Shandi University

Faculty of Graduate studies

Assessment of iron status in pre-eclamptic pregnant ladies attending Omdurman Midwives Hospital

A thesis submitted for partial fulfillment of the requirement to award Master degree in Medical Laboratory sciences majoring Hematology

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قال تعالى:

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صدق الله العظيم

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Dedication

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to my heart.
My humble effort , I dedicate to my sweet and loving

Father & Mother

Whose affection , love , encouragement and prayers make me able to get such success and

Honor.

Along with all hard working and respected

Teachers.

Acknowledgment

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List of abbreviation:

Cl	Confidence interval
OR	Odds Ration
HELLP	Hemolysis elevation of liver enzymes and low platelets
ARDS	Acute respiratory distress syndrome
IUGR	Intrauterine growth restriction
CAD	Coronary artery disease
MPV	Mean platelets
PECAM	Platelet endothelial cell adhesion molecule
SGA	Small for gestation age
T2DM	Type2 diabetes mellitus
LDH	Lactic acid Dehydrogenase
TIBC	Total iron binding capacity
UIBC	Unsaturated iron binding capacity
FR	Free radical
LDL	Low density lipoprotein
ROS	Reactive oxygen species
CD36	Cluster of differentiation
ng/ml	nanograms per milliliter
mg/dl	Micrograms per deciliter
Umol/L	Micromoles per liter

الخلاصة

أجريت هذه الدراسة على السيدات الحوامل اللواتى يعانين من تسمم الحمل و حضرن لمستشفى أم درمان للقابلات خلال الفترة من فبراير حتى يونيو 2018.

أدرجت في هذه الدراسة 50 من سيدات تسمم الحمل بالإضافة إلى 30 من السيدات الحوامل الأصحاء اللواتي يتطابقن معهن فى العمر تم اختيارهم كمجموعة ضابطة في هذه الدراسة.

هدفت هذه الدراسة إلى الكشف عن التباين في مستوى الحديد ، الفيريتين ، والقدرة الكلية للربط الحديدي والنسبة المئوية لتشبع الترانسفيرين وربط التغيرات المكتشفة مع العمر ، والتاريخ السابق لتسمم الحمل وبروتين البول بالاضافة الى مؤشر كتلة الجسم

وجد أن مستوى الهيموغلوبين أقل بكثير في حالات تسمم الحمل (الاختبار: 11.5 ± 0. التحكم: 12.5 ± 0.62 g / dl، .(P-value 0.00وكانت هناك زيادة في مستوى الحديد (الاختبار: 140.6 ± 51.3 mg / dl، التحكم: 71.7 ± 19.7 mg / dl ، (P-value0.008،ومستوى الفيريتين (الاختبار: 76.7 ± 27.3 mg / dl، التحكم: 62.9 ± 28.2 mg / dl ، (P-value0.034، والنسبة المئوية للتشبع في الترانسفيرين (الاختبار: 62.5 ± 28.7٪ ، التحكم: 27.5 ± 5.9٪ (P-value0.034وتقلل القدرة الكلية للربط الحديدي بشكل كبير في (الاختبار: 247.6 ± 57.7 mg / dl، التحكم: 270.0 ± 33.9 mg / dl، .(P-value0.047

لا توجد آثار ذات دلالة احصائية للتغير في العمر ، مؤشر كتلة الجسم أو بروتين البول على أي من المعلمات المقاسة أعلاه.

هذه النتائج تتفق مع نتائج البحوث المنشورة.

Abstract

This study was carried out on pre-eclamptic pregnant ladies attending Omdurman Midwives Hospital during the period from February till June 2018.

50 pre-eclamptic pregnant ladies in addition to 30 control healthy pregnant ladies matching for age were included in this study .

This study aimed to detect the variation in the level of serum Iron, Ferritin, Total iron binding capacity and percentage saturation of Transferrin in pre-eclamptic pregnant women compared with control and to correlate the detected variation with the age , previous history of pre-eclampsia , urine protein and body mass index.

It had been found that the haemoglobin level a significantly lower in pre-eclampsia (Test :11.5± 0.84g/dl , control :12.5±0.62g/dl , P-value 0.00) . There was significant increase in serum iron (Test : 140.6±51.3mg/dl , Control : 71.7±19.7 mg/dl, P-value0.008) , serum ferritin (Test : 76.7±27.3mg/dl , Control : 62.9±28.2 mg/dl, P-value0.034) , percentage saturation of transferrin (Test : 62.5± 28.7% , Control : 27.5±5.9%, P-value0.034) and the total iron binding capacity was significantly reduced in pre-eclamptic pregnant ladies (Test : 247.6± 57.7mg/dl, Control : 270.0± 33.9mg/dl, P-value0.047).

No significant effects for variation in age , BMI or urine protein on any of the above measured parameters .

Previous history of pre-eclampsia affect significantly haemoglobin level (P-value 0.026) , serum iron (P-value 0.046) , transferrin and TIBC (P-value 0.037 & 0.012).

These findings agree with the published literature .

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Chapter one

Introduction

1. Pre-eclampsia

In the past, the definition of preeclampsia has been inconsistent and this has led to difficulty in comparing studies on treatments or outcomes .There is now a widely accepted classification system of hypertensive disorders in pregnancy which defines pre-eclampsia as at least 140/90 mmHg recorded on at least two separate occasions and at least 4 hours apart and in the presence of at least 300 mg protein in a 24 hour collection of urine, arising de novo after the 20th week of pregnancy in a previously normo- tensive woman and resolving completely by the sixth postpartum week [(1)].Pre-eclampsia called also toxemia of pregnancy and gestosis .It usually resolves after delivery of the fetus and placenta. However, women who develop preeclampsia appear to be at increased risk for developing cardiovascular complications later in life [(2)],this condition occurs in 5% to 8% of pregnancies and may involve other systems, such as the liver.

Preeclampsia occurs in 5 to 8 per cent of pregnant women worldwide [(3,4)] .

Chronic hypertension (with or without renal disease) existing prior to pregnancy can predispose to the later development of superimposed pre-eclampsia. Even in the absence of superimposed pre-eclampsia, chronic hypertension is associated with increased maternal and fetal morbidity and pregnancies complicated by chronic hypertension should therefore be regarded as high risk.

Non-proteinuric gestational hypertension, i.e. hypertension arising for the first time in the second half of pregnancy and in the absence of proteinuria, is not associated with adverse pregnancy outcome. Every effort therefore should be made to clearly distinguish it from pre-eclampsia [4].

Abnormal placentation related to immune mechanisms and mal-adaptation of the placenta may be the first step in the etiology and development of pre-eclampsia [5,6] .

Pre-eclampsia is a major cause of maternal mortality (15-20% in developed countries) and morbidities (acute and long-term), perinatal deaths, pre-term birth, and intrauterine growth restriction [7] ,Pre-eclampsia occurs in an estimated one in 20 pregnancies. It can develop into eclampsia, or convulsive fits, which account for up to 10 percent of maternal deaths. From another public health perspective, it is alarming that the rate of pre-eclampsia has increased in worldwide especially in developed countries by 40% between 1990 and 1999 due to an increase in number of older mothers and multiple births, conditions known to increase the risk of pre-eclampsia [8] .In developed countries, where maternal mortality attributable to pre-eclampsia has been reduced, the condition primarily affects fetal well-being through intrauterine growth retardation, preterm birth, low birth weight, and peri-natal death [9,10].

Pre-eclampsia is a common condition, but the etiology remains unknown. Despite numerous basic, clinical, and epidemiologic studies that have been conducted over the past half-century, knowledge of the etiology and pathogenesis of preeclampsia remains elusive. It may be placental in origin and may also be influenced by maternal factors such as obesity, diabetes

[11]. Pre-eclampsia appears to have a genetic component through the father as well as mother [12] . Currently, women who are at increased risk for preeclampsia are identified on the basis of epidemiologic factors [13] .

1.1.Epidemiology

Pre-eclampsia is more common in primi-gravid women. It is thought that the normal fetal–maternal transfusion that occurs during pregnancy and particularly during delivery exposes the mother to products of the fetal (and hence paternal) genome, protecting her in subsequent pregnancies. In line with this, the protective effect of first pregnancy seems to be lost if a woman has a child with a new partner. Overall the recurrence risk in a subsequent pregnancy is 20 %, but is much higher if severe pre-eclampsia developed at an extremely early gestation in the first pregnancy. There also appears to be a maternal genetic predisposition to pre-eclampsia as there is a three- to four-fold increase in the incidence of pre-eclampsia in the first degree relatives of affected women . Incidence Pre-eclampsia complicates approximately 2–3 % of pregnancies, but the incidence varies depending on the exact definition used and the population studied. In the most recent Confidential Enquiry (2003–2005), there were 18 deaths due to preeclampsia, making this the second most common cause of direct death in pregnancy and the puerperium in the UK [14] .

1.2.Risk factors for pre-eclampsia

1.2.1.Maternal-specific risk factors

1.2.1.1.Maternal age (years), Maternal height (in cm) and Body mass index

There is a conflicting data on the relationship of age with pre-eclampsia. Some studies have reported association between age and preeclampsia especially in elderly women above the age of 35 years, while others have shown an association of pre-eclampsia with younger age groups. Advancing maternal age as well as young maternal age is a risk factor for pre-eclampsia [15-18] .Amongst the complications during pregnancy, pregnancy induced hypertension was commonest complication in elderly primi- gravidas [19] . A high proportion of pre-eclampsia cases occur in those at the extreme ends of the reproductive age [20] . Women above 40 years had twice the risk of pre-eclampsia, whether they were pimiparous or multi-parous women [21] .

Shorter maternal height is associated with higher risk of preeclampsia [22] . There is evidence of strong and consistent relationship between high pre-pregnancy body mass index and pre-eclampsia [23, 24]. Studies have shown that obesity is a definitive risk factor for pre-eclampsia risk.

1.2.1.2.Past history of pre-eclampsia in multi-parous women

Mothers who had pre-eclampsia in the first pregnancy are known to be at a substantially higher risk to develop pre-eclampsia in a subsequent pregnancy [25,260] . Multi-parous patients with a past history of severe pre-eclampsia are a high risk population which should be identified early in pregnancy [27].

1.2.1.3.Maternal blood group

With respect to blood group O, A, B and Rh type, no statistically significant correlation with severe pre-eclampsia has been found. However in one study an increased risk of preeclampsia for mothers with blood type AB (adjusted odds ratio = 3.07; 95% confidence interval 1.48-6.36) has been found out. Although these results should be considered with caution, they support the hypothesis of a linkage mechanism involving blood group in the inheritance of susceptibility to pre-eclampsia [28,29] .

1.2.1.4. Interval between pregnancies (in years):

Some researchers have found that a long time to pregnancy is associated with pre-eclampsia, supporting the hypothesis that some factors delaying clinically recognized conception may also be in a causal pathway for pre-eclampsia [30-33] . The risk in a second or third pregnancy was directly related to the time that had elapsed since the preceding delivery, and when the inter birth interval was 10 years or more, the risk approximated that among nulliparous women. After adjustment for the presence or absence of a change of partner, maternal age and year of delivery, the odds ratio for pre-eclampsia for each one-year increase in the inter birth interval was 1.12 (95%CI; 1.11 to 1.13) [34] . In a cross sectional study, women with more than 59 months between pregnancies had significantly increased risk of pre-eclampsia compared with women with intervals of 18-23 months [35] .

1.2.1.5. Medical history of any autoimmune disease

Women with rheumatic disease had significantly higher rates of pre-eclampsia and cesarean section. The relative risk of pre-eclampsia was particularly high in women with connective tissue disease [36] .

1.2.1.6. Family history of hypertension and diabetes among first blood relations

There are consistent findings of a positive association between family history of diabetes and hypertension and pre-eclampsia risk [37-39] . Family history of hypertension is a proxy measure for hereditary factors as well as common environmental or behavioral exposures that may underlie pre-eclampsia risk. Women's family history of chronic hypertension is an important and easy to acquire clinical risk marker of pre-eclampsia compared to the biochemical markers. The family history of hypertension questions can be used as screening tool to identify pregnant women who need closer monitoring for the signs of pre-eclampsia during early pregnancy.

1.2.1.7. Family history of Pre-eclampsia

In an primi-gravida , a family history of pre-eclampsia is associated with a fourfold increased risk of severe pre-eclampsia. This clinical history identifies a group who warrant close clinical surveillance during pregnancy and who may be suitable for trials of prophylactic interventions [40] . Genetic factors are important in the development of pre-eclampsia as well as gestational hypertension. In efforts to identify women with elevated risk of developing pre-eclampsia during pregnancy, a question about family history of pre-eclampsia is important [41,42] . The findings from these studies are biologically plausible for reason that epidemiological and clinical data document a close association between insulin resistance, type 2 diabetes, and hypertension [43] .

1.2.2. Exogenous factors

1.2.2.1.Stress & Working women status : (Work-related psychosocial strain)

Work related stress is also a risk factor for pre-eclampsia. Pre-

eclamptic women were also more likely to work during pregnancy (adjusted OR, 2.1; 95% Cl, 1.1 to 4.4) [44-45] . Working women had 2.3 times the risk of developing pre-eclampsia compared with nonworking women . Epidemiologic studies show that relative risk for pre-eclampsia is increased in many stressful situations [46-47] . Many risk factors for pre-eclampsia are stress-related. Low-stress situations, on the contrary, are protective. Stress in pregnancy corroborates all physio-pathologic theories for pre-eclampsia [48] .

1.3.Etiology and pathophysiology

Pre-eclampsia only occurs in pregnancy, but has been described in pregnancies lacking a fetus (molar pregnancies) and in the absence of a uterus (abdominal pregnancies) [49] , suggesting that it is the presence of trophoblast tissue that provides the stimulus for the disorder. Placental bed biopsies have demonstrated that trophoblast invasion is patchy in pre-eclampsia and the spiral arteries retain their muscular walls. This is thought to prevent the development of a high flow, low impedance utero-placental circulation. The reason why trophoblast invades less effectively in these pregnancies is not known but may reflect an abnormal adaptation of the maternal immune system .It is widely believed that defective trophoblast invasion results in relative under-perfusion of the placenta and that this releases a factor(s) into the maternal circulation that targets the vascular endothelium, The nature of this factor has not been identified, although numerous candidates have been proposed including a variety of growth factors, cytokines and products of oxidative stress caused by hypoxic-reperfusion injury in the placenta [49].
As the target cell of the disease process, the vascular endothelial cell, is so ubiquitous, pre-eclampsia is a truly multisystem disease, affecting multiple organ systems, often concurrently .

Normal pregnancy is characterized by marked peripheral vasodilatation resulting in a fall in total peripheral resistance despite an increase in plasma volume and cardiac rate. Pre-eclampsia is characterized by marked peripheral vasoconstriction, resulting in hypertension. The intravascular high pressure and loss of endothelial cell integrity results in greater vascular permeability and contributes to the formation of generalized edema .In the kidney, a highly characteristic lesion called 'glomerulo-endotheliosis' is seen. This is relatively specific for pre-eclampsia (it is not seen with other hypertensive disorders) and is associated with impaired glomerular filtration and selective loss of intermediate weight proteins, such as albumin and transferrin, leading to proteinuria. This, in turn, causes a reduction in plasma oncotic pressure and exacerbates the development of edema [49] .
In the liver, sub-endothelial fibrin deposition is associated with elevation of liver enzymes. This can be associated with hemolysis and a low platelet count due to platelet consumption (and subsequent widespread activation of the coagulation system).The presence of these findings is called HELLP syndrome (hemolysis, elevation of liver enzymes and low platelets). HELLP syndrome is a particularly severe form of pre-eclampsia, occurring in just 2–4 per cent of women with the disease. It is associated with a high fetal loss rate (of up to 60 per cent) [49] .

1.4. Clinical presentation

The various changes and symptoms that occur with pre-eclampsia vary according to the organ system or systems that are affected. These changes can affect the mother only, baby only, or more commonly affect both mother and baby. Some of these symptoms give the woman warning signs, but most do not .The classic symptoms of pre-eclampsia include a frontal headache, visual disturbance and epigastric pain. However, the

majority of women with pre-eclampsia are asymptomatic or merely complain of general, vague ‘flu-like’ symptoms [50] .

The most common symptom and hallmark of pre-eclampsia is high blood pressure. This may be the first or only symptom. Blood pressure may be only minimally elevated initially, or can be dangerously high; symptoms may or may not be present. However, the degree of blood pressure elevation varies from woman to woman and also varies during the development and resolution of the disease process. There are also some women who never have significant blood pressure elevation. The kidneys are unable to efficiently filter the blood (as they normally do). This may cause protein to be present in the urine. The first sign of excess protein is commonly seen on a urine sample obtained in the health care professional's office. Rarely does a woman note any changes or symptoms associated with excess protein in the urine. In extreme cases affecting the kidneys, the amount of urine produced decreases greatly. Swelling (especially in the hands and face) was originally considered an important sign for a diagnosis of pre-eclampsia. However, because swelling is a common occurrence in pregnancy, its utility as a distinguishing factor in pre-eclampsia is not high. Pitting edema (unusual swelling, particularly of the hands, feet, or face, notable by leaving an indentation when pressed on) can be significant . Rapid weight gain over a few days (more than 2 pounds a week) .

Nervous system changes can include blurred vision, seeing spots, severe headache , convulsions, and even occasionally blindness. Any of these symptoms require immediate medical attention.

Changes that affect the liver can cause pain the upper part of the abdomen and may be confused with indigestion or gallbladder disease. Other more subtle changes that affect the liver can affect the ability of the platelets to cause blood to clot; these changes may be seen as excessive bruising.

Changes that can affect the baby can result from problems with blood flow to the placenta, and therefore, the baby does not receive proper nutrients. As a result, the baby may not grow properly and may be smaller than expected, or worse the baby will appear sluggish or seem to have decreased activity for example baby's movement decrease [51,52] .

1.5.Complication of preeclampsia

Most women with chronic hypertension do well in pregnancy. In normal pregnancy, blood pressure falls at the end of the first trimester and then increases to pre-pregnancy values in the third trimester. For the majority of women with chronic hypertension, blood pressure follows the same pattern. Some women, however, experience a rise in blood pressure during pregnancy, which can in-crease their risk for stroke and other complications and may therefore require more aggressive antihypertensive treatment .A more worrisome complication of chronic hypertension is the development of superimposed pre-eclampsia .pre-eclampsia is more likely to occur in women who have poorly controlled hypertension, underlying renal disease and diabetes mellitus. At present, there is no treatment for pre-eclampsia except for delivery of the baby; therefore, babies of women who have this condition are frequently born prematurely. Another complication of chronic hypertension that may cause premature birth is placental abruption. An abruption is an early separation of the placenta from the wall of the uterus, usually leading to strong contractions, bleeding, and early delivery .

Complications of pre-eclampsia can affect both the mother and the fetus. Acutely, pre-eclampsia can be complicated by eclampsia, the development of HELLP syndrome, hemorrhagic or ischemic stroke, liver damage and dysfunction, acute kidney injury, and acute respiratory distress syndrome (ARDS) [51,52] .

The development of convulsions in a woman with pre-eclampsia is defined as eclampsia . Vasospasm and cerebral edema have both been implicated in the pathogenesis of eclampsia. Retinal hemorrhages , exudates and papilla-edema are characteristic of hypertensive encephalopathy and are rare in pre-eclampsia, suggesting that hypertension alone is not responsible for the cerebral pathology [49] . It is a sign that the underlying pre-eclamptic condition is severe and is associated with high rates of perinatal and maternal morbidity and mortality [51] .

HELLP syndrome is defined as hemolysis (microangiopathic), elevated liver enzymes (liver dysfunction), and low platelets (thrombocytopenia). This condition may occur in 10–20% of patients with severe pre-eclampsia and eclampsia [53] and is associated with increased maternal and fetal morbidity and mortality. In 50% of instances, HELLP syndrome develops preterm, while 20% of cases develop in late gestation and 30% during the post-partum period [54] .

Lowered blood supply to the fetus in pre-eclampsia causes lowered nutrient supply, which could result in intrauterine growth restriction (IUGR) and low birth weight [55] . The fetal origins hypothesis states that fetal under nutrition is linked with coronary heart disease later in adult life due to disproportionate growth [56] .

There are a wide variety of reasons why a baby may be born small including congenital anomalies, fetal infections and chromosomal abnormalities. However, most babies that are born small are either constitutionally small (i.e. healthy, but born to small parents and fulfilling their genetic growth potential) or are small secondary to abnormal placenta function and have Fetal growth restriction ,it is a major cause of neonatal and infant morbidity and mortality [59]. Because pre-eclampsia leads to a mismatch between the maternal energy supply and

fetal energy demands, pre-eclampsia can lead to Intrauterine Growth Restriction (IUGR) in the developing fetus [57] .Infants suffering from IUGR are prone to suffer from poor neuronal development and in increased risk for adult disease according to the Barker hypothesis. Associated adult diseases of the fetus due to IUGR include, but are not limited to, coronary artery disease (CAD), type 2 diabetes mellitus (T2DM), cancer, osteoporosis, and various psychiatric illnesses [58] . The risk of pre-eclampsia and development of placental dysfunction has also been shown to be recurrent cross-generationally on the maternal side and most likely on the paternal side. Fetuses born to mothers that were born small for gestational age (SGA) were 50% more likely to develop pre-eclampsia while fetuses born to both SGA parents were three-fold more likely to develop pre-eclampsia in future pregnancies [60] .

1.6. Hematological changes of preeclampsia

The hematological changes that appear in Pre-eclamptic pregnancy are divided into three major groups

1.6.1. Numerical and functional platelets anomalies

Up to 50% of pre-eclampsia associate thrombocytopenia is generally proportional to the severity and may precede clinical manifestations [61,62] . Thrombocytopenia may be severe, potentially life threatening. The major role played by the thrombocyte in the patho-physiology of pre-eclampsia is related to the release of thromboxane A2, with subsequent increase of thromboxane/ prostacyclin ratio [63-64] .Thromboxane A2 promotes vasospasm, induces supplementary platelet aggregation and endothelial damage, which add an important contribution to maintaining platelet dysfunction and promoting platelet consumption (activation, aggregation, micro-angiopathic hemolysis induced by severe vasospasm),resulting in thrombocytopenia, which is an important sign of severe/aggravating preeclampsia.

Therefore, excessive platelet activation is associated with endothelial dysfunction, thrombosis in microcirculation, end organ degenerative necrosis, placental infarction (IUGR). The response of a normal bone marrow is followed by the release of young elements, with in-creased mean platelet volume (MPV) ; studies show that MPV is significantly increased in women with preeclampsia [65] and it is even linked to the cases with altered uterine Doppler velocymetry predictive for pre-eclampsia [66] . Studies also advocate a more extensive platelet activation in pre-eclamptic pregnancies, suggested by an increased expression of P-selectine , CD63 and PECAM – platelet surface glycol-proteins, markers of platelet activation [67,68] .

1.6.2. Alterations of hemoglobin and erythrocyte parameters

Most frequently hemo-concentration manifested with increased hematocrit [69] due to increased endothelial permeability; anemia may also be present in rare cases. The anemia that is strictly connected to pre-eclampsia (and not in the context of the pregnancy, such as due to hemo-dilution, bleeding and deficient iron balance) is most frequently associated with HELLP syndrome and it is due to micro-angiopathic intravascular hemolysis – physical destruction of erythrocytes in the microcirculation affected by disseminated micro-thrombosis . The anemia will be slight/medium, normochromic, normocytic, with a hemolytic pattern (increased bilirubin – unconjugated fraction, increased Lactic Acid Dehydrogenase (LDH) , increased reticulocyte count), fragmented erythrocytes and micro-spherocytes or peripheral blood smear [70] , and, in severe forms, hemoglobinuria and hemoglobinemia .

1.6.3. Iron profile

A very small quantity of iron is present in most of the cells of the body, in plasma and in other extra cellular fluids .The body

conserves iron supply, so that less than 0.1% of the body iron is lost daily, mostly in desquamated Cells .The iron exist in two oxidized states ferric (Fe+3) and ferrous(Fe+2) Ionic forms. The mucosal cells take up ferric form of iron during its absorption. The iron (Fe+3 /Fe+2) act as cofactor for many biochemical activities of the cell.

1.6.3.1.Serum iron

is a medical laboratory test that measures the amount of circulating iron that is bound to transferrin ,65% of the iron in the body is bound up in hemoglobin molecules in red blood cells .About 4% is bound up in myoglobin molecules. Around 30% of the iron in the body is stored as ferritin or hemosiderin in the spleen, the bone marrow and the liver. Normal range :

Men: 65 to 176 μg /dL
Women: 50 to 170 μg/dL
Newborns: 100 to 250 μg/dL
Children: 50 to 120 μg/dL
μg/dL = micrograms per deciliter

1.6.3.2.Transferrin

are iron-binding blood plasma glycoproteins that control the level of free iron (Fe) in biological fluids [71] .Transferrin glycoproteins bind iron tightly, but reversibly. Although iron bound to transferrin is less than 0.1% (4 mg) of total body iron, it forms the most vital iron pool with the highest rate of turnover (25 mg/24 h). It has a molecular weight of around 80 kDa and contains two specific high affinity Fe(III) binding sites. The affinity of transferrin for Fe(III) is extremely high [72] but decreases progressively with decreasing pH below neutrality (pH 7.4).When not bound to iron, transferrin is known as "apo-transferrin".

Percent Saturation of Transferrin %= S Iron \TIBC X 100

Normal range of Transferrin saturation:

Male 20–50%

Female 15–50% [73] .

1.6.3.3.Ferritin

is a globular protein complex consisting of 24 protein subunits forming a nano cage with multiple metal–protein interactions [74] . It is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes. Ferritin that is not combined with iron is called apo ferritin.
Ferritin is found in most tissues as a cytosolic protein, but small amounts are secreted into the serum where it functions as an iron carrier. It is produced by almost all living organisms, including bacteria, higher plants and animals [75] .
Normal range :

Men :	18–270 ng/ml
Women :	18–160 ng/mL
Children (6 months to 15 years) :	7–140 ng/mL
Infants (1 to 5 months) :	50–200 ng/mL
Neonates :	25–200 ng/mL

ng/mL = nanograms per milliliter

1.6.3.4.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 [76] .It is performed by drawing blood and measuring the maximum amount of iron that it can carry, which indirectly measures transferrin [77] . Increased TIBC levels are seen in Iron deficiency anemia ,Acute viral

hepatitis .

Decreased TIBC levels are seen in Liver related inflammation, neoplasia and chronic diseases , Severe malnutrition [78] .

Normal range

TIBC : 250–370 μg/dL (45-66 μmol/L)

μg/dL = micrograms per deciliter , μmol/L = micromoles per liter

1.6.3.5.Unsaturated Iron Binding Capacity (UIBC)

It denotes the amount of transferrin unbound to iron. It is 2/3rd of TIBC [79]

TIBC = UIBC + S. IRON

1.7.Relation of iron profile with pre-eclampsia

When tissues become ischemic, reactive oxygen species (ROS) such as superoxide and hydrogen peroxide are produced, but these ROS may not be able to initiate any cellular damage directly. The transition metal ions such as iron, arising from ischemic placenta by destruction of red blood cells from thrombotic, necrotic and hemorrhagic areas can generate highly reactive hydroxyl radical by Fenton reaction. This radical can initiate lipid per-oxidation which if uncontrolled results in endothelial cell damage [79-80] .

$Fe(ll) + H_2O_2 \rightarrow Fe(lll) + OH + OH^-$ (Fenton reaction) .

An imbalance between pro-oxidants and anti -oxidants results in oxidative stress which increases the potential for the development of pre-eclampsia [81]. There is raised serum iron, percent transferrin saturation, serum ferritin and decreased TIBC, UIBC and serum transferring in pre-eclamptic women compared to the normal healthy pregnant.

The elevated serum iron levels are due to hemolysis caused by physical destruction of RBC as a result of vaso-spasm or abnormal endothelial cell erythrocyte interactions. Excess iron is a causative factor of oxidative stress (i.e., in its radical form) involved in the pathogenesis of pre-eclampsia [82-84] . The excess iron released from destruction of RBCs can react with free radicals produced from cell membrane (as it is rich in polyunsaturated fatty acids) and circulating lipoproteins initiates lipid per-oxidation [85] . In addition to this , the damaged placenta is a site for release of free radicals (FR) in pre-eclampsia. The elevation or excess iron can also react with these released FR of placenta and can initiate and propagate lipid per-oxidation both in placenta and vasculature. This is one of the significant etiologic factor in the endothelial cell damage of pre-eclampsia [86] .

The doubling of percentage transferrin saturation is due to raised serum iron and decreased serum transferrin levels. Even at low concentrations iron components such as hemoglobin and heme can increase low density lipoprotein (LDL) oxidation, suggesting a role of iron in LDL oxidation of preeclampsia [87] .

The decreased TIBC correlates well with decreased serum Transferrin levels. This effect can cause increased proliferation of bacteria in blood due to excessive availability of free iron, and it also causes increased production of hydroxyl radicals in the tissues.

The reduced transferrin levels also leads to proteinuria in pre-eclampsia [88] . In pre-eclampsia unaccompanied by proteinuria, albumin and transferrin levels are similar to those found in the normal pregnant women , but there are significant falls in α_2-macroglobulin and IgG. When pre-eclampsia is accompanied by proteinuria there is a marked fall in albumin and transferrin , and an increase in α_2-macroglobulin .

The decreased UIBC contributes to release of iron free radicals from ischemic placenta in pre-eclampsia. The decreased UIBC, Serum transferrin levels suggests a role of iron in the development of endothelial dysfunction seen in pre-eclampsia [89] .

The increased ferritin levels of serum in pre-eclampsia women are thought to be due to hepatic damage, resulting in leakage of ferritin into circulation [90] . James M. Roberta et al. quoted that serum ferritin increase in pre-eclampsia as a marker of acute phase reaction. Because inflammatory responses are increased in pre-eclampsia, these results in alterations in iron homeostasis [91] . The hyper ferritinemia seen in pre-eclampsia is due to hepatic cell damage and increased ferritin synthesis by placenta. Thus, hepato-cellular damage is the likely reason for the increase in ferritin levels of women with pre-eclampsia [92] . The raised serum ferritin suggest the intensification of free radical oxidation, results in disturbance of anti-oxidant defence . The enzymatic function of transferrin contributes to an increase in vascular resistance and development of endothelial dysfunction [93-95] .

Chapter Two

Literature Review

Many studies have been performed to determine the change in iron status among pre-eclamptic pregnant ladies . In 2007 , at Department of Biochemistry, Frontier Medical College, Abbottabad with collaboration of Department of Obstetrics and Gynecology , Ayub Medical Complex, Abbottabad , one hundred pregnant women of age ranging between 15-35 years and having gestational age between28 to 34 weeks were studied .Fifty obstetric patients were identified as having pre-eclampsia. Serum iron, serum ferritin and transferrin saturation were significantly higher ($P<0.001$) in pre-eclamptic in comparison with control group. Total iron binding capacity and unsaturated iron binding capacity were significantly lower ($P<0.001$)in pre-eclamptic group when compared to the control group. Correlation coefficient between serum iron, total iron binding capacity (TIBC), serum ferritin, unsaturated iron binding capacity (UIBC) , systolic and diastolic blood pressure in pre-eclamptic group showed no significant positive correlation in any parameter [(96)] .

An article written by Kandi and Sabitha, et al. about pre - eclampsia and iron Status , published in the American Journal of Medical and Biological Research showed that the existing literature indicates that in preeclampsia, there is a possibility of vasospasm which may result in the destruction of RBC's leading to anemia and raised serum iron levels. Excess iron reacts with free radicals of cell membrane and lipoproteins initiating lipid peroxidation. This causes a change in the serum activities of ferritin, transferrin, ceruloplasmin and TIBC which may be responsible for hepatic dysfunction, increased vascular resistance and endothelial dysfunction. Therefore early identification of pre-eclampsia associated with genetic predisposition,

physiological and other associated contributing factors will help in better management and reduction in the morbidity and mortality to mother and fetus [97,98].

In 2002 , another study was conducted to investigate iron status parameters in pre-eclampsia with a view to exploring their possible contribution to the aetiology . serum samples from 40 pre-eclamptic women and matched pregnant control subjects at the John Radcliffe Hospital, Oxford, a number of iron status parameters were measured. Statistical analysis was by the Wilcoxon signed rank test and linear regression. Serum iron concentration, ferritin, and percent saturation of transferrin were significantly higher in the pre-eclamptic patients than in control subjects, whereas unsaturated iron- binding capacity and apotransferrin levels were significantly lower. No difference was found in hemopexin concentrations in the two groups. Gestationalage at the time of sampling was correlated (positively) with only two parameters, total and unsaturated iron-binding capacity, but only in the preeclampsia group. Eighteen percent of pre-eclamptic subjects had percent transferrin saturation levels in the region associated with iron overload [99] .

In Sudan , a study a conducted in Al-Neelein University to assess iron Status in Pregnant Ladies with Pre-eclampsia . This study found all iron status (Iron, Ferritin, Transferrin) was significant higher in pre-eclampsia, while TIBC was significant lower [100].

Rational

Pre-eclampsia is still one of the leading causes of maternal and fetal morbidity and mortality . Pre-eclampsia is a serious condition that affects between 5% - 10% of pregnant women .

Recent evidences suggest there may be several underlying causes leading to endothelial dysfunction and causing the sings of hypertension, Proteinuria and edema- findings that allow the diagnosis of the syndrome of Pre-eclampsia. It had been found that high ferritin is associated with increase risk for pre-term delivery and neonatal asphyxia, while the lower ferritin level was associated with decrease risk of Preeclampsia, pre-labour rupture of membranes , so this study aims to determine the changes in iron profile associated with pre-eclampsia among Sudanese pregnant ladies to focus on it's significance for early diagnosis and therefore proper intervention to avoid fatal complication.

Objectives

General Objective :

Assessment of iron status in pre-eclamptic pregnant ladies attending Omdurman Midwives Hospital during the period from February till June 2018.

Specific Objective :

-To detect the variation in the level of serum Iron, Ferritin, Total iron binding capacity and percentage saturation of Transferrin in pre-eclamptic pregnant women compared with control .

-To correlate the detected variation with the age , previous history of pre-eclampsia , urine protein and body mass index .

Chapter Three

Materials and Methods

3.1-Study type and design :

This is a descriptive cross - sectional study.

3.2-Study area:

Patients and control were recruited from Midwives Hospital , Omdurman .

3.3-Study population:

Obstetric patients were identified as having pre-eclampsia according to specific standard criteria (Blood pressure and proteinuria).

Gestational hypertension was defined as an increase of 30 mm Hg systolic or 15 mm Hg diastolic blood pressure compared with values obtained before 20 weeks gestation or an absolute blood pressure >140/90 mm Hg after 20 weeks gestation if earlier blood pressure were not known.

Proteinuria was defined as >500 per 24 hrs urine collection or >2+ on a voided or >1+ on a random urine specimen.

Healthy pregnant subjects were taken as controls, having uncomplicated pregnancy and were normotensive throughout gestation and without proteinuria.

3.4-Sample size:

50 pre-eclamptic pregnant ladies in addition to 30 control healthy pregnant ladies were included in this study .

3.5-Methods and tools of data collection :

Data was collected from each patient using structured questionnaire (see the appendix) in addition to reviewing the hospital form.

3.5.1.Sample collection and transport :

5 ml of veinous blood sample was collected from each patient and control using sterile disposable syringe and applying aseptic , standardized and non-traumatic vein puncture technique .The sample was then emptied into an EDTA vacutainers . Blood was mixed with the anticoagulant by gently inverting the container several times and labelled with patient name and serial number. After measuring the haemoglobin level ,plasma was separated by centrifugation at 3000rpm/5min .It was then used for the estimation of Iron, Ferritin and TIBC**.**

Patient was also instructed to collected random urine sample.

3.5.2.Measurement of Haemoglobin level:

Haemoglobin level was measured automatically by exposing the well mixed EDTA anticoagulant veinous blood sample to a well-controlled fully automated haemocytometer (Mindray BC-3000 plus).

3.5.3. Estimation of serum iron level and total iron binding capacity :

serum concentration of iron and TIBC were estimated using Ferrozine method via semi-automatic chemistry analyzer (Mindray BA-88A). This is based on the principle that iron bound to Transferrin is released in an acidic medium and the Ferric ions are reduced to Ferrous ions . The Fe(II) ions react with Ferrozine to form a violet colored complex. Intensity of the complex formed is directly proportional to the amount of iron present in the sample . For TIBC ,the serum is treated with excess of Fe (II)to saturate the iron binding sites on transferrin. The excess Fe (II) is adsorbed and precipitated and the Iron content in the supernatant is measured to give the TIBC .

For iron Assay , the following method was applied :

-Into clean dry test tubes labelled as Blank (B), Standard (S), Sample Blank (SB) and Test(T), the following were pipetted :

	B (ml)	S (ml	SB (m	T (ml)
Iron Buffer Reagent(L1)	1.0	1.0	1.05	1.0
Distilled water	0.2	_	_	_
Iron Standard(S)	_	0.2	_	_
Sample	--	--	0.2	0.2
Iron Colour Reagent(L2)	0.05	0.05	--	0.05

- The content were mixed well and incubated at room temperature for 5 min. The absorbance of the Blank (Abs .B), Standard(Abs. S), Sample Blank (Abs.SB) and Test Sample (Abs. T) against D.W.

Calculation :

- Iron in Mg/dl = Abs.T – (Abs.SB + Abs.B)/ Abs. S - Abs.B × 100

For TlBC assay , into a clean dry test tube , the followings were pipetted :

Serum	0.5 ml
TIBC Saturating Reagent (L1)	1.0ml

-This was mixed well and allowed to stand at R.T. for 10 min followed by addition of :

TIBC Precipitating Reagent (L2)	Approx. 50 mg

- Mixed well and allowed to stand at R.T. for 10min.Centrifuged at 2500-3000rpm for 10min to obtain a clear supernatant.
- The Iron content in the supernatant was then measured as above mentioned in the Iron Assay

Calculation :

TIBC in Mg/dl =Abs.T - (Abs. SB + Abs.B)/ Abs. S - Abs. B × 300

3.5.4. Estimation of serum ferritin level :

The AIA-PACK is a two-site immuno enzyme-metric assay which is performed entirely using the AIA-PACK F test cups .

Ferritin present in the test sample bound with monoclonal antibody immobilized on magnetic solid phase enzyme labelled monoclonal antibody in the test cups . washing was performed to remove the unbound enzyme label monoclonal antibody and then incubated with flaorogeme substrate . 4 methylumbellifery phosphate (4MUP) .The amount of enzyme labelled monoclonal antibody that bound to the beads was directly proportional to the ferritin concentration in the test sample. A standard curve was constructed, and unknown sample concentration was calculated using the curve .

3.5.5. Semi-quantitative measurement of urine protein :

This was performed by dipping urine testing strip in patient's sample and compare the colour changed with a standard label .

3.6-Data analysis:

Data obtained was analyzed using the statistical package of social science (SPSS) program version 21 and the result was presented in form of charts and tables .

3.7-Ethical consideration:

This study was approved from the ethical committee of Shandi University , Faculty of graduate studies and the administration of Midwives Hospital , Omdurman as well as consent from each patient .

Chapter Four

Result

50 pre-eclamptic pregnant ladies in addition to 30 control healthy pregnant ladies were included in this study . The frequency of their age interval is demonstrated in figure (4.1) .

Data as collected using structured questionnaire , the frequency of previous history of pre-eclampsia and number of pre-eclamptic pregnancies are shown in figures (4.2) and (4.3) . Their BMI status and urine protein level are demonstrated in figures (4.4) and (4.5) .

Iron profile as assessed in all test and control samples , the obtained result are shown in table (4.1) . The P-value for the effects of age variation , previous history of pre-eclampsia , No. Of pre-eclamptic pregnancies , BMI and urine protein on the measured parameters are demonstrated in tables (4.2) up to (4.6) respectively .

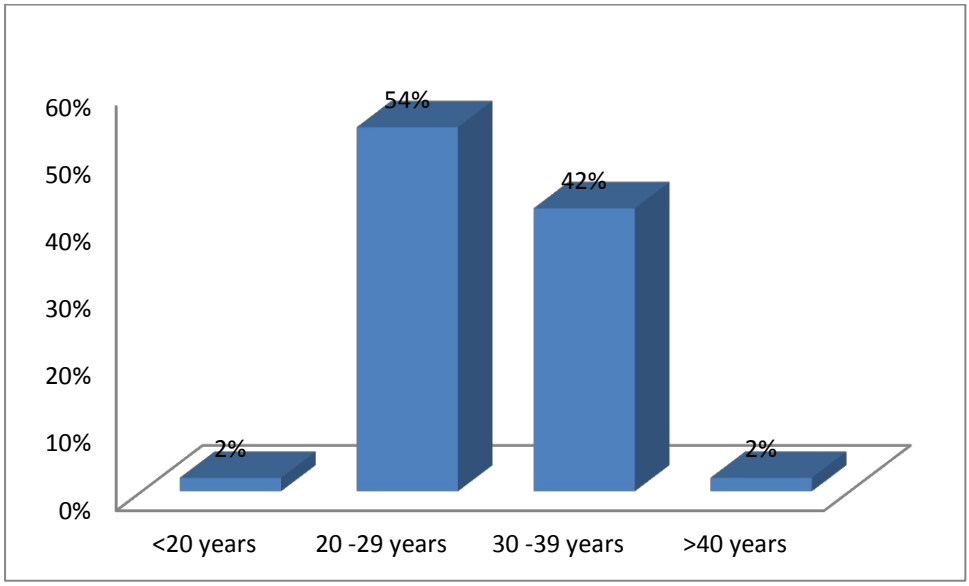

Fig.(4.1) : Frequency of age intervals of the study population

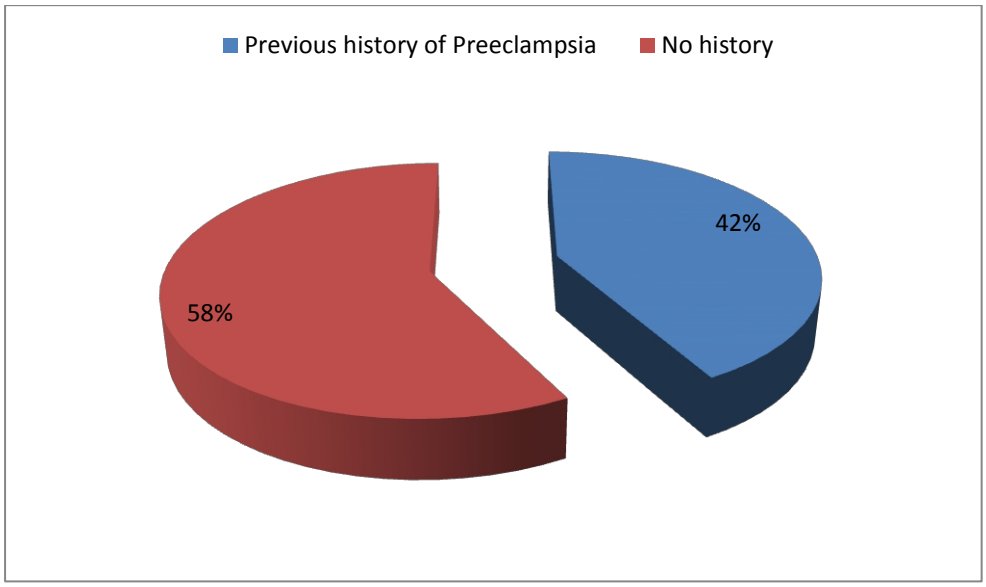

Fig.(4.2) : Frequency of previous history of pre-eclampsia in the study population

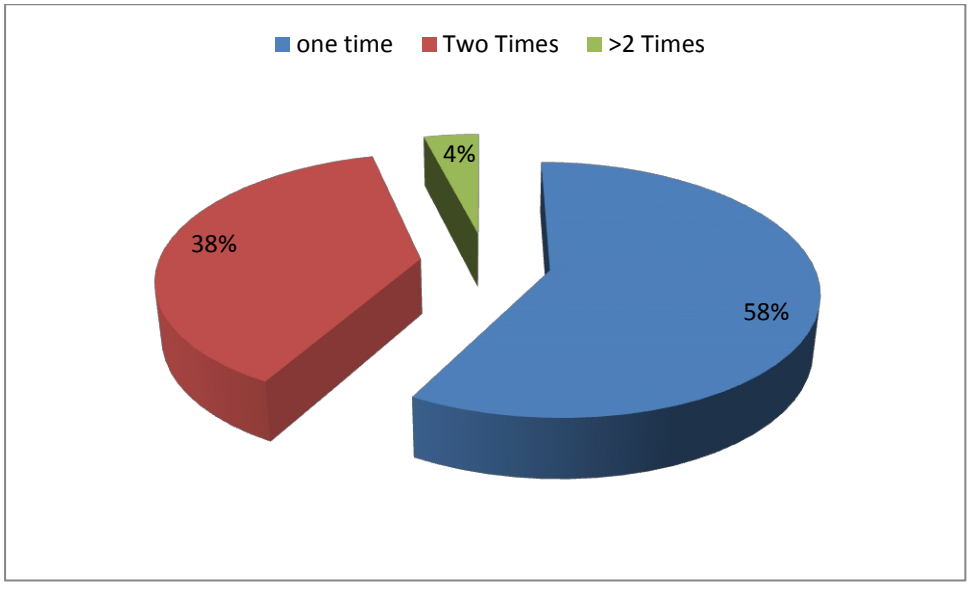

Fig.(4.3) : Frequency of number of pre-eclamptic pregnancies in the studied women

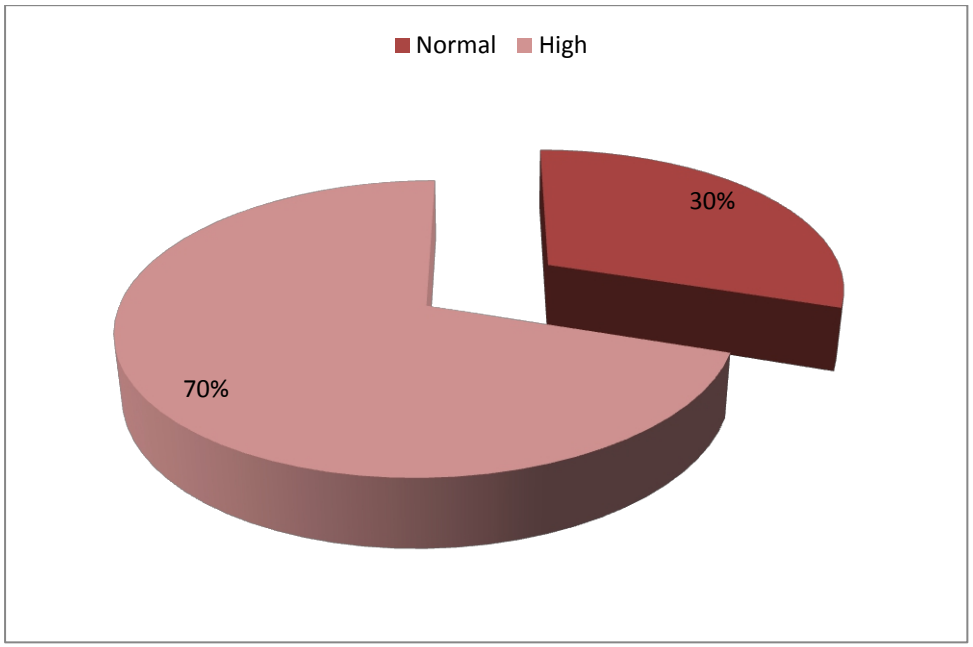

Fig.(4.4) : Frequency of Normal and high BMI of the study population

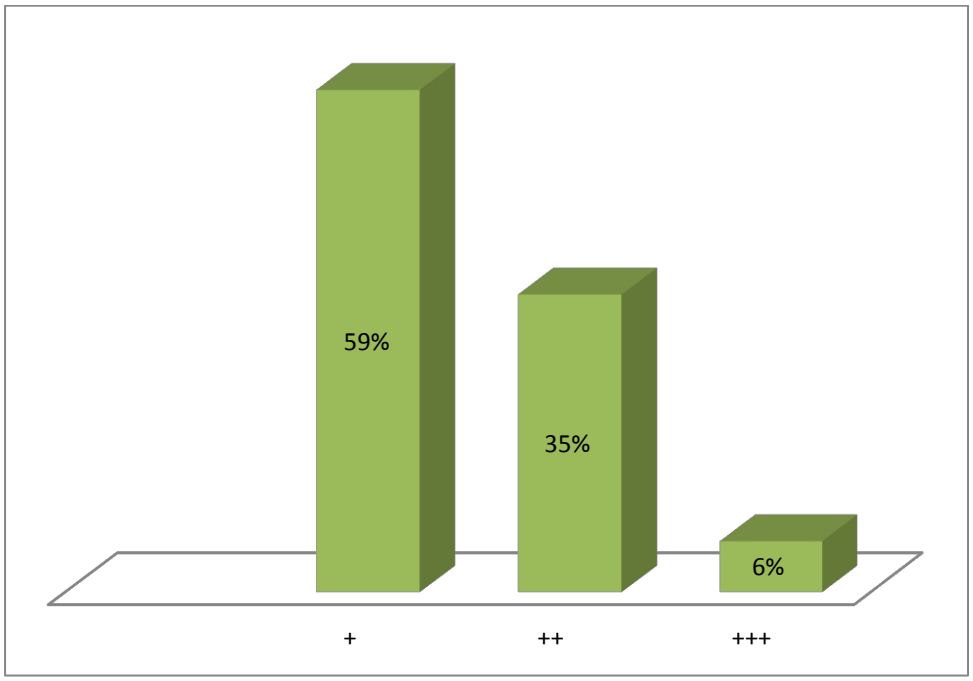

Fig.(4.5) : Frequency of urine protein level of the study population

Table (4.1) : Iron profile findings of the study population and the control group .

	Pre-eclamptic women	Control group	P-value
Hb level (g/dl)	11.5±0.84	12.5±0.65	0.00*
Serum iron level (mg/dl)	140.6±51.3	71.7±19.7	0.008*
Serum ferritin level (mg/dl)	76.7±27.3	62.9±28.2	0.034*
TIBC	247.6±57.7	270.0±33.9	0.047*
Percentage saturation of Transferrin level (%)	62.5±28.7	27.5±5.9	0.000*

Table (4.2) : P-value for the effect of age variation on the measured parameter s

Age	Hb	S. iron	Ferritin	Transferrin	TIBC
<20 years	0.655	0.089	0.175	0.416	0.411
20-29 years	0.994	0.219	0.070	0.089	0.324
30-39 years	0.784	0.570	0.403	0.333	0.112
>40 years	0.626	0.573	0.804	0.746	0.477

Table (4.3): P-value for the effect of previous history of pre-eclampsia on the measured parameters

Previous history	Hb	S. iron	Ferritin	Transferrin	TIBC
No	0.255	0.436	0.516	0.433	0.202
Yes	0.026*	0.046*	0.076	0.037*	0.012*

Table(4.4): P-value for the effect of number of previous pre-eclamptic pregnancies on the measured parameters

No. Of previous pre-eclamptic pregnancies	Hb	S. iron	Ferritin	Transferrin	TIBC
1	0.662	0.273	0.297	0.425	0.839
2	0.592	0.842	0.882	0.890	0.720
>2	0.865	0.109	0.217	0.261	0.284

Table (4.5) : P-value for the effect of number of BMI on the measured parameters

BMI	Hb	S .iron	Ferritin	Transferrin	TIBC
Normal	0.610	0.326	0.477	0.433	0.224
High	0.552	0.414	0.542	0.549	0.277

Table(4.6): P-value for the effect of urine protein level on the measured parameters

Urine protein	Hb	S. iron	Ferritin	Transferrin	TIBC
+	0.319	0.231	0.319	0.416	0.767
++	0.518	0.808	0.873	0.924	0.870
+++	0.520	0.931	0.924	0.893	0.770

Chapter Five

Discussion

Pre-eclampsia is still one of the leading causes of maternal and fetal morbidity and mortality. The etiology of this disorder remains exclusive to human pregnancy is an enigma. In this study , 50 pre-eclamptic pregnant ladies in addition to 30 apparently healthy pregnant ladies matching for age elected a control group , were investigated .

They were at different age intervals :<20 years old (2%) , 20-29 years old (54%) , 30-39 years old (42%) and >40 years (2%) . 58% of them had no previous history of pre-eclampsia while 42% had a history , of whom 58% had one pre-eclamptic pregnancy before , 38% had two pre-eclamptic pregnancies and 4% had a history of more than two . 30% of the study population had normal BMI while 70% had high BMI .

Iron profile , including the measurement of serum iron , ferritin , percentage saturation transferrin and total iron binding capacity were performed in all samples and the result obtained was compared with that of the control group .

It had been found that there is a significant reduction in haemoglobin level in pre-eclamptic pregnant ladies when compared with the control (P-value 0.00). The serum iron ,ferritin and percentage saturation of transferrin levels were significantly elevated (P-value 0.008 , 0.034 and 0.00 respectively) while as TIBC was significantly reduced (P-value 0.047).

Normal women has a decrease in serum iron and ferritin during the third trimester of pregnancy as their stores of iron are depleted because of feto- placental demand and required expansion of red cell

mass[22,23]. However, elevated level of serum iron is observed in pre-eclampsia compared to normal pregnant women, a study supported [96].Total iron binding capacity (TIBC) is low in pre-eclamptic group as compared to control. A measure of the iron binding reserve of serum is also significantly lower in women with preeclampsia relative to normal pregnancy .Serum ferritin is found elevated in pre-eclamptic group, which is in agreement with studies conducted [96-100]. Serum ferritin is a reliable indicator of total body iron status in non-diseased individuals, with low concentration diagnostic of iron deficiency. However a high ferritin does not always signify iron excess . High ferritin was associated with increased risk for preterm delivery and neonatal asphyxia, while the lower ferritin level was associated with decreased risk of preeclampsia, pre - lab our rupture of membranes . Increased concentration of serum ferritin during third trimester may be part of an acute phase response, which suggests maternal infection and is increased risk of poor pregnancy outcome .Increased percent saturation of transferrin in pre-eclamptic group is observed, which is in agreement with data collected [97,98].

No significant effects for variation in age , BMI or urine protein on any of the measured parameter although it had been found that the presence of previous history of pre-eclampsia affect significantly haemoglobin level (P-value 0.026) , serum iron (P-value 0.046) , transferrin and TIBC (P-value 0.037 & 0.012). Nothing is detected in the literature concerning the previous mentioned variables on these measured parameters .

Chapter six

Conclusion and Recommendations

6.1. Conclusion

-Haemoglobin level a significantly lower in pre-eclampsia (Test :11.5± 0.84g/dl , control :12.5±0.62g/dl , P-value 0.00) .

-There was significant increase in :

-serum iron (Test : 140.6±51.3mg/dl , Control : 71.7±19.7 mg/dl, P-value0.008) .

-serum ferritin (Test : 76.7±27.3mg/dl , Control : 62.9±28.2 mg/dl, P-value0.034).

-Percentage saturation of transferrin (Test : 62.5± 28.7% , Control : 27.5±5.9%, P-value0.034).

-Total iron binding capacity a significantly reduced in pre-eclamptic pregnant ladies (Test : 247.6± 57.7mg/dl, Control : 270.0± 33.9mg/dl, P-value0.047).

- No significant effects for variation in age , BMI or urine protein on any of the above measured parameters .

- Previous history of pre-eclampsia affect significantly haemoglobin level (P-value 0.026) , serum iron (P-value 0.046) , transferrin and TIBC (P-value 0.037 & 0.012).

6.2. Recommendation

-Future studies can be performed using larger sample size.

-Early identification of pre-eclampsia through measuring certain parameters including the assessment of iron profile associated together with genetic predisposition, physiological and other associated contributing factors will help in better management and reduction in the morbidity and mortality to mother and fetus.

- Iron status of pregnant women should be assessed before giving iron supplements as these may cause more harm than benefit.

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Shandi University

Faculty of Graduate studies

Assessment of iron status in pre-eclamptic pregnant ladies attending Omdurman Midwives Hospital

Prepared by: Somia Abd El Halim Abdullah

Supervisor: Dr. Mouna Adel Samaan

1. Name: ……

2. Age: ……...

3. Tel .No: ……

4.Previous history of Pre-eclampsia ;

Yes ☐ **No** ☐

5.Number of previous pre-eclamptic pregnancies :

once ☐ **twice** ☐ **>2 times** ☐

5.BMI :……..……..

6.Urine protein:

(+) ☐ **(++)** ☐ **(+++)** ☐

7. Serum iron :.....

8. Serum Ferritin :.....

9. Serum Transferrin :.....

10.TIBC :.....