூக மருத்துவத் துறைகளால் மேற்கொள்ளப்படும் ஆய்வு இது. கர்ப்பகால நீரிழிவு நோயில் இரத்தத்தில் இரும்பு அளவிற்கும் இன்சுலின் எதிர்ப்பிற்கும் தொடர்பு இருக்கிறதா என்று பார்ப்பதே ஆய்வின் நோக்கம்.

கர்ப்பகால நீரிழிவு நோயில் இரும்புக்கும் இன்சுலின் எதிர்ப்பிற்கும் உள்ள தொடர்பைப் பற்றி மருத்துவர்களுக்கு நன்கு புரிந்துகொள்ள இந்த ஆய்வு உதவும்.

இந்த ஆய்வின் நோக்கத்திற்காக, உங்களிடம் இருந்து எங்களுக்கு 6 மில்லி இரத்த மாதிரி தேவைப்படுகிறது, இது உங்கள் குளுக்கோஸ் சகிப்புத்தன்மை சோதனைக்கு இரத்தம் எடுக்கப்படும் அதே நேரத்தில் சேகரிக்கப்படும். நீங்கள் ஆய்வில் பங்கேற்க தயாராக இருக்கிறீர்களா என்பதை அறிய விரும்புகிறோம்.

இரத்த சேகரிப்பால் உங்களுக்கோ அல்லது உங்கள் குழந்தைக்கோ எந்தத் தீங்கும் ஏற்படாது. சேகரிக்கப்பட்ட இரத்த மாதிரிகள் ஆராய்ச்சி நோக்கங்களுக்காக மட்டுமே பயன்படுத்தப்படும். இந்த ஆய்வு முடிந்தபிறகு ஏதேனும் மாதிரி இருந்தால், எதிர்காலத்தில் மாதிரிகள் சேமிக்கப்பட்டு தொடர்புடைய ஆய்வுகளுக்கு பயன்படுத்த உங்கள் அனுமதியை நாங்கள் விரும்புகிறோம்.

நீங்கள் வழங்கிய அனைத்து தகவல்களும் ரகசியமாக வைக்கப்படும்.

இந்த ஆய்வில் பங்கேற்பதனால் உங்களுக்கு எந்த நேரடி பயனும் கிடையாது. ஆய்வில் பங்கேற்பதனால் உங்களுக்கு எந்தவித சிறப்பு சலுகைகளும் அளிக்கப்படமாட்டாது. உங்கள் கர்ப்பகால சுகாதார பரிசோதனையின் ஒரு பகுதியாக மருத்துவர் கேட்கும் அனைத்து வழக்கமான சோதனைகளுக்கும் நீங்கள் பணம் செலுத்த வேண்டும். இருப்பினும், இந்த ஆய்வின் ஒரு பகுதியாக மேற்கொள்ளப்பட்ட சோதனைகளுக்கு நீங்கள் பணம் செலுத்த வேண்டியதில்லை.

நீங்கள் ஆய்வில் பங்கேற்க விரும்பவில்லை என்றால், நீங்கள் அவ்வாறு செய்ய சுதந்திரமாக இருக்கிறீர்கள். இது எந்த வகையிலும், நீங்கள் மருத்துவமனையில் பெறும் சிகிச்சையை பாதிக்காது.

உங்கள் மேலும் கேள்விகளுக்கு, கீழே கொடுக்கப்பட்டுள்ள எண்களில் எங்களை தொடர்பு கொள்ளவும்.

டாக்டர் லோயிஸ் சாரா ஜேம்ஸ், பி.ஜி. பதிவாளர், உயிர் வேதியியல் துறை, சி.எம்.சி, வேலூர், தொடர்பு எண்: 8860164833

டாக்டர் மோலி ஜேக்கப், பேராசிரியர், உயிர் வேதியியல் துறை, சி.எம்.சி, வேலூர், தொடர்பு எண்: 0416 - 2284267

டாக்டர் ஜோ வர்கீஸ், பேராசிரியர், உயிர் வேதியியல் துறை, சி.எம்.சி, வேலூர், தொடர்பு எண்: 0416 - 2284267

உயிர் வேதியியல் மற்றும் சமூக மருத்துவத் துறைகள்,

கிறிஸ்டியன் மருத்துவக் கல்லூரி, வேலூர்.

ஆய்வு எண்.

பங்கேற்பாளர்க்கான தகவலறிந்த ஒப்புதல் படிவம்

ஆய்வு தலைப்பு: கர்ப்பகால நீரிழிவு நோயில் இரும்பு தொடர்பான அளவுருக்கள் மற்றும் இன்சுலின் எதிர்ப்பு ஆகியவற்றுக்கு தொடர்பு இருக்கிறதா?

பங்கேற்பாளரின் முழு பெயர்:

பிறந்த தேதி / வயது:

மேற்கண்ட ஆய்வுக்கு ..... தேதியிட்ட தகவல் தாளை நான் படித்து புரிந்து கொண்டேன் என்பதையும் கேள்விகளைக் கேட்கும் வாய்ப்பையும் பெற்றேன் என்பதை உறுதிப்படுத்துகிறேன். [ ]

ஆய்வில் நான் பங்கேற்பது தன்னார்வமானது என்பதையும், எந்த காரணமும் தெரிவிக்காமல், எனது மருத்துவ கவனிப்பு அல்லது சட்ட உரிமைகள் பாதிக்கப்படாமலும், எந்த நேரத்திலும் ஆய்வில் இருந்து விலக சுதந்திரமாக இருக்கிறேன் என்பதையும் நான் புரிந்துகொள்கிறேன்.[ ]

நான் ஆய்வில் பங்கேற்கும்போது, என்னிடமிருந்து ஒரு இரத்த மாதிரி (6 எம்.எல்) சேகரிக்கப்படும் என்பதை நான் புரிந்துகொள்கிறேன். [ ]

சேகரிக்கப்பட்ட இரத்த மாதிரி ஆராய்ச்சி நோக்கங்களுக்காக மட்டுமே பயன்படுத்தப்படும் என்பதை நான் புரிந்துகொள்கிறேன். இந்த ஆய்வு முடிந்தபின் மீதமுள்ள மாதிரி ஏதேனும் இருந்தால், எதிர்காலத்தில் மாதிரியை சேமித்து இதுபோன்ற ஆய்வுகளுக்குப் பயன்படுத்த நான் அனுமதி அளிக்கிறேன். [ ]

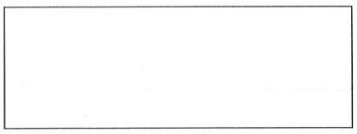
நான் ஆய்வில் இருந்து விலகினாலும், தற்போதைய ஆய்வு மற்றும் அது தொடர்பாக மேற்கொள்ளப்படக்கூடிய எந்தவொரு ஆராய்ச்சியையும் பொறுத்தவரை, எனது சுகாதார பதிவுகளைப் பார்க்க நெறிமுறைக் குழு மற்றும் ஒழுங்குமுறை அதிகாரிகளுக்கு எனது அனுமதி தேவையில்லை என்பதை நான் புரிந்துகொள்கிறேன். இதை நான் ஒப்புக்கொள்கிறேன். இருப்பினும், மூன்றாம் தரப்பினருக்கு வெளியிடப்படயிருக்கும் எந்தவொரு தகவலிலும் எனது அடையாளம் வெளிப்படுத்தப்படாது என்பதை நான் புரிந்துகொள்கிறேன். [ ]

இந்த ஆய்விலிருந்து எழும் எந்தவொரு தரவையும் அல்லது முடிவுகளையும் பயன்படுத்துவதை தடுக்கமாட்டேன் என்று நான் ஒப்புக்கொள்கிறேன், அத்தகைய பயன்பாடு அறிவியல் நோக்கத்திற்காக மட்டுமே. [ ]

மேற்கண்ட ஆய்வில் பங்கேற்க ஒப்புக்கொள்கிறேன். [ ]

பங்கேற்பாளர் கையொப்பம் (அல்லது கட்டைவிரல் ரேகை)/ சட்டப்படி ஏற்றுக்கொள்ளத்தக்கது

தேதி: _____/_____/_____



கையொப்பமிட்டவரின் பெயர்: .....

புலனாய்வாளரின் கையொப்பம்: .....

தேதி: _____/_____/_____

ஆய்வு ஆய்வாளரின் பெயர்: .....

சாட்சியின் கையொப்பம் அல்லது கட்டைவிரல் எண்ணம்: .....

தேதி: _____/_____/_____

சாட்சியின் பெயர் & முகவரி: .....

अध्ययन के लिए प्रतिभागी सूचना पत्र

बायोकेमिस्ट्री विभाग और सामुदायिक चिकित्सा विभाग, सीएमसी, वेल्लोर

परियोजना का शीर्षक: क्या गर्भावधि मधुमेह की बीमारी में लोहे से संबंधित मापदंडों और इंसुलिन प्रतिरोध के बीच एक संबंध है?

तारीख:

यह सीएमसी, वेल्लोर में जैव रसायन और सामुदायिक चिकित्सा विभाग द्वारा किया जाने वाला एक अध्ययन है। अध्ययन का उद्देश्य यह देखना है कि क्या गर्भावस्था में रक्त और इंसुलिन प्रतिरोध में आयरन के स्तर के बीच कोई संबंध है, जो गर्भावस्था के मधुमेह (जेस्टेशनल डायबिटीज) में हो जाता है।

यह अध्ययन डॉक्टरों को गर्भावधि के मधुमेह (जेस्टेशनल मेलेटस) में आयरन और इंसुलिन प्रतिरोध के बीच लिंक की बेहतर समझ प्रदान करने में मदद करेगा।

इस अध्ययन के उद्देश्य के लिए हमें प्रत्येक प्रतिभागी से 6 मिलीलीटर रक्त के नमूने की आवश्यकता होती है, जिसे उसी समय एकत्र किया जाएगा, जब रक्त आपके ग्लूकोज सहिष्णुता परीक्षण के लिए तैयार हो। हम जानना चाहेंगे कि क्या आप अध्ययन में भाग लेने के लिए तैयार हैं और इसके लिए रक्त का नमूना उपलब्ध कराना चाहते हैं।

रक्त के संग्रह से आपको या आपके बच्चे को कोई नुकसान नहीं होगा। एकत्र किए गए रक्त के नमूनों का उपयोग केवल अनुसंधान उद्देश्यों के लिए किया जाएगा। यदि इस अध्ययन के पूरा होने के बाद कोई नमूना शेष है, तो हम चाहेंगे कि आपके नमूने भविष्य में संबंधित अध्ययन के लिए संग्रहीत और उपयोग किए जाएं।

आपके द्वारा दी गई सभी जानकारी को गोपनीय रखा जाएगा।

इस अध्ययन में भाग लेने से आपको सीधे लाभ नहीं हो सकता है। अध्ययन में भागीदारी आपको रियायत या किसी अन्य विशेष उपचार का अधिकार नहीं देती है। आपको उन सभी नियमित परीक्षणों के लिए भुगतान करना होगा जो डॉक्टर आपको अपने प्रसवपूर्व स्वास्थ्य जांच के हिस्से के रूप में करने के लिए कह सकते हैं। हालांकि, आपको इस अध्ययन के हिस्से के रूप में किए गए परीक्षणों के लिए भुगतान करने की आवश्यकता नहीं है।

यदि आप अध्ययन में भाग लेने के इच्छुक नहीं हैं, तो आप ऐसा करने के लिए स्वतंत्र हैं। यह किसी भी तरह से, आपके द्वारा अस्पताल में प्राप्त उपचार को प्रभावित नहीं करेगा।

अपने आगे के प्रश्नों के लिए, कृपया नीचे दिए गए नंबरों पर हमसे संपर्क करें।

डॉ. लोइस सारा जेम्स, पीजी रजिस्ट्रार, बायोकेमिस्ट्री विभाग, सीएमसी, वेल्लोर, संपर्क नंबर: 8860164833

डॉ. मौली जैकब, प्रोफेसर, बायोकेमिस्ट्री विभाग, सीएमसी, वेल्लोर, संपर्क नंबर: 0416 – 2284267

डॉ. जो वर्गीस, प्रोफेसर, बायोकेमिस्ट्री विभाग, सीएमसी, वेल्लोर, संपर्क नंबर: 0416 – 2284267

बायोकेमिस्ट्री और सामुदायिक चिकित्सा विभाग, क्रिश्चियन मेडिकल कॉलेज, वेल्लोर

अनुसंधान के प्रतिभागियों के लिए सूचित सहमति प्रपत्र

अध्ययन संख्या:

अध्ययन का शीर्षक: क्या गर्भावधि तथा मधुमेह की बीमारी में आयरन संबंधित मापदंडों और इंसुलिन प्रतिरोध के बीच संबंध है?

प्रतिभागी का नाम:

जन्म तिथि / आयु:

मैं पुष्टि करती हूं कि मैंने उपरोक्त अध्ययन के लिए दिनांक _____ की सूचना पत्र को पढ़ा और समझा है और मुझे प्रश्न पूछने का अवसर मिला है। [ ]

मैं समझती हूं कि अध्ययन में मेरी भागीदारी स्वैच्छिक है और मैं किसी भी समय मेरी चिकित्सा देखभाल या कानूनी अधिकारों को प्रभावित किए बिना, बिना किसी कारण के, नाम वापस लेने के लिए स्वतंत्र हूं। [ ]

मैं समझता हूं कि जब मैं अध्ययन में भाग लूंगा, तो मुझसे रक्त का नमूना (6 एमएल) एकत्र किया जाएगा। [ ]

मैं समझती हूं कि एकत्र किए गए रक्त के नमूनों का उपयोग केवल अनुसंधान उद्देश्यों के लिए किया जाएगा। यदि इस अध्ययन के पूरा होने के बाद कोई नमूना शेष है, तो मैं नमूनों को संग्रहीत करने और भविष्य में संबंधित अध्ययन के लिए उपयोग करने की अनुमति देती हूं। [ ]

मैं समझती हूं कि वर्तमान अध्ययन और किसी भी आगे के संबंध में, मेरे स्वास्थ्य रिकॉर्ड को देखने की अनुमति, अनुसंधान जो इसके संबंध में आयोजित किया जा सकता है, भले ही मैं परीक्षण से वापस ले लूं, मैं इसके लिए सहमत हूं। हालाँकि, मैं समझती हूँ कि तीसरे पक्ष या प्रकाशित करने के लिए जारी की गई किसी भी जानकारी में मेरी पहचान उजागर नहीं की जाएगी। [ ]

मैं सहमत हूं कि इस अध्ययन से उत्पन्न किसी भी डेटा या परिणामों के केवल वैज्ञानिक उद्देश्य के लिए उपयोग को प्रतिबंधित नहीं किया गया है। [ ]

मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं। [ ]

प्रतिभागी / कानूनी रूप से स्वीकार्य हस्ताक्षर (या अंगूठे का निशान)
दिनांक ___/___/_____

हस्ताक्षरकर्ता का नाम::_____

अन्वेषक के हस्ताक्षर:
दिनांक ___/___/_____

अध्ययन अन्वेषक का नाम::_____

साक्षी का हस्ताक्षर या अंगूठे का निशान::

दिनांक ___/___/_____

गवाह का नाम और पता::_____

APPENDIX 3- PARTICIPANT'S PROFORMA

Proforma for patient's details

Departments of Biochemistry and Community Medicine, Christian Medical College, Vellore-632 002

Study title: Is there a link between iron-related parameters and insulin resistance in gestational diabetes mellitus?

Information collected by: **Date:**

Subject's full name:

Date of birth /age:

Hospital number:

Address with phone number:

Occupation:

Name of husband:

Occupation:

Gestational age on day of recruitment (as per LMP):

Last menstrual period (LMP):

Expected date of delivery:

History of any previous pregnancy/abortion/miscarriages: Yes/No

If yes, brief details:

Any previous illnesses/current illnesses:

On any medications currently (including traditional medicines): Yes/No
If yes, brief details

On iron and folic acid supplements: Yes/No

How regular in taking these supplements:

On examination

Height: Weight:

Mid arm Circumference:

Pulse rate: BP:

Pallor: Pedal edema:

Urine for glucose and protein:

Results of laboratory tests
Haemoglobin/haematocrit:

Results of oral glucose tolerance test: plasma glucose (mg/dL)
Fasting:
1-hour post OGTT:
2 hours post OGTT:

Biochemical parameters of interest in the study

Investigations	Results
Ferritin	
Iron, TIBC, transferrin saturation	
Hepcidin	
Glucose	
Insulin	
hs-CRP	

Outcomes of pregnancy
BP at time of admission for delivery:

Any pregnancy-related complication that developed:

Gestational age at delivery:

Mode of delivery:

Sex of baby:

Birth weight:

Length of baby:

APGAR score: