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ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Assessment of the association between iron status and Gestational Diabetes Mellitus: A hospital-based case-control study.

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A thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfilment of the Requirements for the Degree of Master of Science in Food Science and Nutrition.

November, 2020

Addis Ababa, Ethiopia

Declaration

I, the undersigned, declare that this is my original work and has never been presented in this or any other university, and that all the source materials used for this thesis have been dully acknowledged.

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LIST OF ABBREVIATIONS /ACRONYMS

ADA	American Diabetes Association
CBC	Complete Blood Count
CRP	Carbon Reactive Protein
DM	Diabetes mellitus
Fe	Iron
GDM	Gestational Diabetes Mellitus
HbA1c	Glycated haemoglobin
HCT	Haematocrit
HGB	Haemoglobin
ID	Iron Deficiency
IDA	Iron Deficiency Anaemia
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
OGTT	Oral Glucose Tolerance Test
RBC	Red Blood Cell
RBS	Random Blood Sugar
SPSS	Statistical Package for Social Sciences
TfR	Serum Transferrin Receptor
TIBC	Total Iron Binding Capacity

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Abstract

Background: Gestational diabetes mellitus (GDM) is the glucose intolerance that results in hyperglycemia, which is first recognized during pregnancy. Recently, attention on GDM pathophysiology has been focused on the relation between iron overloads and glucose intolerance. In most developing countries including Ethiopia, pregnant women are exposed to adverse pregnancy outcomes related to iron. However, evidence is lacking regarding the association of iron in the development of gestational diabetes mellitus.

Objective: the objective of the study was to assess the association between iron status of pregnant women and GDM.

Method: Hospital based matched case control study design consisting 100 pregnant women, 50 cases diagnosed with GDM and 50 controls with non-GDM. Both groups were matched for maternal age and gestational week. Following case and control identification, participants in both groups were assessed back in time for the exposure status (haemoglobin and other red blood cell indices) measured at their first antenatal visit. Independent sample t-test and logistic regression analysis were computed to identify significantly associated variables.

Result: During early pregnancy (at first trimester), higher concentrations of multiple indices haemoglobin (Hgb), haematocrit (HCT), and mean cell haemoglobin concentration (MCHC) were shown a significant association with GDM occurrence. Regarding iron status indices measured at third trimester, significantly higher values of HGB and HCT were associated with GDM. Whereas, only higher value of HCT was associated with GDM in the second trimester. The mean Hgb concentrations among cases (13±1 g/dl) and controls (12.5±1.1 g/dl) (p=0.003) were observed at first trimester. Regression analysis of the present study demonstrated that for every unit increase in Hgb concentration, the odds of GDM occurrence nearly doubles (OR = 1.97, 95% CI=1.216 - 3.209, p=0.006). Furthermore, in comparison to those with Hgb concentration of < 13 g/dl, pregnant women with Hgb concentration of > 14 were 4.6 times more likely to develop GDM.

Conclusion: Higher iron status during pregnancy is associated with GDM. Either HGB alone or in combination with HCT concentrations at early pregnancy could be used as an iron status indicator in predicting the potential occurrence of GDM.

Key-words: *Gestational Diabetes Mellitus, iron status, Hemoglobin, pregnancy.*

1. INTRODUCTION

1.1. Background

Iron deficiency is the most common micronutrient deficiency in the world and disproportionately affects pregnant women and young children (Georgieff *et al.*, 2019). Pregnancy poses a large risk of negative iron balance to a woman. In comparison to the non-pregnant, during pregnancy women require one gram of additional iron. This iron demand is mainly utilized in: (i) the fetoplacental growth and development during gestation. From the required 1gm, 360 mg is transferred from the mother to the fetus, particularly during the third trimester when growth is most rapid—in order to maintain a content of 75 mg of iron per kg body weight of the fetus; (ii) the expansion of plasma and blood volumes by pregnant women to maintain proper circulation and oxygen delivery to her own organs as well as to the placenta. The blood volume expansion consumes 450 mg of the additional iron required during pregnancy (Fisher & Nemeth, 2017).

Increasing evidence supports the concept that postnatal iron status at 9 months of age depends on proper fetal iron store during pregnancy. Therefore, the goals of maintaining iron sufficiency during pregnancy are to reduce maternal morbidity, promote fetal health, and to set up the new-born with adequate nutrient stores for early postnatal life. With this regard, iron supplementation programs have been successful in reducing this health burden. However, iron supplementation of iron-sufficient individuals is likely not necessary and may carry health risks (Zhao *et al.*, 2015).

Iron sufficiency during pregnancy results in better pregnancy outcomes for the mother and the child (Georgieff *et al.*, 2019). However, there exists controversy with respect to the routine iron supplementation in apparently iron-sufficient (i.e., non-iron-deficient) women during pregnancy. The US Preventive Services Task Force (2015) stated that there was insufficient evidence to advocate routine iron supplementation during pregnancy. A similar statement from the European Food Safety Authority (2015) concluded that iron supplementation during pregnancy should be reserved for those at risk for or with documented iron deficiency. A randomized placebo-controlled trial by Ziaei *et al.* (2007) have assessed the effect of iron supplementation on women with high hemoglobin concentrations (i.e., >13.2 g/dL) during the second trimester leads to even higher hemoglobin concentrations and found an increased rate of maternal preeclampsia and fetal growth

restriction. Zhangz & Rawal (2017) observed that iron supplementation in pregnant women has also been linked to a greater risk of gestational diabetes mellitus.

GDM is a serious disease, which may cause fetal death and complications such as preeclampsia, increased cesarean rates, macrosomia in mothers and neonates, shoulder dystocia, neonatal hypoglycemia, respiratory distress syndrome, and childhood obesity (Fattah *et al.*, 2020). The American Diabetes Association (2000) defines gestational diabetes mellitus as any degree of glucose intolerance with onset or first occurrence during pregnancy. Among the various suggested causes for GDM occurrence, studies have been showing significant association of GDM with high levels of haemoglobin that was measured in first trimester (Yerebasmaz *et al.*, 2015; Georgieff *et al.*, 2019). Pregnant women in Ethiopia, like in most other developing countries, have been receiving additional iron from a universal iron supplementation irrespective of their iron status. However, iron status of pregnant women was not so far studied for any association with the occurrence of GDM in the Ethiopia context.

Therefore, the present study aimed to assess whether GDM in recent days in Ethiopia is related to maternal iron status during pregnancy through a hospital-based case-control study.

1.2. Statement of the Problem

Gestational diabetes mellitus (GDM) is posing a serious complication to the maternal and child health/nutrition status particularly in resource limited settings that are challenged with conducting early detection and management of the problem. Limited studies have assessed the prevalence of GDM from developing countries including Ethiopia. For instance, in Ethiopia, the existing few studies showed an increasing trend in GDM from a prevalence of 3.7% in 1999 to 12.8% in 2019 as demonstrated in cross-sectional studies conducted in northern Ethiopia by Seyoum *et al.* (1999) and Muche *et al.* (2019) respectively.

Furthermore, those studies have pointed out factors associated with the occurrence of GDM including overweight/obesity, family history of diabetes, previous history of GDM and increase in number of pregnancies (Wolka et al., 2018; Muche et al., 2019). However, contribution of maternal iron status during pregnancy to GDM remains to be studied in the Ethiopian context.

1.3. Significance of the study

Globally, GDM is rising, but it is a neglected health threat to mothers and their children in low resource countries. The present study tries to evaluate the association between maternal iron status during pregnancy with the risk of occurrence to GDM. The finding of the study will provide a baseline evidence to suggest what has to be done in the existing antenatal care to avert an increasing trend in GDM and will serve as a spring board for further future studies.

1.4. Objective of the study

1.4.1. General Objective

- To assess the association between iron status of pregnant women and the occurrence of Gestational Diabetes Mellitus.

1.4.2. Specific Objectives

- To evaluate an association between iron status (hemoglobin levels) and GDM
- To determine an association between Red blood cell indices: HCT, MCV, MCH, MCHC with GDM.

2. LITERATURE REVIEW

2.1. Iron

2.1.1. Historical Background and Importance of Iron

From ancient times, man has recognized the special role of iron in health and disease. Iron had early medicinal uses by Egyptians, Hindus, Greeks, and Romans. For many years, nutritional interest of iron focused on its role in hemoglobin formation and oxygen transport. Nowadays, low iron intake and/or bioavailability are responsible for most anemias in industrialized countries. Infectious and inflammatory diseases, blood loss, and other nutrient deficiencies (vitamin A, riboflavin, folic acid, and vitamin B12) are also important causes (Nazanin, Richard and Roya, 2014).

Iron is a trace mineral that is vital for growth and development. It plays a key role as a cofactor for enzymes involved in oxidation reduction reactions, which occur in all cells during metabolism. It is also important for proper production and catabolism of several neurotransmitters mostly during fetal and early childhood (Edelstein,2011). Iron is a mineral that is naturally present in many foods, added to some food products, and available as a dietary supplement. It is an essential component of hemoglobin, an erythrocyte protein that transfers oxygen from the lungs to the tissues (Wes sling *et al.*, 2014).

2.1.2 Iron metabolism and Absorption

With respect to the mechanism of absorption, there are two kinds of dietary iron: heme iron and non-heme iron. In the human diet the primary sources of heme iron are from consumption of meat, poultry, and fish whereas non-heme iron is obtained from cereals, pulses, legumes, fruits, and vegetables. The average absorption of heme iron from meat-containing meals is about 25 percent and can vary from about 40 percent during iron deficiency to about 10 percent during iron repletion. Heme iron can be degraded and converted to non-heme iron if foods are cooked at a high temperature for too long. Calcium is the only dietary factor that negatively influences the absorption of heme iron and does so to the same extent that it influences non-heme irons (Hallberg & Gramatkovski, 1997).

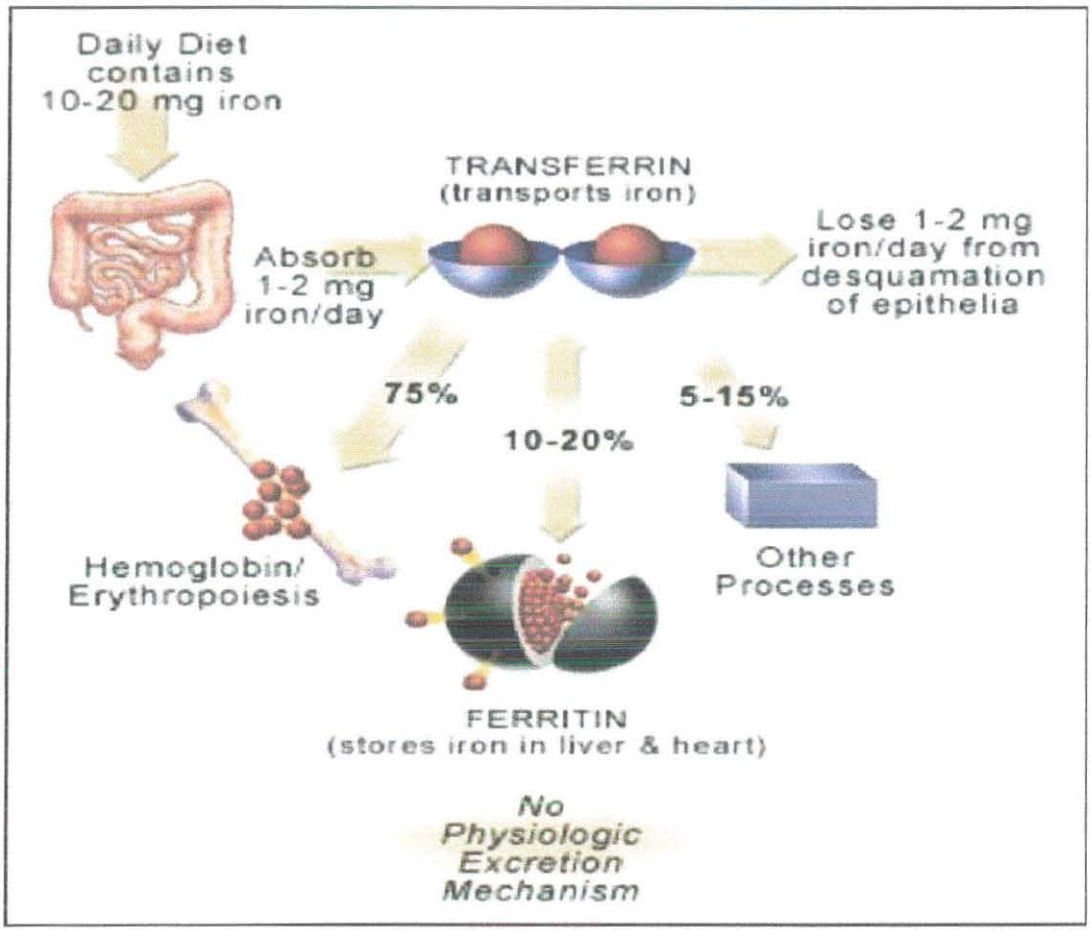

Figure 1: Iron absorption, transport and storage (Abbaspour *et al.*, 2014).

Iron is bound and transported in the body via transferrin and stored in ferritin molecules (Figure 1). Once iron is absorbed, there is no physiologic mechanism for excretion of excess iron from the body other than blood loss, that is, pregnancy, menstruation, or other bleeding (Abbaspour *et al.*, 2014).

2.2. Pregnant women and Iron Inadequacy

The highest probability of suffering iron deficiency is found in those parts of a population that have inadequate access to foods rich in absorbable iron during stages of high iron demand. During pregnancy, plasma volume and red cell mass expand due to dramatic increases in maternal red blood cell production. As a result the needs of the fetus and placenta, the amount of iron that women need increases during pregnancy. Iron deficiency during pregnancy increases the risk of maternal and infant mortality, premature birth, and low birth weight (WHO, 2001).

In the last trimester of pregnancy, the fetus requisitioned from the mother at an average rate of 3 to 4 mg/day. Each pregnancy extracts about 1000 mg of iron which exceeds the normal iron stores. Hence iron must be supplemented in such cases to avoid iron deficiency anemia. So that, it is vital to determine iron levels in all patients with anemia, since there are disorders such as thalassemia that may be present and misdiagnosed as iron deficiency (Chatterjea, 2011). Infants especially those born preterm or with low birth weight or whose mothers have iron deficiency are at risk of iron deficiency because of their high iron requirements due to their rapid growth (Domellöf, 2011).

During pregnancy, there is a significant increase in iron requirement due to the rapid growth of the placenta and the fetus and the expansion of the globular mass. In contrast, adult men and postmenopausal women the amount of iron in a normal diet is usually enough to cover their physiological requirements (Vist *et al.*, 2011).

2.3. Iron status and gestational diabetes risk

Unlike pre-gestational diabetes, gestational diabetes has not been clearly shown to be an independent risk factor for birth defects. Birth defects usually originate sometime during the first trimester of pregnancy, whereas GDM gradually develops and is least pronounced during the first and early second trimester. Studies have shown that the offspring of women with GDM are at a higher risk for congenital malformations (Allen *et al.*, 2007).

Studies on body iron stores in early pregnancy and subsequent GDM risk are light. A case–control study conducted in participants from the Danish National Birth Cohort iron stores measured in early pregnancy are associated with GDM risk. Plasma biomarkers of iron stores, including ferritin and soluble transferrin receptor (sTfR) suggesting that plasma ferritin measured in early pregnancy is significantly and positively associated with GDM risk (Bowers *et.al*, 2016).

Free radicals have a role in GDM. As there are little data about iron status in GDM, A transitional metal, especially iron, which is abundant in the placenta, is important in the production of free radicals which is risk of GDM (Afkhami, 2009).

There was a relationship between serum ferritin concentration in early pregnancy and the risk of GDM. Diagnosis of GDM was done by 75 oral glucose tolerance tests between 24-28wks Women who developed GDM had a higher concentration of serum ferritin than women who

did not develop GDM. The risk of GDM with these high levels of ferritin was 1.4 fold higher than that for subjects with lower concentrations. So that High serum ferritin can be regarded as a significant risk factor for the development of gestational diabetes (Soheilykhah S.*et al.*, 2017).

Association of iron overload with GDM may inform the health debate. One meta-analysis investigating the association of iron biomarkers and dietary iron exposure with GDM identified 33 eligible studies (N= 44,110), did not find any differences in Total Iron Binding Capacity (TIBC) or transferrin concentration in women with and without GDM. Accumulating evidence suggests that circulating and dietary iron biomarkers among pregnant women are associated, which is a good predictor for the development of GDM (Galal and Salah, 2015).

There is one study show opposite from above studies. In a total 100 subjects: 43 with healthy non diabetic and non-anemic pregnant women (Control group) and 57 with pregnant women having GDM (cases), serum iron concentration was significantly lower in GDM group as compared to Control. So that, GDM does not seem to be associated with iron deficiency or elevated body iron store (Sarker *et al.*, 2011). Some Studies showed that higher iron stores are also associated with an increased risk of type 2 diabetes in healthy populations, independently of known diabetes risk factors (Jiang *et al.*, 2004).

2.4. Iron Supplementation and absorption

Iron deficiency anemia occurs mainly in infants, children, and fertile women. For this reason, a variety of foods, including infant formula and infant cereals is fortified with iron. Ferrous sulfate is a form of iron that is most readily absorbed by the gut, but when added to dry cereals it can promote their spoilage and rancidity. For this reason, dry cereals are fortified with elemental iron particles, ferric pyrophosphate, or ferrous fumarate (Chatterjea, 2011).

Randomized trials of iron prophylaxis during pregnancy have demonstrated positive effects on reducing low hemoglobin and hematocrit, and increasing serum ferritin, serum iron and other measures, including bone marrow iron (Shukla, *et.al*, 2014).

Iron is available in many dietary supplements. Multivitamin/multimineral supplements with iron, especially those designed for women; typically provide 18 mg iron.

Multivitamin/multimineral supplements for men frequently contain less or no iron. Frequently used forms of iron in supplements include ferrous and ferric iron salts, such as ferrous sulfate, ferrous gluconate, ferric citrate, and ferric sulfate (Oslon *et al.*, 2005).

Because of its higher solubility, ferrous iron in dietary supplements is more bioavailable than ferric iron. High doses of supplemental iron (45 mg/day or more) may cause gastrointestinal side effects, such as nausea and constipation. Other forms of supplemental iron, such as heme iron polypeptides, carbonyl iron, iron amino-acid chelates, and polysaccharide-iron complexes, might have fewer gastrointestinal side effects. For oral iron supplementation, ferrous iron salts (ferrous sulfate and ferrous gluconate) are preferred because of their low cost and high bioavailability (Oslon *et al.*, 2005). Iron supplementation during pregnancy is advisable in developing countries, where women often enter pregnancy with low iron stores (CDC, 2002).

2.5. Iron supplementation and GDM

High levels of iron biomarkers in the body are associated with an increased risk of GDM in pregnant women, raising questions about routine recommendations of iron supplementation (Rawal *et al.*, 2017). The role of iron excess from iron supplementation in the pathogenesis of GDM needs to be examined (Afkahami, 2009). However, some studies have shown no association between iron supplementation and GDM (Milman, 2006).

In investigating the association between maternal iron status and the risk of developing gestational diabetes, results from a pooled analysis of case-control studies there was an association between the increased risk of gestational diabetes and higher levels of ferritin, serum iron, and dietary heme iron intake. But, not result from short exposures to iron supplements during pregnancy, however is associated with higher intakes of dietary heme iron during the preconception and early pregnancy period (khambalja, 2016).

Iron and folic acid supplementation during pregnancy in developing countries has improved the maternal and fetal outcome. There is a study on improvement in overall nutritional status and an increase of iron supplement in pregnancy may be responsible for the increasing prevalence of GDM in developing areas. Assessment of hemoglobin status in all pregnant women, and prescribing iron supplements to only those with anemia is done indicating that routine iron supplementation should be avoided in women with GDM (Zargar *et al.*, 2004).

There are a number of other studies which suggest a significant association between iron overload and GDM (Stephansson *et al.*, 2000).

One prospective study was conducted to determine if pre-pregnancy dietary and supplemental iron intakes are associated with the risk of GDM. Among 13,475 women who reported a singleton pregnancy between 1991 and 2001, a total of 867 incident GDM cases were reported. The result show, dietary heme iron intake was positively and significantly associated with GDM risk. No significant associations were observed between total dietary, nonheme, or supplemental iron intake and GDM risk (Bowers, 2011, Zhao *et al.*, 2017).

A case control study of 107 GDM cases and 214 controls done to assess several biomarkers of iron status, including plasma hepcidin, ferritin, and soluble transferrin receptor (sTfR), and these data were used to calculate the sTfR:ferritin ratio, which captures both cellular iron need and availability of body iron stores. The result suggested that, higher maternal iron stores may play a role in the development of GDM starting as early as the first trimester, and raise potential concerns about the recommendation of routine iron supplementation among pregnant women who already have sufficient iron (Rawal *et al.*, 2017).

2.6. Iron overload and Health Risks

Iron overload is the accumulation of excess iron in body tissues, and it usually occurs as a result of a genetic tendency to absorb iron in excess but also caused by an increase ingestion of iron supplements or multiple blood transfusions. In advanced stages of iron overload disease (hemochromatosis), the iron accumulates in several organs particularly in liver, followed by the heart and pancreas; this condition can lead to organ dysfunction and even death (Pietrangelo, 2004). Excess ferrous iron forms free hydroxyl radicals that cause damage to tissues through oxidative reactions with lipids, proteins, and nucleic acids. Thus, dietary iron absorption and factors affecting bioavailability in the body are strongly regulated wherever possible (Thomas and Martin, 2018).

Iron is also potentially toxic, that it can catalyze the conversion of hydrogen peroxide into free radicals. Free radicals can cause damage to a wide variety of cellular structures, and ultimately kill the cell. To prevent that kind of damage, all life forms that use iron bind the iron atoms to proteins. This binding tolerates cells to benefit from iron while also limiting its ability to do harm (Stewart, 2003).

There is no homeostatic mechanism for disposing of excess iron chronic iron overload occurs in three situations: In hereditary hemochromatosis , Patients with intractable anemia, (for example, thalassemia) and repeated blood transfusions and People who regularly drink alcohol beverages with high iron content receive excess absorbable iron by mouth (Stewart, 2003). However, acute intakes of more than 20 mg/kg iron from supplements or medicines can lead to pains, especially if food is not taken at the same time (Aggett, 2012).

It has been previously reported that iron overload promotes inflammatory processes by inducing free radical formation through an oxidative mechanism (Fernandez *et al.,* 2002). Iron overload is believed to reduce placental perfusion and result in preeclampsia, low birth weight and preterm birth (Stephansson, Dickman and Johansson *et al.* 2000).

2.7. Body iron storage and storage compounds

Iron is found in two forms, essential iron for normal function of the body and the reserve for times of needs. The essential iron is mostly haemo proteins and is present in hemoglobin or erythrocyte and is the major part of the body iron. The second largest fraction is myoglobin. The other important position of essential iron is in enzymes required for mitochondrial function and DNA Synthesis (Wriggles worth & Baum, 1980).

Of the body's total iron content; about 400 mg is devoted to cellular proteins that use iron for important cellular processes. 3-4 mg circulates through plasma bounding to transferrin. Because of its toxicity, free soluble iron is kept in low concentration in the body (Camaschella and Schrier, 2018). Iron functions as a component of proteins and enzymes. Almost two thirds of the iron in the body (approximately 2.5 grams of iron) is found in hemoglobin, the protein in red blood cells that carries oxygen to tissues, and about 15 percent is in the myoglobin of muscle tissue (Miret, 2003).Transporting iron from one organ to another is accomplished by the reversible binding of iron to the transport protein, transferrin, which will then form a complex with a highly specific transferrin receptor (TfR) located on the plasma membrane surfaces of cells. Ferritin is the major iron-storage compound: its production increases in cells as iron supplies increase. All cells are capable of storing iron, the liver, spleen, and bone marrow cells are primary iron-storage sites in people (Trumbo, 2001).

Under steady state conditions, serum ferritin concentrations relate with total body iron stores (Hunt, 2001). Thus, serum ferritin is the most convenient laboratory test to estimate iron

stores. Apart from iron losses due to menstruation, other bleeding or pregnancy, iron is highly preserved and not readily lost from the body (Hunt, Zito and Johnson, 2009). Free iron is toxic and catalysis the conversion of oxygen to hydroxyl radicals. Iron bound to ferritin is nontoxic (Chatterjea, 2011).

Hemosiderin is an iron storage complex that less readily releases iron for body needs. Evidence suggests that hemosiderin is derived from ferritin. Hemosiderin contains a larger fraction of its mass and exists as microscopically visible iron staining particles. Hemosiderin is usually seen in states of iron overload or when iron is in excess. Iron in hemosiderinis available for formation of Hgb, but mobilization of iron is much slower from hemosiderin than ferritin (Chatterjea, 2011).

2.8. Iron deficiency Anemia (low iron)

Anemia is the condition of low levels of hemoglobin in the blood. This results in a reduced amount of oxygen being transported in the body. Iron is a main component of hemoglobin and iron deficiency is estimated to be responsible for over half of all anemias globally. Other causes of anemia include malaria, hookworm and other helminths; other nutritional deficiencies such as vitamin A, vitamin B12 or folic acid deficiency; chronic infections; genetic conditions and high fertility (WHO, 2001).

Ferritin index has been proposed as a useful tool in the diagnosis of iron deficiency. Anemia also defined as hemoglobin level <12 g/dL in women and<13 g/dL in men. Hemoglobin is a commonly used, well validated, and widely accepted indicator for anemia. Mean hemoglobin is one useful way to present this indicator. However, anemia is also commonly presented based on cutoffs (Enjuenes, *et al.*, 2016).

2.9. Markers of iron status and measurements

2.9.1. Markers of iron status

Markers that have been used to assess iron status are categorized according to whether they represent: the distribution (transport and supply) of iron to tissues, iron deposits in tissues and a functional use of iron in the synthesis of hemoglobin. There are a number of hematological and biochemical indicators which are used to assess iron adequacy, deficiency and excess. These include:

Iron in tissues: serum transferrin (transport iron capacity),

Functional iron biomarkers: serum ferritin (systemic iron depots), hemoglobin (functional iron) concentration, zinc protoporphyrin (hemoglobin synthesis), red blood cell indices (MCV and MCH).

Tissue iron supply: serum transferrin receptors (tissue needs for iron). Most assessments combine ferritin and hemoglobin concentrations as markers of the deposition of iron in tissues and iron utilization (Calder, 2010).

Iron status is essentially a qualitative concept. It cannot be precisely quantified because of difficulties in determining accurate thresholds for adaptive responses and for adverse events associated with iron deficiency or excess. Assessment of status for iron, as for other micronutrients, requires an approach based on integrated use of the markers according to their functional significance and potential confounders. However, because they each measure different aspects of iron metabolism, the iron related markers do not always correlate well. (Calder, 2010).

No single marker of iron metabolism is considered ideal for the assessment of iron deficiency, adequacy or excess, as all the individual indices have limitations in terms of their sensitivity and specificity. However, in accordance with practical decisions made by others such as WHO, hemoglobin (functional iron) and ferritin (iron depots), in combination, are considered to be the most useful indicators of iron status. Although these markers are useful for field work, they need to be used critically in developing and monitoring interventions in practice, and in developing policy. A relationship between iron markers and outcomes can only be expected to exist in iron deficiency or increased iron needs (WHO /CDC, 2004).

2.9.2. Measures of iron status markers

Many different measures of iron status are available, and different measures are useful at different stages of iron depletion. Measures of serum ferritin can be used to identify iron depletion at an early stage (Gibson, 2005). The measures are most informative when multiple measures of iron status are examined and evaluated in the context of nutritional and medical history (WHO/CDC, 2004).

Quantitative estimates of body iron enhance the evaluation of iron status and the sensitivity of iron intervention trials in populations in which inflammation is uncommon or has been excluded by laboratory screening. The method is useful clinically for monitoring iron status in those who are highly susceptible to iron deficiency (Cook, 2003).

The clinical application of body iron measurements is limited by the numerous disorders that affect serum ferritin and sTfR levels independently of iron status, although the most important ones can be detected by elevated levels of C-reactive protein. At present, clinicians depend on serum ferritin level to determine the adequacy of iron stores and on the hemoglobin concentration to identify advanced iron deficiency. However, there is no reliable laboratory method at present for detecting tissue iron deficiency before the onset of anemia (Cook, 2003).

Hematocrit is also commonly used as an indicator of anemia. Hematocrit and hemoglobin are interchangeable using the following conversion factors (100g hemoglobin=6.2mmol hemoglobin = 0.20 l/l (WHO, 2001). neither hemoglobin nor hematocrit are indicators of iron deficiency. Iron deficiency is measured using serum ferritin which requires techniques and laboratories. Assessment of adult populations should include questions related to smoking habits including what type of smoking and how many per day, high altitudes (<1000 feet) (Parker, *et al.*, 2018).

Iron in tissues and Tissue iron supply test

Several methods are used to measure iron and related analyses. Serum iron concentration measures the amount of ferric iron (Fe3+) bound mainly to serum transferrin but does not include the divalent iron contained in serum as hemoglobin. Elevated concentrations of serum iron occur in iron-loading disorders such as hemochromatosis. Serum iron is not a good indicator of iron stores and is not a sensitive measure of iron deficiency because of daily fluctuations. Serum iron measurements are used in conjunction with TIBC measurements. Normally, because one third of the iron-binding sites of transferrin are occupied by Fe3+, serum transferrin has considerable reserve iron-binding capacity (WHO, 2001).

Another indicator of iron status is the concentration of TfR in serum. Since TfR is mostly derived from developing RBCs, it reflects the intensity of erythropoiesis and the demand for iron (WHO/CDC, 2004). The major advantage of TsfR as an indicator is estimating the magnitude of the functional iron shortage once iron stores are depleted (Baynes, 1996).

The ratio of TfR to ferritin (TfR/ferritin) was designed to evaluate changes in both stored iron and functional iron and was thought to be more useful than either TfR or ferritin alone. TfR/ferritin has been used to estimate body iron stores in both children and adults (Cook *et al.*, 2005). However, the high cost and the lack of standardization have limited the applicability of the method (Yang *et al.*, 2008). But ferritin levels may be artificially high in cases of anemia of chronic disease and inflammatory acute phase protein and not a marker for iron overload (Theil, 2012).

If ferritin is high, there is iron in excess or else there is an acute inflammatory reaction in which ferritin is mobilized without iron excess. Ferritin is also used as a marker for iron overload disorders, such as hemochromatosis or hemosiderosis (Kennedy *et al.* 2004).The measurement of ferritin through immunoassay or immune-turbidimeteric methods may also be picking up these isoferritins thus not a true reflection of iron storage status (Tran J. *et al.*, 2013). Total iron-binding capacity (TIBC) or sometimes transferrin iron-binding capacity is a medical laboratory test that measures the blood's capacity to bind iron with transferrin (Yamanishi *et al.* 2003).

Functional iron biomarkers test

Rather than examining the Hgb content of the entire RBC population that may be anywhere between 1 and 120 days old, the reticulocyte Hgb content provides a picture of how much iron was available for RBC production in a clinically relevant time frame. The reticulocyte Hgb content is widely available on many of the same multichannel hematology analyzers that does the complete blood count (Wish, 2006).

Another alternative iron marker is percentage of hypochromic red blood cells, which is a test of the concentration of Hgb in RBC, as opposed to the Hgb content as in the reticulocyte Hgb content. Because percentage of hypochromic red blood cells is based on the Hb concentration in RBC; it takes into account the absolute amount of Hgb as well as the size of the cell. The big problem with the utility of this test is that RBC tends to expand while they are stored (Wish, 2006). Another iron test related tests are CBC, especially: total red blood cell count, Hgb, MCV, MCH or MCHC (Buttarello & Plebani, 2008).

Various Red blood cell indices (parameters calculated from other CBC results) are often reported in addition to cell counts and hemoglobin. Automated hematology analyzers calculate the MCH and MCHC of Hgb within each red blood cell. Average red blood cell size

MCV and shape (RDW) are also calculated to provide additional diagnostic information (Tan *et al.*, 2016).

Depletion of iron concentration leads to inhibition of Hgb synthesis Taking this into consideration estimation of iron concentration and Hgb level from blood samples of 96 working women volunteers from various age groups is carried out. 37 cases show normal Hgb but are deficient in iron concentration which may be predisposed to anemic condition in future. This condition may be due to dehydration which causes falsely high Hgb and iron concentration which disappears when proper fluid balance is restored. Even if there is enough iron in blood, the Hgb can be low or vice versa as there is no direct correlation between iron concentration and Hgb level in blood (Rajurkar, *et. al.*, 2012).

2.10. Effects of pregnancy on hormones

Pregnancy hormones and other factors are thought to interfere with the action of insulin as it binds to the insulin receptor. Because insulin promotes the entry of glucose into most cells, insulin resistance prevents glucose from entering the cells properly. As a result, glucose remains in the bloodstream, where glucose levels rise. More insulin is needed to overcome this resistance (Carr and Gabbe, 1998). Pregnancy is attributed to increased maternal adiposity and insulin desensitizing effects of placental products such as human placental lactogen, estrogen and prolactin (Gilmartin *et al.*, 2008).

A decrease in insulin sensitivity (i.e. an increase in insulin resistance) is normally seen during pregnancy to spare the glucose for the fetus. This is attributed to the effects of placental hormones. In a few women the physiological changes during pregnancy result in impaired glucose tolerance which might develop GDM. Although the majority of women with GDM return to normal glucose tolerance immediately after delivery, a significant number will remain diabetic or continue to have impaired glucose tolerance (Al-Noaemi, 2011).

Emerging evidence has pointed to a possible link between higher iron stores and abnormal blood sugar control (including type 2 diabetes) in non-pregnant individuals (Rawal *et al.*, (2016).

Clinicians are more aware of the need to precisely identify and manage metabolic dysfunction in pregnancy manifested especially by abnormal glucose metabolism. This has led to an increased focus on the ability to predict and prevent many potential fetal and

maternal complications in the guide pregnancy (Hod *et al.*, 2015). Certain factors including having a family history of diabetes, over 25 years of age, obese, belonging to a particular ethnic group and having previously given birth to a baby weighing 4 kg or more (microsomal), put women at greater risk of developing GDM (ADA, 2010).

2.11. Prevalence of GDM

Approximately 7% of all pregnancies are complicated by GDM, resulting in more than 200,000 worldwide cases annually and the prevalence may range from 1% to 14% of all pregnancies depending on the population studied and the diagnostic tests employed (Sue Kirkman and Schaffner, 2012). Determining a country's GDM prevalence can assist with policy guidelines regarding GDM screening, management and focus areas requiring research (Macaulay, Dunger and Norris, 2014).

The effects of urbanization have not only had impact on developing countries' economies but also on public health. The transition from rural to urban ways of life is often associated with changes in eating habits, body mass and composition, and reduction in physical activity. The movement towards more Westernized diets that consumption of fats, sugars and refined carbohydrates. As a result, low or middle-income countries are going through a rapid increase in overweight and obesity as well as non-communicable diseases, such as diabetes, that accompany such conditions (Popkin and Adair, 2012). Developed countries are almost low prevalence for example, (Denmark (2–3%), UK (2–3%), Germany (0.3–0.8%) (Macaulay, Dunger and Norris, 2014).

GDM in African

Diabetes was essentially unknown in Africa in 1901, until now in 2013 19.8 million people were reportedly living with the condition and this number is predicted to increase to 41.5 million in 2035 equating to a 109% increase (IDF, 2013).

Systematic review of the literature search identified 466 unique records in Africa. Prevalence was obtained for six African countries; Ethiopia (n=1), Morocco (n=1), Mozambique (n=1), Nigeria (n=6), South Africa (n=4) and Tanzania (n=1). Prevalence figures ranged from 0% (Tanzania) to 13.9% (Nigeria) with some studies focusing on women with GDM risk factors. In the result six countries, equating to 11% of the African continent, were represented in this systematic review. This indicates how little is known about GDM in Africa and highlights the

need for further research to allow effective intervention programs (Macaulay, Dunger and Norris, 2014).

GDM in Ethiopia

GDM in rural pregnant mothers of Ethiopia determines the prevalence of gestational diabetes mellitus 3.7% this prevalence was low compared to other study (Berhane *et al.*, 1999).

Study led by Management Sciences for Health revealed that about 11% of women in Mekelle have GDM high even for Africa with a rate of about 5 percent. An estimated 80 % of cases in Ethiopia remain undiagnosed (Abraha *et al.*, 2014). Universal or selective screening is routine in most high-income countries. But in Ethiopia, as in most low-income countries, screening is the exception and not the rule. International GDM guidelines were successfully adapted and implemented within the local context in Tigray, providing the basis for scale up across Ethiopia (Abraha *et al.*, 2014).

The possible exposure of the general population in this area are, chronic malnutrition as a result of long famine, drought and war, to the high prevalence of gestational diabetes mellitus permits further study (Seyoum *et al.*, 1999). Recent study from southern Ethiopia, from a total of 518 pregnant women with duration of 24-28 weeks of pregnancy were examined for gestational diabetes mellitus by using WHO 2013 criteria, The overall prevalence of gestational diabetes mellitus was 4.2%,(4%) among urban residents and (4.9%) among rural (wolka,2019).

2.11.1. Prevalence and risk factors of GDM

Pregnancies affected by GDM create a risk for complications such as the need for Caesarean sections due to fetal macrosomia. Macrosomia occurs as a result of accelerated fetal growth fuelled by maternal hyperglycemia (Lindsay, 2009). In addition, research into the long-term effects of poor maternal glucose metabolism on the fetus has exposed that offspring born to mothers with GDM are susceptible to obesity. (Hillier *et al.*, 2007). With these associations it would be important to identify pregnant women at risk for GDM. So that, prevention management such as lifestyle modifications can be implemented (Forsbach-Sanchez *et al.*, 2005).

As urbanization and lifestyle transitions, the double burden of under and over nutrition is a cause for concern. Therefore, epidemiologists, public health specialists, health professionals,

and policy leaders need to place GDM and macrosomia as key elements in their maternal and child health framework. Thus, enabling policies and practice to minimize the risk of maternal impaired glucose metabolism during pregnancy is a solution (Al-Noaemi, 2011).

2.12. Criteria used for diagnosis of GDM

A systematic review was undertaken focusing on short-term pregnancy and prenatal outcomes and effects on pregnancy when both the International Association of Diabetes and Pregnancy Study Groups (IADPSG) and the WHO criteria (which had different diagnostic cutoffs) were used.

2.13. Association of iron status and GDM (high iron)

During pregnancy, increased maternal red cell synthesis and transfer of iron to the developing fetus cause a greater demand for iron. Unless iron supplements are given, iron stores generally fall, with accompanying falls in serum ferritin and serum iron, and rises in serum transferring and total iron binding capacity (WHO. 2003). Both iron deficiency and iron overload have serious health consequences. Iron overload, on the other hand, is the result of genetic disorders that affect the control of iron absorption and hematological conditions (Andrews, 2008).

Oxidative stress induced from excess iron accumulation can cause damage to and death of pancreatic beta cells which produce insulin and, consequently, contribute to impaired insulin synthesis and secretion. In the liver, high iron stores may induce insulin resistance by means of damaging insulin signaling as well as by limiting the capacity of the liver to extract insulin (Rawal, 2017). Thus, those patients who have limited beta cell capacity for the compensation of pregnancy induced insulin resistance due to variations in the genes involved in insulin secretion and utilization of carbohydrates is likely to develop GDM (Sonagra *et al.*, 2018).

Some authors suggest that iron overload can cause insulin resistance. An interesting study demonstrated that iron can affect insulin synthesis and secretion by decreasing glucose utilization in muscles and increasing hepatic gluconeogenesis .Furthermore, it has been shown that elevated serum ferritin levels are associated with a two-fold increase in the risk of developing type 2 diabetes mellitus and higher plasma ferritin levels (Javadian *et al.*, 2014).

2.14. The role of iron in the induction of diabetes

Although the exact mechanism of iron-induced diabetes is uncertain, it is likely, as discussed below, to be mediated by three key mechanisms: insulin deficiency, insulin resistance, and hepatic dysfunction. An understanding of the pathogenic pathways of iron-induced diabetes is derived mainly from studies on animal models of hemochromatosis (Fernandez-Real, 2005).

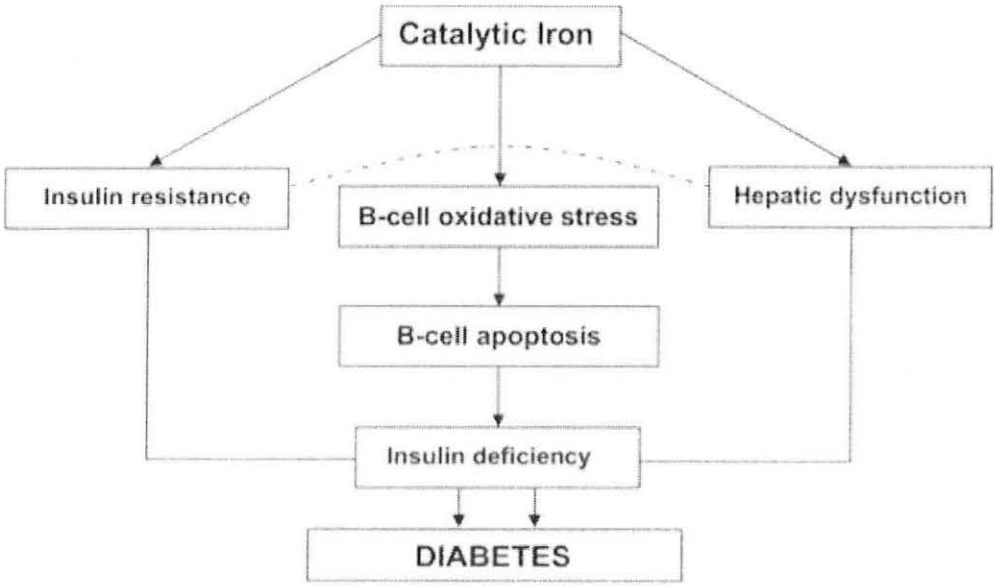

Figure 2: Pathogenic pathways for iron induction of diabetes (Swaminathan *et al.*, 2007)

Systemic iron overload could contribute to abnormal glucose metabolism was first derived from the observation that the frequency of diabetes is increased in classic hereditary hemochromatosis. However, with the discovery of novel genetic disorders of iron metabolism, it is obvious that iron overload, irrespective of the cause or the gene involved, results in an increased incidence of type 2 diabetes. The role of iron in the pathogenesis of diabetes is suggested by: An increased incidence of type 2 diabetes in diverse causes of iron overload and Reversal or improvement in diabetes (glycemic control) with a reduction in iron load achieved using either phlebotomy or iron chelation therapy (Fernandez-Real, 2005).

2.15. Association of Hemoglobin (functional iron) and GDM

Pregnancy has various effects on hematologic parameters, it is well known that Hgb levels decrease during the first trimester, reaching minimum in late second trimester and tend to increase during third trimester (Laflamme, 2011). So that, Hgb levels must be screened

during pregnancy because, both low and high Hgb levels are related with adverse pregnancy outcomes (Cakmak, 2011).

Another interesting finding is the risk of adverse pregnancy outcomes in pregnancies with high Hgb levels (both total erythrocyte number and plasma volume increase), but Hgb levels decrease according to the higher increment in plasma volume. This condition provides placental perfusion with reduced blood viscosity. As a consequence, these pregnancies can be complicated with pregnancy-induced hypertension, fetal growth restriction, and perinatal death (Mani & Duffy, 1995). The association between Hgb levels and adverse pregnancy outcomes differs by trimesters. However, it is more evident in early pregnancy for low Hgb levels, and it is evident in all trimesters for high Hgb concentrations (Dewey, 2017).

There are limited data about the relationship between adverse pregnancy outcomes and Hgb levels in the first trimester of pregnancy. In Turkish population one study evaluated the effect of first trimester Hgb levels. Pregnancy outcomes were compared between the groups. The main prenatal outcomes were accepted as stillbirth, Increased Hgb and ferritin have been associated with GDM (Kaur *et al.*, 2015).

Examining the relationship between high maternal Hgb concentration at first antenatal visit and occurrence of GDM in the third trimester in no anemic women conclude that, high maternal Hgb (more than 13 g/dL) is an independent risk factor for GDM in Chinese women. This may reflect a better nutritional status in these women (Lao *et al.*, 2002).

The retrospective cohort study including 131 GDM and 300 non- GDM pregnant mothers result found out that the mean Hgb value and MCV were significantly higher in the GDM group. After the analysis of covariance for maternal age, body mass index, parity, birth weight there were significant differences in Hgb values and mean corpuscular volume values between two groups were significantly higher. The study demonstrated GDM was associated with high levels of Hgb that was measured in first trimester (yerebasmaz *et al.* 2015).

Adequate nutrition is critical in pregnancy. One study assesses statuses of the essential micronutrient (iron, copper and zinc) in normal pregnancy and in pregnancies complicated GDM. The study included three groups: non-pregnant (n = 44), healthy normal pregnancy (n = 47) and pregnancy complicated with GDM (n = 42). It concluded that normal pregnancy and GDM are associated with imbalance in iron (Amani *et al.*, 2018).

3. MATERIAL AND METHODS

3.1. Study Area

The study was conducted in Tikur Anbessa specialized teaching hospital, a tertiary referral hospital located in Addis Ababa. Tikur Anbessa specialized teaching hospital is the largest with 800 beds. The hospital provides a tertiary level of health care service for more than 400,000 patients per year in all wards. In the obstetrics and gynecology department, over 4,600 deliveries were attended each year. Furthermore, the hospital has a well-organized diabetes center to look after pregnant women with gestational diabetes millets (Eshetu *et al.*, 2019).

3.2. Study Design

Hospital based matched case control study design was employed.

3.3. Study period

Study was conducted from November 1, 2019 to February 25, 2020.

3.4. Source Population

All records of mothers with gestational diabetes at Tikur Anbessa Specialized Hospital diabetes center for cases. Control source population are those non-GDM pregnant women attending ANC at the same hospital matched for gestational age to cases.

3.4.1. Study Population

All records of singleton and non-smoker mothers with GDM which fulfill inclusion criteria and controls which sign consent.

3.5. Inclusion criteria

Case groups: Records of pregnant mother with gestational diabetes at Tikur Anbessa Specialized Hospital diabetes center Positive on the basis of OGTT (Fasting and 2 hour after 75 g Oral glucose) following the WHO criteria (2006) (Sarker *et al.*, 2011), do not have pre-existing hypertension, renal disease, previous GDM

Control Group: Healthy non diabetic pregnant women at antenatal care clinic who are signed informed consent to participate in the study. Non-GDM pregnant women attending

ANC at the same hospital, Matched for age & gestational age to cases and do not have pre-existing hypertension, renal disease

3.6. Exclusion criteria

Mothers' cards with incomplete records of study variable, diabetic pregnant women with chronic diabetic complications, previous GDM, Pregnant subjects with renal disease and pregnancy with acute or chronic medical connective tissue diseases and chronic hypertension (Sarker *et al.*, 2011).

3.7. Sample size determination

In a case control study where the exposure status is measured in a quantitative continuous unit, the following sample size calculation formula is suggested (Charan & Biswas, 2013)

$$\text{Sample size (each group)} = \frac{r+1}{r}\frac{(SD)^2\,(Z_\beta + Z\alpha/_2)^2}{d^2}$$

r = Ratio of control to cases, $r = 1$ for equal number of case and control;

SD = Standard deviation that can be taken from previous studies

Z_β = Standard normal variate for power = for 80% power is 0.84 and for 90% value is 1.28

$Z_{\alpha/2}$ = Standard normal variate for level of significance (LS) (at 5% LS = 1.96 or at 1% = 2.58)

d = Expected mean difference between case and control (from previous studies)

The present study considered (i) equal number of sample size, $r = 1$, (ii) standard deviation and mean difference were 1.64g/dL and 1.26g/dL, respectively taken from previous study by Afkhami-Ardekani and Rashidi (2009), (iii). Standard normal variate at 1% level of significance ($Z_{\alpha/2}$=2.58) and power of 90% (Z_β=1.28). Finally, the resulting sample size for each group was found 50.

3.8 Sampling procedure

The required data were extracted from admission registration and GDM registration in diabetes center. Card numbers were obtained and document of all women who have GDM during the study period were searched and checked for completeness of the required data. Then those cards which had a complete record of data (58 cards) were identified and consecutively reviewed whether they satisfy the inclusion criteria to define cases (those

having GDM). Again, from ANC follow-up registry, pregnant women having no-GDM and tested for iron status at similar gestational age (trimester) to that of cases (88 cards) were identified as controls. From the lists of eligible women (sampling frame), cases and controls were randomly chosen [systematic random sampling method].

3.9. Study Variables

3.9.1. Dependent Variable

Pregnant women presented: with or without GDM

3.9.2. Independent Variables

Iron (hemoglobin) status of the mother, HCT, MCV, MCH, and MCHC, Age, Gestational Age (gestational age at time of sampling)

3.10. Operational and conceptual definitions of variables

Gestational Diabetes Mellitus: is any degree of glucose intolerance with onset or first recognition during pregnancy and disappears by the end of the prepartum.

Pre gestational diabetes: diabetes diagnosed prior to onset of pregnancy this can be type 1/2.

Type 1 Diabetes: Metabolic disorder resulting from absolute insulin deficiency.

Type 2 Diabetes: Metabolic disorder resulting from defective insulin secretion or resistance.

Hemoglobin: a protein that carries oxygen that show status of iron.

MCV defines the size of the red blood cells; MCH quantifies the amount of hemoglobin per red blood cell. MCHC indicates the amount of hemoglobin per unit volume. In contrast to MCH, MCHC correlates the hemoglobin content with the volume of the cell.

3.11. Data collection tools and procedure

Cases information and CBC results were collected through document review in Diabetic center from patient card of mothers who has GDM during the study period and checked for completeness of the data. From those mothers who have complete documents, the data

were collected by the principal investigator. Controls were collected from normal pregnant mothers from antenatal clinic.

Sample type: whole blood was collected with anticoagulated test tube (purple top) from non GDM pregnant mothers who are positive for consent form by laboratory technologists. CBC was done within half to an hour after collection with an automated hematology analyzer in central laboratory of TASH.

Laboratory Diagnosis Methods:

A total of 4mls of venous blood was collected from each (depending on the accessibility to the vein) by the laboratory technologists and investigator. The blood sample in anti-coagulated vacutainer was analyzed for RBC, HGB, HCT, MCV, MCH, MCHC and other parameters, using Sysmex hematology analyzer (SN, kx-5014, Japan) at Tikur Anbesa Specialized Hospital half to one hour after collection. To avoid any possible transmission of blood borne diseases, all lancets and vacutainers to draw blood were disposable. For the safety of the study participants' antiseptic solution (70% alcohol with cotton) was used before the vein puncture in order to prevent infections. Hgb measurement was adjusted for altitude (WHO, 2011).

Hgb correction = -0.032 (altitude $\times$ 0.0032808) + 0.022 (altitude $\times$ 0.0032808)^2 for the average altitude of Addis Ababa (2350 meter above sea level) = +1.06 g/dl unit

Principle of sample Analysis

To perform the mentioned test for controls whole blood samples are taken and performed in the central laboratory. The RBC count and platelets are taken by the RBC detector block. The haemoglobin detector block measures the haemoglobin concentration using the non-cyanide haemoglobin method (Sysmex KX-21N™ Sysmex Corporation, Kobe, Japan).

3.12. Data quality control

To ensure the quality of the data and checking the existence of required variable, before the actual data collection, pre- test was done, and then appropriate modifications were made on the procedures after checking. The principal investigator checks the data collection process and ensures the completeness and consistency of the gathered information.

3.13. Data processing and analysis

All data were analysed using SPSS for windows version 22 (Chicago, IL; SPSS Inc.). Data normality test was done by Shapiro-Wilk test. Descriptive statistics was used. Categorical variables were presented and continuous variables were expressed as mean and standard deviation. Comparisons between cases and control were done using independent sample t-test. Relationship between HGB and other iron status indicators like RBC and HCT, MCV, MCH and MCHC were examined between case and controls using Pearson's correlation coefficient (r). Binary logistic regression was used to assess the association among the predictor variables and gestational diabetes in the comparison group. A bivariate and multivariate logistic regression (crude and adjusted odds ratio) analysis was performed to identify associated factors related to GDM. A p-value of <0.05 was considered as significant. The results were presented in tables, graphs. P-value <0.05 were used to declare statistical significance.

3.14. Ethical Consideration

The protocol of this study was approved for implementation by the Institutional Review Board of College of Natural and Computational Science, Addis Ababa University (Reference number: CNS/DO/185/12/19 and Minute number: IRB/40/2019). All the participants were informed about the general purpose of the study, their participation in the study would not involve any risks and they have a full right to refuse or discontinue the study. Following this information, volunteer participants were given their written consent. Participants' information was kept confidential by excluding names and other personal identifiers–instead, codes were used to indicate participants' card and collected specimen.

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1. Characteristics of the participants

This matched case-control study consisted of 100 pregnant women, 50 cases diagnosed with GDM and 50 controls with non-GDM. Both groups were matched for maternal age and gestational week. Age of the participants ranges from 19 to 39 years and about ninety percent of all the participants were aged ≤ 35 years. Age group difference was not statistically significant (p=0.056) between cases and controls. Similarly, gestational age of participants presented with insignificant between group (p=0.914) where, more than a quarter of participants were in the first trimester (Table 1).

Table 1: Characteristics of cases and controls (N=100).

Variables	Case N (%)	Control N (%)	p-value
Age (Year)			**0.056**
≤ 35 years	44 (88)	49 (98)	
> 35 years	6 (12)	1 (2)	
Gestational age (Trimester)			**0.914**
First	14 (28)	15 (30)	
Second	18 (38)	16 (32)	
Third	18 (36)	19 (38)	
Gestational age (Week) (Mean ± SD)	24.66 ± 8.13	23.86 ± 8.04	**0.620**

4.1.2. Iron status indicators and GDM

As a part of routine diagnostic parameters of pregnancy, iron status indicators like haemoglobin and red blood cell indices such as, HCT, MCV, MCH, and MCHC were available from patient record. Aggregate mean comparison of haemoglobin concentration presented with significantly higher in cases (13.93±1.02) than the controls (13.38±0.80). Likewise, significantly higher values were observed among cases for HCT and MCHC (Table 2).

Table 2: Mean comparison of various iron status indicators between cases and controls (N=100).

Variables	Case (n=50)		Control (n=50)		P value
	Mean	SD	Mean	SD	
RBC (x10^6/ mm3)	5.11	1.22	5.05	1.47	0.807
HGB (gm/dl)	13.93	1.02	13.38	0.80	**0.004**
HCT (%)	40.98	3.26	37.81	2.50	**<0.001**
MCV (fl)	87.27	4.90	86.89	2.50	0.633
MCH (pg)	30.52	2.25	30.80	1.27	0.445
MCHC (gm/dl)	34.59	1.44	35.66	2.92	**0.023**

Data expressed as mean ± Standard Deviation (SD), n=number of samples, RBC=Red Blood Cell, HGB= Haemoglobin, HCT= haematocrit, MCV = mean cell volume, MCH = mean cell haemoglobin, MCHC= mean cell haemoglobin concentration. P-values were derived from independent sample t-test

In correlation analyses, occurrence of GDM was correlated negatively with MCHC (r=-0.227, p=0.023) and correlated positively with HCT (R=0.482, P<0.001), age (r=0.328, p=0.001) and HGB (r=0.289, p=0.004) concentrations (Table 3). In addition, haematocrit value was positively correlated with haemoglobin concentration (0.673, p<0.001).

Table 3: Pearson Correlation of iron status indices to GDM occurrence

Variables	RBC	HGB	HCT	MCV	MCH	MCHC	Age	Gestational age
r	0.025	0.289	0.482	0.048	0.077	-0.227	0.328	0.05
P-value	0.807	**0.004**	**0.000**	0.633	0.445	**0.023**	**0.001**	0.620

RBC=Red Blood Cell; HGB= Haemoglobin, HCT= haematocrit, MCV = mean cell volume, MCH=mean cell haemoglobin, MCHC=mean cell haemoglobin concentration, r=Pearson's correlation coefficient, p=significance difference.

4.1.3. Iron status indicators and GDM - disaggregated by gestational age

In first trimester a statistically significant mean differences were observed for RBC (4.9 ± 0.3 in cases and 5.9 ± 1.8, p=0.038), HGB (13.0 ± 1.0 in cases, 12.5 ± 0.8 in controls, p=0.003), HCT (41.3± 2.7 in cases and 37.9 ± 2.9 in controls, p=0.003) and MCHC (34.5 ± 1.5 in cases and 35.7 ± 1.1 in controls, p=0.021), respectively. While MCV and MCH (p=0.841 and 0.735) are not significant (Table 4). In second trimester, HCT was the only parameter demonstrated a significant between group difference (40.9 ± 3.4 in cases and 38.2± 2.6 in controls, P=0.015) (Table 5). In third trimester, both HGB and HCT values were show statistically significant between group differences (Table 4).

Table 4: Mean comparisons between cases and control at first and third trimesters (N=64).

Variables	First trimester			Third trimester		
	Case (n=14)	Control (n=15)	P value	Case (n=16)	Control (n=19)	p-value
RBC ($x10^6$/ mm3)	4.9 ± 0.3	5.9 ± 1.8	**0.038**	4.8 +0.4	4.8 ± 1.4	0.818
HGB (gm/dl)	13.0 ± 1.0	12.5 ± 0.8	**0.003**	13.2 ± 1.1	12.6 ± 1.0	**0.001**
HCT (%)	41.3 ±2.7	37.9 ±2.9	**0.003**	41.2 + 3.7	37.4 ± 2.2	**0.001**
MCV (fl)	86.4± 2.2	86.3 ± 2.8	0.841	88.6 +6.7	86.7 ± 2.5	0.256
MCH (pg)	30.6 ± 2.6	30.8 ± 1.0	0.735	30.9 ± 2.3	30.5 +1.4	0.554
MCHC (gm/dl)	34.5 ± 1.5	35.7 ± 1.1	**0.021**	34.6 ± 0.9	36.5 ± 4.7	0.242

Data expressed as mean ± Standard Deviation (SD), n=number of samples, RBC=Red Blood Cell; HGB= Haemoglobin, HCT= haematocrit, MCV = mean cell volume, MCH = mean cell haemoglobin, MCHC= mean cell haemoglobin concentration. P-values were derived from independent sample t-test.

Table 5. Mean comparison between cases and control at second trimester (N=36).

Variables	Second trimester		
	Case (n=18)	Control (n=18)	P value
RBC ($x10^6$/ mm3)	8.3 ± 1.5	4.5 ± 0.7	0.071
HGB (gm/dl)	13.9 ± 1.2	13.6 ± 0.7	0.453
HCT (%)	40.9 ± 3.4	38.2 ± 2.6	**0.015**
MCV (fl)	86.9 ± 4.5	87.8 ± 2.2	0.475
MCH (pg)	30.4 ± 2.1	31.1 ± 1.3	0.225
MCHC (gm/dl)	34.8 ± 1.7	35.2 ± 0.6	0.412

Data expressed as mean ± Standard Deviation (SD), n=number of samples, RBC=Red Blood Cell; HGB= Haemoglobin, HCT= haematocrit, MCV = mean cell volume, MCH = mean cell haemoglobin, MCHC= mean cell haemoglobin concentration.

Generally, among participants across the three trimesters, haemoglobin concentration was higher in cases than controls. Except in the second trimester, the observed differences were statistically significant in the other trimesters (Figure 3). Haematocrit value was shown a consistent significant increase in cases across all the trimesters.

Figure 3: Dynamics of Haemoglobin concentrations across the gestational age among cases and controls.

4.1.4. Risk factors associated with gestational diabetes mellitus.

Logistic regression was performed to define the strength of variables associated to GDM. HGB, HCT, MCHC were found to be associated with GDM. Factors associated with GDM were identified by using binary logistic regression model. In binary logistic regression the following variables were significantly associated, HGB (OR: 1.976, 95%CI: 1.216-3.209, p =0.006), HCT (OR: 1.471; 95% CI: 1.232-1.756, P< 0.001), MCHC (OR: 0.598; 95% CI: 0.405-0.884, P= 0.01). This indicates that, iron (HGB) status of pregnant mother has an association with mothers who did not have GDM. On the other hand, RBC (OR: 1.038; 95%CI: 0.774-1.391, P=0.805), MCV (OR: 1.026; 95% CI, 0.925-1.137, P=0.630) and MCH (OR: 0.918; 95% CI: 0.405-0.884, P= 0.442) were not significantly associated with outcome or GDM (Table 6).

In age adjustment for the variables associated with GDM, RBC (AOR: 1.007; 95% CI: 0.735-1.381, p=0.963), HGB (AOR: 1.896; 95% CI; 1.130-3.180, p=0.015), HCT (AOR; 1.473; 95% CI: 1.217-1.782, p<0.001), MCV (AOR: 1.061;95% CI; 0.948-1.189, p=0.303), MCH (AOR: 0.964; 95% CI: 0.762-1.022, p=0.760), MCHC (AOR:0.671; 95% CI; 0.444-1.014, p=0.580) (Table 6).

In comparison to those with Hgb concentration < 13 g/dl, pregnant women with Hgb concentration > 14 were 4.6 times more likely to develop GDM. Furthermore, for every unit increase in Hgb concentration, the odds of GDM occurrence increases by 1.97 times.

Table 6: Logistic regression analysis of factors associated with Gestational Diabetes Mellitus

Variables	Bivariate Analysis		Multivariate Analysis	
	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
RBC($x10^6/mm^3$)	1.038 (0.774, 1.391)	0.805	1.007 (0.735, 1.381)	0.963
HGB (gm/dl)	**1.976** (1.216, 3.209)	**0.006**	**1.896** (1.130, 3.180)	**0.015**
HCT (%)	**1.47** (1.232, 1.756)	**<0.001**	**1.473** (1.217, 1.782)	**<0.001**
MCV (fl)	1.026 (0.925, 1.137)	0.630	1.061 (0.948, 1.189)	0.303
MCH (pg)	0.918 (0.737, 1.143)	0.442	0.964 (0.762, 1.220)	0.760
MCHC (g/dl)	**0.598** (0.405, 0.884)	**0.010**	0.671 (0.444, 1.014)	0.580
Haemoglobin (g/dl)				
< 13	1			
13 - 14	1.538 (0.523, 4.523)	0.434	-	-
> 14	**4.600** (1.424, 14.856)	**0.011**	-	-

OR= odds ratio, RBC=Red Blood Cell, HGB= Haemoglobin, HCT= haematocrit, MCV =mean cell volume, MCH=mean cell haemoglobin, MCHC=mean cell haemoglobin concentration.

4.2. Discussion

The purpose of this study was to assess the association between iron status and GDM. Various biochemical and haematological markers are used in recent days to measure iron status, of which, HGB and RBC indices remain in the routine blood tests to evaluate basic health/anaemia status of pregnant mothers during antenatal care in most developing countries including Ethiopia. The present study used this opportunity to assess how do iron status during pregnancy is associated with GDM through making use of those routine iron status indicators.

During early pregnancy (at first trimester), higher concentrations of multiple indices (HGB, HCT and MCHC) were show a significant association with GDM occurrence. Regarding iron status indices measured at third trimester, significantly higher values of HGB and HCT were associated with GDM. Whereas, only higher value of HCT was associated with GDM in the second trimester. The mean Hgb concentrations among cases (13±1 g/dl) and controls (12.5±1.1 g/dl) were observed at first trimester (p=0.003) in the present study. This finding is in line with recent studies that reported a high prevalence of GDM among women with high haemoglobin levels in early pregnancy (Lao, 2004, Wang *et.al,* 2012, Yerebasmaz *et. al.*, 2015; Chen *et al.*, 2018; Wang *et al.* 2018; Rayis *et al.*, 2020).

High maternal HGB has not received the same attention as anemia because it is more looks as a symbol of good nutrition status (Rasmussen *et al.*, 2005). However, there are studies that show health problems including GDM associated with increased maternal iron status or HGB (Scholl, 2005, Nastaran & Nourossadat, 2012, Mehrabian & Hosseini, 2013 and Fernandez-coa *et. al*, 2016). In line with present studies, particularly show that high levels of iron status during first trimester are more exposed to GDM (Alamolhoda, *et al.*, 2010, Yerebasmaz *et al.*, 2016).

How high haemoglobin levels can predispose pregnant women to GDM is unclear and seems to have context-specific; however, generally, a high haemoglobin level indicate high iron and ferritin levels (Afkhami-Ardekani and Rashidi, 2009; Kaur *et al.*, 2015). Iron overload and high ferritin levels are thought to play a role in the development of GDM through several potential mechanisms: (i) free iron can promote several cellular reactions that generate reactive oxygen species and increase the level of oxidative stress as a strong pro-oxidant; (ii) oxidative stress induced from excess iron accumulation can cause damage to and death of pancreatic beta cells and, consequently, contribute to reduced insulin synthesis and secretion;

(iii) in the liver, high iron stores may induce insulin resistance by means of damaging insulin signaling as well as by limiting the capacity of the liver to extract insulin (Khambalia *et al.*, 2016; Rawal, 2017). Generally, an increase in oxidative stress is part of normal pregnancy, routine iron supplementation in women without iron depletion might contribute to further oxidative stress. Furthermore, increased iron stores in the general population have been associated with increased incidence of diabetes (Scholl *et al.*2005).

Regression analysis of the present study demonstrated that for every unit increase in Hgb concentration, the odds of GDM occurrence nearly doubles (OR = 1.97). Furthermore, in comparison to those with Hgb concentration < 13 g/dl, pregnant women with Hgb concentration > 14 were 4.6 times more likely to develop GDM.

Previous study by Lao *et al.* (2002) reported that a high maternal haemoglobin levels (> 13 g/dl) led to a higher risk of developing GDM. In settings where the prevalence of anaemia is higher, even a smaller rise in iron status during pregnancy shown to predispose GDM. This is evidenced in Sudanese study by Rayis *et al.* (2020) that showed women with Hgb > 10.8 g/dl were at risk of having GDM (with the 2.5 times more odds). Likewise, Tarim *et al.* (2004) indicated that pregnant women with haemoglobin levels of 12.2 g/dl had a significant risk of developing GDM. These findings further suggest the variation in Hgb cut-off as a risk for having GDM is partly dependent on prevalence of anaemia among women of reproductive age in a particular setting.

Iron and folic acid supplementation during pregnancy in developing countries has improved the maternal and fetal outcome in Ethiopia as well (Desta *et al.*, 2019). Iron supplementation during pregnancy increases maternal iron status including hemoglobin, serum iron, MCV, transferrin saturation, and serum ferritin. (Shukla P, Xiao X, Mishra R.,2014). Interestingly, this iron supplementation increases lipid peroxidation possibly through triggering inflammatory processes. (Lachili B, Hininger I, Faure H, *et al.*, (2001). Therefore, risk of GDM in pregnant women becomes raising questions about routine recommendations on iron supplementation in pregnancy (Rawal *et al.*, 2017). The study on improvement in overall nutritional status and excessive use of supplementation in pregnancy may be responsible for the increasing prevalence (predictor) of GDM in developing areas. So, this requires the assessment of hemoglobin status in all pregnant women, and prescribing iron supplements to only those with anemia to prevent an iron overload (Zargar *et al.*, 2004).

The association between HGB levels and adverse pregnancy outcomes differs by trimesters. However, it is more evident in early pregnancy for low HGB levels, and it is evident in all trimesters for high Hgb concentrations (Dewey, 2017, Laflamme, 2011). However, in current study, at first trimester, means of, HGB, HCT and MCHC have statistically significant association with GDM. HCT is significant at second trimester (p=0.015). At third trimester HGB and HCT are significant (p=<0.001). This indicates that high iron status predicts GDM or it seems that high levels of hemoglobin or iron status during the first trimester may be a warning sign for development of gestational diabetes over the next gestational weeks of pregnancy. HGB and HCT level in GDM might probably due to an increase in blood glucose level which probably favors the proliferation of red blood cells (Hope *et al.*, 2019). The result agrees with the study carried out by Dhirendra *et al.*, 2016 and Nasiri *et al.*, 2011. So that, HGB and HCT levels must be screened during pregnancy because both low and high HGB levels are related with adverse pregnancy outcomes on maternal and fetal well-being (Çakmak, 2011, Nasiri *et al.*, 2011).

The first trimester is an important lifestyle to reduce the risk of adverse outcomes in GDM and there are many studies to analyse serum biomarkers either alone or in conjunction with other maternal risk factors to predict GDM in first trimester. However; even though the studies, there is not yet a method and single biomarker that predicts the disease (GDM) accurately at first trimester (Yeral *et al.*, 2014 and Ozgu-Erdinc *et al.*, 2015). HGB levels assessed in second trimester of gestations affected by the iron supplementation received during the second half of pregnancy and probably obscured the true difference of hemoglobin level among the groups (Galal & Salah, 2015 and Gungor *et al.*, 2007).

In line with the current study, one prospective study of non-anaemic pregnant mothers in china at third trimester also shows high maternal HCT levels could be used in predicting GDM (Wang *et al.*, 2012). Hematocrit is also commonly used as an indicator of anemia. But, Hematocrit and hemoglobin are interchangeable using the following conversion factors (100g hemoglobin=6.2mmol hemoglobin = 0.20 l/l (WHO. 2001).

Studies focusing on status of HGB levels and pregnancy outcomes like GDM are limited, and the findings from those studies again present with some variation due to variations in study designs, sample sizes, populations and the time of HGB testing or gestational weeks. For instance, the study by Galal & Salah (2015) showed there was no significant difference in HCT concentrations between pregnant mothers who developed GDM when compared to who

did not develop. In other case control study, there is no difference between GDM and control groups with respect to HCT (Rajurkar *et al.*, 2012; Javadian *et al.*, 2014). This condition may be due to dehydration which causes falsely high HGB and iron concentration which disappears when proper fluid balance is restored (Rajurkar *et al.*, 2012). HGB levels decrease according to the higher increment in plasma volume (Mani & Duffy, 1995).

Limitation of the study

- Assessment of serum ferritin status (iron depot) and other iron specific indicators are not measured together due to that it is not done for cases.
- Iron specific measures serum iron/ferritin are considered as an ideal/gold standard measure of iron store/status than haemoglobin concentration. However, studies that used multiple iron status indicators including serum iron, ferritin and haemoglobin concentrations in relation to GDM occurrence have shown that haemoglobin concentration was well resonate with iron-specific indicators – with similar pattern of iron status was observed among participants in comparison to iron specific indicators. Moreover, in resource poor settings haemoglobin is the convenient and easier to conduct proxy indicator of iron status that is not affected by infection/inflammation.
- Even though nearly all pregnant women supplemented with iron-folate pill, the level of adherence to the supplemental iron was not assessed among participants. However, the present study partly tried to capture iron status and GDM association at early pregnancy (at first trimester) before the start of iron supplementation.
- All the required data were obtained from patient records and this limited us to from obtaining additional information regarding family history of diabetes, number of pregnancies, body weight/composition-based indices (like MUAC during pregnancy or pre-pregnancy BMI).
- Limited number of pregnant women notice their pregnancy earlier and visit the health facilities for follow-up. Whereas most of them do their first ANC visit beyond the first trimester of pregnancy and hence we could not find iron status of all pregnant women at early pregnancy.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusion

Higher iron status during pregnancy is associated with GDM. Haemoglobin concentrations at early pregnancy could be used as a proxy iron status indicator in predicting the potential occurrence of GDM. Haematocrit level have shown a consistent level across the three trimesters and an elevated level of this indicators could also be used as an additional predictor. With the exception of MCHC, the other RBC indices (MCV, MCH) were not associated to GDM occurrence.

5.2. Recommendations

Based on current study we would like to forward the following recommendations for the concerned bodies:

- **To healthcare practitioners:** As a part of routine antenatal follow-up, the practice of measuring haemoglobin concentration has to be strengthened. Those women presented with a higher haemoglobin concentration at early pregnancy may signal other health problems including GDM as the pregnancy progresses and therefore closer medical attention has to be given to those subjects. Similar caution has to be paid in prescribing supplemental iron that will overload with additional iron.
- **To policy makers:** Blanket iron supplementation recommendation has to be revisited as per individual's iron status. Furthermore, universal iron fortification with wheat flour or other vehicles has to consider both target intervention groups and those at risk of developing secondary health problems.
- **To researchers:** We recommend further future study with a prospective/cohort study design in larger samples to capture various other covariates affecting the association between iron status and GDM – including body wight/composition, family history of diabetes, number of pregnancies, previous GDM, level of adherence to iron supplementation – encompassing iron-specific indicators along with haemoglobin concentrations at early pregnancy. Determine cut-off value of how high haemoglobin levels or other iron status indicators can predispose pregnant women to GDM.

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ANNEXES

Annex 1: Consent form and information sheet – English Version

Dear participant!

In the case of promotion of mothers' health understanding the association of iron status and Gestational Diabetes Mellitus is needed. You are chosen to participate in this study. The choice is made consecutively.

In order to attain the goal effectively, I request your will and full cooperation. Confidentiality is strictly protected. It is your right to participate or to refuse in the study, if you do not won't to participate in the study, you can discontinue at any time. But your honest participation will have contribution to generate valid information that can be used for investigation design. So please be cooperative to give the sample. If there is anything that require clarification please ask the facilitator.

Do you wish to participate in the study?

Yes I want to participate ( )

No I do not want to participate ( )

Thank you!

Information sheet

Title: Assessment of the association between iron status and Gestational Diabetes Mellitus: A hospital-based case-control study at TASH. Ethiopia.

Principal investigator: **Dereje Meskela**

Institution: Addis Ababa University; center for food science and nutrition

Introduction: Diabetes is one of the most common medical problems that complicate pregnancy. Diabetes in pregnancy can be gestational or pre-gestational. Globally, Gestational Diabetes Mellitus remains a serious challenge especially in developing country. Ethiopia and background comorbidities compound the problem.

It is prevalent in most of the developing world and it is probably nutritional association. High maternal hemoglobin concentrations have been reported to have a positive correlation with adverse pregnancy outcome. Excess iron has been shown to affect insulin synthesis and secretion, increase lipid oxidation, and decrease glucose utilization in muscle; hepatic gluconeogenesis is also stimulated and a state of insulin resistance is induced.

Procedures

If you agree to participate, I will collect the 4ml blood for analysis.

Risks

Unless few pain, nothing harmful will come from your participation.

Benefits

There are no direct benefits to you. However the results will possibly help others. Based on the finding I will inform the authorized person to work on it and help others like you.

Compensation

There will be no compensation to you for participating.

Participant's right

If I have said things that are not clear to you, you may ask me without any fear, and I will give you answer and explanation .You may feel free and ask questions. Your participation in the study is entirely volunteer and up to you to decide. There is no penalty if you don't agree to participate. You can say no without worry. The hospital and the health care provider will continue to give care for you as usual.

Confidentiality By excluding names and other identifying numbers from the questionnaire confidentiality of information will be assure. Test results and any information about your child will be kept privet.

Persons to contact

If you have any question, you can ask at any time, if you have additional questions about the study you may contact: Dereje meskela.Email,deremesk@gmail.com. Phone No. 0922573157.

Do you wish to participate in the study?

Yes I want to participate (_) Name _____Signature _____Date

No I do not want to participate (_)

Thank you for your cooperation!

Annex 2: Consent form and information sheet – Amharic Version

አዲስ አበባ ዩኒቨርስቲ ስነ የምግብ ሳይንስና ኒውትሬሽን ማዕከሌ

የሦስተኛ ዓመት የድህረ ምረቃ ተማሪ የሚያደርጉት ጥናት

የጥናት ፈቃድ ፎርም

በጥቁር አንበሳ ስፒሻሊይዝ ሆስፒታሌ የሄሞግልቢን መጠን እና GDM ህመምተኞች ሊይ የሚካሄድ ጥናት::

ዋና ተመራማሪ:- Dereje Meskela , ከአዲስ አበባ ዩኒቨርስቲ ስነ የምግብ ሳይንስና ኒውትሬሽን ማዕከሌ

ማብራሪያ:- አይረን የሚባሇው ንጥረ ነገር ሇሰውነታችን በጣም አስፈሊጊ በመሆኑ በተሇይም ደግሞ የነርቭ ስርዓት እናም የአከስጅን ዝውውር ሰውነታችን በሚፈሌገው መጠን እንዲከናወን ያደርጋሌ:: በመሆኑም በይበሌጥ ሇሾችግሩ ተጋሊጭ በመሆናቸው ምክንያት ጥናቱ በእነሱ ሊይ እንዲሆን አድርጎታሌ:: እንዲሁም በአይረን የሚጠቁ ቁጥር በከፍተኛ ደረጃ ቁጥራቸው እየጨመረ በምጣቱ ችግሩን አውቆ መፍትሄ ሇመስጠት ያስችሌ ዘንደ የሚደረግ ጥናት ነው:

መመሪያዎች:-

ጥናቱ ሊይ ሇመሳተፍ ከተስማማሽ 4 ሲሲ ደም ከሌጅሽ ሊይ ሇምርምር ይወሳሌ

ስጋት:-

ያንቺ መሳተፍዎ ምንም አይነት ጉዳት አያደርስም

ጥቅም:-

ምንም አይነት ቀጥተኛ ጥቅም ሊንቺ አይኖርም:: ግን በተዘዋዋሪ ሊንቺ የጥናቱ ውጤት ሊይ ተመርኩዞ ሇ ወጥ ሉያመጡ የሚችለ ሰዎች በማሳወቅ እንዲሰሩበት ያስችሊሌ::

ማካካሻ:-

በማሳተፍዎ ምንም አይነት ማካካሻ አይኖረውም::

የተሳታፊ መብቶች:-

ምንም አይነት ግሌጽ ያሌሆነ ነገር ካሇ የፈሇግሽውን ጥያቄ ካሇምንም ፍርሀትና ጭንቀት መጠየቅ ትችያሇሽ:: ተሳታፊ በሙለ ፍቃደኝነት ሊይ የተመሰረተ ነው::

ባሇመሳተፍዎ የሚደርስብሽ ምንም አይነት ቅጣት አይኖረውም:: ሆስፒታለም ሆነ የጤና ባሇሞያዎቹ የሚያስፈሌገውን አገሌግልት ሌክ እንደበፊቱ ይሰጣለ::

ሚስጥራዊነቱ ማንኛውም ገሊጭ የሆኑ ነገሮች ማሆትም ስም ቁጥር የመሳሰለትን በማስወገድ ማንኛውን መረጃ በሚስጥር ይጠበቃሌ::

ጉዳዩ የሚመሇከታቸው ሰዎች አድራሻ

ምንም አይነት ጥያቄ ወይም አስተያየት ቢኖራችሁ Dereje Meskela (deremesk@gmail.com) ስሌክቁጥር : 0922573157 ብሆው ይጻፉሌን/ይደወለሌን::

ጥናቱ ሊይ ሇመሳተፍ ፍቃደኛ ኖት

ስም ---------- ፊርማ ---------- ቀን ----------

የጥናቱ አስተባባሪ ፊርማ ----------

ስለትብብርዎት እናመሰግናሇን::

Annex 3: Letter of Ethical approval

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University
OFFICE OF THE DEAN
የተፈጥሮና ኮምፒውቲንግ ሣይንስ ኮሌጅ
አዲስ አበባ ዩኒቨርሲቲ
የዲን ጽ/ቤት

Ref. No.
የጥ/
Date
ቀን

CNSDO/185/12/19
November 13, 2019

To Whom It may Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on October 18 /10/2019 Minute No. IRB/40/219 has examined the project proposal entitled " **Assessment of the association between iron status and Gestational Diabetes Mellitus" : a case-control study**
by Dereje Meskela (PhD) from the Addis Ababa University.

The proposal is approved for implementation.

With regards,

Dr. Addisalem Abaineh
Dean , College of Natural & Computational Science

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