# HEMOGLOBIN RESPONSE TO INTRAVENOUS IRON THERAPY IN NONDIALYTIC CHRONIC KIDNEY DISEASE PATIENTS WITH ANEMIA

*Dissertation submitted in partial fulfilment for the*

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# CERTIFICATE

This is to certify that this dissertation entitled "**HEMOGLOBIN RESPONSE TO INTRAVENOUS IRON THERAPY IN NONDIALYTIC CHRONIC KIDNEY DISEASE PATIENTS WITH ANEMIA**" submitted by **Dr V.Murugesan** to the Faculty of Nephrology, The Tamilnadu Dr.MGR Medical University, Guindy, Chennai-600032, in partial fulfilment of the requirement for the award of DM Degree, Branch III (Nephrology) is a bonafide work carried out by him under my direct supervision and guidance for the academic period from October 2012 to February 2015.

**Dr V. Balaraman MD DM,**
Professor and HOD,
Department of Nephrology,
Kilpauk Medical College ,
Chennai.

**Dr. R.Narayana Babu, MD DCH,**
Dean,
Kilpauk Medical College,
Chennai.

# DECLARATION

I, **Dr.V.Murugesan** declare that I carried out this work on "**HEMOGLOBIN RESPONSE TO INTRAVENOUS IRON THERAPY IN NONDIALYTIC CHRONIC KIDNEY DISEASE PATIENTS WITH ANEMIA**" at the Department of Nephrology, Kilpauk Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree and diploma to any university, board either in India or abroad.

This is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfilment of the rules and regulation for the D.M. Degree examination in Nephrology.

Place : Chennai Dr. Murugesan V.

Date :

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# CONTENTS

# ABBREVIATION

| | | |
|---|---|---|
| WHO | - | World Health Organisation |
| CKD | - | Chronic Kidney Disease |
| RBC | - | Red Blood Cells |
| EPO | - | Erythopoietin |
| KDIGO | - | Kidney disease Improving Global Outcome |
| TSAT | - | Transferrin saturation |
| TIBC | - | Total iron binding capacity |
| IV | - | Intra Venous |
| ESA | - | Erythropoiesis Stimulating Agents |
| USA | - | United States of America |
| DRIVE | - | Dialysis patient's Response to IV iron with Elevated ferritin level |
| RES | - | Reticulo Endothelial System |
| Hb | - | Hemoglobin |
| ESRD | - | End Stage Renal Disease |
| GFR | - | Glomerular Filtration Rate |
| RRT | - | Renal Replacement therapy |
| DM | - | Diabetes Mellitus |
| HT | - | Hypertension |
| CGN | - | Chronic Glomerulo Nephritis |
| CIN | - | Chronic Interstitial Nephritis |
| ADPKD | - | Autosomal Dominant Polycystic Kidney Disease |

| | | |
|---|---|---|
| RVD | - | Renal Vascular Disease |
| HD | - | Hemo Dialysis |
| PD | - | Peritoneal Dialysis |
| NGO | - | Nongovernmental organisation |
| Govt. | - | Government |
| RIA | - | Radio Immuno Assay |
| TCA | - | Tricarboxilic acid |
| CNS | - | Central Nervous System |
| ANS | - | Autonomic Nervous System |
| PNS | - | Peripheral Nervous System |
| BMT-1 | - | Bivalent metal transporter-1 |
| FP-1 | - | Ferroportin-1 |
| IL-6 | - | Interleukin-6 |
| PTH | - | Parathyroid hormone |
| DNA | - | Deoxy ribo nucleic acid |
| ACE | - | Angiotensin converting enzyme |
| GIT | - | Gastro intestinal tract |
| EPO-R | – | Erythropoietin Receptor |
| HIF | - | Hypoxia Inducible Factor |
| HMW | - | High molecular weight |
| DPO | - | Darbepoetin |
| ECG | - | Electro cardiogram |
| HCV | - | Hepatitis C virus |
| DOPPS | – | Dialysis outcomes and practice pattern study |

# INTRODUCTION

Anemia is a common complication in chronic kidney disease (CKD) patients. Anemia causes left ventricular hypertrophy and congestive cardiac failure. It increases cardio vascular morbidity and mortality[1]. It is also a non-traditional risk factor for progression of CKD[2]. Anemia also causes fatigue, weakness, dizziness, breathlessness, decreased exercise tolerance, decreased muscle strength, falls and decreased quality of life[3]. Iron deficiency is one of the major causes of anemia associated with CKD. Iron deficiency may be absolute or functional deficiency. According to Kidney Disease Improving Global Outcome (KDIGO) guidelines on anemia management in CKD a trial of parenteral iron therapy or 1-3 month trial of oral iron therapy is suggested if TSAT is < 30% and serum ferritin< 500 ng/ ml[6]. Following a black box warning on Erythropoiesis stimulating agents (ESAs) regarding concerns about malignancies and cerebro vascular events and also due to problems in reimbursements there is a trend towards reduced usage of ESAs and increased usage of IV iron in USA. Many centres in USA have started using higher iron dosage in spite of having ferritin levels > 500 ng/ dl when TSAT is < 30% based on Dialysis patient's Response to IV iron with Elevated ferritin level (DRIVE) trial. According to DRIVE study group intravenous iron therapy in dialylic patients with ferritin between

500- 1200 ng/ ml and TSAT < 25% caused significant improvement in anemia and reduced ESA dosage need[7]. The intra venous (IV) iron usage increased from 55% in 2010 to 77% in 2011 and stabilised at 66%-68% in 2012 in USA. Higher threshold for ferritin (>800 ng/ml) is the norm in many US centres. In 25% of dialysis centres 2/3 of patients had ferritin level > 800 ng/ ml and 15% had ferritin 1200 ng/ ml[8]. But these measures led to increased need for blood transfusions. Also there is concern about iron overload.

# AIMS OF THE STUDY

1. To assess the Hb response to treatment with intravenous iron in nondialytic CKD patients with anemia.

2. To assess whether the serum ferritin, serum iron, Total iron binding capacity, transferrin saturation (calculated) alone or in combination can predict the response to iron therapy.

# REVIEW OF LITERATURE

## Stages of CKD

CKD is defined as kidney damage or GFR <60 ml/ min/ 1.73 $m^2$ presenting for > 3 months with implications on health[9]. Kidney damage is diagnosed on the basis of presence of certain markers like persistent proteinuria, active urine sediments, serum abnormalities, imaging studies and histology[10].

**Table - 1 : STAGES OF CKD**

| STAGE | DESCRIPTION | eGFR (ml/min/1.73 $m^2$) | PLAN OF TREATMENT |
|---|---|---|---|
| 1 | Kidney damage with normal or increased GFR | ≥ 90 | Diagnosis and treatment of comorbidities |
| 2 | Kidney damage with mildly decreased GFR | 60-89 | Estimate progression |
| 3 | Kidney damage with moderately decreased GFR | 30-59 | Evaluate and treat complications |
| 4 | Kidney damage with severely decreased GFR | 15-29 | Preparation for RRT |
| 5 | ESRD | <15 | Dialysis or transplantation |

In India the incidence of ESRD is 151/ million population[11]. Diabetes mellitus (DM 31.2%) forms the primary causative factor in India just like the world over followed by undetermined cause (16.4%), CGN(13.8%), HT(12.8%), others(11.7%), CIN(7%), obstructive uropathy (3.4%), ADPKD (2.5%), RVD(0.8%) and graft loss(0.3%)[12]. Due to the growing epidemic of DM the incidence of CKD & ESRD are increasing. In India only 10-20% of ESRD patients get RRT in the form of renal transplantation, HD or PD mainly through private sectors, NGOs and Govt. Medical College Hospitals.

**Complications of CKD**

1. Cardio vascular disease.
2. Anemia.
3. Nervous system (CNS/ PNS/ ANS) abnormalities.
4. Mineral and bone disorder.
5. Metabolic abnormalities (hyper/hypoglycaemia, hyperlipidemia, hypertriglyceridemia, protein catabolism)
6. Other endocrine abnormalities
    - decreased libido, sexual function and depression in men, reduced fertility in women
    - growth retardation in children
    - Vitamin D deficiency,
    - hypertension

## RELATIONSHIP OF ANEMIA AND OTHER COMPLICATIONS.

### Cardiovascular disease

Cardiac disease is major cause of morbidity and mortality in CKD patients. Uremic toxins especially parathyroid hormones and anemia cause cardiac muscle hypertrophy and cardiac failure.

### Central nervous system disease

Many of uremic symptoms like fatigue, weakness, dizziness, cognitive impairment and decreased muscle strength initially thought of due to CNS involvement are mainly due to anemia. Anemia also causes impaired mobility and increased risks of falls.

### Bone and mineral disease

Marrow fibrosis caused by uremic toxins like parathyroid hormones may aggravate anemia.

### Anemia

Anemia is defined as a state characterised by decrease red blood cell and haemoglobin mass resulting in decreased oxygen carrying capacity and decreased oxygen delivery to the tissues. Anemia is diagnosed when haemoglobin level less than 13 g/dl in men and less than 12 g/dl in women[6]. The NICE guidelines advocate a higher Hb target (> 13.5 g/ dl

in males). Renal anemia was defined as anemia occurring in patients with GFR < 45ml/ min/ 1.73 $m^2$ with DM and <30 ml/ min/ $m^2$ without DM[14]. The prevalence of anemia increases with progression of CKD. According to 2nd annual report of Indian CKD Registry prevalence of anemia is 67.4% in CKD stage 1, 72.5% in stage 2, 83.5% in stage 3, 93.8% in stage 4, 98.7% in stage 5. According to United States Renal Data System (USRDS) the prevalence of anemia is 43% in CKD stages 1-2 and 57% in CKD stages 3-5[15]. In contrast prevalence of anemia is 12% to 14% of subjects at risk for kidney disease (Kidney Early Evaluation Programme [KEEP]) and 5% to 6% in general population (National Health and Nutrition Examination Survey [NHANES] 1999 to 2004)[16]. Over half of adults with CKD stage 3 / 4 are affected with anemia[17]. According to predialysis survey on anemia management (PRESAM) 20% patients had Hb > 12g/ dl and 2/3 had Hb < 11g/ dl[18]. In India prevalence of anemia is 33% in general population and 41% in CKD patients[19].

**Features of renal anemia**

1. Anemia is rare in CKD stages 1-3(GFR > 30 ml/min/1.73 $m^2$).
2. In diabetic CKD patients anemia starts early (eGFR <45 ml/min/1.73 $m^2$)

3. Anemia is severe in diabetic CKD patients, younger women and older men.

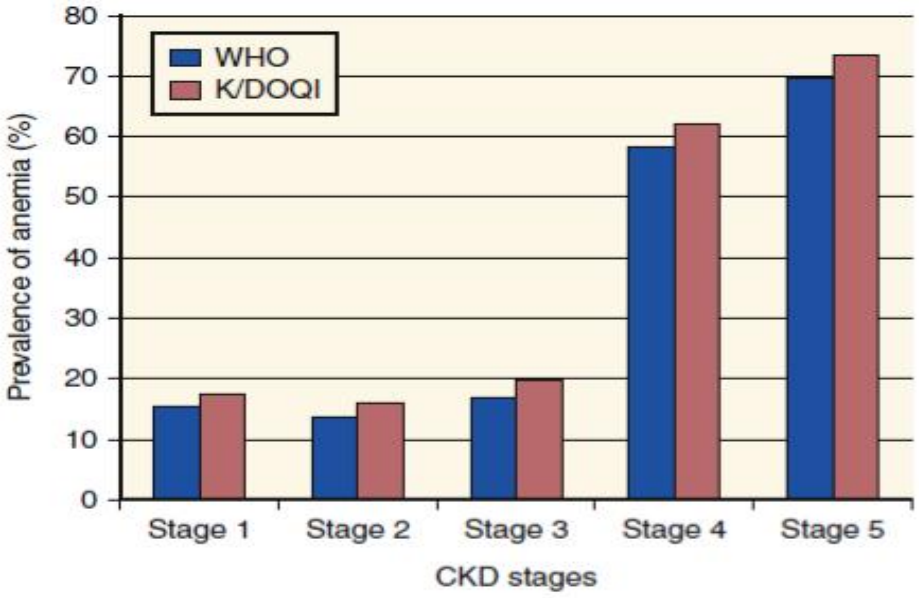



**Figure – 1 : Prevalence of anemia in CKD**

**Historical perspectives of anemia.**

- 1825- First human to human blood transfusion was given in London by James Blundell who transfused a man's blood into his wife for postpartum haemorrhage[20].
- 1836- renal anemia was first described by Richard Bright[21].
- 1906- Carnot and Deflandre discovered that serum from rabbits (hemopoietins) when injected into other rabbits stimulated RBC production.

- 1948- Bonsdorff and Jalavisto named the hemopoietic factor as erythropoietin.
- 1977- Purification of EPO was done.
- 1979- RIA of EPO.
- 1985-Cloning and expression of EPO gene.
- 1986-87- clinical trials of recombinant human EPO.
- Till 1980s blood transfusion and androgen were the treatment modalities for anemia[13].

**Normal erythropoiesis**

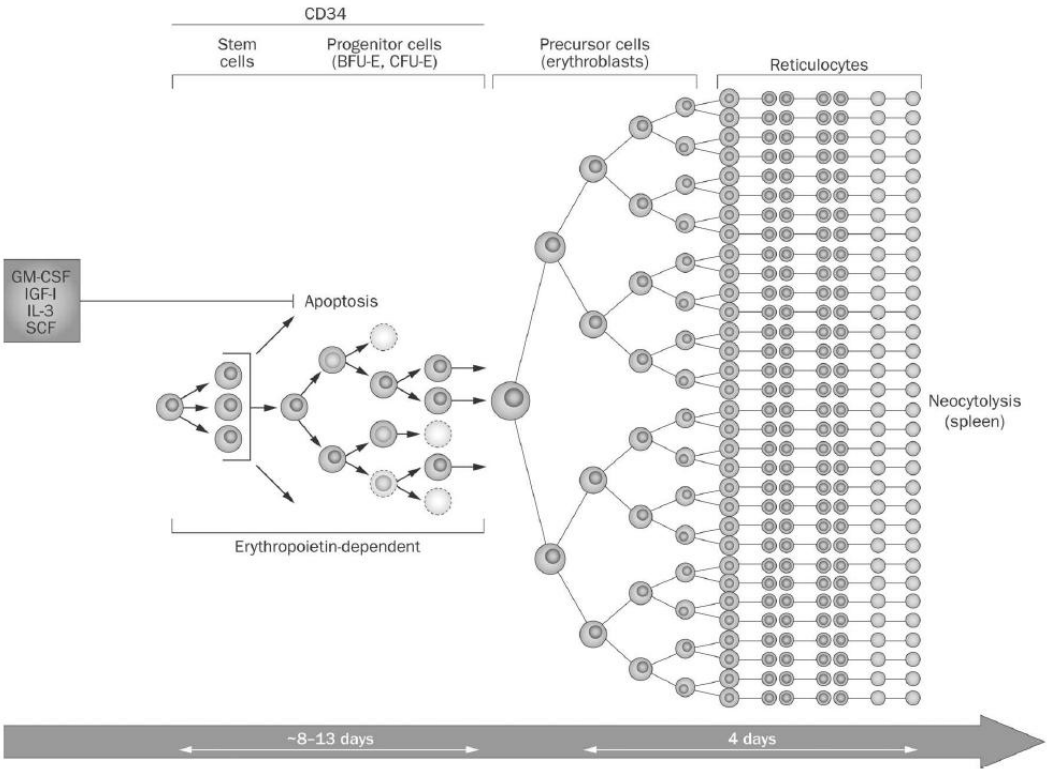



**Figure - 2 : Normal Erythropoiesis**

Erythropoiesis occurs mainly in bone marrow in adults. RBC production starts with EPO independent maturation of multipotent stem cells into colony forming unit – granulocyte, erythrocyte, monocyte, macrophage (CFU-GEMM). CFU- GEMM develops into burst forming unit Erythroid (BFU-E) which is the first cell to have EPO-R which again develops into CFU- E from which erythroblasts, basophilic, polychromatic, orthochromatic normoblasts, reticulocytes and mature RBCs develop.

**EPO**

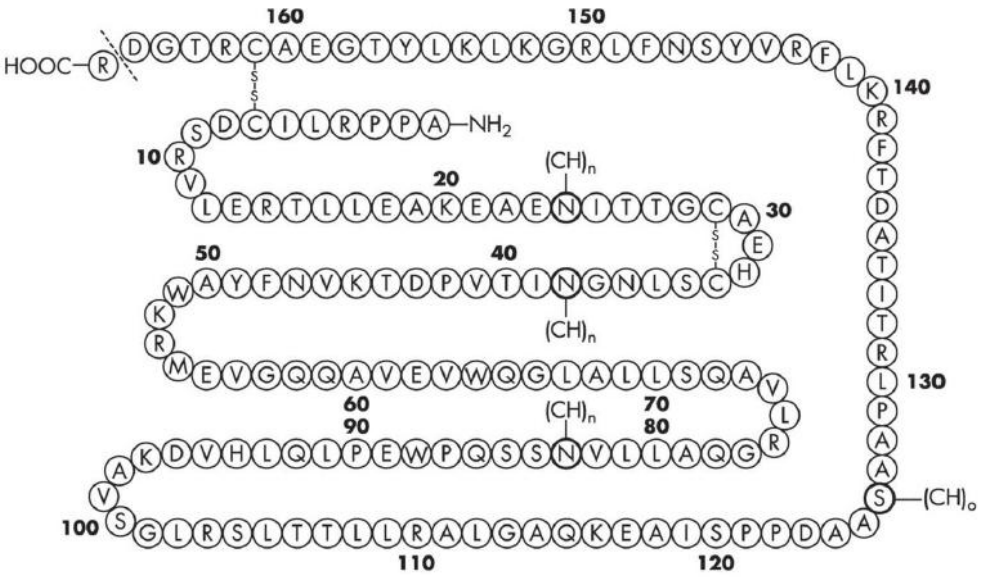



**Figure - 3 : Structure of EPO**

EPO is a glycoprotein containing 165 aminoacids with a molecular weight of 30 kDa. Kidney is the major site of EPO synthesis in adults. 90% of EPO is secreted in the peritubular fibroblasts in the cortex and outer medulla, the areas susceptible to hypoxia. Moderate hypoxia stimulates additional EPO from liver, mainly hepatocytes and to some extent from stellate or Ito cells. EPO is essential for maturation of RBCs. EPO deficiency leads to neocytolysis of immature RBCs[22].

**EPO-R**

EPO-R is also a glycoprotein containing 484 amino acids. It is synthesised in endoplasmic reticulum. It binds with tyrosine kinase and Janus kinase-2. The entire complex associates with type 2 transferrin receptor (TfR-2) for trafficking to cell membrane.

**HIF**

Tissue hypoxia in the kidney provides the major erythropoietic stimuli.

Hypoxia inducible factors are helix transcription factors consisting $O_2$ regulated $\alpha$ subunits and constitutively expressed $\beta$ subunits. Under normoxia, O2, $Fe^{++}$ and ascorbic acid activate prolyl 4-hydroxylase domain (PHD) proteins and degrade HIF. Hypoxia and high concentrations of TCA cycle intermediates inhibit PHD proteins allowing

HIF-α to translocate to nucleus and activate hypoxia response elements (HRE). HRE regulates numerous cellular processes like erythropoiesis, angiogenesis and anaerobic metabolism. There are 3 HIFs. HIF-2 plays an important role in erythropoiesis. HIF-1 is important in regulating glycolysis. HIF-3 splice variants may actually inhibit transcription. HIF proteins activate the transcription of PHD-2 and PHD-3 resulting in their own degradation by negative feedback.

**Iron**

Iron is essential for erythropoiesis. Total body iron is 50mg/kg. 65% of iron is in Hb; 10% in Mb, cytochromes and enzymes. Remainder is in RES, liver and bone marrow. Differentiating erythroblasts need 20-30 mg/ day of iron. Most of this iron is derived from recycling of senescent RBCs.

Daily intake of iron is 10- 20 mg/ day of which only 10% ($Fe^{++}$) is absorbed by enterocytes. Non heme iron presenting as $Fe^{3+}$ needs to be converted to $Fe^{2+}$ by ferrireductase in conjunction with Vitamin C. $Fe^{2+}$ is transported by Divalent Metal Transporter - 1 (DMT-1) into enterocytes which is delivered to circulation via ferroportin-1, oxidized to $Fe^{3+}$ and bound to transferrin.

Iron enters erythroblast when 2 transferrin molecules bind TfR-1 which then undergoes endocytosis into a clathrin coated sclerosome. $Fe^{3+}$ is released and again reduced to $Fe^{2+}$ by ferrireductase and exported to cytoplasm via DMT-1. Cytosolic Iron enters mitochondria where ferrochelatase causes its insertion into protoporphyrin IX to form heme. Apotransferrin: TfR-1 complex is released into circulation for recycling.

**Iron regulation**

1. When the iron storage is low there is upregulation of BMT-1, FP-1, TfR-1 and increased absorption of iron from duodenal enterocytes.

2. When iron is adequate hepcidin is secreted by liver which combines with FP-1 in enterocytes, macrophages and hepatocytes and degrades it thereby prevents iron absorption. Hepcidin is downregulated by HIF in hypoxia, by soluble hemojuvelin in Iron deficiency, by EPO during erythroblast formation.

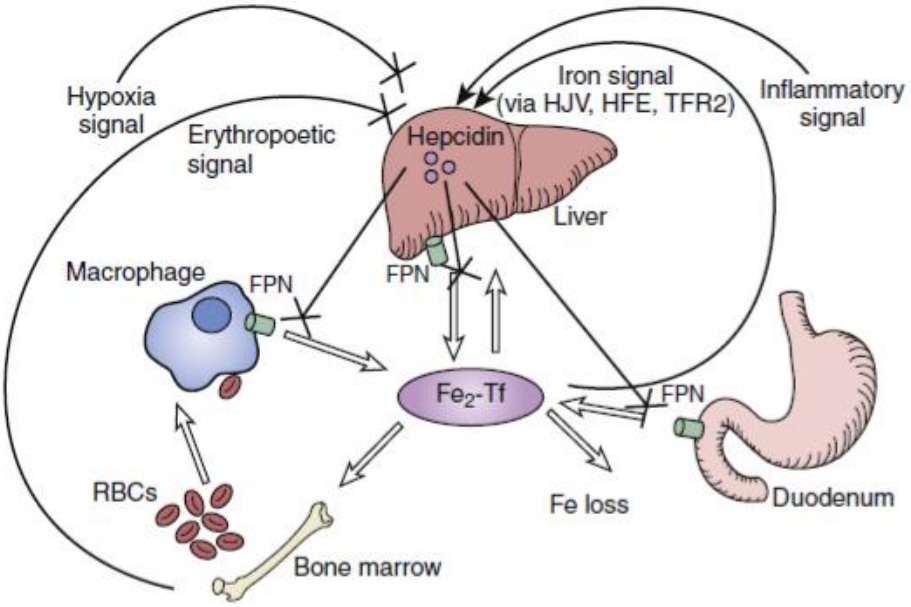



**Figure – 4 : Regulation of iron**

HFE – human hemochromatosis protein 3. Inflammation and infections through IL-6, bone morphogenic proteins and lipopolysaccharides inhibit hepcidin expression, increase BMP-1 expression by macrophages and release of apolactoferrin and neutrophil gelatinase- associated lipocalin(NGAL). These prevent release of iron from RES thereby decrease its availability to iron dependent microorganisms.

4. Iron availability is regulated by iron response elements (IRE) in a negative feedback mechanism.

**Other functions of iron**

- Essential for cellular respiration and synthesis
- Converts harmless superoxide and hydrogen peroxide (Fenton reaction)[23] into toxic hydroxyl ions which causes damage of adjacent macromolecules[24].
- Can increase intracellular labile iron which causes lipid peroxidation, DNA as well as membrane damage and immunological disturbance[25].
- Taken by iron dependant bacteria and precipitates infection.

**Vitamin $B_{12}$ and folic acid**

Vitamin $B_{12}$ and folic acid are essential for many enzymatic reactions in erythropoiesis.

**Causes of anemia in CKD**

1. EPO deficiency relative to the degree of anemia.
   - Adaptation of kidney by attenuation of sodium channels improves oxygenation in outer medulla thereby removes hypoxic stimuli to EPO.
   - Neutralisation of EPO by soluble EPO-R.
   - Inactivation of EPO by desialysation due to uremia.

- Blunting of action of EPO in marrow by absence of permissive factors like IL-3, calcitriol and CD4 cells and presence of inhibitory factors like polyamines, ribonuclease and PTH.

EPO levels are normal or slightly increased in anemia of CKD. Comparing to anemic patients with CKD anemic patients without CKD have 10-100 times EPO levels[26](relative EPO deficiency).

2. Absolute and functional iron deficiency.

Blood loss, inhibition of iron absorption by hepcidin and drugs, poor intake due to anorexia and nausea are the main causes of Iron deficiency. Even though iron deficiency is more common in CKD patients on HD more than 50% of CKD stage 3 or 4 patients also have Iron deficiency[28]. In addition to symptoms of anemia Iron deficiency impairs cellular energy generation in skeletal and cardiac muscles, oxygen storage in myoglobin, T cell proliferation and function, neuron myelination and DNA synthesis[29]. Absolute iron deficiency is the presence of low iron stores (serum ferritin < 100 ng/ml & TSAT <20%)[64]. Functional iron deficiency occurs due to impaired release of iron from RES.

1. Accelerated destruction of young and senescent RBCs.
2. Hemolysis during HD and also due to drugs.

3. Deficiencies of vitamins $B_6$, folic acid, $B^{12}$ and L- carnitine.
4. Marrow fibrosis due to excessive PTH.
5. Chronic inflammation and infections.
6. ACE inhibitors.
7. Malignancy.
8. Pure red cell aplasia.
9. Hemoglobinopathies.
10. Malnutrition.
11. Hepcidin excess.

    Inflammation increases hepcidin production from liver. CKD is a chronic inflammatory state. IV iron may increase hepcidin further and aggravate anemia[30].
12. Aluminium toxicity, one of the major causes of renal anemia has reduced significantly following improvements in HD and reduced usage of citrates[4,5].

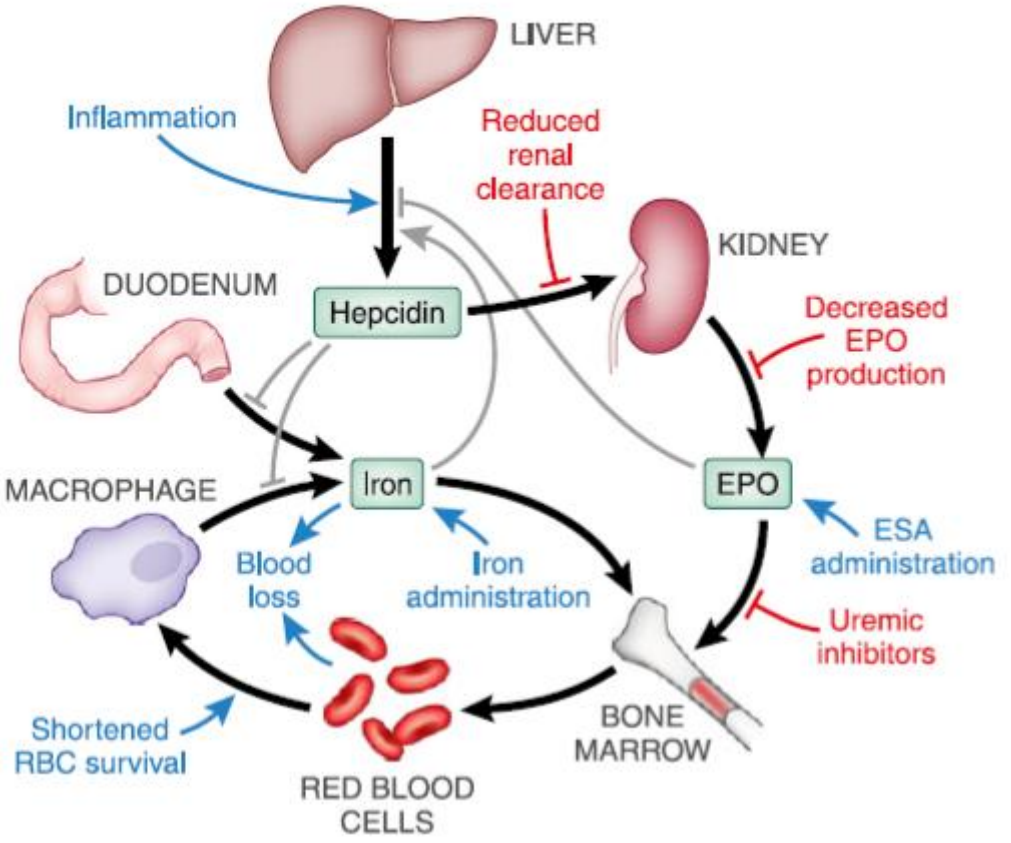



**Figure - 5 : Causes of Anemia in CKD**

**Diagnosis of anemia in CKD**

Anemia is diagnosed when Hb level is below 13 g/dl in men and below 12 g/dl in women[6]. Packed cell volume is not used to diagnose anemia in CKD because of variations due to glucose concentration, storage conditions or analysis techniques. There may be associated thrombocytosis. Peripheral smear study may show normocytic normochromic anemia to indicate anemia of chronic disease or microcytic hypochromic anemia in case of Iron deficiency anemia and macroytic anemia in case of folic acid,vitamin $B_{12}$ deficiency and

fragmented RBCs in hemolysis. Reticulocyte count may be increased in case of hemolysis and decreased in case of marrow failure due to marrow fibrosis. In macrocytosis serum $B_{12}$ and RBC folate level should be measured. Blood loss through GIT and hemolysis should be evaluated.

**Iron indices**

Iron indices show low serum ferritin, low serum Iron levels and low TSAT.

**Serum ferritin**

Serum ferritin level was taken as less than 100 ng/ml in non dialytic patients and less than 200 ng/ml in dialytic patients. Now the target has been increased to less than 500 ng/ml[6]. KDOQI doesn't recommend serum ferritin more than 500 ng/dl. This recommendation is being disputed now. As ferritin is an acute phase reactant it can be elevated in infection and inflammation but supply for erythropoiesis may be low (low TSAT). In 47 non dialytic CKD patients the mean serum ferritin level was 236 ng/dl but 46 patients didn't have detectable bone marrow Iron deposits[60].

Serum iron

Serum iron levels may be modified by diurnal variation and food intake.

Reference values[54]

Males - 65- 175 μg/dl

Females -40- 150 μg/dl

Total Iron binding capacity

Reference value

200-400 μg/dl

Transferrin saturation(TSAT)

TSAT = serum Iron × 100 ÷ TIBC

Target value – 30% - 40%

Initially iron deficiency was treated when TSAT is < 20%. Now target has been increased to < 30%[6].

**Limitations of Iron indices**

1. Acute phase reactant.
2. Diurnal variation
3. Variation due to food intake.

**Correlation of serum ferritin and TSAT with eGFR**

According to NHANES III (1988- 2004) serum ferritin and TSAT didn't correlate with declining renal function in men. In women serum ferritin increases with declining renal function while TSAT decreases with eGFR decline[61].

**Other markers of anemia**

1. Reticulocyte Hb content (CHr).
2. Percentage of hypochromic red cells (PHRC).
3. Soluble transferrin receptor (sTfR).
4. Erythrocyte zinc protoporphyrin.
5. Hepcidin.
6. Bone marrow/ liver biopsy.
7. ESR/CRP.

**Bone marrow iron stores**

Bone marrow iron stores are the gold standard for diagnosis of iron deficiency. Many have demonstrated iron deplete states in marrow of non dialytic CKD patients. Silverberg has demonstrated 90% iron deplete state in CKD patients[60]. Simona stancu observed 48% iron deplete state[65] while it was 23% in Rahman's study[66]. Bone marrow iron stores could not explain functional iron status in CKD patients. Simona stancu et al

demonstrated that in 100 non dialytic CKD patients 1/3 of iron replete (52 iron replete patients) had > 1 g/dl Hb increase 1 month after 1 g Inj. iron sucrose[65].

Both PRBC and CFr appear imperfect but they are stable and accurate[31].

**EPO assays**

EPO assays are done in research laboratories only. Presence of immunogenic EPO fragments[27] are the major limiting factors for EPO assays.

**Hb variability**

Hb variability is defined as fluctuation in Hb levels above or below the original value within a short period even though the mean value may be within the target range[32]. The standard deviation of Hb in dialytic CKD patients in US population is 1.1 to 1.3 g/ dl. It is believed to be due to intermittent short nonbiologic burst of EPO especially in HD patients. Causes may due to iron- EPO imbalance (EPO without iron) and vice versa, infections, inflammation and blood loss. It is said to be a predictor of mortality. To avoid this variation Hb should at least be measured weekly once.

**Treatment of anemia in CKD**

Target Hb

When Hb is below 10 g/dl CKD patients should be treated with EPO after a loading dose of IV iron. Hb target should be between 11-12 g/dl. Target should not be more than 13 g/dl to prevent thrombosis related events (stroke, angina, MI and vascular access failure). A higher dosage of ESAs associated with higher Hb target is associated with increased cardiovascular mortality[33].

ESA administration

ESA is administered when Hb is less than 10 mg/ dl to keep the Hb between 11-12 g/ dl.

ESA dosage

Epoetin- alfa or beta is started in the dosage of 20- 50 IU/ kg weekly thrice , DPO with 0.45μg/kg SC/ IV weekly once dose or 0.75 μg/kg SC fortnightly and CERA with 0.6 μg/kg SC/IV fortnightly in non dialytic patients or 1.2 μg/kg SC monthly once in CKD stage 5D. Women and diabetic patient may need high ESA dosage.

Types of ESA

First generation ESAs

- Epoetin alfa
- Epoetin beta

Second generation ESAs

- Darbepoetin alfa- modified form of erythropoietin wih a carbohydrate moiety which increases the half-life to 25 hours by IV and 48 hours by SC injection[45]. In US it is usually given mainly in non dialytic patients[46] as monthly injections.

Third generation ESAs

- methoxy polyethylene glycol–epoetin beta (Mircera) has a long half-life of 130 hours[47]. Half-life is same for IV as well as SC forms[48].

Side effects of ESAs[49]

- HT
- Seizures
- Dialysis access clotting

ESA resistance

ESA resistance is defined as continued need for rHuEPO at doses of > 300 IU/kg/week SC or > 400 – 450 IU/kg/week IV or darbepoetin at a dose of 1.5 μg/kg/week SC[50].

ESA resistance occurs in 10-20% of patients.

Causes of ESA resistance

- Inflammation
- Iron deficiency
- Marrow fibrosis
- Hemolysis
- Nutritional deficiencies – folic acid, vitamin $B_6$ , $B_{12,}$ L- carnitine
- Miscellaneous – pure red cell aplasia, aluminium, ACEI/ARB.

Novel agents

HIF prolyl- hydroxylase inhibitors, orally active prevent degradation of HIF-α, thereby stimulate EPO release. In experimental studies it increases EPO levels 3 times more than normal[51].

**Management of iron deficiency**

Iron deficiency is treated when serum ferritin level is less than 500 ng/ ml and TSAT is less than 30%[6]. In dialytic patients parenteral iron should be administered. In predialytic patients a 3 month trial of oral iron or parenteral iron may be administered.

Oral iron therapy

Ferrous sulphate, chloride, fumarate, gluconate and heme iron polypeptide (HIP) are the oral iron preparations available. 200 mg of elemental iron is administered daily. Advantages are cheap, easy administration and less systemic side effects. Disadvantages are GI side effects, inability to administer in case of persistent vomiting and less reliable absorption due to hepcidin and other medications especially in dialytic CKD patients. Not effective in patients on EPO[34]. Ferric citrate has in addition phosphate binding properties. In many trials oral Heme Iron Polypeptide produce comparable Hb response[63].

IV Iron therapy

Iron dextran, Iron sucrose, ferric gluconate, ferrumoxytol, ferric carboxy maltose and Iron isomaltoside 1000 are the available IV Iron formulations. Due to increased anaphylactic reactions Iron dextran especially HMW is not preferred now. Advantages of IV Iron are utility in dialytic patients, reliable absorption and compliance. It bypasses absorption difficulties even in the presence of high hepcidin[35]. Disadvantages are cost, need for saline and infusion sets and allergic reactions. Other concerns are proteinuria due to proximal tubular injury, peroxidation of lipids, infections, iron overload and hemosiderosis.

Hemosiderosis is rare when serum ferritin level is less than 2000 ng/ ml. In animal models iron administration may precipitate infections[36,37,38,39]. IV Ferric carboxy maltose can rarely produce hypophosphstemia due to its effect on FGF-23 especially in renal transplant recipients[59].

Oral vs IV iron therapy

IV iron is indicated in dialytic patients. In non dialytic CKD patients a trial of oral iron or IV iron is indicated. In non dialytic patients 2 doses of 510 mg IV Ferumoxytol given 5 ± 3 days gives good Hb improvement in 35 days as against elemental oral iron 200 mg given for 21 days; Safety profile was good; can be given within a minute in fact within 20 seconds[40]. IV iron therapy can reduce the need for higher EPO dosages in dialytic patients and may delay the start of EPO administration. Hb concentration and iron indices rise in short time comparing to oral iron[41,42,43]. Less stable oral iron preparations like ferrous sulphate can release iron rapidly and cause oxidative damage while iron sucrose and ferric carboxy maltose are more stable.

**Studies comparing Hb response to oral/IV iron therapies**

1. Agarwal et al in 2006 compared oral (ferrous sulphate) vs IV iron (sodium ferric gluconate) therapy in 75 patients and showed that Hb response was more in IV iron therapy (0.4± 0.8 g/dl) than oral iron (0.2± 0.9 g/dl)[55].

2. Qunibi et al in 2008 involving 188 patients over 8 weeks compared oral (ferrous sulphate) vs IV iron therapy (ferric carboxy maltose) and observed that Hb response to oral iron was 0.76±1.1 g/dl whereas to that of IV iron was 1.16± 1.1 g/dl[56].
3. Spinowitz et al in 2008 involving 188 patients compared oral iron (ferrous fumarate) with IV iron (ferrumoxytol) observed that Hb response to IV iron was 0.62± 1.02 g/dl and to oral iron was 0.13± 0.93 g/dl[57].
4. Von wyck in 2005 compared 161 patients with oral ferrous sulphate with IV iron sucrose and observed 0.7 g/dl Hb increase in IV iron group and 0.4 g/dl Hb increase in ferrous sulphate group[58].

**Other studies assessing Hb response**

1. Gabriel Mircescu et al from 'Dr Carol Davila' Teaching Hospital of Nephrology, Bucharest, Romania treated 60 predialytic patients with Hb less than 11 g/dl and serum ferritin levels between 20 ng/ml and <500 ng/ml with 200 mg monthly IV iron sucrose for 12 months (total dose 2400 mg). Mean pre-treatment Hb was 9.7 g/dl. Mean Hb increase was 0.8 g/dl after 3 months and 1.3 g/dl after 1 year. Serum ferritin increased from 98 ng/ ml to 156 ng/ml (3 months) and 442.5 ng/ml (1 year). Serum iron increased from (73.9

μg/dl) to 74.8 μg/dl (3 months) and 442.5 μg/dl (1 year). TSAT increased from 21.6% to 24.9% (3 months) and 33.6% (1 year)[52].

2. Silverberg et al administered 200 mg monthly IV iron sucrose for 5 months (total 1000 mg) and noted 1.9 vol% increase in Hct I month after last iron dose[53] in non dialytic patients from Switzerland.

**Iron and hepatitis C infection**

Hepcidin is synthesized by liver. Persistent hepatitis C infection causes low hepcidin due to STAT3 activation which leads to accumulation of iron in liver and augments liver injury (fibrosis, cirrhosis and hepato cellular carcinoma). Iron removal by phlebotomy improves liver function tests and liver histology. Iron depletion also augments HCV eradication by antiviral therapy and decreases the development of hepato cellular carcinoma (HCC)[44].

**Adjuvant therapies**

- Before the advent of ESAs androgens were used to treat anemia which are not advocated now.
- Routine use of vitamins B, C,D, L-Carnitine and pentoxyphylline are not advocated now.

**Blood transfusions**

Blood transfusions should be avoided except in urgent indications like blood loss, urgent preoperative corrections and unstable coronary artery disease to avoid blood borne infections and also to prevent sensitization in patients on renal transplant work up.

**Management plan for anemia in CKD patients**

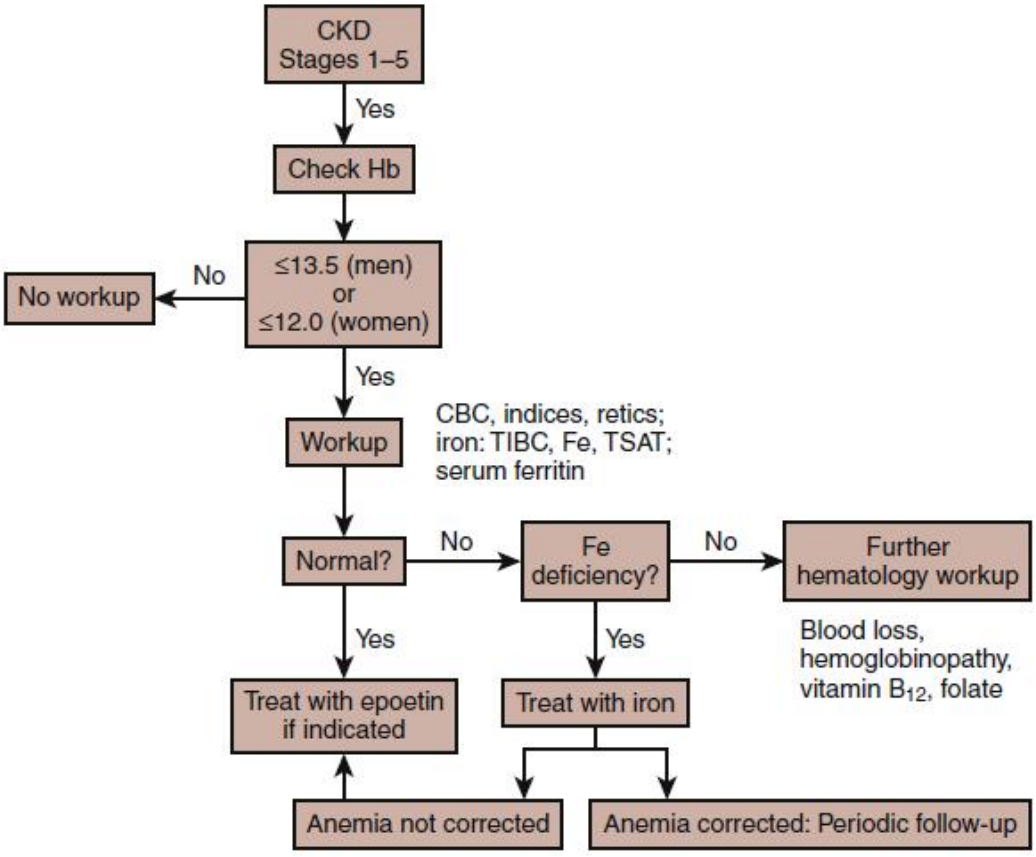



**Figure - 6 : Management protocol for CKD**

# MATERIALS AND METHODS

**Study design:**

Prospective study.

**Study Place :**

Kilpauk Medical College Hospital

**Study Population**

**INCLUSION CRITERIA:**

- All CKD patients more than 18 years with
  1. GFR < 60 ml/min/1.73 m2.
  2. Hb < 10 g/dl.

**EXCLUSION CRITERIA:**

- Patients with active infections/ active bleeding**.**
- Patients with recent myocardial infarction
- Patients with congestive cardiac failure.
- Patients with previous H/O allergic reactions to IV iron sucrose.
- Patients on maintenance hemodialysis.
- Patients on oral iron therapy or erythropoietin therapy.
- Unwilling patients.

**Sample size : 50**

**Methodology:**

50 newly detected CKD patients more than 18 years who attended nephrology outpatient department and admitted in medical and nephrology wards in Govt. Kilpauk Medical College, Chennai were included in this study after obtaining institutional ethical committee permission from the period between December 2014- February 2015. They were explained about the procedure and informed consent was obtained. GIT bleeding was ruled out by doing stool for occult blood test. Infections were ruled out by doing CBC, X- ray chest, urine and blood C&S tests. 10 injections of iron sucrose were administered daily for 10 days in 100 ml NS over 1 hr. Pre-treatment Hb and Iron indices (serum ferritin, serum iron, Total iron binding capacity and TSAT%(calculated) were compared with 1 month post treatment values. Three patients developed minor allergic reactions (rigors and itching) which were treated with antihistamines. They were withdrawn from the study. 2 discontinued treatment due to personal reasons. Five patients didn't return for follow up study in spite of many reminders through mobile phones. At the end 40 patients were assessed the Hb and iron indices response to IV iron sucrose.

Quantitative determination of serum iron.

Principle – colorimetry

Reagent - ferrozine (spinreact)

Iron is dissociated from Transferrin- iron complex in weakly acid medium. Liberated $Fe^{3+}$ is reduced to $Fe^{2}+$ which gives Ferrozine, the reagent used, a coloured complex.

Transferrin $(Fe^{3+})_2 + e^- \rightarrow 2\ Fe^{2+}$ + transferrin

$Fe^{2+} \rightarrow$ coloured complex

Total iron binding capacity

Principle – saturation – precipitation

Serum transferrin is saturated with an excess of $Fe^{2+}$ and the unbound portion is precipitated with magnesium carbonate. Total amount of iron is then determined. Difference between total iron binding capacity and initial serum iron yields unsaturated iron binding capacity.

Ferritin

Principle – enzyme immune assay.

Reagent – pathozyme ferritin (omega diagnostic)

Specific anti-Ferritin antibodies are coated on to microtitration wells. Test sera are applied. Then monoclonal anti – Ferritin labelled with Horseadish peroxide enzyme is added. Ferritin if present is sandwitched between Ab in the wells and Abs labelled with enzymes.

TSAT is calculated from serum Iron and TIBC

TSAT = serum Iron ×100 ÷TIBC

eGFR was calculated using MDRD formula.

Conflict of interest: Nil

Financial grants: Nil

Statistical Analysis: was done using t test, paired t test, chi-square test and ANOVA.

# RESULTS

## ASSESSMENT OF Hb RESPONSE TO INTRAVENOUS IRON THERAPY

Pre-Treatment & Post-Treatment Hb

**Paired samples t-test**

| | |
|---|---|
| Sample 1 | Hb_pre_<br>Hb(pre) |
| Sample 2 | Hb_post_<br>Hb(post) |

| | Sample 1 | Sample 2 |
|---|---|---|
| Sample size | 40 | 40 |
| Arithmetic mean | 7.4500 | 8.2625 |
| 95% CI for the mean | 7.0449 to 7.8551 | 7.8159 to 8.7091 |
| Variance | 1.6041 | 1.9496 |
| Standard deviation | 1.2665 | 1.3963 |
| Standard error of the mean | 0.2003 | 0.2208 |

**Paired samples t-test**

| | |
|---|---|
| Mean difference | 0.8125 |
| Standard deviation | 0.7858 |
| 95% CI | 0.5612 to 1.0638 |
| Test statistic t | 6.539 |
| Degrees of Freedom (DF) | 39 |
| Two-tailed probability | P < 0.0001 |

Mean pretreatment Hb is 7.45 g/dl while mean post treatment Hb is 8.26 g/dl.

Maximum pre-treatment Hb is 9.7 g/dl. Maximum post treatment Hb is 10.8 g/ dl.

Minimum pre-treatment Hb is 5 g/ dl while minimum post treatment Hb level is 5.1 g/ dl.

Median pre-treatment Hb level is 7.45 g/ dl while median post-treatment Hb level is 8.3 g/ dl.

One month after administration of 1000 mg Inj. Iron Sucrose there is increase in Hb which is statistically significant ($p < 0.0001$). . Mean post-treatment increase in Hb is 0.8 g/dl.

**Description of dot and line diagram**

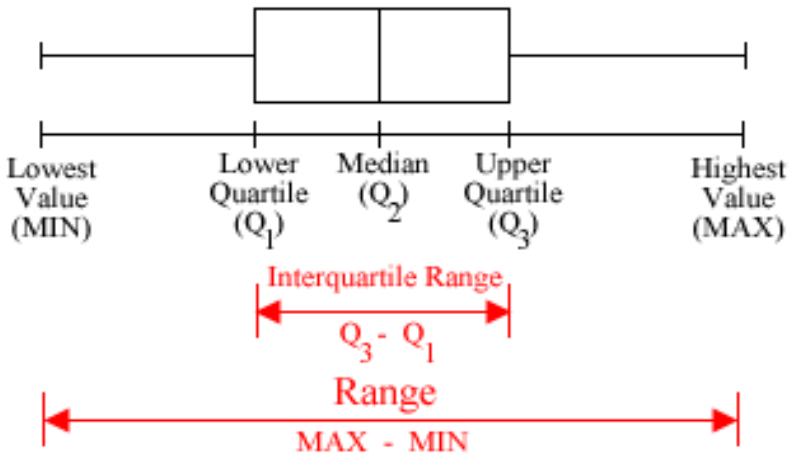



**Figure - 7 : Graphical representation of dot and line diagram.**

**DOT AND LINE DIAGRAM**

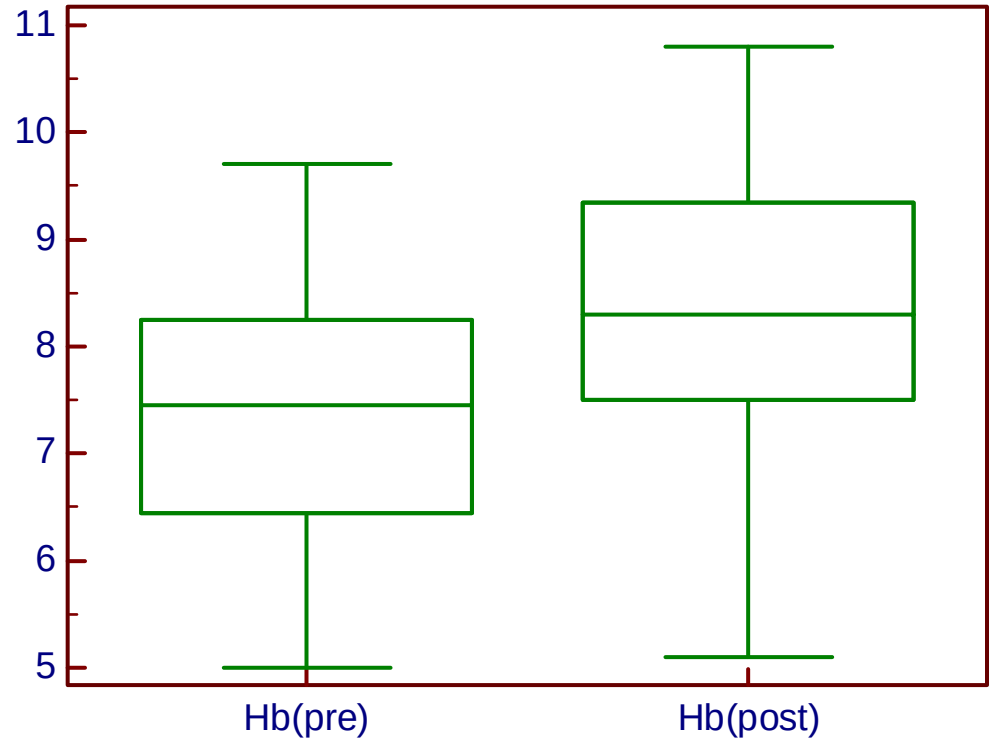



**Figure – 8 : Dot and line diagram showing minimum, maximum and median pre-treatment and post-treatment Hb values.**

**DISTRIBUTION OF Hb INCREASE**

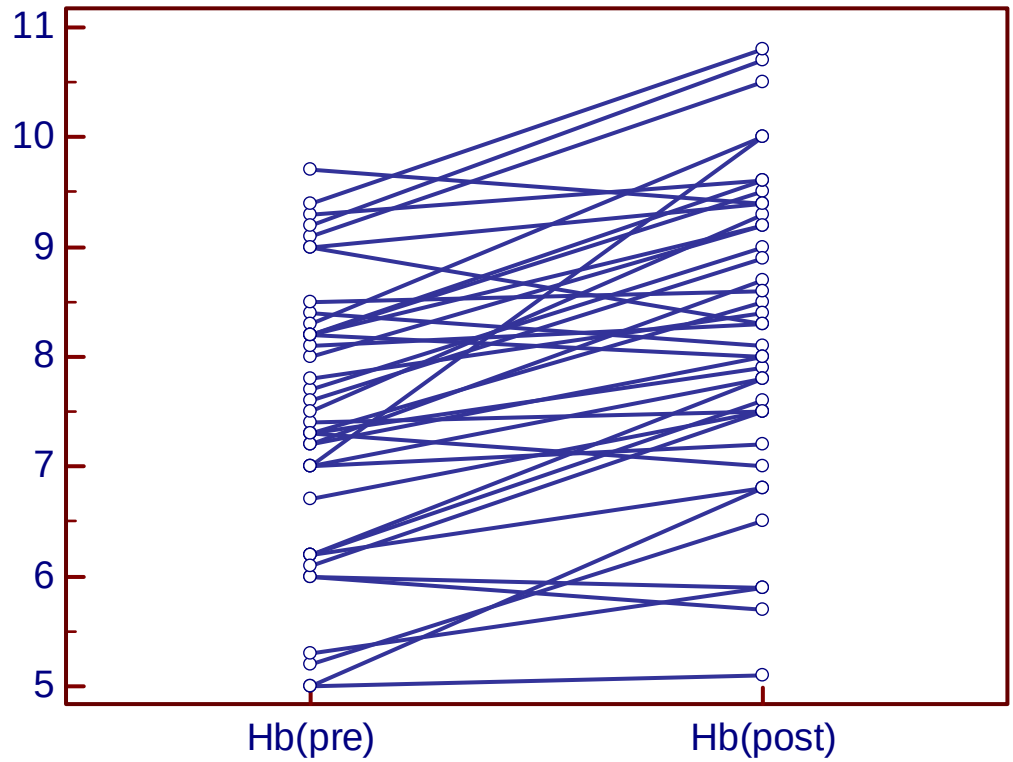



**Figure – 9: Distribution of Hb increase**

## AGE GROUP WISE Hb INCREASE

For statistical analysis patients are divided into 4 age groups.

Group 1 – age < 40 years

Group 2 – age 41-50 years

Group 3 – age 51- 60 years

Group 4 – age 61- 70 years

Mean age – 55.825 years

Median age – 58 years

Maximum age – 70 years

Minimum age – 19 years

| | | | | | | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|---|
| | | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound |
| Hb diff. | 1 | 4 | 1.175 | .8655 | .4328 | -.202 | 2.552 |
| | 2 | 7 | .829 | .7296 | .2758 | .154 | 1.503 |
| | 3 | 12 | .817 | .9203 | .2657 | .232 | 1.401 |
| | 4 | 17 | .644 | .8054 | .1953 | .230 | 1.058 |
| | Total | 40 | .781 | .8182 | .1294 | .520 | 1.043 |

**Table - 2**

There are 4 patients aged less than 40 years. Hb increase is more in the age group < 40 years. Mean Hb increase in the age group < 40 years is 1.175 g/dl.

There are 7 patients in the age group between 41 – 50 years. 12 patients are in the age group between 51 – 60 years. The mean Hb increase in the age groups 41-50 years and 51-60 years come in between. The mean Hb increases in the age groups 41—50 years is 0.83 g/dl and mean Hb increase in the age group between 51 – 60 years is 0.82 g/dl.

Patients aged more than 61 years form the major part of study group. There are 17 patients in the age group more than 61 years. The mean Hb increase is lower in the age group between 61-70 years. The mean Hb increase in the age group between 61 years to 70 years is 0.644 g/dl.

**Correlation of age & Hb response**

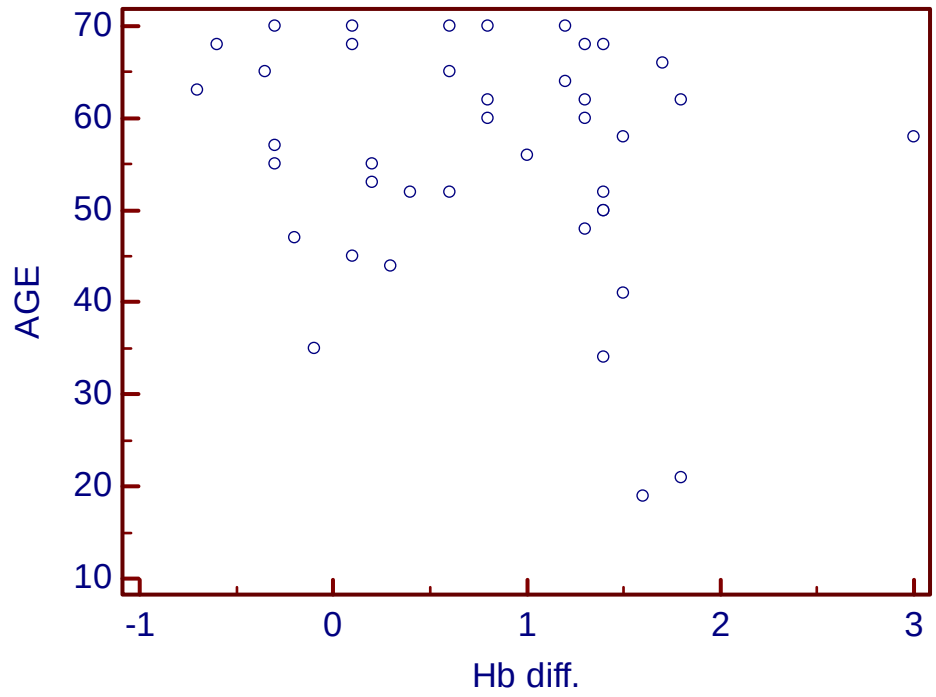
| | |
|---|---|
| Variable Y | AGE |
| Variable X | Hb_diff.<br>Hb diff. |

| | |
|---|---|
| Sample size | 40 |
| Correlation coefficient r | -0.2053 |
| Significance level | P=0.2038 |
| 95% Confidence interval for r | -0.4857 to 0.1135 |

Age group wise mean Hb increase is statistically not significant (p-0.2038).

**Figure – 10 : Distribution of age group wise Hb increase**

**Scatter diagram**



The above scatter diagram clearly shows more number of patients in the age group more than 61 years and less number of patients in the age group less than 40 years. Age groups 41-50 years and 51-60 years come in between.

The scatter diagram also shows the Hb difference distribution. Seven patients have reduced post treatment Hb level. Fifteen patients have less than 1 g/dl increase in Hb level. Seventeen patients have 1-2 g/dl increase in Hb levels. One patient has 3 g/dl increase in Hb level.

One patient in the age group less than 40 year has reduced post treatment Hb level. One patient in the age group 41- 50 years, one patient in the age group 51 – 60 years and four patients more than 61 years also have reduced post treatment Hb levels. Two patients more than 61 years have more than 0.5 g/dl decrease in Hb level.

Two patients in the age group 41-50 years, six patients in the age group 51-60 years and seven patients have upto 1 g/dl increase in Hb levels.

Three patients below 40 years have mean Hb increase of 1-2 g/dl. Two patients in the age group 41-50 years, four patients in the age group 51-60 years and eight patients in the age group more than 61 years have 1-2 g/dl increase in Hb level.

One patient in the age group 51-60 year has 3 g/dl increase in Hb level.

**MEANS PLOTS**

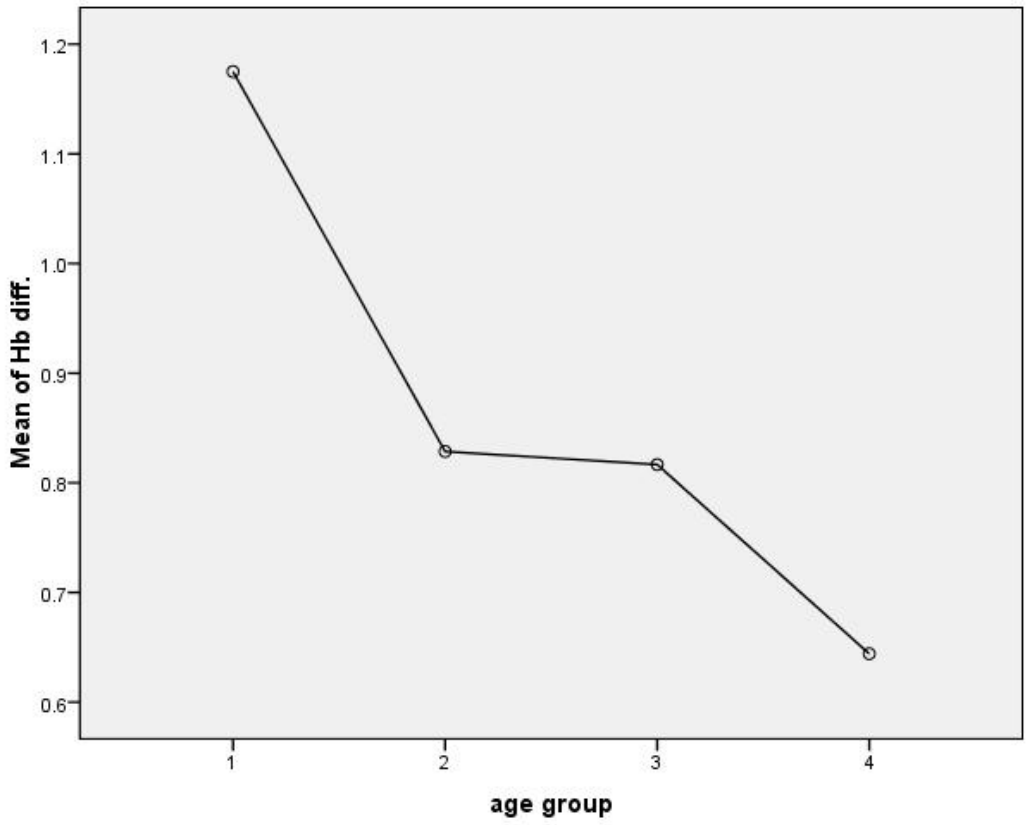



**Figure - 11**

Group 1 - < 40 years

Group 2 – 41-50 years

Group 3 – 51-60 years

Group 4 – 61- 70 years

The above mean plot diagram shows highest mean Hb increase in age group less than 40 years, lowest Hb increase in age group more than 61 years and intermediate Hb increase in age groups 41-60 years.

**SEX WISE Hb INCREASE**

1- Male

2- Female

| | SEX | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Hb diff. | 1 | 24 | .725 | .7450 | .1521 |
| | 2 | 16 | .866 | .9364 | .2341 |
| DIFF | 1 | 24 | 6.17 | 6.790 | 1.386 |
| | 2 | 16 | 2.76 | 18.897 | 4.724 |

**Table - 3**

There are 24 males and 16 females. The mean Hb increase is more in females comparing to males. The mean increase in Hb males is 0.725 g/dl while in females it is 0.866 g/dl.

Hb increase between males and females is statistically not significant.

**CKD STAGEWISE Hb INCREASE**

**SERUM CREATININE**

Mean – 4.825 mg/ dl

Median – 5.2 mg/ dl

Maximum – 6 mg/ dl

Minimum – 1.7 mg/ dl

| | | | | | | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|---|
| | | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound |
| Hb diff. | 3 | 1 | 1.700 | . | . | . | . |
| | 4 | 11 | .791 | .8080 | .2436 | .248 | 1.334 |
| | 5 | 28 | .745 | .8321 | .1573 | .422 | 1.067 |
| | Total | 40 | .781 | .8182 | .1294 | .520 | 1.043 |
| DIFF | 3 | 1 | 15.90 | . | . | . | . |
| | 4 | 11 | 6.69 | 5.553 | 1.674 | 2.96 | 10.42 |
| | 5 | 28 | 3.67 | 14.934 | 2.822 | -2.12 | 9.46 |
| | Total | 40 | 4.80 | 12.938 | 2.046 | .67 | 8.94 |

**Table - 4 : CKD STAGES 3, 4 AND 5.**

**eGFR**

Mean – 13.4275 ml/ min/ 1.73 $m^2$

Median – 11.45 ml/ min/ 1.73 $m^2$

Maximum – 32 ml/ min/ 1.73 $m^2$

Minimum – 7.8 ml/ min/ 1.73 $m^2$

There is only one patient in CKD stage 3. Eleven patients are in CKD stage 4 and twenty two patients are in CKD stage 5. Hb increase in CKD stage 3 is 1.7 g/dl. Mean Hb increase is 0.8 g/dl and 0.74 g/dl in CKD stages 4 and 5 respectively.

| | | | |
|---|---|---|---|
| | | **Minimum** | **Maximum** |
| Hb diff. | 3 | 1.7 | 1.7 |
| | 4 | -.7 | 1.8 |
| | 5 | -.6 | 3.0 |
| | Total | -.7 | 3.0 |
| DIFF | 3 | 16 | 16 |
| | 4 | -1 | 15 |
| | 5 | -59 | 19 |
| | Total | -59 | 19 |

**Table - 5**

The minimum Hb increase is –0.7 g/dl in CKD stage 4 and -0.6 g/dl in CKD stage 5. The maximum Hb increase is 1.8 g / dl in CKD stage 4 and 3 g/dl in CKD stage 5.

**MEANS PLOT DIAGRAM**

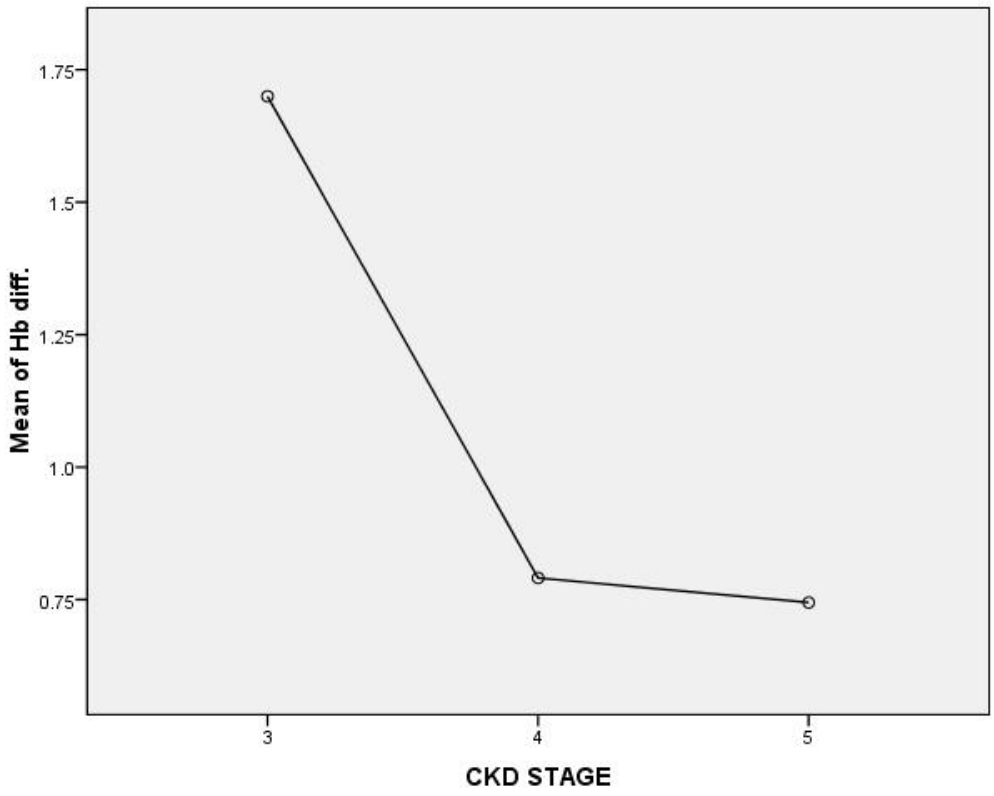



**Figure - 12 : The above graph showing more mean pre-treatment Hb increase in early stages of CKD**

## CORRELATION BETWEEN Hb INCREASE AND INCREASE IN SERUM FERRITIN

### Correlation

| | |
|---|---|
| Variable Y | Ferritin_Diff<br>Ferritin Diff |
| Variable X | Hb_diff.<br>Hb diff. |

| | |
|---|---|
| Sample size | 40 |
| Correlation coefficient r | 0.01421 |
| Significance level | P=0.9306 |
| 95% Confidence interval for r | -0.2986 to 0.3243 |

There is no statistically significant correlation between post treatment increase in Hb and post treatment increase in serum ferritin.

### Scatter diagram

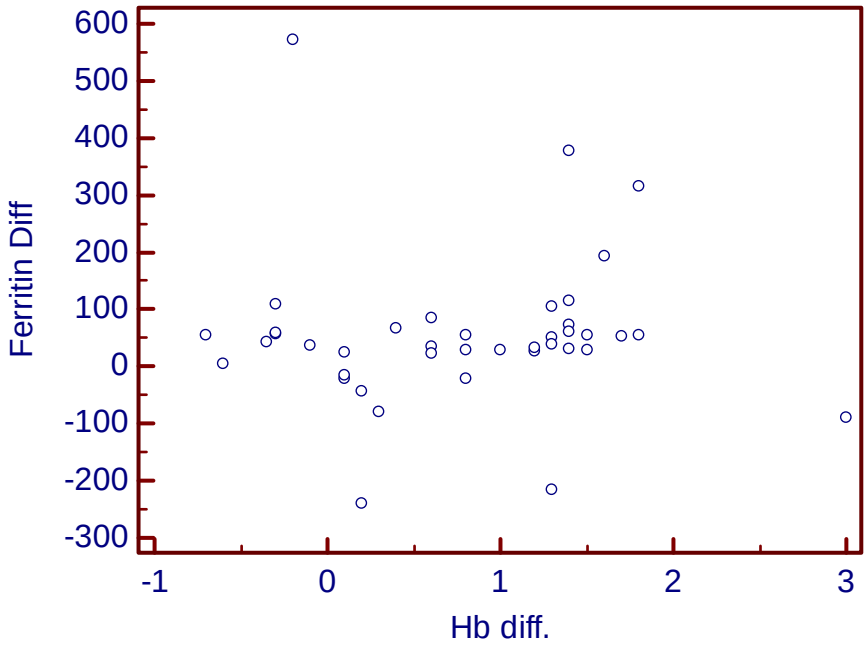



**Figure – 13 : The above scatter diagram showing more of ferritin increase than decrease. Most of the patients have upto 100 ng/ ml increase in serum ferritin levels.**

**Correlation between Hb response and TSAT% differencce**

**Correlation**

| | |
|---|---|
| Variable Y | TSATDIFF |
| Variable X | Hb_diff.<br>Hb diff. |

| | |
|---|---|
| Sample size | 40 |
| Correlation coefficient r | 0.2458 |
| Significance level | P=0.1263 |
| 95% Confidence interval for r | -0.07117 to 0.5177 |

There is no statistically significant correlation between post treatment increase in Hb and post treatment increase in TSAT%.

**Scatter diagram**

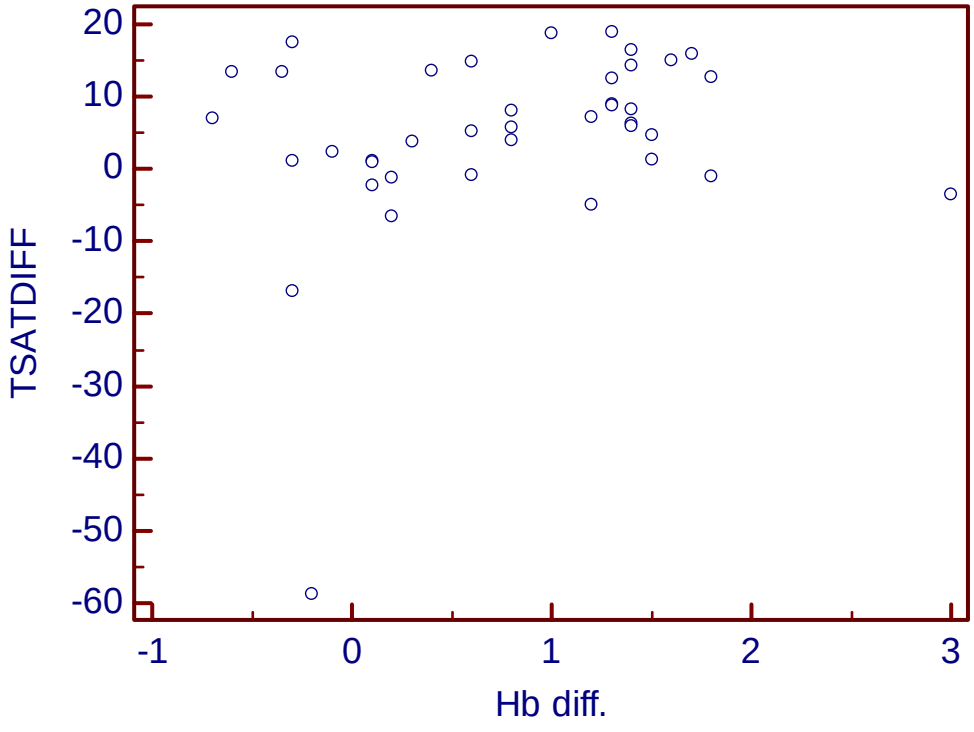



**Figure 14 The above scatter digram showing more of TSAT% increase than decrease. Most patients have 10% - 20% increase in TSAT% post-treatment.**

## ASSESSMENT OF INDIVIDUAL IRON INDICES RISE

### Assessment of Serum ferritin

**Paired samples t-test**

| | |
|---|---|
| Sample 1 | Ferritin_pre_ |
| Sample 2 | Ferritin_post<br>Ferritin post |

| | Sample 1 | Sample 2 |
|---|---|---|
| Sample size | 40 | 40 |
| Arithmetic mean | 311.0600 | 336.6200 |
| 95% CI for the mean | 241.5705 to 380.5495 | 281.4788 to 391.7612 |
| Variance | 47210.6327 | 29727.1693 |
| Standard deviation | 217.2801 | 172.4157 |
| Standard error of the mean | 34.3550 | 27.2613 |

**Paired samples t-test**

| | |
|---|---|
| Mean difference | 25.5600 |
| Standard deviation | 142.3275 |
| 95% CI | -19.9585 to 71.0785 |
| Test statistic t | 1.136 |
| Degrees of Freedom (DF) | 39 |
| Two-tailed probability | P = 0.2630 |

**SERUM FERRITIN (ng/ml)**

| | PRE-TREATMENT | POST- TREATMENT |
|---|---|---|
| Mean | 311 | 336.6 |
| Median | 233.55 | 318.4 |
| Maximum | 982.2 | 798.6 |
| Minimum | 68.8 | 82.6 |

There is increase in mean serum ferritin levels of 25.56 ng/ dl which is statistically insignificant (p = 0.2630).

**DOT AND LINE DIAGRAM**

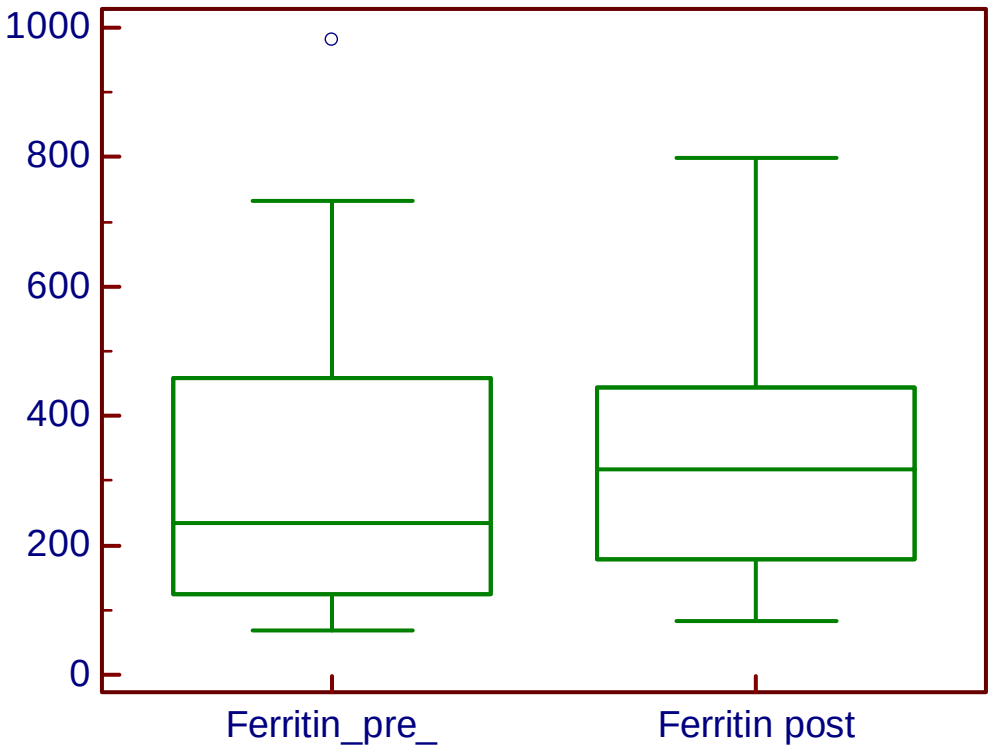



**Figure - 15 : The above diagram showing no significant ferritin increase in spite of having increase in maximum post-treatment ferritin value and median post-treatment ferritin value**

## CORRELATION OF AGE & FERRITIN DIFFERENCE

### Correlation

| | |
|---|---|
| Variable Y | AGE |
| Variable X | Ferritin_Diff<br>Ferritin Diff |

| | |
|---|---|
| Sample size | 40 |
| Correlation coefficient r | -0.3553 |
| Significance level | P=0.0245 |
| 95% Confidence interval for r | -0.6004 to -0.04923 |

There is significant correlation (p= 0.0245) between age group and post treatment ferritin rise.

## SCATTER DIAGRAM

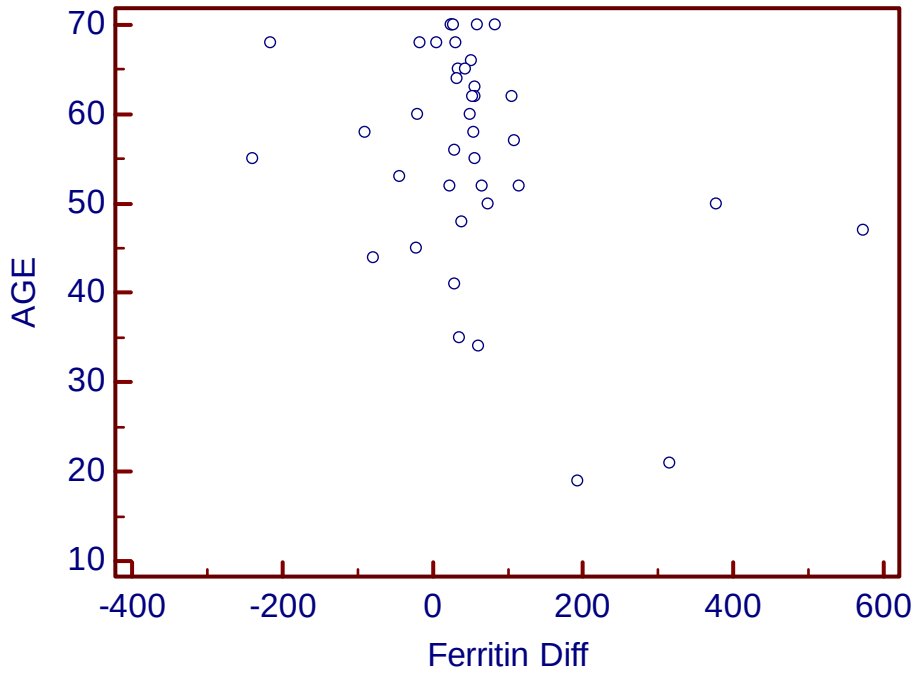



**Figure - 16 : The above scatter diagram showing upto 100 ng/ml increase in post-treatment ferritin in many patients.**

**SERUM IRON**

**Paired samples t-test**

| | |
|---|---|
| Sample 1 | serum_Iron<br>serum Iron |
| Sample 2 | Iron_post_<br>Iron(post) |

| | Sample 1 | Sample 2 |
|---|---|---|
| Sample size | 39 | 39 |
| Arithmetic mean | 120.5026 | 134.0795 |
| 95% CI for the mean | 104.1450 to 136.8601 | 122.3607 to 145.7983 |
| Variance | 2546.3055 | 1306.8896 |
| Standard deviation | 50.4609 | 36.1509 |
| Standard error of the mean | 8.0802 | 5.7888 |

**Paired samples t-test**

| | |
|---|---|
| Mean difference | 13.5769 |
| Standard deviation | 38.8589 |
| 95% CI | 0.9803 to 26.1735 |
| Test statistic t | 2.182 |
| Degrees of Freedom (DF) | 38 |
| Two-tailed probability | P = 0.0354 |

**SERUM IRON (µg/dl)**

| | **Pre-treatment** | **Post-treatment** |
|---|---|---|
| Mean | 120.5 | 134 |
| Median | 118.35 | 138 |
| Maximum | 283 | 211.8 |
| Minimum | 36.3 | 42 |

There is post treatment increase in serum iron (13.57 µg/dl) which is statistically significant (p= 0.0354).

**DOT AND LINE DIAGRAM**

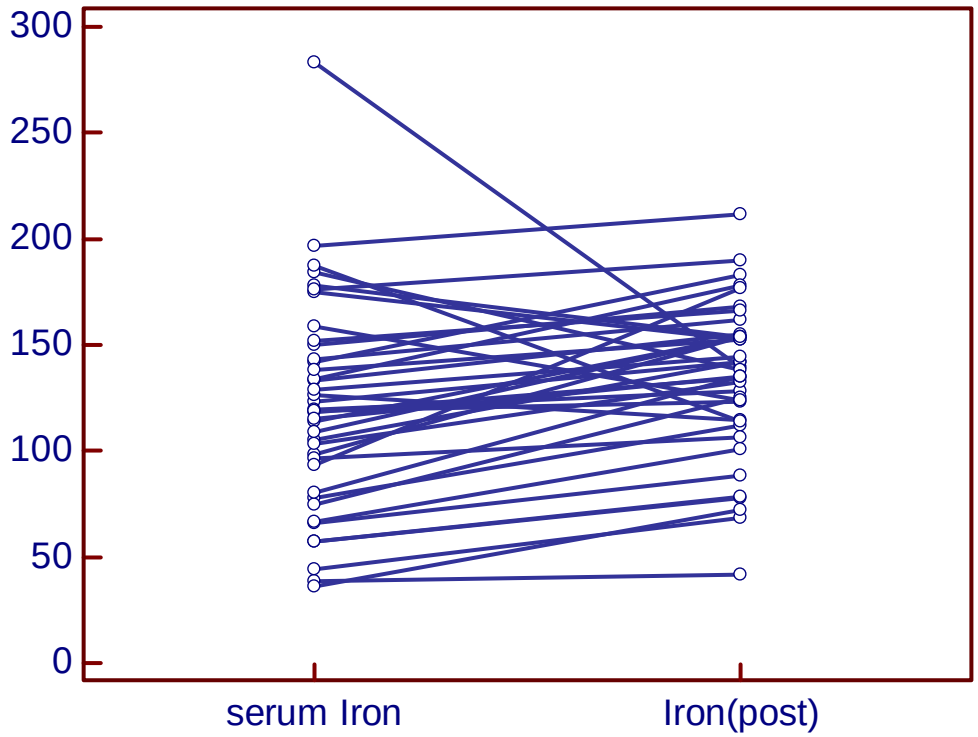



**Figure - 17 : The above diagram showing distribution of pre and post-treatment serum iron.**

**TIBC**

**Paired samples t-test**

| | |
|---|---|
| Sample 1 | TIBC_Pre_<br>TIBC(Pre) |
| Sample 2 | TIBC_post_<br>TIBC(post) |

| | Sample 1 | Sample 2 |
|---|---|---|
| Sample size | 40 | 40 |
| Arithmetic mean | 435.3593 | 405.2555 |
| 95% CI for the mean | 399.2723 to 471.4462 | 373.2151 to 437.2959 |
| Variance | 12732.1673 | 10036.8461 |
| Standard deviation | 112.8369 | 100.1841 |
| Standard error of the mean | 17.8411 | 15.8405 |

**Paired samples t-test**

| | |
|---|---|
| Mean difference | -30.1037 |
| Standard deviation | 73.0883 |
| 95% CI | -53.4785 to -6.7290 |
| Test statistic t | -2.605 |
| Degrees of Freedom (DF) | 39 |
| Two-tailed probability | P = 0.0129 |

**TIBC (µg/ dl)**

| | **PRE-TREATMENT** | **POST-TREATMENT** |
|---|---|---|
| Mean | 435.3 | 405.2 |
| Median | 412 | 384.2 |
| Maximum | 765.9 | 652.8 |
| Minimum | 217.2 | 196.8 |

There is post treatment decrease in TIBC (30.1 µg/ dl) which is statistically significant (p= 0.0129).

**DOT AND LINE DIAGRAM**

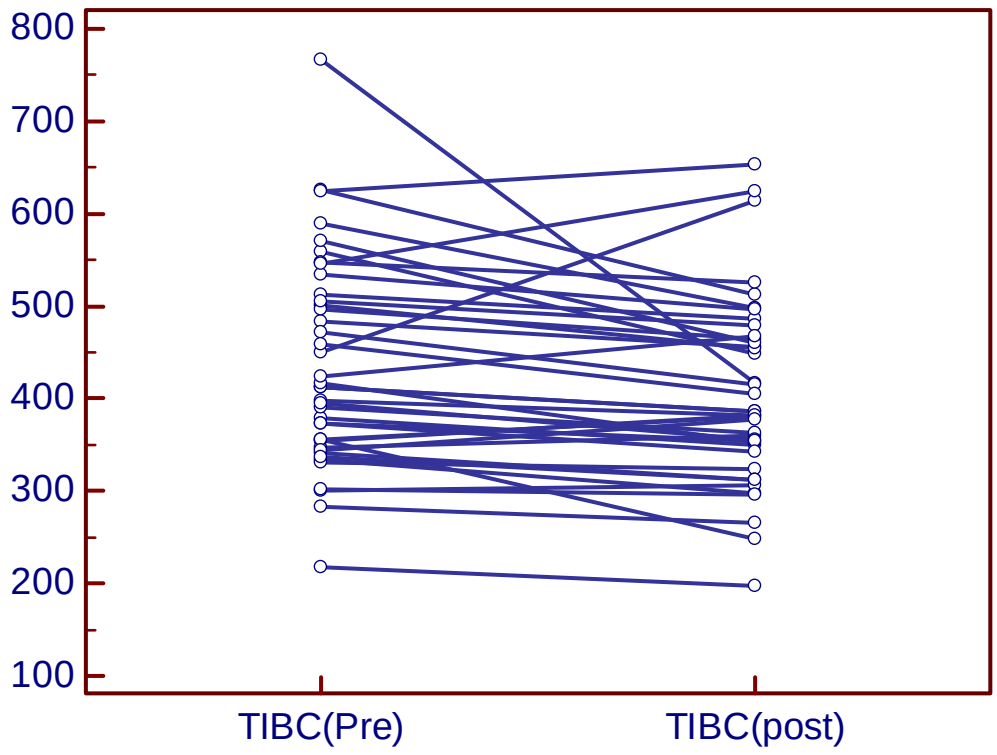



**Figure - 18 : The above diagram showing distribution of pre, post-treatment TIBC.**

**TSAT%**

**Paired samples t-test**

| | |
|---|---|
| Sample 1 | TSAT%_pre_ TSAT%(pre) |
| Sample 2 | TSAT%_post_ TSAT%(post) |

| | Sample 1 | Sample 2 |
|---|---|---|
| Sample size | 40 | 40 |
| Arithmetic mean | 29.1975 | 33.9665 |
| 95% CI for the mean | 25.4102 to 32.9848 | 30.7912 to 37.1418 |
| Variance | 140.2331 | 98.5741 |
| Standard deviation | 11.8420 | 9.9285 |
| Standard error of the mean | 1.8724 | 1.5698 |

**Paired samples t-test**

| | |
|---|---|
| Mean difference | 4.7690 |
| Standard deviation | 13.4142 |
| 95% CI | 0.4789 to 9.0591 |
| Test statistic t | 2.248 |
| Degrees of Freedom (DF) | 39 |
| Two-tailed probability | P = 0.0303 |

**TSAT%**

| | **PRE-TREATMENT** | **POST- TREATMENT** |
|---|---|---|
| Mean | 29.19 | 33.9 |
| Median | 28.7 | 33.35 |
| Maximum | 76 | 60.4 |
| Minimum | 10.8 | 12.9 |

There is post treatment increase in TSAT% (4.769) which is statistically significant (p= 0.0303).

**Correlation**

| | |
|---|---|
| Variable Y | AGE |
| Variable X | TSATDIFF |

| | |
|---|---|
| Sample size | 40 |
| Correlation coefficient r | 0.05729 |
| Significance level | P=0.7255 |
| 95% Confidence interval for r | -0.2588 to 0.3623 |

There is no statistically significant (p= 0.7255) age related TSAT rise.

**SCATTER DIAGRAM**

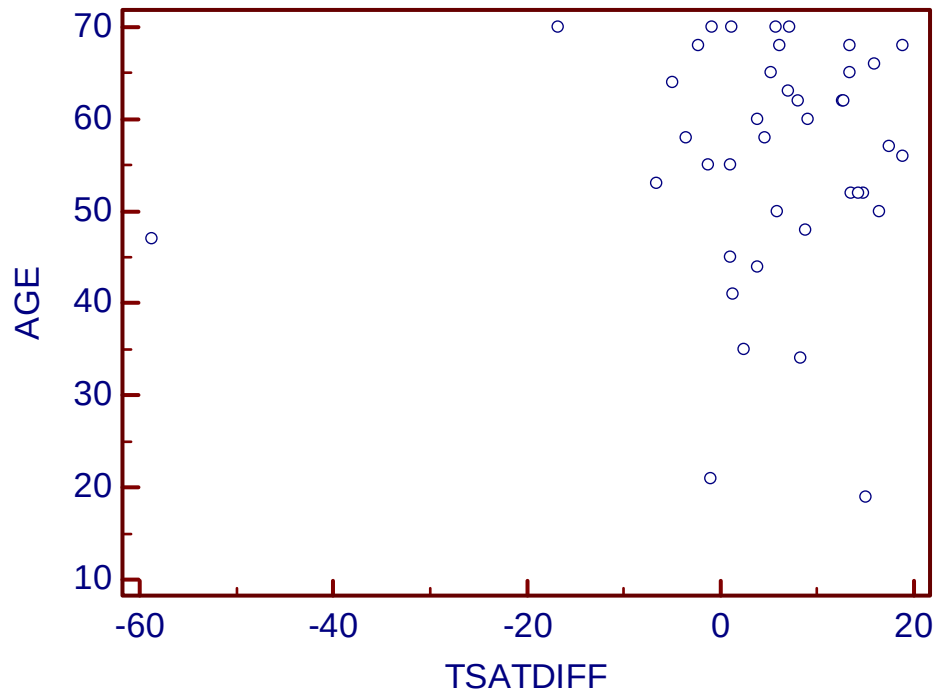



**Figure - 19 : The above scatter diagram showing 10-20% increase in post-treatment TSAT in many patients.**

**DOT AND LINE DIAGRAM**

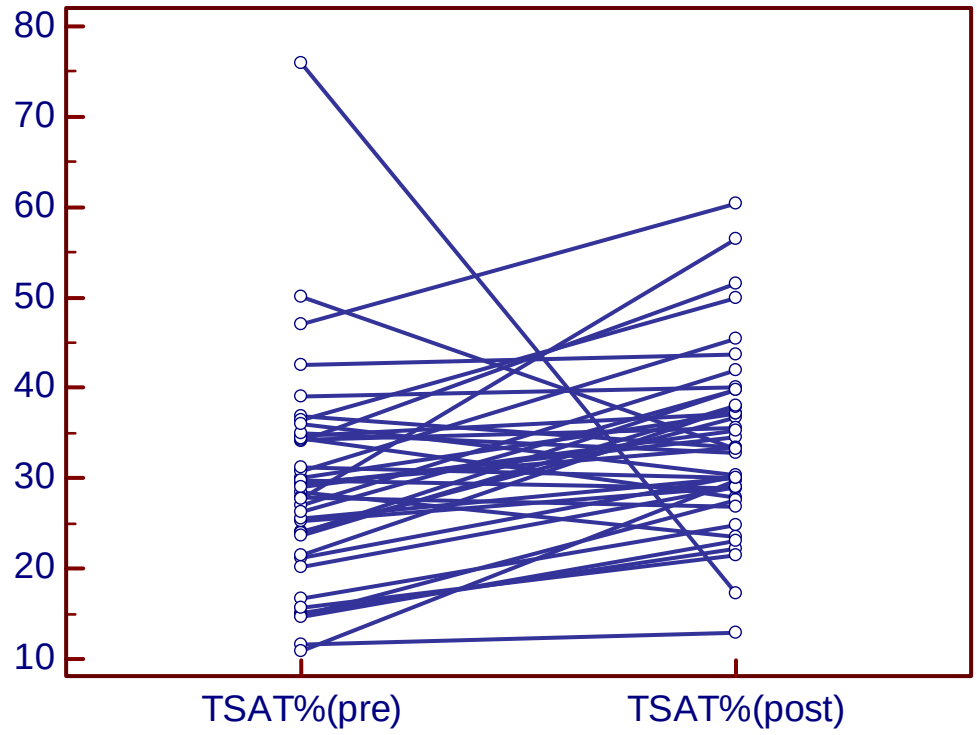



**Figure - 20**

**CORRELATION OF Hb RESPONSE AND PRE-TREATMENT IRON INDICES**

**Correlation between pre-treatment serum ferritin and Hb response**

To assess the correlation between pre-treatment serum ferritin and Hb response patients were divided into three groups according to pre-treatment serum ferritin levels.

1. Serum ferritin levels less than 200 ng/ml.
2. Serum ferritin levels between 200 – 500 ng/ml.
3. Serum ferritin levels more than 500 ng/ml

**Descriptive**

Hb diff.

| | | | | | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound |
| 1 | 17 | 1.1059 | .74117 | .17976 | .7248 | 1.4870 |
| 2 | 15 | .7433 | .86373 | .22301 | .2650 | 1.2216 |
| 3 | 8 | .1625 | .53436 | .18892 | -.2842 | .6092 |
| Total | 40 | .7813 | .81820 | .12937 | .5196 | 1.0429 |

**Table - 6**

**Descriptive**

Hb diff.

| | | |
|---|---|---|
| | Minimum | Maximum |
| 1 | -.70 | 1.80 |
| 2 | -.35 | 3.00 |
| 3 | -.30 | 1.30 |
| Total | -.70 | 3.00 |

**Table – 7**

**ANOVA**

Hb diff.

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 4.876 | 2 | 2.438 | 4.248 | .022 |
| Within Groups | 21.232 | 37 | .574 | | |
| Total | 26.108 | 39 | | | |

**Table - 8**

There are 17 patients with ferritin levels less than 200 ng/ml, 15 patients with ferritin levels between 200-500 ng/ml and 8 patients with ferritin levels more than 500 ng/ ml.

Mean increase in Hb is 1.1 g/ dl, 0.74 g/ dl and 0.16 g/ dl in groups 1, 2 and 3.

**Means Plots**

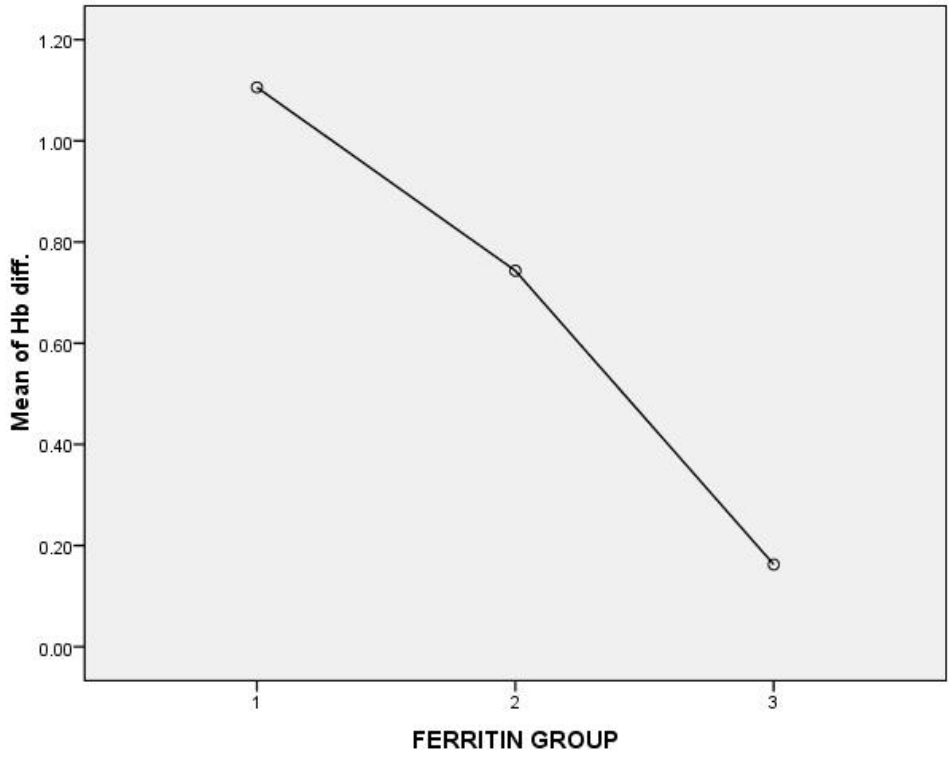



**Figure - 21 : The above means plot diagram clearly shows the increase in Hb response in patients with lower pre-treatment ferritin levels.**

**Correlation between pretreatment serum iron and Hb response**

For statistical purposes patients are divided into 2 groups depending on pre-treatment serum iron levels.

Group 1 – pre-treatment serum iron below the reference value (< 175 μg/dl in males and < 150 μg/dl in females).

Group 2 – pre-treatment serum ron above 175 μg/dl in males and > 150 μg/dl in females.

**Descriptives**

Hb diff.

| | | | | | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound |
| 1 | 33 | .7909 | .72860 | .12683 | .5326 | 1.0493 |
| 2 | 7 | .7357 | 1.23177 | .46557 | -.4035 | 1.8749 |
| Total | 40 | .7812 | .81820 | .12937 | .5196 | 1.0429 |

**Table 9**

**Descriptives**

Hb diff.

| | | |
|---|---|---|
| | Minimum | Maximum |
| 1 | -.70 | 1.80 |
| 2 | -.35 | 3.00 |
| Total | -.70 | 3.00 |

**Table - 10**

**ANOVA**

Hb diff.

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | .018 | 1 | .018 | .026 | .874 |
| Within Groups | 26.091 | 38 | .687 | | |
| Total | 26.108 | 39 | | | |

**Table – 11**

There are 33 patients within the reference value of serum Iron. 7 patients are having serum Iron above reference values.

**Means Plots**

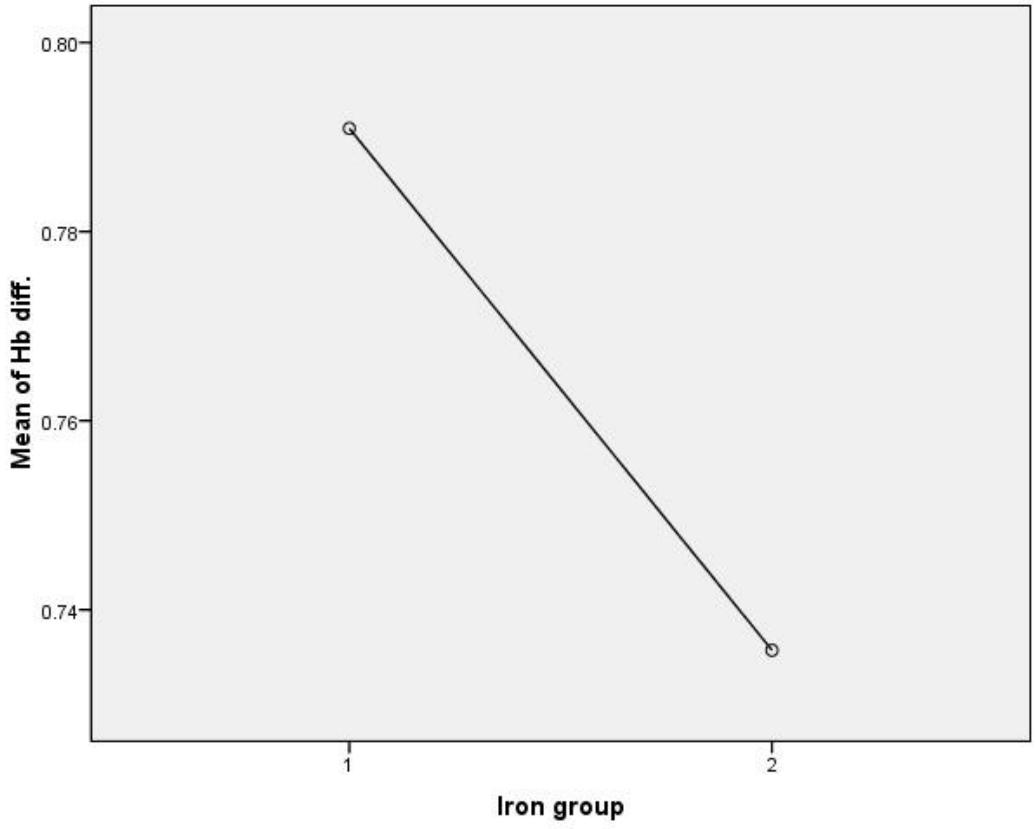



**Figure - 22 : The above diagram clearly showing the inverse relationship between pre-treatment serum iron and Hb response**

**Correlation between pre-treatment TIBC and Hb response**

For statistical analysis patients are divided into 2 groups depending upon the pre-treatment TIBC.

Group 1 – pre-treatment TIBC in the reference value (200-400 μg/dl).

Group 2 – pre-treatment TIBC above 400 μg/dl .

**Descriptives**

Hb diff.

| | | | | | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound |
| 1 | 19 | .5263 | .77520 | .17784 | .1527 | .9000 |
| 2 | 21 | 1.0119 | .80435 | .17552 | .6458 | 1.3780 |
| Total | 40 | .7812 | .81820 | .12937 | .5196 | 1.0429 |

**Table 12**

**Descriptives**

Hb diff.

| | | |
|---|---|---|
| | Minimum | Maximum |
| 1 | -.70 | 1.60 |
| 2 | -.35 | 3.00 |
| Total | -.70 | 3.00 |

**Table – 13**

**ANOVA**

Hb diff.

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 2.352 | 1 | 2.352 | 3.762 | .060 |
| Within Groups | 23.756 | 38 | .625 | | |
| Total | 26.108 | 39 | | | |

**Table – 14**

There are 19 patients having TIBC within reference value and 21 patients having TIBC above reference value. Mean Hb increase in group is less (0.52 g/ dl) than in group 2(1.01 g/dl).

**Means Plots**

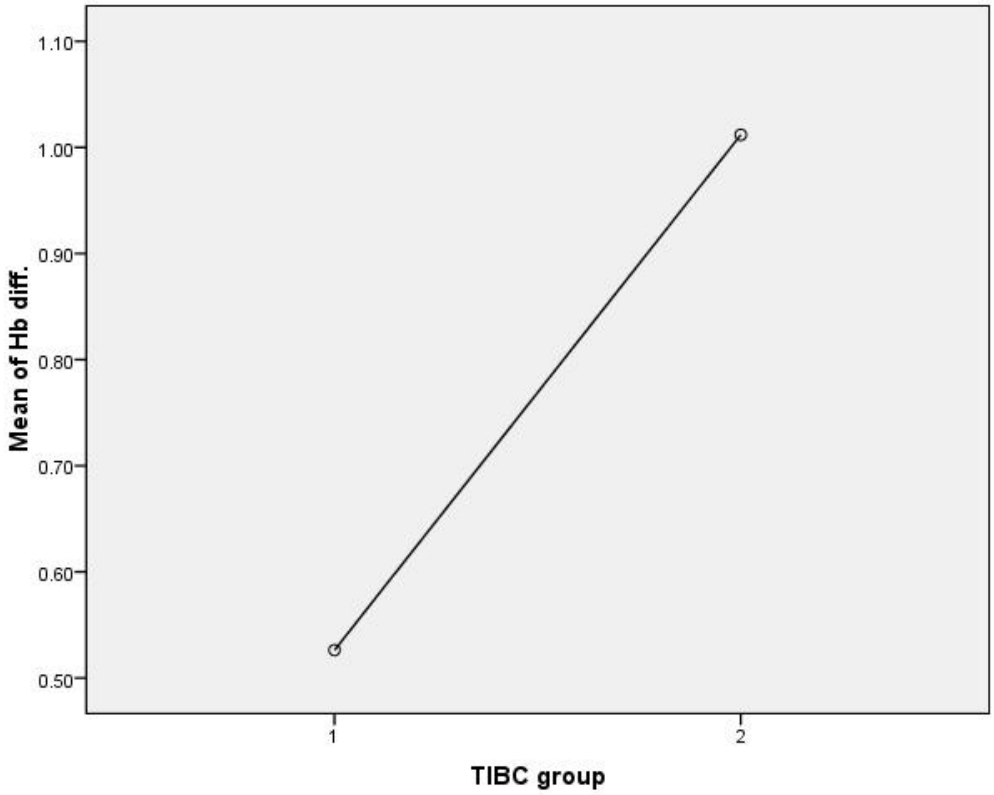



**Figure - 23 : The above means plot diagram showing direct relationship between Hb response and pre-treatment TIBC.**

**Correlation between Hb response and pre-treatment TSAT**

To assess the correlation between pre-treatment serum ferritin and Hb response patients were divided into three groups according to pre-treatment TSAT levels.

1. TSAT levels less than 20%
2. TSAT levels between 20% – 30%
3. TSAT levels more than 30%

**Descriptives**

Hb diff.

| | | | | | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound |
| 1 | 7 | 1.3429 | .30472 | .11518 | 1.0610 | 1.6247 |
| 2 | 17 | 1.1529 | .45705 | .11085 | .9179 | 1.3879 |
| 3 | 16 | .1406 | .85969 | .21492 | -.3175 | .5987 |
| Total | 40 | .7813 | .81820 | .12937 | .5196 | 1.0429 |

**Table 16**

**Descriptives**

Hb diff.

| | | |
|---|---|---|
| | Minimum | Maximum |
| 1 | .80 | 1.80 |
| 2 | .30 | 1.80 |
| 3 | -.70 | 3.00 |
| Total | -.70 | 3.00 |

Table - 17

7 patients have pre-treatment TSAT <20%, 17 patients have 20-30% and 16 patients have pre-treatment TSAT > 30%. In patients with pretreatment TSAT < 20% there is greater increase in post treatment Hb (mean increase 1.34 g/dl) , patients with TSAT 20-30% have 1.15 g/dl increase whereas in patients with pretreatment TSAT > 30% there is not much increase in post treatment HB (mean difference 0.14 g/dl).

Hb diff.

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 11.123 | 2 | 5.561 | 13.731 | .000 |
| Within Groups | 14.986 | 37 | .405 | | |
| Total | 26.108 | 39 | | | |

**Table – 18 : ANOVA**

## Means Plots

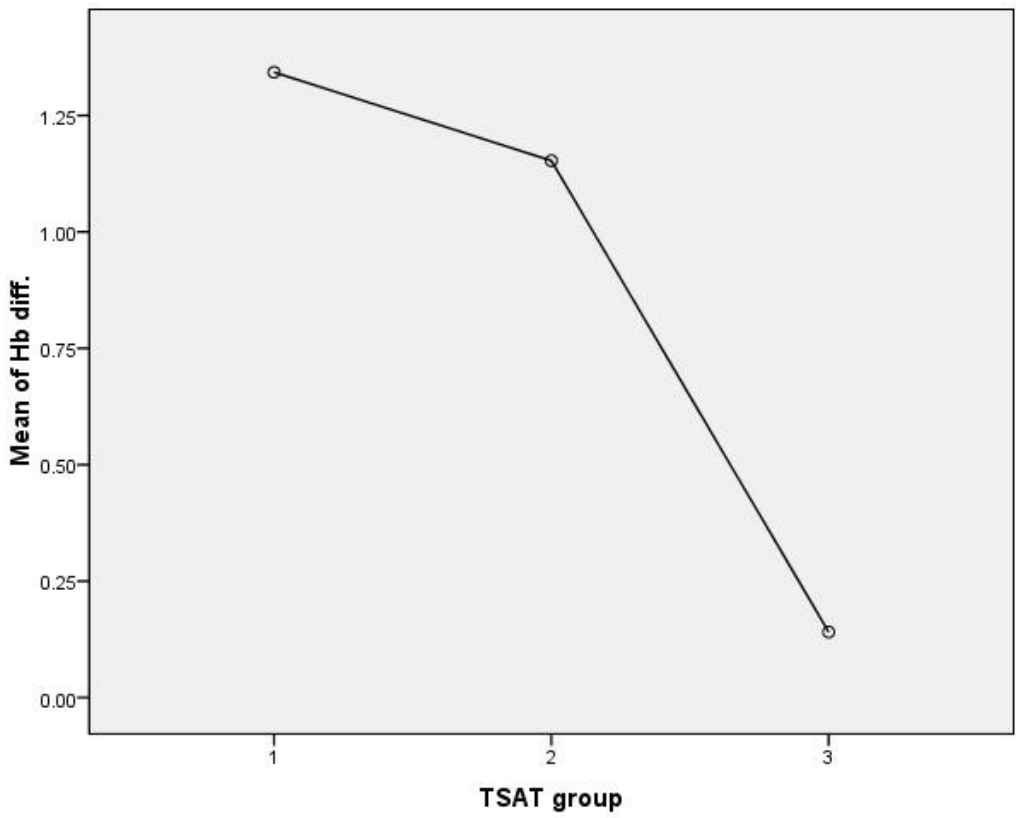



**Figure – 24 : There is inverse relationship between Hb response and pre-treatment TSAT.**

**Correlation of Hb response with pre-treatment serum ferritin and TSAT levels**

For statistical analysis patients are divided into 9 groups depending upon pre-treatment serum ferritin and TSAT levels.

| groups | Pre-treatment serum ferritin(ng/ml) | Pre-treatment TSAT |
|---|---|---|
| Group 1 | < 200 | <20% |
| Group 2 | <500 | <30% |
| Group 3 | >500 | >30% |
| Group 4 | <200 | <30% |
| Group 5 | <200 | >30 |
| Group 6 | <500 | <20% |
| Group 7 | <500 | >30% |
| Group 8 | >500 | <20% |
| Group 9 | >500 | <30% |

**Table – 19**

Hb diff.

| | | | | | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | N | Mean | Std. Deviation | Std. Error | Lower Bound | Upper Bound |
| 1 | 4 | 1.3750 | .41932 | .20966 | .7078 | 2.0422 |
| 2 | 6 | .9500 | .35637 | .14549 | .5760 | 1.3240 |
| 3 | 5 | -.1400 | .20736 | .09274 | -.3975 | .1175 |
| 4 | 9 | 1.4667 | .18708 | .06236 | 1.3229 | 1.6105 |
| 5 | 4 | .0250 | .78475 | .39238 | -1.2237 | 1.2737 |
| 6 | 2 | 1.3000 | .14142 | .10000 | .0294 | 2.5706 |
| 7 | 7 | .4071 | 1.16348 | .43975 | -.6689 | 1.4832 |
| 8 | 1 | 1.3000 | . | . | . | . |
| 9 | 2 | .3500 | .07071 | .05000 | -.2853 | .9853 |
| Total | 40 | .7813 | .81820 | .12937 | .5196 | 1.0429 |

**Table - 20**

**Descriptives**

Hb diff.

| | | |
|---|---|---|
| | Minimum | Maximum |
| 1 | .80 | 1.80 |
| 2 | .60 | 1.50 |
| 3 | -.30 | .20 |
| 4 | 1.30 | 1.80 |
| 5 | -.70 | .80 |
| 6 | 1.20 | 1.40 |
| 7 | -.35 | 3.00 |
| 8 | 1.30 | 1.30 |
| 9 | .30 | .40 |
| Total | -.70 | 3.00 |

**Table - 21**

**ANOVA**

Hb diff.

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 14.499 | 8 | 1.812 | 4.840 | .001 |
| Within Groups | 11.609 | 31 | .374 | | |
| Total | 26.108 | 39 | | | |

**Table - 22**

There are 4,6,5,9,4,2,7,1 and 2 patients in groups 1,2,3,4,5,6,7,8 and 9 respectively.

Mean increase in post-treatment Hb is 1.375 g/dl, 0.95 g/dl, - 0.14 g/ dl, 1.46 g/dl, 0.025 g/ dl, 1.3 g/dl, 0.4 g/ dl, 1.3 g/dl and 0.35 g/ dl respectively in groups 1,2,3,4,5,6,7,8 and 9 respectively.

There is greater increase in post-treatment Hb in patients with low serum ferritin and low TSAT.

The Hb response decreases (mean Hb increase – 0. 14 g/ dl in Iron replete patients (serum ferritin > 500 ng/ dl and TSAT > 30%).

When the pre-treatment TSAT is < 20 % the Hb response is good; 1.375 g/ dl with ferritin < 200 ng/ dl: 1.3 g/ dl with ferritin 200- 500 ng/ dl and > 500 ng/ dl.

**Means Plots**

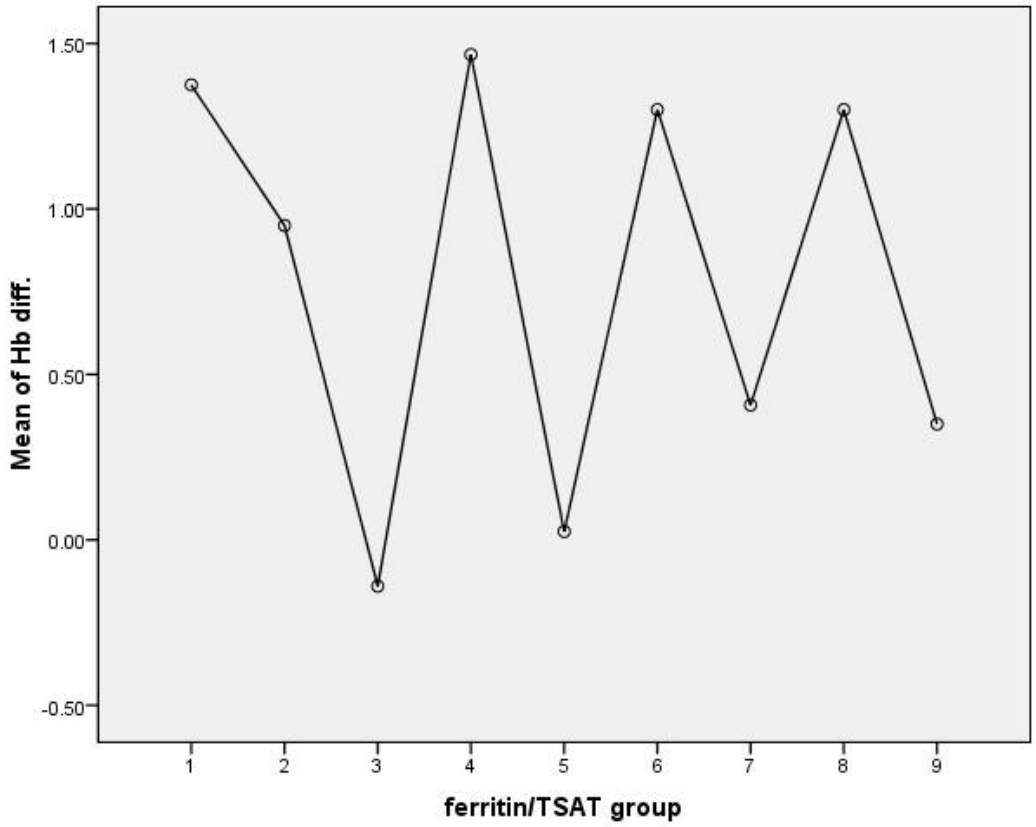



**Figure - 25 : The diagram showing negative response in Iron replete state and good response in patients with TSAT < 20 % and intermediate response in other patient groups.**

# DISCUSSION

## Absolute and relative ron deficiency

There is only one patient with absolute iron deficiency (serum ferritin < 100 ng/ dl and TSAT < 20%). But there are 20 patients with functional iron deficiency (serum ferritin < 500 ng/ml and TSAT < 30%). 4 patients (10%) have serum ferritin level less than 100 ng/dl and seven (17.5%) patients have TSAT < 20%. According to NHANES III (1988-96) 46% of women and 19% men had TSAT < 20%. 47% women and 44% of men had ferritin < 100 ng/dl.

## Hb response

There is statistically significant increase in Hb level 1 month after 1 G Inj. iron sucrose IV in most of the patients. Mean increase in Hb post treatment is 0.81 g/dl. In many similar study groups the Hb response is between 0.4 g/ dl to 1.4 g/ dl after IV iron therapies. In similar studies Agarwal has observed 0.4 g/dl Hb response in 75 patients. Qunibi observed 1.16 g/dl Hb response in 188 patients, Spinowitz 0.62 g/dl in 188 patients, Von wyk 0.7 g/dl in 188 patients, Anuradha 0.94 g/dl in 56 patients, Panesar 1.3 g/dl in 32 patients, Bennett 0.99 g/dl in 36 patients, Atray 1.16 g in 22 patients and Post 1.4 g/dl in 5 patients.

**AGE GROUP**

The increase in Hb response is more in younger age group which is statistically insignificant due to small number of patients in younger age (<40 years). Majority of patients are from 61-70 years (17 numbers). This may be due to more conservative non dialytic approach in older peoples comparing to younger patients. Decreased response to IV iron in older age group may be due to age related cytopenias.

**SEX**

Males are expected to show higher Hb response than females due to the influence of androgens over erythropoiesis. In our study females fare slightly better than their male counterparts. This may be again due to majority of patients being from older age group.

**CKD STAGE**

Majority of patients are from CKD stages 4, 5 than CKD stage 3. In general population CKD stage 3 are more than stage 4 or 5. But our study was conducted in outpatient as well as in patients of tertiary health care centre where the symptomatic CKD stage 4, 5 patients constitute the majority of patients. As there is only one patient in CKD stage 3 we could not assess the Hb response according to the CKD stages.

There is no statistically significant age, sex and CKD stage related Hb response.

## CORRELATION BETWEEN Hb RESPONSE AND FERRITIN AND TSAT

In our study there is no correlation between Hb response and ferritin and TSAT response. In a similar study Silverberg et al has observed that there is no correlation between Hb response and ferritin and TSAT response in CKD patients from Switzerland.

## ASSESSMENT OF FERRITIN, IRON, TIBC AND TSAT RESPONSES

In our study serum ferritin increased from 311 ng/dl to 336.6 g/dl, iron from 120.5 μg/dl to 134 μg/dl and TSAT from 29.19% to 33.9%. TIBC decreased from 435.3 μg/dl to 405.2 μg/dl. In a similar study by Gabriel Mircescu from Romania involving 60 patients serum ferritin increased from 98 ng/ ml to 156 ng/ml a 3 months after 600 mg IV iron Sucrose and 442.5 ng/ml 1 year after 2400 mg IV iron Sucrose. Serum iron increased from (73.9 μg/dl) to 74.8 μg/dl (3 months) and 442.5 μg/dl (1 year). TSAT increased from 21.6% to 24.9% (3 months) and 33.6% (1 year)[52].

There is no statistically significant serum ferritin response. But statistically significant age related ferritin response is present.

There are statistically significant serum iron, TIBC and TSAT responses present. But there is no age related response.

**CORRELATION BETWEEN PRETREATMENT FERRITIN, TSAT AND Hb RESPONSE**

When the TSAT is < 20% the Hb response is more irrespective of serum ferritin levels. When serum ferritin is less than 200 ng/ dl the Hb response is good if TSAT is < 30%. If the TSAT is > 30% there is no significant Hb response in spite of having low serum ferritin levels (< 200 ng/ dl).When the serum ferritin is > 500 ng/ml or TSAT is >30% (Iron replete state) there is negative Hb response. This is the basis for more emphasis on TSAT rather than serum ferritin. This has been shown in the 2012 DOPPS study where more than half of dialytic patients have serum ferritin >800ng/ml.

# LIMITATIONS OF THE STUDY

1. Small sample size.
2. No control group.
3. No long term follow up.
4. Hb is measured only once before and after treatment with parenteral Iron therapy. Hb variability is not studied.
5. Gold standard bone marrow iron stores were not compared due to ethical concerns.

# CONCLUSIONS

1. There is increase in Hb after one month of intravenous iron therapy.
2. Pre-treatment transferrin saturation (TSAT) level < 20% is the most important predictable factor to assess Hb response.
3. If TSAT is < 30%, Hb response is good even with high serum ferritin level (>500 ng/dl).
4. Serum ferritin response is not statistically significant.
5. Iron, TIBC, TSAT responses are statistically significant
6. Response in Hb, ferritin and TSAT did not correlate with each other.
7. There is a significant correlation between Hb response and pre-treatment ferritin and TSAT levels.
8. There is no correlation between Hb response and pre-treatment serum iron and TIBC.
9. IV iron sucrose has very good safety profile except for a few minor adverse effects.

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# INSTITUTIONAL ETHICAL COMMITTEE
# GOVT.KILPAUK MEDICAL COLLEGE,
# CHENNAI-10
## Protocol ID.No.3/10/2014
## CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "Haemoglobin response to intravenous iron therapy in nondialytic chronic kidney disease patients with anemia submitted by Dr.V. Murugesan, Dept. of Nephrology, PG Student, KMC, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

GOVT. KILPAUK MEDICAL COLLEGE
01 DEC 2014
Kilpauk, Chennai-10.

CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,Chennai

# PROFORMA

NAME: AGE: SEX:

ADDRESS MOBILE

D.O.A: D.O.D IP.NO:

HISTORY

Breathlessness pedal edema facial puffiness easy fatiguability

anorexia Nausea/vomiting Hematemesis malena

hematochezia hemoptysis Hematuria Epistaxis

menorrhagia/scanty flow

Passing worms Barefoot walking

Drugs

DM SHT CAD

GENERAL EXAMINATION

Conjunctiva tongue Hand skin crease

Nail changes pedal edema

PR BP Temp RR

Weight

CVS RS ABDOMEN

INVESTIGATIONS

Hb

Pre-treatment post-treatment

Complete blood count

pretreatment post treatment

Peripheral smear study

Blood sugar urea creatinine
eGFR (MDRD formula)

Na + K+

URINE albumin deposits

URINE C&S BLOOD C&S

Motion for occult blood

X-Ray chest ECG
ECHOcardiography

USG KUB

Serum ferritin
Pretreatment post treatment

Serum iron
Pretreatment post treatment

Total iron binding capacity
Pretreatment post treatment

Transferrin saturation
Pretreatment post treatment

# MASTER CHART

| SI.NO | AGE | SEX | CREATININE(mg% | eGFR(ml/min/1.73m2 | CKD STAGE | Hb(pre)g/dl | Hb(post)g/dl | Hb diff. |
|---|---|---|---|---|---|---|---|---|
| 1 | 57 | 1 | 5.8 | 10.8 | 5 | 9.7 | 9.4 | -0.3 |
| 2 | 60 | 1 | 5.5 | 11.3 | 5 | 7.7 | 9 | 1.3 |
| 3 | 58 | 2 | 5.2 | 9 | 5 | 7 | 10 | 3 |
| 4 | 70 | 2 | 3 | 16.4 | 4 | 7.3 | 8.5 | 1.2 |
| 5 | 41 | 1 | 5.8 | 11.5 | 5 | 7.2 | 8.7 | 1.5 |
| 6 | 62 | 1 | 3.1 | 21.8 | 4 | 7.6 | 8.9 | 1.3 |
| 7 | 35 | 2 | 5.3 | 10 | 5 | 6 | 5.9 | -0.1 |
| 8 | 65 | 2 | 5.8 | 7.8 | 5 | 7.3 | 7.9 | 0.6 |
| 9 | 48 | 1 | 4.2 | 16.2 | 4 | 8.2 | 9.5 | 1.3 |
| 10 | 60 | 1 | 5.1 | 12.4 | 5 | 7 | 7.8 | 0.8 |
| 11 | 55 | 1 | 4.3 | 16 | 4 | 8.4 | 8.1 | -0.3 |
| 12 | 70 | 2 | 5.2 | 8.7 | 5 | 5 | 5.1 | 0.1 |
| 13 | 64 | 1 | 5.4 | 11.4 | 5 | 8 | 9.2 | 1.2 |
| 14 | 63 | 1 | 2.8 | 24.4 | 4 | 9 | 8.3 | -0.7 |
| 15 | 66 | 2 | 1.7 | 32 | 3 | 8.3 | 10 | 1.7 |
| 16 | 52 | 1 | 2.8 | 25.4 | 4 | 7.8 | 8.4 | 0.6 |
| 17 | 53 | 1 | 6 | 10.5 | 5 | 7 | 7.2 | 0.2 |
| 18 | 68 | 2 | 5.2 | 8.7 | 5 | 5.2 | 6.5 | 1.3 |
| 19 | 19 | 2 | 5.2 | 11.3 | 5 | 6.2 | 7.8 | 1.6 |
| 20 | 65 | 2 | 5.6 | 8.1 | 5 | 6 | 5.7 | -0.35 |
| 21 | 62 | 1 | 6 | 10.1 | 5 | 5 | 6.8 | 1.8 |
| 22 | 45 | 1 | 5.2 | 12.8 | 5 | 7.4 | 7.5 | 0.1 |
| 23 | 70 | 1 | 5.4 | 11.2 | 5 | 5.3 | 5.9 | 0.6 |
| 24 | 68 | 1 | 5.6 | 11.8 | 5 | 6.2 | 6.8 | -0.6 |
| 25 | 68 | 1 | 4.8 | 12.9 | 5 | 8.5 | 8.6 | 0.1 |
| 26 | 44 | 2 | 3.9 | 13.3 | 5 | 9.3 | 9.6 | 0.3 |
| 27 | 21 | 2 | 3.5 | 17.5 | 4 | 7.5 | 9.3 | 1.8 |
| 28 | 55 | 1 | 5.3 | 20.8 | 4 | 8.1 | 8.3 | 0.2 |
| 29 | 68 | 1 | 4 | 15 | 4 | 9.1 | 10.5 | 1.4 |
| 30 | 70 | 1 | 5.8 | 10.3 | 5 | 6.7 | 7.5 | 0.8 |
| 31 | 58 | 1 | 3.5 | 19.2 | 4 | 9.2 | 10.7 | 1.5 |
| 32 | 52 | 1 | 3.8 | 17.9 | 4 | 9 | 9.4 | 0.4 |
| 33 | 62 | 2 | 5.2 | 8.9 | 5 | 7.2 | 8 | 0.8 |
| 34 | 56 | 2 | 5.6 | 8.6 | 5 | 8.2 | 9.2 | 1 |
| 35 | 50 | 1 | 5.2 | 12.5 | 5 | 8.2 | 9.6 | 1.4 |
| 36 | 34 | 1 | 5.4 | 13 | 5 | 6.2 | 7.6 | 1.4 |
| 37 | 47 | 2 | 5.6 | 8.7 | 5 | 8.2 | 8 | -0.2 |
| 38 | 70 | 2 | 4.8 | 9.5 | 5 | 7.3 | 7 | -0.3 |
| 39 | 52 | 2 | 5.4 | 8.8 | 5 | 6.1 | 7.5 | 1.4 |
| 40 | 50 | 1 | 6 | 10.6 | 5 | 9.4 | 10.8 | 1.4 |

Sex 1 - Male 2 - Female

| Ferritin(pre)ng/ml | FERRITIN GROUP | ferritin (post)ng/ml | ferritin Diff | Iron(pre)µg/dl | Iron group | Iron(post)µg |
|---|---|---|---|---|---|---|
| 599 | 3 | 708 | 109 | 114 | 1 | 154 |
| 127.3 | 1 | 178 | 50.7 | 133.4 | 1 | 155 |
| 433.8 | 2 | 343.8 | -90 | 283 | 2 | 139.4 |
| 225.4 | 2 | 252 | 26.6 | 57.4 | 1 | 78 |
| 125.5 | 1 | 155 | 29.5 | 38.4 | 1 | 42 |
| 138 | 1 | 243 | 105 | 142 | 1 | 183 |
| 575.9 | 3 | 612 | 35.9 | 123.2 | 1 | 142 |
| 418.9 | 2 | 453 | 34.1 | 150.2 | 2 | 168 |
| 136 | 1 | 175 | 39 | 78 | 1 | 112 |
| 374.8 | 2 | 354 | -20.8 | 143.2 | 1 | 162 |
| 486 | 2 | 542 | 56 | 152 | 1 | 166 |
| 409.5 | 2 | 434 | 24.5 | 119.2 | 1 | 128 |
| 312.6 | 2 | 345 | 32.4 | 178 | 1 | 154 |
| 98.4 | 1 | 154 | 55.6 | 118 | 1 | 134.6 |
| 124.6 | 1 | 176.4 | 51.8 | 98.6 | 1 | 154 |
| 154.7 | 1 | 178 | 23.3 | 104.9 | 1 | 142 |
| 478 | 2 | 434 | -44 | 184 | 2 | 138 |
| 504.5 | 3 | 289 | -215.5 | 60.4 | 1 | 133,6 |
| 100.1 | 1 | 293 | 192.9 | 96.2 | 1 | 106.5 |
| 310.8 | 2 | 354 | 43.2 | 196.5 | 2 | 211.8 |
| 68.8 | 1 | 124.6 | 55.6 | 74.4 | 1 | 124.8 |
| 415 | 2 | 393.6 | -21.4 | 118.7 | 1 | 123.4 |
| 368 | 2 | 452 | 84 | 134 | 1 | 178 |
| 77.4 | 1 | 82.6 | 5.2 | 103.3 | 1 | 132.8 |
| 202.4 | 2 | 186.2 | -16.2 | 175 | 1 | 152.5 |
| 558 | 3 | 478.6 | -79.4 | 138 | 1 | 152.4 |
| 121.2 | 1 | 437 | 315.8 | 159 | 2 | 123.8 |
| 641.5 | 3 | 412.6 | -238.9 | 176.1 | 2 | 189.7 |
| 123 | 1 | 154.2 | 31.2 | 115 | 1 | 134.8 |
| 96 | 1 | 124 | 28 | 126.5 | 1 | 114.6 |
| 231.7 | 2 | 286.8 | 55.1 | 129 | 1 | 144.2 |
| 732 | 3 | 798.6 | 66.6 | 57.3 | 1 | 78.4 |
| 103 | 1 | 156.8 | 53.8 | 66 | 1 | 88.4 |
| 235.4 | 2 | 254 | 28.6 | 93.2 | 1 | 176.8 |
| 125 | 1 | 198.6 | 73.6 | 80.4 | 1 | 134.8 |
| 437 | 2 | 498.6 | 61.6 | 44.4 | 1 | 68.4 |
| 982.2 | 3 | 409.7 | 572.5 | 36.3 | 1 | 71.9 |
| 519.4 | 3 | 578.4 | 59 | 187.3 | 2 | 114.2 |
| 108.2 | 1 | 224 | 115.8 | 109.2 | 1 | 154 |
| 163.4 | 1 | 540.7 | 377.3 | 66.3 | 1 | 100.9 |

Ferritin group 1- < 200 ng/ml, 2- <500 ng/ml, 3- >500 ng/ml

Iron group 1- <175 µg/dl in males, < 150 µg/ml in females,

2- >175 µg/dl in males, >150 µg/dl in females

| TIBC(Pre) | TIBC group | TIBC(post) | TSAT%(pre) | TSAT group | TSAT%(post) | TSAT DIFF(µg/ml) | ferritin/TSAT group |
|---|---|---|---|---|---|---|---|
| 333.9 | 1 | 298 | 34.1 | 3 | 51.6 | 17.5 | 3 |
| 626.4 | 2 | 512 | 21.2 | 2 | 30.2 | 9 | 4 |
| 765.9 | 2 | 416.4 | 36.9 | 3 | 33.4 | -3.5 | 7 |
| 378.9 | 1 | 350 | 15.1 | 1 | 22.2 | 7.1 | 6 |
| 330.5 | 1 | 324 | 11.6 | 1 | 12.9 | 1.3 | 1 |
| 589 | 2 | 498 | 24.1 | 2 | 36.7 | 12.6 | 4 |
| 354.1 | 1 | 382 | 34.7 | 3 | 37.1 | 2.4 | 3 |
| 512 | 2 | 486 | 29.3 | 2 | 34.5 | 5.2 | 2 |
| 412 | 2 | 386 | 20.2 | 2 | 29 | 8.8 | 4 |
| 483.8 | 2 | 456 | 29.5 | 2 | 33.4 | 3.9 | 2 |
| 356 | 1 | 380 | 42.6 | 3 | 43.68 | 1.08 | 7 |
| 347.1 | 1 | 360 | 34.3 | 3 | 35.5 | 1.2 | 7 |
| 624.6 | 2 | 652.8 | 28.4 | 2 | 23.5 | -4.9 | 2 |
| 390.8 | 1 | 362.6 | 30.1 | 3 | 37.12 | 7.02 | 5 |
| 412 | 2 | 386 | 23.9 | 2 | 39.8 | 15.9 | 4 |
| 340.8 | 1 | 312 | 30.7 | 3 | 45.5 | 14.8 | 5 |
| 534.6 | 2 | 496.2 | 34.4 | 3 | 27.8 | -6.6 | 7 |
| 559.2 | 2 | 448.5 | 10.8 | 1 | 29.7 | 18.9 | 8 |
| 355.2 | 1 | 248.79 | 27 | 2 | 42 | 15 | 4 |
| 416.7 | 2 | 352 | 47 | 3 | 60.4 | 13.4 | 7 |
| 500.6 | 2 | 453.8 | 14.8 | 1 | 27.5 | 12.7 | 1 |
| 299.7 | 1 | 306.7 | 39 | 3 | 40 | 1 | 7 |
| 450 | 2 | 614.8 | 29.7 | 2 | 28.9 | -0.8 | 2 |
| 282.9 | 1 | 265.9 | 36.5 | 3 | 49.9 | 13.4 | 5 |
| 497.1 | 2 | 464.8 | 35 | 3 | 32.8 | -2.2 | 7 |
| 546.9 | 2 | 524.8 | 25.2 | 2 | 29 | 3.8 | 9 |
| 570 | 2 | 460.89 | 27.8 | 2 | 26.86 | -0.94 | 4 |
| 546.4 | 2 | 624.48 | 31.2 | 3 | 30 | -1.2 | 3 |
| 397.5 | 1 | 382.4 | 29 | 2 | 35.2 | 6.2 | 4 |
| 344.4 | 1 | 378 | 36 | 3 | 30.3 | 5.7 | 5 |
| 505 | 2 | 478.8 | 25.5 | 2 | 30.1 | 4.6 | 2 |
| 217.2 | 1 | 196.8 | 26.3 | 2 | 39.8 | 13.5 | 9 |
| 394.8 | 1 | 356.2 | 16.7 | 1 | 24.8 | 8.1 | 1 |
| 336.3 | 1 | 312.6 | 27.7 | 2 | 56.5 | 18.8 | 2 |
| 372.9 | 1 | 354.8 | 21.5 | 2 | 37.9 | 16.4 | 4 |
| 301.8 | 1 | 296.2 | 14.7 | 1 | 23 | 8.3 | 6 |
| 471.9 | 2 | 415.56 | 76 | 3 | 17.3 | -58.7 | 3 |
| 373.2 | 1 | 342.9 | 50.1 | 3 | 33.3 | -16.8 | 3 |
| 458.1 | 2 | 404.8 | 23.7 | 2 | 38 | 14.3 | 4 |
| 424.17 | 2 | 467.7 | 15.6 | 1 | 21.5 | 5.9 | 1 |

TIBC groups 1- < 400 µg/dl,  2- > 400 µg/dl

TSAT groups 1- < 20%,  2- < 30%  and 3 -> 30%

# KEY TO MASTER CHART

| Ferritin & TSAT groups | Pre-treatment serum ferritin(ng/ml) | Pre-treatment TSAT |
|---|---|---|
| Group 1 | < 200 | <20% |
| Group 2 | <500 | <30% |
| Group 3 | >500 | >30% |
| Group 4 | <200 | <30% |
| Group 5 | <200 | >30 |
| Group 6 | <500 | <20% |
| Group 7 | <500 | >30% |
| Group 8 | >500 | <20% |
| Group 9 | >500 | <30% |

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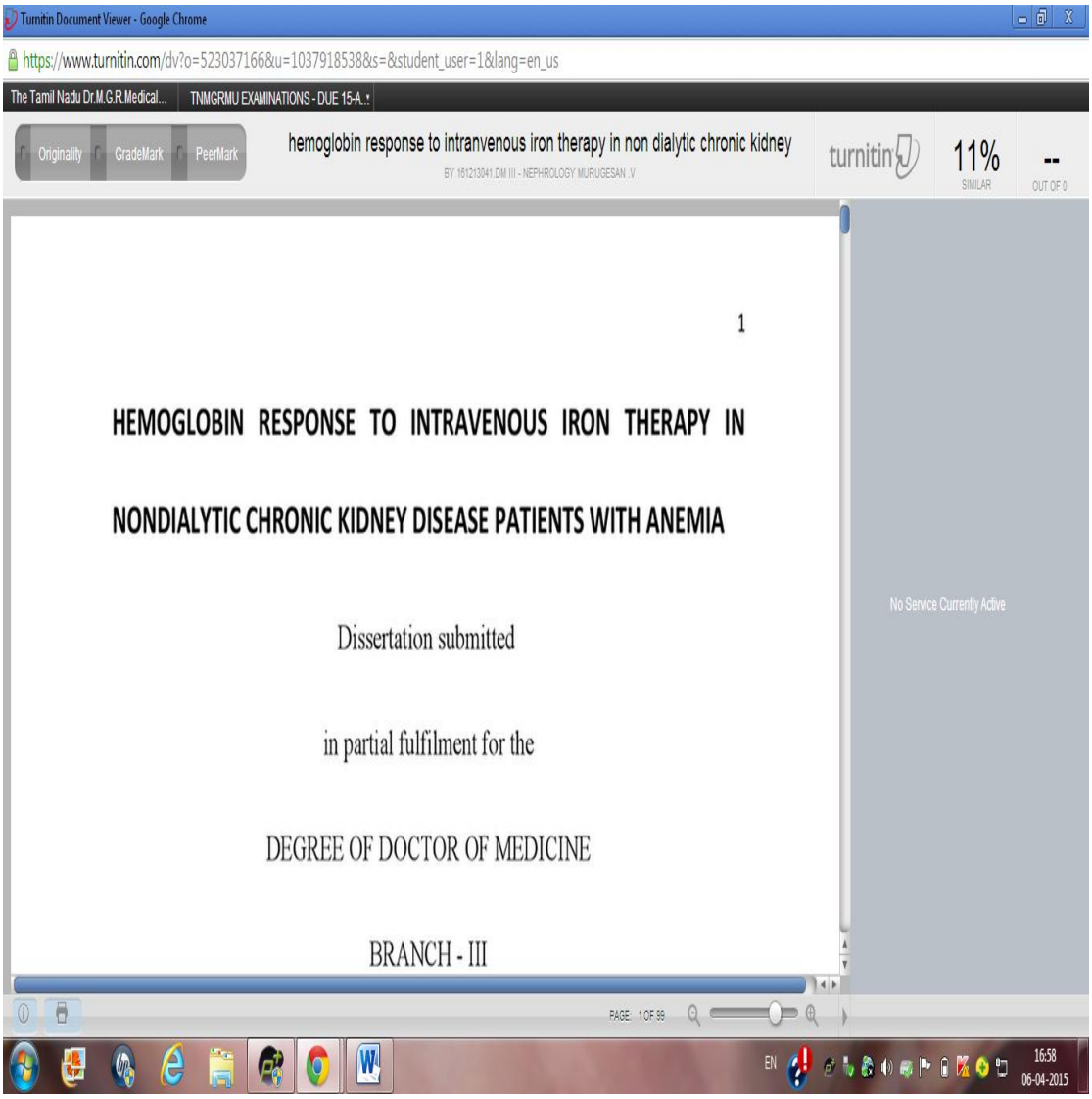
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