COMPARATIVE STUDY OF THE ROLE OF SERUM IRON AND OXIDATIVE STRESS IN IRON DEFICIENCY ANAEMIA IN PREGNANCY AND IRON DEFICIENCY ANAEMIA IN PREECLAMPSIA

By

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Under the Guidance of Dr. Sheela .S.R Professor and HOD

And

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Dr. KRITHIKA RAJ

LIST OF ABBREVATIONS USED

WHO- world health organization

IDA- Iron deficiency Anaemia

PE- preeclampsia

ROS- reactive oxygen species

MDA- malondialdehyde

NTBI- non-transferrin-bound iron

OS- oxidative stress

NS- nitrosative stress

$O_2^{\bullet -}$ - superoxide

H_2O_{2-} hydrogen peroxide

HO – hydroxyl radical

RNS- reactive nitrogen species

NO- Nitric oxide

$ONOO^-$ - peroxynitrite

SOD- superoxide dismutase.

ER- endoplasmic reticulum

ETC- electron transport chain

COX-2 – cyclooxygenase 2

XO – Xanthine Oxidase

NOX- NADPH oxidase

CTV- villous cytotrophoblasts

EVT- extra villous trophoblast

SCT- Syncytiotrophoblast

ACOG – College of obstetrics and gynaecology American

Hb- haemoglobin

PCV- packed cell volume

MCV- mean corpuscular volume

MCH- Mean corpuscular haemoglobin

MCHC- Mean corpuscular haemoglobin concentration.

RDW- Red cell distribution width.

MCHC- microcytic hypochromic anaemia

ABSTRACT

INTRODUCTION: According to World Health Organization (WHO), the prevalence of anemia in pregnant women is approximately 38.2%. In India, approximately 54% pregnant women were with hemoglobin concentrations < 11%.

Preeclampsia (PE) is a multisystem disease, characterized by new onset hypertension and proteinuria after 20 weeks of gestation

The aim of the study is evaluate the serum Iron content, hemoglobin, along with oxidative stress condition in iron deficiency anemia pregnancy and iron deficiency anemia in preeclampsia women.

METHOD: It is a comparative study with 120 study population enrolled after 20 weeks of gestation with singleton pregnancy among 60 IDA(iron deficiency anaemia) normotensives and 60 IDA preeclamptic. Serum ferritin and serum MDA levels were measured in the study population and results were recorded. Each and every patient was followed up until postdelivery and outcome of pregnancy and complications if any were noted down. The results were statistically analyzed using parameters like mean, standard deviation, median and chi square test.

RESULTS: The median ferritin levels in IDA in normotensives was 4.63 and IDA in preeclampsia was 27.30 which was statistically significant ($p< 0.001$). The mean MDA levels in IDA normotensives was 0.43 ± 0.23 and IDA in preeclamptic was 0.65 ± 0.21 which was statistically significant ($p<0.001$). This indicates presence of oxidative stress in both the groups. Low birth weight infants were found to be significant in IDA normotensives and IUGR significant in IDA preeclamptic women.

CONCLUSION: The present study concludes that, the elevated oxidative stress in terms of MDA and more ferritin observed in IDA with preeclampsia group compared to IDA in normotensive pregnant. IDA preeclampsia group confined to Primigravida whereas IDA normotensives was confined to multigravida as parity is concerned. The study outcomes on follow up of the subjects associated with IUGR in preeclamptic group and Low birth weight in anaemia.

Therefore, determination of MDA and ferritin level at early stage may serve for the purpose of understanding preeclampsia complications.

KEYWORDS: serum ferritin, MDA levels, oxidative stress, preeclampsia, Iron deficiency anaemia, Preeclampsia.

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INTRODUCTION

INTRODUCTION

Anaemia, especially maternal anaemia is a serious global health concern. According to World Health Organisation (WHO) report, approximately about 32.4 million women who are pregnant suffer from anaemia worldwide, out of which 0.8 million pregnant suffer from severe anaemia. Moreover, 50% cases of anaemia can be recognised to be Iron deficiency anaemia. According to World Health Organization (WHO), the prevalence of anaemia in pregnant women is approximately 38.2%. In India, approximately 54% pregnant women were with hemoglobin concentrations < 11%.[1]. During pregnancy and reproductive age, Iron deficiency is common. In pregnancy, Iron requirement is more due to increased red cell mass and for the development/growth of feto-placental unit. Consequently, this situation worsens when there is an inadequate iron supply from the diet of the mother[2]. Studies reported that, iron deficiency is a risk factor for low birth weight, preterm delivery and perinatal mortality [3-5]

Preeclampsia (PE) is a multisystem disease, characterized by new onset hypertension and proteinuria after 20 weeks of gestation[6]. Preeclampsia affects 10% of pregnancies and it's also a major cause of maternal and fetal morbidity[7]. In India, the incidence of preeclampsia is as high as 8-10%. Preeclampsia and eclampsia accounts for about 24% of all maternal mortality[8]. The neonatal mortality rate which is associated with preeclampsia and eclampsia is around 43 per 1000 live births in India[9].

Preeclampsia is disease of many theories, the exact cause for the pathogenesis of preeclampsia is unknown[10]. Oxidative stress has been proposed to contribute significantly to the pathophysiology of preeclampsia[11]. Studies have reported that maternal immune maladaptation is one of the crucial factors leading to abnormal

placentation and altered endothelial function[12]. Reduced placental perfusion aggravates placental ischemia-reperfusion injury, which stimulates reactive oxygen species (ROS) production. Further, increased ROS generation, lipid peroxidation and/or reduced antioxidant status exacerbates endothelial dysfunction[13].

Placenta in pregnancy is rich in mitochondria, and this condition favours oxidative stress. Iron which unlike other transitional metals is particularly abundant in the placenta in important in production of free radicals. In order to protect the fetus, protective mechanisms against free radical generation and damage continuously increases throughout the pregnancy, however some amount of oxidative stress is been subjected on the fetus. Oxidative stress peaks by the second trimester of pregnancy, which unfortunately is the vulnerable period for gestational progress and fetal wellbeing.

Gestational iron requirements and of the iron proportion being absorbed from different iron supplemental doses, it could be concluded that with the present supplements, the intestinal mucosal cells are in constant exposure to unabsorbed iron causing excess iron and there by leading to oxidative stress. A study carried out on nonanemic women belonging to Mexico City at mid- trimester showed that had an increased risk of haemoconcentration when given 60mg/day leading to low birth weight and premature babies. The same was not seen when another group of nonanemic women were supplemented with 120mg iron once or twice in a week.[14,15]

This was the condition with women having normal haemoglobin levels. A developing country like India, where nutritional anaemia is prevalent especially iron deficiency anaemia (IDA) particularly in pregnancy a question arises with supplementation which is given routinely to every woman irrespective to the

haemoglobin levels. With this background, the present study aimed to detect oxidative stress marker MDA, in iron deficiency anaemia pregnancy which can be quantified by serum iron content from both IDA pregnancy and IDA in preeclampsia, and to record the outcome of pregnancy.

Evidences are insufficient to screen Iron deficiency anemia in pregnancy causing oxidative stress. The iron supplementation given irrespective of the Haemoblogin levels also can contribute to the increase in serum ferritin levels which can catalase the Fenton reaction. Therefore, an attempt is made to evaluate the serum Iron content, hemoglobin, along with oxidative stress condition in iron deficiency anemia pregnancy and iron deficiency anemia in preeclampsia women.

AIMS & OBJECTIVES

OBJECTIVES:

1. To detect Serum Iron content (serum Ferritin) in Iron deficiency anaemia in pregnancy group and iron deficiency anaemia in preeclampsia group.
2. To detect Oxidative stress parameters (MDA- malondialdehyde) in Iron deficiency anaemia in pregnancy group and iron deficiency anaemia in preeclampsia groups.
3. To record the outcome of pregnancy in both groups.

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

Pregnancy is a condition where there is increase susceptibility to oxidative stress due to the disturbance in the balance of oxidants-antioxidants, which is more in favour to oxidants which ultimately lead to potential damage. Before going to the topic of oxidative stress, there is a need to know further about oxidants and antioxidants- how they are formed and the damage/protection they can cause.

Oxidants are formed normally, as a product of aerobic metabolism. In certain pathophysiological conditions, oxidants are produced abundantly. Molecular oxygen can be reduced to water. This oxygen reduction process has an intermediate step which leads to the formation of superoxide anion radical, hydrogen radical and the hydroxyl radical. There is also formation of one, two and three electrons corresponding to the steps of reduction of oxygen. Other oxidants like alkyl or peroxyl radicals from lipids, peroxynitrate which is a nonradical reactive species formed from nitric oxide and superoxide anion radicals are generated. Radiations like ultraviolet light, ultrasound, microwave radiations, X-irradiation and even shear stress is known to generate oxidants. The half-lives of major reactive oxygen species differ from each other vastly, thereby requiring different types of defence mechanism. Rate constants for the reaction also differs, rate constant for hydroxyl radical takes place practically at the site of generation whereas peroxyl radicals are relatively stable (half-life is in the range of seconds), diffuse away from their site of generation and transport the radical or oxidant function to other target sites. In cell metabolism, an oxidant- clandestine can be transported to a distant target sites where it exerts it oxidant activity. Certain compounds or enzymes with activities

that are non-toxic in one environment but can be activated to generate oxidants under other conditions. The human diet also contains oxidant and antioxidants. [16]

Halliwell and Gutteridge in 1989, stated that an antioxidant is "any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate"[16]. The definition included compounds of non-enzymatic as well as enzymatic in nature.

There seem to be a lot of defence mechanisms (prevention, interception and repair), but the first line of defence and the most important defence-prevention. Enzymes like cytochrome oxidase carries out most of the cellular oxygen reduction, due to this mechanism of prevention does not release superoxide or other radical, though it contains iron and copper ions. In the same way, enzyme ribonucleotide reductase prevents tyrosyl functioning subunit B from spreading to the environment by forming a 'cage' around it.[16]

For a chain reaction to occur, binding of metal ions, in particular iron and copper ions is required, which can be prevented as well. Metal chelation is considered major in controlling lipid peroxidation and DNA fragmentation. Hence, the metal-binding proteins like ferritin, transferrin, ceruloplasmin, metallothionine, are of utmost importance to control potential radical generating reactions. Likewise, another strategy to increase the resistance to metal ion dependant oxidation is to modify the target site. However, all the above preventive mechanism is not 100% efficient.

Interception- It is another preventive strategy of antioxidation where harmful product is converted to less harmful product, hence lowering the risk of further damage. For example, intestinal mucosal cells. These cells

are exposed to a variety of reactive intermediates and xenobiotics, rate of accumulation of products of oxidative damage in these cells seems to be high. Thus, the turnover and elimination of these cells prevent further spread of the challenging species.

Repair – the effects of oxidants can be repaired after the damage has occurred. Since the processes such as prevention and interception are not completely effective, products of damage are continuously formed in low amounts and hence may get accumulated. This may cause DNA damage, in the form of damaged bases or single-strand or double-strand breakage, membrane damage, in the form of a variety of phospholipid oxidation products, and damage to proteins and other compounds as well. Hence, there are multiple enzyme systems involved in DNA repair and lipolytic and proteolytic enzymes capable of functioning as restitution or replenishment.

With this background, oxidative stress in pregnancy and its relation with iron is discussed further. The trophoblastic microvilli of the maternal surface of the human placenta is extremely rich in transferrin receptors. In between these microvilli there are coated pits which are believed to result from receptor-mediated endocytosis, where low pH releases the iron from maternal transferrin into the cytoplasm and this is further transferred to the syncytiotrophoblast and finally into the fetus [16]. As pregnancy progresses, different mechanisms like thinning of the syncytiotrophoblast , increase in placental blood flow and in transferrin receptors enhance the transfer of iron to the placenta and the fetus[17,18]. In the placenta of a guinea pig, the transfer of maternal iron to the syncytiotrophoblast is extremely fast, which may suggest that non-transferrin-bound iron (NTBI) must have been involved in placental transfer of iron[19]. However, there are no studies that directly indicate this nor whether more

NTBI reaches the placenta when high daily iron supplements are ingested or in other circumstances. But it is important to note that elevated NTBI has been detected shortly after the ingestion of iron supplements in plasma and in umbilical cord blood[20].

Iron-mediated oxidative stress has been demonstrated in the intestinal mucosa, liver, spleen, bone marrow and placenta. When the intestinal mucosa is exposed to excess intake of iron, it retains a large proportion of iron as ferritin, which enhances the possible effects of excessive free iron. Nonetheless, it is susceptible to oxidative damage secondary to the constant presence of relatively small excess amount of iron intake [21]. The iron that gets accumulated in the intestinal mucosa leads to intestinal abnormalities and injuries which was observed in patients who were receiving therapeutic iron [22].

Few evidences, point out that serum Iron level in preeclampsia is more than normal. the increased iron in the serum stimulates lipid peroxidase activity and there by induces endothelial cell damage.

In a study, daily iron doses equivalent to 120 mg/d in humans was administered to rats, the iron accumulation lead to mucosal necrosis, reduction of microvillus height (and even complete mucosal erosion) and evidence of oxidative stress was been demonstrated [23]. There was production of hydroxyl and methoxyl radicals in both the luminal contents and mucosal contents of the gastrointestinal tract which authenticates the role of iron in free radical damage. The same abnormalities were noticed more prominently iron-deficient animals and supplementation with α-tocopherol or a combination of α-tocopherol and ascorbic acid protected these animals [24-26]

In a study, one group had Iron-normal and the other group had iron-deficient rats. Both were subjected to twice daily iron supplementation for 24 days providing a total daily dose 10 times their normal food iron intake (comparable to 120 mg/d doses in humans). At first the rats absorbed 10% and 21% of the supplemental iron, respectively. Absorption dropped to stabilize at approximately 6% by day 7 of supplementation in both groups. The rats were then dissected on day 24, to only notice duodenal mucosa was elevated 5–6 times, ileal mucosa 2–4 times and liver 3–4 times above the levels observed in normal, rats who were not given any supplementation. Iron levels in the liver was already normal or slightly above normal by day 3 of supplementation, after which the previously iron-deficient rats always showed higher iron levels in the liver that continued to increase beyond the normal levels [27].

Henceforth, this information can lead to the question of whether previously iron-deficient pregnant women are more susceptible to oxidative stress resulting from excessive iron absorption, particularly when given daily pharmacological doses of iron [28].

Oxidative stress (OS) is defined as "an imbalance between oxidants and antioxidants, leading to a disruption of redox signalling and control and/or molecular damage".[29] OS includes reactive oxygen species (ROS), which are starting from the most common species being superoxide ($O_2^{\bullet -}$) , hydrogen peroxide (H_2O_2), and the hydroxyl radical (•HO). In the same way, a process where there is an imbalance in the ratio of nitrosants to antioxidants is known as nitrosative stress (NS). NS essentially involves the reactive nitrogen species (RNS) which are nitric oxide (•NO) and peroxynitrite ($ONOO^-$). In cases of aerobic organisms, there will be a controlled

production of ROS and RNS depicting a physiological phenomenon, which is essential to play an important role in cellular metabolism and cell signal cascade. However, when there is an imbalance created between the formation of oxidizing substances and antioxidant molecules Oxidative stress (OS) and Nitrosative stress (NS) which ultimately promotes detoxification. Once this imbalance is established the highly reactive ROS and RNS species formed due to OS and NS respectively can potentially cause structural and physiological damage to the most vital DNA, RNA, proteins, and lipids, and also cell membrane-bound lipids.

ROS and RNS can be generated by various cellular compartments or metabolic pathways.

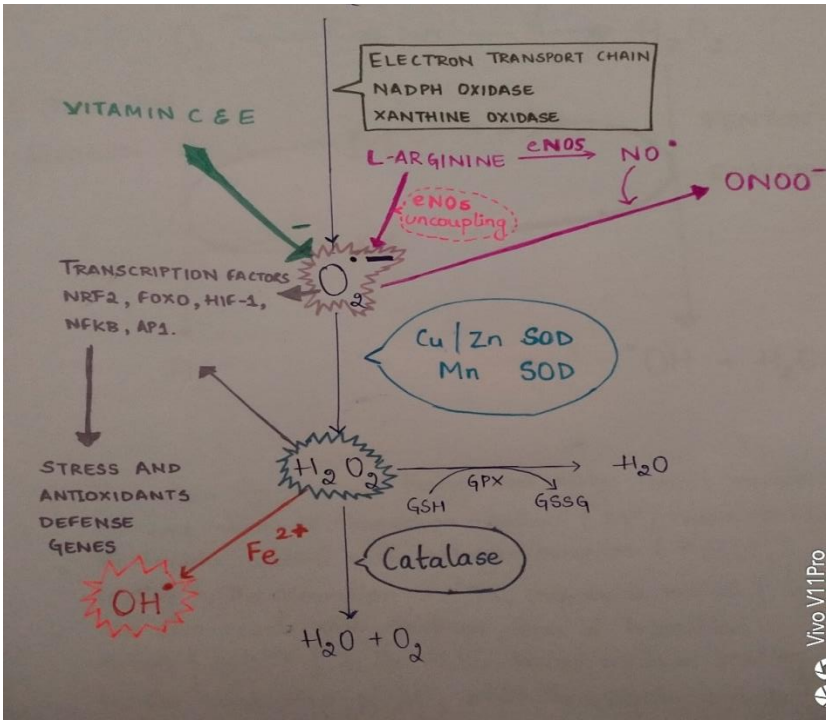


FIGURE 1: Role of oxidative stress in the pathophysiology of preeclampsia

The above flow chart depicts that, oxidative stress plays a central role in the pathophysiology of preeclampsia. The mitochondria, endoplasmic reticulum (ER), and nuclear membrane produce anions as a by-product of the auto-regulation of

electron transport chain (ETC) components. ROS are also produced as a consequence of arachidonic acid metabolism by cyclooxygenase 2 (COX-2), Lipoxygenases, Xanthine Oxidase (XO), and Cytochrome P450. NADPH oxidases (NOX) are another significant source of ROX. NOX generates superoxide ($O_2^{\cdot -}$) by transferring electrons from NADPH inside the cell across its membrane and coupling them to O_2. eNO synthase(eNOS) can generate $O_2^{\cdot -}$ and H_2O_2 specifically when the concentrations of its substrate, L- Arginine, or its cofactor, tetrahydrobiopterin (BH4) are low. When intracellular ROS production increases (especially $O_2^{\cdot -}$ ions), •NO may react with ROS to form peroxynitrate ($ONOO^-$) a major cause for nitosative stress[30]. Superoxide is rapidly dismutated to H_2O_2 by superoxide dismutase (SOD). H_2O_2 can be transformed into H_2O and O_2 by catalase and glutathione peroxidase (GPX). However, in the presence of Fe^{2+}, and through Fenton's reaction H_2O_2 can generate the highly reactive radical hydroxyl ($OH^{\bullet}$)

With the following molecular biology knowledge, oxidative stress that takes place in the placenta will be looked into. The human placenta is composed of abundant cell types, as shown in a study by Tsang et al that showed twelve major cell clusters identified by their expression profile[31]. Among them, the amplest cells are stromal cells, endothelial cells, villous cytotrophoblasts (CTV), and extra villous trophoblasts (EVT). Syncytiotrophoblast (SCT), which is formed from the fusion of villous trophoblasts, was separated from the other cells of trophoblastic lineage. Syncytiotrophoblasts synthesis a mRNA that encodes for the Chorionic Gonadotrophin β polypeptide, which, together with Chorionic Gonadotrophin α polypeptide, constitutes human Chorionic Gonadotrophin (hCG) hormone. Synthesis of hCG by the placenta as early as implantation and syncytialization, roughly around 8 days post-fertilization in humans. Remarkably, clusters of immune cells were also

identified (Macrophages, Dendritic cells, T cells), which could be responsible for generating oxidative stress.

Oxidative stress can be induced when there is low oxygen partial pressure, hyperoxia, or alternations of hypoxia and reoxygenation, as seen in various highly vascularized tissues such as the brain or eye[32,33]. In a human placenta, during the first trimester normally there is hypoxia generated which is a physiological condition and can be measured by in vivo methods[34]. This normally occurring phenomenon fogs the distinction between regular physiology and possible destructive effects of hypoxia at specific stages of placental development. Aside from trophoblast cells as mentioned above, oxidative stress could originate in endothelial cells (ECs) present in the placental tissue, stromal cells of the villi, or immune cells (Hofbauer cells). Generally, trophoblasts cells get modified through gene expression due to oxidative stress. On achieving this, the placenta makes the extracellular vesicles that get emitted into maternal circulation and thereby influence the gene expression in both maternal endothelial and immune cells.

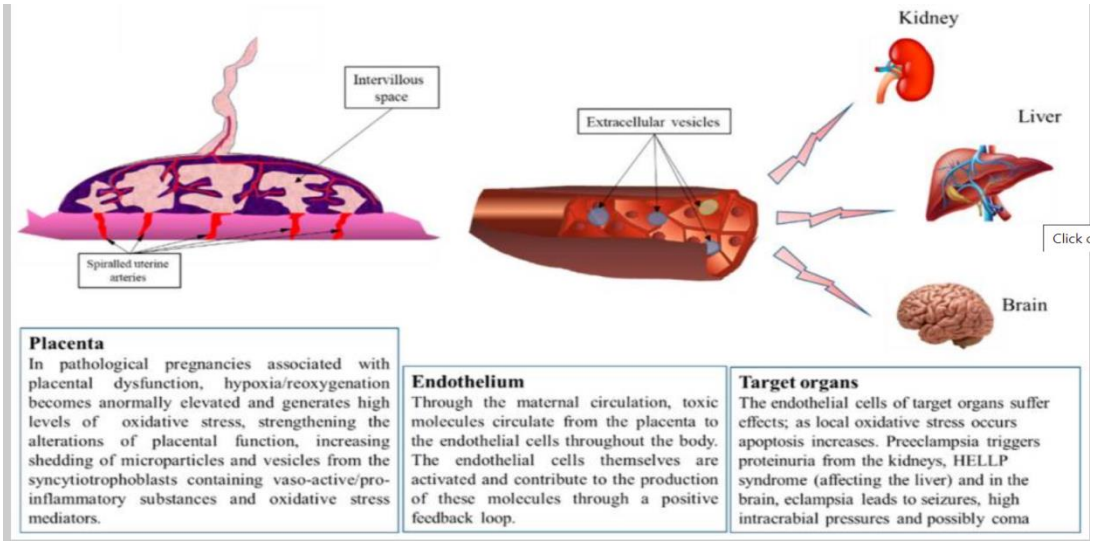


Figure 2: Pathophysiology of preeclampsia

Human placenta creates an interface between mother and fetus, playing the multifactorial role as the boundary between the two adjoined organisms, regulating immunological discourse and tolerance, nutrient and gas exchange, and producing hormones vital for pregnancy. During the first trimester, there is general hypoxia that occurs in the intervillous space, as numerous maternal spiral arteries get plugged with extravillous trophoblast cells, thereby preventing maternal red blood cells from passing into this space. Following 12–14 weeks of normal gestation, these placental extravillous trophoblasts, they colonize into the maternal spiral arteries to the extent of involving the proximal third of the myometrium. Thus, this unique innervation leads to the relaxation of arteries and further lose their contractile properties, which in turn increases blood flow and oxygen partial pressure. Hence, reversing the previously hypoxic environment which was present initially. This proves that oxidative stress in general is present in healthy human placenta and in fact plays prominent role for organogenesis. When there is abnormal /incomplete placentation, the oxidative stress occurs at an extreme level, leading to an elevated discharge of placental debris and vesicles into the human maternal circulation. Extracellular vesicles that where released transfer active molecules (proteins of microRNA) generated by stressed placental cells. Once they are transferred to blood, they meet maternal endothelial cells and potentially transfer their contents, leading to transcriptome alterations and inflammation. Eventually, the endothelium of maternal organs is affected. In the case of preeclampsia, most commonly this phenomenon occurs in the kidney, liver, and brain.

To interpret the origin of oxidative stress, many studies have been carried out on trophoblast cell models, specifically HTR8/SVneo cells that had been exposed to hypoxia/reoxygenation (H/R). The HTR-8/SVneo cell line was developed from first

trimester extra-villous trophoblast infected with SV40 Large T antigen. Recent evidence points out that it may be a heterogeneous cell line, which includes pure trophoblasts (marked by CK-7) and mesenchymal cells (marked by vimentin)[36], which has been extensively used as a sufficient model of extravillous trophoblasts. In context of this model, the oxidative stress downregulates N-acetylglucosaminyltransferase III[37], Special AT-rich sequence Binding protein 1 (SATB1, localized in placental trophoblast cells[38]), and abnormal regulation of Parkinson Associated deglycase 7 (PARK7)[39]. SATB1 has been shown to inhibit the Wnt-β-Catenin pathway, and thereby the migration and invasion of the trophoblast. H/R treatment in HTR-8/SVneo cells also prompts alterations of the Phospho Inositol 3 Kinase/Protein Kinase B (AKT) pathway. With serum deprivation, it triggers an increase in necrotic cell death, which was able to be saved by relaxin, an insulin superfamily hormone expressed by the maternal reproductive system. It has also been shown that trichloroethylene (TCE) or flame retardants such as Polybrominated diphenyl ethers (PBDE, such as BDE-47) amends the retort to oxidative stress in this model [40,41]. The TCE metabolite DCVC (S-(1,2-dichlorovinyl)-l-cysteine) generates an inflammation reaction by releasing IL-6, which is formed as a result of ROS generation , thereby disrupting the mitochondrial function.

In trophoblast cell lines, antioxidant molecules have the capability of reversing the oxidative stress cascades formed. This is similarly seen in Resveratrol [42], which stabilised the activity of SOD formed in the HTR8-SVneo H/R stressed cells, the concentration of Malondialdehyde (a marker of oxidative stress targeting unsaturated fatty acids), and lessened apoptosis. Likewise, when Sildenafil citrate was applied to H/R HTR-8/SVneo, it proved to support cell survival, in the company of NO and cGMP [43]. Oxidative stress-induced apoptosis can also be attenuated by HBEGF

(heparin- binding EGF-like growth factor) treatment when administered to the first trimester human placental explants, as well as on the HTR-8/SVneo cells [44]. Exposure to oxygen peroxide induced down-regulation of HLA-G expression, a trophoblast immunomodulatory molecule that facilitates implantation and placental development. It normally leads to proliferation inhibition, apoptosis, and decreased cell invasion 45. Therefore, through exposure to OS, the EVTs of the placenta may trigger an excessive immune response via the decidua cells. This can lead to a decreased endometrium invasion and faulty uterine spiralled artery assembly, the latter being a hallmark feature of preeclampsia. Consistently, Preimplantation Factor (PIF*), a 15 amino-acid linear peptide secreted early by viable embryos, was discovered as an enhancer of implantation and has recently been shown to protect against oxidative stress, thereby enhancing HLA-G expression and fostering better implantation [46]. Extracellular vesicles are exosomes, which measure about 30–100 nm, and the microvesicles (which are small in number) measure about 100–1000 nm. The placenta releases large cell debris including nuclear aggregates and apoptotic bodies which measure about 20–500 μm and 1–4 μm, respectively. Similar events occur in preeclampsia and in a larger scale to be precise.

Lipid peroxidation is increased in preeclampsia (PE) that expressed as elevated malondialdehyde (MDA) serum levels, marker of lipid peroxidation. Therefore, high lipid peroxidation and elevated lipid profile in preeclamptic women might be the cause of increased risk of coronary artery disease in PE [47].

Paul et al, conducted a study where 40 cases where preeclampsia and 40 cases were normotensives. They came to a conclusion that serum ferritin was more in preeclampsia compared to normotensives and chronic hypertensives. They concluded that serum ferritin was found to be useful as a marker of iron status parameters reflecting in preeclampsia. This also could mean that the iron acts as a catalyzer of oxidative stress and lipid peroxidation in the pathophysiology of preeclampsia.[48]

Aquirre et al, recruited around 31 women with preeclampsia and 30 healthy pregnant women. Ferritin level was above the upper normal limit in only 4 patients of their preeclampsia group who were receiving prophylactic iron.[6]

Taheripanah et al, conducted a study with 33 preeclamptic and 33 normal pregnant women before parturition. The serum ferritin levels in preeclamptic women was found to be 3.6 times to the normal ferritin levels. Out of the 33 preeclamptic women 15 women (45.5%) had abnormal levels of ferritin levels. They found a strong relation between ferritin and preeclampsia. They came to conclusion that identifying high risk subjects and diagnosing preeclampsia before the obvious signs set in could be done by evaluation of ferritin and Total Iron binding capacity(TIBC). In the end, they went on to question the rationale of iron supplementation given to non-anaemic women.[49]

According to Rayman et al, 40 preeclamptic women were included in the study and serum Ferritin level was compared with control and noticed that the median serum ferritin was 6-fold raise than the normal pregnant women. They came to a conclusion that raised serum iron and ferritin could have the potential to be used diagnostically as caution towards incipient preeclampsia.18% of their preeclamptic subjects showed increased levels of transferrin suggesting an overload of iron, also they concurred that iron supplements could be an aggravating factor in women with increased susceptibility for iron overload.[50]

Tiwari et al, observed in their study of 130 pregnant with iron deficiency anaemia were recruited and observed that there was increased level of lipid peroxidation products such as LPO, PC, CD. The escalated lipid peroxidation process was credited to over production of reactive oxygen species (ROS) or could be due to deficiency of antioxidant defense. There was decrease in ferritin levels and decrease in SOD activity as the ROS formed inhibit SOD thereby increasing oxidative stress.[51]

Bhale et al, conducted a study comprising of 50 anaemic and 50 non anaemic women were taken. MDA levels were found to be significantly increased in iron deficiency anaemia group. They concluded that iron deficiency anaemia was associated with generation of free radical.[52]

Maitra et al, conducted a study of 33 anaemic women and 20 normal pregnant women without anaemia. They concluded that iron deficiency anaemia had higher levels of MDA compared with normal pregnancies[53]

In a study , the mean of serum MDA level was significantly higher in women with pre-eclampsia when compared with that of women without pre-eclampsia, whereas mean levels of both serum total glutathione and vitamin E(antioxidants) were significantly lower in women with pre-eclampsia when compared with those of women without pre-eclampsia concluding pre- eclampsia exhibits an imbalance between blood oxidants and antioxidant levels that includes higher circulatory levels of MDA and lower levels of total glutathione and vitamin E and concluding that MDA, vitamin E and blood total glutathione may be possible candidate markers to predict pre-eclampsia. [54]

A research article showed, Women with early onset pre-eclampsia showed a higher O2 radical total and a non-significant O2 radical as compared to those with a mild late onset pre-eclampsia, indicating that women with more severe pre-eclampsia have a higher NAD(P)H

oxidase mediated O2 radical production. The presence of a functional and highly active NAD(P)H oxidase in placental tissue from normotensive pregnant women and those with pre-eclampsia, which could be an important source of O2 radical generation. [55]

A study showed preeclampsia, a heightened level of oxidative stress encountered is been attributed to the placenta. production of free radicals with maternal leukocytes and maternal endothelium being the contributing factors to this process. The study showed NADPH oxidase produced superoxide, and which is present in placental trophoblast concluding, Women with early onset of preeclampsia have been found to have a higher superoxide production compared with those with late onset disease [56]

Higher Serum ferritin levels, were associated with PE. The incidence of thrombocytopenia was higher in preeclamptic women, however, the remaining haematological parameters were similar in both groups. They also concluded by saying that iron supplementation irrespective of Haemoblogin levels which has been a practice for very long time, can cause unnecessary iron toxicity, and can put the pregnant women to a risk of Preeclampsia [6]

Normal pregnancies were characterized by increased levels of Copper and ROS(reactive oxygen species) production and enhanced antioxidant protection, while preeclampsia was associated with increase of iron and imbalance in oxidative homeostasis. Such an imbalance could be detected even in the beginning of the preeclamptic disorder inducing a significant increase of total serum antioxidant capacities. It is still unknown whether significantly higher serum Fe levels are associated with preeclampsia as a cause or as a consequence of this disorder. In both cases, the results indicated that the Iron supplementation for non-anemic pregnant women might have harmful effects as a risk of developing preeclampsia. [57]

Ischaemic placental tissue could be a primary source of potentially toxic iron in preeclampsia and the released iron species may contribute to the aetiology and would exacerbate lipid

peroxidation and endothelial cell injury. Increased iron can further promote oxidative stress by decreasing serum antioxidant capacity [58]

There is no evidence of deficiency in the maternal protective antioxidant systems or increased production of lipid peroxidation products, LPO (lipid peroxide) and MDA (malondialdehyde) in African women with pre-eclampsia as compared with normal pregnancy. However, there was evidence of increased cord plasma concentrations of MDA and vitamin E in eclampsia as compared with normal pregnancy and pre-eclampsia. The placenta may be effective in removing MDA. The antioxidant uric acid serves as a protective role whilst the antioxidant and oxidant capacity in the different study groups remained unchanged [59]

Increased maternal serum TAS, TOS and arylesterase levels are significantly associated with the presence of severe preeclampsia. Furthermore, elevated maternal serum TAS, PON (PON- paraoxonase; TAS-total antioxidant status; TOS- total oxidant status) and arylesterase levels are significantly and positively correlated with adverse perinatal outcomes. We suggest that in preeclampsia increased oxidative status may cause adverse perinatal outcomes and antioxidants may be increased in order to protect the fetus against oxidative damage [60]

Increase in serum iron in preeclampsia was not due to haemoconcentration was noted .In Preeclampsia iron stores are already depleted, release of iron from ferritin as a result of liver damage may not contribute to raised serum iron level in preeclampsia, and raise in iron level cannot be due to the possibility of the cessation of erythropoiesis as reticulocyte count was found to be more .Hence, haemolysis can be a major contributory factor for the increased levels of serum iron in pre-eclampsia[61]

In a study significant association with oxidative stress seem to overlap those linked to anemic condition. Similarity between complications of anemia and that of oxidative stress is more observed at 28 - 32 weeks of gestation. This strongly suggests that major correction in both anemic and oxidative status should be initiated long before this landmark.[62]

In a study, the perinatal and maternal complications are significantly associated with severity of anemia in preeclampsia women. Anemia being an easily detectable and modifiable risk factor, detection of anemia in early gestation can be a key to prevent or decrease the severity of preeclampsia[63]

ANAEMIA

Anaemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status.

Iron deficiency is thought to be the most common cause of anaemia globally, although other conditions, such as folate, vitamin B12 and vitamin A deficiencies, chronic inflammation, parasitic infections, and inherited disorders can all cause anaemia.

In its severe form, it is associated with fatigue, weakness, dizziness and drowsiness. Pregnant women and children are particularly vulnerable.

DEFINITION:

The definition of iron deficiency anaemia (IDA) in pregnancy is indistinctive as a result of pregnancy-induced changes in plasma volume and haematocrit, differences in haemoglobin (Hb) concentration through the trimesters, differences in diagnostic tests, and ethnic variation.

According to the World Health Organization (WHO), a pregnant woman is considered to be anaemic if her Hb concentration is < 11gm/dl , whereas centres for disease control and prevention (CDC) guidelines define anaemia as haemoglobin less than 11 gm/dl in the first trimester and less than 10 gm/dl in the second or third trimester [64].

INCIDENCE:

IDA remains the most common nutritional deficiency globally, with about 32 million pregnant women categorised as anaemic and about 0.75 million pregnant women categorised

as severely anaemic. India is one of the countries with the highest prevalence of anaemia in the world. According to the Indian National Family Health Survey, the prevalence of IDA in pregnancy ranges from 23.6–61.4%.[65] The incidence of IDA in India was estimated at 60.0% in the urban population and 69.0% in the rural population, and IDA resulted in approximately 326,000 maternal deaths with an associated disability-adjusted life years of 12,497,000 [65] Diverse cultures, religions, food habits, lifestyles, and traditions pose a great challenge to the implementation of various government health programmes in India.

CAUSES FOR IDA:

Scarce quantity of iron-rich foods, poor environmental sanitation, unsafe drinking water, iron is lost due to parasites (e.g., malaria or intestinal worms), and adolescence, teenage pregnancies anaemia, along with teenage pregnancies and recurrent pregnancies in low resource countries

PATHOPHYSIOLOGY OF IRON DIFFICIENCY:

Normally in pregnancy due to a relatively greater expansion of plasma volume by 30–40% in comparison to a 20–25% raise in Hb mass and erythrocyte volume leading to Physiological or dilutional anaemia. This results in an uncertain decrease in Hb levels, creating a low viscosity state, which promotes oxygen transport to the placenta and fetus. The iron requirement in Pregnancy significantly increases as there is increase in demand for iron to balance the physiological requirements of increased haematocrit, developing the fetoplacental unit, and for losses during delivery and lactation.

The Institute of Medicine made an assessment where the total iron loss which is linked with pregnancy and lactation was approximately 1,000 mg, hence, the daily recommended dietary allowance(RDA) for nutritional iron in pregnancy of 27 mg [66] If

this RDA of iron in pregnancy is not met, it would result in depleted iron stores developing IDA.

Recent research into iron metabolism in humans has paved the way for the discovery of a unique, peptide hormone, hepcidin, that acts by controlling iron efflux into the plasma, acting as a homeostatic regulator of systemic iron concentration. Hepcidin levels in pregnant women are lower when compared with a nonpregnant healthy women, and further decreases as pregnancy advances. Lowest levels of hepcidin were found to be seen in the third trimester.[67]With this decreasing trend of hepcidin levels being expressed, the absorption of dietary iron is elevated, resulting in amplification of mobilisation of iron from body stores modulated by ferroportin. Inflammatory states, including pre-eclampsia, malaria infection, and obesity, have been noticed to have higher hepcidin during pregnancy compared to healthy controls, suggesting that maternal and fetal iron bioavailability could be compromised.

MATERNAL AND FETAL OUTCOME:

So far, all the literature points out that, if there is no success in meeting the desired iron requirements in pregnancy, leading to both maternal and fetal adverse consequences. An estimate of about 591,000 perinatal deaths and 115,000 maternal deaths worldwide was attributed to IDA directly or indirectly[67]. Likewise, correcting IDA of any severity reduces the risk of maternal death by about 20% for each 1 g/dL increase in Hb. Keeping all these facts in mind, newer health policies need to focus on mild-to-moderate anaemia in pregnancy, along with severe anaemia, for a greater public health impact.

Iron deficiency in pregnancy causes maternal morbidity accounting to increase susceptibility of abortion, increased vulnerability to infection due to defective macrophage phagocytosis

and lymphocyte replication, physical weakness, pre-eclampsia, preterm labour, heart failure, increased risk of postpartum haemorrhage due to impaired myometrial contractility resulting from hypoxia-induced enzymatic and cellular dysfunction, puerperal sepsis, and postnatal depression.

According to a study by Lone et al., [68] IDA in pregnancy can lead to increased risk of preterm birth by 4-fold, low birth weight babies by 2.2-fold, and low Apgar score in newborn babies by 1.8-fold in comparison to nonanaemic women. Additionally, maternal iron depletion also depletes fetal iron stores and thereby, accounting to the risk of neonatal anaemia and perinatal morbidity. This concludes that correction of iron deficiency has proved to have beneficial effects on both the mother and the fetus.

SCREENING:

More than 80% of antenatal women who are diagnosed with IDA, routine screening of all pregnant women becomes a standard practice in India. Indian National Rural Health Mission (NRHM) guidelines recommends a compulsory Haemoglobin which should be estimated for all pregnant women by the cyanmethaemoglobin or by photocalorimeter method at 14–16 weeks, 20–24 weeks, 26–30 weeks, and in gestational age between 30–34 weeks (a minimum of four Hb estimations). The interval between two estimations should be at least 4 weeks apart. A patient could be referred to a specialised institution when Hb level of less than 7 g/dl at a gestational age of 14 weeks, 20-24 weeks gestation, and 26-30 weeks gestations, or a Hb of less than or equal to 9 g/dl at 30-34 weeks of gestation[69.]

CLINICAL SYMPTOMS AND SIGNS

In pregnancy, the signs and symptoms are nonspecific and IDA mirrors normal pregnancy changes, until the anaemia becomes severe.

Fatigue - most common symptom, followed by varying degrees of pallor, lethargy, headache, palpitations, dizziness, dyspnoea, lack of concentration, and irritability. Pica develops in rare cases.

INVESTIGATIONS: Investigations specific for IDA:

Complete blood count: it is a preliminary step for diagnosis of IDA. It provides a complete blood picture where in low Hb, mean cell Hb (MCH), mean cell volume (MCV), and mean cell Hb concentration (MCHC); a peripheral smear showing presence of microcytic hypochromic red cells and characteristic anisopoikilocytosis marking the diagnosis of IDA.

Red Cell Distribution Width: Elevated red cell distribution width infers alteration in red blood cell (RBC) volume distribution, and helps in differentiating IDA from thalassaemia and other haemoglobinopathies.

Serum Ferritin: Estimation of serum ferritin precisely reflects iron stores and is commonly the first laboratory test to become abnormal as iron stores decline. Serum ferritin values are the best parameter to assess diminished iron stores in pregnancy, and they are impassive by any recent iron ingestion. A concentration of less than 15mcg/L signifies iron exhaustion in all stages of pregnancy. Assessment of serum ferritin in pregnancy becomes the most important investigation when a woman is suspected of having thalassaemia or haemoglobinopathy, whose anaemia would fail to respond to a 2-week trial of oral iron, and before any parenteral iron replacement.

Serum Iron, Total Iron Binding Capacity, and Transferrin:

Saturation of Serum iron is a variable indicator of the iron available at tissue level because of wide variations in serum iron levels due to recent ingestion of iron or infection, or diurnal rhythm.

Total iron binding capacity is more with iron deficiency and an indirect measure of bioavailability of iron-binding sites and transferrin levels.

Zinc Protoporphyrin : Zinc protoporphyrin levels elevate when iron availability decreases as zinc is incorporated into the protoporphyrin ring instead of iron. Serum zinc protoporphyrin is not influenced by plasma dilution thereby having greater sensitivity and specificity for iron depletion. The disadvantage is that this test is not easily made available.

Bone Marrow Iron: It is considered the gold standard test for the assessment of marrow iron stores. Here, the bone marrow sample is stained with Prussian blue for identification. The major drawback is that this test is invasive, and should be restricted for complicated cases where the causal of anaemia could not be diagnosed by simpler means.

A TRIAL OF IRON THERAPY

It is both diagnostic and therapeutic for IDA. If demonstrable rise of Hb level is noted by 2 weeks, it confirms deficiency of iron stores, thereby making it both time and cost effective. Serum ferritin levels are estimated for patient who are at higher risk for haemoglobinopathy or whose status is unknown. Iron therapy can be started while screening is being performed. Even after 2 weeks of iron therapy, when there is no significant raise in haemoglobin, the pregnant women can be referred to a higher centre to contemplate other causes of anaemia.

WHO and CDC guidelines are to estimate serum ferritin/ serum transferrin receptor in association with Hb in the absence of any kind of infection postulates the best estimation of iron store status.[70] In a low- resource setting country as India, these tests are either not easily affordable or not easily available. In such conditions, RBC indices will hold equal importance for primary diagnosis, which could be cost effective for the patient. Out of all the available

indices, the MCV:RBC ratio which is also called Meltzer index has been proved to be the most reliable indicator with corresponding highest sensitivity.[71]

According to Indian National Rural Health Mission guidelines, [69] IDA is managed by Hb estimation by cyanmethemoglobin method using a semiautoanalyser or photocalorimeter, which is mandatory in all institutions. Peripheral smear, MCV:RBC ratio, serum iron binding capacity, and Hb electrophoresis is to be performed in medical colleges, District Headquarter hospitals, and other secondary care institutions with facilities for these tests. Urine assessment for albumin, sugar, and deposits, and a urine culture if pus cells are detected, to rule out refractory anaemia.

SUPPLEMENTATION AND PROPHYLAXIS

Iron-rich food like meat from cattle, fish, poultry, legumes and green leafy vegetables. The required dietary intake of iron during the second half of pregnancy is 30 mg. Absorption of iron increases to 3-fold by third trimester and requirement increases from 1–2 mg to 6 mg per day.[72] This rapid requirement cannot be met by dietary modification alone thereby ending up in maternal anaemia in most women.

The WHO strongly recommends daily oral supplementation of iron and folic acid as an integral part of antenatal care to lower the chances of low birth weight babies, anaemia in pregnancy, and iron deficiency. The International Nutritional Anemia Consultative Group (INACG), as well as the WHO, recommends that 60 mg elemental iron has to be given as prophylaxis to all antenatal women in countries where the prevalence of anaemia is >40%.1 In India, where prevalence is as high as 58%, Ministry of Health and Family Welfare (MOHFW) recommends elemental iron supplementation of 100 mg elemental iron for 100 days along with 500 μg of folic acid which should be initiated from 14–16 weeks. When

compared to the recommended 60 mg , 100mg elemental iron causes gastrointestinal side effects like nausea, vomiting, and constipation. For nonanaemic patients with iron deficiency, the dose as low as 20–60 mg can be given.

Iron salt	Dose per tablet	Elemental iron
Ferrous sulphate	300 mg	60 mg
Ferrous sulphate (dried)	200 mg	65 mg
Ferrous fumarate	322 mg	100 mg
Ferrous gluconate	300 mg	35 mg
Ferrous succinate	100 mg	35 mg

FIGURE 3 : Elemental iron content in each tablet of different oral iron preparation.

TREATMENT

National Institute for Health and Care Excellence (NICE), British Society of Haematology (BSH), and Australian Guidelines all recommend a trial of oral iron for 2 weeks in women diagnosed with anaemia during the antenatal period [73] This treatment can be started at the community level and a sufficient rise in Hb is considered to be diagnostic of IDA. If the haemoglobinopathy state is not known, haemoglobinopathy screen should be planned simultaneously iron therapy should be started. In order to replenish the iron stores iron therapy should be continued until when the patient's Hb rises to normal and continued for 3 more months or up to 6 weeks of postpartum. Parenteral iron should be taken into account when patient has severe gastrointestinal side effects or Intolerance to oral iron malabsorption, or IDA unresponsive to oral iron, or Absolute noncompliance, particularly if mother is near term.

Among the various non oral iron preparations, iron carboxymaltose is the most preferred drug, which rarely causes anaphylaxis. This drug is administered as total drug infusion and is marketed at concentration of 50 mg/mL of elemental iron. The dose is calculated on the basis of pre-pregnancy or booking weight, aiming for a target Hb of 11 g/dL. Infused over 15

minutes, 1,000 mg in 20 mL is diluted in 250 mL of 0.9% sodium chloride. The patient is observed for 30 minutes after administration and oral iron is not advisable for the next 5 days. Repeat Hb is tested post 2–3 weeks and depending on which a second dose can be considered. A single dose should not surpass 1,000 mg of iron per week. In India, oral iron therapy is initially started in all women with Hb ≤9 g/dL. In patients with Hb 7–9 g/dL, parental iron to be given after 32–34 weeks for early rise, ensuring 100% compliance. In developing countries like India, the most prevalent iron preparation is iron sorbitol citrate complex, the disadvantage of this preparation being painful injections and cause permanent skin staining. The injection is given in a Z-track injection technique.

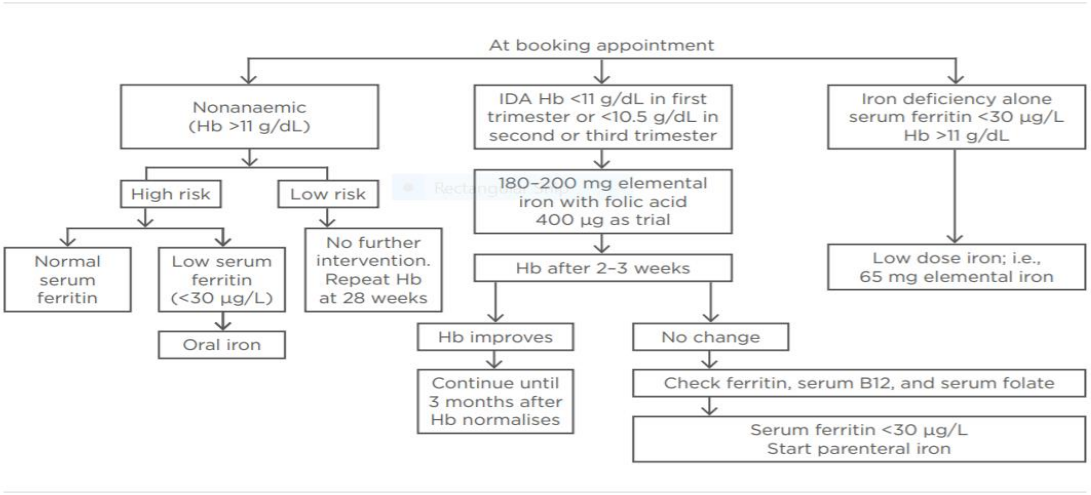


FIGURE 4: Management of iron deficiency anaemia in pregnancy in accordance to Royal college of Obstetricians and Gynaecologists (RCOG) Green-top Guidelines.

The recent Royal College of Obstetricians and Gynaecologists (RCOG) blood transfusion guideline recommends blood transfusion in labour or the immediate postpartum period if Hb is<7 g/dL. In Western countries, provision of cell salvage should be considered at the time of caesarean section.

HISTORY IN PREECLAMPSIA:

Hypertensive disorders in pregnancy dates back to ancient history. Several hypothesis have been made on pre-eclampsia and eclampsia.

Bernhart (1939), stated that eclampsia has been mentioned in ancient Chinese, Indian, Egyptian and Greek mythology. Even in writings of Atharva Veda and Sushrutha there is a mention on hypertensive disorders in pregnancy.

In 4 th century BC, Hippocrates stated the significance of convulsions, head ache and drowsiness in the pregnant mother.

On this basis, several other concepts have emerged stating that pre-eclampsia presents as various degrees of toxaemia in which hypertension, fluid retention, albuminuria with or without convulsions.[15]

Lever's (1843) discovered testing of urine for proteinuria, which is considered as a precursor for eclampsia. Cook and Briggs (1903) stated increase in blood pressure as the earliest sign of impending convulsions.

Zangemeister (1916), postulated that sudden increase in maternal weight is a warning sign for development of pre-eclampsia and eclampsia.[15]

From the perspective of few German authors, the age-old reports dated from 2200 BC referring to eclampsia was observed in papyri of ancient Egypt [74]. The word eclampsia was derived from the Greek éklampsis meaning "bright light" [74]. For nearly 2000 years, eclampsia was assumed as a disease characterized by convulsive seizures, distinctive of late gestation, that usually ended at termination of pregnancy. Scientists from the late 19th century, encountered the the similarity between the swollen appearance of women who had seizures and the edema of Bright's disease, an abrupt glomerulonephritis onset characterized

by proteinuria. Thereafter, urinary alterations in childbearing women with seizures was sought for, which concluded in finding proteinuria in them. With the dawn of noninvasive blood pressure measurement at that time, it was noticed that these women had increased blood pressure levels. Later on, they went on to prove that proteinuria and arterial hypertension preceded the onset of seizures. [74-76] thus, was the advent of defining "preeclampsia".

DEFINITIONS: Hypertension is diagnosed when systolic Blood pressure exceeds 140 mm Hg or diastolic blood pressure exceeds 90 mm Hg in previously normotensive patients in two occasion at least 6 hours apart, after 20 weeks of gestation.

To define diastolic pressure korotkoff phase V (i.e., disappearance of sounds) is used. Blood pressure is checked either in sitting position or in left lateral position with arm at the level of the heart with the appropriate sized cuff.[77]

Gestational Hypertension

Elevation of the blood pressure to 140/90 mm of Hg or greater for the first time after the mid pregnancy with absent proteinuria and the blood pressure returns to normal after 12 weeks postpartum.[77]

Preeclampsia superimposed with Chronic Hypertension

It is the sudden increase in proteinuria or BP or platelet count less than 1 lakh per ml in women with hypertension and proteinuria before 20 weeks of Gestation.[77]

Preeclampsia and Eclampsia syndrome

It is best described as the pregnancy specific disorder which causes multi organ dysfunction. It is characterised by increased blood pressure during pregnancy after 20 weeks of gestation associated with proteinuria (> 300 mg per 24 hours or Dip stick > +1).[77]

Eclampsia

Presence of new onset Grand mal seizures in a woman with pre existing Preeclampsia excluding other causes of seizures. Eclampsia can occur before, during or after labour.[77]

RISK FACTORS:

According to NICE 2010, risk factors are classified as

High risk	Moderate risk
1.History of any hypertensive disorders in Previous pregnancies	1. Primigravida
2.Chronic kidney disease	2. Women aged 40 years or or older
3.Autoimmune disease such as systemic lupus erythematosus or antiphospholipid antibody syndrome	3. Interdelivery interval greater than 10 years
4.diabetes Type 1 and 2	4. When BMI is greater than 35 kg/m^2 at the beginning of prenatal care
5.Chronic arterial hypertension	5. a family history of PE
	6. Multiple gestations.

PATHOGENESIS:

Though the complete pathogenesis is not been fully explained, there has been tremendous progress in the last decades. The placenta has always been an essential factor in the etiology of preeclampsia because when the placenta is removed it is necessary for symptoms to revert[78,79]. Numerous placental infarcts and sclerotic narrowing of arterioles were noticed when the placenta of advanced preeclampsia was examined[80].

ABNORMAL TROPHOBLASTIC INVASION:

The hypothesis of defective trophoblastic invasion and associated uteroplacental hypoperfusion could cause preeclampsia is universally accepted and is supported by animal and human studies [81]. Defective trophoblastic invasion is explained in two stages: stage 1- incomplete spiral artery remodelling in the uterus that contributes to placental ischemia, stage 2- the release of antiangiogenic factors from the ischemic placenta into the maternal circulation that contributes to endothelial damage. During the process of implantation, placental trophoblasts invade the uterus and bring about remodelling of the spiral arteries, in parallel to this, there is also obliteration of the tunica media of the myometrial spiral arteries; permitting the arteries to contain the increased blood flow, there by not depending on maternal vasomotor changes to nourish the developing fetus. Part of this remodelling to occur, the trophoblasts need to embrace an endothelial phenotype and its various adhesion molecules. If this remodelling does not take place, the placenta is denied oxygen, leading to a state of relative ischemia and an increase in oxidative stress during states of intermittent perfusion. Hence complicating pregnancies with conditions like intrauterine growth restriction, gestational hypertension, and preeclampsia.

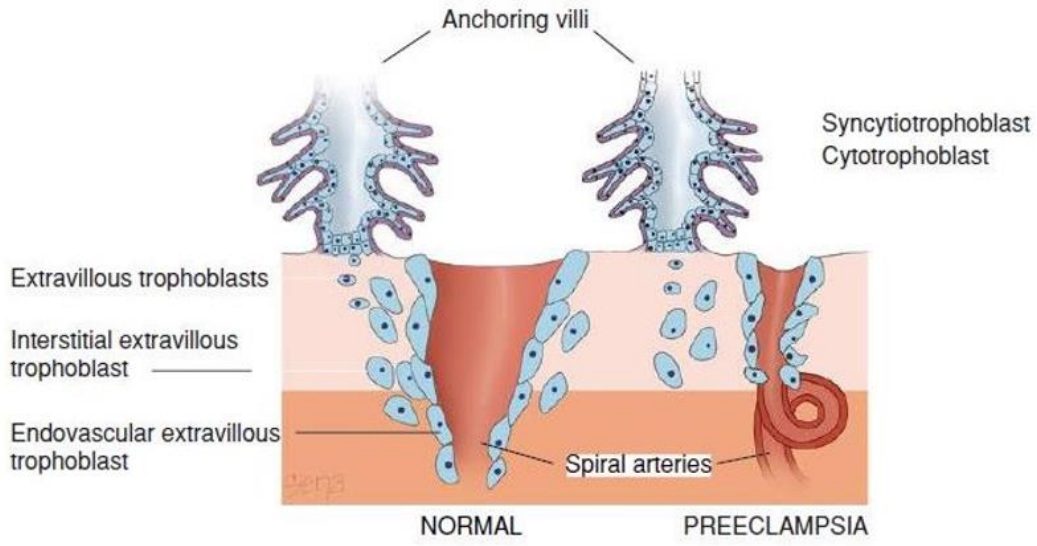


FIGURE 5: Depicting Abnormal trophoblastic invasion

ANGIOGENIC FACTORS:

sFlt-1(soluble fms- like tyrosine kinase 1) is a splice variant of the vascular endothelial growth factor (VEGF) receptor fms–like tyrosine kinase 1. Since sFlt-1 does not hold any cytoplasmic and membrane domains of the receptor, it circulates around and bind to VEGF and placental growth factor (PIGF), thereby preventing their binding to the cell surface receptor fms-like tyrosine kinase 1 (VEGF receptor 1). In a study, sFlt-1 was injected into rats using an adenovirus. following which when rat was examined, the rat happened to develop significant hypertension and albuminuria and histologic changes was found harmonious with preeclampsia (*i.e.*, glomerular enlargement, endotheliosis, and fibrin deposition within the glomeruli). Thus, it was concluded that sFlt-1 seemed to be a crucial mediator in the development of preeclampsia[82]. In the same way, another placenta–derived protein, soluble endoglin (sEng), found to be upregulated in preeclampsia just like sFlt-1. sEng, a circulating coreceptor of TGF-β, and binds with TGF-β in the plasma. Antagonizing TGF-β, a proangiogenic factor, is similar to sFlt-1 antagonizing VEGF. Similar to sFlt-1, raised levels of sEng in the blood also is proved to produce signs of severe preeclampsia in

pregnant rats. Of late, a multicentre trial among 14 countries studied the significance of these markers is their ability to predict adverse maternal and fetal outcome seen in high–risk pregnant in their second and third trimesters using angiogenic markers, concluded that a sFlt-1-to-PlGF ratio of 38 or lower drawn at 24–37 weeks of gestation could predict the lack of preeclampsia and fetal adverse outcomes within 1 week.. therefore, Hence, the amalgamation of angiogenic markers could help to risk stratify women with women of high suspicion for preeclampsia. Likewise, these angiogenic markers have also demonstrated to be useful in differentiating when in doubt with diagnosis like chronic hypertension, CKD, and lupus nephritis.

HEME OXYGENASE PATHWAY:

Currently, studies seem to be concentrating on the proximal pathways of sFlt-1 induction. One of the pathways is heme oxygenase (HO). The HO enzyme, exists in two forms, which are Hmox1 and Hmox2, breaks down heme into carbon monoxide (CO) and other products. In conditions of hypoxia and ischemic states, the Hmox gets upregulated. Carbon monoxide which is formed due to heme degradation, acts as vasodilator and thereby decreasing the perfusion pressure in the placenta. Trophoblast expresses HO, and its inhibition leads defective trophoblast invasion in vitro. It is proven that levels of Hmox are decreased in patients with preeclampsia. The increased gene expression of Hmox was shown to decrease circulating levels of sFlt-1. Fascinatingly, CO levels is increased in smokers, which may explain the smoking paradox, because smoking seems to confer a protection against preeclampsia.

HYDROGEN SULFIDE PATHWAY:

The hydrogen sulfide (H_2S) –generating system also shows association in the pathogenesis of preeclampsia. H_2S is a gas known to have vasodilatory, cytoprotective, and angiogenic

properties similar to CO. H_2S is created by three enzymes, cystathionine γ-lyase, cystathionine β-synthase, and 3-mercaptopyruvate sulfurtransferase, using the substrates cystathionine, homocysteine, cysteine, and mercaptopyruvate. It is observed that H_2S levels are decreased in preeclampsia, and also modify levels of sFlt-1 and sEng. This mechanism may be dependent on VEGF. During clinical trials on rats, it showed decreased proteinuria, hypertension, and glomerular injury. On the contrary, decreased levels of the precursor molecules of H_2S have been found in patients with preeclampsia. Prolonged administration of a cystathionine γ-lyase inhibitor, DL-propargylglycine, to pregnant mice proved to have elevated mean BP, liver damage, and decreased fetal growth. Nevertheless, subsequent administration of an H_2S-generating compound to these pregnant mice constrained the sFlt-1 and sEng levels and restored fetal growth. The closest existing compound to H_2S in clinical use is sodium thiosulfate. On the other hand, practically sodium thiosulfate has been mainly used for the treatment of calciphylaxis and resulted in case reports of severe anion gap metabolic acidosis *via* an unknown mechanism. Although, H_2S may offer benefits in preeclampsia, its safety profile is not yet ascertained.

NITRIC OXIDE PATHWAY:

The nitric oxide (NO)/nitric oxide synthase (NOS) system also seemed to be deranged in preeclampsia. NO is a potent vasodilator that achieves relaxation in vascular smooth muscle cells *through* a cyclic guanosine monophosphate pathway. Decreased levels of NO and increased levels of arginase (which disintegrates a precursor molecule in the NOS pathway) has also been noted in preeclampsia. In preeclampsia, conditions like hypertension, proteinuria and platelet dysfunction are accounted for the deficiency in NO pathway. NO deficiency induced uteroplacental changes distinctive to preeclampsia in pregnant mice, including decreased uterine artery diameter, spiral artery length, and uteroplacental blood

flow. Hence, an intact NOS system is crucial for normal spiral artery remodelling and pregnancy.

OXIDATIVE STRESS

During early pregnancy, the placenta assumes a form of oxidative stress resulting from increased placental mitochondrial activity and formation of reactive oxygen species (ROS), mainly superoxide anion. The raised levels of oxidative stress in preeclampsia and source of this stress has been attributed to the placenta, where free radical synthesis occurs, with maternal leukocytes and the maternal endothelium likely to be contributors[83]. The trophoblast of the human placenta shows the presence of superoxide-producing enzyme NADPH oxidase, which would cause oxidative stress. Conditions like Early onset of preeclampsia have been discovered to have higher superoxide production compared with those with late-onset disease[82]. Nonetheless, clinical trials of antioxidant therapy with vitamins C (1000 mg) and E (400 IU) have been not yielding and were associated with an increased number of low–birth weight babies in the treatment arm[84]. The doses of antioxidants required to antagonise the effect of ROS system is unknown, higher doses though permitted in pregnancy, the risk of unknown side effects is high.

ANGIOTENSIN RECEPTOR 1 AUTOANTIBODIES

The autoantibodies to angiotensin receptor 1 (AT1-AAs) autoantibodies seem to be pathogenic in a different of pathways. The AT1-AAs which were isolated from the serum of preeclamptic women causes upregulation of ROS and the NADPH oxidase components as well as NK-κB. When there is a block in the angiotensin receptor 1 (AT1) receptor blocker, such as losartan, was able to weaken these changes. surprisingly, the same group when infused with an endothelin antagonist into an AT1-AA–infused hypertensive rat achieved to decrease its BP. Hence, another possible pathway of AT1-AA–induced hypertension could

be *through* endothelin. When there was a transfer of purified human AT1-AA from women with preeclampsia into pregnant mice produced a similar clinical phenotype of preeclampsia, goes on to prove its pathogenicity. This phenotype was avoided by the coinjection of losartan, which is an AT1 receptor antagonist, or an antibody neutralizing peptide. Nevertheless, the only available class of medication that seemed to improve AT1-AA–induced preeclampsia is the angiotensin receptor blocker, which is teratogenic. Hence, the usage safe blockers of the AT1 system requires further research. There is evidence of a relationship between AT1-AA and angiogenic factors. The presence of AT1-AA in rats seemed to induce sFlt-1 release *by* activation of the calcineurin/nuclear factor of activated t cells pathway. Also, AT1-AA induces TNF-α which stimulates sFlt-1 and sEng and helps in overcoming its negative regulator, HO. Few studies when tested on humans found a correlation between AT1-AA and sFlt-1 levels, while few didn't, hence it remains questionable whether AT1-AA and sFlt-1 levels share the same pathophysiologic mechanism.

MISFOLDED PROTEINS:

Preeclamptic placentas have been shown to store clusters of misfolded protein, which could contribute to the pathophysiology of the disease. In a study, urine samples in preeclampsia showed congophilia, a well- known marker of protein instability and misfolding.[85]. The urine congophilic material comprises of proteoforms of ceruloplasmin, Ig free light chains, serpin peptidase inhibitor 1, albumin, IFN–inducible protein 6–16, and Alzheimer β-amyloid. Urine congophilia was found to be significantly elevated in high-risk women with severe preeclampsia and medically indicated deliveries with respect the healthy or chronic/gestational hypertension pregnant women as controls.[85]hence suggesting that congophilia plays a pathophysiologic role early in the disease and could be used as a predictive marker[86].

DIAGNOSTIC CRITERIA:

ACOG (2019): practice bulletin no 202:

ACOG defined preeclampsia as a "Preeclampsia is a disorder of pregnancy associated with new-onset hypertension, which occurs most often later, after 20 weeks of gestation and frequently near term. Although often accompanied by new-onset proteinuria, hypertension and other signs or symptoms of preeclampsia may present in some pregnant in the absence of proteinuria".

Diagnostic Criteria for Preeclampsia

Blood pressure

a. Systolic blood pressure of 140 mm Hg or more or diastolic blood pressure of 90 mm Hg or more on two occasions at least 4 hours apart after 20 weeks of gestation in a woman with a previously normal blood pressure
b. Systolic blood pressure of 160 mm Hg or more or diastolic blood pressure of 110 mm Hg or more. (Severe hypertension can be confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy)
c. **Proteinuria**
d. 300 mg or more per 24-hour urine collection (or this amount extrapolated from a timed collection) or
e. Protein/creatinine ratio of 0.3 mg/dL or more or
f. Dipstick reading of 2+ (used only if other quantitative methods not available)
g. Or in the absence of proteinuria, new-onset hypertension with the new onset of any of the following:
h. Thrombocytopenia: Platelet count less than $100{,}000 \times 10^9$/L

i. Renal insufficiency: Serum creatinine concentrations greater than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease

j. Impaired liver function: Elevated blood concentrations of liver transaminases to twice normal concentration

    i. Pulmonary edema

    ii. New-onset headache unresponsive to medication and not accounted for by alternative diagnoses or visual symptoms

***Severe* Features:**

1. Systolic blood pressure of 160 mm Hg or more, or diastolic blood pressure of 110 mm Hg or more on two occasions at least 4 hours apart (unless antihypertensive therapy is initiated before this time)
2. Thrombocytopenia (platelet count less than $100{,}000 \times 10^9$/L)
3. Impaired liver function as indicated by abnormally elevated blood concentrations of liver enzymes (to twice the upper limit normal concentration), and severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnoses
4. Renal insufficiency (serum creatinine concentration more than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease)
5. Pulmonary edema
6. New-onset headache unresponsive to medication and not accounted for by alternative diagnoses
7. Visual disturbances

MANAGEMENT OF PREECLAMPSIA

According to the ACOG criteria (2013), the first consideration in the management of non-severe Preeclampsia is safety of both mother and the fetus where as in case of severe Preeclampsia safety of the mother is given more importance.

Non severe Preeclampsia

- Once the diagnosis of mild Gestational hypertension has been established, further management depends upon the maternal and Fetal investigations, Gestational age, presence of fetal membranes, stage of labour, vaginal bleeding and other co morbidities.
- CRITERIA FOE HOME MANAGMENT OF MILD PREECLAMPSIA [85]

  -Ability to comply with the recommendations

  -DBP <100 mm of Hg

  -SBP <150 mm of Hg

  -normal laboratory tests and no maternal symptoms

  -reassuring fetal status with appropriate growth

  - Urine protein 1 gm or less in 24 hours.

If the patient does not fall into the above-mentioned criteria then the patient is advised hospital admission and managed from thereafter.

MANAGEMENT OF NONSEVERE PREECLAMPSIA:

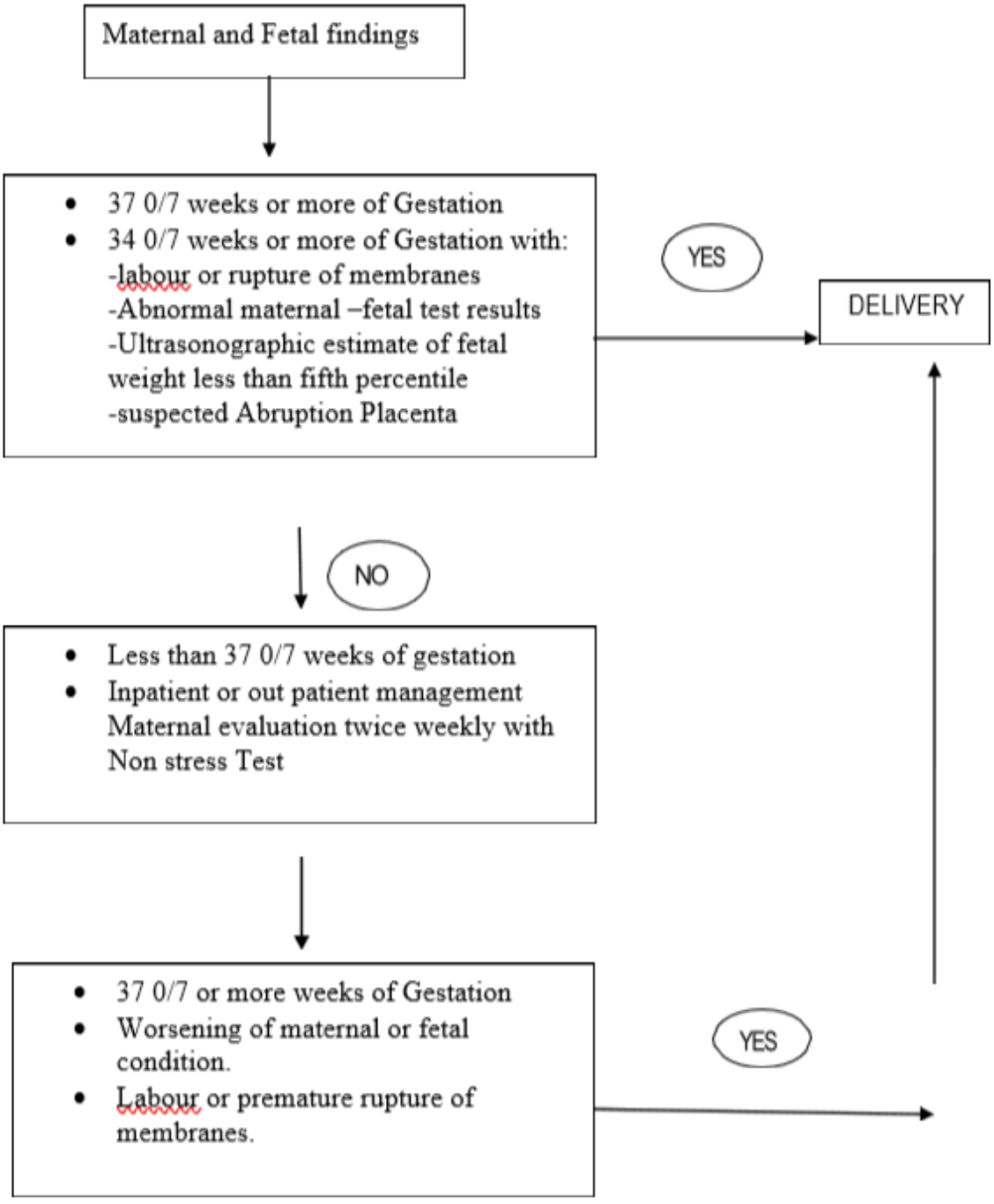


FIGURE 6: Flowchart depicting the management of non-severe preeclampsia

MANAGEMENT OF SEVERE PREECLAMPSIA:

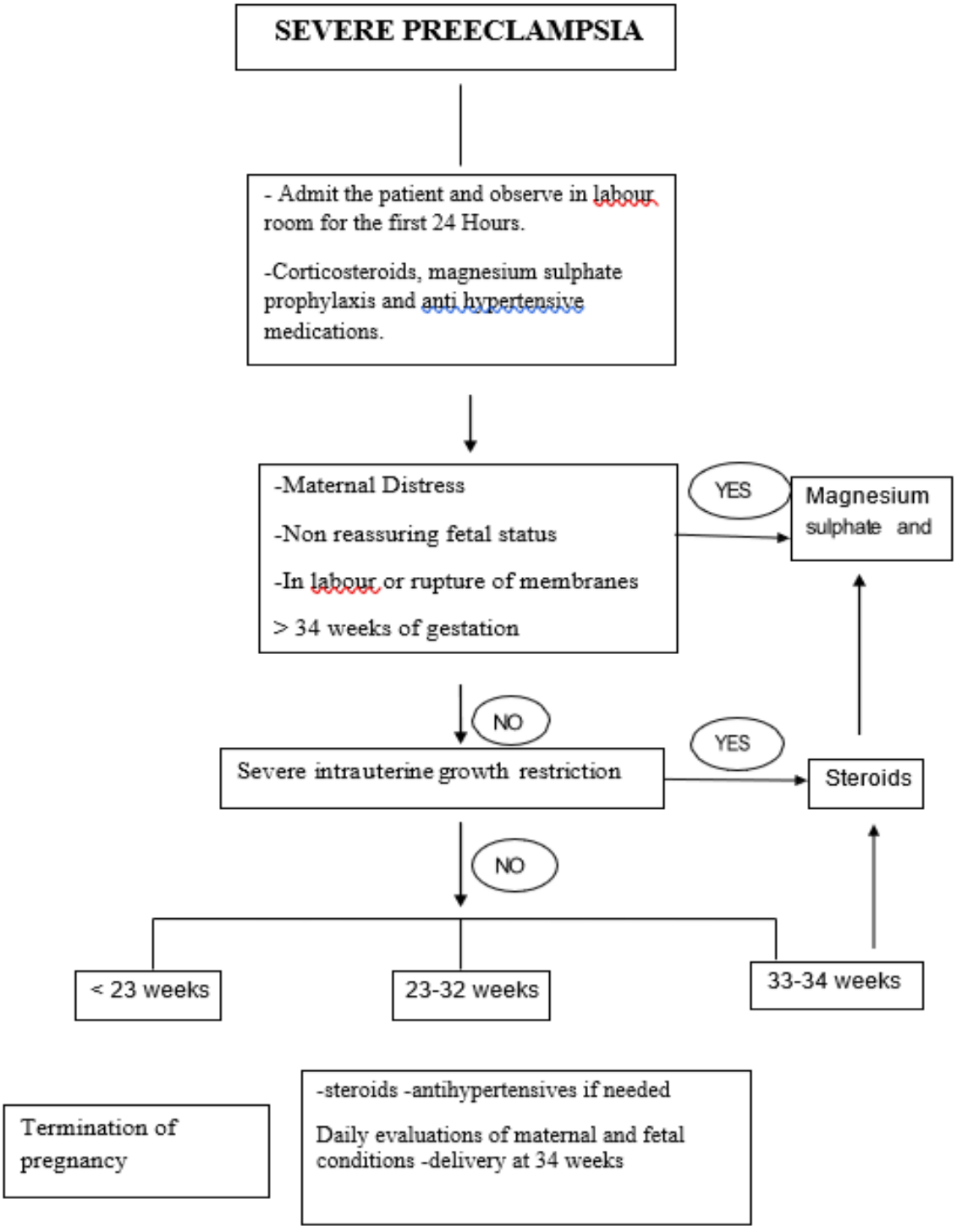


FIGURE 7: Flowchart depicting the management of severe preeclampsia

ANTI HYPERTENSIVE AGENTS USED FOR ACUTE CONTROL OF HYPERTENSION IN PREGNANCY

DRUG	DOSE	COMMENTS
Labetalol	10-20 mg IV, then 20-80 mg every 20- 30 min to a maximum dose of 300 mg or constant infusion of 1-2mg/Hr.	-First line of agent Tachycardia is less common contraindicated in patients with asthma, heart disease
Hydralazine	5 mg IV or IM,then 5-10 mg IV every 20-40 min or constant infusion 0.5-10 mg/Hr ,.	Higher dosage associated with maternal hypotension, fetal distress, head aches
Nifedipine	10-20 mg orally, repeat in 30min if needed, then 10-20 mg every 6 to 8 hours	Reflex tachycardia and head aches

Common oral anti-Hypertensive agents in pregnancy

DRUG	**DOSAGE**	**COMMENTS**
Labetalol	200-2,400 mg/day orally in two to three divided doses	Well tolerated Potential Broncho constrictive effects
Nifedipine	30-120 mg/day orally of a slow release preparation	Do not use sublingual form
Methyl dopa	0.5 -3 gm/day orally in two to three divided doses.	May not be as effective in control of severe Hypertension
Thiazide Diuretics	Depends on agents	Second line agent
Angiotensin converting receptor blockers		Associated with fetal anomalies, hence contraindicated in pregnancy and preconceptional period.

SERUM FERRITIN:

Ferritin (storage form of Iron) is a red-brown water-soluble protein which is present in the tissues of human beings. It stores Fe (III) form of iron in a soluble, nontoxic, and readily available form.

Ferritin contains 17-23% iron as a dense core of hydrated ferric oxide ~ 7nm in diameter surrounded by a protein coat made up of twenty-four subunits of molecular mass of 17 to 21-kDa . the ferritin cores are readily visible in the electron microscope, and ferritin is used as a labeling reagent in microscopy.

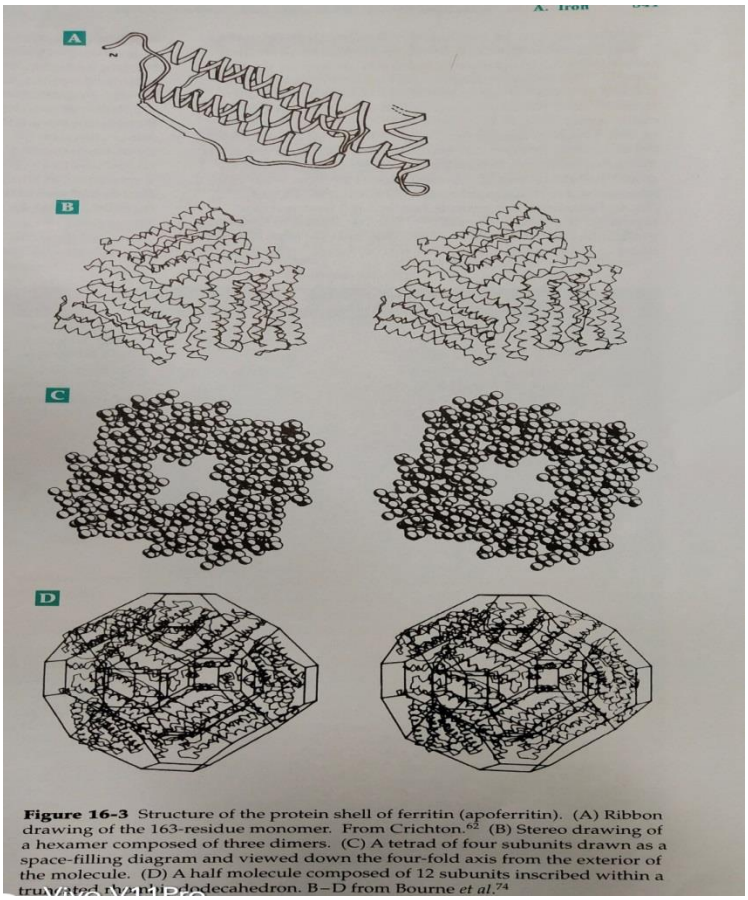


Figure 16-3 Structure of the protein shell of ferritin (apoferritin). (A) Ribbon drawing of the 163-residue monomer. From Crichton.[62] (B) Stereo drawing of a hexamer composed of three dimers. (C) A tetrad of four subunits drawn as a space-filling diagram and viewed down the four-fold axis from the exterior of the molecule. (D) A half molecule composed of 12 subunits inscribed within a truncated rhombi dodecahedron. B–D from Bourne *et al.*[74]

FIGURE 8: structure of the protein shell of ferritin (apoferritin). A- Ribbon drawing of the 163- residue monomer. B- Stereo drawing of a hexamer composed of three dimers. C- A tetrad of four subunits drawn as a space-filling diagram and viewed down the four-fold axis from the exterior of the molecule. D- a half molecule composed of 12 subunits inscribed within a truncated rhombi dodecahedron.

Iron is deposited in ferritin, when an apoferritin stands with Fe (II) salt in the presence of a suitable oxidant, which could be O_2. The physiological transfer of Fe (III) from transferrin (transfer form of iron) to ferritin requires prior reduction to Fe (II). Furthermore, as mentioned earlier iron is stored in Fe (III) form, once the reduction of Fe (III) to Fe (II) occurs, Fe (II) enters ferritin and further undergoes reoxidation of O_2 to Fe (III) and this form finally gets deposited into the ferritin core. This reoxidation process is catalyzed by Ferrooxidase sites.

$$2\ Fe^{2+} + O_2 + 4\ H_2O \longrightarrow 2\ Fe\ (O)\ OH_{core} + H_2O_2 + 4\ H^+$$

Ferooxidase sites is a dinuclear iron center containing 2 iron ions (probably Fe^{2+}) are bounded to each other. They can be later on converted to Fe^{3+} ions by O_2 that may bind initially to Fe^{2+}, forming a transient intermediate which is thought to be blue in colour and having a peroxodiferric structure.

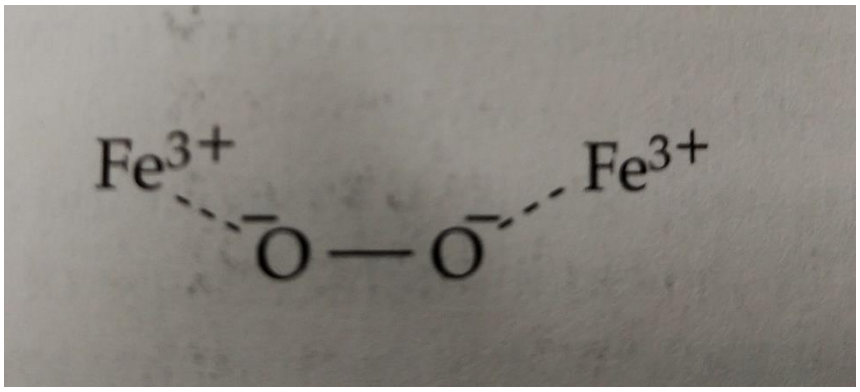


FIGURE 9- The above figure shows – ferooxidase site.

Similarly, if Fe (III) is to be removed from storage in ferritin cores, it requires reduction to Fe (II) again, possibly by the help of ascorbic acid or glutathione.[87]

Iron deficiency occurs when the iron stores fail to meet the needs for metabolism. Initially there is inadequate stores, which leads to iron deficient erythropoiesis and progressive iron deficiency can result in iron deficient erythropoiesis and ultimately leading to iron deficiency

anaemia. Iron deficiency can occur if there is insufficient iron intake or absorption, when there is excessive loss of iron and increased demand for Iron in cases like Pregnancy. [88]

Gold standard for diagnosis of iron deficiency is evaluation of bone marrow iron content. However, this is an invasive and expensive technique and hence it is not used in routine practice. Therefore, a non-invasive method- serum ferritin level evaluation steals the limelight. Ferritin measurement in the plasma/serum is used to reflect iron stores in healthy individuals. A low ferritin level therefore indicates iron deficiency anemia.[88]

MELONDIALDEHYDE:

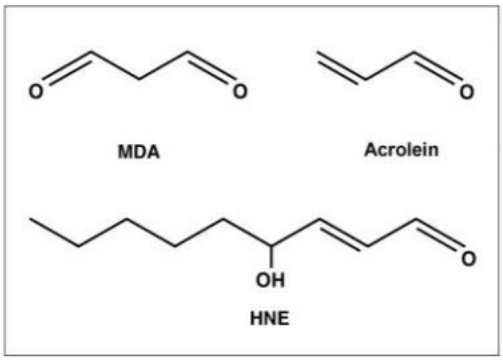


FIGURE 10: Chemical structure of MDA. (for simplicity, stereochemistries are not indicated)

MDA is a physiologic ketoaldehyde produced by peroxidative decomposition of unsaturated lipids as a byproduct of arachidonate metabolism.[89] MDA seems to be the most mutagenic product when compared with the other byproducts of lipid peroxidation. (Lipid peroxidation or reaction of oxygen with unsaturated lipids produces a wide variety of oxidation products). The increased amount of MDA produced due to tissue injury, unite with free amino groups of proteins (MDA reacts mainly with Lys residues), generating MDA-modified protein adducts. Once these proteins get modified due to the combination with MDA, their biological properties also undergo a change. Furthermore, these MDA-modified proteins are immunogenic, and autoantibodies against MDA-modified Lys residues have been identified in the sera of rabbits and humans.

MDA, an end-product by the breakdown of arachidonic acid and larger Polyunsaturated fatty acids (PUFA) can be produced through enzymatic or nonenzymatic processes.

MDA by Enzymatic Processes: MDA can be created in vivo as a by-product of enzymatic processes during the biosynthesis of thromboxane A_2(TXA_2). TXA_2 is a biologically active metabolite of arachidonic acid generated due to the action of the thromboxane A2 synthase, on prostaglandin endoperoxide or prostaglandin H2 (PGH_2) which is formerly produced by the action of cyclooxygenases on arachidonic acid.

MDA Production by Nonenzymatic Processes: A blend of lipid hydroperoxides is formed during lipid peroxidation process. The intermediate free radicals formed after cyclization process can cyclize again to form bicycle endoperoxides, which are similar to prostaglandin structurally, undergoes break down to produce MDA. By means of nonenzymatic oxygen radical-dependent reaction, arachidonic acid is the main precursor of bicyclic endoperoxide, which then goes through a series of reactions with or without the contribution of other compounds to form MDA. another pathway is through other eicosanoids which are in turn generated through nonenzymatic oxygen radical-dependent reaction, becoming the precursor of bicyclic endoperoxide and MDA.

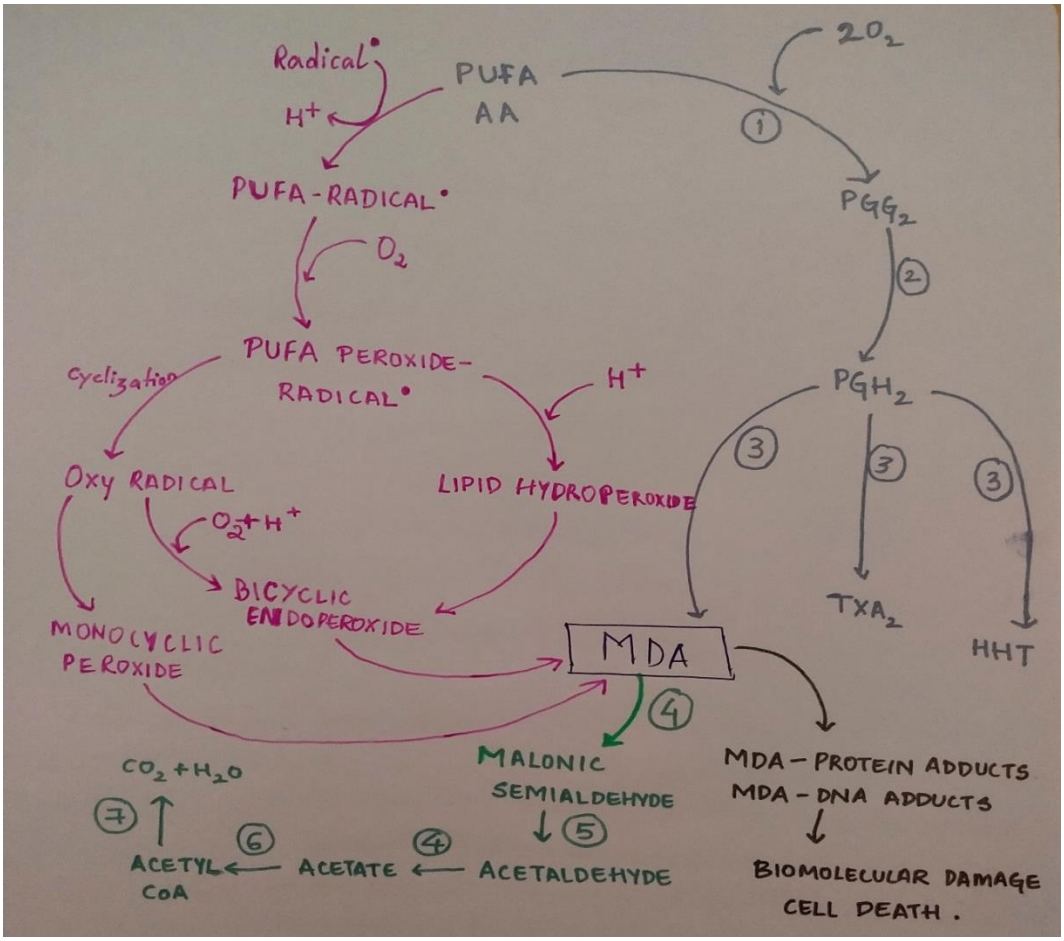


FIGURE 11: The following chart depicts MDA formation and metabolism. MDA can be generated in vivo by decomposition of arachidonic acid (AA) and larger PUFAs (polyunsaturated fatty acids) as a side product by enzymatic processes during the

biosynthesis of thromboxane A_2 (TXA_2) and 12-1-hydroxy-5,8,10-heptadecatrienoic acid (HHT)- blue pathway or through nonenzymatic processes by bicyclic endoperoxides produced during lipid peroxidation – pink pathway. Once formed MDA can be enzymatically metabolized – green pathway. Key enzymes involved in the formation and metabolism of MDA: cyclooxygenases (1), prostacyclin hydroperoxidase (2), thromboxane synthase (3), aldehyde dehydrogenases (4), decarboxylase (5), acetyl CoA synthase (6), and tricarboxylic acid cycle (7).

MDA Metabolism: MDA which is formed initially, undergoes enzymatic metabolism or may react on cellular and tissue proteins or DNA to form adducts causing biomolecular damages. Earlier it was thought that, a possible biochemical route for MDA metabolism could be its oxidation by mitochondrial aldehyde dehydrogenase followed by decarboxylation to produce acetaldehyde, which is oxidized by aldehyde dehydrogenase to acetate and further to CO_2 and H_2O. On the contrary, the phosphoglucose isomerase is perhaps responsible for metabolizing cytoplasmic MDA to methylglyoxal (MG) and further to D-lactate by enzymes of the glyoxalase system by using GSH as a cofactor. A portion of MDA is excreted in the urine as various enaminals (RNH-CH–CH-CHO) such as N-epsilon-(2-propenal) lysine, or N-2-(propenal) serine.

Because MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations, and due to MDA's high reactivity and toxicity underlying the fact that this molecule is very relevant to biomedical research community.

MATERIALS & METHODS

METHODOLOGY:

Materials:

Study designed was a comparative study conducted in Department of Obstetrics and Gynaecology of R L Jalappa hospital and Research centre, Tamaka, kolar. A teaching Hospital of Sri Devaraj URS medical college, a constituent of Sri Devaraj Urs Academy of higher Education and research. The study population consists of clinically proven sixty iron deficiency anaemia cases with preeclampsia developed after 20 weeks of gestation who were admitted to the labour room were recruited in the study after obtaining patient information consent. Similarly, sixty normotensive iron deficiency anaemia pregnant women after 20 weeks of gestational age were admitted in the labour room were recruited in the study as controls.

The study design and patient recruitment criteria was approved by institution Ethics Committee

The Study period covered is between January 2018- December 2018.

CALCULATION OF SAMPLE SIZE:

- **Sample Size calculation:** To detect 40% difference between two groups with 80% power and 90% Confidence Interval. As observed in a study where a Comparative analysis of iron status and other hematological parameters in preeclampsia was done. [6]

  60 samples of each group were calculated.

FORMULA USED

- FORMULA

$$n = \frac{2Sp^2 [Z_{1-\alpha/2} + Z_{1-\beta}]}{\mu^2 d}$$

- Where,
- $\mu^2 d$ – Mean difference between two samples
- 1-β – Power
- α – significant level

SAMPLE COLLECTION:

Four milliliter of Venous blood samples was collected from Ante Cubital vein under aseptic condition into a plain vacutainer from iron deficiency anaemia in normotensive pregnant and iron deficiency anaemia with preeclampsia after 20 weeks of gestational age .The blood samples were allowed to retract at room temperature for 10 minutes, and were centrifuged at 3000 rpm for 10 minutes to obtain clear serum. Thus, obtained clear serum samples were stored at – 20 ^{0}C and until analysis of serum ferritin and MDA levels.

Inclusion Criteria: the iron deficiency pregnant women with or without preeclampsia who visited for antenatal check-up after 20 weeks of gestational period who visited Department of OBG were included in the study. The inclusion criteria consist of pregnant women age 18-35 years, Singleton pregnancy, Gestational age after 20 weeks, All Pregnant with Hb less than 11gm/dl but more than 5 gm/dl, non-smokers and non-alcoholics and also those who were not suffering from any acute infections or chronic illnesses were considered.

Exclusion Criteria: the exclusion criteria consists of Pregnant who were transfused with packed red blood cells in the last 30 days, having multiple pregnancies, elderly primigravida, family history of preeclampsia, Had a previous diagnosis of hemochromatosis ,chronic hypertension, gestational diabetes or an active infectious process and also megaloblastic anaemia, aplastic , pernicious and haemolytic anaemia etc were excluded from the study.

Pregnant clinically fulfilling the inclusion criteria were enrolled in the study.

Detailed Demographic history regarding age, parity, gestational age, menstrual history, obstetric history and any complications in present pregnancy was taken. General clinical examination, complete obstetric examination and necessary routine investigations were done.

Methods:

Serum ferritin levels are measured by Chemiluminescence immunoassay method (CLIA method).

Serum MDA levels are measured by TBARS Method

Procedure

FERRITIN: The serum ferritin concentration is verified by CLIA method as per the procedure supplied by Vitros kit, India.

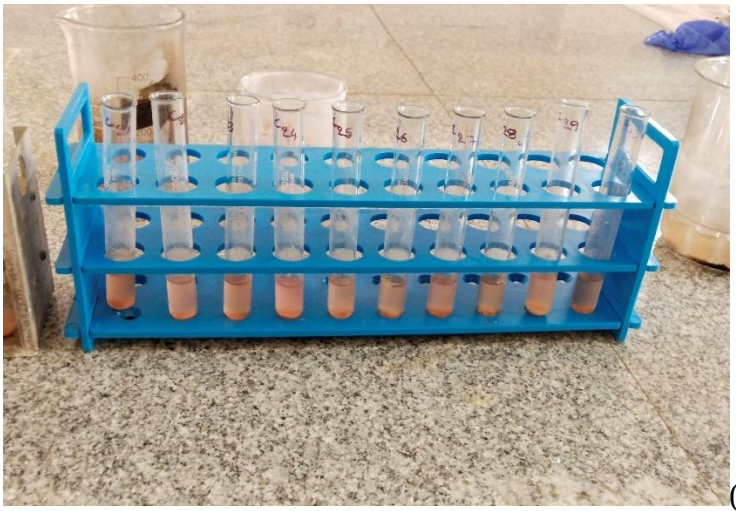
FIGURE 12: showing ferritin cartridge of Vitros ferritin kit

MDA:

Malondialdehyde reacts with thiobarbituric acid to form a pink colored complex. The absorbance was measured at 535 nm spectrophtometrically.[90]

Protein carbonyl reacts with 2,4, di-nitro phenyl hydrazine (DNPH) forming a Schiff base to produce yellow hydrazine. The absorbance was measured spectrophotometrically at 370nm.[91]

(a)



(b)

FIGURE 13(a)and(b): measurement of MDA levels by TBARS method.

STATISTICAL ANALYSIS

Apgar score, ferritin, MDA level, NICU admission were considered as primary outcome variables Gravida, parity, live birth, abortion, lab parameters Were considered as Secondary outcome variables.

Study group (Anemia Vs. Anemia+ Pre-eclampsia) was considered as Primary explanatory variable.

All Quantitative variables were checked for normal distribution within each category of explanatory variable by using visual inspection of histograms and normality Q-Q plots. Shapiro- wilk test was also conducted to assess normal distribution. Shapiro wilk test p value of >0.05 was considered as normal distribution.

For normally distributed Quantitative parameters the mean values were compared between study groups using Independent sample t-test (2 groups). For non-normally distributed Quantitative parameters, Medians and Interquartile range (IQR) were compared between study groups using Mann Whitney u test (2 groups).

Categorical outcomes were compared between study groups using Chi square test /Fisher's Exact test (If the overall sample size was < 20 or if the expected number in any one of the cells is < 5, Fisher's exact test was used.)

P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis

RESULTS

RESULTS:

The study population comprised **120 subjects, were grouped into**

Iron deficiency normotensive pregnant (n=60) and

Iron deficiency pregnant with preeclampsia (n=60)

were included in the final analysis.

The results obtained presented in the tabular form

Table1: Descriptive analysis of group in the study population (N=120)

Group	Frequency	Percentages
Anaemia	60	50.0%
Anaemia+ pre-eclampsia	60	50.0%

Among the study population 69(50%) participants had anaemia and remaining 60(50%) participants had anaemia+ pre-eclampsia. (as shown in Table 1& figure1)

Figure1: Bar chart of group in the study population (N=120)

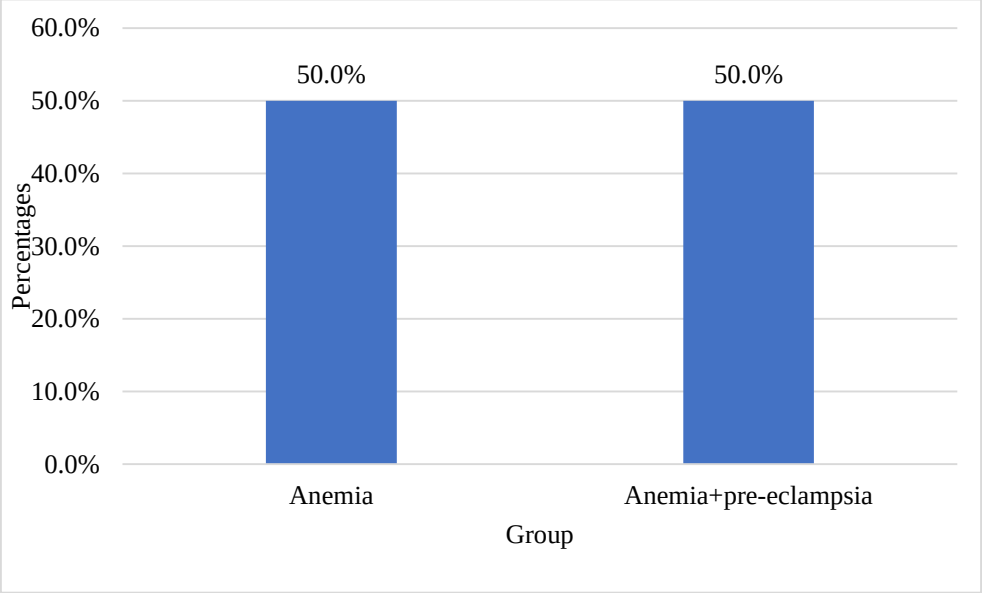


Table2: Comparison of mean age between study population (N=120)

Parameter	Group (Mean± SD)		P value*
	Anaemia (N=60)	Anaemia+preeclampsia (N=60)	
Age	24.48 ± 3.14	25.05 ± 4.56	0.429

***P value < 0.05 was considered statistically significant**

The mean age in anaemia group was 24.48 ± 3.14 years, it was 25.05 ± 4.56 years in anaemia + pre-eclampsia group. The difference in age between two groups was statistically not significant. (P value 0.429). (as shown in Table2)

Table3: Distribution of parity among the study population. (N=120)

Gravida	Group		Chi square	P value*
	Anaemia (N=60)	Anaemia+ Pre-Eclampsia (N=60)		
Primigravida	23 (38.33%)	34 (56.67%)	4.043	0.044
Multigravida	37 (61.67%)	26 (43.33%)		

***P value < 0.05 was considered statistically significant**

Among people with anaemia 23 (38.33%) had primigravida, 37 (61.67%) had multigravida. Among people with anaemia+ Pre-eclampsia 34 (56.67%) had primigravida, 26 (43.33%) had multigravida. The difference in the proportion of gravida between two groups was statistically significant. (P value 0.044). (as shown in Table3 & figure2)

Figure2: Cluster bar chart for distribution of parity among the study population (N=120)

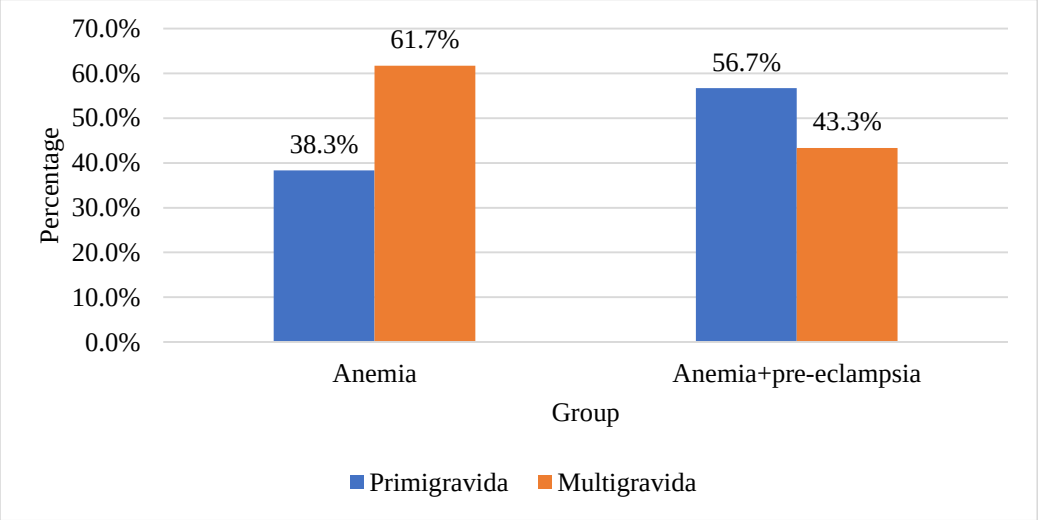


Table 4: Comparison of mean gestational age in study population (N=120)

Parameter	Group (Mean± SD)		P value*
	Anemia (N=60)	Anemia+ pre-eclampsia (N=60)	
Gestational age in weeks	37.05 ± 3.25	36.93 ± 2.91	0.833

***P value < 0.05 was considered statistically significant**

The mean gestational age in people with anaemia was 37.05 ± 3.25 weeks, it was 36.93 ± 2.91 weeks in people with anaemia + pre-eclampsia group. The difference in the mean of gestational age between two groups was statistically not significant. (P value 0.833). (as shown in Table 4)

Table 5: Comparison of mean blood pressure between study population (N=120)

Parameter	Group (Mean± SD)		P value*
	Anemia (N=60)	Anemia+ pre-eclampsia (N=60)	
Systolic blood pressure	113.47 ± 11.71	162.18 ± 19.74	<0.001
Diastolic blood pressure	74.43 ± 9.33	104.33 ± 12.32	<0.001

***P value < 0.05 was considered statistically significant**

The mean systolic blood pressure in people with anaemia was 113.47 ± 11.71 mm hg, it was 162.18 ± 19.74 in people with anaemia + pre-eclampsia group. The mean diastolic blood pressure in people with anaemia was 74.43 ± 9.33 mm hg, it was 104.33 ± 12.32 in people with anaemia + pre-eclampsia group. The difference in systolic and diastolic blood pressure between two groups was statistically significant. (P value <0.001). (as shown in Table 5)

Table 6: Comparison of median ferritin levels between study population (N=120)

Parameter	Group Median IQR		Mann Whitney P value*
	Anemia (N=60)	Anemia+ pre-eclampsia (N=60)	
Ferritin	4.63(3.44, 5.22)	27.30(11.05,61.29)	<0.001

***P value < 0.05 was considered statistically significant**

The median ferritin in people with anaemia was 4.63(IQR 3.44 to 5.22), it was 27.30(IQR 11.05 to 61.29) people with anaemia + pre-eclampsia. The difference in ferritin between two groups was statistically significant. (P value <0.001). (as shown in Table 6)

Table 7: Comparison of mean MDA levels between study population(N=120)

Parameter	Group (Mean± SD)		P value*
	Anemia (N=60)	Anemia+ pre-eclampsia (N=60)	
MDA levels	0.43 ± 0.23	0.65 ± 0.21	<0.001

***P value < 0.05 was considered statistically significant**

The mean MDA levels in people with anaemia was 0.43 ± 0.23, it was 0.65 ± 0.21 people with anaemia + pre-eclampsia. The difference in MDA levels between two groups was statistically significant. (P value <0.001). (as shown in Table 7)

Table 8: Comparison of mode of delivery between study population (N=120)

Mode of delivery	Group		Chi square	P value*
	Anemia (N=60)	Anemia+ Pre-Eclampsia (N=60)		
Normal delivery	38 (63.33%)	18 (30%)	13.393	<0.001
caesarean	22 (36.67%)	42 (70%)		

***P value < 0.05 was considered statistically significant**

Among people with anaemia 38 (63.33%) had normal delivery. Among people with anaemia + pre-eclampsia 18 (30%) had anaemia. The difference in the proportion of anaemia between two groups was statistically significant. (P value <0.001). (as shown in Table 8 & figure 3)

Figure 3: Cluster bar chart of Comparison of mode of delivery between study population (N=120)

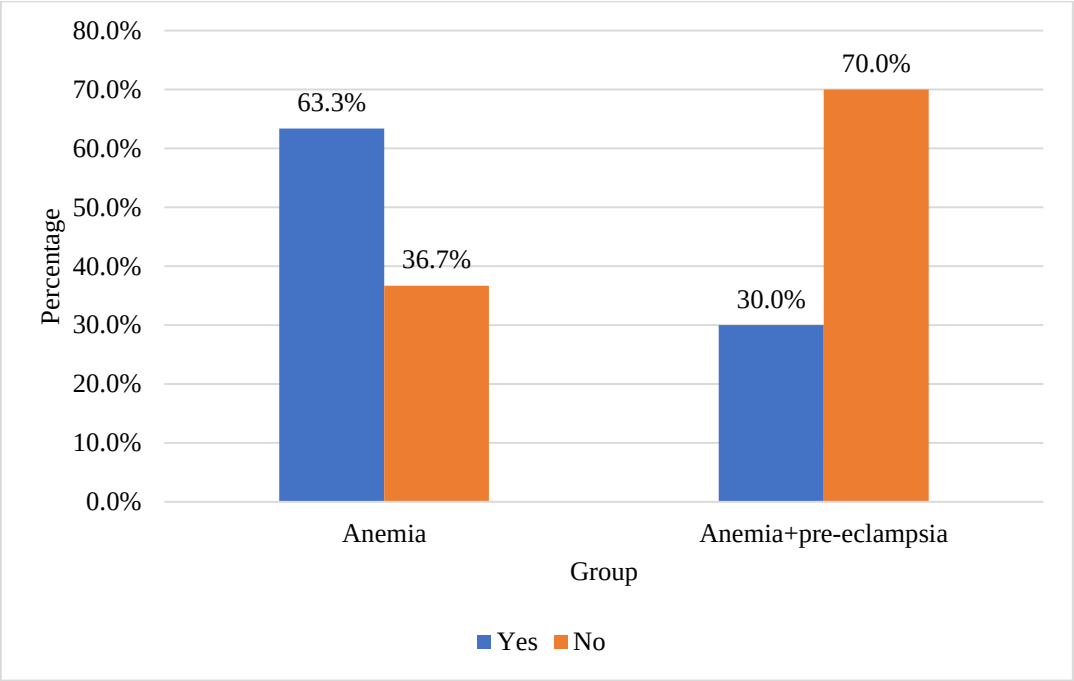


Table 9: Comparison of indication for Caesarean delivery between study population (N=120)

Indication for Caesarean delivery	Group	
	Anemia (N=60)	Anemia+ Pre-Eclampsia (N=60)
Previous LSCS	6 (10%)	0 (0%)
Previous LSCS+ Oligo	0 (0%)	1 (1.89%)
Non-Progression of Labour	1 (1.69%)	1 (1.89%)
Severe Oligo	1 (1.69%)	0 (0%)
Fetal Distress	0 (0%)	7 (13.21%)
IUGR	0 (0%)	6 (11.32%)
Meconium Stained	2 (3.39%)	1 (1.89%)
Maternal Desire	3 (5.08%)	1 (1.89%)
Oligo	0 (0%)	3 (5.66%)
Previous LSCS+ PE	1 (1.69%)	4 (7.55%)
Increased BP readings	0 (0%)	1 (1.89%)

Breech	0 (0%)	1 (1.89%)
Contracted Pelvis	0 (0%)	2 (3.77%)
CPD	0 (0%)	3 (5.66%)
CPD+ Severe PE	0 (0%)	1 (1.89%)
Imminent Eclampsia	0 (0%)	1 (1.89%)
Imminent Eclampsia+ CPD	0 (0%)	1 (1.89%)
Previous LCSC+ Maternal Desire	1 (1.69%)	0 (0%)
Previous LSCS+ Impending Scar Rupture	1 (1.69%)	0 (0%)
Severe PE	0 (0%)	2 (3.77%)
Severe PE+ HTN crisis	0 (0%)	1 (1.69%)
Severe PE+ Oligo	0 (0%)	2 (3.77%)
Severe PE+ Previous LSCS + IUGR	0 (0%)	1 (1.69%)
Severe PE+ IUGR	0 (0%)	1 (1.69%)
Transverse lie	1 (1.69%)	0 (0%)
Previous 2 LSCS	5(8.33%)	0(0%)

Table10: Comparison of APGAR score between study population (N=114)

Apgar	**Group**		**Chi square**	**P value***
	Anemia (N=57)	**Anemia+ Pre-Eclampsia (N=57)**		
Normal	56 (98.25%)	46 (80.7%)	9.314	0.002
Abnormal	1 (1.75%)	11 (19.3%)		

****6 people were not applicable**

***P value < 0.05 was considered statistically significant**

Among people with anaemia 56 (98.25%) had normal Apgar, 1 (1.75%) had abnormal Apgar. Among people with anaemia + pre-eclampsia 46 (80.7%) had normal Apgar and 11 (19.3%) had abnormal Apgar. The difference in the proportion of Apgar between two groups was statistically significant. (P value 0.002). (as shown in Table10 & figure 4)

Figure 4: Cluster bar chart of Comparison of APGAR score between study population (N=114)

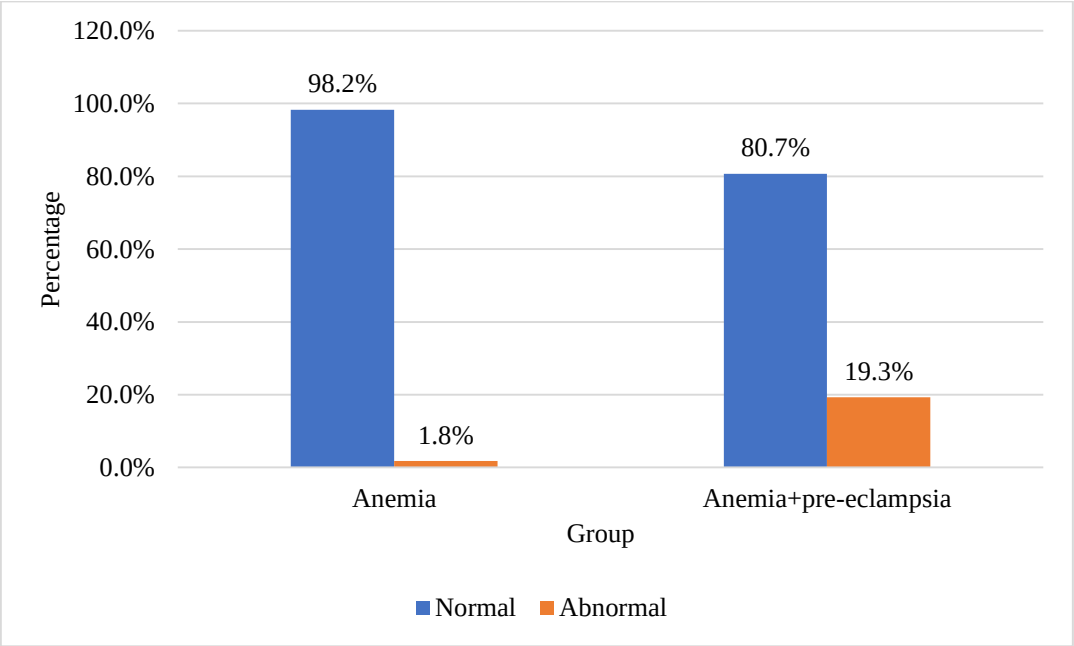


Table 11: Comparison of NICU admission between study population (N=120)

NICU Admission	Group		Chi square	P value*
	Anemia (N=60)	Anemia+ Pre-Eclampsia (N=60)		
Yes	16 (26.67%)	33 (55%)	9.968	0.002
No	44 (73.33%)	27 (45%)		

*P value < 0.05 was considered statistically significant

Among people with anaemia 16 (26.67%) babies were admitted in NICU. Among people with anaemia + pre-eclampsia 33 (55%) were admitted in NICU. The difference in the proportion of NICU admission between two groups was statistically significant. (P value 0.002). (as shown in Table11 & figure 5)

Figure 5: Cluster bar chart of comparison of NICU admission between study population (N=120)

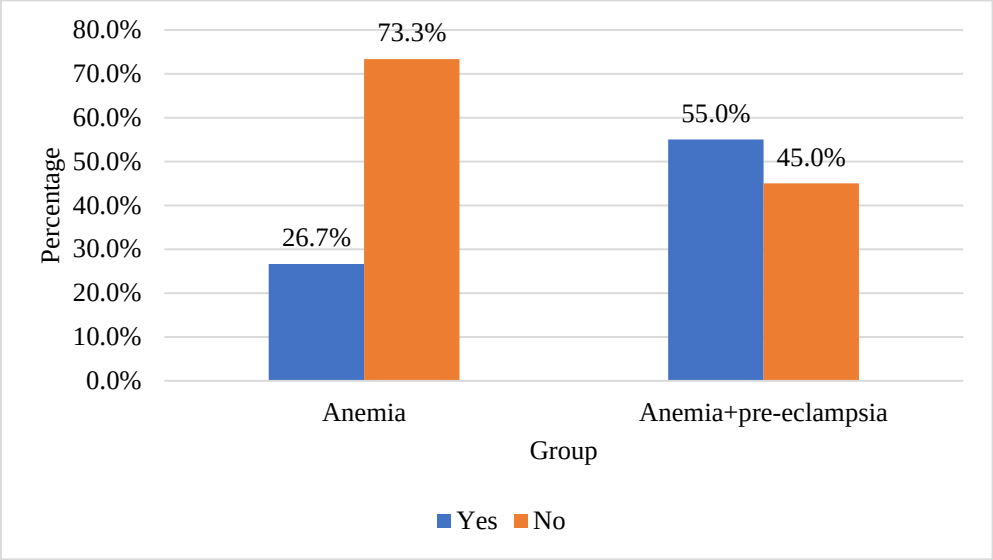


Table12: Comparison of livebirth between study population (N=120)

Livebirth	Group		Chi square	P value*
	Anemia (N=60)	Anemia+ Pre-Eclampsia (N=60)		
0	26 (43.33%)	40 (66.67%)	8.199	0.042
1	22 (36.67%)	10 (16.67%)		
2	10 (16.67%)	7 (11.67%)		
3	2 (3.33%)	3 (5%)		

***P value < 0.05 was considered statistically significant**

Among people with anaemia 22 (36.67%) had single live birth, 10 (16.67%) had two live births, 2 (3.33%) had three live births. Among people with anaemia + pre-eclampsia 10 (16.67%) had single live birth, 7 (11.67%) had two live births, 3 (5%) had three live births. The difference in the proportion of live birth between two groups was statistically significant. (P value 0.042). (as shown in Table 12)

Table13: Comparison of perinatal complications among study population (N=120)

Perinatal Complications	Group	
	Anemia (N=60)	Anemia+ Pre-Eclampsia (N=60)
IUD	2 (3.33%)	2 (3.33%)
Preterm, respiratory distress	0 (0%)	4 (6.67%)
IUGR	0 (0%)	6 (10%)
IUGR +pre term care	0 (0%)	2 (3.33%)
low birth weight	5 (8.33%)	4 (6.67%)
low birth weight, preterm	1 (1.67%)	3 (5%)
pre term	7 (11.67%)	1 (1.67%)
respiratory distress	3 (5%)	9 (15%)
Still born	1 (1.67%)	1 (1.67%)
respiratory distress, low birth weight	1 (1.67%)	0 (0%)
LGA	0 (0%)	1 (1.67%)
perinatal asphyxia	0 (0%)	1 (1.67%)
respiratory distress, IUGR	0 (0%)	1 (1.67%)
SGA	0 (0%)	1 (1.67%)
Nil	40 (66.67%)	24 (40%)

*No statistical test was applied- due to 0 subjects in the cells

Among people with anaemia majority of 7 (11.67%) had pre term, 5 (8.33%) had low birth weight. Among people with anaemia + pre-eclampsia majority of 9 (15%) had respiratory distress, 6 (10%) had IUGR, low birth weight. (as shown in Table13)

Table14: Comparison of maternal complications between study population (N=119)

Maternal Complications	Group	
	Anemia (N=60)	Anemia+ Pre-Eclampsia (N=60)
Blood Transfusion	19 (31.67%)	1 (1.67%)
Postpartum Eclampsia	0 (0%)	4 (6.67%)
Postpartum Haemorrhage	2 (3.33%)	3 (5%)
Nil	39 (65%)	52 (86.67%)

*No statistical test was applied- due to 0 subjects in the cells

Among people with anaemia 19 (31.67%) had anaemia- blood transfusion, 2 (3.33%) had postpartum haemorrhage. Among people with anaemia+ pre-eclampsia 1 (1.67%) had anaemia- blood transfusion, 4 (6.67%) had Postpartum Eclampsia and 3 (5%) had post-partum haemorrhage. (as shown in Table14)

Table15: Comparison of mean lab parameters between study population (N=120)

Parameter	Group (Mean± SD)		P value*
	Anemia (N=60)	Anemia+ pre-eclampsia (N=60)	
Hemoglobin in gm	8.34 ± 1.22	9.84 ± 1.17	<0.001
PCV (%)	27.53 ± 3.26	29.83 ± 3.65	<0.001
MCV (Fl)	71.73 ± 5.96	75.5 ± 5.59	<0.001
MCH(Pg)	23.21 ± 3.21	25.25 ± 3.25	<0.001
MCHC ()	29.21 ± 2.44	30.74 ± 1.97	<0.001
RDW ()	19.91 ± 3.82	17.81 ± 2.42	<0.001

***P value < 0.05 was considered statistically significant**

The mean hemoglobin in people with anaemia was 8.34 ± 1.22, it was 9.84 ± 1.17 in people with anaemia + pre-eclampsia. The mean PCV in people with anaemia was 27.53 ± 3.26, it was 29.83 ± 3.65 in people with anaemia + pre-eclampsia. The mean MCV Fl in people with anaemia was 71.73 ± 5.96, it was 75.5 ± 5.59 in people with anaemia + pre-eclampsia. The mean MCH Pg in people with anaemia was 23.21 ± 3.21, it was 25.25 ± 3.25 in people with anaemia + pre-eclampsia. The mean MCHC in people with anaemia was 29.21 ± 2.44, it was 30.74 ± 1.97 in people with anaemia + pre-eclampsia. The mean RDW in people with anaemia was 19.91 ± 3.82, it was 17.81 ± 2.42 in people with anaemia + pre-eclampsia. The difference in lab parameters between two groups was statistically significant. (P value <0.05) (as shown in Table15).

DISCUSSION

DISCUSSION:

Anaemia prevalence, in developing countries is 35-75% whereas, in developed countries amounts for 18% as per the WHO report. The prevalence of iron deficiency anaemia happens to be exceeding the prevalence of anaemia in general.[92] During iron deficiency anaemia, major proportion of Iron in the body is utilised for heme synthesis in turn for erythropoiesis in bone marrow. However, recent studies indicated that during iron deficiency anaemia creates a Hypoxic environment, in turn elevates oxidative stress and results causes eryptosis/Erythrocyte apoptosis. The onset of oxidative stress varies in different types of anaemia. There is increased Oxidative stress and lipid peroxidation in Iron deficiency Anaemia.[53]

in the pregnancy period, there is a probable mechanism to increase oxidative stress due to raise in prooxidants that have deleterious effect on biomolecules such as oxidation-susceptible lipids and also decrease in antioxidant concentration. In preeclampsia, there is an abnormal iron metabolism which in turn causes increase in prooxidant availability and leading to oxidative stress.[92] In the similar line of observation, in our study also observed elevated oxidative stress measured as MDA and decreased ferritin in IDA. Even though, few studies reported about elevated oxidative stress and low ferritin level in IDA group and compared to non-anaemic group. But less information is available about oxidative stress and its relation with ferritin level in IDA in normotensives and IDA in preeclampsia groups.

The demographic data of our study revealed the mean age and mean gestational age were similar in both the groups. However, it was observed that IDA in normotensives was more prevalent in multigravidae. On the contrary, there was an evidence about increased number of Primigravidae in those observed with IDA in preeclampsia group. The statistical analysis of

parity between IDA normotensive pregnant and IDA preeclamptic group show significance. ($p<0.044$). The above fact was supported by a study conducted by Paul and his co-workers where they reported more than fifty percent of preeclampsia cases falling in primigravidae group. [48]

The present study, noticed mean value of blood pressure in both the groups was statistically significant. ($P <0.001$).

Ferritin levels

In the present study, median ferritin levels of IDA in normotensives was low (4.63) when compared to IDA in preeclampsia women (27.30) and was found to be statistically significant (P value< 0.001). This proves that there is depletion of iron stores in IDA with normotensives as their ferritin levels are low. However, when compared to women with IDA in preeclamptic there seem to be elevated levels of ferritin as much as 6-fold raise than normotensives and the remaining haematological parameters were similar in both the groups.

The higher levels of ferritin in preeclamptic group could be due to haemolysis. This fact is supported by a study conducted by Gupta S et al, where they noticed increased levels of ferritin in their preeclamptic group. They explained that increased ferritin levels were not because of haemoconcentration (volume contraction) as the relationship of Hb and BUN (blood urea nitrogen): creatinine ratio was not significant in their study population. Though liver damage is a well-documented feature in preeclampsia there was no significant raise in GGT (gamma glutamyl transferase) an enzyme of liver cells hence proving liver damage was not the cause of elevated ferritin levels. They also went on to prove that cessation of erythropoiesis also was not the cause of raised ferritin levels as reticulocyte count was found to be increased. Their study showed raise in reticulocyte count, plasma free haemoglobin as

well as conjugated bilirubin thereby proving that the increase iron was possibly due to increased haemolysis. [61]

Similarly, Paul et al conducted a study where serum ferritin was more in preeclampsia compared to normotensives and chronic hypertensives. The mean serum ferritin level of cases(167.11+ 10.43) was almost 10 times higher than that of controls(17.0 + 3.03) and around 15 cases had an abnormal serum ferritin level of more than > 210 ngm/ml. [48] Contrast to this, our study showed median serum ferritin level of cases was almost more than 6 times elevated than that of normotensives.

A study by Aquirre et al, ferritin level was above the upper normal limit in only 4 patients of their preeclampsia group (13%) and one patient (3%) of the control group. However, there was a statistical significance (p= 0.019) between their study population with mean ferritin level of 36.5 of their preeclampsia group and 20.95 mean ferritin levels in their normotensives. They concluded that higher serum ferritin though in the normal range was associated with preeclamptic women receiving prophylactic iron. [6]

Almost similar to our study, Siddiqui et al, conducted a study where mean ferritin level was found to be 32.56±11.72 in preeclamptic women and around 19.89± 8.86 in normotensives. [54]

Rayman et al, showed that median serum ferritin 53.1 in preeclamptic while for controls it was found to be 9.4 the statistical significance of $p<0.001$.[50]

On the contrary, Taheripanah et al, conducted a study where serum ferritin levels were 123.8± 146.0 preeclamptic women and 334±16.2 in normal pregnant with $p<0.001$ significance. Serum ferritin level in their preeclamptic was nearly 4-10 times that of normal pregnancy. They further suggested that, there was no relation between serum ferritin level changes and anaemia in pregnancy as they failed to establish a relationship between TIBC (total iron binding capacity) and ferritin levels in their normotensive population.[49]

Study	year	Ferritin levels in IDA in preeclampsia
Paul et al	2018	167.11±10.43 (mean± SD)
Aquirre et al	2016	36.5(median)
Siddiqui et al	2011	32.56± 11.7(mean± SD)
Taheripanah et al	2007	123.8±146.0 (mean± SD)
Rayman et al	2002	53.1(median)
This study		27.30(median)

MDA levels:

In our study, the mean MDA levels seen in Iron deficiency anaemia in normotensives was found to be 0.43±0.23 and in the group with iron deficiency anaemia with preeclampsia was noticed to be 0.65±0.21(almost similar MDA levels in both the groups), with a statistical significance of $p<0.001$. Thereby confirming that there is oxidative stress in preeclampsia which is a proved theory. However, the raise of MDA levels in Iron deficiency anaemia in normotensives also demonstrates oxidative stress (MDA potent marker for oxidative stress). It is emphasized that IDA could cause preeclampsia if untreated.

In support of our study, a study conducted by Maitra et al reported MDA levels were significantly higher in their anaemia group (5.69±1.25) when compared to their normal pregnancy group (1.30±1.25). They also noticed that their anaemia group had significantly lower levels of serum iron. In addition to this, MDA levels and advanced oxidation protein products (AOPP) was also estimated which were found to be statistically significant. [53]

Rafeeinia et al, estimated mean MDA levels in preeclamptic women to be 4.62±1.17 and in healthy pregnant to be 2.95±1.41 which was statistically significant with $p<0.0001$. this

observation was similar to our study. In contrast to our study, they estimated the levels of trace elements which are Copper and Zinc, copper levels were elevated in preeclamptic women when compared to normal pregnancy while Zinc levels were found to be similar in both the groups. They concluded that raised levels of copper along with MDA could be a marker for the risk assessment of preeclampsia before the onset of clinical features. [93]

Serder et al, showed iron levels in Healthy pregnant to be 73±31, in mild preeclamptic to be 98±48 and in severe preeclampsia was 136±50 which was statistically significant. The MDA levels was found to be higher in preeclampsia group specifically in severe preeclampsia. they compared increased iron levels and MDA levels in severe preeclampsia and concluded that released iron species may link to lipid peroxidation and endothelial injury and thus contributes to the cause of preeclampsia.[58]

Bhale et al, conducted a study where MDA levels were found to be significantly increased in iron deficiency anaemia group (mean MDA level of 7.56) when compared with non-anaemic group (mean MDA level of 0.561). They concluded that iron deficiency anaemia was associated with generation of free radical, abnormalities and peroxidation of vital body molecules that could be a risk to develop preeclampsia.[52]

Study	Year	MDA levels
Maitra et al	2016	5.69± 1.25(mean)
Rafeeinia et al	2014	4.62±1.17(mean)
Bhale et al	2013	7.56(median)
This study		0.65 ± 0.21(mean)

Outcome of pregnancy:

In this study, out of 60 pregnant women having iron deficiency anaemia, 38 women (63.33%) had a normal delivery and 18 (30%) preeclamptic with iron deficiency anaemia women delivered vaginally. It was observed that caesarean deliveries that occurred in iron deficiency anaemia was seen in 22 women (36.67%) and the major indication being previous LSCS and followed by maternal desire. It was also observed there was more of caesarean deliveries in iron deficiency anaemia+ preeclampsia group (42 women-70 %) with the major indication for caesarean was fetal distress followed by IUGR (Intrauterine growth restriction) fetus.

The APGAR score (it is a scoring system is an evaluative measure of a newborn's condition at birth and the need for immediate attention. New-borns are evaluated based on five variables- heart rate, respiratory effort, muscle tone, reflex irritability, and colour.) APGAR score is said to be normal only when 1 minute and 5-minute evaluation was as follows: 1' - 5/10 and 5'- 9/10. In the present study, was found to be normal in neonates born to about 56 women (98.25%) with iron deficiency anaemia and about 46 neonates born to women (80.7%) belonging to IDA + preeclampsia group had normal APGAR score. This signifies that the oxidative stress present in IDA+ preeclampsia group could be one of the factors for fetal distress.

Our study also observed that, Intrauterine death (IUD) and still born were similar in both the groups. 7 infants born to IDA in normotensives were preterm and required NICU admission for a prolonged duration. 9 infants (15%) of IDA in preeclamptic women had respiratory distress and also required NICU admission.

Furthermore, NICU (neonatal Intensive Care Unit) admission seen in neonates born to Anaemic group was seen 16 neonates with major complications being preterm followed by low birth weight. NICU admission seen in neonates born to Anaemia+ preeclampsia group accounting to about 33 women (55%) with major perinatal complication being fetal distress followed by IUGR with low birth weight.

This was supported by a study conducted by Aquirre et al, it was observed that 5 infants born to IDA in preeclamptic women had low APGAR score and 2 infants born to IDA in normotensives had low APGAR score. 2 deaths were seen in preeclampsia group[6].

Out of 60 Iron deficiency anaemic women, 19 women (31.67%) underwent blood transfusion, 2 women had postpartum haemorrhage. Out of 60 Iron deficiency anaemia+ preeclampsia, 4 women (6.67%) had postpartum eclampsia, 3 women (5%) had post-partum haemorrhage and 1 woman (1.67%) had blood transfusion. Our study did not notice any maternal mortality among the study population.

SUMMARY

SUMMARY

It is comparative study conducted in department of Obstetrics and Gynaecology of R L Jalappa Hospital and Research centre, Tamaka, Kolar from January 2018- December 2018. After applying the inclusion/exclusion criteria and taking informed consent,60 were taken as IDA in normotensives and 60 were taken as IDA in preeclampsia patients. Detailed history regarding age, parity, gestational age, menstrual history, obstetric history, any other complications in the present pregnancy was asked and noted down. General clinical examination, complete obstetric examination and necessary investigations are done. Four milliliter of venous blood is taken in a plain vacutainer for the analysis of ferritin and oxidative stress marker MDA levels. Each woman was followed up until delivery and the maternal and fetal outcome is noted and parameters involved in IDA and Oxidative stress where are noted. Serum ferritin level was measured by Chemiluminescence immunoassay method (CLIA method) and MDA level is measured by TBARS method.

The following are the results and observation made in this study:

- Ferritin levels were found to be low in IDA in normotensives, depicting the low iron stores. Ferritin levels was noticed to be elevated in IDA in preeclamptic group which can be due to the hemolysis that occurs in preeclamptic women.
- The elevated MDA levels in both the groups was statistically significant, contributing to the fact that there is oxidative stress in both the groups. However, the MDA levels when compared, was higher in IDA in preeclamptic women.
- The prevalence of IDA in normotensives was seen in multigravidae, on the contrary, the prevalence of IDA in preeclampsia was seen in primigravidae.
- There were a greater number of cesarean deliveries in IDA in preeclamptic group, as the indication for cesarean most of the time was fetal distress followed by IUGR fetus.

- There were increased number of low birth weight infants born to IDA in normotensive mothers.
- 16 infants born to IDA in normotensives required NICU admission with the chief reason being preterm and low birth weight. Similarly, 33 infants born to IDA in preeclamptic required NICU admission major reason being respiratory distress and IUGR.
- 19 women required blood transfusion post-delivery in IDA in normotensives and 4 women had postpartum eclampsia in IDA in preeclamptic women.

Strengths of the study:

Preeclampsia is the most common obstetric complication characterised by maternal and perinatal mortality and morbidity. The exact cause for the onset of preeclampsia is poorly understood and is unclear. This poses a great challenge for health care delivery system. Identification of any newer marker for early assessment of onset of preeclampsia is the most prerequisite criteria in the management of preeclampsia. Several studies report that oxidative stress as one of the conditions associated with hypoxic environment thereby, determination of oxidative stress served essential since oxidative is implicated in damage of cell and cellular components.

Previous studies done, where the evaluation MDA levels was not correlated with maternal and fetal outcome. In our study, the correlation of the oxidative stress and ferritin levels in IDA in normotensives and IDA in preeclampsia with maternal and fetal outcome was recorded.

Study results propose that determination of MDA and ferritin level at an earlier stage may serve for the purpose of understanding preeclampsia complications.

The measure parameters are in direct correlation with maternal and fetal outcome, hence these parameters can be used as diagnostic and prognostic tool in preeclampsia complications

There is no Maternal mortality observed during the study period.

LIMITATIONS:

The Limitation of the study with respect to less sample size and lack of measurement of complete iron profile and antioxidants.

CONCLUSION

CONCLUSION:

The present study concludes that, the elevated oxidative stress in terms of MDA and more ferritin observed in IDA with preeclampsia group compared to IDA in normotensive pregnant. IDA preeclampsia group confined to Primigravida whereas IDA normotensives was confined to multigravida as parity is concerned. The study outcomes on follow up of the subjects associated with IUGR in preeclamptic group and Low birth weight in anaemia.

Therefore, determination of MDA and ferritin level at early stage may serve for the purpose of understanding preeclampsia complications.

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BIBLIOGRAPHY:

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ANNEXURES

ANNEXURES

CASE PROFORMA

NAME: IP NO:

AGE: DOA:

OCCUPATION: DOD:

ADDRESS:

EDUCATION:

HUSBANDS OCCUPATION:

SOCIOECONOMIC STATUS:

CHIEF COMPLAINTS:

HISTORY OF PRESENT ILLNESS:

OBSTETRIC HISTORY:

Marital life: Consanguinity:

Gravida: Para: living: Abortion: Dead:

Details of previous pregnancy:

Details of present pregnancy:

MENSTRUAL HISTORY:

Last menstrual period: Age of menarche:

Expected delivery date:

Period of gestation:

Period of gestation according to early scan:

Past menstrual cycles:

PAST HISTORY:

HTN/DM/BA/TB/BLOOD DYSCRASIAS/EPILEPSY/THYROID DISORDER/CARDIAC DISEASE/ALLERGY

H/O blood transfusions:

H/O Surgeries or hospitalization:

PERSONAL HISTORY:

Sleep and appetite:

Diet:

Bowel and bladder:

FAMILY HISTORY:

DRUG HISTORY:

GENERAL EXAMINATION:

General condition: Fair/ moderate/ Poor

Built: Nourishment:

Ht: cms Wt: kgs BMI:

Pallor: Icterus:

Cyanosis: Clubbing:

Lymphadenopathy: Edema:

VITALS:

Pulse rate:

Respiratory rate:

Blood pressure

Temperature:

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Central nervous system:

Per abdomen: Uterus size:

Relaxed / Irritable / Acting

Presentation: cephalic/ Breech/ other

FHS:

LOCAL EXAMINATION:

Per vaginum: Effacement:

Dilatation:

Station:

Membranes:

Pelvis:

PROVISIONAL DIAGNOSIS:

INVESTIGATIONS:

Blood group and Rh typing:

CBC: HB: HIV:

PCV: HbsAG:

RBC: VDRL:

WBC:

PLT:

RBS:

Urine analysis: Albumin-

Sugar-

Microscopy-

OBSTETRICS SCAN:

DELIVERY DETAILS:

Mode of delivery: Vaginal delivery/ Caesarean section

CAESAREAN-

Indication:

DETAILS OF NEONATE:

Sex: Date: Time:

Birth weight:

APGAR : 1'- 5'-

Admission to NICU:

MATERNAL COMPLICATIONS:

Hypertension

Convulsions

Premature rupture of membranes

Antepartum hemorrhage

Postpartum hemorrhage

Uterine hyperstimulation

FETAL COMPLICATIONS:

Respiratory distress

Admission to NICU

CONDITION AT DISCHARGE:

Mother:

Baby:

SRI DEVARAJ URS MEDICAL COLLEGE & RESEARCH CENTRE, TAMAKA, KOLAR

PATIENT INFORMATION SHEET

Study title COMPARATIVE STUDY OF IRON DEFICIENCY ANAEMIA IN PREGNANCY AND IRON DEFICIENCY ANAEMIA IN PREECLAMPSIA

Study location: R L Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar.

Please read the following information and discuss with your family members. You can ask

Patients who are of visiting OBG department OPD of R L Jalappa hospital attached to Sri Devaraj Urs medical college are recruited in the study after obtaining patient information consent.

All patients recruited are screened for serum ferritin, haematological picture and Oxidative stress.

4 ml of venous blood is collected from the study subjects for serum iron, hematological picture and oxidative stress

any question regarding the study. If you agree to participate in the study, we will collect information (as per proforma) from you or from a person responsible for you or both. Relevant history will be taken. This information collected will be used only for dissertation and publication.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. This study has been reviewed by the Institutional Ethics Committee and you are free to contact the member of the Institutional Ethics Committee. There is no compulsion to agree to this study. The care you will get will not change if you don't wish to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participate in this study.

For further information contact

Dr. Krithika Raj

Post graduate, Department of obstetrics and GynaecologyR L Jalappa hospital, Kolar. Phone no: 8197210231.

PATIENT CONSENT FORM

Case no:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I have understood that I have the right to refuse consent or withdraw it at any time during the study and this will not affect my treatment in any way. I consent voluntarily to participate in this study

"COMPARATIVE STUDY OF THE ROLE OF SERUM IRON AND OXIDATIVE STRESS IN IRON DEFICIENCY ANEMIA IN PREGNANCY AND IRON DEFICIENCY ANEMIA IN PREECLAMPSIA"

Name of Participant_____

Signature/ thumb print of Participant _____

Date _____

Statement by the researcher/person taking consent:

I have accurately read out the information sheet to the potential participant and to the best of my ability made sure that the participant understands that the following will be done:

5 ml venous blood sample taken for serum ferritin levels estimation, oxidative stress in anemia group and preeclampsia group.

I confirm that the participant was given an opportunity to ask questions about the study and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been forced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Name and Address of Principal Investigator: Dr.Krithika Raj

R.L Jalappa Hospital

Tamaka, Kolar.

KEY TO MASTER CHART

AGE:

1= 18-25 years

2= 26-33 years

3= 34- 40 years

Gestational age in weeks:

1= </= 30 weeks 6 days (but more than 20 weeks)

2= 31 weeks – 35 weeks 6 days

3= 36 weeks – 40 weeks 6 days

4= 41 weeks – 42 weeks 6 days

Mode of delivery:

1= Normal delivery

2= caesarean delivery

INDICATION FOR CESAREAN DELIVERY:

1= Previous LSCS

2= Previous LSCS+ oligohydramnios

3= Non progression of Labour

4= severe oligohydramnios

5= fetal distress

6= IUGR

7= Meconium stained

8= maternal desire

9= Oligohydramnios

10= Previous LSCS+ PE

11= Increased BP readings

12= Breech

13= Contracted Pelvis

14= Cephalopelvic disproportion (CPD)

15= CPD+ Severe PE

16= Imminent eclampsia

17= Imminent eclampsia+ CPD

18= Previous LSCS+ maternal desire

19= Previous LSCS+ Impending scar rupture

20= severe PE

21= severe PE+ HTN crisis

22= Severe PE+ previous LSCS+IUGR

23= Severe PE+ IUGR

24= Transverse lie

25= previous 2 LSCS

26= severe PE+ oligohydramnios

APGAR SCORE:

1= Normal

2= abnormal

NICU ADMISSION:

1= yes

2= No

PERINATAL COMPLICATIONS:

1= preterm

2= low birth weight

3= preterm+ low birth weight

4= respiratory distress

5= still born

6= IUD

7= IUGR

8= respiratory distress+ low birth weight

9= Small for gestational age

10= Large for gestational age

11= Preterm+ respiratory distress

12= IUGR+ preterm

13= preterm+ low birth weight

14= perinatal asphyxia

15= IUGR+ respiratory distress

MATERNAL COMPLICATIONS:

1= blood transfusion

2= postpartum eclampsia

3= postpartum haemorrhage

MASTER CHART

anaemia																				
Name	hospital no.	Age	score	gestational age	Systolic blood pressure	Diastolic blood pressure	haemoglobin(gm%)	PCV(%)	haematological picture	MCV(fl)	MCH(pg)	MCHC(%)	RDW(%)	normal delivery	indications for cesearean delivery	APGAR	NICU admission	ferritin	MDA levels	perinatal complications	maternal complications
Suma	593997	1	G2P1L1	3	120	80	7.9	27.8	mchc	62.9	17.9	28.4	19.1	1		1	2	2.99	0.261		
Amreen	513145	1	G4P1L1A2	3	112	78	9.7	30.3	mchc	77.3	25.6	30.2	17	2	1	1	2	4.22	0.301		
Gowthami	591657	1	G2A1	4	110	70	9.6	31.2	mchc	67.4	20.7	30.8	17.8	2	3	1	2	5.13	0.247		
Monika	591006	1	G2P1L1	2	110	80	6.8	23.9	mchc	65.3	18.6	28.5	18.8	2	4	1	1	6.38	0.126	1	1
puneetha	592604	1	G2P1L1	2	90	60	8.1	24	mchc	80	28	31	16.9	1		1	2	3.92	0.49		
Nagamani	575428	1	primi	3	130	80	8.5	26.8	mchc	61	18	29.5	17.52	1		1	2	5.8	0.962		
Mala	591584	1	primi	3	110	60	8.4	28.3	mchc	71.3	21.2	29.7	21.5	1		1	2	6.11	0.765		
Anjali	598550	1	primi	2	100	90	8	25	mchc	68.4	19.1	25.9	21.8	1		1	1	5.35	0.261	1	
Manjula	593609	1	G2P1L1	3	118	70	9.1	30	mchc	71.4	21.7	30.3	16.4	2	1	1	2	5.29	0.96		
Lakshmi	598499	1	primi	2	124	82	6.9	23.4	MCHC	62.4	18.4	29.5	21.3	1		1	1	5.5	0.908	1	1
Arshiya	523324	1	G4P1L1A2	3	126	90	8	30.1	mchc	61.2	20.3	33.2	16.6	1		1	2	5.21	0.9		1
Anitha	629986	2	G4P1L1A2	1	130	80	10.5	31.6	MCHC	82.9	26	30	19.05	2	1	1	2	4.92	0.282		
Vidhyashree	706603	2	primi	3	130	80	8.4	28	MCHC	70.7	21.2	30	19.9	2	7	1	2	3.68	0.256		
Nasreen Taj	757638	1	G2P1L1	3	120	70	9.3	30.2	mchc	70.1	21.6	30.8	16.3	2	18	1	2	4.29	0.9		1
Priyanka	756666	2	G2P1L1	3	110	80	9.8	30.5	mchc	72.8	23.4	30	18	2	19	1	2	5.23	0.339		
Hemalatha	757632	1	G2P1L1	3	120	70	9.5	30	mchc	75.6	23.9	30.7	15.5	1		1	2	4.79	0.493		
Lakshmi	756683	2	G5P3L3A1	1	130	70	10.1	33.9	mchc	77.7	23.5	30	17.7	1				4.24	0.366	5	
Roja	755720	1	G3P1L1A1	3	90	60	8.6	27.7	mchc	72.5	22.5	30	19.5	2	7	1	2	6.02	0.286		3
Nagaveni	756651	1	primi	3	110	70	6.3	17.4	mchc	67.9	21	28	17	1		1	2	6	0.276		3
Ramulamma	756625	2	primi	3	100	80	7.3	24.4	mchc	73.9	22.1	29.9	21.5	1		1	1	2.69	0.52	2	1
Lakshmi	755683	1	G3P2L2	3	90	70	6	21	mchc	60.3	20	25.5	17	2	1	1	1	5.33	0.268	2	1
Manjula	758485	1	primi	3	100	90	10	32.8	mchc	77.7	26.1	30.5	26	1		1	2	3.43	0.267		
Shabana	758352	2	primi	3	110	70	7.4	25.5	mchc	65.4	19	29	20.7	1		1	2	5.24	0.98		
kavitha	758450	1	G3P2L2	3	100	76	9.6	28.9	mchc	71.7	23.8	30.3	18.8	2	25	1	2	3.97	0.316		1
komala	759636	1	primi	2	90	40	10	29.1	mchc	67	26	28	16.6	1		1	1	4.95	0.521	3	
bhavya	623896	1	G4P3L2D1	3	110	60	7	20.3	mchc	65	23	27	19.7	1		1	1	5.27	0.497	2	1
Sameena Taj	631855	1	G2P1L1	2	120	80	8.5	27.9	mchc	62.8	19.1	30.5	16.6	1		1	1	3.3	0.368	1	
Anitha	540092	1	G2A1	3	130	70	8.9	28.9	mchc	74.6	23.2	31	19.6	2	8	1	2	4.83	0.338		
amaravathi	656619	1	primi	3	110	80	9.3	28.8	mchc	77.6	25.1	30.9	16.9	1		1	2	2.32	0.42		
ayesha	630356	1	G4P2L1D1A1	3	104	70	10.8	29	mchc	78.1	27.8	30.6	15	2	25	1	1	3.24	0.399	4	
shafiya	627582	1	G3P2L2	3	120	70	7.6	23.9	mchc	71.3	22.7	30.4	19.89	2	10	1	2	2.74	0.294		1
yamuna	712195	1	primi	3	110	70	8.2	26.5	mchc	75.2	21.8	29	26.5	1		1	2	5.72	0.331		
shilpa	712187	2	G3P2L2	3	110	70	7.2	20.7	mchc	78.8	29.4	28.8	26	1		1	2	3.44	0.286		1

Name	hospital no.	Age	score	gestational age	Systolic blood pressure	Diastolic blood pressure	haemoglobin(gm%)	PCV(%)	haematological picture	MCV(fl)	MCH(pg)	MCHC(%)	RDW(%)	normal delivery	indications for cesearean delivery	APGAR	NICU admission	ferritin	MDA levels	perinatal complications	maternal complications
nagarathna	710424	1	primi	3	110	70	9	29.3	mchc	74.7	23	30.7	22.9	1		2	1	4.83	0.3	4	
jyothi	706972	2	G3P1L1A1	3	130	80	8.8	25.6	mchc	81.7	27	30	17	1		1	2	4.92	0.238		1
sugana	708390	2	G3P2L1	3	130	70	9.7	28.8	mchc	78	28.3	28.8	19.7	2	25	1	2	3.39	0.262		
chanamma	706960	3	G2A1	3	130	80	8.4	28.8	mchc	72	21	29.2	21.8	1		1	2	3.63	0.292		
varalakshmi	656512	1	primi	3	120	80	8.4	28.8	mchc	74.1	24.9	30	19.9	1		1	2	5.24	0.435		
radha	532994	1	G3P2L2	3	130	86	7.2	24.1	mchc	74.6	22.3	29.9	25.3	2	25	1	2	3.35	0.717		1
malini	551247	1	G2P1L1	3	120	80	8.6	28.5	mchc	71.8	28.5	30.2	16.5	1		1	2	2.83	0.247		
mubeenaTaj	705543	1	G2P1L1	2	130	80	8.9	29.2	mchc	68.1	20.7	30.5	29.8	2	8	1	2	4.79	0.532		
fousiya	593982	1	G3P2L2	3	100	70	9.3	30.9	mchc	78	23.5	30.1	19.2	2	25	1	2	3.39	0.299		
radha	601507	1	primi	3	120	70	8	24.6	mchc	63.2	20.6	32.5	17.7	1		1	2	4.82	0.23		
Narayanamma	585629	2	G4P3L2D1	3	116	80	8.5	30.5	mchc	65.9	30.2	27	18.2	2	8	1	2	4.58	0.409		
mallika	656205	2	G3P2L2	3	100	70	10.3	31.8	mchc	80.9	26.2	30.9	16.9	1		1	2	3.92	0.255		
anitha	633745	2	G3P1L1A1	3	120	90	9.3	30.3	mchc	76.5	24.4	31.9	22.2	1		1	2	4.93	0.312		
radhamma	675544	1	primi	1	120	80	8	28.6	mchc	67	23	26	21	1		1	1	4.52	0.273	1	
farhana	700282	2	primi	1	100	90	7	29	mchc	70	19	25	22	1				3.99	0.316	6	1
mubeena	715534	1	primi	1	128	80	7	26	mchc	65	25	20	22	1				4.72	0.235	6	
suma	593981	1	primi	3	110	70	6	21	mchc	67.9	21.7	21.4	20	1		1	1	3.45	0.492	2	1
nagamani	589491	1	primi	3	110	70	8.5	29.1	mchc	75.4	22	29.2	30.1	1		1	2	4.72	0.356		
anjali	598150	1	primi	2	100	60	7	26	mchc	77.5	25.5	28.8	23	1		1	2	6.28	0.285		1
ramya	592122	1	G2P1L1	3	110	70	8.3	26.6	mchc	78	24.3	29.9	32.8	1		1	2	4.39	0.797		
radhamma	588601	1	G3P2L2	4	120	80	10.3	30.7	MCHC	79.8	30.2	32	13	2	24	1	2	4.99	0.502		1
asma	725341	1	G2P1L1	4	110	74	8.2	26.6	MCHC	64.6	19.9	30.8	17.6	1		1	1	3.5	0.371	4	
lakshmidevi	599932	2	G4P3L3	3	120	80	6	26	mchc	67	21	22	22.5	1		1	1	3.3	0.409	2	1
mala	556689	2	primi	2	110	70	7	28	mchc	69	23	29	21	1		1	1	2.8	0.301	1	
jyothi	722588	2	primi	2	100	60	6.5	29	MCHC	79.9	29.9	30.3	18	1		1	1	4.69	0.261	8	1
kouser taj	766989	2	G2P1L1	2	100	90	7	29	MCHC	76	24	28.9	21	2	1	1	1	3.29	0.779	1	1
madhavi bhai	760760	2	G4P3L3	3	140	80	10.5	19	mchc	78	23.9	29.8	20.1	1		1	2	37.9	0.547		
roja	760522	1	primi	3	150	98	10.6	35.5	mchc	80.3	26.2	31.7	15	2	14	1	2	85.2	0.9		
heena	761411	1	primi	2	140	90	7.9	25.5	MCHC	70.8	21.9	31	16.4	2	6	1	1	29.33	0.485	7	
arshiya	757137	1	primi	2	170	130	8	26.2	mchc	58.1	17.7	30.5	19.5	2	6	2	1	22.63	0.405	7	
seema	758495	2	primi	3	144	110	10.8	35.9	mchc	75.3	27	35.9	22	1		1	1	100	0.579	2	
ashwini	759114	1	primi	3	180	100	10.2	31	mchc	75.1	25.7	32.9	20	2	5	1	1	3.96	0.51	4	
sindhu	759613	1	G2A1	3	180	120	11.2	33.5	mchc	81	29.2	30	16.2	2	17	1	2	24	0.738		
sahida	600699	1	primi	3	180	120	9.4	30.3	mchc	75.9	23.6	31	18.8	2	26	1	2	4.52	1.015		
nageena	759135	2	G4P3L2D1	2	150	110	8	29.7	mchc	77.5	22.5	30.8	19.9	2	2			11.2	0.933	6	
mallika	590557	1	primi	3	156	100	10.2	32.7	MCHC	76.6	23.9	31	22.5	2	5	1	1	28.2	0.418	4	
chandrakala	590232	2	G2A1	4	150	110	9.2	26.8	mchc	80	30	31	19.3	2	20	1	2	92.3	0.855		

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asma	596209	1	primi	2	170	90	11	35.5	mchc	75.1	23.5	31.3	17.3	2	14	1	2	44.68	0.598		
shilpa	598154	1	primi	3	170	110	10.4	28.7	mchc	77	29.8	30.9	17.8	2	13	1	2	92.9	0.344		
veena	628421	1	primi	3	140	80	10	28.7	mchc	70	28.7	29.9	17	2	14	1	2	49.3	0.87		
neha Taj	655268	2	primi	3	160	120	11.3	35.4	mchc	71.1	22.7	30.9	15.6	2	11	1	2	17.7	0.375		2
ayesha	655753	1	primi	2	170	110	8.4	25.3	mchc	67.1	22.3	33.2	23.4	2	5	1	1	406	0.524	9	
anitha	633744	2	G3P1L1A1	3	140	90	10.3	32.3	mchc	76.5	24.4	31.9	22.2	1		1	2	24.9	0.596		
ashwini	705029	1	primi	3	220	110	10.1	29.5	mchc	81	30	34.2	17.3	2	7	1	1	10.1	0.656	4	
dayana	705696	1	G2P1L1	3	160	100	10	31.3	mchc	72.5	23.1	31.9	15.2	2	16	1	2	44.4	0.784		
rabeena	704744	1	primi	3	150	70	9.8	30.7	mchc	79.7	25.5	31.9	15	2	3	1	1	7.16	0.98	4	
deepa	602505	3	G4P2L1D1A1	3	180	104	8.3	25.1	mchc	76.6	27.6	29.6	20	2	10	1	2	154	0.626		
gayathri	626126	1	primi	3	150	100	10.8	31.8	mchc	77.6	26.3	31	15.2	1		1	2	55.89	0.36		
nagamma	621748	2	primi	2	140	100	10.6	31	mchc	78.1	26.7	31	14.5	2	5	1	1	8.62	0.506	11	
bindu	706474	1	G2P1L1	3	160	110	10.8	33.45	mchc	78.6	25.4	30.2	17	1		1	2	7.84	0.766		
shoba	655365	2	primi	3	150	100	11	32.4	mchc	79	29.7	29.7	18	2	13	1	1	53.6	0.439	10	
shwetha	605813	1	primi	3	150	100	10.5	29.9	mchc	82.8	29.9	30	15.7	2	20	1	2	50.2	0.395		
venkatalakshmi	629105	2	primi	3	160	100	11	33.7	mchc	78.2	25.5	30	16.2	2	15	1	2	75.9	0.538		
deepa	544023	1	primi	2	150	110	10.2	31.8	mchc	78.7	24.9	30.8	14.9	2	23	1	1	78	0.56	12	
nagamani	621748	1	primi	1	170	120	8	29	mchc	77.09	28.8	29.8	19	2	6	1	1	54	0.86	12	
ravali	712198	1	G5P2L2A2	3	170	110	10	29	mchc	74.9	25.7	34.3	15	1		1	1	22.7	0.548	4	
veena	706652	3	G2P1L1	3	150	90	11	31	mchc	80	26.9	32	18.2	2	10	1	2	18.6	0.626		
salma	707935	3	G3P2L2	3	160	100	10.8	31	mchc	82.4	28.7	34.8	15	1		1	1	24.5	0.359	2	
nagamani	706901	1	G3P2L2	3	160	110	9.3	27	mchc	82.5	28.6	30	17.6	2	6	1	1	53.6	0.287	7	
bindu	715474	1	G2P1L1	3	160	110	10.8	33.4	mchc	80	27.7	29.7	16.7	1		1	2	7.98	0.88		
divya	595260	1	primi	3	150	110	10	31.4	mchc	73.9	23.5	30.7	18.5	1		1	2	12.3	0.877		
supriya	599913	1	primi	3	140	90	11	30	MCHC	77	25	29	19	2	5	1	1	18.4	0.864	2	
shwetha	586510	2	G4P3L3	3	190	110	7	20	mchc	79.08	27.8	26.7	18.9	1		2	1	9.24	0.872	4	15
shabana Taj	600108	2	G2P1L1	2	150	110	7.6	26.6	mchc	65.3	18.8	28.5	21.3	2	22	1	1	44	0.645	3	1
umera	602510	1	G2P1L1	3	155	112	11	32.8	mchc	77	25.8	30.9	17.9	2	6	2	1	20.2	0.947	7	
suganya	600709	2	primi	1	160	110	9.4	24.6	mchc	76.5	23.5	29.8	18.8	1				7.05	0.489	5	2
Bibi fathima	590218	1	G2A1	3	160	110	10	23	mchc	77.2	25	31	16	2	9	2	1	99.6	0.296	4	
rukmini	590392	1	primi	2	186	140	10	30	MCHC	78	20	26.8	20	2	21	1	1	84.2	0.993	7	
shravanthi	740765	1	primi	3	160	100	9.6	31.2	mchc	58.4	18	30.8	21.8	2	9	2	1	5.1	0.57	7	
nasreen	737572	3	G6P2L2A3	3	160	90	8.2	26.4	mchc	60.8	18.9	31.1	20.4	2	5	2	1	8.1	0.66	4	3
madhiya	596380	1	primi	2	140	96	11	32	MCHC	80	21	24	19	2	26	1	1	22.8	0.565	3	
sabiha	593100	2	G3P2D2	3	150	110	11	29	MCHC	81	28.6	31	18	2	5	1	1	15.3	0.5	11	
kalyani	708415	1	primi	3	150	110	10.2	31	mchc	67.8	27.6	28.9	16.8	1		2	1	11	0.831	11	
shamala	601391	1	G2P1L1	3	150	100	10	29.4	mchc	79.5	27	34.8	14	2	10	1	2	6	0.974		

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nagaveni	597041	2	primi	3	140	90	7.3	21.7	mchc	76.8	26.7	31.4	15.6	1		1	2	129	0.839		1
madhiya	596481	1	primi	2	150	110	8.8	29	mchc	78.4	26.4	29.6	16.4	1		1	1	68.1	0.989	1	
aishwarya	743687	2	primi	1	190	110	10.3	35	mchc	77.7	26.5	31.1	11.7	1		2	1	23.1	0.768	11	
manjula	743944	2	G3P1L0A1	3	160	90	11	28.9	mchc	69.2	20.9	29.9	18.8	2	10	1	2	41	0.84		
aruna	744128	2	G2P1L1	2	170	110	9.1	29.7	mchc	67.7	20.7	30.6	22.1	2	12	2	1	26.4	0.833	11	
amrutha	745079	1	primi	1	190	90	10.9	33.5	mchc	72.3	24.2	33.5	15.2	1				63.1	0.395	6	2
sandhya	745078	1	G2A1	3	140	90	10.3	33.7	mchc	76.3	28.3	31.8	16.5	2	9	1	1	9.6	0.558	4	
asma	596207	2	primi	2	220	100	9.3	28.6	mchc	77.4	26.4	29.6	17.5	2	6	2	1	28.9	0.491	3	
geetha	769319	2	G4P3L3	3	180	110	7	29	mchc	75.8	28.4	27.5	16	1		1	1	35.1	0.487	2	3
shobha	593577	1	G3P2L2	2	150	120	11.1	28.2	MCHC	77	28	32	17	1		2	1	84	0.561	14	2
heena	624792	1	primi	3	220	120	10.2	32.8	mchc	79	25	29.5	19.8	1		1	2	105	0.346		
Narayanamma	585529	2	G4P3L2D1	3	190	110	8.6	29.4	mchc	65.8	19.2	29.3	18	2	8	1	2	3.85	0.759		