" MATERNAL MYOCARDIAL PERFORMANCE IN FIRST AND SECOND TRIMESTERS OF PREGNANCY WITH IRON DEFICIENCY ANEMIA".

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PADMAJA TANGEDA

Reg No – 11PHD010

Department of Physiology

SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE
VIJAYAPUR- 586103.

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B.L.D.E.UNIVERSITY

VIJAYAPUR, KARNATAKA, INDIA.

CERTIFICATE

This is to certify that this thesis entitled "MATERNAL MYOCARDIAL PERFORMANCE IN FIRST AND SECOND TRIMESTERS OF PREGNANCY WITH IRON DEFICIENCY ANEMIA" is a bonafide work of Mrs. Padmaja Tangeda and was carried out under our supervision & guidance in the department of Physiology, Shri B.M.Patil Medical College , Hospital & Research Centre, vijayapur, Karnataka, India.

Dr. Sumangala Patil
Guide
Professor in department of Physiology
Shri B.M.Patil Medical College ,
Hospital & Research Centre
Vijayapur.

Dr. Neerja Shastri
Co- guide
Associate Professor in
department of Physiology
Prathima Institute of Medical
Sciences, Karimnagar.

Dr.Manjunatha Aithala
Professor & Head
Department of Physiology
Shri Patil Medical College ,
Hospital & Research Centre
Vijayapur

Dr.S P Guggarigoudar
Dean & Principal
Faculty of medicine
Shri B.M.Patil Medical College
Hospital & Research Centre
Vijayapur.

DECLARATION

I declare that the thesis entitled " Maternal myocardial performance in first and second trimesters of pregnancy with iron deficiency anemia" has been prepared by me under the guidance of Dr. Sumangala patil, department of Physiology, B.L.D.E.University's Shri B.M.Patil Medical College, Hospital & Research Centre, vijayapur, Karnataka, India. No Part of this has formed the basis for the award of my degree or fellowship previously

Date :

Place: Vijayapur.

T.PADMAJA
Ph.D Scholar
Department of Physiology
Shri B.M.Patil Medical College,
Hospital & Research Centre
Vijayapur, Karnataka, India.

DEDICATION

I dedicate this thesis to

My Father

Late Shri T. Venakat Ram Rao

&

My Family members & my Teachers

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Date: **T. PADMAJA**

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LIST OF ABBREVIATIONS

Abbreviations

AV	Atrio Ventricular
BP	Blood Pressure
BPM	Beats per minute
BSA	Body Surface Area
BV	Blood Volume
CBC	Complete Blood Picture
CMIA	Chemiluminscence Microparticulate Immuno Assay
COP	Cardiac Output
CI	Cardiac Index
CL	Chloride
DL	Desi Liter
DBP	Diastolic Blood pressure
DPG	Di Phospho Glycerate
ECG	Electro cardiograph
EDV	End Diastolic Volume
EDD	End Diastolic Diameter
ESV	End Systolic Volume
ESD	End Systolic Diameter
EF	Ejection Fraction
2D Echo	Echocardiogram
ESVI	End Systolic Volume Index
EDTA	Ethylene Diamine Tetra Acetic Acid
FGR	Fetal Growth Restriction

FS	Fractional Shortening
Fe++	Ferrous
Fe+++	Ferric
gm	Grams
Hb	Hemoglobin
HIV	Human immuno deficiency Virus
HR	Heart Rate
HT	Height
ISE	Ion Sensitive Electrode
IDA	Iron Deficiency Anemia
LV	Left ventricular
LA	Left Atrium
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MAP	Mean Arterial Pressure
Na	Sodium
NO	Nitric Oxide
O2	Oxygen
PCV	Packed Cell Volume
PGF2	Prostaglandin F2
PGI2	ProstaglandinI2
PR	Pulse Rate
PR	Peripheral Resistance
PIH	Pregnancy Induced Hypertension
PP	Pulse Pressure
PWT	Posterior Wall Thickness

QRSD	QRS Duration
RBC	Red Blood Cell
RCT	Random control trials
RA	Right Atrium
RR	Respiratory rate
SV	Stroke Volume
SBP	Systolic Blood Pressure
SD	Standard Deviation
SIDA	Severe Iron Deficiency Anemia
SF	Serum Ferritin
SI	Serum Iron
SPECT	Single Photon Emission Computed Tomography
TB	Tuberculosis
TDI	Tissue Doppler Image
TIBC	Total Iron Binding Capacity
TVR	Total Vascular Resistance
TPR	Total peripheral Resistance
TSAT	Transferrin Saturation
WHO	World Health Organization
WT	Weight

ABSTRACT

Back Ground: Anemia during pregnancy in developing countries continues to be a public health problem of significant proportion. Most of the maternal deaths are from rural areas. Anemia affects various organs in body including the heart. Iron depletion and the amount of stored iron are reduced in iron deficiency anemia which limits red cell production. In anaemia, oxygen carrying capacity of blood decreases. However, the studies which show the effect of anemia on myocardial function during pregnancy are few in India.

Objectives: To know the effect of degrees of iron deficiency anemia on cardiac function in first & second trimesters of pregnancy & to compare the relationship between left ventricular function & haematological, biochemical parameters in both anemic pregnant and normal pregnant women.

Methods:

Total 160 pregnant women were included , in which 80 pregnant women in each trimester were selected. Out of 80 pregnant women 3 groups - normal pregnant women (control) and pregnant women with moderate & severe iron deficiency anemia in each trimester depending on the severity of anemia were selected. In first trimester normal (Group-I n=30), Moderate (Group-II n=27) & severe (Group-III n=23) , in second trimester normal (Group-I n=30), Moderate (Group-II n=29) & severe (Group-III n=21) were selected. In second trimester pregnant women without anemic treatment were selected as study groups.

Haematological parameters including CBP, Haemoglobin concentration were determined by an automated cell counter method. Biochemical parameters include estimation of serum ferritin, serum iron ,serum Total iron binding capacity & % Transferrin saturation were estimated. Serum electrolyte profile was estimated by ISE method. Echocardiograms were recorded using MEGAS CVX and MEGAS GPX equipped with ADV4 software from Italy. Two dimensional and Doppler echocardiographic examinations was performed using 3.5 MHz . Left ventricular parameters like EDD, ESD %EF, %FS were assessed. Electrocardiogram was recorded using Philips twelve channel ECG machine model TC20 in both control and study groups to evaluate myocardial performance.

Analysis of Variance (One way ANOVA) was used for comparison between normal pregnant women and anemic pregnant women in first & second trimesters of pregnancy. Bonferroni method was used in posthoc for multiple comparison. The data was analysed by SPSS 17. Left ventricular findings like EDD, ESD, EF, FS &SV were correlated with Hb%, serum ferritin & serum iron using pearson's co-relation co-efficient method.

Results: The prevalence of anemia was more in rural, illeterates, house wives, agriculture labors with low income than employees. Also with non nutritional diet, with more number of children & less pregnancy gap in study groups of both trimesters. We found a significant increase in SBP by 6%,14% ($p<0.001$), 2% & 19% ($p<0.001$) , a significant decrease in DBP by 2% & 8%($p=0.001$)(2^{nd} trimester), MAP by 11% ($p=0.03$), 3%,9% ($p=0.1$), and decrease in TPR ($p<0.001$,$P=0.005$) was observed in study groups when compared to control group of first & second trimester pregnant women. Haemoglobin %, SF, SI also shown a statistically significant decrease ($p<0.001$) in study groups when compared to controls of both trimester pregnant women. A statistically significant increase was observed in HR by 16.13%,20.12% ($p=0.001$), 10.3%,18.36%($p=0.001$) ,SV by 4.59%,8.61%($p=0.01$) , COP by 1.6%, 2.3% ($p=0.001$),EDD & ESD in both trimesters. EF% was significantly in increased in group II by 2.89% (0.001), then decreased significantly in group III by 1.82%, 1.45% in both trimesters. A statistically significant decrease was observed in by 0.29%, 0.52% ($p=0.001$), 0.15 %,) 0.41 %($p=0.01$). QRSD, QT intervals also shown significant decrease in 1^{st} & 2^{nd} trimesters.

Conclusions: In this study echocardiography findings showed decrease in contractile (EF, FS) and pumping function of heart in pregnant woman with severe iron deficiency anemia. These findings suggest that as serum ferritin & serum iron levels are decreased there is a hyperkinetic state of heart depending on degrees of iron deficiency anemia. Detection of moderate anemia in first & second trimester of pregnancy will help the clinicians in proper treatment & prevention of further complications in 3rd trimester. Besides anti-anaemic drugs, cardio tonic facilities and drugs, should be included which improve the metabolism of myocardium & thus prevents further complications to mother in third trimester.

PURPOSE OF THE STUDY

Chapter I: Purpose of the study

1.1. Introduction:

Anemia is the most common nutritional deficiency disorder globally. Among all nutritional deficiency anemias, the predominant one is iron deficiency anemia, which causes major health problem to pregnant women living in developing countries. It has most hazardous effects on pregnant women (Elise M et al., 2011).

40% of maternal deaths which occur in India are due to anemia. WHO estimates that even among the South Asian countries, India has the highest prevalence of anemia. Although, as per the estimations of WHO, the prevalence of anaemia in pregnant women is 65-75 % in India. 14 per cent in rural and 51 per cent in urban areas (DeMayer EM etal., 1998).

K.Kalaivani et al., 2009 had shown the adverse effects of Iron deficiency anemia in pregnancy. It effects not only the new born, but also increase the risk of non communicable diseases even in adult stage. Maternal anemia may also leads to low birth weight depending on its severity.

Insufficient dietary intake of iron, poor iron availability from fiber and phytate rich foods are the predominant cause for increased prevalence of anemia in rural pregnant women. Most of the maternal deaths due to anaemia are from rural areas (61%) according to the survey conducted in Telangana state (Sasikala Mootha et al., 2013).

Heart is affected according to the severity of anemia & presence or absence of secondary circulating changes in the pregnant women. Congestive heart failure occasionally develops only due to anemia (Barbara V et al., 2008). Cardiac complications are the most important factors which may lead to maternal death (MMI 2008). Anaemia is responsible for 20% of maternal deaths in the third world countries (Meseret Alem et al., 2013).

Normal pregnancy is associated with many reversible physiological and cardiovascular changes (Jyotsana et al., 2012). These remarkable changes start with the beginning of pregnancy and proceed through out pregnancy. These compensatory changes may cause a little uncomfort to mother but provides healthy environment to the fetus.

Chapter I: Purpose of the study

Normal pregnancy is accompanied by maternal hemodynamic readjustments like rise in COP, decrease in BP and SVR. These adjustments start in first trimester of pregnancy and reaches high in the second trimester. Other changes that occur are in blood volume, heart rate, stroke volume.

Increase in heart rate is the first maternal hemodynamic adaptation in pregnancy (Campos O etal., 1996). In IDA haemoglobin concentration decreases which leads to tissue hypoxia. To overcome tissue hypoxia hemodynamic & non hemodynamics mechanisms play an important role (Tang YD et al., 2006). Increased COP, increased blood flow to the tissues are the compensatory hemodynamic changes. Rapid production of erythrocytes by erythropoietin hormone, increase in 2, 3, DPG concentration inside the red blood cells are the non hemodynamic mechanisms occur to improve oxygen supply to the tissues.

Increase in left and right ventricular chamber dimensions are also marked maternal hemodynamic adaptations during normal pregnancy (Chesnutt et al., 2004). These alterations occur in order to manage metabolic demands of the fetus.

It is estimated that less than half of the women in population had insufficient iron reserves to maintain pregnancy. Hence the risk with iron deficiency and IDA will increase with gestation age (Scholl TO et al., 2005).

Iron deficiency anemia may occur in pregnancy because iron requirements increase remarkably for expansion of heamatocrit and the exchange of iron to both developing fetus and the placenta. Increased iron requirements in pregnancy mainly depend on the iron reserves & the amount of iron consumed through diet.

In IDA erythrocyte production diminishes due to less iron reserves. Estimation of Serum ferritin has significant diagnosing value for the assessment of stored iron in IDA.

Knowledge of normal maternal physiology is commonly useful for the management of pregnant women with Cardiac diseases. Pregnant women with pre-existing cardiac abnormalities ought to be counselled earlier prior to the chance of risk inpregnancy.

Echocardiography permits one to estimate as well as to evaluate myocardial performance, chamber size in the basal state and in different stages of pregnancy and the perperium.

Chapter I: Purpose of the study

Rubier et al. has stated that echo-Doppler study is the only technique that can be employed with complete safety, repeated frequently and entirely without patient discomfort (Rubier S et al., 1977).

In normal & anemic pregnant women the procedure of echocardiography was performed to assess cardiac function (Mohatvimed S.M.Nouth et al., 2001). With morphological changes in the heart, evaluation of total peripheral resistance, hemodynamic changes one may predict maternal & fetal problems(Barbara V et al., 2008)

Ischemic, hypertensive heart diseases and arrhythmias can be diagnosed in the form of graphs with an essential & simple instrument that is recording of electrocardiogram with (Wu J et al., 2003). Previous studies had shown various changes in electrocardiogram of normal anemic subjects (Coats AJS et al., 2004).

Hence, the present study is taken up

1. To evaluate the effect of iron deficiency anemia on myocardial performance in 1^{st}& 2^{nd} trimesters of pregnancy. To correlate echo parameters & haematological, biochemical parameters in both iron deficient anaemic pregnant and normal pregnant women.

2.To know the effect of IDA on ECG during 1^{st}& 2^{nd} trimester pregnant women. To compare ECG changes of iron deficient pregnant women with normal pregnant women in 1^{st}& 2^{nd} trimesters.

Chapter I: Purpose of the study

1.2. OBJECTIVES OF THE STUDY:

1.2.1. Broad Objective:

With above background, the current study aims to evaluate the effect of degrees of IDA on myocardial performance and to correlate cardiovascular function & haematological, biochemical findings in both iron deficient anemic pregnant and normal 1st& 2nd trimester pregnant women.

To evaluate myocardial performance in 1st and 2nd trimesters of pregnant women in relation to their socio economic characteristics & hematological, biochemical parameters.

1.2.2. Specific objectives:

1. To estimate hematological & biochemical parameters in control & study groups for diagnosing iron deficiency anemia (Haemogram, Serum ferritin, Serum iron & Total iron binding capacity levels).

2. To evaluate the relationship between degrees of anemia and role of socio-economic characteristics in anemic and normal pregnant women.

3. To evaluate cardiac hemodynamics and myocardial performance (by echocardiography & electrocardiography) in both pregnant women with iron deficiency anemia & normal pregnant women.

4. To evaluate relationship between degrees of iron deficiency anemia and cardiac performance in normal & iron deficient pregnant women.

5. To correlate the hematological like HB% , biochemical parameters like serum ferritin, serum iron with End Diastolic Diameter, End Systolic Diameter, Fractional Shortening% Ejection fraction% & Stroke volume in normal & iron deficient pregnant women.

1.3. Hypothesis:

Hypothesis I: Iron deficiency anemia in pregnant women is influenced by socio-economic condition.

Hypothesis II: Iron deficiency anemia effects myocardial performance of the pregnant women in various stages of pregnancy.

Hypothesis III: There is a negative correlation between HB% , Serum ferritin, Serum iron with End Diastolic Diameter, End Systolic Diameter, Fractional Shortening %, Ejection fraction % & Stroke volume in first and second trimesters of anemic pregnant women. The degree of iron deficiency anemia negatively influences the cardiovascular performances in pregnant women.

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REVIEW OF LITERATURE

Chapter I I: Review of Literature

2.1. INTRODUCTION:

Pregnancy is thought to be one of the most wonderful experiences in the woman's life.It is the process by which the life of a baby begins in the mother's womb and progresses up to the stage when it is safe to expose the baby to the external world (RL Bijalani et al.,2006)
Pregnancy is characterized by significant adaptations in each administrative systems function in the human body (Ozmen N et al., 2006, B.N.Nandini1 et al., 2014 &J Misra, et al., 1986).

Anemia is a noteworthy general medical issue which requires total elimination. According to UNICEF, 2004 estimations globally 2 billion individuals are suffering from the ill effects of iron deficiency.

Anemia is one of the most commonly experienced medical disorders in pregnancy. Iron deficiency anemia predominates and is a standout amongst the most generally experienced medicinal issue in pregnancy. In India anemia is the major cause for numerous side effects in mother and the fetus. It brings about fundamentally high maternal mortality, intrauterine growth restriction, still birth infant and prenatal death and mortality (MHFW India, 2013, & Meseret Alem et al., 2013).

2.2. Prevalence of Anemia:

As per WHO 1992 &Kennedy E 2005 estimations, about 56% of women living in developing countries are anemic. MHFW 2013, survey showed the prevalence of anemia in highly developed countries is 9%, in low developed countries the estimated prevalence is 43%. According to WHO estimations in India the prevalence of maternal anemia is about 65-75 %(DeMayer EM et al., 1998 & WHO 2004).

NFHS-2 1999, WHO 2004 & Ezzati Met al., 2002 showed that in India 54% of women in rural & 46% of urban women are anemic. In both developed and developing nations most of the pregnant women are noticeably iron deficient. However a large number of women who enter into pregnancy were already anemic. The predominance of anemia in nonpregnant women in developing nations is 43% and 12% in developed countries (WHO 1992). Predominance of iron

deficiency anemia is more prominent than the predominance of other anemias. In India more than 90% of cases are iron deficiency anemia due to the iron necessities in pregnancy are high and it is not fulfilled by dietary intake alone(Letsky EA. Et al.,1991, Fenton V et al.,1977, Galan P et al.,1990 & Galan P, et al ., 1991)

Most of the maternal deaths are from rural areas (61%) according to the survey conducted in Telangana state (SasikalaMootha et al., 2013).

2.2.1. Factors responsible for high prevalence of anemia

Yajnik et al., 2008 studied that in India the major cause for anemia is iron deficiency.
The main cause for high prevalence of IDA is due to
(i) Low dietary intake, poor iron (less than 20 mg /day) and folic acid intake (less than 70 mg/day);
(ii) Poor bioavailability of iron (3-4% only) in phytate and fibre-richIndian diet; and
(iii) Chronic blood loss due to infection such as malaria and hookworm infestations (Toteja GS et al., 2004 & NNMB, 2002).

According to the survey of NNMB 1975-2006 found that the intake of iron and folic acid was very low in all age groups of women living in India. In our country poverty, religious elements or both constitute to Iron deficiency. Many individuals in India have vegetarian food patterns which additionally adds to its contribution to iron deficiency (Galan et al., 1990).Diet alone cannot supplement to 30 –40 mgs of iron which is required for the absorption during pregnancy(Toteja GS et al., 2004 , NNMB. 2002 & DLHS on RCH 2002-2004).

Iron deficiency (with low serum ferritin and absence of stainable iron in bone marrow) develops during the later stages of pregnancy in women who enter into pregnancy with relatively adequate iron stores.

2.3. Definition of Anemia:

At the beginning of 19th century the word Anemia was a clinical term referring to pallor of the skin & mucous membranes. The word anemia was introduced by French Physician Gabriel Andral in 1843.

According to WHO, in pregnant women haemoglobin level less than 11gm/dl is considered as mild anemia and HB level less than 7 gm/dl is considered as severe anemia. The Center for Disease Control and Prevention (1990) considered anemia when HB% less than 11gm/dl in the first and third trimester and less than 10.5gm/dl in second trimester. As per WHO definition anemia is a qualitative or quantitative reduction in red blood cells (RBC) with their content HB resulting reduced oxygen (O_2)-carrying capacity of the blood to organs and tissues.

As per WHO 2001 & J.B.Sharma et al., 2003 definition for diagnosing of iron deficiency anemia in pregnancy is Hb% less than 11 g/dl , hematocrit less than 33% & serum ferritin less than 15 mg/lt. Anemia is present when the Hb% in the blood is below normal level as per the age & sex of the individual.

Although Hb value is estimated & referred to as the major parameter for determining the severity of anemia. Red blood cell counts, PCV & blood indices like MCV, MCH & MCHC provide additional information about anemia (Sharma J.B. et al., 2003 & Sharma J.B. et al., 1998).

2.3.1 . Anemia Classification:

During pregnancy based on the etiology anemia is classified as

A. Inadequate supply of nutrients essential for erythropoiesis such as:

(i) Iron deficiency

(ii) Vitamin B_{12} deficiency

(iii) Folic acid deficiency

B. Depression of erythropoietic activity:

C. Anemia associated with chronic disorders, such as infections, renal failure, chronic inflammatory diseases, aplastic anemia (decreased activity of bone marrow) and infiltration of bone marrow

Genetic - haemoglobinopathies – sickle cell disease, thalassaemia, etc

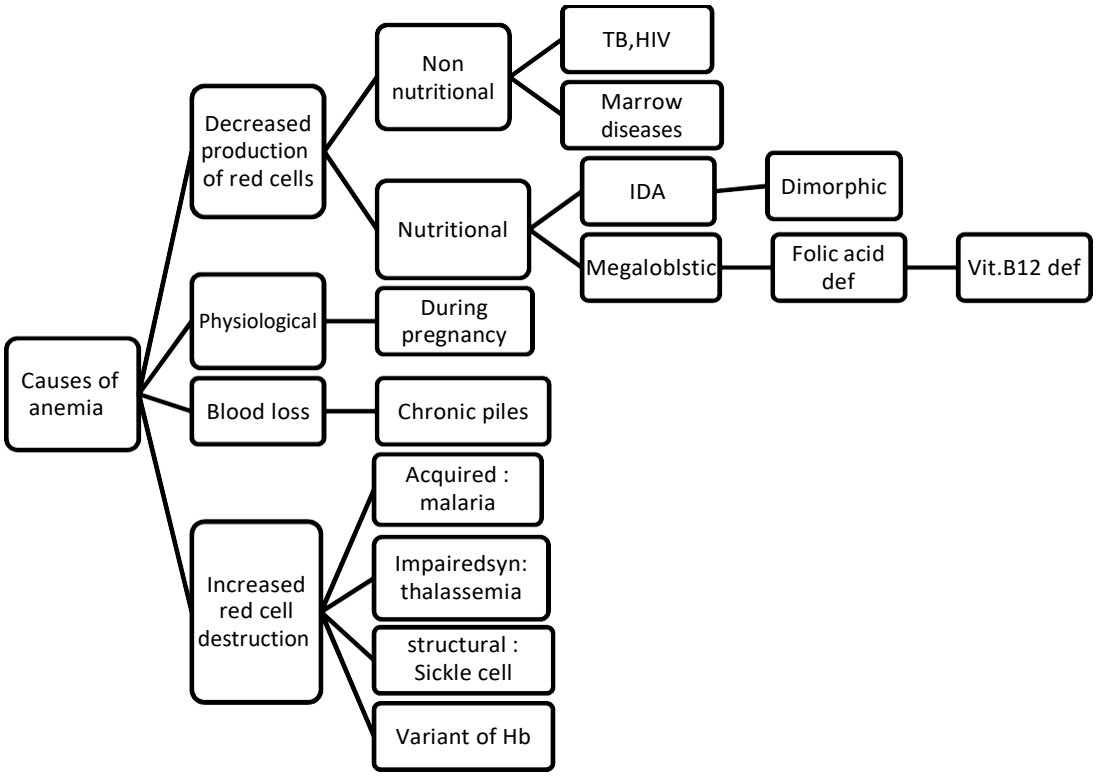

Figure1 Classification of Anemia (J.B.Sharma et al.,2010)

According to WHO Tehnical reports (1992-1993) anemia in pregnancy can be classified as mild, moderate & severe. As per the guidelines of ICMR data in India the prevalence of mild, moderate, and severe anemia are 13%, 57% and 12%respectively.

Table 1: Classification of anemia

Category	Severity of anemia	Hb level gm/dl
1	Mild	10.0-10.9 gm/dL
2	Moderate	7-9.9 gm/dL
3	Severe	<7 gm/dL

Production and maturation of RBC is called erythropoiesis. Various factors are required for production of RBCs. They include proteins, minerals (iron), trace elements (zinc, cobalt and copper), vitamins (folic acid, vitamin B12, vitamin C, pyridoxine; and riboflavin), and hormones (erythropoietin, androgens and thyroxin).

2.4. Morphology of Erythrocyte:

. NORMAL SIZE: Diameter of each RBC is 7.2 µm (range 6.9 - 7.4 µm).

. Thickness in the periphery is 2 µm and in the center 1 µm.

. Surface area of each RBC is about 120 - 140 μm^2.

. Volume is about 80 μm^3 (range 78 – 86 μm^3).

. NORMAL SHAPE: Circular, biconcave discs.

. In females, the count varies from 4.5 – 5.5 millions/ mm^3.

2.4.1. Formation of haemoglobin:

Synthesis of Hb begins in the intermediate normoblast stage of erythropoiesis & continues upto the reticulocyte stage of the red blood cells. Each molecule of heme combines with a globin, synthesized by ribosome's forming a sub unit of Hb chain. Molecular weight of each Hb chain is about 16,000. HbA is the most common form of Hb in adult human being. It has combination of 2α and 2β chains. Each Hb chain contains a heme prosthetic group containing an atom of iron

which binds loosely with one molecule of O2. Each molecule of hemoglobin carries 4 molecules of oxygen can be transported by each Hb molecule (Guyton & Jhon E Hall et al 2006).

2.5. Iron deficiency anemia(IDA):

According to Centers for disease Control 1989 &J.B.Sharma, et al., 2010, Iron deficiency anemia is one of the most common type of anemia during pregnancy. Nutritional status of iron depends on iron reserves which is favoured by dietary intake of sufficient amounts of iron or through iron supplementation.

2.5.1. Pathogenesis of iron deficiency anemia:

Negative iron balance causes decreased iron supply to the bone marrow & hence the anemia. Various factors responsible for negative iron balance are:

Causes:

1. Decrease intake – milk fed infants.
2. Increased loss:

(i) Acute hemorrhage

(ii) Chronic hemorrhage

3. Increased demand: in growing children, Pregnancy & Menstruation.
4. Defective utilization due to decreased absorption in diseases of stomach and duodenum.

2.5.2. Laboratory Finding:

The development of anemia progresses in 3 stages.

1. Firstly, storage iron depletion occurs during which iron reserves are lost without compromising the iron supply for erythropoiesis.

2. The next stage is iron deficient erythropoiesis during which the erythroid iron supply is reduced without the development of anemia.

3. The final stage is the development of frank iron deficiency anemia when the red cells become microcytic & hypochromic.

The following laboratory tests can be used to assess the varying degree of iron deficiency (Bhatt RV et al., 1997, Sharma J.B.et al., 2001 & R.L.Bijlani 3rd ed).

2.5.3. Blood picture and red cell indices:

A. Hemoglobin:

The essential feature is a fall in Hb concentration up to a variable degree.

B. Red blood cells:

The RBCs in the blood smear are hypochromic & microcytic, and there is anisocytosis & poikilocytosis. Hypochromia generally precedes microcytosis. RBC count is below normal but is generally not proportionate to the fall in hemoglobin value. When iron deficiency is associated with severe folate or vitamin B_{12} deficiency, a dimorphic blood picture occurs with dual population of red cells macrocytic as well as microcytic hypochromic.

C. Reticulocyte count:

Reticulocyte count is normal or reduced but may be slightly raised (2-5%) in cases after hemorrhage.

D. Absolute values:

The red cell indices reveal a diminished MCV (below 50 (μm^3), diminished MCH (below 15pg) and diminished MCHC (below 20%).

E. Leucocytes:

The total and differential white cell counts are usually normal.

F. Platelets:

Platelet count is usually normal.

2.5.4. Bone marrow findings:

Bone marrow examination is not essential in such cases routinely but is done in complicated cases so as to distinguish from other hypo chromic anemia.

2.5.5. Biochemical findings:

In addition to blood & bone marrow examination, the biochemical tests are of value.

A. The serum iron level is low often under 50micrograms/dl (Normal 50-175 micrograms/dl).

B. Total iron binding capacity (TIBC) is high (normal250-450micrograms/dl).

C. The serum ferritin is very low (normal 4.6-204ng/ml) indicating poor tissue iron stores.

D. The red cell protoporphyrin level is very low (normal 20-40g/dl) due to its accumulation within the red blood cells because of insufficient iron supply to form haem.

2.5.6. Characteristic features of Iron Deficiency Anemia:

1. Red Blood Cells appear as Microcytic hypochromic

(i) Count decreases or normal.

(ii) MCV, MCH, MCHC and CI decrease.

(iii) Life span – normal.

2. Bone marrow findings shows – Normoblastic hyperplasia.

3. WBC and platelets counts are – normal.

4. Nails become - dry, soft, spoon shaped. Later develop longitudinal striations.

5. Tongue becomes– red.

6. Cardiovascular/ respiratory problems include – Early breathlessness, repeated chest infections.

7. Nervous system – Irritability, loss of concentration, headache, generalized body ache, impotence.

Table 2: Laboratory findings of IDA

Hb	> 10gm%
RBC	> 4 million/mm^3
PCV	> 30%
MCHC	> 30%
MCV	> $75mm^3$
MCH	> 25pg
Serum iron	Below 30mg/100ml
TIBC	Above 400mg/100ml
%Transferrin	10% or less
Serum ferritin	Below 10mg/lt
Serum bilirubin	No increase

2.6. Iron requirement during pregnancy:

The iron necessities for pregnancy is altogether more prominent than in non pregnant stage in spite of brief reprieve from iron loses acquired during menstrual cycle Thomas et al.,2000.

Iron requirement also increases markedly in the midterm of pregnancy for the formation of fetal blood. Iron is also required for the formation of mothers own blood cell and also for the exchange of iron to developing baby and the placental structures. During second trimester, iron necessities start to increase and keep on increasing throughout the rest of pregnancy.

During parturition iron is additionally lost through maternal blood. During pregnancy the amount of iron necessities mainly depends on iron reserves & the rate of absorption of dietary iron (Thomas et al., 2000). Iron deficiency anemia in pregnancy shows, that the physiological adjustments are regularly inadequate for the expanded necessities. Therefore, iron supplementation may require during pregnancy.

Around 1000 mgs of iron is required during pregnancy (Milman N et al., 1999). 500-600 mg for the development of red blood cells, 300 mg for embryo and placenta and the remaining for developing uterus. Pregnant women need 40mg/day (ICMR 1990) of iron. Diet alone cannot give the additional iron. But, IDA occurs if iron stores are already inadequate. If the iron demand were spread evenly throughout gestation, then there will be a sustained increase in iron absorption rate. However, iron requirement show variations throughout pregnancy. Iron requirement diminishes in the 1st trimester of pregnancy because no loss of blood as menstruation stops, which represents a median saving of 160 mg of iron per day in pregnancy (Thomas et al., 2000).

The main iron losses from the body during this period are by means of the gut, skin, and pee(Green R et al.,1968). There is also some evidence Taylor DJ, 1979 that, red cell production may also decreased during this period, with a diminishment in the number of reticulocytes (Hallberg L et al., 1996), and a rise in serum ferritin concentration (Hallberg L et al., 1996 &Kaufer M, 1990).The above hematologic changes are due to improve in O2 utilization by mother and fetus.

Most of the studies (Bothwell TH197) showed that women with iron supplementation cause changes in total blood volume with expansion in plasma volume and increase in red blood cell mass. de Leeuw NKM et al., 1966 also observed rise in HB%. As pregnancy advances, iron requirements for the development of fetus increase consistently as per the weight of the baby (Widdowson EM 1951).

Iron necessities are reduced during 1st trimester, but in 2nd & 3rd trimesters, iron requirements increase around 4 and 6 mg, respectively (Requirements of vitamin A, 1988). Since major changes of red blood cells begins just in the latter half of the 2nd trimester (Hallberg L et al., 1996 &Kaufer M, 1990 &Lund CJ et al., 1967).

Iron prerequisites may increase especially during the last 6–8 wk of pregnancy (Hallberg L et al., 1992). In the latter stage of pregnancy it is clear that, dietary absorption of iron even from the most ideal diet also can't be met daily iron requirements (Bothwell TH1979).

2.7. Iron metabolism:

Iron is important not only for the formation of haemoglobin but also for other essential elements in the body like myoglobin, cytochromes, cytochrome oxidase, and peroxidise catalase. Most of the body iron is present in hemoglobin of circulating RBC's (D.M.Vasudevan et al., 2004).

2.7.1. Absorption of iron: Iron is absorbed by upper part of duodenum. 15-30% of iron is absorbed through haem iron. Half of the iron is absorbed in the form of haem iron in iron deficiency state. Iron absorption decreases to 5-8% with rich haem diet. Cereals, seeds, vegetables, milk and eggs are the nonheam iron sources. The following factors affect this absorption of iron: Only ferrous (Fe++) form (reduced form) is absorbed. Ferric (Fe+++) form is not absorbed. Iron absorption can be increased by enhancers likes haem, proteins, ascorbic acid and fermentation and decreased by inhibitors like phytic acid, fibres, calcium, tannins, tea, coffee, chocolate and herbal infusion, which form insoluble iron salts(Sharma J.B. 2003 & Hallberg L 1972).

Less amount of iron can be absorbed with little bioavailable form of iron (Hallberg L et al., 1996, Hallberg L et al., 1992) as it is present in cereals which is regular food in many developing countries. Intake of meat and ascorbic acid is restricted in many regions of our country.

2.7.2. Absorption of iron from the intestinal tract:

Iron is absorbed through all parts of the small intestine by the following mechanisms.The liver secretes moderate amount of apotransferrin in to the bile, which flows into duodenum through the bile duct. In the duodenum apotransferrin combines with free iron & also certain iron compounds. Iron enters in to the mucosal cells in ferrous state. Then iron binds with

receptors in the membranes of the intestinal epithelial cells. Transferrin molecule along with iron is released in to the blood capillaries in the form of plasma transferrin.

2.7.3. Regulation of total body iron:

Iron metabolism is unique because homeostasis is maintained by regulation of the level of absorption & not by the excretion. In iron deficiency anemia the rate of iron absorption increases as iron stores are less. When adequate quantity of iron is stored, absorption is decreased. This is referred as mucosal block of regulation of absorption of iron.

The WHO report states that the absorption of iron takes place through the following routes (WHO.Geneva. 2001, Brabin L, et al., 1998 & DeMaeyer EM, et al 1985).

1. Absorbed in ferrous form (through most iron is available in ferric form).
2. 10% of dietary iron is absorbed normally.
3. If Apoferritin is completely saturated, absorption of remaining iron is deposited as ferritin.
4. When absorption is slowed, the iron is trapped as ferritin & lost via the body as mucosal cells exfoliate.

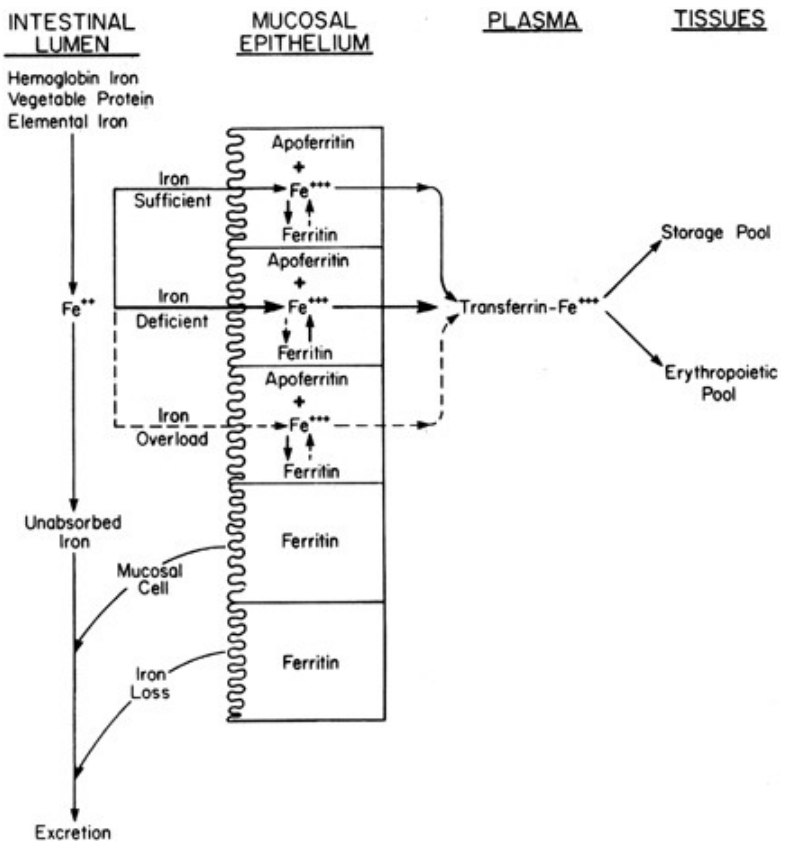

Figure 2.Mechanism of iron absorption from lumen of intestitium to various iron compartments through mucosal epithelium. (Jhon C. Morrison et al., 2016).

2.7.4 .Transport of iron:

Immediately after absorption of iron from the small intestine, in the plasma it combines with a beta globulin, apotransferrin to form transferrin. In the plasma iron is transported in bound form with transferrin. Transferrin is transport form of iron, synthesized by liver cells. In iron deficiency anemia transferrin level is increased, but serum iron level is reduced. One molecule of transferrin binds with ferric iron which can be released to any tissue cell (D.M.vasudevan et al 2004).

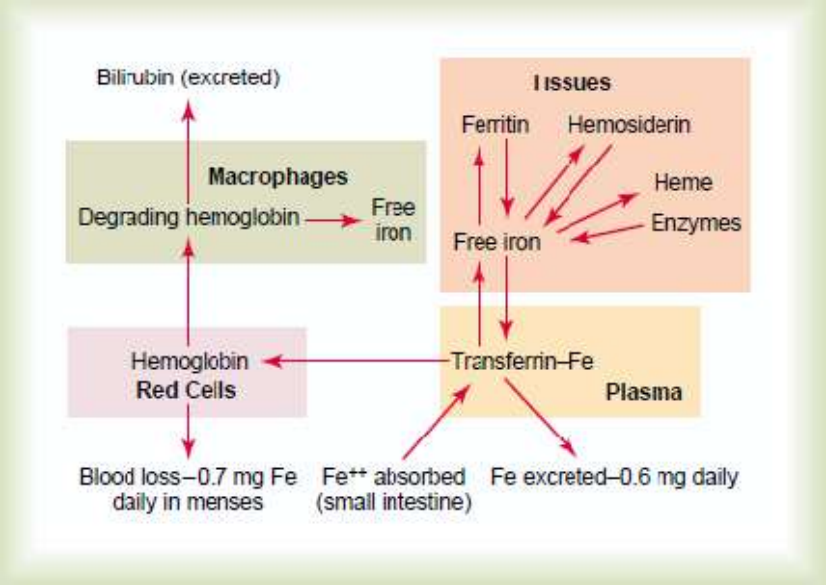

Figure 3. Iron transport & metabolism.(Guyton ,Text book of medical physiology 12ed., pg;425)

2.7.5. Storage of iron:

Iron is stored in liver, spleen and bone marrow. Ferritin is the storage form of iron. In the cell cytoplasm, iron binds with a protein apoferritin to form ferritin. The molecular weight of apoferritin is 460,000. The iron is stored as ferritin. The insoluble form of iron is called hemosiderin. In iron deficiency anemia, ferritin content is reduced. Iron in plasma, attaches to transferrin which takes it& binds to the receptors present in the cell membranes of erythroblasts bone marrow where the iron is incorporated by erythroid cells into hemoglobin. Severe hypochromic anemia develops in people who do not have sufficient quantities of transferrin in their blood and also failure of incorporation of iron in the erythroblasts. Small loss of iron occurs every day through urine, faeces, skin & nails. This loss is replaced by iron absorbed from the diet (Fenton V et al.,1977, Galan P, et al ., 1991).

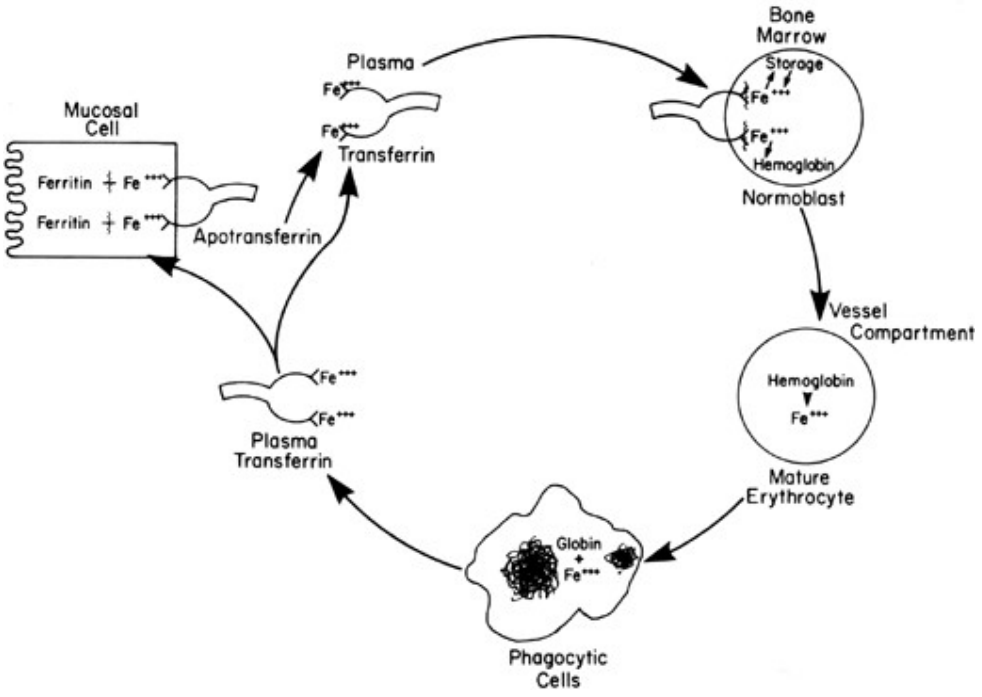

Figure 4. Transport & storage of iron (John C.Morrison et al., 2016)

2.7.6. Regulation of iron transfer to the fetus

Iron transfer from mother to the fetus is supported by a gradual increase in maternal iron absorption during pregnancy which is regulated by the placenta (Green R et al.,1968 &Lee JO et al., 2014)

Iron transfer from mother to the fetus occurs after 30th week of gestation, which corresponds to the time of peak efficiency of maternal iron absorption. Serum transferrin carries iron from the maternal circulation to transferrin receptors located on the apical surface of the placental syncytiotrophoblast. Holotransferrin is transported in to the cells through endocytosis, then iron is released into the cells. Apotransferrin is returned to the maternal circulation. The free iron then bound to ferritin in placental cells where it is transferred as apotransferrin, which enters from the fetal side of the placenta as holotransferrin into the fetal circulation. This placental iron transfer system regulates iron transport to the fetus (Allen L.H et al., 2000) When maternal iron status is poor, the number of placental transferrin receptors increases so that more iron is taken up by the placenta. When mother is iron deficient, the capacity of this system may be insufficient to maintain iron transfer from mother to the fetus (D.M.Vasudevea et al., 2004).

2.8. Assessment of Iron Status in Pregnancy:

In pregnancy assessment of iron status is difficult, because significant maternal hemodynamic adaptations may influence several factors of iron status. During pregnancy expansion in plasma volume leads to a fall in HB% (WHO. Geneva 2001). Assessing Serum ferritin, Serum iron & Total iron binding capacity gives an idea about iron deficiency anemia(Svanberg B, et al., 1975).

Keld-Erik Byg et al., 2000, concluded that Serum ferritin concentration is a reliable, non-invasive diagnosing test for assessing iron status in pregnancy & post partum. They conducted a longitudinal study on healthy pregnant women and compared Ferritin with other iron status markers. They assessed true positive and false positive rates of the iron status markers in the diagnosis of depleted iron stores during normal pregnancy and postpartum. They observed that in general, the true positive rates of other iron status markers in the diagnosis of iron depletion (serum ferritin < 16 &mgr;g/L) were low in pregnancy and postpartum. Transferrin saturation and MCH values has the highest true positive rates. Based on the results they concluded that the sensitivities of other iron status markers were too low and have clinical value in the diagnosis of iron depletion.

In an another cross sectional study by Intragumtornchai T et al ., showed the diagnostic values of serum ferritin and other conventional laboratory tests in patients with liver cirrhosis. They proved that serum ferritin was the most powerful diagnosing test for prediction of iron deficiency.

2.9. Structure of heart:

In human heart, ventricle is one of two large chambers which collect blood from atrial chambers and expel received blood towards the peripheral beds inside the body and lungs. The atrial chamber (an adjacent /upper heart chamber that is smaller than a ventricle) act as primary pump. The right ventricle pumps blood into the pulmonary circulation and the left ventricle pumps blood into the systemic flow through the aorta.

2.9.1. Functions of ventricle:

Ventricles have thicker walls than atria. The physiologic load on the ventricles requires high pressure to fill the ventricle, for the pumping of blood throughout the body and lungs. Further, the left ventricle has thicker wall than the right since it needs to pump blood to the greater part of the body while the right ventricle pumps to the lungs.

During systole, the ventricles contract & pump the blood to entire body. During diastole, the ventricles relax and load with blood once again. The left ventricle receives oxygenated blood from the left atrium through mitral valve and pumps the blood to the aorta through aortic valve as systemic circulation. The left ventricular muscle must relax and contract rapidly to increase or decrease its pumping capacity under the control of the nervous system. After every contraction in the diastolic stage, ventricles enlarge quickly in order to fill with the oxygenated blood. Similarly in the systolic stage, the left ventricle must contract quickly and forcibly to pump filled blood into the aorta, by overcoming higher aortic pressure (G.K.Pal et al., 2016). Extra pressure is also required for the expansion of aorta and other arteries to accommodate blood volume.

The right atrium receives deoxygenated blood from entire body through superior & inferior vena cavae and pumps the blood to right ventricle through tricuspid valve. The blood from right ventricle goes to the pulmonary trunk via the pulmonary valve, as pulmonary circulation.

The function of the ventricles can be measured with several volumetric parameters, like End-Diastolic Volume , End-Systolic Volume , Stroke Volume and Ejection Fraction(Akihiro Kurita et al ., 2008 &Schlosser et al., 2005).

The dimensions of the heart and its performance can be measured with M-Mode echocardiography.In this regards, echocardiography is most reliable non invasive technique for pregnant women without uncomfort to the fetus (Jyotsana R et al., 2012 & Katz R et al., 1978). Echocardiography is noninvasive imaging test used to evaluate left ventricular function as well as for diagnosing cardiac disease in pregnant women (Weiner CP et al., 1994 & Thornburg KL et al., 2000). During pregnancy progressive enlargement of all heart chambers, small pericardial effusion and mild tricuspid and pulmonic valve regurgitation can be evaluated. However, if any

echocardiographic abnormality is detected then a thorough clinical evaluation should be done in pregnancy.

Echocardiography was utilized to estimate & evaluate myocardial performance, chamber size in the basal state and during different stages of pregnancy and in the perperium is done by using echocardiography (Bamfo JE etal., 2007) . Rubier et al.has stated that echo-Doppler study is the only technique which can be employed with complete safety, repeated frequently and entirely without patient discom fort(Jyotsana R et al., 2012and Tasneem Naqvi et al.,2012).

The studies on diastolic function in normal pregnancies assessed by tissue Doppler methods had shown variable results (Ueland K et al., 1979, Schrier RW et al., 1991, Maynard SE etal., 2004). They found that diastolic LV function was unaltered during normal pregnancy. Kametas et al., 2007 measured left ventricular function by using conventional doppler measurements. They observed an increase in mitral peak E-wave velocity and ratio of E-wave/A-wave velocities in the first two trimesters, then a decline in the third trimester. Zentner et al. 2009 reported that there was deterioration in both systolic and diastolic function in late normal pregnancy.

During pregnancy changes in systolic & diastolic functions may lead to development of several signs and symptoms which can mimic the signs and symptoms of heart disease (Chesnutt et al., 2004). Stewart Hunter et al., 1992 studied that major maternal cardiovascular adaptations develop in normal pregnancy and the knowledge of these changes is essential to manage cardiovascular disease in pregnant women. During pregnancy cardiac output increases but the extent of increase and the causes for its increase was not known. The earliest indirect Ficks and dye dilutions methods have in accuracy, potential hazards and ethical problems. To overcome these problems investigators have found non invasive methods of M mode echocardiography and impedance. Echocardiography was first performed in 1960s and 1970s. Cardiac output was measured serially without risk, uncomfort to the subject. A poorcorrelation was observed with impedance cardiograph when compared with thermo dilution techniques. deSwiet et al 1986 had raised doubt on this method. Later M mode echocardiography introduced for longitudinal studies in pregnancy. Stroke volume was calculated by multiplying the left ventricular internal dimensions. With the help of this method left ventricle structure &

functions can be measured easily. This method was valid when ventricular function and structure

are normal. During pregnancy heart volume as well as end diastolic dimension both increase, so in that aspect this method may not be valid.

Later Echo Doppler methods have introduced and accepted for the way of calculating stroke volume and cardiac output. Then this technique was applied on pregnant women for serial assessment of cardiac output.

In 1st trimester increase in cardiac output was observed and the increase was continued to 2nd trimester. The increase in cop was 45% above the non-pregnant level. Thereafter no further significant change was found. Both heart rate and stroke volume contributed to increased cop. The increase in heart rate was seen in early weeks of gestation and continued till third trimester. The increase in stroke volume occurred a little late at eight weeks and reached to maximum at about 20 weeks of gestation.

S.R.Ommen et al., reported that Doppler echocardiography provides useful information about the status of the left ventricular (LV), diastolic filling than cardiac catheterization. Tissue Doppler imaging of the mitral annulus and mitral inflow velocity curves provides better estimations of LV filling pressures than other methods (pulmonary vein, preload reduction).

S. M. NOUH et al proved Non-invasive evaluation of cardiac changes in normal pregnant women, their clinical significance and implications by echocardiography, Doppler color studies, resting electrocardiogram and 24 -hour Holter monitoring in healthy pregnant women. Doppler color flow studies detected that some cases with trivial mitral regurgitation and some cases with combined mild mitral and tricuspid regurgitation. While other cases had mild tricuspid regurgitation. Mitral and tricuspid regurgitation was diagnosed by color Dopplerstudies. And no arryhthrnias were detected in 24-hour ambulatory Holter monitoring.N-hours Holter monitoring showed no ventricular or supraventricular arrhythmias.

Rizwana Solanki etal.,2011 assessed Cardiovascular Hemodynamics & its outcomes in Preeclamptic pregnant women by echocardiography.He found that in preeclamptic subjects mean cardiac output, mean LV diastolic mass, systolic mass ,total vascular resistance were more as compared to normotensive subjects. Women with preeclampsia delivered smaller babies as compared to normotensive controls. With these findings they concluded that women with preeclampsia have significant systolic and diastolic dysfunction compared to normotensive

controls. Blood pressure monitoring alone is insufficient to identify effectively, risk of cardiovascular complications in these subjects.

W. Y. FOK et al., 2006 evaluated maternal diastolic function by using tissue doppler imaging technique. Tissue Doppler imaging is echocardiographic technique for evaluating diastolic function which is relatively independent of preload. Load –independent methods give more accurate results about diastolic functions because loading conditions change significantly during pregnancy. Myocardial relaxation velocity change throughout pregnancy. Load independent methods can detect high risk pregnancies and early signs of cardiac failure to prevent further deterioration.

Sherif F. Nagueh et al., 2009 recommended echocardiography is the best method for the evaluation of left ventricular diastolic function. They concluded that assessment of left ventricular (LV) diastolic function should give essential information especially in heart failure patients. Half of the patients with heart failure have normal or near normal global ejection fractions. The assessment of LV diastolic function and filling pressures are clinically useful to distinguish heart failure from other diseases such as pulmonary disease and to identify underlying cardiac disease for its best treatment.

Tasneem Z. Naqvi, et al., 2012, used comprehensive measures of cardiac function with conventional as well as newer methods of assessment by echocardiography. They determined longitudinal segmental myocardial function during pregnancy with tissue Doppler strain and strain rate imaging and radial and circumferential myocardial function by 2D speckle tracking. They found increase in left ventricular (LV) and right ventricular chamber size as well as in left atrial size and increased LV volumes during both systole and diastole. These changes lead to no overall change in LV ejection fraction or in fractional shortening during pregnancy; however, cardiac stroke volume, cardiac output, and stroke work increased and peripheral vascular resistance decreased during gestation. Significant echocardiographic changes started in 2nd trimester, and reached to peak levels in the 3rd trimester. These changes recovered in the postpartum state. There was a significant reduction in global and segmental longitudinal deformation both of the left and right ventricle. Changes in cardiac dimensions, mass, and globularity, which became manifest in the second trimester, decrease in longitudinal deformation

only occurred in the third trimester, when cardiac volume increase was the highest and normalized in the postpartum state.

Martin Hutyra et al., 2010 found accuracy in echocardiography measurement like left ventricular ejection fraction (LVEF), enddiastolic volume (EDV) and endsystolic volume (ESV) compared with gated Single photon emission computed tomography. They proved that, Echocardiography is accurate tool for assessment of EF and LV volumes compared with gated SPECT in patients with LV systolic dysfunction.

Dr. D. V. Thakker et al., 2010**,** suggested that volume overload in pregnancy is the risk factor for left ventricular contractility functions. Increased values of stroke volume, cardiac output & cardiac index can give information about compensatory pressure overload or elevated venous return. The first relaxation abnormality is left ventricular diastolic dysfunction with left ventricular hypertrophy. These abnormalities can be detected by Doppler echocardiography.

2.10. Electrocardiography:

Electrocardiography is the recording of transthoracic (over the thorax or trunk) electrical movement of the heart over a time period. These changes can be picked up by the electrodes which are attached to the surface of the skin and recordings were shown in graphical form (Bamfo JE etal., 2001). The graphical recording produced by this non-invasive method is named an electrocardiogram. It is also called as ECG or EKG.

An ECG is used to measure the heart's electrical conduction system (kametas et al., 2007). It picks up electrical impulses generated by the polarization and depolarization of myocardial tissue and converts them into a graphical form. These graphs helps to assess the rate and consistency of heartbeats, size and position of the chambers, the existence of any damage in the heart, and the effects of drugs or devices such as pacemaker to manage the heart. ECGs are mostly used for diagnostic and research purposes on human hearts. ECGs may also used for animal studies to find out heart irregularities.

2.11. Hemodynamic changes during pregnancy:

The anatomical, physiological and biochemical adjustment to pregnancy are significant. These significant changes start immediately after fertilization and continue throughout pregnancy and most occur because of physiological stimuli given by the fetus. During pregnancy these changes which occur in mother helps to maintain good environmental for the fetus without distubing mothers health, but these alterations may create little uncomfort to the mother (Zenter etal., 2009,Chesnutt et al., 2004).Many of these physiological changes could be seen as abnormal in the non pregnant women. Physiologic changes especially cardiovascular changes develop in pregnancy. (Kametas NA et al., 2007, Vlahovic stipac a et al., 2010 found that maternal heart disease can importantly affect both the mother and fetus. They also found that increased circulatory burden on pregnancy can un recognize heart disorders and may easily worsen the condition of the pregnant women towards deadly circumstance. Early maternal hemodynamic adaptations include vasodilation, expansion of the plasma volume and red blood cell 2,3-diphosphoglycerate(DPG) concentrations (Hall berg L etal., 1996, Weidemann F et al., 2002).

During pregnancy the other hemodynamic adaptations includes an increase in heart rate, cardiac output and intravascular volume (voigt JU et al., 2003). These changes are made to improve metabolic demands as well as to increase oxygen delivery to the fetus (Jurcut R et al., 2008).

Fujitani etal., 2005 assessed hemodynamic changes in pregnant and peripartum patients and stated that changes in the physiology of pregnant woman is necessary to maintain homeostasis for both mother and fetus, especially in critical illness, which may cause complex pathophysiology. Understanding the normal physiologic changes during pregnancy, intrapartum, and postpartum is essential to manage patients with critically ill with underlying medical diseases and pregnancy-related complications.

Oana Savu et al 2012, Shirley Rubler, et al., 1977 Concluded that Pregnancy is a physiological process associated with increased cardiac performance and progressive LV remodeling. These changes are considered to describe systolic function, such as ejection fraction and longitudinal deformation.

2.11.1. Blood Volume:

One of the major hemodynamic changes in pregnancy is blood volume. The blood volume usually increases in early stages of pregnancy and continues to increase rapidly until in the mid-pregnancy and subsequently the rate of increase but at a slower rate in 3rd trimester. Because of these significant variations in the blood volume, during pregnancy it may increase from 20 to nearly 100 percent of non-pregnant values. During pregnancy the increase in blood volume was found to correlate directly with fetal weight. Along with BV red cell mass also increases. 40% to 50% increase in blood volume was observed during normal pregnancy. The reason for increased volume is due to effect of aldosterone and estrogens. These hormones levels are greatly increased during pregnancy (Zenter et al., 2009, Chesnutt etal., 2004).

This adaptive physiological increase in BV helps to maintain BP along with reduced vascular resistance, facilitates exchange of respiratory gases, nutrients and metabolites from mother to baby. These changes are also helpful to protect the mother from hypotension, and also prevent the hazards related with hemorrhage at delivery Marciniak A, et al., 2007. In normal pregnancy along with BV there is also marked increase in plasma volume which causes dilution of many circulatory factors. The predominant rise in plasma volume causes reduction in HB concentration. Increase in HB level during pregnancy is due to hemodilution which is also termed as "physiologic anemia of pregnancy" (Tasneem et al 2012,Weiner et al., 1994) The decrease in blood viscosity due to less haematocrit increases blood flow to the tissues as a compensatory mechanism(Dr. Toral M. Goswami et al .,2014).

During pregnancy mother is under the risk of getting nutritional deficiency anaemia. Increased production of maternal hormones like estrogen and progesterone contribute in expansion of plasma volume (Kathleen et al., 2011). Progesterone stimulates aldosterone production by increasing plasma renin activity. Rennin-angiotensinaldosterone system stimulates renal sodium absorption and water retention, (Weiner CP et al., 1994, Schrier RW et al., 1991). And also during pregnancy the concentration of plasma adrenomedullin rises which is also responsible for significant increase in BV (Weiner CP et al., 1994, Schrier RW et al., 1991).

Bone marrow also becomes significantly active for the production of extra red blood cells to substantiate with extra fluid volume. RBC mass reduces especially in the early stages of pregnancy, then gradually increases in mid trimester and undergoes a further rise of 30% above

the prepregnancy volume at term. Elevated erythropoietin hormone, progesterone, prolactin and placental lactogen contribute to rise in RBC volume (Cramariuc et al., 2010, Henein MY et al., 1999).With these changes at the time of delivery, the mother has about 1 to 2 litres of extra blood in her circulatory system (Henein MY et al ., 1999).

2.11.2 Cardiac Output:

It is defined as the volume of blood ejected by each ventricle per minute.

Normal cardiac output in adult male is 5-6 lt/min.

Cardiac output is the product of stroke volume and pulse rate (G.K.Pal et al 2016 pp-616).

Stroke volume is the volume of blood ejected by each ventricle per beat. Normal SV is about 70ml.

Cardiac index (CI) is Cardiac output expressed per square m. of BSA. Normal CI is 2.8 ± 0.3 $L/min/m^2$.

. Factors affecting COP:

COP depends on 3 major factors:

1. Preload (degree of ventricular filling or EDV)
2. The inotropic state (myocardial contractility)
3. The after load (resistance offered to the ventricular output)

The EDV is considered as the preload. EDV is defined as the volume of blood present in the ventricles at the end of diastole. EDV is directly proportional to SV. When EDV increases SV also increases &. Similarly decreased EDV decreases SV. Concomitant increase in myocardial contractility may also contribute to increase in the cardiac output (Jyosthna et al., 2012, Katz R. Karliner 1978).

At term pregnancy nearly 625 milliliters of blood flows per each minute to the placenta through maternal circulation. Metabolisms also increase in mother which further increases cardiac output. During pregnancy 30%to 50% increase in cardiac output was observed. The rise in cardiac output is mainly by 3 factors: (1) an increase in venous return due to increased blood volume; (2) reduction in after load due to reduced systemic vascular resistance; and (3) a rise in

maternal heart rate. Stroke volume also increases during the 1st& 2nd trimesters of pregnancy(Desai et al., 2004, Rizwana et al., Schrier RW et al 1991, v Jyosthna et al ., 2012).

2.11.3. Peripheral resistance (After Load):

It is defined as the resistance offered to the flow of blood by the blood vessels. When PR is increased suddenly, it causes an increase in pressure in the aorta. The immediate effect is a drop in CO ("Anrep effect").

There is a direct relation between HR and cardiac output. An increase in HR increases the force of contraction (on the basis of stair case phenomenon). During pregnancy one of the most important hemodynamic alterations is change in cardiac output. It is generally accepted that cardiac output starts to increase in the early stage of pregnancy. Then it increases rapidly until mid trimester and subsequently, it continues to increase up to the term but at a slower rate.

The increase in cop in early stage of pregnancy is disproportionately greater than the rise in HR rather than the SV. As pregnancy advances the heart rate increases and becomes more predominant factor for increasing cardiac output. But in the term pregnancy the stroke volume reduces to normal, prepregnancy level.

2.11.4. Heart Rate:

During pregnancy Heart rate increases with a mean increase of about 10-20 bpm at term. Mean values of HR vary from 78 to 89 bpm. It decreases slightly with a change in position from the supine to the lateral. Some investigators observed that HR increased in early stage of pregnancy and elevated similarly until term.

2.11.5. Systemic Arterial Blood Pressure

Defintion:

The lateral pressure exerted by the column of blood on the walls of arteries (G.K.pal et al., 2016 pp-647).

During pregnancy a slight reduction in systolic arterial blood pressure and also a considerable decrease in diastolic pressure was observed. The decline in BP begins in the 1st trimester and reaches to peak level especially in the mid half of pregnancy. Before term BP returns to normal level. The changes in SBP and DBP usually begin in the early weeks of pregnancy due to vasodilation.

2.11.6. Oxygen Consumption

During pregnancy a subsequent rise in resting oxygen consumption occurs and peak increase of 20-30% can be observed at term. D.V.Thakker et al, Brugada R et al., 2005 observed that the rise in oxygen consumption may be due to the increased metabolic demands of the mother and her growing fetus. Oxygen carrying capacity of the blood decreases in anemia. To compensate anemia both hemodynamic & non hemodynamic mechanisms operate to provide normal or near normal oxygen supply to the tissues (Walraven et al., 2011). In anemia erythropoietin may be enhanced by hormone erythropoietin & increased O2 extraction (Anju Grewal et al., 2010).

Table 3. Summary of hemodynamic changes of pregnancy

Hemodynamic Changes	Gestation	Risks
Cardiac output increases to 30–50%	2nd trimester	Pregnant women with diminished left ventricular which may cause congestive heart failure
Stroke volume increases to 20%	2nd trimester	Preload increassd preload which is risk for obstructive lesions and ventricular dysfunction
Heart rate increases to 10–20%	Third trimester	Tachycardia occurs which may impairs ventricular filling
Blood volume increases to 40%	2nd trimester	Physiologic anemia of pregnancy develops due to reduction in erythrocyte mass
Peripheral vasodilation	Throughout	Hypotension

Jyotsana R. et al., 2012, studied hemodynamic changes in normal Indian primigravids and also observed the recovery of these changes. In early weeks of gestation TPR and MAP decreases, COP increases in the 2nd& 3rd weeks of pregnancy. Left ventricular mass was

increased gradually in 1st& 2nd weeks of gestation and the peak value was observed in 3rd trimester of gestation. Ejection fraction and fractional shortening were same throughout pregnancy and at 6 weeks postpartum.

Desai et al., 2003 studied cardiovascular hemodynamic changes in normal pregnancy assessed by echocardiography. Significant correlation was observed between maternal cardiac output and maternal body surface area, fetal birth weight. Similar findings were observed by Jyotsana R. Et al., 2012.

Terence G.Hennessy et al., 1996, determined the direction of change in cardiac output (COP) during pregnancy. The E wave represented the early passive ventricular filling, whereas the A wave represented the active ventricular filling secondary to atrial contraction. Finally diastolic ventricular function was assessed with E/A ratio.

Poppas A et al., 2007 reported the role of arterial compliance and pulsatile arterial load changes during normal pregnancy .These adaptive changes were developed to accommodate the increased BV. They found in late gestation there was a small increase in LV muscle mass and end-diastolic chamber dimension, with no alterations in myocardial contractility. During pregnancy cardiac output was increased and the total vascular resistance was decreased. The other changes include rise in global arterial compliance, reduction in peripheral wavereflection were observed during various stages of pregnancy Finally, they proved that these coordinated changes especially in the pulsatile arterial load and left ventricular properties were responsible for maintaining the capacity of left ventricle -to-arterial system energy transfer.

2.12. Pathophysiology of Iron deficiency anemia & pregnancy:

The incidence of heart diseases gradually increases from 1% to more in pregnancy. The important cardiac diseases include stenotic valvular lesions, cyanotic disorders, and lesions associated with pulmonary hypertension. These abnormalities may lead to increase the rate of fetal and maternal morbidity and mortality. To avoid this very close monitoring is required during pregnancy. Sahar Naderi. et al.,2014 concluded that the physician also should be familiar with common cardiac disorders in pregnancy with cardiovascular drugs and their effects on the pregnant ladies and fetus. With this awareness cardiac disorders which are relative and absolute

contraindications to pregnancy due to high rates of maternal mortality can be prevented.

In a variety of pathophysiological conditions, LV load changes as per the changes in LV shape changes through a procedure of remodelling (Opie H et al., 2006). In volume over-burden states, LV dilatation and eccentric hypertrophy are compensatory mechanisms frequently related with changes in the LV from an ellipsoid to a more-round shape. Such structural and loading changes can modify functional assessment of the LV by classical parameters, such as, EF or shortening fraction.(NNMB 2006, WHO Geneva 2001, Cramariuc et al.,2010).

Nikita Hegde et al.,2006 in her study concluded that with severe iron-deficiency anemia, left ventricular function deteriorates. Myocardial contractility was determined by of %FS, which reduces when haemoglobin concentration falls below 7g/dl. To evaluate cardiac contractility the ratio of end-systolic wall stress to end-systolic volume index (ESWS/ESVI) also used. Reduction in this ratio suggesting functional compensation especially in patients with HB levels were below 6 g/dL. Iron deficiency anemia, especially when hemoglobin concentration is under 5 g/dl causes left ventricular dysfunction.

2.12.1. Birth weight and Maternal anemia:

The correlation between maternal anemia and birth weight had reviewed extensively in several studies. A high haemoglobin level abnormally show poor plasma volume expansion that leads to high risk of low birth weight. Rate of birth weight was improved in large number of iron-deficient women with iron supplementation. Some authors found that there was a negative correlation between maternal serum ferritin with birth weight and positive correlation with preterm delivery (Bhargava M et al., 1991).

2.12.2. Maternal anemia and infant health:

Correlation between maternal anemia and lower infant birth scores were reported in some studies. In India many studies have observed that the pregnant women with higher maternal HB levels were associated with healthy birth scores with a lower risk of birth asphyxia. Bothwell TH et al., 2000 reported that pregnant women who were treated with iron & their birth scores were significantly improved. Maternal iron deficiency leads to premature birth which has great effect on development of infant health. Preterm infants are likely to have

many perinatal complications, stunned growth, low stores of iron and other nutrients. A survey conducted on perinatal mortality, in Jamaica in 1986 found that the rate of mortality was increased in the children, whose mothers were not treated with iron supplements during pregnancy (Lindsay H Allen et al., 2000). With this survey, the importance of iron intake during pregnancy and the effects of iron deficiency on the health and development of the infant can be understood.

K. M. GODFREY et al., 2000 studied the effect of maternal iron deficiency anaemia on fetal weight. They also observed the ratio of fetal weight to placental weight. They found that placental weight was increased in association with low maternal HB concentration. During pregnancy iron deficiency is associated with large placental weight and a high ratio of placental weight to birthweight. The effects of maternal IDA include 1. Preeclampsia may be due to malnutrition & hypoprotenaemia; 2. Infections – Anemia affects immune system of the body and also decreases resistance to infection. 3. Cardiac failure 4. Pre term labour. Neonates of anemic mother, the fetal effects include 1. Decreased iron stores in infants due to depletion of maternal iron stores. 2. Adverse perinatal outcome like still birth and small-for-gestational-age babies .3. Increased perinatal mortality rates were found especially in neonates whose mothers are anemic (K. Kalaivani et al., 2009)

Mild eccentric hypertrophy develops in normal pregnancy due to the enlargement of maternal left ventricle in response to volume loading. These conditions may be observed in preeclampsia due to deviations of left ventricular (LV) transverse systolic function (D.V.Thakker et al., Chia .P et al 2002). Henein MY et al., 1999 observed the ischemic or pressure load effects on subendocardial fibers of LV which are more susceptible than the circumferential strands.

Dominica ZENTNER et al 2009 observed deterioration of cardiac systolic and diastolic function in late normal human pregnancy. They used echocardiography to assess left ventricular mass, cop, systolic and diastolic velocities, and wall stress. In this study they found that the pregnant women with 37 weeks of gestation had high pulse rates, in association with greater ventricular wall stress, systolic and diastolic lateral wall myocardial velocities. But no difference in SBP, cardiac output was observed. They concluded that, in term pregnancy,

increased ventricular wall stress can cause deterioration of cardiac function.

Mohammed A-Biltagi concluded that IDA (Iron Deficiency Anemia) was associated with diastolic dysfunction. There was significant increase in Left Atrial volume, decrease in LA longitudinal peak strain and increase in LA stiffness. Tissue Doppler imaging was able to detect even subclinical structural and functional alteration of the atrial myocardium. Results in our study denote that atrial dysfunction in iron deficiency anemia patients was due to hemodynamic abnormality. Recent echocardiography modalities (Tissue Doppler) are useful in early detection of subclinical myocardial deformation.

In non pregnant patients with cardiac abnormalities first long-axis systolic dysfunction develops. (Alam et al., 1990, Takela et al., 2001). However in normal pregnancies the few investigations found that both diastolic and longaxis systolic function give variable outcomes.

E. M. ESCUDERMO. et al.,1998 conducted a study on LV morphological and functional characteristics in pregnancy-induced hypertension (PIH). Echocardiograms were used to measure left ventricular systodiastolic diameters and wall thickness. Based on the results they concluded that PIH does not influence remarkable structural changes in the left ventricular cavity beyond those already caused by adaptation to pregnancy. Systolic function changes may be secondary effects of adrenergic activity. But diastolic function was not changed with increase in the left ventricular mass in PIH group. Cecily Mary Majella et al., 2013 also showed that diastolic function is affected adversely in pathologic high output states during pregnancy. Since Tissue Doppler Imaging is relatively load independent it may be the most useful method to assess diastolic dysfunction in high output states.

Mohammed Abdul Hussein conducted study to evaluate the relationship between anemia and diastolic dysfunction of the Heart. He found that anemic group, had diastolic dysfunction. Left ventricular hypertrophy had observed in anemic group. Statistically significant correlation was observed between left ventricular hypertrophy and diastolic dysfunction. So anemia was identified prior to prevent diastolic dysfunction of the heart.

In healthy pregnant women Schanwell C.M et al., 2002 observed left ventricular hypertrophy and diastolic dysfunction. They concluded that in normal pregnancy the natural

volume overload leads to reversible physiological left ventricular hypertrophy. And also observed significant short-term decrease in systolic diastolic left ventricular diastolic functions. To determine left ventricular diastolic function, mitral inflow and pulmonary venous flow profiles were used. And fractional shortening was calculated. The results found in the course of pregnancy were decrease in the left ventricular fractional shortening and a disturbed diastolic relaxation pattern was documented.

JEAK Bamfo and NA Kametas et al., 2007 were conducted studies on normotensive women with pregnancies complicated by severe fetal growth restriction (FGR).They studied maternal left ventricular systolic and diastolic function to know the central haemodynamics with the help of two-dimensional and M-mode echocardiography methods of the left ventricle. Increased total vascular resistance (TVR), reduced systolic function leads to lower the cardiac output, stroke volume, heart rate, ejection time and septal and lateral long-axis shortening were observed in study groups.

Prominent a (atrial) and v (ventricular) waves and brisk x and y waves were observed in pregnant women neck veins. Pregnancy additionally brings about various changes in ECG. The effect of normal pregnancy on ECG was a subject of great enthusiasm since the beginning of electrocardiography.

A normal ECG recording consists of a P wave, a QRS complex, a T wave, and a U wave, which is usually invisible because it is hidden by the T wave and upcoming new P wave. In a normal healthy heart, the baseline on ECG graph is equivalent to the isoelectric line (0 mV). The baseline represents the phases of cardiac cycle. It also represents the direction of flow of current towards either the positive or negative ends of ECG electrodes.

Interpretation of the ECG gives an idea about different electrodes (I, II, III, aVR, aVL, aVF and the chest leads) & also the view of heart from different angles. ECG has two advantages. First, electrodes can recognise the problems & the area of the heart where it is affected. Second, the direction of current flow that is the wave of depolarisation. This is termed the cardiac axis.

Figure 5 Lead groups (TaklaGeirge et al 2006)

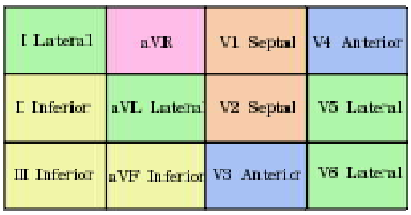

Table 4. Leads & their activity (Electrocardiography Wikimedia foundation 2017)

Category of the lead	Leads	Activity
Inferior leads	Leads II, III, aVF	Shows electrical activities from the inferior surface of the heart
Lateral leads	I, aVL, V1 and V6	Shows Electrical activities from lateral wall of left Ventricle

During normal pregnancy physi logical and cardiovascular adaptations may change the physical findings. Thus these changes n isleading the diagnosis of heart disease. Preg ancy also shows various changes in ECG. So M Set al .2014 had undertaken the study to highlight the effect of normal pregnancy on QRS axis, Q wave and T-wave of the Electrocardiogram. This study distinguished normal ECG changes from that of pathological ECG changes. They concluded that the changes in ECG during pregnancy gives an idea about ecg on normal pregnancy with the help of this one can manage cardiac diseases effectively.

Serum ferritin and iron levels also affect the QT interval in a variety of medical problems, especially in fatal cardiac arrhythmias. Krzysztof Laudanski, et al .,2009 had estimated serum levels of iron, ferritin, Na^+, K^+, Mg^{2+}, Ca^{2+} and total iron-binding capacity in patients with acute illness & were correlated with ECG variables. They observed the serum ferritin level correlated strongly with QT/QTs interval, indicating that serum ferritin and iron levels affect the QT interval in a variety of medical conditions.

K Singh, S Sood et al., 1996 found out changes in mean electrical axis of heart in chronic severe anemia. They observed that Heart rate was found to be increased in anemia. It was found

that despite cardiomegaly the Mean Electrical Axis of heart remains unaffected in chronic severe anemia.

B.N.Nandini et al., 2014 increased QTc interval was observed in various stages of pregnancy, which may be due to tachycardia. And they also observed decrease in QRS frontal axis in 1st, 2nd and 3rd trimesters of pregnancy when compared to non pregnant women. During pregnancy heart position changes from vertical to intermediate axis indicating left shift of heart. Occurrence of Q wave in lead III may be either due to an increased levels of circulating vasopressor agents or may be due to reflect diaphragmatic changes associated with pregnancy.

NN.Iwobi et al., 2002 studied the effect of pregnancy on HR, RR interval, qrs axis, qrs complex ,duration of the ECG in Nigerian women during normal pregnancy. This study showed that pregnancy had no significant effect on HR, RR, qrs axis & qrs duration of the ECG. But, significant left axis deviation was observed in qrs axis of pregnant women when compared to non pregnant women. As pregnancy advances then the magnitude of deviation also increases.

Vitthal H. Khode et al., 2000, observed a shortened QTc in the severely iron deficient anemic group. Sympathetic over activity due to hyper dynamic circulation was the main cause for shortened QTc. Significant positive correlation between serum ferritin levels and the QTc intervalwas observed in SIDA group.

Many electrocardiographic (ECG) changes associated with pregnancy and labour are right and left shifts in the QRS axis and nonspecific anterior T wave changes. During pregnancy if these changes show any variations in ECG one can specify cardiovascular disease. Iron deficiency anemia effects different organs including the heart. Hyper dynamic condition of heart is the best example for effect of IDA on heart. IDA affects the heart by diminishing the O2 supply to myocardium. Therefore supply demand myocardial mismatch leading myocardial ischemia or infarction. A number of compensatory mechanisms play an important role to overcome tissue hypoxia related with anemia. One of the changes is an increase in Cardiac Output and reduction circulation time. These cardiovascular disturbances depend on the severity of anemia and these progressions can be quickly reversed with correction of iron deficiency.

Iron plays a vital role in oxygen delivery, free radical generation production and immunity. Data from human subjects recommend that excessive iron storage is related with

cardiac arrhythmias (Rosenquist et al., 1989, Miskin et al., 2003). Iron is deposited predominantly in myocardial cells of heart. This enhances improper production and proliferation of electrical impulses at myocardial membrane(Aerssens et al.,2005, Yorchheimer et al.,2005).It had suggested that excessive intracellular iron disturbs electrical function of the heart, either by producing more amounts of free radicals or by causing selective dysfunction of Na^+ channels Brungada et al.,2005, Aerssens et al.,2005. An abnormal function of Na^+ and K^+ channels may also responsible for prolonged QT syndrome, ventricular tachyarrhythmias and atrial fibrillation (Brungada et al., Aerssens et al.,2005, Yorchheimer et al.,2005).

Additionally, an abnormal impulse proliferation, such as delayed impulse conduction, is important in the development of a variety of bradyarrhythmias Desai et al., 2004, OpieH et al., 2006.These effects are most likely independent of concomitant structural abnormalities of the heart, myocardial hypertrophy or cardiac ischemia – pathologies frequently seen in a siderotic heart (Rosenqwist et al ., 1989,Fitchett et al., 1980,Wolfe et al., 1985,Dubin et al., 2000).

The impact of abnormal indexes of serum iron metabolism is associated with a development of an intense medical pathology have an effect on the frequency of abnormalities found in the electrocardiogram (ECG), independence of electrolyte disturbances (Krozyzolf et al.,2009). Based on the available literatures ECG reports of anemia shows different opinions (NNMB 2008, WuJykors et al., 2003).

2.12.3. Immune status of anemic pregnant women:

Immuno depression is more common when Hb levels below 8gm/dl. In anemic patient this immune depression causes infections which further leads to morbidity. The adverse effect of anemia is increased morbidity & maternal death (Prema Ramchandran et al 1992).

Serum electrolytes like sodium, potassium & chloride play an important role to maintain osmotic pressures. Serum potassium is the major intracellular cation, and maintains intracellular osmotic pressure. The depolarisation & contraction of the heart require potassium is required. At rest, membranes are more permeable to potassium than other ions. Normal potassium level is 3.5=5mEq/L. Excretion of potassium is through urine. After formation of urine in glomerulus,

K+ is reabsorbed in proximal tubules & then actively secreted in distal tubules. Aldosteron & corticosteroids increase the excretion of K+. Chloride concentration in plasma is 96-106mEq/L. It is excreted through urine & is parallel to sodium (D.M.Vasudevan et al 2004Text book of biochemistry; section D. Nutrition pg 306-307).

During pregnancy hemodynamic changes play an important role to reduce cardiac arrhythmias. Schlosser et al 2005 reported increased frequency of arrhythmias during pregnancy. A comprehensive understanding of cardiovascular adjustments in pregnancy is fundamental for the management of cardiovascular disorders. Heart diseases significantly increase the rate of maternal mortality throughout the world. Heart diseases during pregnancy remain a difficult issue.

Subsequently the current study is choose to know the effect of IDA on electrocardiography during 1^{st} and 2^{nd} trimesters of pregnancy .To compare ECG changes of iron deficiency anemic pregnant women with ECG changes of normal pregnant ladies in first and second trimesters.

In present review we have decided to determine the effect of IDA on left ventricular hemodynamic functions in 1^{st}& 2^{nd} trimesters of pregnancy. The present study makes an attempt to describe the pattern of cardiovascular changes by echocardiography in non anemic and anemic females in first and second trimester pregnancy.

To the best of our knowledge such kind of study was not being done especially in Telangana rural Population. So the present study was carried out with the aim to assess the changes in hemodynamic, i. e. heart rate, cardiac output, mean arterial pressure, total peripheral resistance and left ventricular systolic functions in normal pregnant women in a prospective manner and to compare with iron deficient anemic pregnant women in first & second trimesters

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MATERIALS AND METHODS

3.1. Study population:

i) The study was conducted on pregnant women attending the antenatal OPD & Cardiology departments of Prathima Institute of Medical Sciences karimnagar, Telangana,India.

3.1.2. Study period:

Study was conducted between November 2012 to October 2015.

3.2. Sample size: 160 pregnant women final sample size.

Sample size estimation:

The estimated sample size for this study according to equation was 81. 20% was for non response and incomplete responses bringing the total to 97 which was rounded off to 100.

The prevalence of anemia in Indian pregnant women is 70% as per study conducted by National Family Health Survey (NFHS-3; 2005-2006).

The following sample size calculation formula was used for this study.

$$n = 1.96 \times 1.96 \times p \times q / d2.$$

Where n= sample size.

P= Prevalence of anemia in pregnant women.

The results are presented with the level of 95% confidence intervals.

q = (1-p) and

d= Precision 10%.

3.3. Inclusion & exclusion criteria:

3.3.1.Inclusion criteria:

Pregnant women with normal clinical cardiovascular history, normal physical findings, electrocardiogram, and 2D echocardiographic findings were included in the study.

3.3.2.Exclusion criteria:

Pregnant women with known diabetes, known hypertensives, maternal cardiovascular disease, pregnant women with history of polycystic ovary or any other chronic diseases, with other endocrine disorders (Thyroid, Adrenal) and preeclampsia were excluded from the study.

3.3.3.Criteria for discontinuation:

. Participant refusal

. Participants who could not undergone ECG & Echocardiogram.

3.4. Ethics:

3.4.1. Informed consent

Informed written consent was obtained for participation in the study.

3.4.2. Institutional approval

The study protocol was approved by the ethical committees of B.L.D.E.U Shri BM Patil Medical College, Bijapur, Karnataka, India (IEC/29/2012) and Prathima Institute of Medical Sciences (Ref number: IEC/PIMS/2013/001).

3.5. Study Design:

A total of 160 pregnant women were included in the study. 80 pregnant women in first & 80 pregnant women in second trimester were selected. In which, out of 80 pregnant women 3 groups normal pregnant women (control) and pregnant women with moderate & severe iron deficiency anemia in each trimester depending on the severity of anemia were selected. 60 healthy pregnant women of first and second trimester (30 each) with normal pregnancy enrolled in antenatal clinic having no history of cardiac disease.

We had taken separate groups of moderate & severe iron deficient anemic pregnant women (first and second trimester) without any ante anemic treatment. This helps us to know the effect of iron deficiency anemia on cardiac performance

The first group (group-I) comprised of 30 normal pregnant women in first trimester were compared with 27 pregnant women with moderate iron deficiency anemia (group –II) & 23

pregnant women with severe iron deficiency anemia in first trimester[(10-14) weeks of gestation] were included in group –III.

Again 30 healthy pregnant women in second trimester (group-I) were compared with 29 pregnant women with moderate iron deficiency anemia (group –II) & 21 pregnant women with severe iron deficiency anemia in second trimester [(10-14) weeks of gestation] were included in group –III.

Pregnant women with moderate & severe iron deficiency anemia in first & second trimesters coming for the first time without any ante anemic treatment were included in this study(as these pregnant women have not undergone with anemic treatment. So anemia is persisting were included in second trimester).

3.5.1. Distribution of study subjects:

	Group-I (n=30)	Group-II (n=27)	Group-III (n=23)
First trimester pregnant women	Healthy pregnant women	Pregnant women with moderate iron deficiency anemia	Pregnant women with severe iron deficiency anemia

	Group-I (n=30)	Group-II (n=29)	Group-III (n=21)
Second trimester pregnant women	Healthy pregnant women	Pregnant women with moderate iron deficiency anemia	Pregnant women with severe iron deficiency anemia

3.6. STUDY PROTOCOL:

i) The subjects were selected from antenatal OPD department of Prathima Institute of Medical Sciences & also motivated from health camps.

The subjects were first informed about the purpose of the study and the procedures involved. Once they volunteered for the study, the detailed procedure was explained to the subjects as outlined in the informed consent. Informed consent was taken from the volunteered subjects.

ii) At the onset of the study, a proforma was filled to evaluate age, educational status, nativity, other factors like information regarding menstrual and marital status, history of pregnancy, number of children & pregnancy gap, food habits, monthly income of family, educational status, general health history of the subject, along with personal and family history of the study participants were collected by using structured questionnaire.

iii) Baseline investigations haematological parameters (CBP) were estimated for diagnosing anemia.

Gestation of the subject was confirmed by last menstrual period and ultra sound measurement of the fetal crown-rump-length was done.

iii) A detailed history was taken from all the selected women and a complete physical examination and obstetric examination was performed at the time of recruitment.

iv) In the second stage, anthropometric & physiological parameters of the selected pregnant women were recorded.
Body Surface Area (BSA) of the selected pregnant women was recorded with the help of height and weight.

v) In the third stage, to assess hematological & biochemical parameters 10 ml of venous blood was collected in an evacuated tube containing EDTA solution from each selected subject.

iv) The selected subjects were divided into 3 groups in first & second trimesters of pregnancy based on the severity of anemia

vi) In the final stage, electrocardiography & echocardiography were performed on selected pregnant women to evaluate myocardial performance.

vii) All the recordings were entered.

3.7. Parameters: In first and second trimester:

. Transabdominal Ultrasound examination

.

Anthropometric (Age, Ht,Wt & BSA)& physiological parameters(SBP,DBP ,MAP) were recorded.

. Hematological & biochemical parameters include

. Complete blood picture

. Estimation of serum ferritin.

. Estimation of serum iron.

. Estimation of serum Total iron binding capacity.

. % Transferrin saturation.

. Blood cell indices (MCV, MCH and MCHC)

. Estimation of serum electrolytes.

. Echocardiogram

. Electrocardiogram (ECG)

3.8. Methods:

3.8.1. Anthropometric measurements:

Anthropometric parameters measured in the subjects were:

Height in cm, Body Weight in kg&Body Surface Area in kg/m2.

a. Height

Height of the subject was recorded by using a stadiometer with subject standing erect and is expressed in centimeters (cms).

b. Weight

Weight of the subject was measured with subject standing erect on a human weighing machine in light clothing & is expressed in Kilograms (Kg).

c. Body Surface Area (BSA)

Body surface area was calculated by using the Dubois Formula (Dubois D etal.,) .

BSA = (WEIGHT) 0.425(Kg) x (HEIGHT) 0.725 (cm) x 0.007184

3.8.2. Physiological parameters

a. RECORDING OF BLOOD PRESSURE:

Both systolic and diastolic blood pressures were recorded in each subject by using a Sphygmomanometer in sitting posture.

Blood pressure was measured using standard auscultatory method with help of pneumatically operated mercurial type random zero sphygmomanometer. Blood pressure was measured sitting position with arm at the level of heart.

Principle: The cuff of the sphygmomanometer is wrapped around the arm of the subject. Then the bag is inflated until the air pressure in the cuff dominates the arterial pressure and occludes the arterial lumen. This is confirmed by palpating the radial pulse that disappears when the cuff pressure is raised above the arterial pressure. The pressure is then raised further by about 20mm Hg and then slowly reduced. When the pressure in the cuff reaches just below the arterial pressure, blood escapes beyond the occlusion into the peripheral part of the artery and the pulse starts reappearing. This is detected by the appearance of sounds in the stethoscope and is taken as the systolic pressure. Subsequently, the quality of the sound changes and finally, disappears. The level where sound disappears is taken as the diastolic pressure. The sound disappears because the flow in the blood vessels becomes laminar (G.K.Pal et al.,pp 203-207).

Method of recording:

We checked the level of mercury column in the sphygmomanometer (the upper meniscus of the mercury coincides with the zero of the mercury manometer). The cuff of sphygmomanometer was wrapped around the middle of the arm (over the brachial artery) in such a way that the lower edge of the cuff remains at a minimum distance of one inch above the cubital fossa.

Then we placed the diaphragm of the stethoscope lightly on the brachial artery in the cubital fossa, that is, over the upper part of the forearm medial to the tendon of the biceps. Raised the

pressure of the mercury manometer by compressing the rubber bulb which is operated with other hand over and above 120 mm Hg, then lowered the pressure at the rate of 2-4 mmHg per second. We should note the appearance of the sound, the change in character of the sound and finally the cessation of the sound, while the pressure in the cuff is progressively lowered.

When pressure in the cuff is progressively lowered, the sounds undergo a series of changes in their quality and intensity. These sounds are known as korotkoff sounds (described by the Russian scientist Korotkoff in 1905).They is heard in different phases.

While recording BP appearance of sound (Phase I Korotkoff) and disappearance of sound (Phase V) was recorded as systolic and diastolic BP respectively.

First recorded blood pressure by the palpatory method by palpating the radial artery then recorded by auscultatory method (G.K.Pal et al.,pp 203-207)

b. Mean Arterial pressure:

Mean arterial pressure is the average pressure produced during the cardiac cycle(Ganong 2005).

Mean arterial pressure was calculated using the formula:

$$MAP = \frac{2(\text{Diastolic blood pressure}) + (\text{Systolic blood pressure})}{3}$$

c. Pulse pressure:

Pulse Pressure is the pressure that maintains the normal pulsatile nature of the flow of blood in the vascular compartment.

PP) was calculated as difference of systolic and diastolic blood pressure (G. K. Pal text book 646-48).

Collection Of Blood Samples For Haematological And Biochemical Analysis:

Venous blood sample (10 ml) was drawn from each pregnant women for haematological and biochemical analysis. The blood sample was collected in EDTA tube for haematological and biochemical analysis

. Recording of Haematological& Biochemical Parameters:

. Haematological parameters include
. Complete blood picture
. Determination of Packed Cell Volume
. Blood cell indices (MCV, MCH and MCHC) were calculated based on RBC count Hb concentration & packed cell volume.
. Biochemical parameters include
. Estimation of serum ferritin.
. Estimation of serum iron.
. Estimation of serum Total iron binding capacity.
. % Transferrin saturation.

3.8.3. Estimation of Hemogram:

Haematological parameters were determined by automated method using SYSMEX Automated Haematology Analyzer XT-2000i/XT-1800i (Valeri L.Hill et al., 2009).

Haemoglobin is the main constituent of the red blood cells and carries out the important function of transportation of oxygen from lungs to various parts of the body. When fully saturated each gram of haemoglobin holds approximately 1.34ml of oxygen.

Haematology analyzer technology:

Haematology analyzers are used widely in patients and research settings to count and characterize blood cells for disease detection and monitoring. Basic analyzers return a complete blood count (CBC) with a three part differential white blood cellcount.

The 3 main physical technologies used in haematology analyzers are: electrical impedence, flow cytometry and fluroscent flow cytometry. These are worked in combination with chemical reagents which lyse or change blood cells to expand the measurable parameters.

Electrical impedence is used in every hematology analyzer. Whole blood is passed between 2 electrodes through an aperture so narrow that only one cell can pass through at a time.The

change in impedence is proportional to cell volume, resulting in a cell count and measure of volume.

Counting rates of up to 10,000 per second can be achieved and a typical impedence analysis can be carried out in less than a minute.

3.8.4. Determination of packed cell volume:

Measured hematocrit by automated technique done by using electronic cell counters.

Principle: Anti coagulated blood is taken, filled to the graduation mark of pipette and then centrifuged for prescribed length of time (de Gruchy et al., pp24-26).

Then the readings are taken from the graduations of the pipette.

3.8.5. Calculation of blood indices:

The values for RBC count, Hb count and PCV can be used to obtain certain RBC indices (also called absolute values of blood index). These blood indices indicate the size and hemoglobin concentration within in the RBCs and thus help in diagnosing the type of anemia.

The .various blood indices are (de Gruchy et al., pp24-26):

a. Mean Corpuscular Volume (MCV):

MCV is defined as the volume of a single RBC in cubic microns (μm^3).

It can be computed as:

$$MCV \quad (\mu m^3) = \frac{PCV\ per\ 100\ ml\ blood}{RBC\ count \quad in\ million \quad / cumm} X\ 10$$

$$= \frac{45}{5} X\ 10 = 90\ (average)$$

Normal range = 78-94 μm^3 $(86 \pm 8\ \mu m^3)$

1. RBCs with normal MCV are called normocytes.

2. RBCs who's MCV exceed normal range are macrocytes

3. RBCs with MCV below normal range are microcytes.

b. Mean Corpuscular Hemoglobin (MCH):

MCH is defined as the average amount of Hb in a single RBC in picogram (10^{-12} gm) or micro-micro gram. It can be computed as:

$$MCH\ (pg) = \frac{Haemoglobin\ in\ gms\%}{RBC\ count\ in\ million/mm^3} X\ 10$$

$$= \frac{15}{5} X\ 10 = 30\ (average)$$

Normal range = 28-32 pg (29.5 ± 2.5 pg)

(Note: This blood index is not used clinically)

c. Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC is defined as the amount of Hb expressed as percentage of the volume of a RBC or it is the Hb conc. in a single RBC.

$$MCHC = \frac{Haemoglobin\ in\ gms\ \%}{PCV\ \ per\ 100\ ml\ blood} X\ \ 100$$

$$= \frac{15}{45} X\ 100 = 33\ (average)$$

Normal range = 32 % - 38 % ($35 \pm 3\ \%$)

1. If the MCHC is within normal range, the RBC is normochromic.
2. If the MCH is below normal range, the RBC is hypochromic. This usually indicates that on individual is suffering from iron deficiency.
3. MCHC values never exceed 38% because RBC can't hold HB beyond its saturation point. Therefore anemic can never be hyper chromic.

3.8.6. Estimation of serum feritin:

. Serum Ferritin was quantitatively determined by Chemiluminscence Microparticulate Immuno Assay (CMIA) (Serum Ferritin ARCHITECT SYSTEM.Abbott Ireland Diagnostics Germany 2010, Sheena Blackmore et al., 2008).

Principle:

The Ferritin assay is a two step immunoassay to determine the presence of ferritin in human serum and plasma using chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. First, sample and anti – ferritin coated paramagnetic microparticles were combined. Ferritin present in the sample binds to the anti - ferritin coated microparticles. After washing, anti – ferritn acridinium labeled conjugate was added in the second step. Pre- Trigger and Trigger solutions were added to the reaction mixture; then the resulting chemiluminescent reaction was measured as relative light units (RLUs). A direct relationship exists between the amount of ferritin in the sample and the RLUs detected by the ARCHITECT I optical system.

Reagents:

- ARCHITECT Ferritin Reagent Kit Contains -.MICROPARTICLES 6.6 mL Anti Ferritin coated Microparticles in TRIS buffer with protein stabilizers. Preservative; anti microbial agent. CONJUGATE: 5.9mL Anti Ferritin acridinium labeled conjugate in MES buffer with protein stabilizers. Architect assay diluents.
- Other reagents :

ARCHITECT PRE-TRIGGER SOLUTION containing 1.32% hydrogen peroxide.

ARCHITECT TRIGGER SOLUTION containing 0.35 N sodium hydroxide

ARCHITECT Wash buffer containing phosphate buffered saline solution

- Other requirements:

ARCHITECT reaction vessels, sample cups, septum and replacement caps

Procedure:

Before loading the ARCHITECT Ferritin Reagent kit on the system for the first time, the microparticle bottle mixed to resuspend micriparticles, 30 times. The ARCHITECT ferritin reagents were loaded on the ARCHITECT system. ARCHITECT ferritin calibrators and ferritin controls were mixed by gentle inversion. Prior to use, hold the bottles vertically and dispensed 4 drops were dispended of each calibrator or 3 drops of each control into each respective sample cup. Then the samples were loaded. When we pressed run the ARCHITECT system performed the following function. Moved the sample to the aspiration point, loaded a reaction vessel (RV) into the process route, aspirated & transferred into the Reaction Vessel, advanced & transferred micro particles in to the RV, mixed, incubated and washed the reaction mixture. Then pre-trigger and Trigger solutions were added. Chemiluminescent emission was measured to determine the quantity of ferritin in the sample. The contents of RV to liquid waste were aspirated and unloaded RV to solid waste. Finally the results were calculated. Dilution factor in the patient order screen was entered. The system used this dilution factor to calculate the concentration of the sample before dilution. This gave the reportedresult.

3.8.7. Estimation of serum iron & total iron binding capacity:

Serum iron & total iron binding capacity were estimated by Serum Ferrozine Method (Siedel .J et al., 1989).Iron & TIBC Kit was used for the determination of Iron and Total Iron Binding Capacity in serum.

PRINCIPLE:

Iron, bound to Transferrin, is released in an acidic medium and the Ferric ions are reduced to ferrous ions. The Fe (II) ions react with Ferrozine to form a violet coloured complex. Intensity of the complex formed is directly proportional to the amount of Iron present in the sample. For TIBC, the serum is treated with excess of Fe (II) to saturate the iron binding sites on transferrin. The excess Fe (II) is adsorbed and precipitated and the Iron content in the supernatant is measured to give the TIBC.

Acidic Medium
Fe (III) ----------> Fe (II)

Fe (II) + Ferrozine ----------→ Violet Coloured Complex

Contents	35ml	75ml
Iron reagents		
L1: Iron Buffer Reagent	35ml	75ml
L2: Iron Colour Reagent	2ml	4ml
S: Iron Standard(100µg/dl)	2ml	2ml
TIBC Reagents	**35ml**	**75ml**
SR: TIBC Saturating Reagent	35ml	75ml
PR:TIBC Precipitating Reagent	2gm	4gm

Reagent Preparation:

Reagents are readymade to use.

Procedure

Wavelength/filter: 570 nm (Hg 578 nm) wave length was adjusted. Yellow colour filter was used. Room temperature and light path of 1cm was adjusted.

Materials Required

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

a. Iron Assay:

Four clean dry test tubes were taken and labeled as Blank (B), Standard (S), Sample Blank (SB) and Test (T). Pipetted the solutions into clean dry labelled test tubes in following sequence (serum iron ferrozine method).

Addition Sequence	Blank (B) (ml)	Standard (S) (ml)	Sample Blank(SB) (ml)	Test(T) (ml)
Iron Buffer Reagent (L1)	1.0	1.0	1.05	1.00
Distilled Water	0.2	--	--	--
Iron Standard (S)	--	0.20	--	--
Sample	--	--	0.20	0.20
Iron Colour Reagent (L2)	0.05	0.05	--	0.05

- Mixed well and incubated at room temperature for 5 minutes.
- Measured the absorbance's of the Blank (Abs.B), Standard (Abs.S), Sample Blank (Abs.SB) and Test Sample (Abs.T) against distilled water.

b. TIBC Assay:

- Pipette into a clean dry test tube Serum 0.5 ml TIBC Saturating Reagent (SR) 1.0 ml.
- Mixed well and allowed to stand at room temperature for 10 min. and added TIBC
- Precipitating Reagent (PR) Approx. 50 mg.
- Once again mixed well and allowed to stand at Room Temperature for 10 minutes.
- Centrifuged at 2500-3000 rpm for 10 minutes to obtain a clear supernatant.

Determined the Iron content in the supernatant as mentioned in the iron assay (serum iron ferrozine method).

c. Percentage Transferrin saturation:

% Transferrin Saturation (TSAT): Transferrin saturation is defined as the ratio of serum iron to the total iron-binding capacity (TIBC, a measure of circulating transferring) x 100.

$$\frac{\text{Serum iron} \times 100}{\text{TIBC}}$$

TSAT indicates the percentage of binding sites on transferring that are occupied by iron and is therefore a measure of circulating iron that is immediately available for erythropoiesis.

Areduction in TSAT suggests an inadequate supply of iron to the developing erythrocyte. However, a reduced TSAT does not always indicate iron deficiency since other disorders can cause iron deficient erythropoiesis (Cook JD et al., 1992).

3.8.8. Estimation of serum electrolytes:

Electrolyte profile was analysed by ion selective electrode.
Serum electrolytes were estimated by electrolyte analyzers HY-LYTE 200 with ISE (Ion selectivity electrode) method(Ernopungor et al., 1998).

Principle:

These are used for quantitative measurements of biologically relevant anions & cations. The electrode (ISE) permits measurement of the activity of a specific ion under the presence of a given amount of other ions. The selective transport of a certain ion species from the solution into the membrane phase of electrode allows a potential difference that can be calculated and the ion concentration can be deducted thereof (D.M.Vasudevan et al).

3.8.9. Recording of Electrocardiogram:

Traditionally ECG is in the form of a transthoracic (over the thorax or trunk) interpretation of the electrical movement of the heart over a time period, and the electrical movements are distinguished by electrodes attached to the surface of the skin. These movements can be recorded by a machine. (Ashwin kumar et al.,2010). The recording produced by this non-invasive system is named an electrocardiogram (likewise ECG or EKG).

An ECG is applied to measure the heart's electrical conduction system(Tarek Ajam, et al., 2017).It gets electrical impulses created by the polarization and depolarization of cardiovascular tissue and converts into a waveform. The waveform is then used to quantify the rate and consistency of heartbeats, and also the size, position of the chambers, the presence of any damage in the heart, and the impacts of drugs or gadgets used to manage the heart, such as a pacemaker.

Principles

The ECG device detects and amplifies the tiny electrical changes on the skin that are caused when the heart muscle depolarizes during each heartbeat. At rest, each heart muscle cell has a negative charge across its cell membrane, called the membrane potential. Decreasing this negative charge toward zero, via the influx of the positive cations, Na^{+} and Ca^{++}, is called depolarization, which activates the mechanisms in the cell that cause it to contract. During each heartbeat, a wave of depolarisation is generated by the cells in the sinoatrial node, spreads out through the atrium, and passes through the atrioventricular node then spreads all over the ventricles. This is recognized as tiny rises and falls in the voltage between two electrodes those are placed either side of the heart, which is displayed as a wavy line either on a screen or on paper. This display indicates the overall rhythm of the heart and weaknesses in different parts of the heart muscle (G.K.Pal et al.,)

Electrocardiograph was recorded using Philips ECG machine model TC20 in both control & study groups. The instrument used to record electrocardiogram was the twelve channel electrocardiograph HEWLETT PACKARD page writer manufactured by Philips electronics Ltd.

Procedure:

ECG was recorded in supine position after giving 5 minutes of rest to the subject to allay anxiety. ECG was recorded in all 12 leads i.e, 3 Standard Bipolar Limb Leads I: II & III, 3 Unipolar augmented limb leads: aVR, aVL, aVF and 6 Precordial leads: VI to V6, by connecting electrodes to left arm, right arm, left leg and right leg in supine position.

The output of an ECG recorder is a graph with time represented on the *x*-axis and voltage represented on the *y*-axis. An ECG machine would usually print waves on a graph paper which has a background pattern of 1-millimeter squares, with bold divisions every 5 mm in both vertical and horizontal directions.

Placement of electrodes

Twelve electrodes were used for recording a 12-lead ECG. The electrodes usually consist of a conducting gel, embedded in the middle of a self-adhesive pad onto which cables clip. The electrodes were placed on the forearm proximal to wrist of the upper arms(right & left) & lower leg(right & left) proximal to ankle. 6 chest leads/sensors were placed on the patient's chest as follows (Goldberger AL et al., 2006, Bayes de Luna A et al., 2007):

Electrode	Position of Electrode
V_1	Fourth intercostals space at the right border of the sternum
V_2	Fourth intercostals space at the left border of the sternum
V_3	Between leads V_2 and V_4
V_4	Fifth intercostals space in the mid-clavicular line
V_5	Horizontally even with V_4, in the left ant. axillary line.
V_6	Horizontally even with V_4, in the mid axillary line.

Limb leads:

The two types of limb leads unipolar and bipolar. Bipolar leads have a positive and a negative pole. The bipolar leads are limb leads (I, II and III). Unipolar leads are the augmented limb leads aVR, aVL, aVF.

Lead I is the voltage between the left arm (LA) electrode (positive) and right arm (RA) electrode. Lead II is the voltage between the left leg (LL) electrode (positive) and the right arm (RA) electrode. Lead III is the voltage between the left leg (LL) electrode (positive) and the left arm (LA) electrode.

In Augmented limb lead (aVR)' has the positive electrode on the right arm. The negative electrode is a combination of the left arm electrode and the left leg electrode, which augments the signal strength of the positive electrode on the right arm. In aVL the positive electrode is placed on the left arm. The negative electrode is a combination of the right arm and the left leg

electrodes, which makes the signal strength of the positive electrode on the left arm. In aVF lead the positive electrode is placed on the left leg. The negative electrode is a combination of the right arm electrode and the left arm electrode, which add to the signal of the positive electrode on the left leg (Robert J.Bryg et al., 2016).

3.8.10. Echocardiographic examinations and measurements:

Definition:

Echocardiography is a diagnostic test that uses ultrasound waves to create an image of the heart muscle. Ultrasound waves that rebound or echo off the heart can show the size, shape, and movement of the heart's valves and chambers as well as the flow of blood through the heart. Echocardiography may show such abnormalities as poorly functioning heart valves or damage to the heart tissue(Echocardiography medical dictionary, Mathew N et al.,).

Purpose:

Echocardiography is used to diagnose certain cardiovascular diseases. In fact, it is one of the most widely used diagnostic tests for heart disease. It provides useful information, including the size and shape of the heart, its strength for pumping blood. And also diagnose the location and extent of any damage to its tissues. It is especially useful for assessing diseases of the heart valves. The biggest advantage to echocardiography is that it is non-invasive (does not involve breaking the skin or entering body cavities) and has no known risks or side effects (Siddarth Singh et al., 2007).

Method:

Echocardiography generates an image of the heart using ultra-high-frequency sound waves that are too high in frequency to be heard by the human ear. Echocardiograms were recorded using MEGAS CVX and MEGAS GPX equipped with ADV4 software from Italy. Two dimensional and Doppler echocardiographic examinations were performed using 3.5 MHz.

An echocardiography examination generally lasts between 15-30 minutes. The subject was asked to lie down bare-chested on an examination table. A special gel was spread over the chest to help the transducer make good contact and slide smoothly over the skin. The transducer, a small hand-held device at the end of a flexible cable, was placed against the chest. Essentially a

modified microphone, the transducer directs ultrasound waves into the chest. Then the waves get echoed (or reflected) back to the transducer & can be translated into a meaningful image of the heart, which can be displayed on a monitor or recorded on paper(Echocardiography medical dictionary).

Advantages:

The patient does not feel the sound waves in this procedure

The entire procedure is painless.

No known side effects.

. The following indices of cardiac function were evaluated (A.K.Pandey et al ., 2010).

. Left ventricular systolic functions:

. Left ventricular end diastolic (EDD)

. End systolic diameter (ESD)

. Fractional Shortening (FS)

. Pre ejection period (PEP)

. Ejection fraction %(EF)

. Posterior wall thickness (PWT) is measured in M-mode in the long axis para sternal view.

. Left ventricular diastolic functions:

. Peak velocities of both early (E) and late atrial (A) diastolic filling.

. Isovolumetric relation time (IVRT). The E/A ratio: E- wave declaration time (DT);

Stroke volume (SV), cardiac put (CO) and total peripheral resistance were calculated from the measured dimensions according to the American society of Echocardiography (ASE) guidelines(Devereux et al., 2014).

SV= (EDD)3 - (ESD)3

CO (L/min) = SV x HR

$$EF\% = \frac{(EDD)3 - (ESD)3}{(EDD)3} \times 100$$

$$FS\% = \frac{(EDD) - (ESD)}{(EDD)} \times 100$$

TPR was calculated using the formula:

TPR (dyn X sec X cm-5) = mean Bp X 80 / CO (Maynard SE et al., 2004)

End diastolic Volume: The volume of blood remaining in each ventricle at the end of diastole is EDV (Guyton et al., 2007).

Normal value: 130ml.

Ejection Fraction: The percentage of EDV is ejected with each beat is EF.

EF=SV/EDV*100

Normal value: 65% .Good index of myocardial performance.

End systolic Volume: The volume of blood remaining in each ventricle at the end of systole.

Normal value: 50ml.

3.9. Statistical Analysis:

. The obtained data was expressed in mean and standard deviation(Mean±SD).

. Level of significance: A p value of 0.05 or less was considered as statistically significant.$p<0.01$: Highly significant, $p<0.001$: Very highly significant. $p>0.05$, then the results will be considered to be not Significant.

A one way ANOVA was used to compare the difference between between normal pregnant women and anaemic pregnant women in first & second trimesters of pregnancy. Also we used the post hoc comparison using Bonferroni test for multiple comparisons that is to test significant difference between the groups. Descriptive statistics were used to describe the sample and scale characteristics. The data was analysed by SPSS 17.

The one-way analysis of variance (ANOVA) is used to determine whether there are any statistically significant differences between the means of three or more independent (unrelated) groups(Visweswara Rao.K et al., 2007.pg -226)

Left ventricular findings like EDD,ESD,EF,FS &SV were correlated with Hb%, serum ferritin & serum iron using Pearson's co-relation co-efficient(r) method to test the strength and direction of relationships between the variables.

SYSMEX Automated Haematology Analyzer

Estimation of Serum Ferritin by ARCHITECT SYSTEM.

Recording of Blood pressure

Recording of Electrocardiogram

3.10. References:

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RESULTS

Chapter IV: Results

The purpose of study is to evaluate the effect ofIDA on left ventricular function in first & second trimesters of pregnancy & to compare the relationship between left ventricular function &haematological parameters in both iron deficient anemic pregnant and normal pregnant women.

4.1. Prevalence of anemia in first& second trimester pregnant women

Socio economic information like age, marital status, educational status, residence & occupation & other relevant risk factors like number of children, parasitic infections, gestational period, pregnancy gap & iron supplement of the study participants were collected by using structured & pre tested questionnaire.

Table 5: Distribution socio economic characteristics in 1st trimester normal pregnant & anemic pregnant women

CHARACTERISTICS	Normal pregnant women(n=30)	Moderately anemic% (n=27)	Severely anemic % (n=23)
Residence			
Urban	(23)76%	(9)34%	(5)22%
Rural	(07)24%	(18)66%	(18)77%
Monthly Income			
<5000	(4)14%	(20)74%	(18)78%
>5000	(26)86%	(7)26%	(5)22%
Educational Status			
Illiterates	(5)17%	(20)74%	(18)78%
Literates	(25)83%	(7)26%	(5)22%
HOUSE WIFE,	(15)49%	(18)65%	(20)85%
Agricultural Working Women			
Employed women	(15)51%	(09)35%	(3)15%

Table 5 shows distribution socioeconomic characteristics in first trimester normal pregnant & anemic pregnant women. In moderately & severely anemic pregnant women the percentage of anemia is found to be more in rural population 66%,77% respectively than urban population of first trimester. 74% of moderately anemic &78% of severely anemic pregnant women of first trimester belongs to below poverty line. In first trimester 74% of moderately anemic pregnant women are illiterates & 26 % are found to be literates. In severe anemic group 78% are illiterates & 22% are literates. 65% of moderately anemic & 85% of severely anemic pregnant women are house wives in first trimester pregnancy.

Table 6: Distribution socioeconomic characteristics in 1st trimester normal pregnant & anemic pregnant women

CHARACTERISTICS	Normal pregnant women(n=30)	Moderately anemic% (n=27)	Severely anemic % (n=23)
Number Of Children			
None	(15)50%	(10)37%	(05)23%
1 Child	(10)33%	(04)13%	(04)17%
2 Children	(03)10%	(03)12%	(04)17%
3 Or >3 Children	(02)07%	(10)37%	(10)43%
Pregnancy Gap			
2years	(03)10%	(17)64%	(14)61%
3years	(07)23%	(05)18%	(04)18%
>3 Years	(20)67%	(05)18%	(05)21%
Food Habits			
Nutritional Food	(25)83%	(07)26%	(08)35%
Non Nutritional Food	(05)17%	(20)74%	(15)65%

Table-6 shows 37% of pregnant women with moderate anemia & 43% of pregnant women with severe anemia in first trimester has more than 3 children as compared to normal first trimester pregnant women(7%). 61% of pregnant women with severe anemia,64% of moderately anemic group has less than 2 years pregnancy gap . 35% of pregnant women with severe anemia, 26% of pregnant women with moderate anemia & 83% of normal pregnant women in first trimester were on nutritious food .74% of pregnant women with moderate anemia, 65% of pregnant women with severe anemia, 17% of normal pregnant women were on non nutritious diet.

Figure 6: Prevalence of anemia in normal , moderate , severe first trimester pregnant women

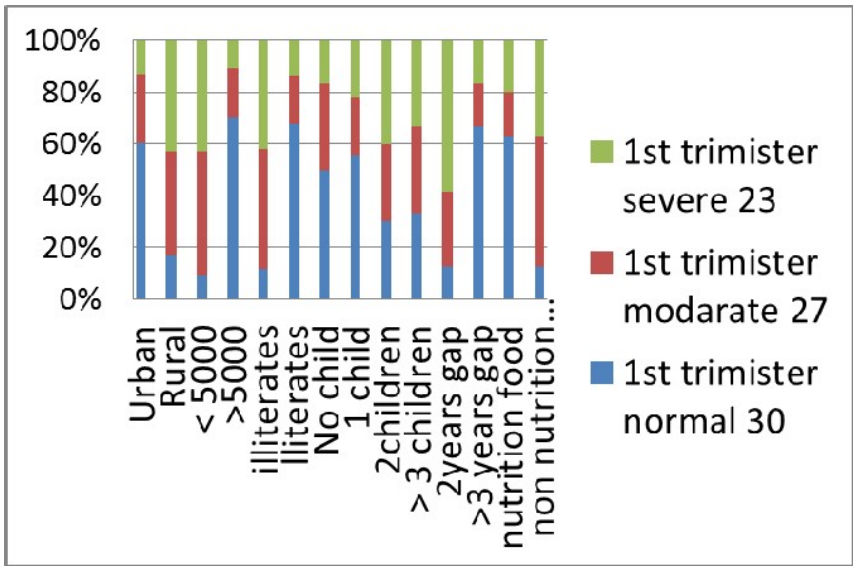


Table 7: Distribution socioeconomic characteristics in 2nd trimester normal pregnant & anemic pregnant women

CHARACTERISTICS	Normal pregnant women(n=30)	Moderately anemic% (n=29)	Severely anemic % (n=21)
Residence			
Urban	(25)83%	(08)31%	(05)24%
Rural	(05)17%	(21)69%	(16)76%
Monthly Income			
<5000	(05)17%	(26)89%	(18)86%
>5000	(25)83%	(03)11%	(03)14%
Educational Status			
Illiterates	(07)23%	(26)89%	(17)81%
Lliterates	(23)77%	(03)11%	(04)19%
House Wife			
Agricultural Working Women	(15)50%	(20)70%	(17)80%
Emoloyed women	(15)50%	(09)30%	(04)20%

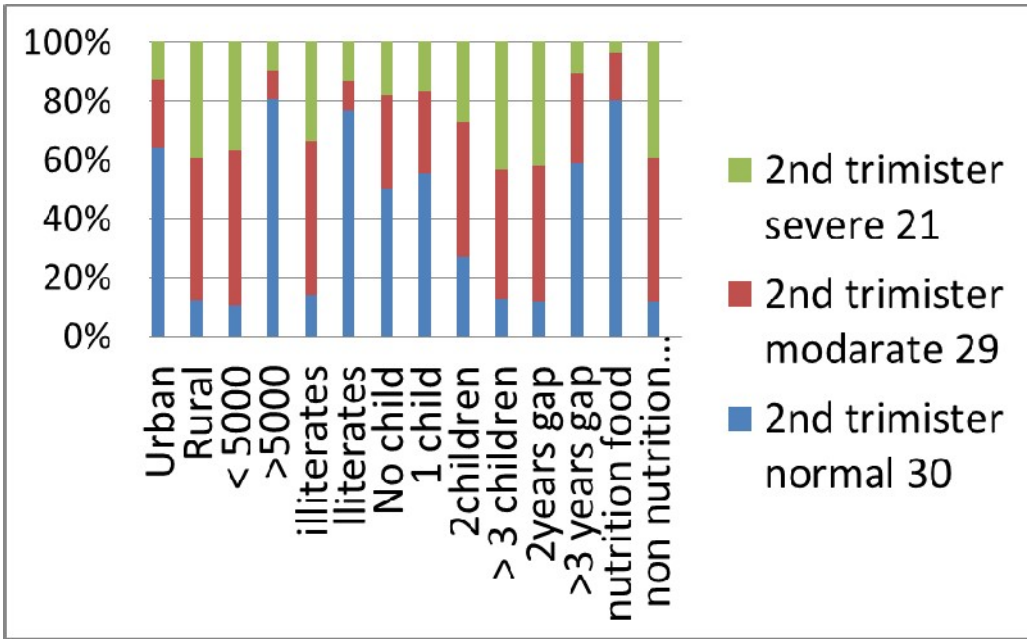
Table 7 shows in second trimester 69% of moderately anemic & 76% of severely anemic pregnant women are from rural area. 89% of moderately anemic pregnant women 86% of severely anemic pregnant women are under low poverty when compared to normal second trimester pregnant women (17%). In educational status 89% of moderately anemic & 81% of severely anemic pregnant women are illeterates as compared to 23% of illiterates or normal second trimester pregnant women.

Table 8: Distribution socioeconomic characteristics in 2nd trimester normal pregnant & anemic pregnant women

CHARACTERISTICS	Normal pregnant women(n=30)	Moderately anemic% (n=29)	Severely anemic % (n=21)
Number Of Children			
None	(14)47%	(09)32%	(05)23%
1 Child	(10)33%	(05)17%	(03)15%
2 Children	(03)10%	(05)17%	(03)15%
3 Or >3 Children	(03)10%	(10)34%	(10)47%
Pregnancy Gap			
2years	(05)17%	(20)69%	(18)86%
3years	(05)17%	(04)14%	(02)9%
>3 Years	(20)67%	(05)17%	(01)5%
Food Habits			
Nutritional Food	(25)83%	(05)17%	(01)5%
Non Nutritional Food	(05)17%	(24)83%	(20)95%

Table 8 shows distribution socioeconomic characteristics in second trimester normal pregnant & anemic pregnant women. In second trimester 34% of pregnant women with moderate anemia & 47% of pregnant women with severe anemia has 3 or more than 3 children . Also 86% & 69% of severely & moderately anemic pregnant women has less than 2 years pregnancy gap.In second trimester 83% of normal pregnant women, 17% of pregnant women with moderate anemia & 5% of pregnant women with severe anemia were on nutritious diet. Whereas 17% of normal pregnant women, 83% of pregnant women with moderate anemia & 95% of pregnant women with severe anemia were on non nutritious diet.

Figure 7. Prevalence of anemia in normal , moderate , severe second trimester pregnant women



7:

4.2. Anthropometric data of first & second trimesters pregnant women

Table 9: Anthropometric parameters of first trimester pregnant women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	F	Pvalue
Age(years)	22.36±1.97	23.60±3.31	23.22±3.35	2.151	0.1(ns)
Gestational age(weeks)	11.67±2.10	11.44±1.86	11.70±1.91	0.127	0.8(ns)
Height(cms)	136.6±2.84	136.93±3.35	137.7±3.53	0.072	0.92(ns)
Weight(kg)	45.1±6.37	43.7±6.52	44.04±6.54	0.361	0.6(ns)
Body surface area(m2)	13.64±1.99	13.25±1.99	13.41±1.73	0.316	0.7(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).					

Table 10: Anthropometric parameters of control & study groups post hoc tables of 1st trimester pregnant women

Post hoc values between Group I(normal) & Group II.

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Pvalue
Age(years)	22.36±1.97	23.60±3.31	0.1(ns)
Gestational age(weeks)	11.67±2.10	11.44±1.86	0.9(ns)
Height(cms)	136.6±2.84	136.93±3.35	0.9(ns)
Weight(kg)	45.1±6.37	43.7±6.52	0.6(ns)
Body surface area(m2)	13.64±1.99	13.25±1.99	1.0(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group I(normal) & Group III(Severe).

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=23) Mean±SD	Pvalue
Age(years)	22.36±1.97	23.22±3.35	0.3(ns)
Gestational age(weeks)	11.67±2.10	11.70±1.91	0.9(ns)
Height(cms)	136.6±2.84	136.7v3.53	0.9(ns)
Weight(kg)	45.1±6.37	44.04±6.54	0.8(ns)
Body surface area(m2)	13.64±1.99	13.41±1.73	1.0(ns)
*** p: <0.001: Very highly significant, ** p: <0.01: Highly significant, *p: <0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group II(Moderate) & Group III(Severe).

Parameter	Group -II=N(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	Pvalue
Age(years)	23.60±3.31	23.22±3.35	0.9(ns)
Gestational age(weeks)	11.44±1.86	11.70±1.91	0.8(ns)
Height(cms)	136.93±3.35	136.7v3.53	0.9(ns)
Weight(kg)	43.7±6.52	44.04±6.54	0.9(ns)
Body surface area(m2)	13.25±1.99	13.41±1.73	1.0(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 9,10, shows Anthropometric parameters of first trimester pregnant women. Age, Body surface area were almost similar in three groups. No statistically significant change was observed in BSA in 2nd & 3rd groups when compared to 1st group , in 3rd group when compared to 1st group of first trimester pregnant women.

TABLE 11: Anthropometric Parameters of second Trimester Pregnant Women

Parameter	Group-I=N (n=30) Mean±SD	Group-II=M.A (n=29) Mean±SD	Group-III=S.A (n=21) Mean±SD	F	P Value
Age(years)	23.53±2.80	23.17±2.60	23.43±2.74	0.1356	0.8(ns)
Gestational age(weeks)	21.27 ±2.63	22.03±3.38	21.33±3.27	0.5319	0.5(ns)
Height (cm)	136.9±3.05	136.9±2.72	137.6±2.61	0.4838	0.6(ns)
Weight(kg)	47.3±4.58	49.21±7.07	50.29±6.84	1.548	0.2(ns)
Body surface area (m2)	14.33±1.43	14.92±2.26	15.33±2.21	1.643	0.2(ns)

*** p <0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

TABLE 12: Anthropometric parameters of control & study groups post hoc tables of 2nd trimester pregnant women.

Post hoc values between normal & moderate groups.

Parameter	Group-I=N(n=30) Mean±SD	Group-II=S.A(n=29) Mean±SD	Pvalue
Age(years)	23.53±2.80	23.17±2.60	0.3(ns)
Gestational age(weeks)	21.27 ±2.63	22.03±3.38	0.9(ns)
Height(cms)	136.9±3.05	136.9±2.72	0.9(ns)
Weight(kg)	47.3±4.58	49.21±7.07	0.8(ns)
Body surface area(m2)	14.33±1.43	14.92±2.26	0.4(ns)

*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

Post hoc values between normal & severe groups.

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=21) Mean±SD	Pvalue
Age(years)	23.53±2.80	23.43±2.74	0.8(ns)
Gestational age(weeks)	21.27 ±2.63	21.33±3.27	0.6(ns)
Height(cms)	136.9±3.05	137.6±2.61	0.9(ns)
Weight(kg)	47.3±4.58	50.29±6.84	0.4(ns)
Body surface area(m2)	14.33±1.43	15.33±2.21	0.4(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between moderate & severe groups.

Parameter	Group -II=N(n=29) Mean±SD	Group-III=S.A(n=21) Mean±SD	Pvalue
Age(years)	23.17±2.60	23.43±2.74	0.9(ns)
Gestational age(weeks)	22.03±3.38	21.33±3.27	0.7(ns)
Height(cms)	136.9±2.72	137.6±2.61	0.6(ns)
Weight(kg)	49.21±7.07	50.29±6.84	0.8(ns)
Body surface area(m2)	14.92±2.26	15.33±2.21	0.7(ns)
*** p <0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 11,12showsAnthropometric parameter of second trimester pregnant women.There was no statistically significant difference observed in age, height & BSA. A non significant increase in weight was observed between control and study groups or within the subgroups of study groups in second trimester pregnant women.

4.3. Changes in Blood pressure & peripheral resistance in first & second trimesters pregnant women.

TABLE13: Blood Pressure & Peripheral Resistance Changes In First Trimester Pregnant Women

Parameter	Group-I=N (n=30) Mean±SD	Group-II=M.A (n=27) Mean±SD	Group-III=S.A (n=23) Mean±SD	F	P Value
Systolic Blood Pressure (mm Hg)	106.6±7.64	112.3±9.92	120.8±10.1	16.01	0.000***
Diastolic Blood Pressure (mm Hg)	70.33 ±9.27	69.62±0.39	67.39±7.51	0.73	0.4(ns)
Mean Arterial Pressure (mmHg)	181.6±19.09	172.72±16.33	170.7±12.67	3.43	0.03*
Total Peripheral Resistance (dyn/sec/cm^{-5})	2.53±0.77	2.01±0.73	1.714±0.413	10.26	0.000***

*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

TABLE 14: Blood Pressure & Peripheral Resistance Changes In First Trimester Pregnant Women (post hoc)

Post hoc intervention of BP & PR between group I & group II

Parameter	Group-I=N (n=30) Mean±SD	Group-II=M.A (n=27) Mean±SD	P Value
Systolic Blood Pressure (mm Hg)	106.6±7.64	112.3±9.92	0.01*
Diastolic Blood Pressure (mm Hg)	70.33 ±9.27	69.62±0.39	1.0(ns)
Mean Arterial Pressure (mmHg)	181.6±19.09	172.72±16.33	0.1(ns)
Total Peripheral Resistance (dyn/sec/cm^{-5})	2.53±0.77	2.01±0.73	0.01*
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc intervention of BP & PR between group I & group III

Parameter	Group-I=N (n=30) Mean±SD	Group-III=S.A (n=23) Mean±SD	P Value
Systolic Blood Pressure (mm Hg)	106.6±7.64	120.87±10.1	0.000***
Diastolic Blood Pressure (mm Hg)	70.33 ±9.27	67.39±7.51	0.7(ns)
Mean Arterial Pressure (mmHg)	181.6±19.09	170.7±12.67	0.05*
Total Peripheral Resistance (dyn/sec/cm^{-5})	2.53±0.77	1.714±0.413	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc intervention of BP & PR between group II & group III

Parameter	Group-II=M.A (n=27) Mean±SD	Group-III=S.A (n=23) Mean±SD	P Value
Systolic Blood Pressure (mm Hg)	112.3±9.92	120.87±10.1	0.01*
Diastolic Blood Pressure (mm Hg)	69.62±0.39	67.39±7.51	0.1(ns)
Mean Arterial Pressure (mmHg)	172.72±16.33	170.7±12.67	1.0(ns)
Total Peripheral Resistance (dyn/sec/cm^{-5})	2.01±0.73	1.714±0.413	0.2(ns)
*** p <0.001: Very highly significant, ** p <0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 13,14 shows Blood pressure & peripheral resistance changes in first trimester pregnant women.

A statistically significant increase in systolic blood pressure from 106.6±7.64 to 112.3±9.92, 120.87±10.1 was observed in 2nd & 3rd groups when compared to 1st group. Diastolic blood pressure was statistically not significant in 2nd & 3rd groups when compared to 1st group of first trimester pregnant women. A statistically significant decrease in Mean arterial pressure from 181.6±19.09 to 172.72±16.33&170.7±12.67was observed in 2nd & 3rd groups when compared to 1st group women was observed. A statistically significant decrease from 2.53±0.77 to 2.01±0.73 & 1.71±0.41 was observed in total peripheral resistance in 2nd & 3rd groups when compared to 1st group of first trimester pregnant women.

TABLE 15: Blood pressure & peripheral resistance changes in 2nd trimester pregnant women

Parameter	Group-I=N (n=30) Mean±SD	Group-II=M.A (n=29) Mean±SD	Group-III=S.A (n=21) Mean±SD	F	P Value
Systolic Blood Pressure (mm Hg)	103.67±9.6	105.86±9.07	122.38±10.91	25.49	0.000***
Diastolic Blood Pressure (mm Hg)	72.33 ±7.27	70.69±7.98	64.76±6.79	6.78	0.001***
Mean Arterial Pressure (mmHg)	179.22±15.72	176.67±17.08	170.79±14.94	1.73	0.1(ns)
Total Peripheral Resistance (dyn/sec/cm^{-5})	2.55±0.81	2.13±0.53	1.97±0.46	5.66	0.005**
*** p <0.001: Very highly significant, ** p <0.01: Highly significant, *p <0.05: Significant, p>0.05: Not Significant (NS).					

TABLE 16: Blood pressure & peripheral resistance changes in2nd trimester pregnant women (post hoc)

Post hoc intervention of BP & PR between group I & group II

Parameter	Group-I=N (n=30) Mean±SD	Group-II=M.A (n=29) Mean±SD	P Value
Systolic Blood Pressure (mm Hg)	103.67±9.6	105.86±9.07	1.0(ns)
Diastolic Blood Pressure (mm Hg)	72.33 ±7.27	70.69±7.98	1.0(ns)
Mean Arterial Pressure (mmHg)	179.22±15.72	176.67±17.08	0.9(ns)
Total Peripheral Resistance (dyn/sec/cm^{-5})	2.55±0.81	2.13±0.53	0.04*
*** p <0.001: Very highly significant, ** p <0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc intervention of BP & PR between group I & group III 2nd trimester.

Parameter	Group-I=N (n=30) Mean±SD	Group-III=S.A (n=21) Mean±SD	P Value
Systolic Blood Pressure (mm Hg)	103.67±9.6	122.38±10.91	0.000***
Diastolic Blood Pressure (mm Hg)	72.33 ±7.27	64.76±6.79	0.002**
Mean Arterial Pressure (mmHg)	179.22±15.72	170.79±14.94	0.2(ns)
Total Peripheral Resistance (dyn/sec/cm^{-5})	2.55±0.81	1.97±0.46	0.006**
*** p <0.001: Very highly significant, ** p <0.01: Highly significant, *p <0.05: Significant, p>0.05: Not Significant (NS).			

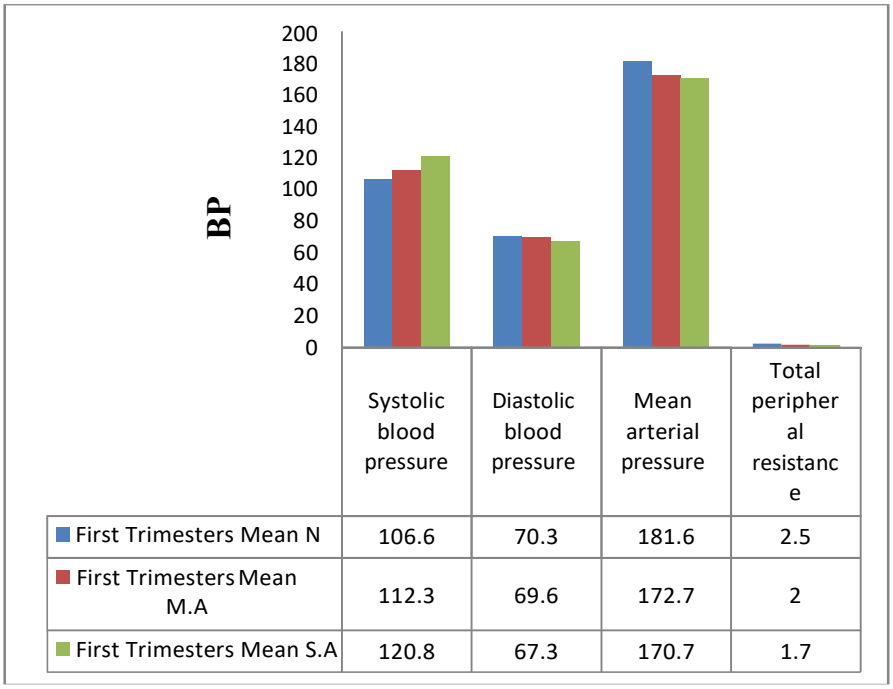
Post hoc intervention of BP & PR between group II & group III

Parameter	Group-II=M.A (n=29) Mean±SD	Group-III=S.A (n=21) Mean±SD	P Value
Systolic Blood Pressure (mm Hg)	105.86±9.07	122.38±10.91	0.000***
Diastolic Blood Pressure (mm Hg)	70.69±7.98	64.76±6.79	0.02*
Mean Arterial Pressure (mmHg)	176.67±17.08	170.79±14.94	0.6(ns)
Total Peripheral Resistance (dyn/sec/cm^{-5})	2.13±0.53	1.97±0.46	0.6(ns)
*** p <0.001: Very highly significant, ** p <0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 15,16 shows Blood pressure & peripheral resistance changes in second trimester pregnant women.

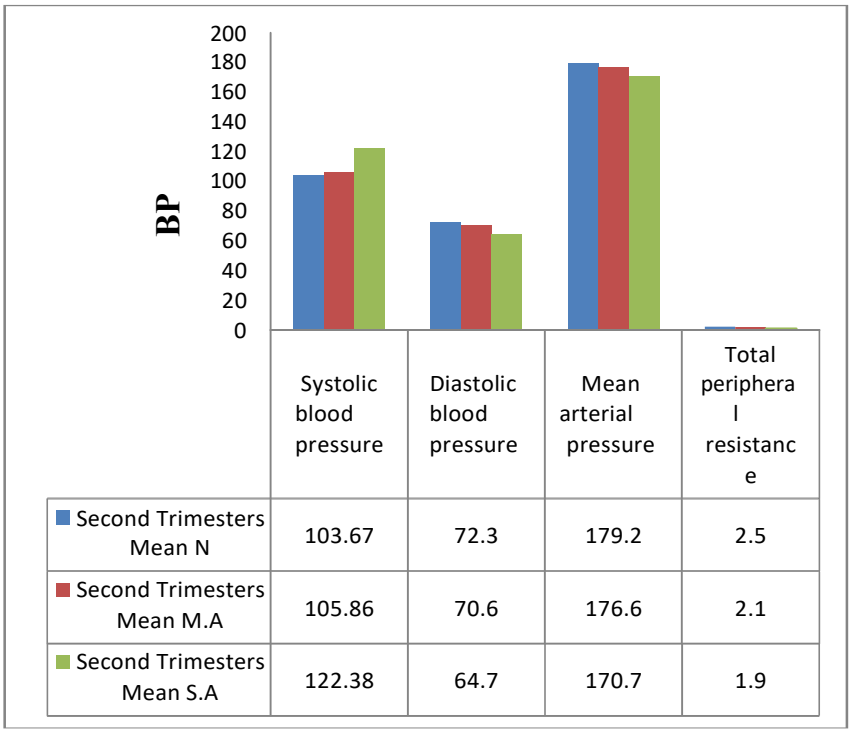
Statistically significant increase (p=000) in systolic blood pressure & statistically significant decrease (p=0.001) in diastolic blood pressure was observed in 2nd & 3rd groups when compared to 1st group. Similarly statistically significant decrease (p=0.005) was observed in total peripheral resistance from 2.55±0.81 to 2.13±0.53 & 1.97±0.46 in 3 groups of second trimester pregnant women.

Figure 8: Comparison of BP & Total peripheral Resistance in between control & study groups 1st trimester pregnant women.



	Systolic blood pressure	Diastolic blood pressure	Mean arterial pressure	Total peripheral resistance
First Trimesters Mean N	106.6	70.3	181.6	2.5
First Trimesters Mean M.A	112.3	69.6	172.7	2
First Trimesters Mean S.A	120.8	67.3	170.7	1.7

Figure 9: Comparisonof BP & Total peripheral Resistance in between control & study groups 2nd trimester pregnant women.



	Systolic blood pressure	Diastolic blood pressure	Mean arterial pressure	Total peripheral resistance
Second Trimesters Mean N	103.67	72.3	179.2	2.5
Second Trimesters Mean M.A	105.86	70.6	176.6	2.1
Second Trimesters Mean S.A	122.38	64.7	170.7	1.9

4.4. Haematological & biochemical findings in both 1st& 2nd trimester pregnant women.

Table 17: Haemogram values in first trimester pregnant women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	F	Pvalue
Haemoglobin gm%	11.49±1.22	8.09±0.70	4.89±0.60	344.1	0.001***
Serum ferritin 4.6-204ng/ml	32.39±27.50	5.33±1.37	4.10±0.52	24.54	0.001***
Serum iron 50-175mg	128.30±15.71	39.81±2.54	39.07±4.34	723.26	0.001***
Total Iron Binding Capacity 250-400mg/dl	364.50±47.86	564.81±15.67	560.43±11.38	367.38	0.001***
Transferrin saturation	35.43±4.25	6.93±0.60	7.15±0.80	102.2	0.001***
*** p <0.001: Very highly significant, ** p <0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).					

Table 18: Post hoc haemogram values in first trimester pregnant women

Post hoc values between normal & moderate groups

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Pvalue
Haemoglobin gm%	11.49±1.22	8.09±0.70	0.001***
Serum ferritin 4.6-204ng/ml	32.39±27.50	5.33±1.37	0.001***
Serum iron 50-175mg	128.30±15.71	39.81±2.54	0.001***
Total Iron Binding Capacity 250-400mg/dl	364.50±47.86	564.81±15.67	0.001***
Transferrin saturation	35.43±4.25	6.93±0.60	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between normal & severe groups

Parameter	Group-I=N (n=30) Mean±SD	Group-III=S.A (n=23) Mean±SD	Pvalue
Haemoglobin gm%	11.49±1.22	4.89±0.60	0.001***
Serum ferritin 4.6-204ng/ml	32.39±27.50	4.10±0.52	0.001***
Serum iron 50-175mg	128.30±15.71	39.07±4.34	0.001***
Total Iron Binding Capacity 250-400mg/dl	364.50±47.86	560.43±11.38	0.001***
Transferrin saturation	35.43±4.25	7.15±0.80	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Moderate & Severe groups

Parameter	Group-II= M.A (n=27) Mean±SD	Group-III=S.A (n=23) Mean±SD	Pvalue
Haemoglobin gm%	8.09±0.70	4.89±0.60	0.001***
Serum ferritin 4.6-204ng/ml	5.33±1.37	4.10±0.52	0.09(ns)
Serum iron 50-175mg	39.81±2.54	39.07±4.34	0.09(ns)
Total Iron Binding Capacity 250-400mg/dl	564.81±15.67	560.43±11.38	0.09(ns)
Transferrin saturation	6.93±0.60	7.15±0.80	0.09(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 17, 18 shows Haemogram values in first trimester pregnant women.

The mean haemoglobin concentration from 11.49 to 8.09 & 4.89,serum ferritin from 32.39 ±27.50 to 5.33±1.37 & 4.10±0.52 (p=0.001) & serum iron from 128.30 to 39.07 & 39.81 (p<0.001), and %trasferrin saturation from 35.43 to 6.93 & 7.15 (p<0.001) were significantly decreased in study groups when compared to control group of first trimester. Statistically significant increase in Total iron binding capacity was observed from 364.50±47.86 to 564.81±15.67 & 560.43±11.38.These observations were statistically significant in 2nd & 3rd groups when compared to 1st group & also in 2nd group when compared to 1st group & also in 3rd group when compared to 1st group.

Table 19: Haemogram values in second trimester pregnant women

Parameter	Group-I=N (n=30) Mean±SD	Group-II=M.A (n=29) Mean±SD	Group-III=S.A (n=21) Mean±SD	F	P Value
Haemoglobin gm%	11.28±0.97	8.27±0.56	6.17±1.63	143.7	0.001***
Serum ferritin 4.6-204ng/ml	43.29±47.96	4.87±1.19	3.91±0.60	16.3	0.001***
Serum iron 50-175mg	134.46±14.90	41.86±2.73	34.33±2.70	976.4	0.001***
Total Iron Binding Capacity 250-400mg/dl	327.73±3.04	572.24±5.13	562.3±7.77	144.5	0.001***
Transferrin saturation	41.41±6.45	7.33±0.52	6.61±0.05	708.60	0.001***

*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

Table 20: Post hoc haemogram values in second trimester pregnant women

Post hoc values between Group I & Group III

Parameter	Group-I=N (n=30) Mean±SD	Group-II=M.A (n=29) Mean±SD	P Value
Haemoglobin gm%	11.28±0.97	8.27±0.56	0.001***
Serum ferritin 4.6-204ng/ml	43.29±47.96	4.87±1.19	0.001***
Serum iron 50-175mg	134.46±14.90	41.86±2.73	0.001***
Total Iron Binding Capacity 250-400mg/dl	327.73±3.04	572.24±5.13	0.001***
Transferrin saturation	41.41±6.45	7.33±0.52	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group I & Group III

Parameter	Group-I=N (n=30) Mean±SD	Group-III=S.A (n=21) Mean±SD	P Value
Haemoglobin gm%	11.28±0.97	6.17±1.63	0.001***
Serum ferritin 4.6-204ng/ml	43.29±47.96	3.91±0.60	0.001***
Serum iron 50-175mg	134.46±14.90	34.33±2.70	0.001***
Total Iron Binding Capacity 250-400mg/dl	327.73±3.04	562.3±7.77	0.001***
Transferrin saturation	41.41±6.45	6.61±0.05	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group II & Group III

Parameter	Group-II=M.A (n=29) Mean±SD	Group-III=S.A (n=21) Mean±SD	P Value
Haemoglobin gm%	8.27±0.56	6.17±1.63	0.001***
Serum ferritin 4.6-204ng/ml	4.87±1.19	3.91±0.60	0.9(ns)
Serum iron 50-175mg	41.86±2.73	34.33±2.70	0.01*
Total Iron Binding Capacity 250-400mg/dl	572.24±5.13	562.3±7.77	0.2(ns)
Transferrin saturation	7.33±0.52	6.61±0.05	0.8(ns)
*** p <0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 19,20 shows Haemogram values in second trimester pregnant women.

Statistically significant decrease was observed in mean haemoglobin concentration, from 11.28 to 8.27 & 6.17 (p=0.001)serum iron from 134.46 to 41.86 &34.33 (p<0.001), serum feritin from 43.29 to 4.87 & 3.9 (p<0.001)and % transferrin saturation from 41.41 to 7.33 & 6.61 (p=0.001) in study groups than in control group of second trimester. Statistically significant increase in total iron binding capacity from327.73 to 572.24 to 562.3 (p<0.001) between control & study groups or within the subgroups of study groups of second trimester was observed.

4.5. Blood indices values in 1st& 2nd trimester pregnant women.

Table 21: Blood indices values in first trimester pregnant women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	F	Pvalue
PCV(<30%)	26.60±2.23	25.70±1.89	24.34±1.43	8.96	0.001***
MCV (78-90µm3)	68.95±4.88[a***]	62.83±6.20	60.54±6.63	17.55	0.001***
MCH (28-30pg)	27.24±3.88	20.83±2.80	18.38±2.62	135.3	0.001***
MCHC (33-35%)	43.45±5.42	30.32±2.76	18.32±2.73	259.18	0.001***

*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

Table 22: Blood indices values in first trimester pregnant women (post hoc)

Post hoc values between Group I & Group II

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Pvalue
PCV(<30%)	26.60±2.23	25.70±1.89	0.2(ns)
MCV (78-90µm3)	68.95±4.88	62.83±6.20	0.001***
MCH (28-30pg)	27.24±3.88	20.83±2.80	0.1(ns)
MCHC (33-35%)	43.45±5.42	30.32±2.76	0.001***

*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

Post hoc values between Group I & Group III

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=23) Mean±SD	Pvalue
PCV(<30%)	26.60±2.23	24.34±1.43	0.001***
MCV (78-90µm3)	68.95±4.80	60.54±6.63	0.001***
MCH (28-30pg)	27.24±3.88	18.38±2.62	0.001***
MCHC (33-35%)	43.45±5.42	18.32±2.73	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group II & Group III

Parameter	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	Pvalue
PCV(<30%)	25.70±1.89	24.34±1.43	0.04*
MCV (78-90µm3)	62.83±6.20	60.54±6.63	0.3(ns)
MCH (28-30pg)	20.83±2.80	18.38±2.62	0.001***
MCHC (33-35%)	30.32±2.76	18.32±2.73	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 21,22 shows blood indices values in first trimester pregnant women.

A statistically significant decrease in blood indices like MCV from 68.95 to 62.83 & 60.54 (p<0.001),MCH from 27.24 to 20.83 & 18.38 (p<0.001) & MCHC from 43.45 to 30.32 & 18.32 (p< 0.001)were observed in in 2nd & 3rd groups when compared to 1st group , in 2nd group when compared to 1st group & also in 3rd group when compared to 1st group of first trimester pregnant women. But MCV was not statistically significant in between 2^{nd}& 3^{rd} groups. Also MCH was not statistically significant in between 1st & 2nd groups of first trimester pregnant women.

Table 23: Blood indices values in second trimester pregnant women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=29) Mean±SD	Group-III=S.A(n=21) Mean±SD	F	Pvalue
PCV(<30%)	33.00±1.01	27.86±2.26	27.76±2.09	74.16	0.001***
MCV (78-90µm3)	81.36±7.27	70.32±3.96	69.54±6.94	31.36	0.001***
MCH (28-30pg)	27.73±2.41	21.06±2.80	15.69±4.95	80.75	0.001***
MCHC (33-35%)	34.20±2.78	29.87±2.81	22.35±6.07	56.49	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).					

Table 24: Blood indices values in second trimester pregnant women(post hoc)

Post hoc values between Group I (normal) & Group II (Moderate)

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=29) Mean±SD	Pvalue
PCV(<30%)	33.00±1.01	27.86±2.26	0.9(ns)
MCV (78-90µm3)	81.36±7.27	70.32±3.96	0.001***
MCH (28-30pg)	27.73±2.41	21.06±2.80	0.001***
MCHC (33-35%)	34.20±2.78	29.87±2.81	0.002**
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group I (normal) & Group III(Severe)

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=21) Mean±SD	Pvalue
PCV(<30%)	33.00±1.01	27.76±2.09	0.001***
MCV (78-90µm3)	81.36±7.27	69.54±6.94	0.001***
MCH (28-30pg)	27.73±2.41	15.69±4.95	0.001***
MCHC (33-35%)	34.20±2.78	22.35±6.07	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group II (Moderate) & Group III (Severe)

Parameter	Group-II=M.A(n=29) Mean±SD	Group-III=S.A(n=21) Mean±SD	Pvalue
PCV(<30%)	27.86±2.26	27.76±2.09	0.9(ns)
MCV (78-90µm3)	70.32±3.96	69.54±6.94	0.9(ns)
MCH (28-30pg)	21.06±2.80	15.69±4.95	0.001***
MCHC (33-35%)	29.87±2.81	22.35±6.07	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 23, 24 shows blood indices values in second trimester pregnant women.

A statistically significant decrease in blood indices like MCV from 81.36 to 70.32 & 69.54 ($p<0.001$), MCH from 27.73 to 21.06 & 15.69 ($p<0.001$) & MCHC from 34.20 to 29.87 & 22.35 ($p< 0.001$)were observed in 2nd & 3rd groups when compared to 1st group & also in 2nd group when compared to 1st group & also in 3rd group when compared to 1st group of second trimester pregnant women. Non significant decrease was observed in MCV between 2^{nd}& 3^{rd} groups of second trimester pregnant women.

4.6. Echocardiographic parameters in both 1st& 2nd trimester pregnant women.

Table 25: Comparison of echo parameters in first trimester preg.women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	F	Pvalue
Heart Rate	82.80±9.96	98.93±15.39	102.92±13.37	20.06	0.001***
EDD(End Diastolic Diameter)	4.38 ±0.22	4.47±0.21	4.58±0.14	6.537	0.002**
ESD(End Systolic Diameter)	2.42±0.21	2.49±0.17	2.60±0.18	5.422	0.006**
EF%(Ejection Fraction)	63.03±2.89	65.92±2.12	61.21±1.65	26.40	0.001***
FS%(Fractional Shortening)	31.46±1.45	32.62±1.20	30.73±0.86	14.73	0.001***
SV(Stroke Volume)	70.33±10.88	74.92±10.94	78.94±8.84	4.597	0.01*
COP(Cardiac output)	5842.4±1263.3	7517.7±1941.6	8173.50±1444	15.91	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).					

Table 26: Comparison of echo parameters in first trimester preg.women (post hoc)

Post hoc values between normal & moderate groups

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Pvalue
Heart Rate	82.80±9.96	98.93±15.39	0.001***
EDD(End Diastolic Diameter)	4.38 ±0.22	4.47±0.21	0.2(ns)
ESD(End Systolic Diameter)	2.42±0.21	2.49±0.17	0.3(ns)
EF%(Ejection Fraction)	63.03±2.89	65.92±2.12	<0.001***
FS%(Fractional Shortening)	31.46±1.45	32.62±1.20	0.002**
SV(Stroke Volume)	70.33±10.88	74.92±10.94	0.2(ns)
COP(Cardiac output)	5842.4±1263.3	7517.7±1941.6	<0.001***
*** p<0.001: Very highly significant, ** p <0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between normal &severe groups

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=23) Mean±SD	Pvalue
Heart Rate	82.80±9.96	102.92±13.37	0.001***
EDD(End Diastolic Diameter)	4.38 ±0.22	4.58±0.14	0.002**
ESD(End Systolic Diameter)	2.42±0.21	2.60±0.18	0.005**
EF%(Ejection Fraction)	63.03±2.89	61.21±1.65	0.01*
FS%(Fractional Shortening)	31.46±1.45	30.73±0.86	0.05*
SV(Stroke Volume)	70.33±10.88	78.94±8.84	0.01*
COP(Cardiac output)	5842.4±1263.3	8173.50±1444	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Moderate & Severe groups

Parameter	Group-II=M.A (n=27) Mean±SD	Group-III=S.A (n=23) Mean±SD	Pvalue
Heart Rate	98.93±15.39	102.92±13.37	0.4(ns)
EDD(End Diastolic Diameter)	4.47±0.21	4.58±0.14	0.1(ns)
ESD(End Systolic Diameter)	2.49±0.17	2.60±0.18	0.1(ns)
EF%(Ejection Fraction)	65.92±2.12	61.21±1.65	0.001***
FS%(Fractional Shortening)	32.62±1.20	30.73±0.86	0.001***
SV(Stroke Volume)	74.92±10.94	78.94±8.84	0.3(ns)
COP(Cardiac output)	7517.7±1941.6	8173.50±1444	0.4(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 25, 26 shows comparison of echo parameters in first trimester pregnant women.

In this study there was significant increase in heart rate from 82.80, 98.93 & 102.9 ($p<0.001$) ESD from 2.42, 2.49 & 2.60 ($p=0.006$), EDD from 4.38 to 4.47 & 4.58, SV from 70.33 to 74.92 & 78.94 & COP from 5842.4 to 7517.7 & 8173.5 ($p<0.001$) in study groups of first trimester pregnant women was observed. A statistically significant increase was observed in above parameters in 2nd & 3rd groups when compared to 1st group , in 2nd group when compared to 1st group & also in 3rd group when compared to 1st group.

In current study a significant increase in EF% from 63.03 to 65.92 ($p<0.001$)in 1st & 2nd groups & a statistically significant decrease ($p=0.01$) in EF% between 1st & 3rd groups were observed.

Table 27: Comparison of echo parameters in second trimester preg.women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=29) Mean±SD	Group-III=S.A(n=21) Mean±SD	F	Pvalue
Heart Rate	84.56±7.08	94.86±12.73	102.92±13.39	13.22	0.001***
EDD(End Diastolic Diameter)	4.33 ±0.29	4.35±0.27	4.54±0.17	4.70	0.01*
ESD(End Systolic Diameter)	2.40±0.24	2.41±0.15	2.60±0.21	7.09	0.001***
EF%(Ejection Fraction)	63.60±2.42	64.48±2.70	61.71±2.45	7.34	0.001***
FS%(Fractional Shortening)	31.26±1.31	32.93±1.27	30.61±0.80	26.25	0.001***
SV(Stroke Volume)	67.92±14.04	69.21±13.48	76.11±11.58	2.60	0.08(ns)
COP(Cardiac output)	5874.4±1223.6	6895.0±1427.3	7960.7±1377.5	17.15	0.001***
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).					

Table 28: Comparison of echo parameters in second trimester preg.women (post hoc)

Post hoc values between Group I& Group II

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=29) Mean±SD	Pvalue
Heart Rate	84.56±7.08	94.86±12.73	0.005**
EDD(End Diastolic Diameter)	4.33 ±0.29	4.35±0.27	0.06(ns)
ESD(End Systolic Diameter)	2.40±0.24	2.41±0.15	0.05*
EF%(Ejection Fraction)	63.60±2.42	64.48±2.70	0.5(ns)
FS%(Fractional Shortening)	31.26±1.31	32.93±1.27	0.001***
SV(Stroke Volume)	67.92±14.04	69.21±13.48	0.9(ns)
COP(Cardiac output)	58745.4±1223.6	6895.0±1427.3	0.005**

*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

Post hoc values between Group I & Group III

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=21) Mean±SD	Pvalue
Heart Rate	84.56±7.08	102.92±13.39	0.001***
EDD(End Diastolic Diameter)	4.33 ±0.29	4.54±0.17	0.03*
ESD(End Systolic Diameter)	2.40±0.24	2.60±0.21	0.05*
EF%(Ejection Fraction)	63.60±2.42	61.71±2.45	0.03*
FS%(Fractional Shortening)	31.26±1.31	30.61±0.80	0.1(ns)
SV(Stroke Volume)	67.92±14.04	76.11±11.58	0.09(ns)
COP(Cardiac output)	58745.4±1223.6	7960.7±1377.5	0.001***

*** p<0.001: Very highly significant, ** p <0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

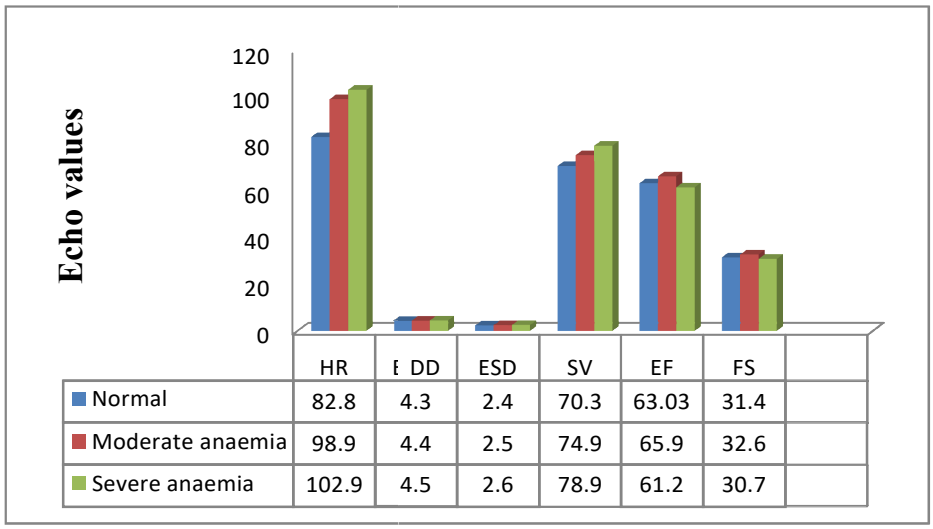
Post hoc values between Group II & Group III

Parameter	Group-II=M.A (n=29) Mean±SD	Group-III=S.A (n=21) Mean±SD	Pvalue
Heart Rate	94.86±12.73	102.92±13.39	0.5(ns)
EDD(End Diastolic Diameter)	4.35±0.27	4.54±0.17	0.07(ns)
ESD(End Systolic Diameter)	2.41±0.15	2.60±0.21	0.06(ns)
EF%(Ejection Fraction)	64.48±2.70	61.71±2.45	0.001***
FS%(Fractional Shortening)	32.93±1.27	30.61±0.80	0.001***
SV(Stroke Volume)	69.21±13.48	76.11±11.58	0.2(ns)
COP(Cardiac output)	6895.0±1427.3	7960.7±1377.5	0.02*
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 27, 28 showscomparison of echo parameters in second trimester pregnant women.

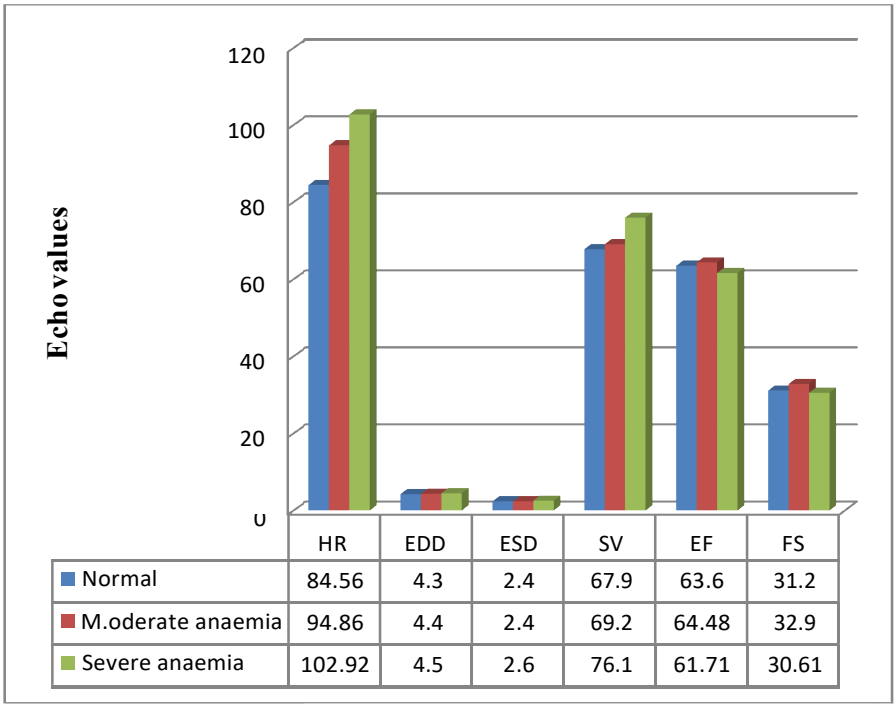
There was statistically significant increase in ESD from 2.40 to 2.41 & 2.60(p<0.001), EDD from 4.33 to 4.35 & 4.54(p=0.01), COPfrom 5745.4 to 6895 & 7960.7 (p<0.001) & HR from 84.56 to 94.86 & 102.92 (p<0.001) was observed in 2nd & 3rd group when compared to 1st group & also in 3rd group when compared to 1st group of second trimester pregnant women . A statistically significant decrease from63.60 to 61.71(p<0.001) in EF% and from 31.26 to 30.61 (p<0.001) in FS% was observed in 3rd group when compared to 1st group of second trimester pregnant women.

Figue 10: Comparision of Mean Echocardiographic Parameters in control & study groups of first trimester pregnant women



	HR	DD	ESD	SV	EF	FS
Normal	82.8	4.3	2.4	70.3	63.03	31.4
Moderate anaemia	98.9	4.4	2.5	74.9	65.9	32.6
Severe anaemia	102.9	4.5	2.6	78.9	61.2	30.7

Figue 11: Comparision of Mean Echocardiographic Parameters in control & study groups of second trimester pregnant women



	HR	EDD	ESD	SV	EF	FS
Normal	84.56	4.3	2.4	67.9	63.6	31.2
M.oderate anaemia	94.86	4.4	2.4	69.2	64.48	32.9
Severe anaemia	102.92	4.5	2.6	76.1	61.71	30.61

4.7. Serum electrolyte findings in first & second trimesters of pregnant women.

Table 29: Serum electrolyte values in first trimester pregnant women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	F	P value
Sodium(Na) 135-145	137.46±2.56	136.92±2.09	134.82±1.96	9.066	0.003**
Potassium(K) 3.5-5	3.93±0.43	3.64±0.27	3.41±0.21	16.1	0.001***
Chloride(Cl) 95-111	103.3±3.49	106±3.8	104.4±3.43	3.78	0.02*
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).					

Table 30: Serum electrolyte values in first trimester pregnant women (post hoc)

Post hoc values between Group I & Group II

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	P value
Sodium(Na) 135-145	137.46±2.56	136.92±2.09	0.7(ns)
Potassium(K) 3.5-5	3.93±0.43	3.64±0.27	0.003**
Chloride(Cl) 95-111	103.3±3.49	106±3.8	0.02*
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group I & Group III

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=23) Mean±SD	P value
Sodium(Na) 135-145	137.46±2.56	134.82±1.96	0.003**
Potassium(K) 3.5-5	3.93±0.43	3.41±0.21	0.001***
Chloride(Cl) 95-111	103.3±3.49	104.4±3.43	0.5(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p <0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group II & Group III

Parameter	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	P value
Sodium(Na) 135-145	136.92±2.09	134.82±1.96	0.004**
Potassium(K) 3.5-5	3.64±0.27	3.41±0.21	0.05*
Chloride(Cl) 95-111	106±3.8	104.4±3.43	0.3(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 29, 30 shows serum electrolyte values in first trimester pregnant women.

In present study a statistically significantdecrease in serum electrolyte values like sodium from 137.46±2.5 to 136.92±2.0 & 134.82±1.9 , potassium from 3.93±0.4 to 3.64±0.2 & 3.41±0.2 were observed in 2nd & 3rd groups when compared to 1st group of first trimester pregnant women. A statistically significant increase in chloride from 103.3 to 106 was observed between 1st& 2nd groups.

Table 31: Serum electrolyte values in second trimester pregnant women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=29) Mean±SD	Group-III=S.A(n=21) Mean±SD	F	P value
Sodium(Na) 135-145	137.7±2.56	138.2±2.09	135.9±1.96	9.066	0.01*
Potassium(K) 3.5-5	3.82±0.45	3.67±0.37	3.41±0.30	4.19	0.01*
Chloride(Cl) 95-111	105.4±3.22	106±3.75	107.4±2.95	1.56	0.2(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).					

Table 32: Serum electrolyte values in second trimester pregnant women (post hoc)

Post hoc values between Group I & Group II

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=29) Mean±SD	Pvalue
Sodium(Na) 135-145	137.7±2.56	138.2±2.09	0.8(ns)
Potassium(K) 3.5-5	3.82±0.45	3.67±0.37	0.3(ns)
Chloride(Cl) 95-111	105.4±3.22	106±3.75	0.4(ns)
*** p<0.001: Very highly significant, ** p <0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group I & Group III

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=21) Mean±SD	P value
Sodium(Na) 135-145	137.7±2.56	135.9±1.96	0.06(ns)
Potassium(K) 3.5-5	3.82±0.45	3.41±0.30	0.01*
Chloride(Cl) 95-111	105.4±3.22	107.4±2.95	0.2(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group II & Group III

Parameter	Group-II=M.A(n=29) Mean±SD	Group-III=S.A(n=21) Mean±SD	P value
Sodium(Na) 135-145	138.2±2.09	135.9±1.96	0.01*
Potassium(K) 3.5-5	3.67±0.37	3.41±0.30	0.2(ns)
Chloride(Cl) 95-111	106±3.75	107.4±2.95	0.8(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 31 shows serum electrolyte values in second trimester pregnant women.

A statistically significant decrease in serum electrolyte valueslike sodium from 137.7±2.8 to 135.82±1.9 , potassium from 3.82±0.4 to 3.67±0.2 & 3.41±0.2 , were observed in 2nd & 3rd groups when compared to 1st group of second trimester pregnant women.

4.8. Electrocardiogram values in pregnant women.

Table 33: Electrocardiogram values in first trimester pregnant women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	F	P value
QRS duration (80-100ms)	82.33±9.18	76.40±5.46	83.60±8.70	6.47	0.003**
QT interval (320-360ms)	364.6±20.09	346.67±17.78	341.5±17.71	11.59	0.001***
QTc interval (350-420ms)	426.97±15.88	430.3±16.96	432.8±17.82	0.10	0.01*
T axis(in degrees) (-33-54)	24.15±16.80	13.48±18.63	16.27±2.60	5.22	0.007*
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).					

Table 34: Electrocardiogram values in first trimester pregnant women(post hoc)

Post hoc values between Group I & Group II

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=27) Mean±SD	P value
QRS duration (80-100ms)	82.33±9.18	76.40±5.46	0.02*
QT interval (320-360ms)	364.6±20.09	346.67±17.78	0.3(ns)
QTc interval (350-420ms)	426.97±15.88	430.3±16.96	0.9(ns)
T axis(in degrees) (-33-54)	24.15±16.80	13.48±18.63	0.06*
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group I & Group III

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=23) Mean±SD	P value
QRS duration (80-100ms)	82.33±9.18	83.60±8.70	1.0(ns)
QT interval (320-360ms)	364.6±20.09	341.5±17.71	0.01*
QTc interval (350-420ms)	426.97±15.88	432.8±17.82	0.01*
T axis(in degrees) (-33-54)	24.15±16.80	16.27±2.60	0.009**
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group II & Group III

Parameter	Group-II=M.A(n=27) Mean±SD	Group-III=S.A(n=23) Mean±SD	P value
QRS duration (80-100ms)	76.40±5.46	83.60±8.70	0.004**
QT interval (320-360ms)	346.67±17.78	341.5±17.71	0.9(ns)
QTc interval (350-420ms)	430.3±16.96	432.8±17.82	0.9(ns)
T axis(in degrees) (-33-54)	13.48±18.63	16.27±2.60	1.0(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 33 shows electrocardiogram values in first trimester pregnant women.

In present study, QRSD showed statistically significant decrease from 82.33 to 76.40 (p=0.02,) in 2nd when compared to 1st group. Statistically significant decrease in QT interval was observed between the control and study groups of first trimester .

A statistically significant increase in QTc interval was observed in between control & study groups. A statistically significant decrease in T axis from 24.13 to 13.85 & 9.47(p<0.006)was observed . This observation was statistically significant in 2nd & 3rd groups when compared to 1st group & also in 3rd group when compared to 1st group.

Table 35: Electrocardiogram values in second trimester pregnant women

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=29) Mean±SD	Group-III=S.A(n=21) Mean±SD	F	P value
QRS duration (80-100ms)	80.20±8.60	75.45±7.70	73.8±7.43	0.18	0.01*
QT interval (320-360ms)	361.33±21.33	345.7±19.84	344.6±21.03	6.31	0.002**
QTc interval (350-420ms)	425.96±18.98	442.7±16.14	448.6±13.88	13.41	0.001***
T axis(in degrees) (-33-54)	20.73±20.40	20.34±18.50	17.90±19.03	0.14	0.8(ns)

*** p <0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

Table 36: Electrocardiogram values in second trimester pregnant women (post hoc)

Post hoc values between Group I & Group II

Parameter	Group-I=N(n=30) Mean±SD	Group-II=M.A(n=29) Mean±SD	P value
QRS duration (80-100ms)	80.20±8.60	75.45±7.70	0.8(ns)
QT interval (320-360ms)	361.33±21.33	345.7±19.84	0.008**
QTc interval (350-420ms)	425.96±18.98	442.7±16.14	0.006**
T axis(in degrees) (-33-54)	20.73±20.40	20.34±18.50	0.9(ns)

*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).

Post hoc values between Group I & Group III

Parameter	Group-I=N(n=30) Mean±SD	Group-III=S.A(n=21) Mean±SD	P value
QRS duration (80-100ms)	80.20±8.60	73.8±7.43	0.01*
QT interval (320-360ms)	361.33±21.33	344.6±21.03	0.01*
QTc interval (350-420ms)	425.96±18.98	448.6±13.88	0.001***
T axis(in degrees) (-33-54)	20.73±20.40	17.90±19.03	0.8(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Post hoc values between Group II & Group III

Parameter	Group-II=M.A(n=29) Mean±SD	Group-III=S.A(n=21) Mean±SD	P value
QRS duration (80-100ms)	75.45±7.70	73.8±7.43	0.9(ns)
QT interval (320-360ms)	345.7±19.84	344.6±21.03	0.9(ns)
QTc interval (350-420ms)	442.7±16.14	448.6±13.88	0.4(ns)
T axis(in degrees) (-33-54)	20.34±18.50	17.90±19.03	0.8(ns)
*** p<0.001: Very highly significant, ** p<0.01: Highly significant, *p<0.05: Significant, p>0.05: Not Significant (NS).			

Table 35 shows s electrocardiogram values in second trimester pregnant women.

A statistically significant decrease (p=0.01) in QRSD from 80.20 to 75.45 & 73.8 was observed in 3rd group when compared to 1st group. Statistically significant decrease (p=0.008) was observed in QT interval between 1st& 3rd groups of second trimester pregnant women. There was statistically significant increase in QTC interval from 425.96 to 442.72 & 448.6 l was observed in second trimester pregnant women. This observation was statistically significant in 2nd & 3rd groups when compared to 1st group & also in 2nd group when compared to 1st group & also in 3rd group when compared to 1st group.

Table 37: Correlation between Haemoglobin.ferritin and serum iron with LV functions of normal first trimester pregnant women

Parameters	Haemoglobin		Serum ferritin		Serum iron	
	R	p	r	P	r	P
Heart rate	0.1393	0.4	0.2231	0.2	-0.072	0.7
EDD(End Diastolic Diameter)	0.076	0.6	0.128	0.5	0.186	0.32
ESD(End Systolic Diameter)	0.240	0.2	0.143	0.4	0.048	0.80
Ejection fraction (%EF)	0.031	0.8	0.129	0.4	0.137	0.4
Fractional Shortening(FS)	0.025	0.4	0.099	0.6	0.103	0.5
Stroke Volume(SV)	0.008	0.9	0.099	0.6	0.185	0.3
Cardiac output(COP)	0.068	0.7	0.048	0.8	0.093	0.6
Correlation is significant at the 0.01 level(2-tailed)(p);r-regression						

Table 37 shows correlation between Haemoglobin, serum ferritin & serum iron with left ventricular functions in normal first trimester pregnant women.

A positive correlation between Hb , SF, SI with HR, SV , EF, FS , COP ,EDD ,ESD was observed in first trimester normal pregnant women . These observations were statistically not significant.

Table 38: Correlation between Haemoglobin,ferritin and serum iron with LV functions of moderately anemic first trimester pregnant women

Parameters	Haemoglobin		Serum ferritin		Serum iron	
	R	p	r	P	r	P
Heart rate	-0.025	0.9	0.036	0.8	-0.200	0.3
EDD(End Diastolic Diameter)	-0.099	0.6	-0.254	0.2	-0.115	0.5
ESD(End Systolic Diameter)	-0.067	0.7	-0.184	0.3	-0.136	0.4
Ejection fraction (%EF)	-0.061	0.7	-0.249	0.2	-0.291	0.1
Fractional Shortening(FS)	-0.140	0.4	-0.201	0.3	-0.390	0.04
Stroke Volume(SV)	-0.102	0.6	-0.195	0.3	-0.138	0.4
Cardiac output(COP)	-0.050	0.8	-0.087	0.6	-0.166	0.4
Correlation is significant at the 0.01 level(2-tailed)(p);r-regression						

Table 38 shows s correlation between Haemoglobin, serum ferritin & serum iron with left ventricular functions of moderately anemic first trimester pregnant women.

Negative correlation between Hb, SF&SI with HR, SV, EF, FS, ESD, EDD was observed in moderately anaemic first trimester pregnant women . These observations were statistically not significant.HR was positivelycorrelated with serum ferritin. This observation was statistically not significant.

Figure 12:Correlation between Haemoglobin & Echo findings in moderately anemic(a1) first trimester pregnant women

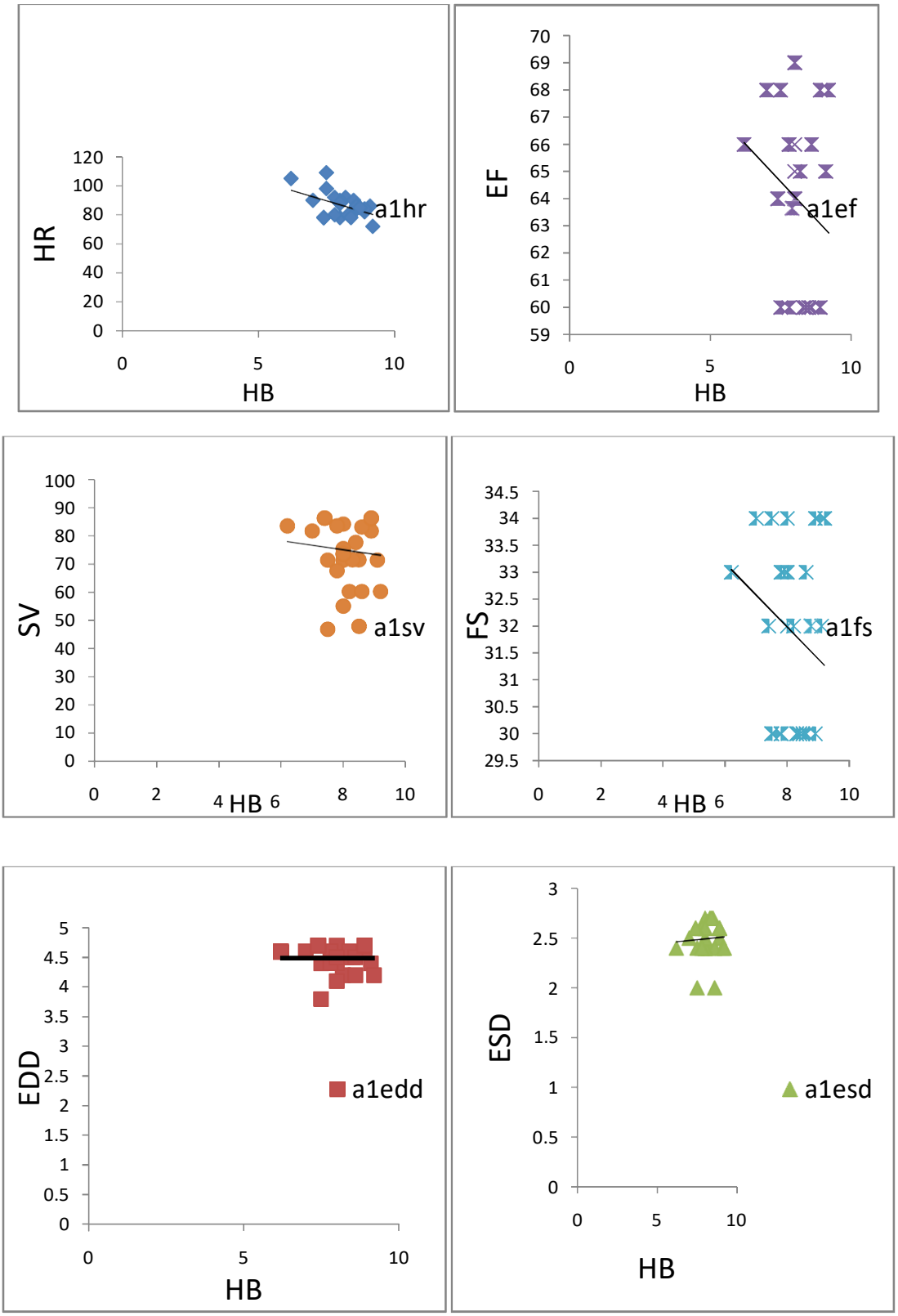


Figure 13:Correlation between Serum ferrtin & Echo findings in moderately anemic(a1) first trimester pregnant women

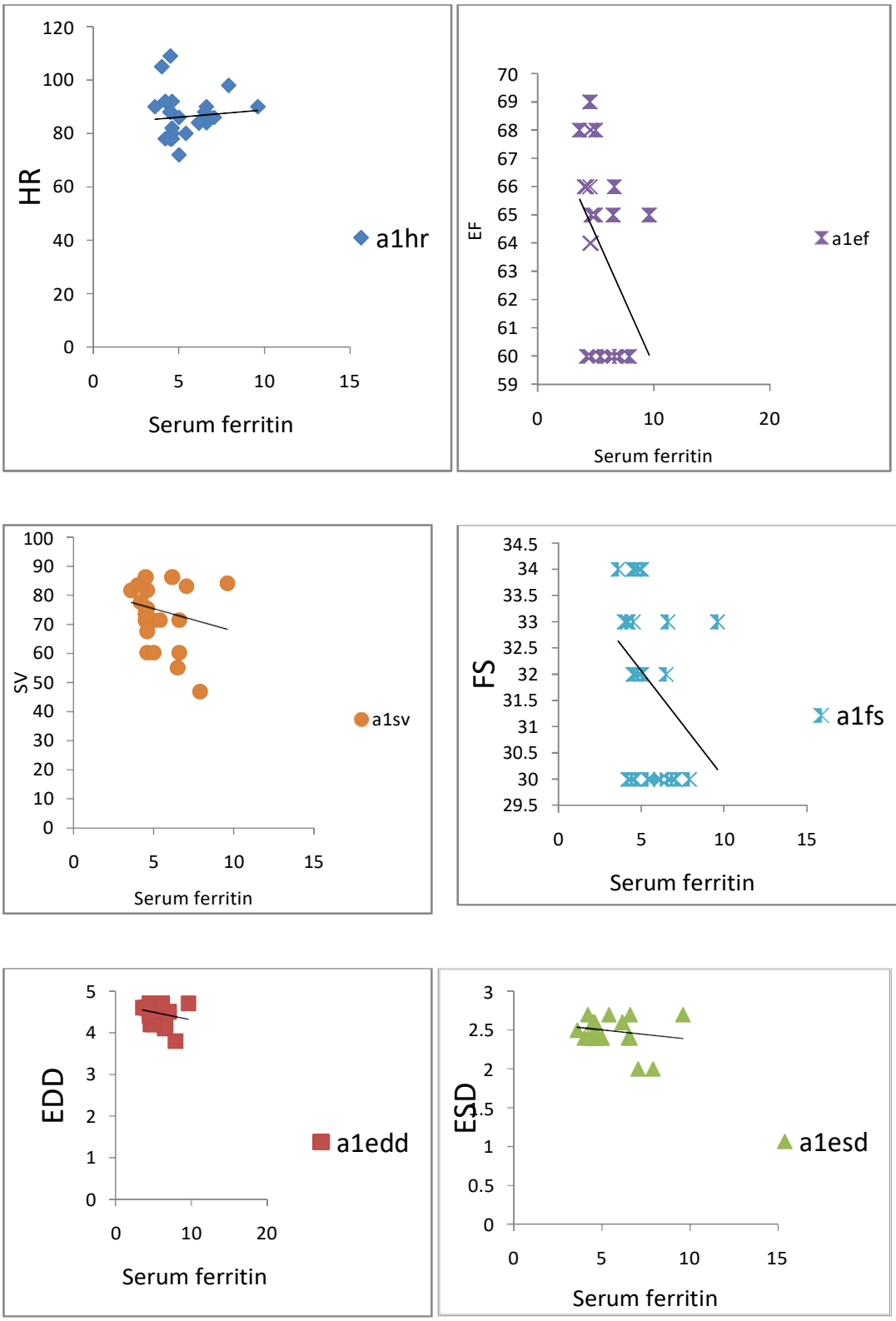


Figure 14:Correlation between Serum iron & Echo findings (HR,SV,EF,FS,EDD,ESD) in moderately anemic(a1) first trimester pregnant women

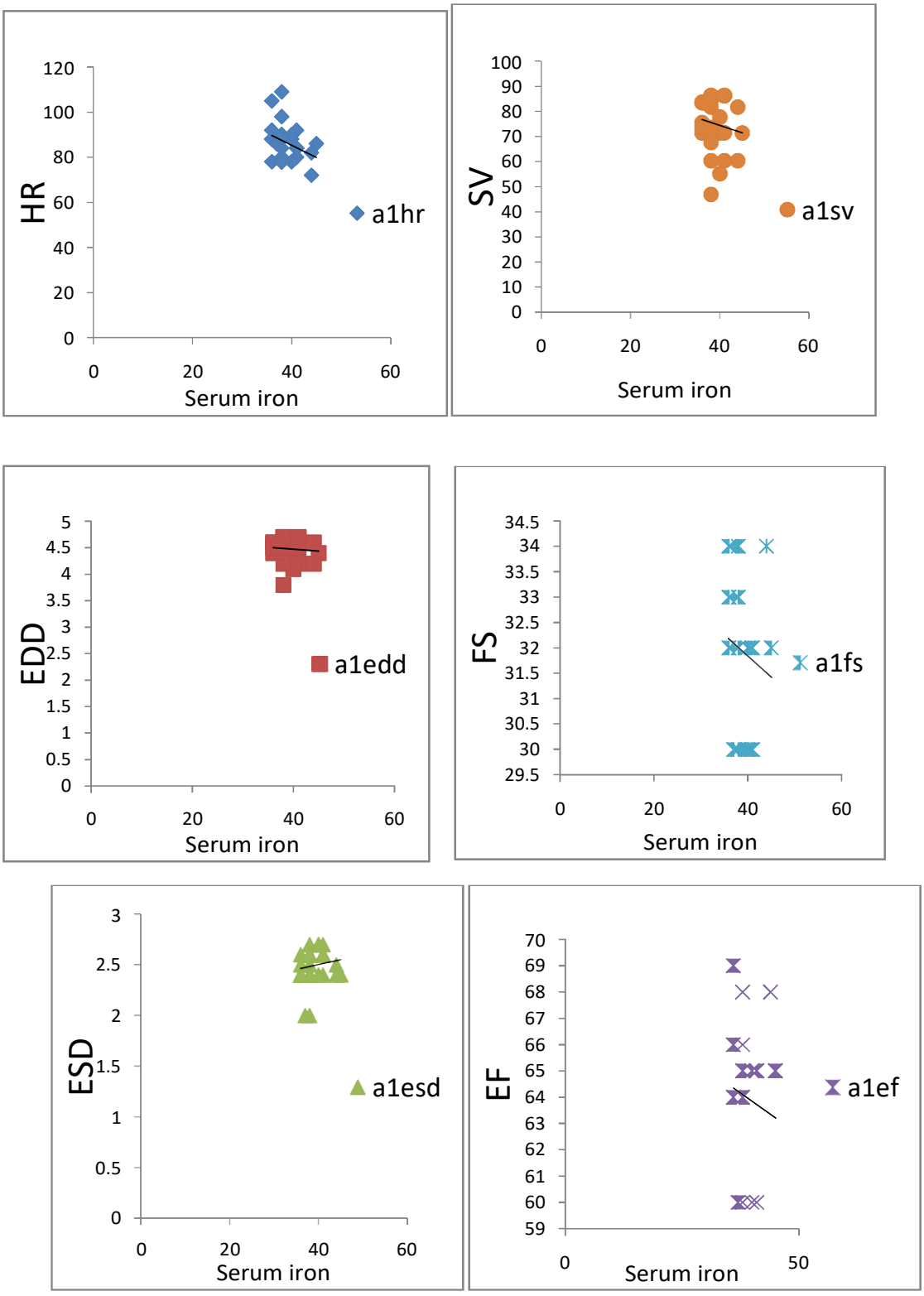


Table 39: correlation between Haemoglobin, serum ferritin & serum iron with echo findings in severely anemic first trimester pregnant women.

Parameters	Haemoglobin		Serum ferritin		Serum iron	
	R	p	r	p	r	P
Heart rate	-0.042	0.8	-0.062	0.7	0.155	0.4
EDD(End Diastolic Diameter)	-0.243	0.2	-0.030	0.8	-0.233	0.2
ESD(End Systolic Diameter)	-0.250	0.2	-0.069	0.7	-0.046	0.8
Ejection fraction (%EF)	-0.094	0.6	-0.098	0.6	-0.324	0.1
Fractional Shortening(FS)	-0.141	0.5	-0.210	0.3	-0.158	0.4
Stroke Volume(SV)	-0.346	0.1	0.040	0.8	0.199	0.3
Cardiac output(COP)	0.163	0.1	0.087	0.6	-0.010	0.9
Correlation is significant at the 0.01 level(2-tailed)(p);r-regression						

Table 39 shows correlation between Haemoglobin, serum ferritin & serum iron with left ventricular functions of severely anemic first trimester pregnant women.

Negative correlation between Hb, serum ferritin,serum iron & HR, SV,EDD,ESD, EF, FS was observed in severely anemic first trimester pregnant women .These observations were statistically not significant. But HR&SV were positively correlated with serum iron. This observation was statistically not significant.

Figure 15:Correlation between HB & Echo findings(HR,SV,EF,FS,ESD,EDD) in severely anemic first trimester pregnant women

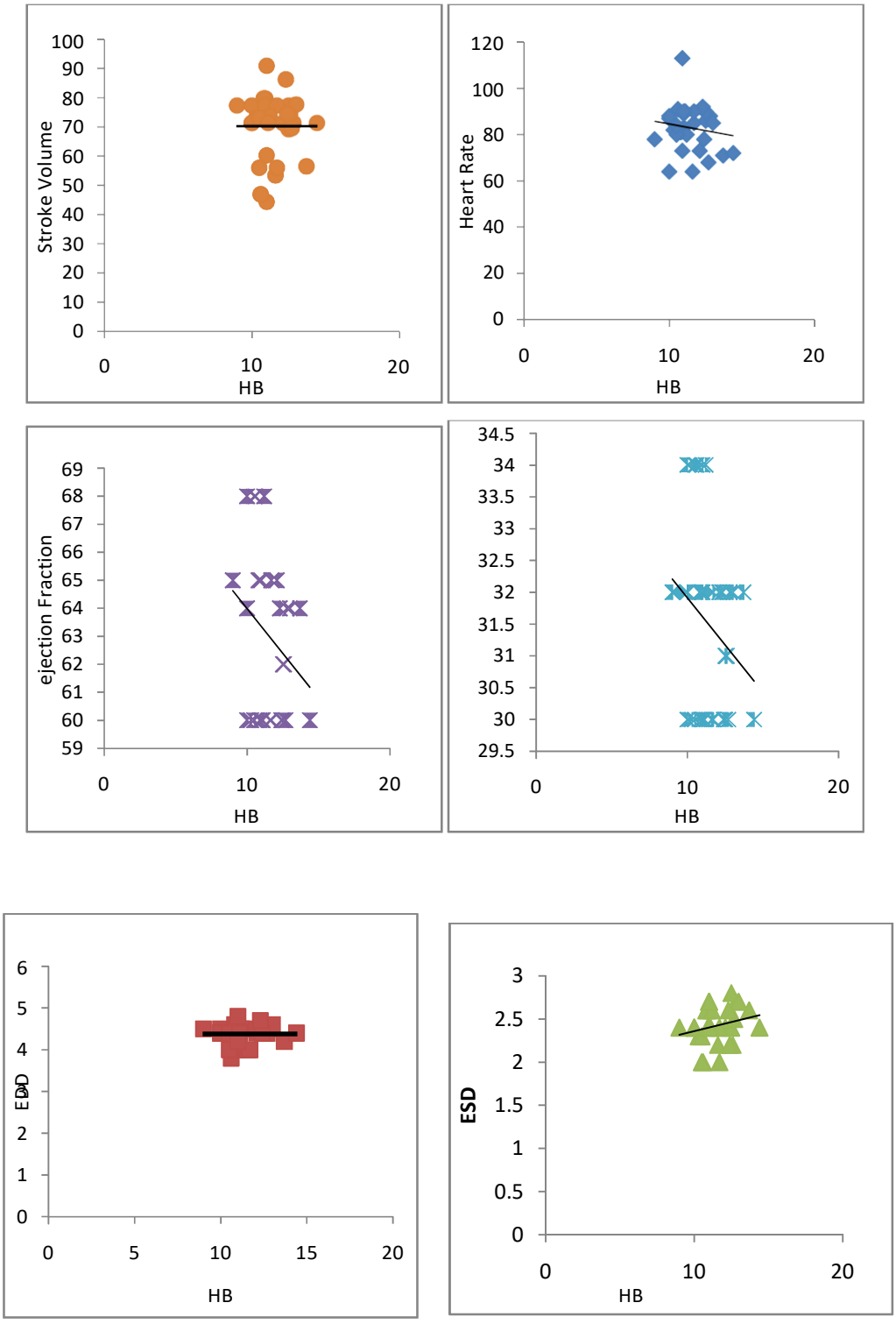


Figure 16:Correlation between serum ferritin & Echo findings(HR,SV,EF,FS,ESD,EDD) in severely anemic first trimester pregnant women

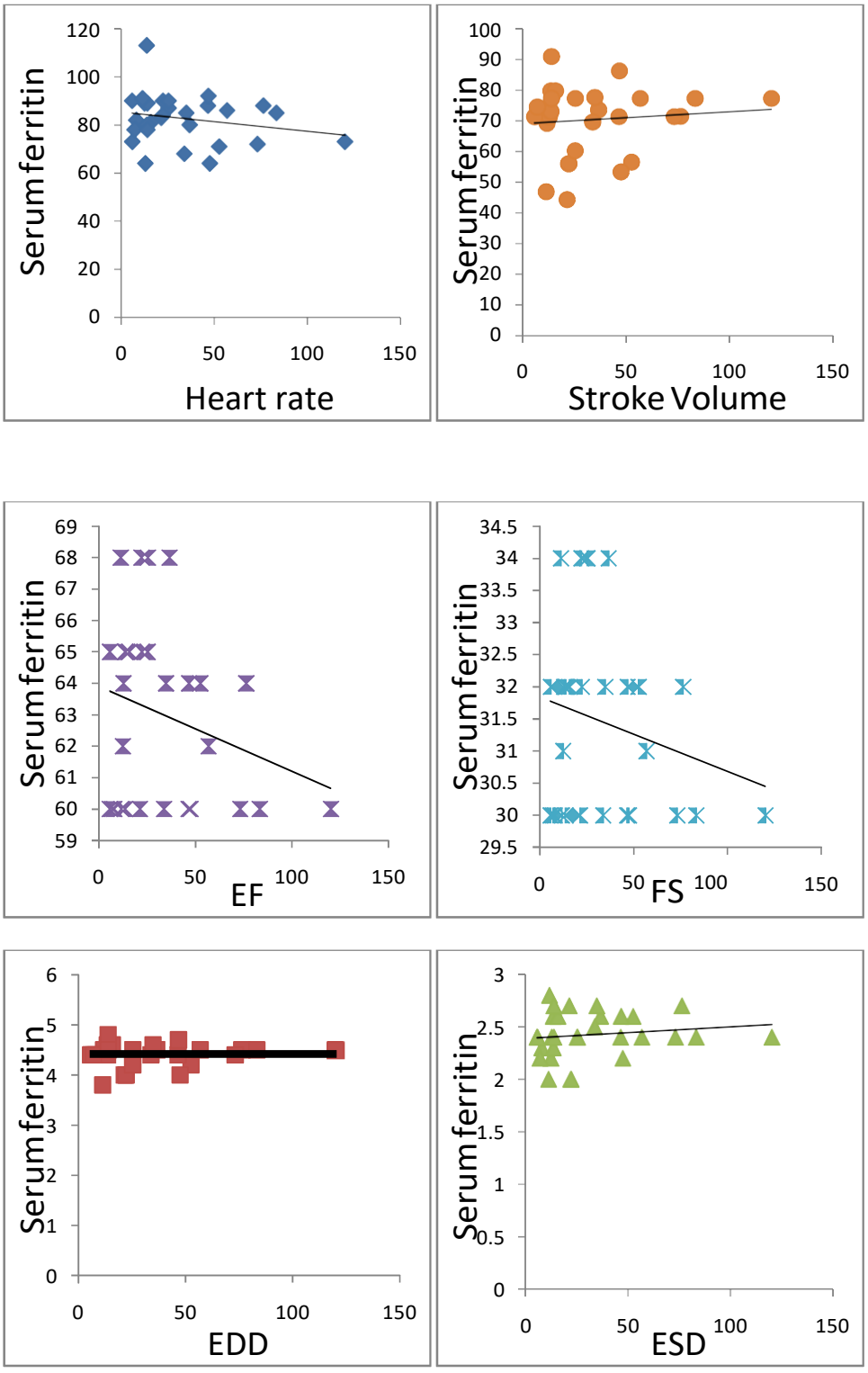


Figure 17: Correlation between serum iron & Echofindings(HR,SV,EF,FS,ESD,EDD) in severely anemic first trimester pregnant women

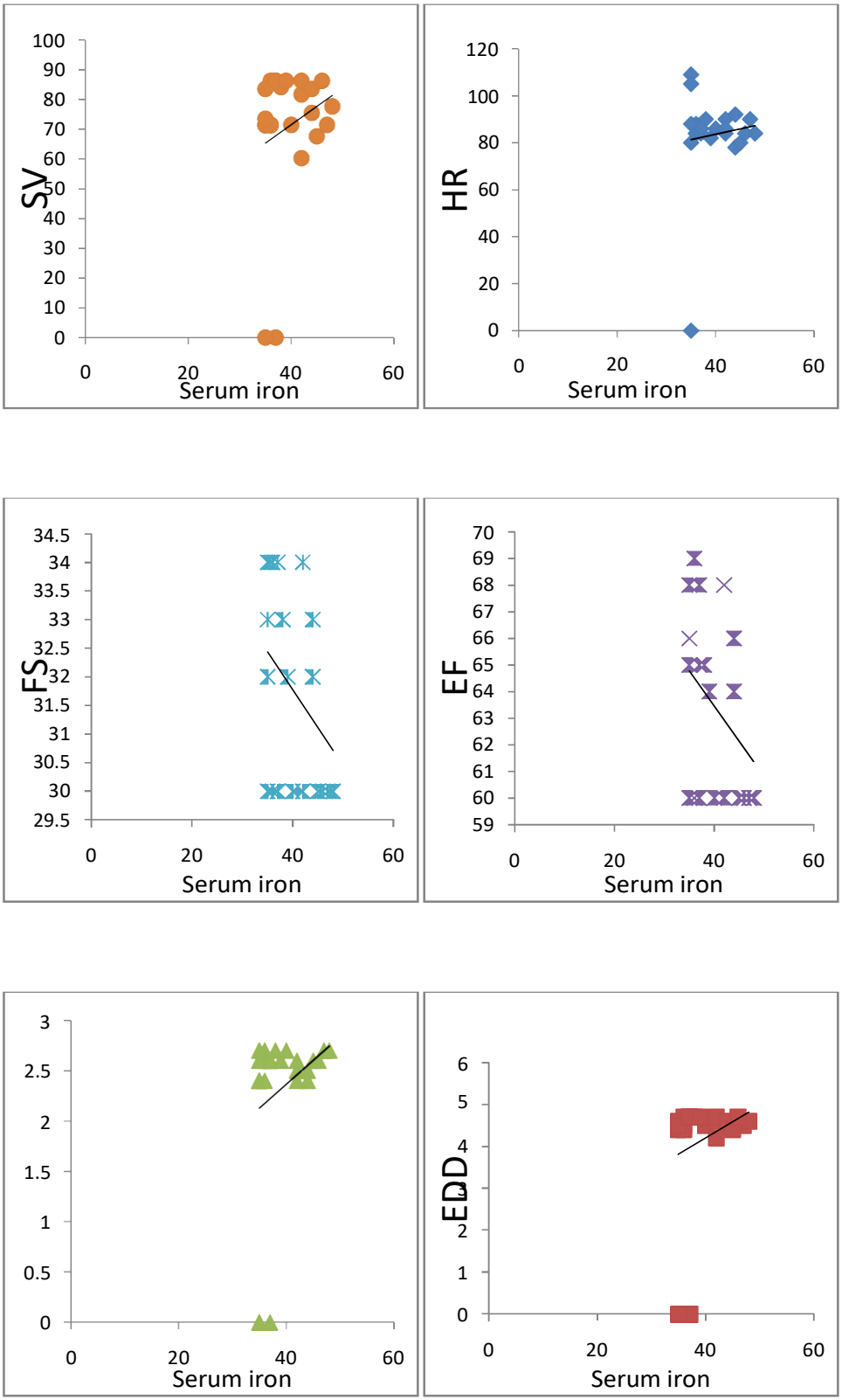


Table 40: Correlation between Haemoglobin, ferritin and serum iron with LV functions of normal second trimester pregnant women

Parameters	Haemoglobin		Serum ferritin		Serum iron	
	R	p	r	p	r	p
Heart rate	0.135	0.5	0.083	0.7	0.217	0.3
EDD(End Diastolic Diameter)	0.102	0.6	0.250	0.8	0.054	0.2
ESD(End Systolic Diameter)	0.322	0.6	0.544	0.2	0.309	0.12
Ejection fraction (%EF)	0.072	0.6	0.119	0.7	0.015	0.2
Fractional Shortening(FS)	0.009	0.8	0.105	0.3	0.113	0.1
Stroke Volume(SV)	0.238	0.7	-0.446	0.6	-0.167	0.3
Cardiac output(COP)	-0.322	0.9	-0.340	0.7	-0.154	0.4
Correlation is significant at the 0.01 level(2-tailed)(p);r-regression						

Table 40 shows correlation between Haemoglobin, serum ferritin & serum iron with left ventricular functions of normal second trimester pregnant women.

Positive correlation between Hb, SF ,SI with HR, SV,EF, FS, EDD,ESD was observed in normal second trimester pregnant women . These observations were statistically not significant.

Table 41: Correlation between Haemoglobin.ferritin and serum iron with LV functions of moderately anemic second trimester pregnant women

Parameters	Haemoglobin		Serum ferritin		Serum iron	
	R	p	r	p	r	P
Heart rate	0.430	025	-0.014	0.9	0.566	0.3
EDD(End Diastolic Diameter)	-0.014	0.6	-0.209	0.2	-0.078	0.6
ESD(End Systolic Diameter)	-0.038	0.7	-0.177	0.3	-0.047	0.8
Ejection fraction (%EF)	-0.184	0.09	0.300	0.2	-0.229	0.5
Fractional Shortening(FS)	-0.085	0.08	-0.285	0.2	-0.229	0.5
Stroke Volume(SV)	-0.003	0.6	0.171	0.3	-0.099	0.6
Cardiac output(COP)	-0.075	0.9	-0.061	0.7	-0.057	0.7
Correlation is significant at the 0.01 level(2-tailed)(p);r-regression						

Table 41 shows correlation between Haemoglobin, serum ferritin & serum iron with left ventricular functions of moderately anemic second trimester pregnant women.
Negative correlation between Hb, serum ferritin, serum iron with EF, FS, ESD,EDD&SV was observed in moderately anemic second trimester pregnant women . This observation was statistically not significant. Ejection fraction & stroke volume were positively correlated with serum ferritin. This observation was statistically not significant.

Figure 18:Correlation between HB & Echo findings(HR,SV,EF,FS,ESD,EDD) in moderately anemic second trimester pregnant women

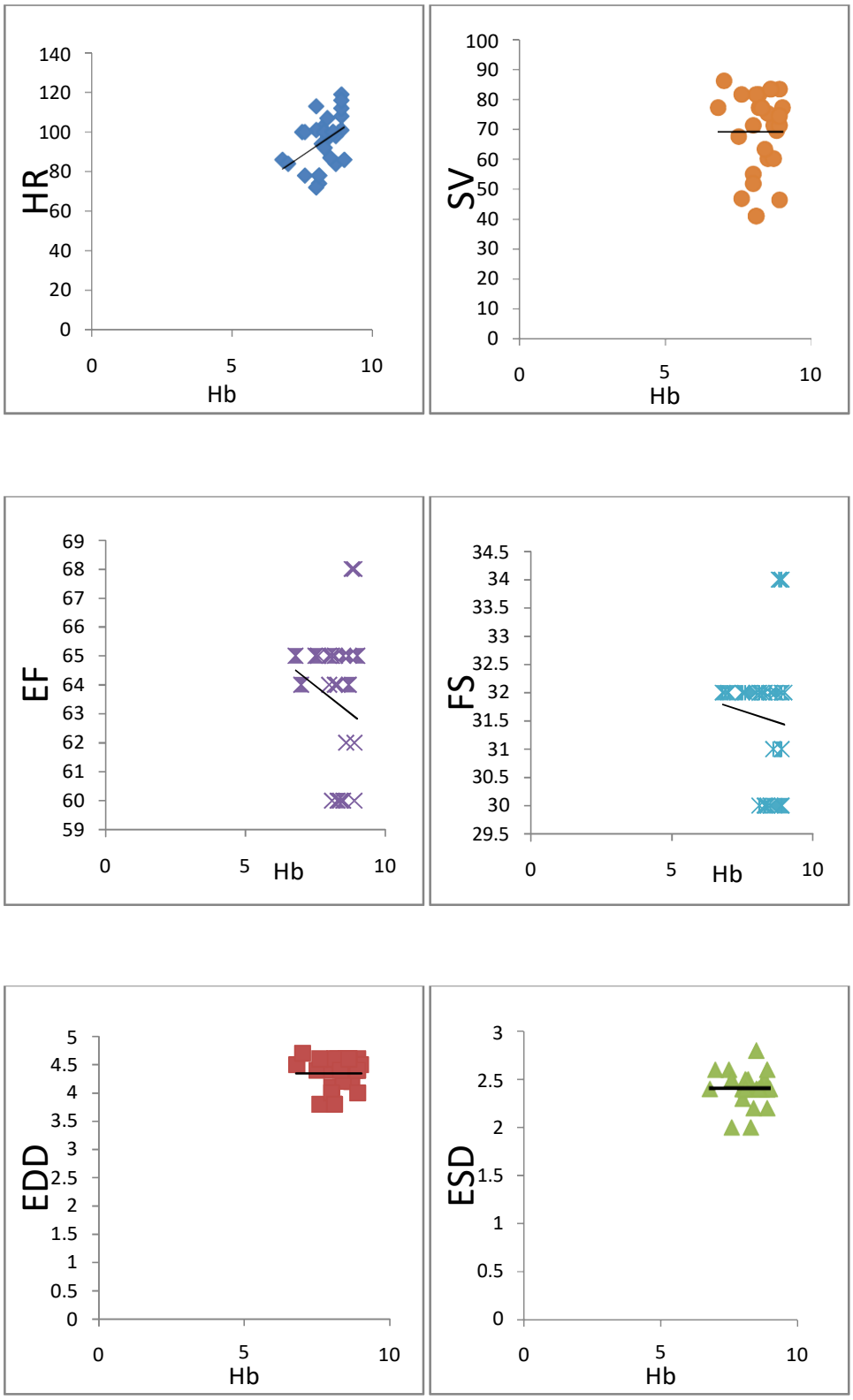


Figure19:Correlation between serum ferritin & Echo findings (HR,SV,EF, FS,ESD,EDD) in moderately(a2) anemic second trimester pregnantwomen

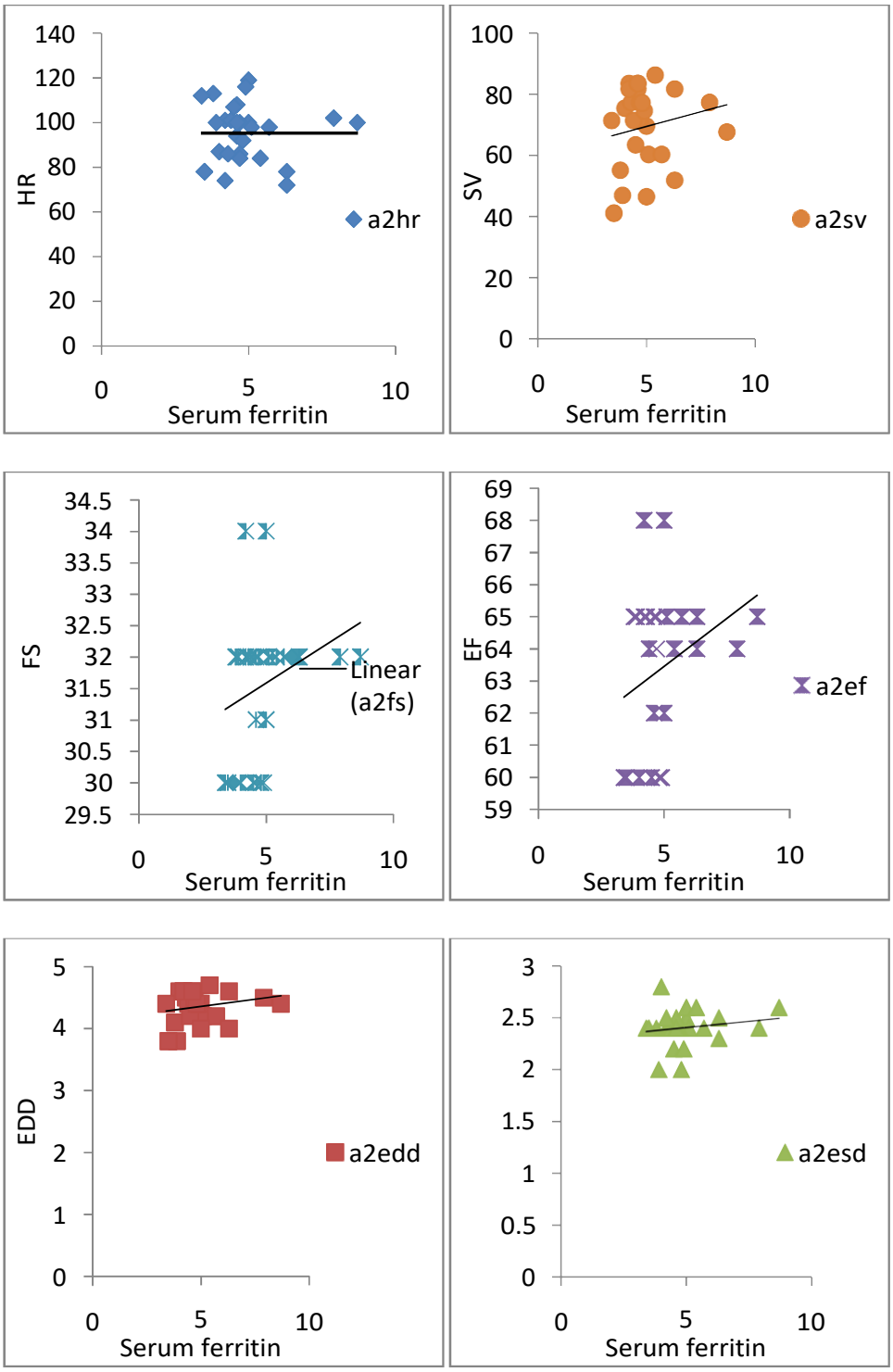


Figure 20: Correlation between serumiron & Echo findings(HR,SV,EF, FS,ESD,EDD) in moderately(a2) anemic second trimester pregnant women

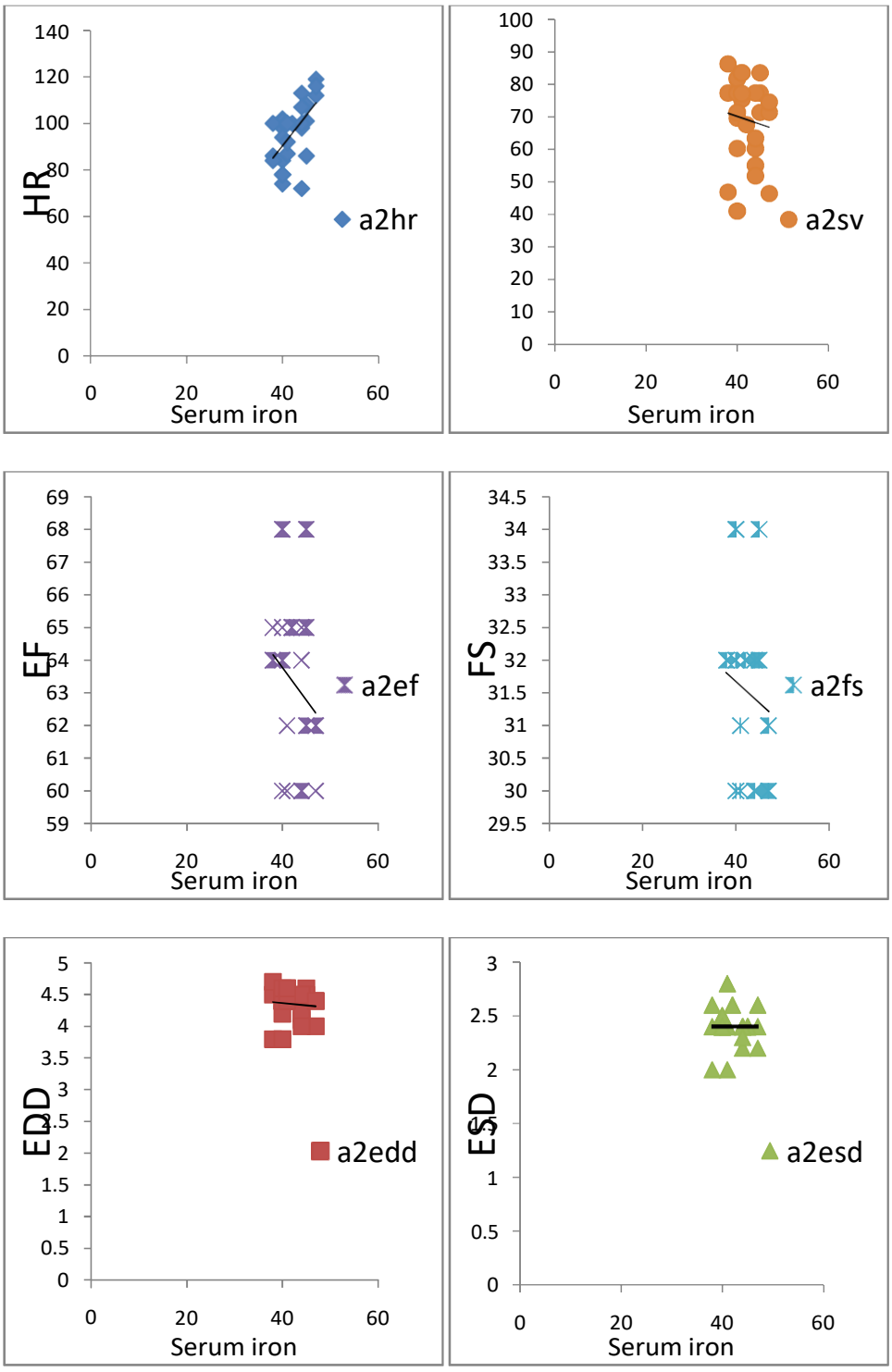


Table 42: Correlation between Haemoglobin.ferritin and serum iron with LV functions of severely anemic second trimester pregnant women

Parameters	Haemoglobin		Serum ferritin		Serum iron	
	R	p	r	P	r	P
Heart rate	-0.223	0.2	0.228	0.2	-0.097	0.6
EDD(End Diastolic Diameter)	-0.008	0.9	-0.067	0.7	-0.006	0.9
ESD(End Systolic Diameter)	-0.052	0.7	-0.071	0.7	-0.117	0.5
Ejection fraction (%EF)	-0.014	0.5	-0.195	0.3	-0.438	0.4
Fractional Shortening(FS)	-0.010	0.9	-0.147	0.4	-0.326	0.07
Stroke Volume(SV)	-0.012	0.9	0.114	0.5	0.026	0.8
Cardiac output(COP)	0.027	0.8	0.166	0.3	-0.005	0.9
Correlation is significant at the 0.01 level(2-tailed)(p);r-regression						

Table 42 shows correlation between Haemoglobin, serum ferritin & serum iron with left ventricular functions of severely anemic second trimester pregnant women.

Negative correlation between Hb, SI with HR, EF, FS, ESD,EDD &SV were observed in severely anemic second trimester pregnant women . Only stroke volume was positively correlated with serum iron. Both observations were statistically not sgnificant.

Negative correlation between serum ferritin & Fractional shortening, Ejection fraction ,EDD,ESD was observed in severely anaemic second trimester pregnant women. HR &SV were positively correlated with serum ferritin. The above observations were statistically not significant.

Figure21:Correlation between HB & Echo findings(HR,SV,EF,FS,ESD,EDD) in severely anemic second trimester pregnant women

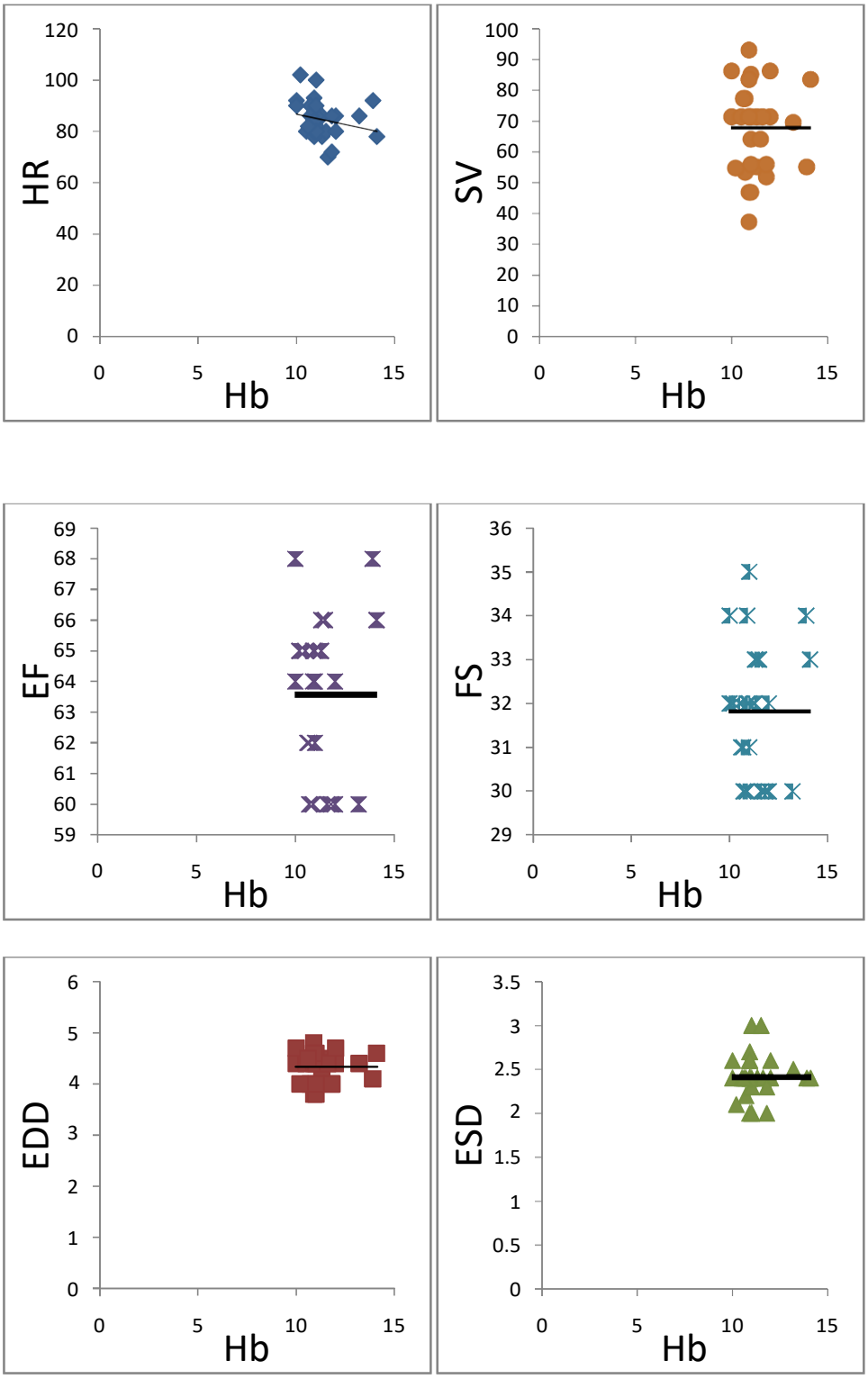


Figure 22:Correlation between serum ferritin & Echo findings(HR,SV,EF, FS,ESD,EDD) in severly anemic second trimester pregnant women

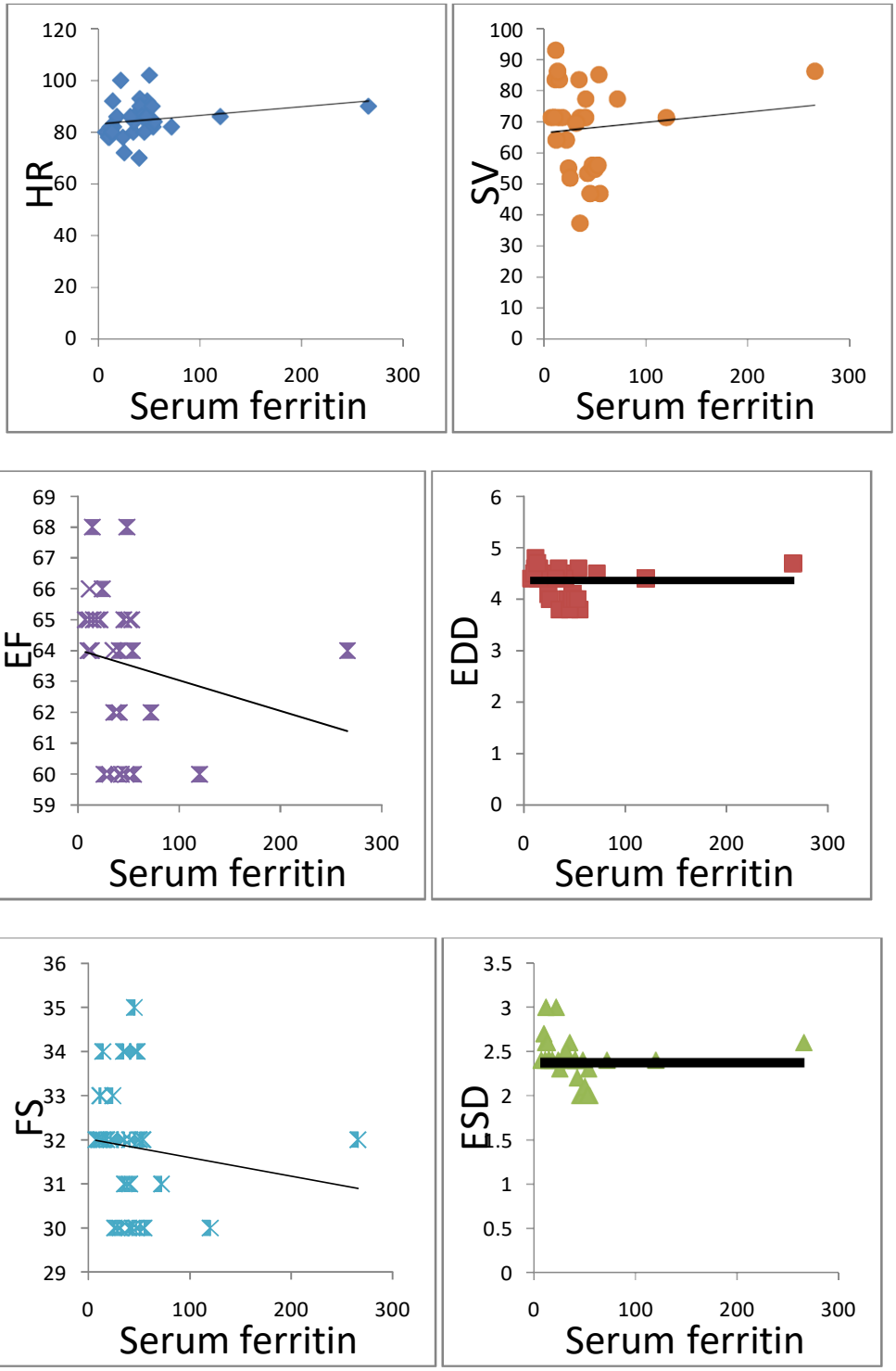
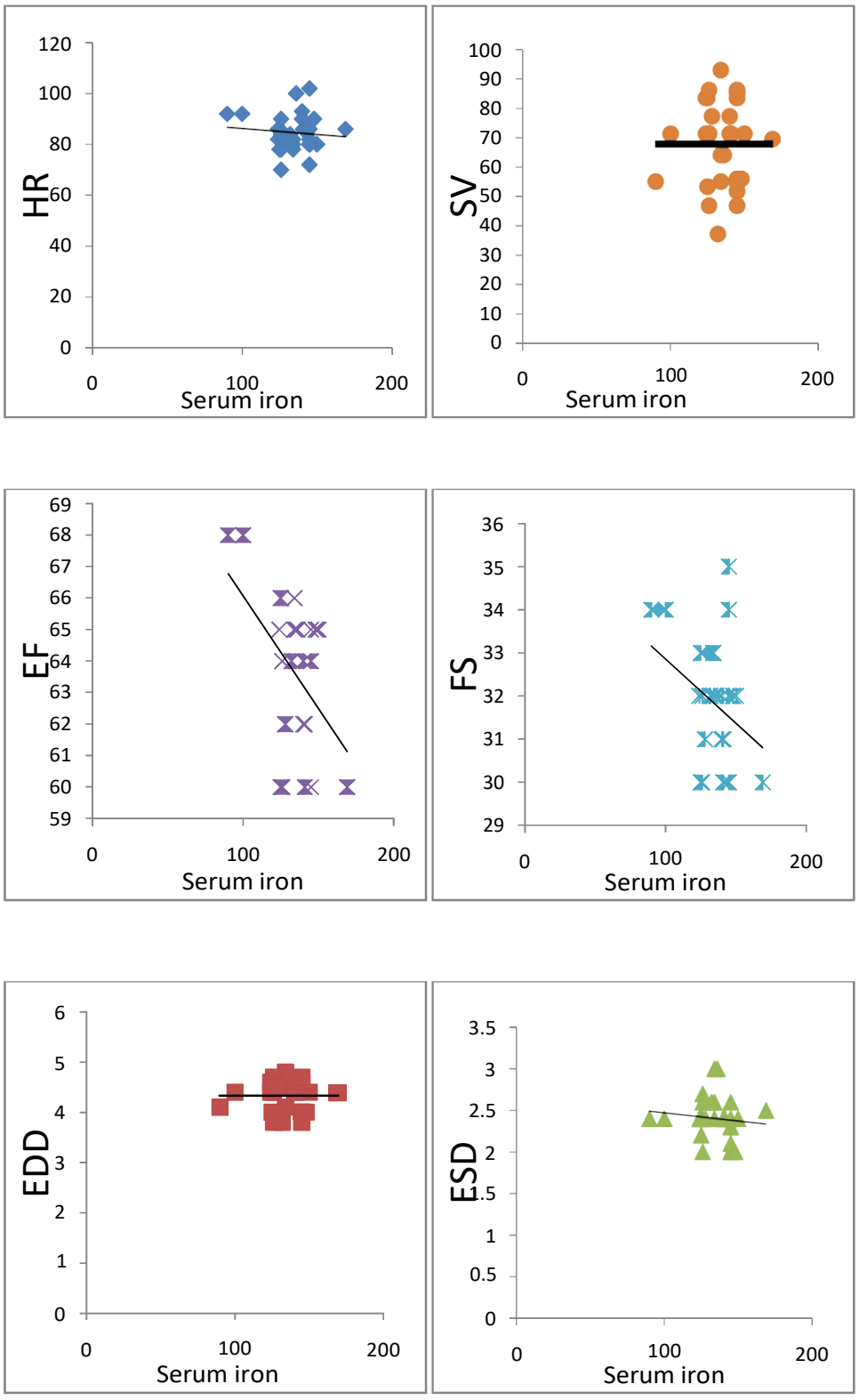


Figure 23:Correlation between serumiron & Echo findings(HR,SV, EF, FS,ESD,EDD) in severely anemic second trimester pregnant women



DISCUSSION

Chapter V: Discussion

5.1. Prevalence of anemia in first & second trimester pregnant women

5.1.1. Discussion**:** In this observational study cardiac function was evaluated in first & second trimesters of pregnant women with iron deficiency anemia.

According to our data the prevalence of anaemia was high among rural population(69%,79% & 66%,77%),illeterates(74%,78% & 89%,81%) , house wives(65%,85% & 70%,80%), agriculture labors with low income group(74%,78% &89%,86%).We also found high rate of anaemia with non nutritional diet(74%,65% &83%,95%) , with more number of children(37%,43% & 34%,47%) & less pregnancy gap (64%,61% & 69%,86%)in both moderate & severely anemic pregnant women in first & second trimester .

5.2. Anthropometric data of first & second trimesters pregnant women

5.2.1. Discussion:

In our study the anthropometric parameters like age,Ht,Wt & BSA were statistically not significant in study & control groups .

A statististically significant change was not observed in weight of control & study groups of pregnant women. Our findings are inagrement with (D C Dutta et al., 2015 & Park's Preventive Medicine 2009, pg 553) anemia with protein energy malnutrition may cause clinically significant weight loss in pregnant women than with only iron deficiency anemia. In second trimester anemic pregnant women non significant increase in weight was observed , may be due to water retention , compensatory changes for hypervolemia (D C Dutta et al.,2015, pg-58).

5.3. Changes in Blood pressure & peripheral resistance in first & second trimesters pregnant women.

5.3.1. Discussion:

In this study statistically significant increase was observed in Systolic blood pressure in first & second trimester (p=0.000, p=0.000)pregnant women indicating that anemia may influence blood pressure. SBP mainly depends on COP & Systemic vascular resistance. Systemic vascular resistance depends on the diameter of the blood vessel, viscosity of the blood. In iron deficiency

anemia blood viscosity decreases. Decreased blood viscosity may be inversely proportional to increased SBP(Ioana Mozos et al., 2015, Guyton et al., 2006 pg-166). In first trimester Diastolic blood pressure was not significantly (p=0.4) decreased. DBP was decreased significantly (p=0.001) in second trimester may be due to decreased vascular resistance (Desai et al., 2004)

Increase in Systolic blood pressure & decrease in diastolic pressure in study groups contributed to decrease in MAP. In present study the MAP was decreased statistically (p=0.03) in first trimester pregnant women may be due to the action of vascular smooth muscle relaxing substance nitric oxide (NO) which acts through cyclic GuanosineMonoPhosphate (cGMP) on blood vessels. Relaxation of vascular smooth muscle cells reduces the vascular resistance, tone of the vessels and decreases MAP. (Weiner CP et al.,1994).

Besides this during pregnancy estrogen and progesterone metabolites enhances Angiotensin II production which in turn stimulate NO production (Magness R et al., 1996).
Not only these increased secretions of ANP by heart in response to atrial distention also contribute to vasodilatation. This is also mediated by cGMP on vascular smooth muscle. Similar observations were found by F Gay Cunningham et al., 2005, Sala C et al.,995.

Total peripheral resistance (TPR) was significantly decreased ($p<0.001$)($p=0.005$) in first & second trimesters of both moderately & severely anemic pregnant women.

Decreased TPR was more in severely iron deficient anaemic pregnant women than normal pregnant women which may be due to reduced vascular resistance as a result of decreased blood viscosity, hypoxia induced vasodilatation. Vasodilatation may be due to hypoxia generated metabolites & endothelial derived relaxing factor. Similar reports were given by(Mabie et al.,1994,Carbillon et al.,2000). Devereux et al.,1986 showed that the decrease in the systemic vascular resistance is the result of a lower resistance circulation in the pregnant uterus.

The other causes for decreased TPR might be hormones (estrogen, prolactin, PGF2, and PGI2) & their effects that take place during pregnancy Athena poppa's et al., 1997. Increased estrogen levels in pregnancy, presence of estrogen receptors in both the vascular smooth muscle and

endothelium & causes vasodilation by potentiating endothelium-dependent (related to acetylcholine) pathways in pregnant women .Williams KJ et al ., 1992 ,Gilligan et al ., 1995.

5.4. Haematological & biochemical findings in both 1st& 2nd trimester pregnant women.

5.4.1 Discussion:

In our study the mean haemoglobin concentration ($p<0.001$) serum iron ($p<0.001$) serum ferritin ($p<0.001$) were significantly decreased study groups when compared to controls as well as within subgroups of first & second trimester pregnant women. Our findings were supported by (J.B.Sharma et al., 2010). The reason for above findings in iron deficiency anaemia may be due to decrease in RBC count, fall of blood viscosity. This decreases resistance to blood flow in the blood vessels. So greater amount of blood passes to the tissues and returns to the heart. Similar reports were given by F Gay Cunningham et al., 2005.

Decreased HB %, SF &SI may cause hemodilution and the mobilization of iron from stores to meet the expanded demands of pregnancy. In iron deficiency hemodilution occurs may be due to reduced hemoglobin concentration, serum iron and ferritin concentrations and iron binding capacity increases which reflects an expansion in the concentration of transferrin in plasma(Fenton v et al.m 1977,de Leenu et al.,1966). During 12 and 25 wk gestation of iron deficient pregnant women, serum ferritin falls markedly due to the iron utilization for expansion of the maternal red blood cell mass(Camaschella C. et al., 2005,Svanbag et al . ,1975).

Michele van vraken et al., 2010 found that serum iron, serum ferritin , trasferrin saturation levels & TIBC are useful in diagnosing iron deficiency anemia. TIBC is the ability of unsaturated trasferrin to bind to iron. This measure is usaually increased in IDA. Ferritin is a complex of iron & the binding protein apoferritin. Ferritin reflects true iron stores Waterbury L et al., 2003.

5.5. Blood indices values in 1st& 2nd trimester pregnant women.

5.5.1. Discussion:

From the result presented in Table 23 & 24 Blood indices values in first trimester pregnant women & Blood indices values in second trimester pregnant women significant decrease was found in PCV of the 2nd& 3rd groups when compared to the 1st group of first & second trimester pregnant women. The decrease in PCV may be due to the increase in plasma volume during

pregnancy which causes hemodilution & hormonal changes during pregnancy. This was supported by Geetanjali Purohit et al., 2015, Rigvardhan et al., 2015. The other blood indices like Mean carpuscular hemoglobin (MCH) and mean carpuscular hemoglobin concentration (MCHC) showed significant decrease ($p<0.001$,$p<0.001$) in all groups of first and second trimesters. It is observed that when hemoglobin concentration reduces in association with a mean corpuscular volume may indicates coexistent of iron deficiency(Geetanjali Purohit et al., 2015). But Crocker et al., 2000 showed that MCV does not change significantly during pregnancy, small increase in MCV was observed in pregnant women. He explained the reason for increased MCV was that, RBC production increased to meet the demands of pregnancy.

5.6. Echocardiographic parameters in both 1st& 2nd trimester pregnant women.

5.6.1. Discussion:

According to the observations of comparison of echo parameters in first and second trimester preg.women in Table 25 & Table 27, we found that there was statistically significant increase in End Systolic Diameter (ESD)($p=0.006$ & $p<0.001$) End Diastolic Diameter(EDD)($p=0.002$&0.01) in severely anemic pregnant women of first & second trimesters suggesting that there was an increased stroke volume. Statistically significant increase was not observed in ESD & EDD between control and study groups(group II) of moderately anemic pregnant women suggesting that adaptation to the changed conditions of hemodynamic in this group. The reason for increased ESD & EDD may due to decreased afterload. Similar to the findings observed by (Mabie et al.,1994). Previous studies have reported similar left ventricular structural alterations in normal pregnancy Katz et al., Duvekot JJ et al., 1993.

The increase in heart chamber diameters (EDD,ESD) was due to structural remodelling as a compensatory mechanism for the volume-overload state (Mesa et al., 1999,Duvekot et al., 1994).

Left ventricular contractility was assessed with the use of percentage ejection fraction and percentage fractional shortening. Present study showed a significant decrease in left ventricular contractility functions like percentage ejection fraction ($p=0.01$,$p=0.03$)and a significant decrease in percentage fractional shortening ($p=0.05$) during first & second trimesters of both

severely anemic groups. We found a statistically significant increase in %EF(p<0.001,) &%FS (p=0.002,p=0.001) in group II .

Mone et al., also observed increase in %EF &%FS in normal pregnancy & this increase was associated with reduction in afterload of left ventricle. Robson et al., & Rubler et al., shown that an increase in %EF in pregnancy was due to increase in heart rate. However, Savu et al., 2012,Clapp JF et al., did not find any significant change in left ventricular %EF, %FS in pregnancy could be due to differences in systemic vascular resistance.

In mild to moderate iron deficiency anemia increase in LV contractility can be best explained by Franks Starlings law which states that initial length of cardiac muscle fiber is directly related to the end diastolic filling of the ventricle within the physiological limits. These findings were supported by D.V.Thakker et al.,.In this study based on above observations increase in %EF,%FS% could be due to decreased after load which may increase venous return(preload) & left ventricular filling pressure leading to increase in left ventricular end diastolic volume .

In current study a significant decrease in %EF & %FS in severe group was observed could be due to increase in preload & heart rate. D.V.Thakker et al.,

As the volume of blood increases in the ventricle the actin & myosin filaments are pulled apart far enough so the strength of each cardiac fiber contraction becomes less. Guyton and Jhon E.Hall suggesting that volume over load during pregnancy decreases left ventricular contractility functions.

In this study we found a statistically significant increase in stroke volume study groups of first trimester pregnant women when compared to control group may be due to enhanced left ventricular contractility. Increased left ventricular contractility was due to increased concentration of catecholamines, noncatecholamines, inotropic factors & increased sympathetic activity Stuart Campbell et al., 2000 & Rosenthal Ds et al., 1992. In second trimester non significant increase in SV between control & study groups was observed.

In current study we found an increase in Cardiac output (p=<0.001)during pregnancy in both moderately & severely iron deficiency anemia may be due to increase in heart rate &

stroke volume because of increased circulating volume, reduced systemic vascular resistance . But in second trimester pregnant women increased cardiac output may be as a result of tachycardia.The echocardiographic study of the heart ,conducted by a number of authors Desai et al, savu et al., 2012 is a witness to the fact that increase COP occurs in healthy pregnant women in response to maintain healthy environment for mother & fetus .

Our findings were supported by (D.V.Thakker et al., Pereira AA et al.,2003) found that to compensate anemia cardiac output increases in order to maintain adequate oxygen supply. In moderately anemic group the increase was within physiological limit but clinically significant increase was observed in severly anemic group.(Fabien Metivier et al., 2000Beard JL et al.,1990,Muller R etal., 1991).

In study groups of first and second trimester as a result of decreased afterload (decreased mean arterial pressure), which is balanced by the increasing ventricular size and allows an increase in stroke volume and cardiac output.

In current study we found that in moderately anemic pregnant women increased ESD,EDD,increased EF% with decreased serum ferritin & serum iron were compensatory mechanisms may be due to anemia.

In present study based on the increased ESD,EDD decreased EF% with decreased serum ferritin & serum iron in severely anemic pregnant women, compensatory restructuring of hemodynamics is observed. In severe anemia due to reduction in the viscosity of blood & arterial dilatation vascular resistance decreases which leads to acceleration of blood flow. Arteriolar dilatation is due to increase in the diameter of arterioles but also involvement of new vessels & formation of collaterals. As a result compensatory hyperplasia develops & may lead to increase of blood flow to the heart and this in turn leads rise in the volume of blood in the left ventricle, i.e. its volume overload occurs.

An alteration in LV wall stress i.e., blood flow or volume overload is the signal for LVH. Prolonged volume overload causes dilatation of the cavity & increase in wall thickness (eccentric hypertrophy) of left ventricle. Continuous effect of hypoxia on the myocardium during severe anemia in pregnant women may lead to disruption of metabolism of heart muscles and developmentof myocardiodystrophy. These findings were supported by Grossman W etal 1980.

These changes in overall hemodynamic function, allowing the cardiovascular system to adjust to the physiological demands of the fetus while maintaining maternal cardiovascular integrity. Similar reports were given by Stuart Campbell et al.,

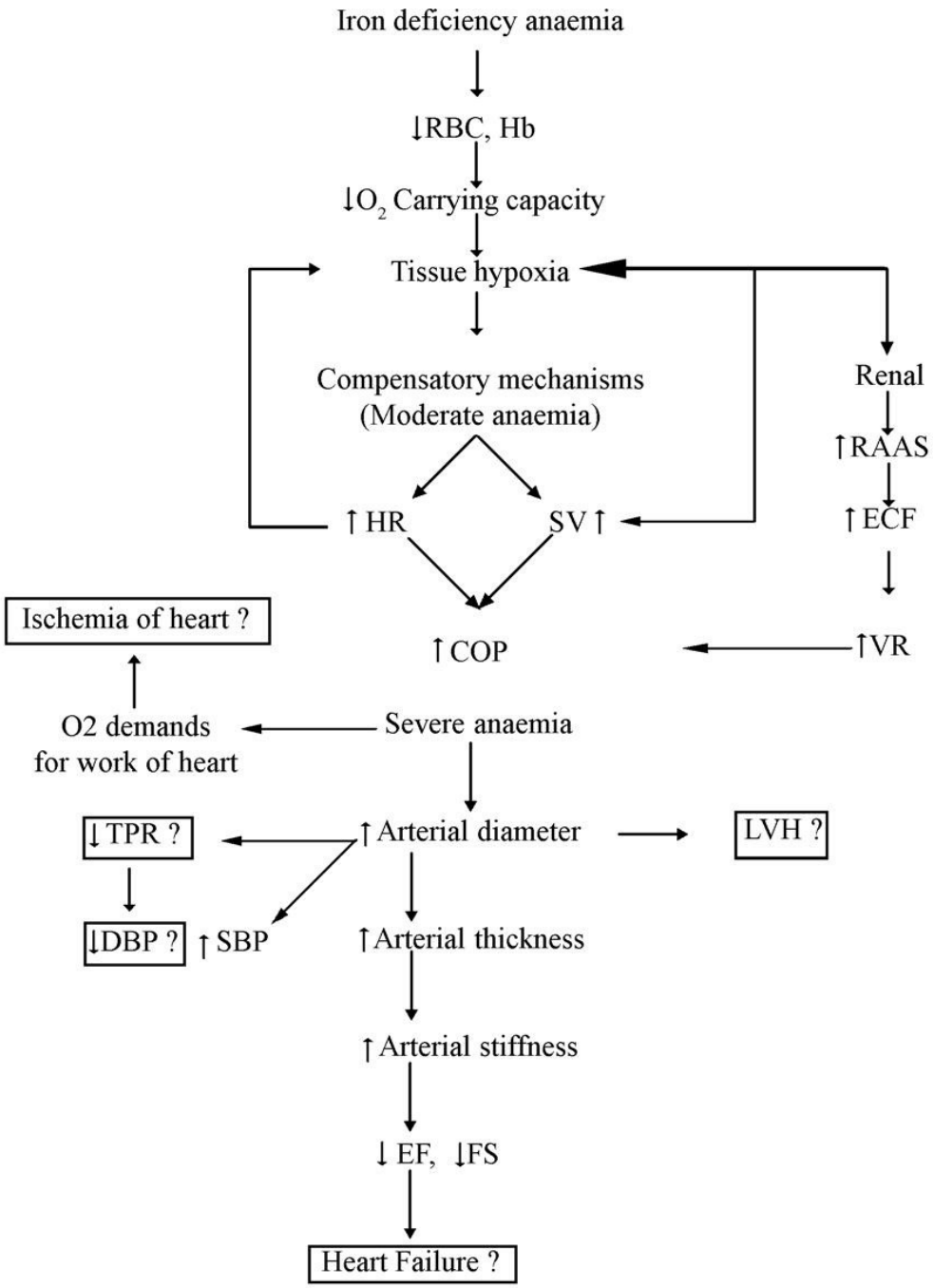


Figure 24: Possible mechanism of degrees of iron deficiency anemia & its effects on cardio vascular function of Heart

5.7. Serum electrolyte findings in first & second trimesters of pregnant women.

5.7.1.Discussion:

In this study Table29, Table31 shows Serum electrolyte values in first trimester & second trimester pregnant women. Serum electrolytes like sodium , potassium & chloride levels were decreased statistically in first trimester pregnant women.The serum sodium & chloride level were increased in 2nd group(moderately anemic) & then decreased in severely anaemic pregnant women of second trimesters.This observation was supported with the findings of other workers (Dutta D.C. et al ., 2015, Kathleen et al ., 2011) & may be due to retension of water & volume overload by the increased activity of progesterone, rennin –angiotensin –aldosteron system.In current study serum sodium levels also decreases(p=0.003,p=0.01) in all groups of first & second trimester pregnant women may be due to polyuria to maintain osmolality. The serum level of potassium (p=0.001,p=0.01) falls during pregnant may be due to persistent vomiting.

In the present study, there was significant decrease in potassium levels in iron deficient anemic pregnant women of both first & second trimesters may inhibit the sodium pump of arterial & arteriolar vascular smooth muscle cells and decreasing the potassium concentration in the intracellular fluid (Blaustein MP et al 2006).

5.8. Electrocardiogram values in pregnant women.

5.8.1. Discussion:

The electrocardiogram during normal pregnancy may show huge variations. Low serum ferritin and haemoglobin levels show changed electrical properties of the myocardium which might be the cause for variations in ECG. ECG recordings show changes with iron deficiency anemia in pregnancy.

The ECG changes observed were significant increase in QTc interval (p=0.01,p=0.001) . QTc interval in ECG reflects the time taken for depolarization and repolarization in the ventricular myocardium. Sunitha M et al., Ozmen N et al., Carruth JE et al., Oram S et al., BN Nandini et al., in their study found prolongation in QTc interval during normal pregnancy. In our study an increase in QTc interval may be due to tachycardia and complex consequence with changes in

regulatory mechanisms during pregnancy. In current study, prolongation of QTc interval may be due to low serum ferritin, serum iron.Because prolongation of QTc predominately depends on K+ rectifier current. It is possible that low levels of ferritin and serum iron might affect ferritin dependent K+ current, both the inward and the outward rectifier current and which may affect the QTc interval (Kuryshev YA et al., 1999 , Aerssens J et al.,).

The other ECG changes noted in our study was LVH, indicating cardiac enlargement in severely anemic group of both trimesters. It was accepted that LV hypertrophy and cardiac enlargement in anemic pregnant women was due to increased work load of heart, but now it has been allocated to inadequate O2 supply to the myocardium Roy SB et al,.

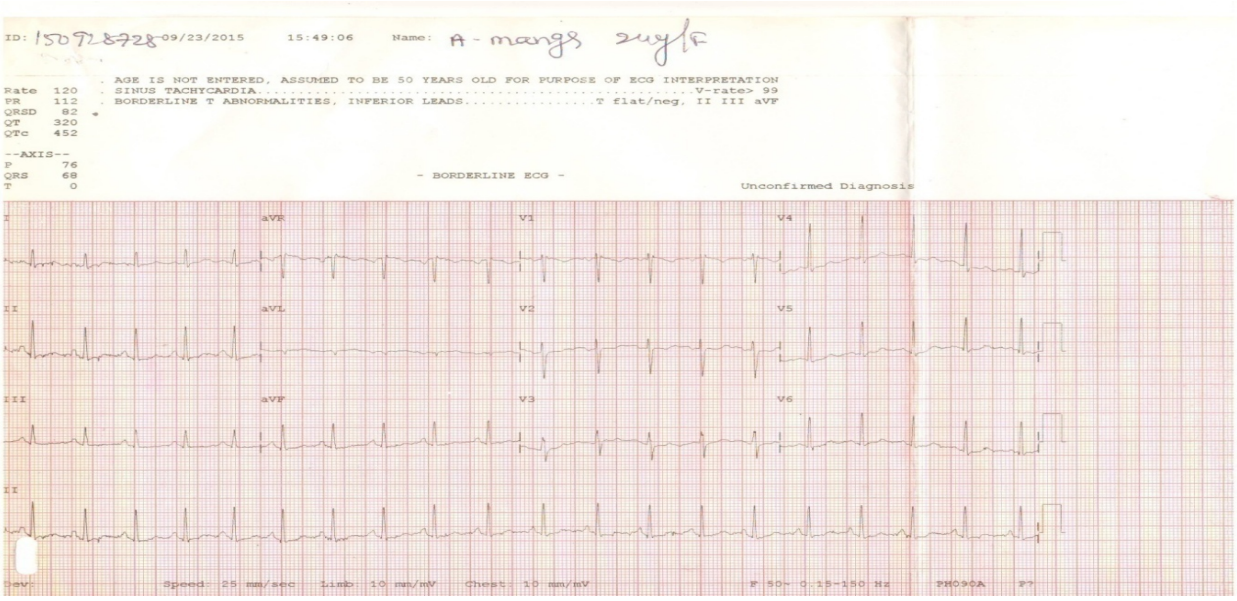
In present study due to iron deficiency anemia both HR and stroke volume were increased, inorder to increase cop, this in turn improves O2 delivery. To accommodate this greater output, LV chamber size, both EDD & ESD were increased (Hayashi R,, G georgieva et al., 1997).

In the current study tachycardia was observed in study group I & II pregnant women, may be due to hypoxia induced stimulation of chemo-receptors & increased sympathetic activity which is due to physiological adjustments in circulation during anemia Rosenthal et al .,1992.

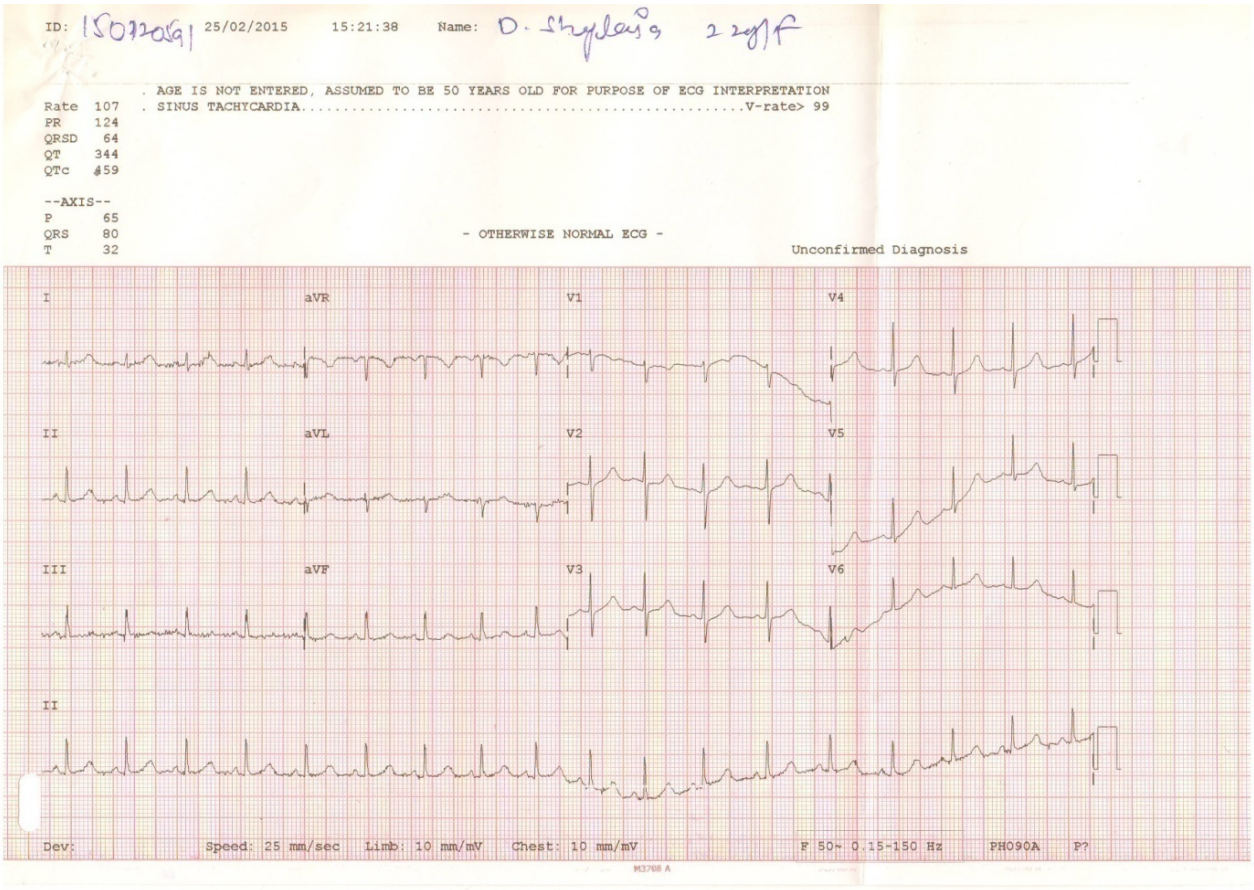
In our ECG findings, QRSD was significantly decreased ($p=0.003$,$p=0.01$) in first & second trimesters of severely anemic pregnant women, may be due to altered circulatory hemodynamics during pregnancy. Changes in QRSD reports were given by Lechmanova et al., during normal pregnancy.

In addition the other ECG changes observed in our study were T wave abnormalities like flattening and inversion in lead II, III, aVF and also in chest leads V2-V4 were more in group III when compared to group I of both first & second trimesters pregnant women. T wave abnormalities(inversion & in some cases flattening) may be disturbances in myocardium due to diminution of oxygen carrying power of the blood which in turn leads to temporary ischemia. Ischemia is represented by T wave inversion .Similar report was given by Zamani M et al., J Misra, Szekely P, in normal pregnant women.

But some studies showed that T flattening is due to enlargement of QRS complex [16]. According to Pereira AA. T wave flattening is due to decreased QRS amplitude and minor degrees of atrioventricular disturbances. Hyponatremea also decreases QRS amplitude (Guyton Jhon E.Hall et al .,)



Abnormal ECG (T wave abnormalities & tachycardia)



Chapter V: Discussion

In current study abnormal pattern of ST segment (depression, in some cases elevation) inferior leads, lead II III aVF may be due to electrolyte imbalance such as hypokalaemia.

In this study serum electrolytes potassium showed statistically significant decrease (p=<0.001, p=0.01) which is also responsible for flattened and inverted T waves in ECG.

It has been suggested that transient ST segment depression is associated withanxiety which may be a provoking stimulus and that can be attributed to an endogenous hypersensitivity. One of the mechanisms in which adrenaline induces hypersensitivity is increasing oxygen demand by the increased muscular action and coronary dilation. Anxiety might be accompanied by an increase in circulating hormonal agents which would directly affect myocardial electrical activity (Georgia et al .,)

We hypothesized that the reduction of haematological & biochemical parameters like Hb, serum ferritin, serum iron in first & second trimesters were negatively & insignificantly correlated with end diastolic volume, end systolic volume, ejection fraction & fractional shortening indicating hyperkinetic heart in pregnancy & small sample size.

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SUMMARY AND CONCLUSION

Chapter VI: Summary and Conclusion

6.1. LIMITATIONS OF THE STUDY

1. In this study only first & second trimester pregnant women were included which is major limitation of the study. Third trimester pregnant women were not included because pregnant women in third trimester without anemic treatment coming for the first visit to hospital is rare & were not available.

2. In present cross sectional study the sample size is small due to non co-operation of subjects as the source of study is rural based & more expensive in financial aspects. So Longitudinal study is required with more sample size.

3. The present study sample size is sufficient for an experimental design, but to infer the clinical outcomes by RCT large sample size is required.

4. This study is confined to know the effect of IDA on cardio vascular function. Follow up was not done in this study because once anemia was diagnosed usually they undergo treatment for anemia in first trimester pregnant women.

6.2. SUMMARY AND CONCLUSION

2.1. The purpose of study is to evaluate the effect of moderate & severe degree iron deficiency anemia on cardiovascular function in first & second trimesters of pregnancy. Also to correlate the relationship between Echo findings [EDD,ESD,%EF ,%FS & SV]& haematological [serum ferritin, serum iron], biochemical parameters in both anemic pregnant and normal pregnant women.

2.2. We hypothesized that the reduction of haematological parameters in first & second trimesters negatively correlates with left ventricular cardiac function. As serum ferritin & serum iron levels are decreased there is an increase in End diastolic volume, End systolic volume and decrease in ejection fraction and fractional shortening suggesting hyperkinetic state of heart.

2.3. The objectives of the study were evaluated by echocardiography, electrocardiography, haematological and biochemical parameters. The participants were pregnant women of first and second trimesters aged between 20-30 years with 10-14, 20-24 weeks of gestation respectively. The selected participants were both normal pregnant, moderate and severely iron deficiency anemic pregnant women of both trimesters. Pregnant women with diabetes, maternal cardiovascular disease, and preeclampsia were excluded from the study.

2.4. Socio economic information like age, marital status, educational status, residence, occupation and other relevant risk factors like number of children, parasitic infections, gestational period, pregnancy gap and iron supplement of the study participants were collected by using structured & pre tested questionnaire.

2.5. In the present study a total of 160 pregnant women were selected in first and second trimester. In that we found Group I(n= 30), Group II(n=27) and group III(n=23) in first trimester &group I (n=30), group II(n=29) & group III(n=21) pregnant women in second trimester. All the selected subjects were examined for anthropometric and physiological parameters.

2.6. According to our findings the prevalence of iron deficiency anemia was more in rural, illiterates, low income, with non nutritional diet, and also with more number of children, less pregnancy gap in both moderate & severely anemic pregnant women in both first & second trimester. Among occupation category, high prevalence of anaemia was found in house wives, agricultural labourers as compared with employees.

2.7. The following haematological & biochemical parameters were tested for diagnosing iron deficiency anemia in pregnant women Hematological parameters include Complete blood picture (CBP). Biochemical parameters include Estimation of serum ferritin, Estimation of serum iron, Estimation of serum, Total iron binding capacity and Transferrin saturation percentage & Estimation of Serum electrolytes. Myocardial performance in pregnant women was evaluated by echocardiography (2D echo) and electrocardiography (ECG).

2.8. We believe that this may be the first observational study that assessed the effect of moderate and severe degree iron deficiency anemia on cardiac function in first & second trimester pregnant women. It is a rare work done in rural areas with 58.36 % of anaemia in pregnant ladies in Telangana, India.

2.9. We found a statistically significant increase in Systolic blood pressure from 106.6 to 112.3, 120.3 was observed in first trimester (p=0.000) pregnant women. Similarly in second trimester SBP increasesd from 103.6 to 105,122 (p=0.009) and DBP decreased to 70.69 & 64.76(p=0.001). These findings suggest that anemia influence blood pressure.

2.10. A statistically significant decrease in total peripheral resistance [TPR] (p=0.001) from 2.53±0.77 to 2.01±0.73 & 1.71±0.41 was observedin moderate & severely iron deficient first trimester pregnant women. Similarly statistically significant decrease in TPR (p=0.0005) from 2.55±0.81 to 2.13±0.53 & 1.97±0.46 was observed in moderate & severely anemic second trimester pregnant women. Reduced vascular resistance as a result of decreased blood viscosity contributed for decreased TPR in severely iron deficient anemic pregnant women than in normal pregnant women.

2.11. Echocardiographic examination carried out showed, that in severe iron deficiency anemic first trimester pregnant women statistically significant increase in ESD from 2.42 to 2.60 (p=0.006), EDD from 4.38 to 4.58(p=0.002) was observed. In second trimester a statistically significant increase in ESD from2.40 to 2.60(p=0.001), from 4.33 to 4.54(p=0.01) was observed. Statistically significant decrease in EF from63.03 to 61.21 (p=0.001) in first trimester, 63.60 to 61.71(p=0.001) in second trimester, & FS from 31.46 to 30.73 (p=0.001) in first trimester, from 31.26 to 30.61 (p=0.001) in second trimester was observed.

The above findings showed, decrease in contractile and pumping function of heart suggesting that these changes lead to volume overload of the left ventricle. This was the novel finding of the study.

2.12. In moderately iron deficient anemic pregnant women increased EDD, ESD were statistically not significant & EF was statistically significantly increased (p=0.001) with decreased Hb%, serum ferritin & serum iron suggesting that these changes can be recovered with correction of anemia.

2.13. A statistically significant decrease was observed in **mean haemoglobin** concentration from 11.49 to 8.09 & 4.89(p=0.0001),**serum ferritin** from 32.39 ±27.50 to 5.33±1.37 & 4.10±0.52 (p=0.0001) &**serum iron** from 128.30 to 39.07 & 39.81 (p=0.0001), and **%trasferrinsaturation** from 35.43 to 6.93 & 7.15 (p=0.0001) in first trimester pregnant women. Statistically significant increase in Total iron binding capacity was observed from 364.50±47.86 to 564.81±15.67 & 560.43±11.38 in first trimester than in control group.

Mean haemoglobin concentration (p=0.0001) serum iron(p=0.0001) and serum ferritin (p=0.0001) were significantly decreased in second trimester pregnant women . These findings confirm iron deficiency anemia & also differentiate other types of anemia.Serum ferritin is stable, unaffected by recent iron intake, reflects iron stores accurately, **and is the first abnormal laboratory test in iron deficiency.**Low Serum ferritin levels indicate iron deficiency.

2.14. A statistically significant decrease in serum electrolyte values like sodium from 137.46±2.5 to 136.92±2.0 & 134.82±1.9 (p=0.003), potassium from 3.93±0.4 to 3.64±0.2 & 3.41±0.2 (p=0.001) were observed in moderate & severe groups of first trimester pregnant women. In second trimester pregnant women a statistically significant decrease in serum sodium from 137.7±2.8 to 135.82±1.9 (p=0.01), potassium from 3.82±0.4 to 3.41±0.2 (p=0.01), were observed.

2.15. Electrocardiogram values in first trimester pregnant women showed statistically significant decrease in QRSD (p=0.02,), QT interval (p=0.001) was observed.A statistically significant increase in QTc interval (p=0.01) was observed in between control & study groups of first trimester . In second trimester pregnant women a statistically significant decrease was observed in QRSD(p=0.01), QT interval (p=0.002) , statistically significant increase in QTC interval from 425.96 to 442.72 & 448.6 (p=0.001).These findings suggest that low levels of ferritin affect the ferritin dependent K+ current which in turn affect the QTc interval. Tachycardia, ST depression, T wave changes, LVH in ECG as seen in the present study evidences that anemia contributes to cardiac stress.

2.16. In first & second trimester Hb, serum ferritin,serum iron negatively & insignificantly correlates with EDD,ESD,EF% and FS% . The significant increase in left ventricular systolic parameters reflects a hyperkinetic heart in pregnancy. Positive correlation between Hb, serum feritin and serum iron, echo findings in normal first & second trimester pregnant women was observed.

2.17. In conclusion, based on the above findings pregnant women with moderate anemia did not show significant changes in EDD,ESD significant increase in EF,FS indicating compensatory cardiac and extracardiac mechanisms occur which will further prevent the development of cardiac abnormalities in them.

2.18. In pregnant woman with severe iron deficiency anemia echocardiography findings showed decrease in contractile (EF, FS) and pumping function of heart. Increased cardiac output, tachycardia, decreased contractility indicates exhaustion of myocardium. These changes depend on the degree of anemia leading to cardiac stress which may be fatal. If detected early & proper treatment is instituted, anemia improves.
Therefore the important message is that early assessment of iron status and treatment of iron deficiency anemia as early as possible in pregnancy isrequired in view of preventing complications later in pregnancy.

2.19. Detection of moderate anemia in first & second trimester of pregnancy will help the clinicians in proper treatment & prevention of further complications in 3rd trimester Besides anti-anemic drugs , cardio tonic facilities and drugs ,should be included which improve the metabolism of myocardium and thus prevents further complications to mother in third trimester..
Diagnosing iron deficiency anemia during first and second trimester of pregnancy can be supported by Echo, ECG changes to avoid misdiagnosis and also has dramatic clinical and recovery can be achieved with anemia correction.

2.20. In rural areas health education should be given to improve the utilization of available facilities to reduce adverse obstetric outcome associated with maternal anemia. Prevention should start even before pregnancy

Echocardiography examination should be included as one of the routine parameter in antenatal profile check up especially in rural areas to prevent complications of iron deficiency anemia in pregnancy.

6.3. FUTURE DIRECTIONS

1. Future studies should be done with larger sample size to get more accurate results.

2. Longitudinal study should be carried to know the outcome of different degrees of anemia in pregnant women.

APPENDICES

APPENDICES

APPENDIX-1

SAMPLE WRITTEN INFORMED CONSENT FORM
PRATHIMA INSTITUTE OF MEDICAL SCIENCES
KARIMNAGAR, TELANGANA.
DEPARTMENT OF PHYSIOLOGY

CONSENT FORM

Title of the Project
MATERNAL MYOCARDIAL PERFORMANCE IN FIRST AND SECOND TRIMESTERS OF PREGNANCY WITH IRON DEFICIENCY ANEMIA.

Principal investigators name: Padmaja Tangeda

1. PURPOSE OF RESEARCH: I have been informed that this study will assess the effect of iron deficiency anemia on maternal myocardial function in first and second trimester of pregnantwomen. This study will be useful academically as well as clinically to establish association of anemia & cardiac function in pregnancy.

2. PROCEDURE: I understand that, the procedure of the study will involve recording of 2decho, ecg , various physiological, haematological and biochemical parameters. The procedure will not interfere withany of my physiological parameters and theyare non invasive.

3. RISK AND DISCOMFORTS: I understand determination of above mentioned tests willnot cause any discomfort to me and do not involve any risk to my health.

4. BENEFITS: I understand that my participation in the study will help me clinically to know about my heart condition & help me go for further treatment. This will improve my fetus development and i may have normal safe delivery.

5. CONFIDENTIALITY: I understand that medical information produced by this study willbecome part of institutional records and will be subject to the confidentiality and privacyregulation of the said institute. Information of a sensitive personal nature will not be apart of medical record, but will be stored in investigators research file and identified onlyby a code number. The code key connecting name to numbers will be kept in a separatesecured location.

If the data are used for publication in the medical literature and for teaching purposesno names will be used and other identities such as photographs, audio and video tapeswill be used only with my special written permission. I understand I may see thephotographs and the video tapes and have the audio tapes before giving this permission.

6. REQUEST FOR MORE INFORMATION: I understand that I may ask more questionsabout the study at any time. Concerned researcher is available to answer my questions orconcerns. I understand that I will be informed of any significant new findings discoveredduring the course of this study which might influence my continued participation.

Ifduring the study or later, I wish to discuss my participation in all concerns regarding thisstudy with a person not directly involved, I am aware that the social worker of theInstitute is available to talk with me. A copy of this consent form will be given to me tokeep for careful re-reading.

7. REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that myparticipation is voluntary and may refuse to participate or may withdraw my consent anddiscontinue participation in the study at any time without prejudice to my present orfuture care at this hospital.

I also understand that researcher may terminate myparticipation in this study at any time after she/he has explained the reasons for doing soand had helped arrange for my continued care by my physician or physical therapist ifthis is appropriate.

8. **INJURY STATEMENT:** I understand that in unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, then medical treatment will be available to me, but no further compensation would be provided. I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to _____(Patient/Relevant guardian) the purpose of the research, procedures required and the possible risk and benefits to the best of my ability.

Investigator Date

I confirm that _____(Name of the PrincipalInvestigator) has explained to me the purpose of research, the study procedure that I willundergo, and the possible risk and discomforts as well as benefits that I may experience.Alternative to my participation in the study have also been to give my consent from.Therefore I agree to give consent to participate as a subject and this research project.

Participant Date:

Witness to signature Date:

(Modified from Portney L.G, Watkins M.P., in Foundation of Clinical Research, Second Edition,New Jersey, Prentice Hall Health 2000.)

విశదీకరించిన ఒప్పంద పత్రము

పరిశోధన కొరకు రోగి యొక్క గుర్తింపు సంఖ్య :
పరిశోదనా శీర్షిక :
ముఖ్య పరిశోధకుడి పేరు : దూరవాణి సంఖ్య :

నాకు అర్థమగు భాషలో ఈ తేదినందు ..... విశదీకరించిన ఒప్పంద పత్రమునందున్న సమాచారమును నాచే వివులంగా చదవబడినది / వివరింపబడినది. నాకు ప్రశ్నలడుగుటకు స్వేచ్ఛ వున్నదని స్పష్టమైనది.

పరిశోధన యొక్క రీతి, కారణము, ఉపద్రవములు, లాభములు, పరిశోధనాకాలము, మరియు ఇతర పరిశోధన వివరములు నాకు వివులముగా తెలియజేయబడింది.

నేను ఈ పరిశోధనలో స్వచ్ఛందంగా పాల్గొనుచు మరియు ఏ సమయంలోనైనా ఇందుండి అకారణముగా నా హక్కులు, వైద్యసహాయాలకు ఉల్లంఘన జరగకుండా వైదొలగుటకు స్వేచ్ఛ వున్నదని స్పష్టమైనది.

నేను పరిశోధనలో పాలుపంచుకొన్న సమయంలో నానుంచి సేకరించిన వివరములు, నా ఆరోగ్య స్థితి వివరములను ప్రతిమ వైద్య సంస్థలోని / సంబంధిత బాధ్యతాయుత అధికారులచే చూడబడుటకు నా సమ్మతిని తెలియజేయుచున్నాను.

ఈ పరిశోధనలో పాల్గొనుటకు సమ్మతించుచున్నాను.

తేది :
స్థలము :

సంతకము :
ఎడమ చేతి వేలి ముద్ర :

పాల్గొను వ్యక్తి యొక్క పేరు :

యొక్క పుత్రుడు / పుత్రిక / భార్య / భర్త :

పూర్తి తపాలా / చిరునామా :

ఈ ఆమోదము నా యొక్క హాజరీలో గైకొనబడినది.

ముఖ్య పరిశోధకుడి సంతకం : తేది :
స్థలము :

సాక్షి - 1 సాక్షి - 2

సంతకము సంతకము

రోగి యొక్క సమాచార పత్రము

సూచనలు : ఈ 'రోగి యొక్క సమాచార పత్రము" ఈ పరిశోధనలో పాల్గొననున్న (రోగి) వ్యక్తి గురించి అయివుండాలి. ఎంచుకున్న పరిశోధనను బట్టి వ్యక్తి యొక్క వివరములు మారవచ్చు. రోగియొక్క/పరిశోధన జరిపే వ్యక్తులు / సాధారణ వ్యక్తులు, వివిధ రకముల ఆమోద పత్రములు తీసుకొనవలెను. ఆ ఆమోదపత్రపు తయారీలో పరిశోధకుడు తప్పనిసరిగా క్రింది సమాచారమును సులభమగు భాషలో తెలుగు మరియు ఆంగ్లమునందు అర్థమగు రీతిలో యుండవలెను.

పరిశోధన శీర్షిక :

ముఖ్య శోధకుని పేరు :

మార్గదర్శకుని పేరు :

పరిశోధన యొక్క ఉద్దేశము :

పరిశోధనా పద్ధతులు :

రక్త నమునా మరియు కణజాల నమూనాల పరిమాణము :

పరిశోధనలో (రోగి) వ్యక్తి పాల్గొనే సమయము :

పరిశోధన వలన రోగి/ఇతరులకు కలుగు ప్రయోజనము :

మందుల స్వభావ పరీక్షలో : మందు యొక్క రసాయనిక నామము, తయారీ తేది, సమూహసంఖ్య :

పరిశోధన వలన రోగికి కలుగు హాని :

పరిశోధన పత్రముల భద్రతపై విశ్వసనీయత :

పరిశోధనకు అగు ఖర్చు మరియు మూలములు:

పరిశోధనా కారక గాయాలకు ఉచిత వైద్య సదుపాయము :

అంగవికలత మరియు పరిశోధనా కారక మరణములకే కాక అనుకోని ఉపద్రవములకు నష్టపరిహారము:

పరిశోదనా కాలపు ఏ సమయంలోనైనా రోగి తన ఇష్టానుసారంగా పరిశోధన నుండి పరిశోధనా లాభాలను నష్టపోకుండా వైదొలగడము :

చిరునామా మరియు దూరవాణి సంఖ్య (పరిశోధకుడి / సహాయ పరిశోధకుడి / మార్గదర్శకుడి):

పరిశోధనా పత్రములపై పరిశోధకుడి సంతకము :

APPENDIX –II

PUBLICATIONS

1. T Padmaja, Sumangala M patil, Neerja Shastri, and Manjunath Aithala.Maternal Left Ventricular Performance in First Trimester of Pregnancy with and Without Anaemia In Pregnancy. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016 March–April, 7(2):1339

2. Padmaja Rao Tangeda, Sumangala Patil, Neerja Shastri, Shah Navid Noorali. Comparison of Electrocardiogram Changes in Second Trimester of Pregnancy With & Without Anaemia. Journal of Clinical and Diagnostic Research. 2016 Mar, Vol-10(3): CC16-CC18.

Original Article

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Maternal Myocardial Performance in Second Trimester of Pregnancy With Iron Deficiency Anaemia

Physiology Section

Padmaja Rao Tangeda[1], Sumangala PaTil[2], neeRja ShaSTRi[3], Shah navid nooRali[4]

ABStrAct

Introduction: Anaemia affects various organs in body including the heart. In anaemia, oxygen carrying capacity of blood decreases. Iron depletion and the amount of stored iron are reduced in iron deficiency anaemia which limits red cell production. However, the studies which show the effect of anaemia on myocardial function during pregnancy are few in India.

Aim: To study the effect of iron deficiency anaemia on myocardial function by ECG during second trimester of pregnancy and to compare ECG changes with normal pregnant women in second trimester.

Materials and Methods: The study was conducted at antenatal OPD between Oct 2014 to Jul 2015. Hundred pregnant women were selected and divided into 2 groups. A total of 50 normal pregnant women (control group) in 2nd trimester (10-14 weeks of gestation) were compared with equal number of pregnant women with anaemia (study group) in 2nd trimester, aged between 20-30 years. Electrocardiogram was recorded using Philips twelve channel ECG machine model TC20 in both control and study groups to evaluate myocardial performance.

Haematological parameters were analysed by SYSMEX auto analyser. Analysis of Variance (One way ANOVA) was used for comparison between study and control groups and the data was analysed by t-tests.

results: In our study a significant decrease in QRS duration and increase in QTc were observed in study group ($p<0.05$). T-wave abnormalities like flat and negative T-waves in lead II, III, avF, V2 – V4 were more frequent ($p<0.05$). 90% of subjects in study group had tachycardia and ECG abnormalities. There was a negative correlation between Hb level, serum ferritin and tachycardia, ECG abnormalities.

conclusion: Pregnancy with Iron deficiency anaemia brings about various changes in ECG, suggesting that anaemia and volume overload in pregnancy is a risk factor that may lead to cardiac hypertrophy.

Keywords: Electrocardiogram, Serum ferritin, Tachycardia.

IntrOductIOn

Globally, anaemia is the most common disease and in developing country like India, iron deficiency anaemia predominates. Although, the prevalence of anaemia in countries with high development is estimated at 9%, in countries with low development the prevalence is 43% [1]. Anaemia affects various organs in body including the heart. True congestive heart failure rarely results from the anaemic state [2]. Similarly, maternal heart disease is the most important non-obstetric cause of death in pregnant women [3].

Pregnancy usually causes dramatic reversible changes in a woman's cardiovascular system. These remarkable changes begin soon after fertilization and continue throughout gestation to maintain healthy environment for the fetus and mother. The first haemodynamic change during pregnancy seems to be a rise in the heart rate [4]. In anaemia, the oxygen carrying capacity of blood decreases. The following mechanisms operate in anaemia to maintain a normal or near normal oxygen supply to the tissues [2]. Haemodynamic mechanism includes increased cardiac output; blood flow and its distribution; the oxygen-carrying capacity of the blood, i.e., haemoglobin concentration; and oxygen extraction. Among all these, the iron requirement also increases during pregnancy for fetal blood formation and iron is required for mothers own blood and cell mass. The degree of iron requirement depends on iron stores and the amount of dietary iron that can be absorbed during pregnancy. Iron depletion and the amount of stored iron are reduced in iron deficiency anaemia which limits the red cell production [5]. Stored iron can be estimated by serum ferritin in iron deficiency anaemia [6].

One of the important and simplest tools for the diagnosis of heart diseases is recording electrocardiogram. Electrocardiography is used to detect ischemic heart diseases, hypertensive heart diseases and asymptomatic arrhythmias [7].

Earlier studies have reported diverse changes on reports of ECG in anaemia [8,9]. Few studies have shown a decrease in QRS amplitude, T wave flattening and minor degrees of atrioventricular (AV) conduction disturbances [10], but these have not been observed in more recent studies [8]. Later studies have reported frequent non-specific ST-T wave changes [11]. However, the studies which show the effect of iron deficiency anaemia on myocardial function during pregnancy are few in India.

Hence, the present study is taken up to know the effect of iron deficiency anaemia on electrocardiograms during second trimester of pregnancy and to compare the ECG changes with normal pregnant women in second trimester.

MAterIAlS And MethOdS

The study was conducted at antenatal OPD, Departments of Physiology and Cardiology of Prathima Institute of Medical Sciences Hospital between Oct 2014 to July 2015. Hundred pregnant women were selected for this study and divided in to 2 groups. Group I included 50 normal pregnant women (control group) in 2nd trimester (10-14 weeks of gestation) with normal clinical cardiovascular history and normal physical findings. Group II included equal number of pregnant women with iron deficiency anaemia (Haemoglobin% is 7-9.9g%, serum ferritin <4.6ng/ml), in 2nd trimester, aged between 20-30 years. Selected pregnant women were informed about the course and aim of the study and signed consent was obtained.

The study protocol was approved by ethical committees of B.L.D.E.U Shri BM Patil Medical College, Bijapur, Karnataka, India (IEC/29/2012) and Prathima Institute of Medical Sciences (Ref number: IEC/PIMS/2013/001. Pre-determined exclusion criteria for the selection of the study population were pregnant women with diabetes, maternal cardiovascular disease and pre-eclampsia.

Complete physical and obstetric examination was performed after taking detailed history from the selected subjects at the time of recruitment. Gestation was confirmed by last menstrual period and ultrasound measurement of the fetal crown-rump-length in selected pregnant women. Study was conducted between 9.00AM to 12PM. Subjects were asked to lie down in supine posture comfortably for 15 min before recording ECG and then electrocardiogram was recorded using Philips ECG machine model TC20 in both control and study groups to evaluate myocardial performance. The instrument used to record electrocardiogram was the twelve channel electrocardiograph HEWLETT PACKARD page writer manufactured by Philips Electronics Ltd. Haematological parameters were analysed using SYSMEX auto analyser. Serum Ferritin was quantitatived by Chemiluminscence Microparticulate Immuno Assay (CMIA).

StAtIStIcAl AnAlySIS

Data was expressed as Mean±SD. Analysis of Variance (One-way ANOVA) was used for comparison between anaemic pregnant women and normal pregnant women. The data was analysed by t-test (MINITAB 14 SOFTWARE). p< 0.05, p< 0.01 was considered statistically significant, p< 0.001 was considered highly significant (HS) and p> 0.05 was considered as not Significant.

reSuItS

[Table/Fig-1] shows demographic characteristic of the study population. Age and Body Surface Area (BSA) were almost similar in the two groups. This observation was not statistically significant (p>0.05). SBP showed an increase in study group when compared to control group. This observation was not statistically significant between control and study groups (p>0.05). DBP showed a decrease in study group when compared to control group. This observation was not statistically significant between control and study groups (p>0.05). [Table/Fig-2] shows comparison of haemoglobin concentration, RBC count between control and study groups. Hb% showed a statisticaliy significant decrease in study group when compared to control group (p<0.001). RBC showed a statistically significant decrease in study group when compared to control group (p<0.1). Serum ferritin showed a statistically significant decrease in study group when compared to control group (p<0.01).

[Table/Fig-3] shows comparison of Mean±SD, significance and range of QRS duration, QT interval, QTc interval and QRS axis between control and study groups of 2nd trimester pregnant women.

Parameter	group –i	group-ii	p-value
	Control (n=50)	Study (n=50)	
Maternal age (years)	23±2	22±3	0.17 (NS)
Gestational age at the time of echo (week)	20 ±2	21 ±2	0.65 (NS)
Weight (kg)	47.40±4.43	50.32±6.28	0.09 (NS)
Height (cm)	140.3±3.5	141.2±4.0	0.2 (NS)
Body surface area	27.33±0.14	29.35±0.16	0.5 (NS)
SBP (mmHg)	101.6±6.62	102.3± 5.19	0.06 (NS)
DBP (mmHg)	68.8± 7.25	64.6±7.48	0.09 (NS)

[table/Fig-1]: The anthropometric data in second trimester pregnant women of control & study groups.
p>0.05: Not Significant (NS), *p: <0.05: Significant,** p: <0.01: Highly Significant, *** p: <0.001: Very Highly Significant.

Parameter	group –i	group-ii	p-value
	Control (n=50)	Study (n=50)	
Hb%	11.57±1.19	8.49±0.75	0.000 (HS)
RBC (millions/cumm)	4.16±0.41	3.89±0.40	<0.1 (HS)
Serum ferritin	41.76±52.25	4.89±1.21	0.002 (HS)

[table/Fig-2]: Comparison of haematological parameters between two groups.
p>0.05: Not Significant (NS), *p: <0.05: Significant,
** p: <0.01: Highly Significant, *** p: <0.001: Very Highly Significant.

Parameter	2nd trimester		2nd trimester	p-value
	Control group i		Study group ii	
	mean±Sd	Range	mean±Sd	
QRS duration	83.04±8.79	80-100ms	76.52±10.76	0.02 (S)
QT interval	365.04±24.89	320-360ms	350.32±17.44	0.06 (NS)
QTc interval	431.44±23.75	350-420ms	449.46±17.33	0.003 (HS)
QRS axis (in degrees)	46.44±16.24	22-82	44.36±21.21	0.7 (NS)
T axis (in degrees)	19.84±20.	-33-54	21.40±18.20	0.78 (NS)

[table/Fig-3]: Comparison of Mean±SD, significance & range of QRS duration, QT interval, QTc interval & QRS axis between control & study groups.
p>0.05: Not Significant(NS), *p: <0.05: Significant,
** p: <0.01: Highly significant, *** p: <0.001: Very highly significant.

QRSD in sec in control 2nd trimester pregnant women without anaemia and in study group 2nd trimester pregnant women with anaemia were 83.04±8.79 and 76.52±10.76, respectively. This observation showed a statistically significant decrease in study group when compared to control group (p<0.02).

QT interval was decreased in study group when compared to control group. This observation was not statistically significant between control and study groups (p<0.06).

QTc interval showed statistically significant increase in study group when compared to control group (p<0.01).

QRS axis showed decrease in study groups. This observation was statistically not significant (p>0.05).

T axis showed an increase in study group when compared to control group. There was no statistically significant decrease between control and study groups (p>0.05).

T-wave abnormalities

In this study, incidence of T-wave abnormalities like flat T-waves and negative or inverted T-waves in lead II, III, avF and also in chest leads V2-V4 were statistically more in study group when compared to control group. This observation was statistically significant at p>0.05.

Pregnant women with anaemia in 2nd trimester (study group) showed sinus tachycardia and was statistically significant p>0.01. There was negative correlation between Hb%, serum ferritin and tachycardia, ECG changes i.e. as the Hb and serum ferritin levels decrease, there was an increase in occurrence of tachycardia and ECG abnormalities.

dIScuSSIOn

Electrocardiography is one of basic tools in the investigation of cardiovascular diseases [12]. Serum ferritin can be used to estimate the amount of stored iron and is conventional test for the diagnosis of iron deficiency anaemia. The other iron status markers such as, serum trasferrin and serum iron are of less clinical value in the diagnosis of iron depletion as there sensitivities were too low and the false positive rates too high. Despite physiological variations due to haemodilution, the serum ferritin concentration is currently the most reliable non-invasive marker of iron status in pregnancy [6,13]. The electrocardiogram during normal pregnancy may show wide variation from the normal non pregnant women. These variations may be due to the changed spatial arrangement of the chest organs as well as changed electrical properties of the myocardium due to low serum ferritin and haemoglobin levels. ECG recordings show changes with anaemia in pregnancy. In the current study tachycardia was observed in anaemic pregnant women, it could be due to increase in heart rate which is due to physiological adjustments in circulation during anaemia. To compensate anaemia cardiac output increases in order to maintain adequate oxygen supply. Cardiac output increases due to increase in blood volume, preload, heart rate, stroke volume along with a decrease in after load [14] Similar reports were given by Roy SB et al., [15].

But according to Gv S et al., Lokhotia M et al., tachycardia observed in their study seems to be is due to low basal parasympathetic outflow [16,17].

In addition the other changes seen in our study were T wave flattening and inversion.

In present study T wave abnormalities in lead II, III, aVF may be due to disturbances in myocardium, but not due to necrosis of heart muscle, resulting from oxygen deficiency caused by diminution of oxygen carrying power of the blood and due to increased workload on heart due to temporary ischemia represented by T wave inversion which is supported by studies J Misra, Szekely P, Zamani M et al., [18-20].

But some studies showed that T flattening is due to enlargement of QRS complex [10].

According to Pereira AA [14] T wave flattening is due to decreased QRS amplitude and minor degrees of atrioventricular disturbances.

QRSD: In present study, QRSD showed statistically significant decrease in study group when compared to control group. Altered circulatory dynamics during pregnancy might have some effect on its duration. Similar reports were given by Lechmanova et al., [21].

QT interval: In current study there was no statistically significant decrease in QT interval when compared between the control and study groups.

QTc interval: QTc interval in ECG reflects the time taken for depolarization and repolarization in the ventricular myocardium. In our study an increase in QTc interval may be due to tachycardia and complex consequence with changes in regulatory mechanisms during pregnancy. Also, supported by Sunitha M et al., Ozmen N et al., Carruth JE et al., Oram S et al., BN Nandini et al., in their studies [22-26]. In current study, prolongation of QTc interval may be due to low serum ferritin because prolongation of QTc is predominately dependent on K+ rectifier current. It is possible that low levels of ferritin might affect the ferritin dependent K+ current, both the outward and the inward rectifier current and that it may affect the QTc interval which was supported by Aerssens J et al., [27,28].

QRS axis: It is a measure of overall direction of depolarization of the ventricles. In current study QRS axis showed no statistically significant decrease in control and study groups.

T axis: In present study there was no statistically significant increase in T axis when compared between the control and study groups.

LIMITATIONS

In this study follow up was not possible because the study participants were from small cities and rural areas with limited medical facilities. Present study is cross-sectional study with ECG changes, however longitudinal study is required with more sample size and in different areas.

CONCLUSION

Pregnancy with iron deficiency anaemia brings about various changes like QRS duration QTc interval, ST depression wave changes and tachycardia in ECG. There was a negative correlation between Hb level, serum ferritin and ECG abnormalities. If anaemia persists for longer time it may lead to cardiac hypertrophy. Although, ECG recovery can be achieved with anaemia correction. This study clinically helps the condition of the heart for early diagnosis.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Physiology, Prathima Institute of Medical Sciences, Nagunur, Karimnagar, Telangana, India.
2. Professor, Department of Physiology, B.L.D.E.U, Shri B.M Patil Medical College, Vijayapur, Karnataka, India.
3. Associate Professor, Department of Physiology, Prathima Institute of Medical Sciences, Nagunur, Karimnagar, Telangana, India.
4. Associate Professor, Department of Physiology, Prathima Institute of Medical Sciences, Nagunur, Karimnagar, Telangana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Padmaja Rao Tangeda,
Assistant Professor, Department of Physiology, Prathima Institute of Medical Sciences,
Nagunur, Karimnaga-505417, Telangana, India.
E-mail: kprao2011@yahoo.com

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Maternal Left Ventricular Performance in First Trimester of Pregnancy with and Without Anaemia In Pregnancy.

T Padmaja[1]*, Sumangala M patil[1], Neerja Shastri[2], and Manjunath Aithala[1].

[1]Department of Physiology, B.L.D.E.U Shri BM Patil Medical College, Bijapur, Karnataka, India.
[2]Department of Physiology, Prathima Institute Of medical Sciences, Nagunur, Karimnagar, Telangana, India.

ABSTRACT

To evaluate & Compare the effect of anaemia on left ventricular function in normal pregnant women & pregnant women with anaemia in first trimester. 50 pregnant women were selected for this study & divided in to 2 groups.25 normal pregnant women (control group)in Ist trimester (10-14 weeks of gestation) were compared with equal number of pregnant women with anaemia(study group) in Ist trimester, aged between 20-30 years . Doppler echocardiography was performed using MEGAS CVX & MEGAS GPX equipped machine in both control & study groups to evaluate left ventricular systolic and diastolic function. Stroke volume (SV), cardiac put (CO) and total peripheral resistance were calculated from the measured dimensions according to the American society of Echocardiography (ASE) guidelines. Haematological parameters were analysed by SYSMEX auto analyser. Analysis of Variance (One way ANOVA) was used for comparison between study and control groups & the data was analysed by t test. $P< 0.05$ was considered statistically significant. In this study mean values of haemoglobin concentration and serum ferritin levels were significantly lower in study group. Whereas mean values of left ventricular parameters like were increased significantly in study groups when compared to the control groups. Reduction of Hb% in study group as compared to control group significantly & negatively correlates with the left ventricular cardiac function. Anaemia and volume overload in pregnancy is a risk factor that may lead to other cardiac problems.

Keywords: Anaemia; Pregnancy; Echocardiography; Left ventricular function.

Corresponding author

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INTRODUCTION

Pregnancy causes dramatic, usually reversible changes in a woman's cardiovascular system. These remarkable changes begin soon after fertilization & continue throughout gestation. These changes help to maintain healthy environment for the fetus without compromising mother's health, although sometimes, these alterations determine small discomfort to the mother. The first hemodynamic change during pregnancy seems to be a rise in heart rate[1]

Cardiac output rises 30%to 50% above baseline during pregnancy. The increase in cardiac output is achieved by 3 factors: (1) an increase in preload because of greater blood volume; (2) reduced after load because of a decrease in systemic vascular resistance; and (3) a rise in the maternal heart rate by 10-15 beats/min. Stroke volume increases during the first and second trimesters. Blood pressure typically falls about 10mm Hg below baseline by the end of the second trimester because of reduction in systemic vascular resistance and the addition of new blood vessels in the uterus and placenta [2]. These changes begin to take place during the first 5-8 weeks of pregnancy and reach their peak in the second trimester. Loading conditions (preload & afterload) change significantly during pregnancy [3].Also changes occur in blood volume.

Blood volume increases 40% to 50% during normal pregnancy. The marked increase in plasma volume associated with normal pregnancy causes dilution of many circulatory factors. This increase may tend to decrease red cell count & its content haemoglobin due to hemodilution (i.e., the anaemia of pregnancy). Thus during the pregnancy the mother is under risk of developing nutritional deficiency anaemia.

In normal pregnancies, there is an increase in the left ventricular end diastolic volume and also increase in the left atria, right atrial and right ventricular diastolic dimensions [4].These marked hemodynamic changes during pregnancy occur as a result of increased metabolic demand of the fetus.

These changes during pregnancy account for the development of several signs and symptoms during normal pregnancy that can mimic the signs and symptoms of heart disease. Maternal heart disease is the most important non obstetric cause of death in pregnant women[2].

A working knowledge of the normal Physiology of pregnancy is often useful in the management of subjects with heart disease. Echocardiography was used to evaluate left ventricular (LV) function in pregnant women [3].Structural changes within the heart, assessment of total vascular resistance, maternal hemodynamics can predict maternal fetal complications [5]. Among all these the iron requirement also increases during pregnancy for fetal blood formation & iron is required for mothers own blood and cell mass. The degree of iron requirement depends on iron stores & the amount of dietary iron that can be absorbed during pregnancy. Iron depletion & the amount of stored iron is reduced in iron deficiency anaemia which limits reduced red cell production [6]. Serum ferritin can be used to estimate the amount of stored iron [7].

Anaemia in pregnancy in developing countries continues to be a public health of significant proportion. At least 50% of the anaemia has been noticed due to iron deficiency. However the changes in the left ventricular function during pregnancy with anaemia studies are few in India.

The aim of this study is to evaluate the effect of anaemia on left ventricular function in first trimester of pregnancy & to compare the relationship between left ventricular function & hematological parameters in both anaemic pregnant and normal pregnant women.

MATERIAL AND METHODS

The study was conducted at antenatal OPD, departments of Physiology and Cardiology of Prathima Institute of Medical sciences hospital between Feb 2012 to Oct 2013.Fifty pregnant women were selected for this study & divided in to 2 groups.25 normal pregnant women (control group) in Ist trimester (10-14 weeks of gestation) were compared with equal number of pregnant women with iron deficiency anaemia(Haemoglobin% is 7-9.9gm%---Moderate anaemia) (study group) in Ist trimester, aged between 20-30 years .Selected pregnant women were informed about the course and aim of the study and signed consent was obtained. The study protocol was approved by ethical committee of Prathima Institute of medical

sciences(Ref number: IEC/PIMS/2013/001). Pregnant women with normal clinical cardiovascular history and normal physical, ECG and 2D echocardiographic findings will be included in the study.

Predetermined exclusion criteria for the selection of the study population were pregnant women with diabetes, maternal cardiovascular disease and preeclampsia.

A detailed history was taken from all the women and a complete physical and obstetric examination was performed at the time of recruitment. Gestation was confirmed by last menstrual period and ultra sound measurement of the fetal crown-rump-length in selected pregnant women.

Height was measured in cm; weight was measured to the nearest Kg by using standard methods. All observations were done by single person. Body surface area was calculated by using the Dubois Formula [8].

$$BSA = (WEIGHT)\ 0.425(Kg) \times (HEIGHT)\ 0.725\ (cm) \times 0.007184$$

Doppler echocardiography was performed using MEGAS CVX & MEGAS GPX equipped with Phillips – HD7 machine in both control & study groups to evaluate left ventricular systolic and diastolic function. Two-dimensional Doppler echocardiographic examinations were performed using 3.5 MHZ. M–mode studies were performed at the level of aorta, left atrium and LV at midposition between the tips of the mitral value and papillary muscles. Systolic parameters studied were left ventricle end systolic diameter (LV ESD), stroke volume (SV), cardiac output (CO), left ventricular mass(LVM), posterior wall thickness (PWT) in long axis parasternal view. Diastolic parameters studied were E wave, A wave, E/A ratio, fractional shortening (FS %) and percentage ejection fraction (%EF).

Stroke volume (SV), cardiac put (CO) and total peripheral resistance were calculated from the measured dimensions according to the American society of Echocardiography (ASE) guidelines [9].

$$SV = (EDD)^3 - (ESD)^3$$

$$CO\ (L/min) = SV \times HR$$

$$EF\% = \frac{(EDD)^3 - (ESD)^3}{(EDD)^3} \times 100$$

$$FS\% = \frac{(EDD) - (ESD)}{(EDD)} \times 100$$

$$LVM\ (ASE) = 0.8[1.04\ (IVS + EDD + PWT)\ 3 - (EDD)\ 3] + 0.6\ g$$

TVR was calculated using the formula:

$$TPR\ (dyn \times sec \times cm\text{-}5) = mean\ Bp \times 80 / CO$$

Blood pressure was measured using standard auscultatory method with help of pneumatically operated mercurial type random zero sphygmomanometer. Blood pressure was measured in left arm in sitting position with arm at the level of heart. While recording BP appearance of sound (Phase I Korotkoff) and disappearance of sound (Phase V) was recorded as systolic and diastolic BP respectively.
Mean arterial pressure was calculated using the formula:

$$MAP = \frac{2(\text{Diastolic blood pressure}) + (\text{Systolic blood pressure})}{3}$$

To evaluate iron deficiency anaemia haematological parameters like Haemoglobin percentage, red blood cells were analysed by SYSMEX auto analyser. Serum Ferritin was quantitatively determined by Chemiluminscence Microparticulate Immuno Assay (CMIA).

Statistical Analysis:

Data was expressed as Mean±SD. Analysis of Variance (One way ANOVA) was used for comparison between anaemic pregnant women and normal pregnant women. The data was analysed by t tests (MINITAB 14 SOFTWARE).

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RESULTS

Table 1 shown demographic characteristic of the study population. Age and body surface area (BSA) were almost similar in the two groups.

Table 1: The anthropometric data of the two study groups

Parameter	Group –I (Control group) (n=25)	Group-II (Study group) (n=25)	P Value.
Maternal age (years)	22±1.9	23± 3.4	0.28 (NS)
Gestational age at the Time of echo (week)	10 ± 4	10 ± 4	0. 7 (NS)
Weight (kg)	42.03±3.40	43.05±4.02	0.6 (NS)
Height (cm)	140.3±3.5	141.2±4.0	0.5 (NS)
Body surface area	20.33±0.04	21.35±0.06	0.5 (NS)

P value of ≤ 0.05 were considered significant; NS-non significant.
p>0.05: Not Significant, *p: <0.05: Significant(S),
**p: <0.01: Highly significant(HS), *** p: <0.001: Very highly significant.

Table 2 shown comparison of hemodynamic parameters between two groups. It shown that SBP was increased & DBP was decreased in study group. This observation was statistically significant. Stroke volume was significantly increased in study group. Cardiac output in the study group was 6314 ±1152 ml/min as compared to 5764±890 ml/min in the control group. This observation was statistically significant at P < 0.05.Total peripheral resistance in control was higher at 1423.6 ± 157 dyne, sec cm5 as compared to 1405 ± 130 dyne, sec cm5 in the study group. This observation was statistically significant at P<0.05.

Table 2: Comparison of hemodynamic data between two groups

Parameter	Group –I (Control group) (n=25)	Group-II (study group) (n=25)	P Value.
SBP(millimitres of mercury)	101.6±6.62	102.3± 5.19	0.01 (S)
DBP(millimitres of mercury)	68.5± 7.92	66.6±7.48	0.001 (HS)
MAP(millimiters of mercury)	77.1±5.89	78.5±6.70	1.00 (NS)
End systolic diameter(cm)	2.47±0.17	2.63±0.08	0.01(S)
End diastolic diameter(cm)	4.25±0.21	4.40±0.90	0.05 (S)
Stroke volume(ml)	61.83±14.22	67.73±8.22	0.01 (S)
Pulse rate(beats/min)	81.09±6.82	87.23±9.93	0.04 (S)
Cardiac output(ml/min)	5764±890	6314±1152	0.04 (S)
Total peripheral resistance (dyn.sec.cm-5)	1423.6±157	1405±130	0.05 (S)

P value of ≤ 0.05 were considered significant; NS-non significant.
p>0.05: Not Significant, *p: <0.05: Significant(S), **p: <0.01: Highly significant(HS),
***p: <0.001: Very highly significant.
SBP: Systolic Blood Pressure;DBP: Diastolic Blood Pressure;
MAP: Mean Arterial Pressure

Table 3 shown comparison of left ventricular contractile parameters between normal and anaemic pregnant women. Isovolumertic relaxation time was increased in study group. This observation was not significant whereas %Ejection fraction & %Fractional shortening were significantly higher in anaemic group.

Table 4 shown comparison of haemoglobin concentration, RBC count & serum ferritin between control & study groups. Haemoglobin concentration showed a statistically significant decrease in study group when compared to control group (P<0.001). RBC showed a statistically significant decrease in study group

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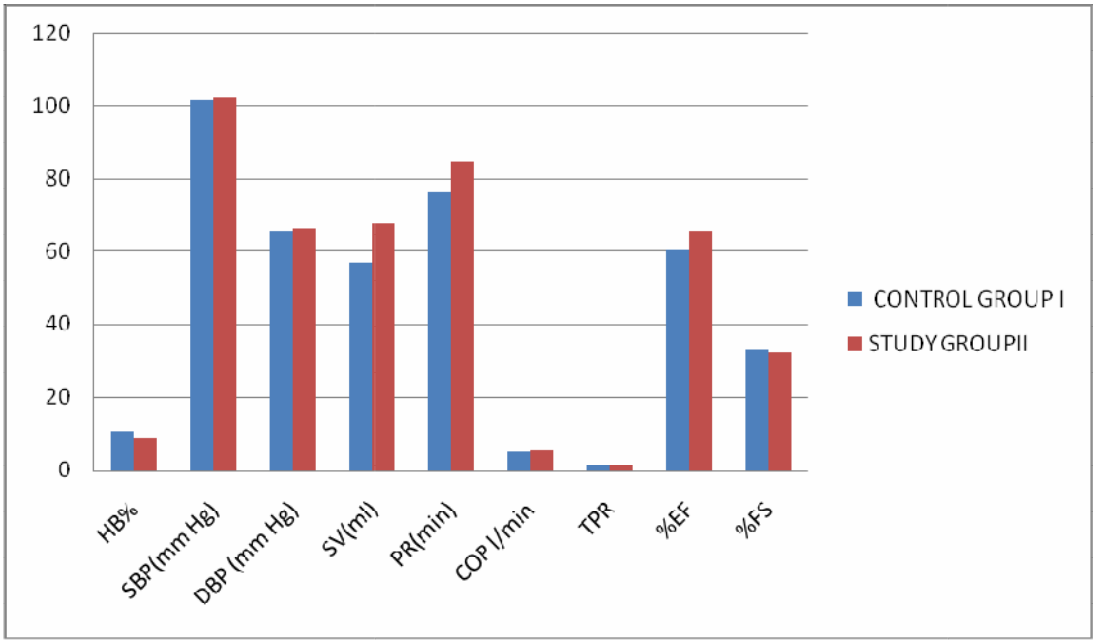
when compared to control group (P<0.1). Serum ferritin showed a statistically significant decrease in study group when compared to control group(p<0.01).

Table 3: Comparison of left ventricular contractile parameters between two groups

Parameter	Group –I (Control group) (n=25)	Group-II (Study group) (n=25)	P Value.
%Ejection fraction	62.42±2.74	64.42±3.17	0.02 (S)
%fractional shortening	31.14±1.31	32.14 ±1.55	0.02 (S)
Isovolumetric relaxation Time	0.89±0.10	0.95±0.08	0.07 (NS)
P value of ≤ 0.05 were considered significant; NS-non significant. p>0.05: Not Significant, *p: <0.05: Significant (S), **p: <0.01: Highly significant(HS), ***p: <0.001: Very highly significant.			

Table 4: Comparison of haematological parameters between two groups

Parameter	Group –I (Control group) (n=25)	Group-II (study group) (n=25)	P Value.
Hb%	11.51±1.11	8.06±0.71	0.000 (HS)
RBC (millons/cumm)	4.16±0.41	3.89±0.40	0.1 (S)
Serum ferritin	35.57±29.59	5.48±1.9	0.000 (HS)
P value of ≤ 0.05 were considered significant; NS-non significant. p>0.05: Not Significant, *p: <0.05: Significant(S), **p: <0.01: Highly significant(HS), ***p: <0.001: Very highly significant.			



DISCUSSION

Normal pregnancy is associated with adaptive changes in the maternal hemodynamics to fulfil the needs of enlarged uterus and to protect mother. Initially marked increase in circulatory blood volume is met

with an increase in stroke volume and a 15%-20% increase in heart rate. The net effect is a 30-50% increase in cardiac output by the end of first trimester[10]. In this study there was significant increase in cardiac output, stroke volume & heart rate in study group. This confirms the earlier studies [11] (Mabie et al.,) and is due to increased circulating volume, reduced systemic vascular resistance & increase in heart rate.

These changes in overall hemodynamic function, allowing the cardiovascular system to adjust to the physiological demands of the fetus while maintaining maternal cardiovascular integrity. Similar reports were given by Stuart Campbell et al., [12]

In this study MAP was not significantly increased and TPR decreased significantly in study group suggesting these changes might have started in the earlier weeks of pregnancy. Thus it supports the cardiac changes suggested by previous studies [13]. In present study a significant increase in left ventricular internal dimensions (ESD &EDD) were observed in study group which in turn showed changes in stroke volume and cardiac output.

Left ventricular contractility was assessed with the use of percentage ejection fraction (EF %) and percentage fractional shortening (FS %). In current study a significant increase in EF% and an increase in FS% were observed in anaemic pregnant women as compared to normal pregnant women. This can be best explained by Franks Starlings law. These observations suggest that volume over load during pregnancy is a risk factor for left ventricular contractility functions. This observation was supported by D. V. Thakker et al [14].,

The mean haemoglobin concentration was significantly decreased in study group than in control group .In anaemia due to low RBC count blood viscosity falls. This decreases resistance to blood flow in the blood vessels. So greater amount of blood passes to the tissues and returns to the heart. So stroke volume, cardiac output and left ventricular contractility functions of the heart increase. Similar reports were given by F Gay Cunningham et al.,[15]

However present results might be influenced by our small sample size, socioeconomic status of the study population, maternal characteristics & methodology. The population size was small. Follow up was not there in this study because the study participants came from small city & rural population.

CONCLUSION

Pregnant women in Ist trimester with low haemoglobin concentration showed greater changes in heart rate, left ventricular dimensions& decrease in total peripheral resistance than in normal pregnant women in Ist trimester.

Increase in left ventricular functions shows hyperkinetic heart. Reduction of Hb in study group as compared to control group significantly & negatively correlates with the left ventricular cardiac function. Anaemia and volume overload in pregnancy is a risk factor that may lead to adverse effects in both mother and fetus. This study clinically helps in assessment of condition of the heart.

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INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 29-12-2011 *at* 10-30 am *to scrutinize the Synopsis/Research projects of postgraduate/undergraduate student/Faculty members of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis/Research project has been accorded Ethical Clearance.*

Title "Maternal myocardial performance in various stages of normal pregnancy and pregnancy with anemia"

Name of P.G./U.G. student/Faculty member Mrs. Padmaja. T. Physiology

Name of Guide/Co-investigator Dr. G. B. Dhanakshirur. Prof Physiology

DR.M.S.BIRADAR,
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

Ref. No. IEC/PIMS/2013/001

Date : 17/01/2013

Mrs. Padmaja Tangeda,

RE: Approval for ethical clearance for a study entitled "Maternal myocardial performance in first and second trimesters of normal pregnancy and pregnancy with anaemia ".

Reference is made to the above heading

I am pleased to inform you that the institutional ethics committee has approved ethical clearance of the above mentioned study based on the recommendation of the committee members.

The validity of this ethical clearance is three years effective from 17th January 2013. You will be required to apply for renewal of ethical clearance if the study is not completed at the end of this clearance. You will be expected to provide six monthly progress reports and a final report upon completion of your study.

17/01/13

Dr. B. Prabhakar Rao,
Chairman,
Institutional Ethics Committee (for human research),
Prathima Institute of Medical Sciences.

Chairman
Institutional Ethics Committee,
Prathima Institute of Medical Sciences

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Annexure-I

PLAGIARISM VERIFICATION CERTIFICATE

1. Name of Student: Padmaja Tangeda Reg. No. 11PHD010
2. Title of the Thesis: Maternal myocardial performance in first and second trimesters of pregnancy with iron deficiency anemia
3. Department: Physiology
4. Name of Guide & Designation: Dr. Sumangala Patil, Professor
5. Name of Co Guide & Designation: Dr. Neerja Shastri, Assoc. Professor.

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