Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 1 |JAN-MAR |2013|14-23

Research Article
Biological Sciences

STUDY OF SERUM IRON, TIBC, TRANSFERRIN SATURATION AND FERRITIN IN IRON DEFICIENCY ANEMIA IN TERTIARY CARE HOSPITAL

A. Veena [1a], Amit D. Sonagra[2b], Rekha M.[3b], Jayaprakash Murthy D.S.[4b]*

[a]*Department of Biochemistry, S.S. Institute of Medical Science, Davangere, Karnataka (India).*

[b]*Department of Biochemistry, JJM Medical College, Davangere-577004, Karnataka (India).*

***Corresponding Author Email:** veena.karatagi@gmail.com

ABSTRACT

Background: *Amongst anemia, Iron deficiency anemia is the most common nutritional deficiency disorder in the world. Iron deficiency leads to giddiness, anorexia, decreased defense activity of the body, decreased alertness, cerebral ischaemia, menstrual irregularities leading to maternal morbidity and mortality.* ***Objectives****: To estimate serum iron, TIBC, transferrin saturation and ferritin in iron deficiency anemic patients.* ***Materials & methods****: 30 anemic patients were selected, 20 controls were taken and ferrokinetic study was done with commercially available kits. Statistical analysis was done by student "t" test to study serum iron, transferrin and serum ferritin levels in iron deficiency subjects.* ***Results****: There was decreased serum iron, transferrin saturation and serum ferritin and increased total iron binding capacity in iron deficient patients.* ***Conclusion****: Estimation of serum iron, TIBC, transferrin saturation and ferritin gives accurate analysis of patients iron status and helps to take necessary interventions in planning, treating and thus prevents the risk of adverse events.*

KEYWORDS

Ferritin, Iron deficiency anemia, serum iron, total iron binding capacity, transferrin saturation %.

INTRODUCTION

Anemia constitutes a common problem in clinical practice and hematological Laboratories. It is neither a diagnosis in itself nor a specific entity but a manifestation of an underlying disease process which is often related to the severity of the disease process. [1]

Iron deficiency is the most common nutritional deficiency disorder in the world, reported to affect 50-60% of young children and pregnant females & 20-30% of non-pregnant females in developing countries. It is the most common microcytic anemia. The consequences of iron deficiency are numerous as iron plays a central part in the transport of oxygen in the body and is also essential in many enzyme systems like cytochrome oxidase, xanthine oxidase. Iron deficiency affects neurotransmitter systems in brain causing changes in behavior such as attention, memory and learning in infants & small children. It also negatively influences the normal defense system against infection. In pregnant women, iron deficiency contributes maternal morbidity & mortality & increases risk of fetal morbidity, mortality & low birth weight.[2]

In children it is frequently caused by dietary deficiency, because milk has low iron content and in adults it is almost always the result of chronic blood loss or child bearing. Iron deficiency anemia develops when there is inadequate iron for hemoglobin synthesis and is the result of an imbalance between iron assimilation and iron loss. [3]

Iron absorbed from diet or released from stores circulates in the plasma bound to transferrin, the iron transport protein. The half-clearance time of transferrin-bound iron is very rapid → 60-90 min. Most of iron transported by transferrin is delivered to the erythroid marrow. So, when erythropoesis is markedly stimulated, the pool of erythroid cells requiring iron increases and the clearance time of the iron from the circulation decreases.

The half-clearance time of iron in the presence of iron deficiency is very short i.e. 10-15min. [5]

The balance of iron in human is tightly controlled and conserve iron for reutilization. Iron is lost from the body by blood loss (via gastrointestional bleeding, menses or other forms of bleeding), loss of epithelial cells from the skin, gut and genito-urinary tract. Only route by which iron comes into the body via absorption from food or from medicinal iron. Iron also enters the body through red cell transfusion or injection of iron complexes.

Amount of iron required from the diet to replace losses averages about 10% of body iron content a year in men & 15% in women of child bearing age. Iron bioavailability is affected by the nature of the food stuff, with heme iron (e.g. red meat) being most readily absorbed.

Infants, children and adolescents require additional iron due to demands of body growth, lower dietary intake of iron. During the last two trimesters of pregnancy, daily iron requirement increases to 5-6mg. [4, 5]

The progression to iron deficiency can be divided into three stages - (**Fig 3**): [6]

1) **First stage**: Negative Iron balance where demand of iron exceed the body's ability to absorb iron from the diet. (Blood loss, pregnancy where the demands for red cell production by the fetus outstrip the mother's ability to provide iron, rapid growth spurts in the adolescent, or inadequate dietary iron intake). During this period- serum ferritin levels decrease. As long as iron stores are present and can be mobilized the serum iron, total iron binding capacity and red cell protoporphyrin levels remain within normal limits. Red cell morphology and indices are normal at this stage.

2) **Second stage**: (Iron deficient erythropoesis) - when iron stores becomes depleted, when marrow iron stores are absent, when s.ferritin levels <15µg/L, the serum iron begins to fall. Gradualll, the TIBC increases. Once the transferrin saturation falls to 15-20%, hemoglobin synthesis becomes impaired. Evaluation of peripheral blood smear reveals the appearance of microcytic cells.[6]

3) **Iron Deficiency Anemia** (IDA): Hemoglobin & hematocrit begin to fall. Transferrin saturation is 10-15% in this stage.[5]

In severe anemia (7-8g/dl), hypochromia and microcytosis is more prominent, target cells and poikilocytes appear in blood smear.[5]

The objective of this study was to study and know the levels of serum iron , TIBC, transferrin saturation% (TSAT) and serum ferritin in relation with Hb and peripheral smear at Bapuji Hospital, Karnataka.

MATERIALS AND METHODS

A prospective study was conducted in Iron deficiency anemia subjects from Bapuji Hospital and Chigateri General Hospital, Davangere (both attached teaching hospitals for J.J.M Medical college, Davangere). The controls were selected from surrounding community. Each participant gave an informed consent and this study was approved by the ethical and research committee of J.J.M. Medical College, Davangere to use human subjects in the research study. The patients and controls voluntarily participated in the study.

A) Selection of study subjects

Based on inclusion and exclusion criteria a total number of 50 subjects (30 cases and 20 controls) were selected for the present study.

Inclusion Criteria used to select the study subjects:

Iron deficiency anemia was detected based on clinical history, Hb estimation and peripheral blood smear study.

Patients having Hb levels equal to or less than 7 gm% percentage, microcytic hypochromic blood picture, and age group between 20-50 yrs were included in the study.

Controls- It included 20 age matched healthy non-anemic people in age group of 20-50 yrs without any major illness and who are not on any medication.

Exclusion Criteria:

Patients with anemia of age group <20yrs and > 50yrs and anemia of chronic disease were excluded from the study.

Based on the inclusion and exclusion criteria, age matched cases and controls were included in the present study after obtaining informed consent. A proforma was used to record relevant information and patient's data.

B) Collection of blood samples:

About 5 ml of venous blood was drawn under aseptic precautions in a sterile bulb from selected subjects. Serum was separated by centrifugation and was used for analysis. Serum Iron, Total Iron Binding Capacity (TIBC), Serum Ferritin and TSAt% were estimated.

Serum Iron and TIBC were estimated by Iron and TIBC kit in semiautoanalyzer, (Erba Chem 5plus) which uses Ferrozine method.[7]

Transferrin saturation was calculated as Serum iron × 100 / TIBC.[3]

Serum ferritin was estimated by Chemiluminescence Immunoassay.[8]

Values were calculated as mean ± SD and the statistical analysis was done using SPSS 17.0 software. Student's unpaired t-test was used to study serum iron, TIBC transferring satutation% and serum ferritin levels in iron deficiency subjects. The p-value of less than 0.05 was considered as statistically significant.

RESULTS

Table 2, 3 shows that serum iron, serum ferritin and transferrin saturation% was decreased significantly and TIBC levels were significantly increased as compared to controls. No significant difference was found in Hb percentage.

Table 4 shows that maximum cases belong to age group of 31-40 yrs, followed by 41-50 yrs and least in 21-30 yrs group. Also Fig 4 shows that maximum IDA cases are of females i.e 18 cases out of total 30 cases.

Table 5 shows that 60% of cases are severly anemic with ferritin levels <6ng/ml.

Table 6 shows that maximum cases of IDA had Hb levels in the range of 6.1-7.0g%; followed by 5.1-6.0g%, 4.1-5.0g%, 3.1-4.0g% and 2.1-3.0g% respectively.

Table 2: Showing descriptive information of subjects.

Groups	Hb%	Serum Iron(µg/dl)	TIBC (µg/dl)	Serum Ferritin(ng/ml)	Transferrin Sat%
Controls	12.3 ± 1.5	76.5 ± 32.5	322.4 ± 32.9	136.8 ± 34.0	24 ± 10.6
Cases	5.3 ± 1.2	28.6 ± 10.3	496.0 ± 99.5	5.1 ± 2.3	6.3 ± 2.7

Table 3: Hb %, serum iron, TIBC, serum ferritin and transferrin saturation in iron deficiency anemic patients.

Groups	Hb%	Serum iron (µg/dl)	TIBC (µg/dl)	Serum ferritin (ng/ml)	Transferrin sat%
IDA	5.3 ± 1.2	28.6 ±10.3	496 ± 99.5	5.1 ± 2.3	6.3 ± 2.7
t-value	1.33	6.71	5.76	25.5	6.96
p-level	0.19, ns	<0.001	<0.001	<0.001	<0.001

"t" = unpaired t-test, p <0.05=significant, p> 0.05= not significant, ns=non significant

Table 4: Age distribution in two groups

Age (yrs)	Controls		IDA	
	No	%	No	%
21-30	4	20	7	23.3
31-40	8	40	14	46.7
41-50	8	40	9	30
Total	20	100	30	100
Mean Age	**36.8 years**		**36.6 years**	

Table 5: Classification of patients with IDA based on severity of ferritin levels.

Severity	No	Percentage
Moderate (Serum Ferritin 6-10 ng/ml)	12	40
Severe (Serum Ferritin <6 ng/ml)	18	60

TABLE 6: Study of IDA at various ranges of hemoglobin percentage.

Hb percentage levels	IDA	
	No	%
2.1-3.0	1	3.2
3.1-4.0	4	12.9
4.1-5.0	7	22.6
5.1-6.0	8	25.8
6.1-7.0	11	35.5
Total	30	100.0

Figure 1: Iron circulation [4]

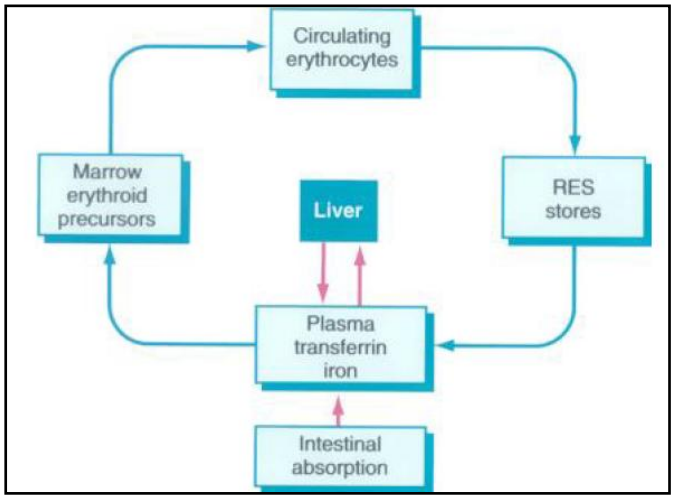


Figure 2: Iron transport in humans.[4]

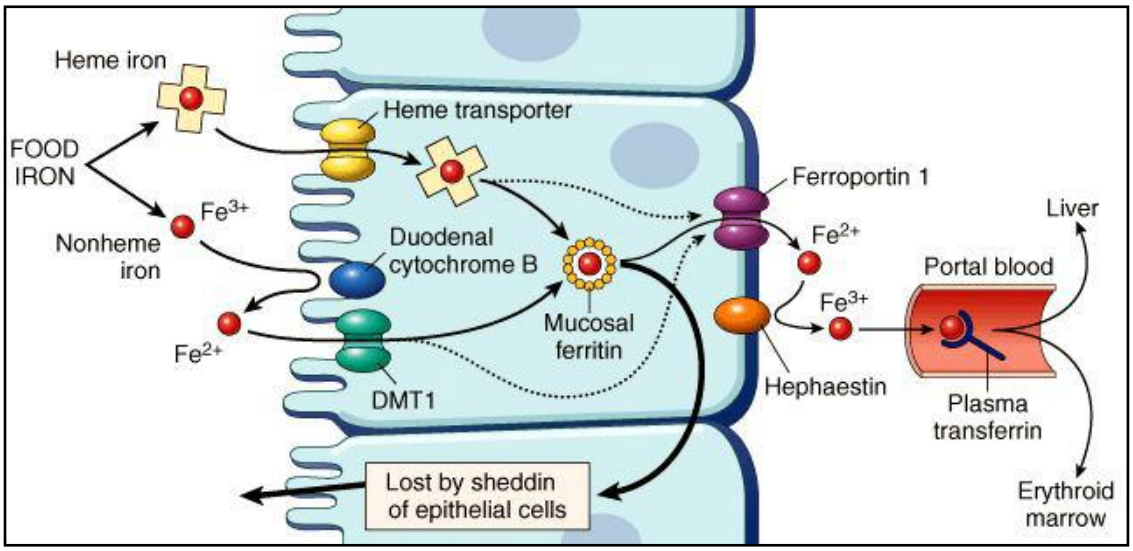
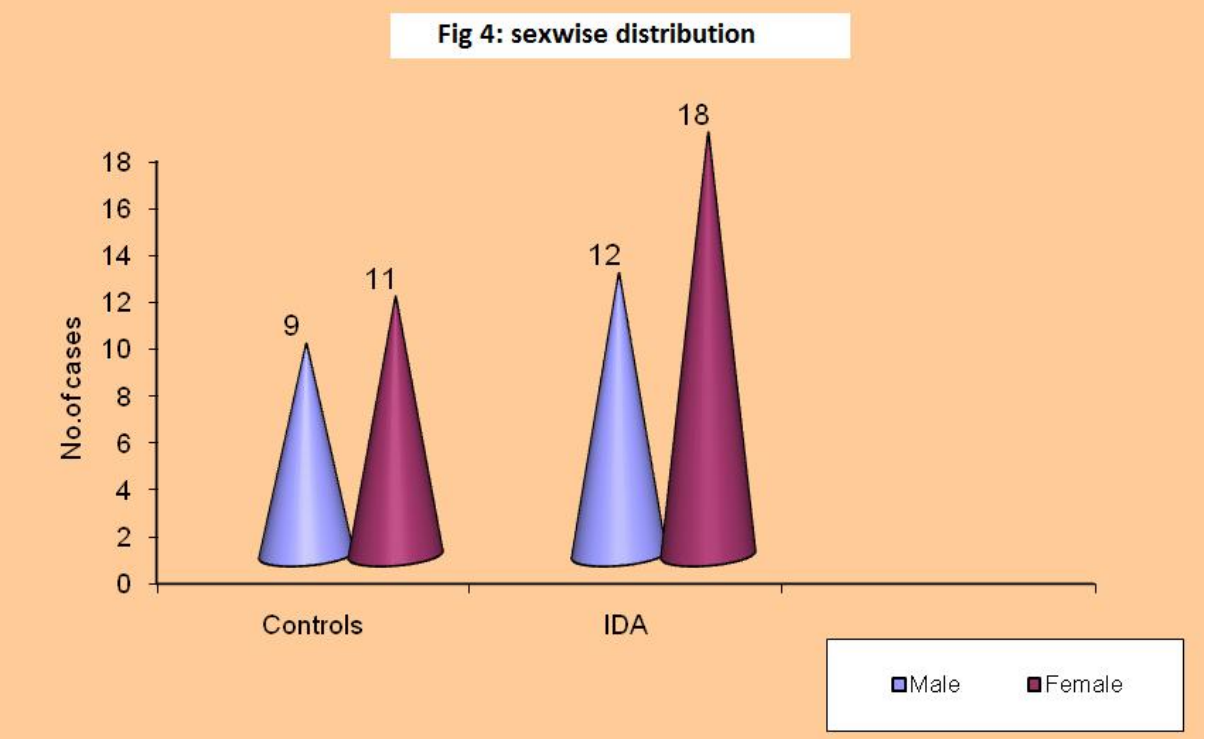


Fig 3: Stages of iron deficiency anemia

	Normal	Negative iron balance	Iron-deficient erythropoiesis	Iron-deficiency anemia
Iron stores				
Erythron iron				
Marrow iron stores	1-3+	0-1+	0	0
Serum ferritin (µg/L)	50-200	<20	<15	<15
TIBC (µg/dL)	300-360	>360	>380	>400
SI (µg/dL)	50-150	NL	<50	<30
Saturation (%)	30-50	NL	<20	<10
Marrow sideroblasts (%)	40-60	NL	<10	<10
RBC protoporphyrin (µg/dL)	30-50	NL	>100	>200
RBC morphology	NL	NL	NL	Microcytic/ hypochromic



DISCUSSION

Iron deficiency anemia is one of the most common anemia worldwide accounting for about two billion people. In developed countries, it accounts for about 2-5% of adult men and post menpausal women and is a common cause of referral to gastroenterology clinic.[8]

In India, it is a major public health problem especially for infants and pregnant women. Deficiency of iron during developmental stage of brain cause irreversible disturbances and damage to GABA neurotransmitters system thus causing poor learning capacity, varying degrees of impairment in cognitive performance etc.[9]

Maximum number of patients in IDA group fall in the Hb range of 6.1 to 7.0 g/dl as compared to 39 patients of Hb range 4.6 to 6.5 g/dl according to Krishna Das [10].

Nutritional inadequacy is the most likely single aetiological factor which is augmented by other factors such as helminthic infestations, blood loss, malabsorption, failure to utilization of nutrients, increased demand by the body, and dietary interactions which hinders iron absorption. Microcytic hypochromic anemia accounts for 76% and macrocytic anemia 7%. Maternal iron deficiency is reflected in the new born as reduction in the serum iron and lower levels of Hb.[10]

Anemia is more common in females. Menstruating, pregnancy and lactation is a form of iron excretion. There is an average loss of 297mg/pregnancy in Indian women. As nearly 150mg of iron are conserved during pregnancy as a result of suppression of menstruation, the average net loss is about 150 mg/pregnancy. This is a recurring loss with each pregnancy and frequently occurs during the adolescent period when their own body need for iron is great due to growth in size of body.[11]. In our study 18 females had IDA out of total 30 cases.

There were two peaks in iron deficiency anemia i.e 21-30 yrs and 41-50 yrs, suggesting that majority of patients with iron deficiency anemia were aged between 20-60yrs.

Capillary fragility is increased in iron deficiency. The role of iron in maintaining the integrity of vascular endothelium and mucosal cells is emphasized. In IDA, there is impairment of cell mediated immunity and is demonstrable by defective lymphocyte transformation. Iron deficiency also interferes with cellular immune mechenisms. Myeloperoxidase is reduced in Iron Defeciency. The cell mediated immune response and bactericidal activity of leucocytes are reduced when hemoglobin falls to 10 g/dl or less.[12]

The typical Indian diet is based on cereals and pulses which contain more than 40% of total phosphorus as phytates and vegetables and plant food contain oxalates, which interfere with absorption of food iron inspite of high dietary intake.[3]

Compared to controls, anemic patients have significantly (p value <0.001) increased levels of TIBC. This is in accordance with studies of Sharma.D.C[3] & Robert Hawkins.C[13].

Trnsferrin saturation % is decreased in IDA cases compared to controls which are in accordance with studies of Sharma. D.C [3], Neeta bahal[14] and Saul Nurko[15].

In IDA patients, there is increase in iron carrying protien transferrin and the amount of iron which is available to bind is reduced causing decrease in transferrin saturation.

In circulation, tiny quantity of ferritin is found which in normal and iron depleted subjects, is in close direct co-relation with total body iron reserve[3].

In our study, 18 patients have ferritin levels <6ng/ml accounting for 60% showing that majority of IDA have ferritin levels less than 6ng/ml which is due to multiple factors including dietary deficiency , poor socioeconomic condition, worm infestation, multiple pregnancy , maternal bleeding etc in our region. The remaining 12 patients have moderate IDA with ferritin levels in range of 6-10ng/ml, accounting for 40%.

The lower the TSAT and serum ferritin , the higher the likelihood that a patient is iron deficient, and higher the TSAT, serum ferritin, the lower the likelihood that a patient is iron deficient.

Estimation of ferritin is non invasive and an excellent alternative to bone marrow stainable iron and showed a significant positive correlation with it.[3]

Complete exhaustion of iron stores is indicated by serum ferritin concentration <12 ng/ml and TSAT <15%.[8]

Ferritin is an acute phase reactant and is increased in inflammatory diseases or cirrhosis. Serum ferritin concentration of <12µg/dl is diagnostic of iron deficiency.[16]

In our study,12 patients belong to moderate IDA with ferritin levels 6-10ng/ml and 18 pateints belong to severe IDA with ferritin levels <6ng/ml which is in accordance with the study by Mohammed Idris[17].

Measurement of serum transferrin receptor is not routinely used in clinical practice as it is expensive and should only be carried out in patients in whom the quantitation of hypochromic red cells is not available or when the determination of serum ferritin , serum iron, transferrin and TSAT does not lead to an accurate classification of type of anemia.[18]

Reticulocyte hemoglobin content (CHr) measures Hb entering reticulocytes during terminal differentiation and hence reflects the effectiveness of erythropoesis. It is highly accurate with lowest coefficient of variability, but is a least widely available assay.[8]

Serum ferritin is to be used as an indicator of the iron stores rebuilt by i.v iron supplementation and to be determined after 2 weeks of last i.v iron dose.

Serum ferritin levels below 25 ng/ml were associated with lower hemoglobin concentration. Levels above 100ng/ml were not consistently associated with higher hemoglobin concentration.[19]

Oral iron therapy is usually the first line therapy for patients with IDA. An increase in the Hb level of 1g/dl (10g/l) should occur every two or three wks on iron therapy and may take up to four months for the iron stores to return to normal after the hemoglobin has corrected. Laxatives, stool softeners and adequate intake of liquids can alleviate the constipating effects of oral iron therapy.

Indications for use of intravenous iron include chronic uncorrectable bleeding, intestinal malabsorption, intolerance to oral iron, non adherence, or a hemoglobin level less than 6g/dl with signs of poor perfusion in patients who would otherwise receive transfusion (eg.those who have religious objections).

Parenteral iron preparations like iron dextran, iron sucrose, sodium ferric gluconate can be used. Its side effects are anaphylactic reactions and delayed reactions like myalgias, headache, arthralgias which occur 24 to 48 hrs after infusion. NSAIDs are used to relieve these symptoms.[20,21]

CONCLUSION

The present study shows decreased serum iron, TSAT and ferritin levels with high levels of TIBC in IDA group whose iron levels may be improved by giving parenteral /oral iron therapy. They can be used as an alternative noninvasive technique to assess iron stores and response to treatment. So, iron level estimations should be done which helps in accurate analysis of patients iron status and thus helps in taking necessary interventions to prevent the risk for adverse events.

ACKNOWLEDGEMENT

We acknowledge all the study subjects for their participation in the study and their cooperation.

REFERENCES

1. MM Eldibany, KF Tonochi: Usefulness of certain red blood cell indices in diagnosing & differentiating thalassemia trait from iron deficiency anemia: *Am J Clin Path* 111:676-682, (1999).
2. Detels R. Iron deficiency. In: McEmen J, Beaglehole R, Tanakai H (edts). Oxford textbook of public health. Food & Nutrition. 4thed. 1999, pg 152- 153.
3. Sharma D, Mathur R, Singh P. Iron metabolism: A Review. *Indian Journal of Clinical Biochemistry*, 8(2): 80-101, (1993).
4. WHO. Techn. Rep. Ser. No. 405(1968).
5. Kumar P. Hematological disease. In: Kumar P, Clark M (edts). Kumar & Clark's Clinical Medicine.7th Ed. Pg.392.
6. Adamson J. Iron deficiency and other hypoproliferative anemias. In: Harrison's principles of internal medicine. 17th ed, pg 628.
7. Seidel J et al. *Clin Chem*.30:975, (1984).
8. Hussain A, Tayyab M, Tasneen T, Ahmed N, Chaudhary. serum Ferritin; An indicator of Bone marrow iron stores in hemodialysed patients. *Kidney international*:152-156,(1999).
9. Goddard A, McIntyre A, Scott B. Guidelines for management of Iron deficiency anemia. *Gut* 46 (suppl IV): IV I-IV S,(2000).
10. Batra J, Seth P. Effect of iron defeciency on developing rat brain. *Indian Journal of Clinical Biochemistry* 17(2) : 108-114, (2002).
11. Krishna Das KV. Nutritional anemias in India. Review article. *Jr.Asso.Phys.Ind* 28:521-533, (1960).
12. Meier P, Olson K, Berg R. Prevetion of iron deficiency in adolescent and adult pregnancies. *Clinical Medicine and Research* 1(1): 29-36,(2002).
13. Besarab A, Horl W, Silverberg D. Iron metabolism, iron deficiency, thrombocytosis and the cardiorenal anemia syndrome. *The Oncologist* 14(suppl 1):22-33,(2009).
14. Hawkins R. Total iron binding capacity or transferrin concentration alone outperforms iron and saturation indices in predicting iron deficiency. *Clinica Chemica Acta* 380: 203-207,(2007).
15. Bahal N, Mara O. Anemia in patients with chronic kidney disease. In brief. *Diabetes Spectrum* 21(1), (2008).

16. Nurko S. Anemia in chronic kidney disease:Causes, diagnosis, treatment. Cleveland *Clinic Journal of Medicine* 73(3): 289-297, (2006).
17. Idris M, Anis-ur-Rehman. Iron deficiency anemia in moderate to severely anemic pts. *J Ayub Med Coll Abbottabad:* 17(3),(2005).
18. Al-Sayes' F, Gari M, Qusti S, Bagation N, Abuzenadah A. Prevalence of Iron deficiency & iron deficiency anemia among females at University stage. *Journal of Medical Laboratory & Diagnosis* 2(1) : 5-11,(2011).
19. Lutter C. Iron deficiency in young children in low income countries and new approaches for its prevention. *J Nutr:* 138: 2523-2528,(2008).
20. Kaltwasser J, Gottschalk R. Erythropoietin and Iron. *Kidney International* (55): suppl 69: s-49-s-56, (1999).
21. Killip S, Bennet J, Mara C. Iron deficiency anemia. *Am Fam Physician*: 75:671-8,(2007).

CONFLICT OF INTEREST: Nil
FUNDS FOR STUDY: Nil

***Corresponding Author:**
Dr. A. Veena. *
Assistant Professor,
Department of Biochemistry,
S.S. Institute of Medical Science,
Davangere, Karnataka.
Email id: veena.karataqi@gmail.com