"STUDY OF EFFECT OF IRON DEFICIENCY ANEMIA ON GLYCATED HEMOGLOBIN (HbAlc)."

By

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Dissertation Submitted to the

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Of the Requirements for the Degree of

M.D

In

GENERAL MEDICINE

Under the guidance of

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APRIL 2011

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Signature

Dr. PAAWAN WADHAWAN

LIST OF ABBREVIATIONS USED

A1 c	Glycated hemoglobin A1 c
ADA	American Diabetes Association
C-2	2nd carbon atom
^{l4}C	Carbon-14 isotope
^{51}Cr	Chromium-51 isotope
CRF	Chronic Renal Failure
dl	Deciliter
DLC	Differential leucocyte count
DMT-1	Divalent metal transporter-1
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
ESR	Erythrocyte Sedimentation Rate
g	Gram
Hb	Hemoglobin
HbA	Adult hemoglobin
HbAl	Glycated Hemoglobin
HbA la	Glycated Hemoglobin la
HbA 1b	Glycated Hemoglobin 1 b
HbAlc	Glycated Hemoglobin 1c
HbA2	Hemoglobin A2
HbC	Hemoglobin C
HbF	Fetal hemoglobin
HbS	Sickle cell Hemoglobin
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography

kDA	Kilo dalton
KFT	Kidney Function tests
LFT	Liver Function Tests
MCH	Mean Corpuscular hemoglobin
MCHC	Mean Corpuscular hemoglobin concentration
MCV	Mean Corpuscular volume
ml	milliliter
$NaBH_4$	Sodium borohydrate
ng	Nanogram
PV	Per vaginum
SFT	Stimulators of iron transport
T 1/2	Half life
TIBC	Total Iron Binding capacity
TLC	Total Leucocyte count
TRP	Transferrin Receptor protein
5-HMF	5-hydroxymethylfurfural
µg	Microgram

ABSTRACT

Introduction- Iron deficiency anemia is most common form of anemia. HbAlc is one of the glycated hemoglobins which is used to assess the glycemic status of an individual over last 2-3 months and is mostly being used in diabetics and in those with impaired glucose tolerance. Certain studies have been done which show that HbAlc levels are affected in hemolytic anemias[43]. However, interest further arose as to what happens to HbAlc levels in more commonly encountered anemias like iron deficiency anemia.

Objective:

1. To study the effect of iron deficiency anemia on HbA1c levels.
2. To study the response of treatment of iron deficiency anemia on HbAlc levels.

Study design: A Prospective clinical non-controlled prognostic study with 50 Iron deficiency anemia patients who were nondiabetics was undertaken from November 2008 to July 2010 in R.L.JALAPPA Hospital. Patients were followed up for a period of 2 months. All Cases were managed as per standard treatment protocol [70]and were treated with iron replacement therapy/blood transfusion as required.

Result: The mean hemoglobin value (g/dl) of patients at baseline was 6.25 (SD= 2.09) which rose to 9.73(SD=1.68) at the end of 1month of treatment and to 12.57 (SD= 1.09) at the end 2 months of treatment. The mean serum ferritin values in patients at baseline was 7.55 (SD= 2.47) ng/ml which rose to 280.79 (82.24) at 2 months .The mean HbAlc value (%) in patients at baseline was 5.79(SD= 7.05), decreased to 5.57(SD= 0.55) and5.42 (SD=0.59) at the end of 1 month and 2 month respectively. There was a significant decrease in the mean HbAlc (%) value after correction of iron deficiency anemia from the baseline value ($p<0.001$).

Conclusion: Iron deficiency should be corrected before any diagnostic or therapeutic decision is made based on HbA1c.

Key words: HbA1c-Glycated hemoglobin.

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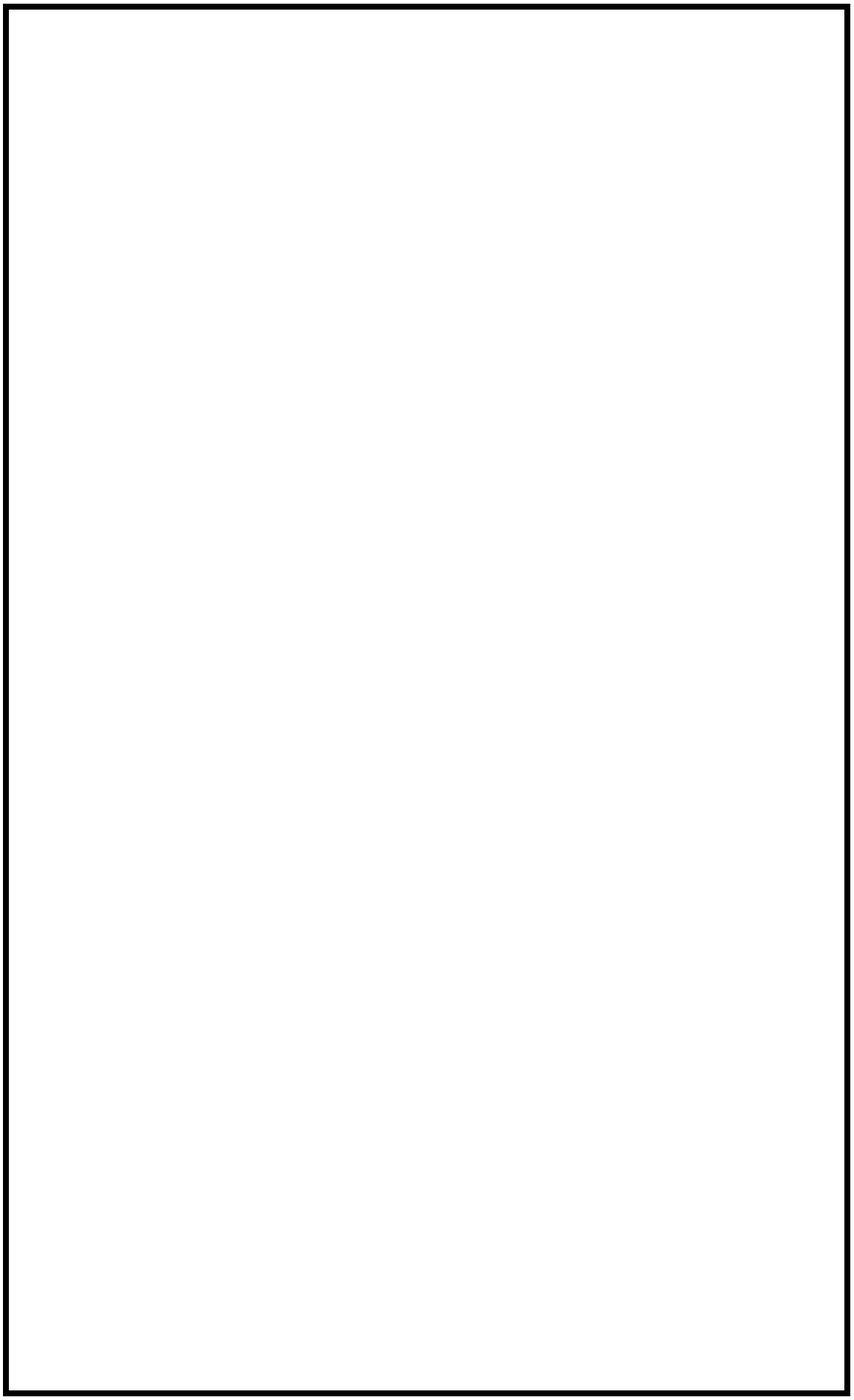


INTRODUCTION

HbAlc, a glycated hemoglobin, is formed by the glycosylation of hemoglobin. Its value represents the glycemic status of a person over last 2-3 months[1] . It is measured in diabetics and in those with impaired glucose tolerance to assess the glycemic status over last 2-3 months. According to the American Diabetes Association Guidelines, 2010[2] , the value of HbAlc should be kept below 7% in all the diabetics. According to the same guidelines, HbAlc is now referred to as Alc only and is now included in the criteria of diagnosis of diabetes[2]. Value greater than 7%, indicates increased chances of progression to diabetic complications. Glycated hemoglobin doesn't include HbAlc alone. It includes other hemoglobins as well and together these constitute the HbAl fraction[1] of adult hemoglobin (HbA). Among the various glycated hemoglobins, HbAlc is the predominant fraction.

It appears from above that, HbAlc is affected by blood glucose levels alone. But certain studies have proved that HbAlc levels are altered in hemolytic anemias and hemoglobinopathies[2]. There are however, very few studies which show the effect of iron deficiency anemia on HbAlc levels. Iron deficiency anemia is the commonest form of anemia[3] it would be prudent to show the effect of the same on HbAlc levels before any decision or guidelines are made based on HbAl c levels.

Aims and Objectives of study:

1. To study the effect of iron deficiency anemia on glycated hemoglobin (HbA1c) levels.
2. To study the response of treatment of iron deficiency anemia on glycated hemoglobin (HbAlc) levels.

REVIEW OF LITERATURE

GLYCATED HEMOGLOBINS:-

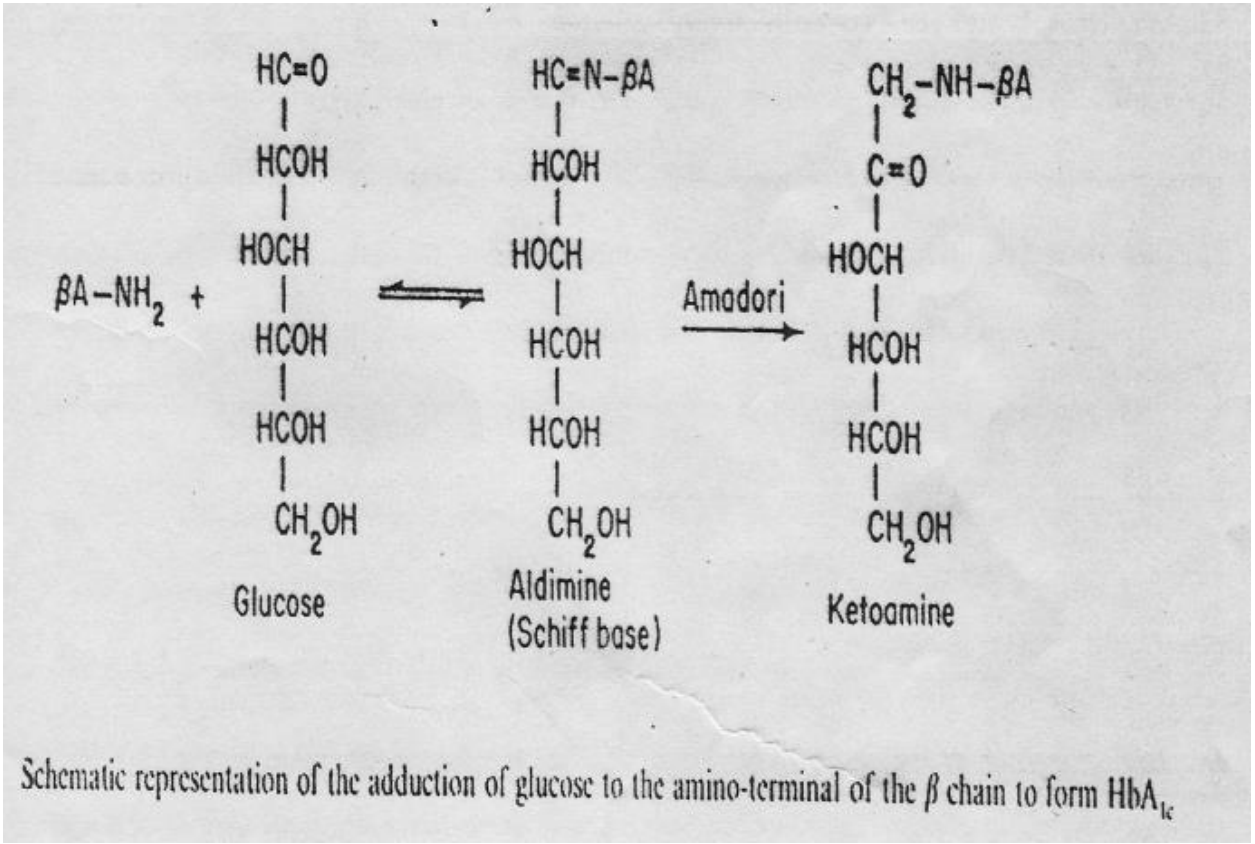
Historical Perspective

Maillard, described the reaction of reducing sugars with amino acids resulting in stable ketoamine adduct formation[4] . In 1958, Allen, Schroeder and Balog demonstrated that normal adult hemoglobin could be separated chromatographically on a cation exchange resin into a main component accounting for more than 90% of the hemoglobin and three negatively charged minor components which they designated HbAla, HbAlb and HbAlc- collectively called as HbAl [5].They never demonstrated the nature of these fractions. Four years later, Huisman and Dozy observed a two to three fold rise in the HbAl fraction in 4 diabetic patients being treated with tolbutamide[6] . In 1968, Rahbar reported similar findings in 2 diabetic patients at Tehran University[7] and later found similar abnormality in 57 diabetic patients[8] . In a larger study of diabetic patients, Trivelli et al found a two fold increase of HbAlc over values seen in non-diabetic subjects[9] . Thus, by mid 1970's it was clear that HbAl and HbAlc were elevated in humans with diabetes mellitus although the mechanism of this abnormality was not understood.

Biochemical considerations

In 1966, it was shown that HbAlc was identical to HbA except that it has an unidentified group, aldehyde or ketone which is attached to the N- terminal of the beta chain. The linkage was reducible by sodium borohydrate ($NaBH_4$)[10] . Later it was

shown by mass spectroscopic analysis that a hexose was attached to the N- terminal valine of both the beta chains [11]. Bunn et al, by subjecting HbAlc to mild acid hydrolysis, were able to isolate sugar-glucose and mannose in a ratio of 3:1 [12]. If the HbA1c was first reduced by titrated (NaBH4) and then exposed to acid hydrolysis, nearly all the radioactivity was recovered as [^{3}H] formic acid. This suggested that in red cell, glucose reacts with N-terminal valine of both the beta chains to form an aldimine linkage which then undergoes an Amadori rearrangement to form a more stable ketoamine link. This also explains why, during acid hydrolysis, both glucose and mannose are released- racemisation at the second carbon atom gives glucose and its C-2 epimer mannose. It was initially believed that aldimine to ketoamine was an irreversible process but it was later proven that it is quite a reversible process[13,14].



Schematic representation of the adduction of glucose to the amino-terminal of the β chain to form HbA_{Ic}

The next major advance in the understanding of the nature of HbAlc was the discovery that it could be formed by incubating either whole blood or purified hemoglobin in the presence of glucose at 37°C[15] . The rate of absorption of [^{14}C] glucose into hemoglobin to form HbAlc was the same whether purified hemoglobin or crude hemoglobin was used, suggesting that the action was not mediated by a red cell enzyme. Studies of the kinetics of the conversion of HbA to HbAlc in vivo gave further weight to the theory that the process is non-enzymatic[16] . Here, injections of ^{59}Fe- bound transferring into a normal volunteer enabled specific radioactivity in the hemoglobin to be measured over a period of 100 days. The activity in the major, HbA0, peak reached a maximum in 15 days and then was relatively constant for the next 80 days consistent with normal eryhropoiesis and a cell viability of approximately 100 days. The specific activities of HbA la, HbA lb and HbAlc increased only gradually, however, continued to rise throughout the 100 days, and exceeded the activity of HbA0 from approximately 60 days onwards. Several other observations were consistent with HbAlc being formed throughout the life span of the red cell as a post synthetic modification of HbA. When human reticulocytes or marrow were incubated with radioactive labeled amino acids, the specific activity of HbAlc was much lower than that of HbA0. Young red cells, isolated by density gradient, have lower levels of HbAlc than older red cells[17] .

The amino terminus of the beta chain is not the only site of formation of glucose adducts with hemoglobin. The amino terminus of the alpha chain is similarly modified, though at an eight to ten fold lower rate, both in vivo and in vitro [18]. Moreover, the modification at that site has an insufficient effect on the charge of the protein to permit separation by ion exchange chromatography in the same way as with HbAlc. There are a number of epsilon amino groups of lysine, throughout the alpha

and beta chains but the conformational structure of the hemoglobin molecule means that some more reactive amino groups may be less accessible to free glucose[19] . The following table shows various modifications of HbA known or presumed to result in minor hemoglobins i.e. HbA1.

Table A :- Showing various glycated hemoglobin and sugar moieties attached to them.

S.NO	HEMOGLOBIN	MODIFICATIONS
1.	HbA0	That fraction that remains after separation of HbAl. Approximately 8-10% of HbA0 appears to be glycated at lysine residues.
2.	HbA1a1	Fructose 1,6 biphosphate attached to amino terminus of beta chain.
3.	HbAla2	Glucose-6-phosphate attached to amino terminus of beta chain.
4.	HbA lb	Either deamidization product of HbA or further modification of HbAlc.
5.	HbAlc	Glucose attached to amino terminus of beta chain.
6.	HbAld	Glutathione attached to amino terminus of beta chain.

Nomenclature

Term 'glycosylated' was used initially but it has been pointed out that the term 'glycosylated' strictly refers to glycosides. Therefore, the Joint Commission on Biochemical Nomenclature has proposed that the term 'glycation' is appropriate for

any reaction that links a sugar to a protein, or in the particular case of the reaction with hemoglobin, 'glycated hemoglobin' [20]. In the American Diabetes Association Guidelines, 2010 HbAlc has been referred to as Alc^2.

Relation of Glycated Hemoglobin to glycemic control in Diabetes

This was first convincingly shown by Koeing et al, who examined the relationship between HbAlc and glycemic control in five poorly controlled diabetic patients [21]. Improvement in glycemic control caused reduction in levels of HbAlc after approximately 4 weeks. In another study of newly diagnosed diabetic patients, it was observed that initially elevated levels of HbAlc decreased gradually in the weeks following the onset of dietary and insulin therapy, tending to level out after approximately 7 weeks [22]. Reports such as these tended to substantiate the view that measurement of glycated hemoglobin would provide an accurate and objective index of glycemic control in the diabetic patients.

Labile HbA1c

In 1979, Svendsen and colleagues demonstrated that short term (6-12 hours) exposure of red blood cells to high glucose concentrations, both in vivo and vitro, led to significant increases in the glycated hemoglobin measured by ion exchange chromatography [23]. At the same time it was demonstrated by Goldstein and colleagues that HbAlc measured by HPLC, increased 2 hours after a standard breakfast, that the increment in HbAlc correlated closely with the plasma glucose increment and that incubating the red cell in 0.9% saline for 5 hours at 37°C before HbAlc assay eliminated the post-prandial increment [24]. This phenomenon is due to the unstable

Schiff base, or aldimine (also called as labile HbAlc), formed as an intermediate step in the glycation reaction and it may be potential source of error in any assay that relies on the effect of glycation on the charge of the molecule. In order for the original concept of glycated hemoglobin as an index of long term integrated glycemia to hold good, the labile fraction should be removed before such assay is performed and this can be achieved by saline incubation, dialysis or some other chemical methods [25]. It is clear that in some patients, the perception of glycemic control, as reflected by glycated hemoglobin could be altered [26]. To eliminate this fraction, the chromatographic kits now contain an additive in their hemolytic reagent which eliminates the labile fraction.

Methods of Measuring glycated hemoglobin

These can be divided into two groups, those that depend on the effect of glycation on the charge of the molecule and those which depend on identifying specific property of the ketoamine linkage in glycated hemoglobin. Those methods which depend on the effect of glycation on the charge of the molecule, measure HbAlc or HbAl but not 'total' glycated hemoglobin i.e. HbAl plus hemoglobin glycated at sites other than the amino terminal valine of the beta chain. Since the other group of methods detects the specific property of ketoamine linkage in glycated hemoglobin, they measure the 'total' glycated hemoglobin.

Methods dependent on charge:-

1. *Ion exchange chromatography*- It has been the most widely used technique. The results are expressed as percentage of total hemoglobin. The original method [9] utilized macrocolumns and enabled separation of the individual fractions HbAl a, HbAlb and HbAlc, which, being more negatively charged than HbA0 elute before HbA0. But this is a tedious process requiring large quantities of buffer, cyanides, and, their pH is critical with small changes affecting the degree of separation of the minor hemoglobins. Minicolumn system has now largely replaced the above system [27,28]. These have the advantage of speed and ease of handling, and they are available in kit form as prepacked columns with prepared buffers, standards and additive to eliminate labile adducts. But with them it is not possible to measure glycated hemoglobin as separate fractions and it is measured as HbA1. This separation is influenced by temperature, for each 1° C rise in temperature, HbA1 increases by 0.25% [29]. So, a constant temperature has to be maintained. Another problem is presence of variant hemoglobins like HbF which coelutes with HbA1 and can give falsely high readings whereas HbC and HbS coelute with HbA and lead to underestimation of HbA1 [30]. So, where there is prevalence of variant hemoglobins, this technique should be used with caution. Labile hemoglobin can also lead to high HbA1. So, it should be eliminated before assay. In most assays the range of HbA1 in non-diabetic subjects is 5-9%, with the levels in diabetic patients ranging up to approximately 20%. Coefficient of variation is usually 2-3% for same-day analysis, while interassay variation is

4-5% [27,29].

2. *HPLC*- It has same principles of measurement of HbAlc and HbAl as ion exchange chromatography but the use of finely divided resins and high flow pressures

result in a more constant flow rate and a faster and a more precise separation [31,32,33]. It is an expensive method.

3. *Isoelectric focusing*- In this technique, hemolysate is applied to a thin layer polyacrylamide gel containing an ampholyte over a pH of 6-8 followed by application of suitable voltage to separate the hemoglobin fractions and finally quantification by high resolution microdensitometry [34,35]. Despite the fact that the difference in Isoelectric points between HbAlc and HbA0 is only 0.02 pH units, precise separation is achieved. Advantage is that HbF, HbC and HbS migrate separately. Interassay variation is 6.9-12.6% which is higher than that of other techniques and is an expensive procedure.

4. Agar gel electrophoresis- In this technique hemolysate is applied to the agar gel at the anodic site and after electrophoresis with a citrate buffer at 60V for 40 minutes, HbA1 moves cathodic to HbA0 and is then quantified by scanning densitometry at 420 nm after the gel has been fixed by heat drying for 20 minutes [36,37].It is essential to eliminate labile component here also. HbC and HbS migrate to points anodic to HbA and do not interfere with its estimation but HbF migrates to same point as HbA1. Intra-assay variation is 1.6-7.3%, and inter assay variation is 2.6-7.3%.

Methods detecting ketoamine linkage [19]:-

1. *Weak-acid hydrolysis*- It is one of the oldest methods. Here glycated hemoglobin is hydrolyzed by a weak acid and the amount of 5-hydroxymethyl furfural (5-HMF) released is quantified colorimetrically after reaction with thiobarbituric acid. It is an inexpensive process but has some disadvantages. 5-HMF is destroyed as it is being released and its production is non-stoichiometric. Glucose itself interferes in the color formation in proportion to its concentration. The hydrolysis step lasts for several

hours. To overcome these disadvantages there must be a scrupulous adherence to rigid assay conditions. By performing the hydrolysis in an autoclave, at increased temperatures and pressures, the yield of 5-HMF is enhanced and more constant in a much shorter period of time. Use of fructose or glycated hemoglobin standards help to correct the variation in hydrolysis between assays. These precautions and modifications lead to the intra and inter assay coefficients of variation to be less than 2% and 3% respectively and shorten the procedure to less than 2 hours. Advantage is that it detects glycation at all sites and there is no interference from labile fraction or hemoglobin variants.

2. *Affinity Chromatography*- Here aminophenyl boronate immobilized on cross-linked agarose provides a suitable matrix for affinity chromatography columns. Glycated hemoglobin adsorbs to the affinity gel, while non-glycated hemoglobin passes through the system. The adsorbed fraction is then removed by elution with a competing ligand at high concentrations, enabling calculation of percentage total glycated hemoglobin. The method is very precise, the coefficient of variation being 2-2.6%. It is sensitive to temperature change and there is conflicting evidence on the interference by labile HbA1. So, it is advisable to remove it. It is less sensitive to pH change and there is no interference from variant hemoglobins.

Clinical use of HbA1c

HbA1c has been used in diabetics. Since HbA1c is formed over a period of 2-3 months, it reflects the glycemic status of a patient over the past 2-3 months [38]. In the ADA guidelines 2010[2], HbA1c has been referred to A1c. These guidelines recommend that A1c should be performed at least two times a year in patients who are meeting treatment goals (and who have stable glycemic control) and quarterly in

patients whose therapy has changed or who are not meeting glycemic goals. ADA guidelines also prescribe the use of point-of-care testing for A1c for timely decisions on therapy changes. The availability if the A1c results at the time when the patient is seen (point-of-care testing) has been reported to result in the frequency of intensification of therapy and improvement in glycemic control [39,40]. There is a correlation between A1c levels and mean plasma glucose levels on multiple testing over 2-3 months [41]. For example, A1c value of 6% corresponds to mean plasma glucose of 135 g/dl.

For A1c, ADA recommends that lowering of A1c reduces the risk of microvascular and neuropathic complications and possibly macrovascular complications. A1c should be kept to less than 7% for patients in general and to less than 6% for individual patients. A1c is the primary target for glycemic control. Guidelines also suggest that post prandial glucose may be targeted if A1c goals are not met despite reaching pre prandial glucose levels.

Confounding medical conditions:-

Accepting glycated hemoglobin as a reflection of integrated glycemia presupposes two basic assumptions- first, that the patient has a normal red cell life and second, that the particular assay method is specific for the non-enzymatic adduction of glucose to hemoglobin. The various confounding medical conditions are as follows:-

1.Acute and chronic blood loss- These decrease the red cell survival. So, they decrease theA1clevels[42].

2.Hemolytic anemias- Again due to decreased red cell survival, A1c is lowered[43].

3.Hemoglobin variants- These can alter the A1c levels as already discussed[44].

4.Blood urea- de Boer et al showed that, in patients with uremia and normal glucose tolerance, glycated hemoglobin measured by ion exchange chromatography was significantly elevated, with seemingly no correlation with the degree of glucose intolerance [45]. It is because excessive amount of cyanate derived from urea causes carbamylation at the N-terminal valine residue and this carbarnylated hemoglobin causes increase in the HbAl(a+b) and hence, increased levels of HbA1 [46]. Agar gel electrophoresis and weak acid hydrolysis should be used in cases of elevated blood urea levels as they do not detect this carbamylated hemoglobin [47]. In CRF patients, there is hemolysis and sometimes gastrointestinal loss of blood which lowers the A1c levels. So, the effect of urea on A1c levels is varying.

5.Pregnancy- There are studies which show that HbA1c levels decrease in second trimester of a normal non-diabetic pregnancy [48,49] and rise in third trimester of pregnancy [50]. These changes are very slight amounting to less than 1% of total hemoglobin. When using glycated hemoglobin as an aid in assessing glycemic control in diabetic pregnancy, these 'physiological' changes should be borne in mind.

6.Other anemias- Their effect is discussed later.

All the confounding factors should be kept in mind before taking any therapeutic decision based on HbA1c levels.

NORMAL VALUE

Normal value is 4%- 6%[51] .

IRON DEFICIENCY ANEMIA

It is the most common form of anemia [3].

IRON [52,53]

Iron is of great importance in human nutrition. The adult human body contains 3-4 gm of iron, of which about 60%-70 % is present in the blood (Hb iron) as circulating iron, and the rest (1-1.5 gm) as storage iron. Each gram of hemoglobin contains about 3.34 mg of iron. Iron is vital for all living organisms because it is essential for multiple metabolic processes, including oxygen transport, DNA synthesis, and electron transport. It is also a component of myoglobin. Iron uptake in the proximal small bowel occurs by 3 separate pathways. These are the heme pathway and separate pathways for ferric and ferrous iron. Heme iron is not chelated and precipitated by numerous constituents of the diet that renders nonheme iron non absorbable. Examples are phytates, phosphates, tannates, oxalates, and carbonates. Heme is maintained soluble and available for absorption by globin degradation products produced by pancreatic enzymes. Heme iron and nonheme iron are absorbed into the enterocyte noncompetitively. Heme enters the cell as an intact metalloporphyrin, presumably by a vesicular mechanism. Heme is degraded within the enterocyte by heme oxygenase with release of iron so that it traverses the basolateral cell membrane in competition with nonheme iron to bind transferrin in the plasma. Ferric iron utilizes a different pathway to enter cells than ferrous iron. This was shown by competitive inhibition studies, the use of blocking antibodies against divalent metal transporter-1 (DMT-1) and beta3-integrin, and transfection experiments using DMT-1 DNA. This indicated that ferric iron utilizes beta3-integrin and mobilferrin, while ferrous iron

uses DMT-1 to enter cells. Which pathway transports most nonheme iron in humans is not known. Other proteins are described that appear related to iron absorption. These are stimulators of iron transport (SFT), which are reported to increase the absorption of both ferric and ferrous iron, and hephaestin, which is postulated to be important in the transfer of iron from enterocytes into the plasma. The relationship and interactions between the newly described proteins is not known at this time and is being explored in a number of laboratories. The iron concentration within enterocytes varies directly with the body's requirement for iron. Absorptive cells in iron-deficient humans and animals contain little stainable iron, whereas this is increased significantly in subjects who are replete in iron. In contrast to findings in iron deficiency, enhanced erythropoiesis, or hypoxia, endotoxin rapidly diminishes iron absorption without altering enterocyte iron concentration. This suggests that endotoxin and, perhaps, cytokines alter iron absorption by a different mechanism. Most iron delivered to nonintestinal cells is bound to transferrin. Daily iron requirements for male is 0.9 mg, for menstruating females is 2.8 mg, in first half of pregnancy is 0.8 mg, in second half of pregnancy is 3.5 mg, during lactation is 2.4 mg and in post menopausal women is 0.7 mg.

SERUM FERRITIN [54]

Ferritin is a globular protein complex consisting of 24 protein subunits and is the main intracellular iron storage protein in both prokaryotes and eukaryotes, keeping it in a soluble and non-toxic form. Ferritin which is not combined with iron is called apoferritin. The Ferritin protein consists of 24 protein subunits. In vertebrates, these are both the light(L) and the heavy (H) type with a molecular weight of 19 kDA or 21

kDA respectively. In plants and bacteria the complex only consists of the H-chain type. Inside the ferritin shell, iron ions form crystallites together with phosphate and hydroxide ions. The resulting particle is similar to the mineral ferrihydrite. Each ferritin complex can store about 4500 iron (Fe3+)ions. Serum ferritin levels are measured in patients as part of the iron studies workup for anemia and for restless leg syndrome. The ferritin levels measured have a direct correlation with the total amount of iron stored in the body. If ferritin is high there is iron in excess, which would be excreted in the stool. If ferritin is low there is a risk for lack in iron which sooner or later could lead to anemia. Low ferritin levels have been associated with symptoms of restless leg syndrome even in the absence of anemia. In the setting of anemia, serum ferritin is the most sensitive lab test for iron deficiency anemia. Ferritin is also used as a marker for iron overload disorders, such as haemochromatosis and porphyria in which the ferritin level may be abnormally raised. As ferritin is also an acute-phase reactant, it is often elevated in the course of disease. A normal C-reactive protein can be used to exclude elevated ferritin caused by acute phase reactions. Normal values of serum ferritin are 10-150 ng/ml in females and 29-248 ng/ml in males [51].

CAUSES OF IRON DEFICIENCY ANEMIA[51,53]

1)Increased demand for iron and/ or hematopoiesis-

a) Rapid growth in infancy or adolescence
b) Pregnancy
c) Erythropoietin therapy

2)Increased iron loss

a) Chronic blood loss

b) Menses

c) Acute blood loss

d) Blood donation

e) Phlebotomy as treatment of Polycythemia Vera

3. Decreased iron intake or absorption

a) Inadequate diet (vegetarianism), pica

b) Malabsorption from disease (sprue, Crohn's disease)

c) Malabsorption from surgery (post-gastrectomy)

d) Acute or chronic inflammation

STAGES OF IRON DEFICIENCY ANEMIA [51,53,70]

There are three stages:-

1. **Stage of negative iron balance**- In this stage the demands for (or losses of) iron exceed the body's ability to absorb iron from the diet. So, here the iron deficit is made up by mobilization of iron from the iron storage sites. During this period, serum ferritin and bone marrow stainable iron decreases. As long as iron stores are mobilized, serum iron, total iron binding capacity (TIBC) and red cell protoporphyrin levels remain normal. Red cell morphology and indices are normal at this stage.

2. **Stage of iron deficient erythropoiesis**- Here the stores are depleted. Serum iron decreases, TIBC begins to rise and so do the erythrocyte protoporphyrin levels. Transferrin saturation falls to 15%-20% and hemoglobin synthesis is impaired. Smear reveals the appearance of microcytic cells and hypochromic reticulocytes.

3.**Stage of iron deficiency anemia-** Here finally, hemoglobin and hematocrit begin to fall and there is anemia. Transferrin saturation at this stage is 10%-15%.When there is moderate anemia (Hb 10-13 g/dl) bone marrow remains hypoproliferative. As the anemia becomes severe (Hb 7-8 g/dl) , microcytosis and hypochromia become more prominent, poikilocytes in the form of cigar- or pencil-shaped cells are seen on the smear and marrow becomes increasingly ineffective.

SYMPTOMS AND SIGNS OF IRON DEFICIENCY ANEMIA [51, 53]

Conditions like pregnancy, adolescence, periods of rapid growth and an intermittent history of blood loss should alert the clinician to possible iron deficiency. Fatigue and diminished capability to perform hard labor are attributed to the lack of circulating hemoglobin; however, they occur out of proportion to the degree of anemia and probably are due to a depletion of proteins that require iron as a part of their structure. Increasing evidence suggests that deficiency or dysfunction of nonhemoglobin proteins has deleterious effects. These include muscle dysfunction, pagophagia, dysphagia with esophageal webbing, poor scholastic performance, altered resistance to infection, and altered behavior. In one study [55] symptoms of fatigue, faintness, palpitations, pain in chest, swelling of ankles, breathlessness, pallor and lack of concentration assessed by method A and symptoms of fatigue, dizziness, palpitations, irritability, headache and breathlessness assessed by method B were correlated with hemoglobin concentration and it was found that only ‘ pallor’ assessed by method A had a significant positive correlation with hemoglobin levels and that to in females. Correlation of other symptoms by both the methods with hemoglobin concentration was not significant. There is pallor of the mucous membranes. A number of abnormalities of epithelial tissues are described in association with iron deficiency anemia. These include esophageal webbing, koilonychia,platynychea,

glossitis, angular stomatitis, and gastric atrophy. Koilonychia and cheilosis are signs of advanced tissue iron deficiency. The exact relationship of these findings to iron deficiency is unclear and may involve other factors. For example, in publications from the United Kingdom, esophageal webbing and atrophic changes of the tongue and the corner of the mouth are reported in as many as 15% of patients with iron deficiency; however, they are much less common in the United States and other portions of the world. Splenomegaly may occur with severe, persistent, untreated iron deficiency anemia. This is uncommon in the United States and Europe.

RED CELL SURVIVAL IN IRON DEFICIENCY ANEMIA

It is believed that life span of red blood cells is normal in iron deficiency anemia. But in certain studies [56,57] it has been shown that life span of red blood cell is decreased. In one study [56], the life span of hypochromic red cells was found to be 46-85 days and the explanation given was due to an intracorpuscular defect. In another such study [57], 33 out of 35 patients had reduced T1/2 ^{51}Cr. In this study it was also shown that patients with microcytic hypochromic picture had greater reductions in red cell survival as compared to patients with normocytic hypochromic picture.

DIAGNOSIS OF IRON DEFICIENCY ANEMIA [51]

The following tests can be used to diagnose iron deficiency anemia and distinguish it from other microcytic hypochromic anemias namely, thalassemias, sideroblastic anemia and anemia of chronic disease:-

1. Serum iron and TIBC- Normal serum iron is 50-150 microgram/ deciliter and normal range for TIBC is 300-360 microgram/ deciliter. Normal transferring

saturation is 25%-50%. Serum iron and transferrin saturation are decreased in later stages of iron deficiency anemia and TIBC increases.

2. Serum ferritin- As described already, it is the first to decrease in iron deficiency anemia even before iron studies become diagnostic. A value less than 15 nanogram/ milliliter is virtually always diagnostic of absent iron stores.

3. Bone marrow iron stores- This is decreased or absent in cases of iron deficiency anemia. In addition to storage iron the marrow iron stain provides information about the effective delivery of iron to the developing erythroblasts. Normally, 20-40% of developing erythroblasts have visible ferritin granules and are called sideroblasts. In case of blockage of release of iron from the storage sites, there are few or no sideroblasts. In myelodysplastic syndromes, accumulation of iron in the mitochondria appears in a necklace pattern around the nucleus of erythroblast. Such cells are called as ringed sideroblasts.

4. Red cell protoporphyrin levels- Protoporphyrin is an intermediate in the synthesis of heme and its accumulation indicates impaired heme synthesis due to inadequate iron supply to erythroid precursors for heme synthesis. Normal value is less than 30 microgram/ deciliter. In iron deficiency anemia, values in excess of 100 microgram/ deciliter are seen.

5. Serum levels of transferrin receptor proteins- Because erythroid cells have the highest numbers of transferrin receptors, and because transferrin receptor protein (TRP) is released by cells into circulation, serum levels of TRP reflect the total erythroid marrow mass. Normal levels are 4-9 microgram/ deciliter. TRP levels are elevated in absolute iron deficiency.

DIFFERENTIAL DIAGNOSIS OF IRON DEFICIENCY ANEMIA [51,70]

The following table shows how to distinguish between various microcytic hypochromic anemias:-

Table B:- Showing differential diagnosis of iron deficiency anemia

Tests	**Iron deficiency**	**Inflammation**	**Thalassemia**	**Sideroblastic anemia**
Smear	Microcytic/ hypochromic	Normal microcytic /hypochromic	Microcytic/ hypochromic with targeting	Variable
Serum iron(ug/dl)	<30	<50	Normal to high	Normal to high
TIBC (ug/dl)	>360	<300	Normal	Normal
Percent saturation	<10	10-20	30-80	30-80
Ferritin (ug/L)	<15	30-200	50-300	50-300
Hemoglobin pattern	Normal	Normal	Abnormal	normal

TREATMENT OF IRON DEFICIENCY ANEMIA [70]

The aim is to find the cause of anemia, treat the cause of anemia and replete the iron stores. Modalities available are:-

1. Blood transfusion- It is given to patients who have symptoms of anemia, cardiovascular instability and ongoing blood loss from any site. It stabilizes the patient while other options are reviewed.

2. Oral iron therapy- Here, drugs with various iron formulations are available. On an average 300 mg of elemental iron is given per day in 2-4 divided doses. Major side effect of oral iron is gastrointestinal side effects which include abdominal pain, nausea, vomiting and constipation which lead to noncompliance. Response occurs in 4-7 days measured by reticulocyte response after initiation of therapy and peaks at one-and-a-half weeks. Serum ferritin rises slowly on oral iron therapy [58].

3. Parentral iron therapy- Here, again various intravenous/ intramuscular formulations are available. Care should be taken while using iron dextran as anaphylaxis is common. Parentral iron can be given either as a single dose or in multiple repeated doses. Serum ferritin rises faster after intravenous iron therapy and reaches peak in 1 week[59].

IRON DEFICIENCY ANEMIA AND GLYCATED HEMOGLOBIN LEVELS

Brooks et al [60] assessed HbA1values in 35 non-diabetic patients having iron deficiency anemia before and after treatment with iron. They observed that HbA1 values were significantly higher in iron deficiency anemia patients and decreased after treatment with iron. The mechanism leading to increased glycated HbA1 levels was not clear. It was proposed that in iron deficiency the quaternary structure of the hemoglobin molecule is altered and that, glycation of the beta globin chain occur more readily in the relative absence of iron. Sluiter et al [61]tried to give explanation for the above findings. They were of the view that the formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbA1 in one erythrocyte will increase linearly with the cell's age. In patients with normal blood glucose values but with red cells that are younger than usual, as after treatment of iron deficiency anemia, HbA1 concentration falls. However, if the iron deficiency has been persisting

for a long time, the red cell production rate falls, leading not only to anemia but also to a higher than normal average age of circulating erythrocytes and, therefore, of increased HbA1. Mitchell et al [62] in 1980, commented upon the study done by Brooks et al. They calculated the absolute amount of HbA1 in each red cell i.e. mean corpuscular HbA1 and found that there was no difference in HbAl before and after iron treatment. They also analyzed the study done by Sluiter et al and were of the view that red cell age was unlikely to be a significant factor in explaining the change in HbA1 during the treatment of iron deficiency anemia. Later Heyningen et al [63] reported no changes in the HbA1c concentrations when compared with controls in non- diabetic patients with iron deficiency anemia before and after treatment .They proposed that the reported differences in the HbAlc concentrations before and after iron supplementation are due to differences in the laboratory methods used for measuring HbA1c.

However Rai et al [64]investigated the different methods and no difference was detected among calorimetric, ion exchange chromatography and affinity chromatography. Hansen et al[65] demonstrated that there was no significant difference in HbA1c concentration in iron deficient, vitamin B-12 deficient and controls. They were of the opinion that in iron deficiency anemia, the erythrocyte survival rate is normal, while in vitamin B-12 deficiency the red cell survival rate is decreased but the hemolytic component is often minor and affects both mature and immature erythrocytes and hence normal levels of glycated hemoglobin are expected. HbAlc levels decreased on treatment of anemia which was probably due to increased bone marrow erythropoiesis on treatment leading to production of new immature erythrocytes.

Further studies [66, 67] showed that HbAlc levels were higher in patients with iron deficiency anemia and decreased significantly on treatment with iron. The probable explanation of elevated HbAlc in iron deficiency anemia is that , if serum glucose is accepted to remain constant, a decrease in the hemoglobin concentration might lead to an increase in the glycated fraction. The exact mechanism still remains elusive.

METHODOLOGY

The present work is a Prospective clinical non-controlled prognostic study conducted in department of medicine, R. L. Jalappa Hospital and Research centre, Kolar attached to Sri Devaraj Urs Medical College, Kolar from November 2008 to July 2010. . A total of 50 patients who fulfilled the inclusion criteria were included in the study and followed up for a period of 2 months.. Clearance was obtained from the Institutional Ethical Committee.

INCLUSION CRITERIA: -

1.Patients with confirmed diagnosis of iron deficiency anemia.

2.Adult patients with age ≥ 18.

EXCLUSION CRITERIA: -

Those with the following conditions will be excluded:

1. Pregnancy
2. All known Diabeteics or fasting blood sugar greater than 110 milligram per deciliter at the beginning during and after the completion of the study.
3. Hemolytic anemia
4. Chronic alcoholic state
5. Renal failure
6. Dyslipidemia

All patients were subjected to a detailed history and physical examination through a preset proforma (Proforma is attached in the annexure).

INVESTIGATIONS

Hemoglobin, MCV, MCH, MCHC, hematocrit, platelet count, TLC, DLC, ESR and peripheral smears were examined at the time of enrollment and then after, 1 week, 1 month and 2 months of starting the treatment.

Based on hemoglobin values, patients were divided into those having mild, moderate or severe anemia. Patients with hemoglobin (g/dl) values between 12-12.9 and 11-11.9 were considered to be having mild anemia in males and females respectively, those with values between 9-11.9 and 8-10.9 in males and females respectively, were taken to be having moderate anemia and those with values less than 9 and 8 in males and females respectively, were considered having severe iron deficiency anemia [68]. Reticulocyte count was measured at baseline and count again repeated after 1 week of starting therapy. Repeat count at 1 week was to check for response. Those with predominantly microcytic (MCV < 80 fentolitre) and hypochromic (MCH<26 picogram/ cell) indices were considered to be having iron deficiency anaemia which was confirmed by measuring serum ferritin. Those who had serum ferritin ≤ 12 nanogram per millilitre were included. Ferritin was measured again at 1 month and 2 month after initiation of treatment. HbAlc was also measured at the time of enrollment and then after 1 and 2 months of starting of treatment.

Other parameters which were recorded are as follows:-

a) Fasting blood sugar levels at the beginning and then, at 1 and 2 months on treatment. This was done to exclude diabetes or impaired glucose tolerance.

Those with fasting blood sugar ≤100 milligram per deciliter were included.

b) Urine pregnancy test was done in females to rule out pregnancy at baseline.
c) KFT's were done at baseline to rule out renal failure.
d) Serum lipid profile comprising of total cholesterol, HDL and triglyceride were done at baseline to rule out dyslipidemia.

All Cases were managed as per standard treatment protocol [70]and were treated with iron replacement therapy/blood transfusion as required.

METHODS USED FOR MEASURING VARIOUS PARAMETERS :-

1.Hemoglobin , TLC, DLC, Platlet count were done both manually and by an automated counter. MCV, MCH, MCHC and hematocrit were measured by automated counter only. Peripheral smear examination, reticulocyte count and ESR were done manually.

2.Reticulocyte count was done by supravital staining and ESR by Wintrobe's method.

3.Serum ferritin:- Serum ferritin levels were measured using the Roche Enhanced chemiluminescence immunoassay (ECLIA) methodology. Kits were stored and used as per desired guidelines. Further details of the kit are present in the kit product insert attached in the annexure.

4.HbAlc:- It was measured using RECOMBIGEN GLYCOSYLATED HEMOGLOBIN Kits. Kits were stored and used as per desired guidelines. Further

details regarding the kit and procedure can be read from the kit product insert attached in the annexure.

5.Renal function test, Lipid profile and blood sugar were measured by an automated counter .

Statistical Methods: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Repeated Measures Analysis of variance (RMANOVA) has been used to find the significance of study parameters between three or more groups of patients.

1. t-test of a correlation coefficient

Objective: To investigate whether the difference between the sample correlation co-efficient and zero is statistically significant.

Limitations: It is assumed that the x & y values originates from a bivariate normal distribution and that relationship is linear. To test an assumed value of population co-efficient other than zero, refer to the Z-test for a correlation co-efficient.

$$r = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sqrt{\sum (x - x)^2 \sum (y - y)^2}}$$

$t = \frac{r\sqrt{(n-2)}}{\sqrt{(1-r^2)}}$ is calculated and follows student t distribution with n-2 degrees of freedom.

2.Classification of Correlation Co-efficient (r)

Up to 0.1	Trivial Correlation
0.1-0.3	Small Correlation
0.3-0.5	Moderate Correlation
0.5-0.7	Large Correlation
0.7-0.9	V.Large Correlation
0.9- 1.0	Nearly Perfect correlation
1	Perfect correlation

3.Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value:$0.01<P \leq 0.05$)

** Strongly significant (P value : $P \leq 0.01$)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

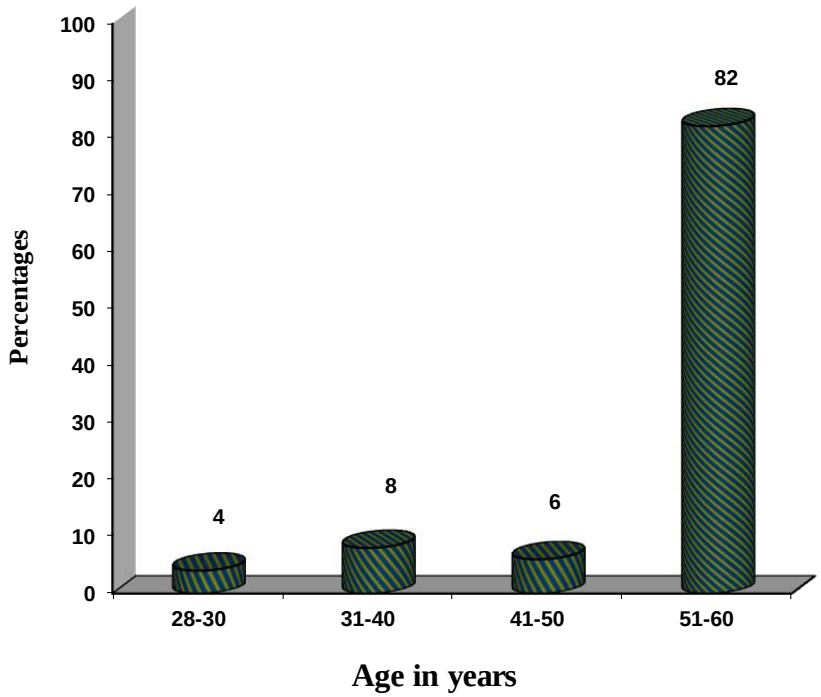
RESULTS

Study design: A Prospective clinical non-controlled prognostic study with 50 Iron deficiency anemia who are nondiabetics is undertaken to study the effect of iron deficiency anemia on glycated hemoglobin (HbAIc) and To study the response of treatment of iron deficiency anemia on glycated hemoglobin (HbA lc).

Table 1: **Age distribution of patients studied**

Age in years	Number of patients	%
28-30	2	4.0
31-40	4	8.0
41-50	3	6.0
51-60	41	82.0
Total	50	100.0

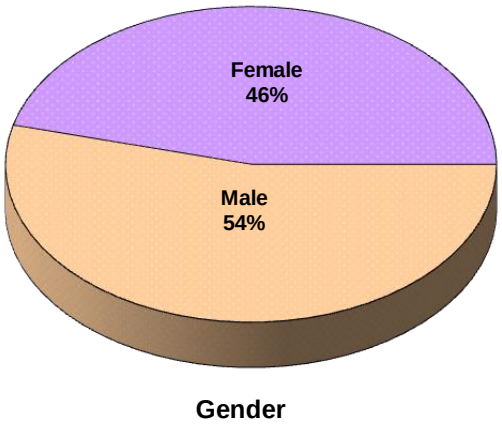
Mean ± SD: 51.66±7.87



The mean age of patients was 51.66. The minimum age in patients was 30 years and maximum age was 60 years.

Table 2: Gender distribution of patients studied

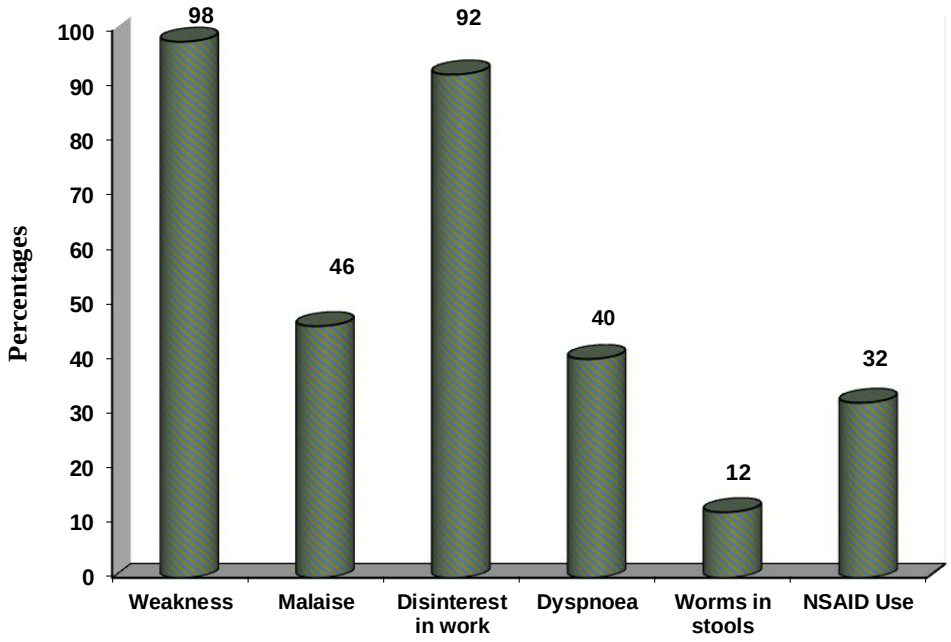
Gender	Number of patients	%
Male	27	54.0
Female	23	46.0
Total	50	100.0



There were 23 females (46%)and 27 (54%)males among the patients.

Table 3: Clinical features of patients studied

Clinical features	Number of patients (n=50)	%
Weakness	49	98.0
Malaise	23	46.0
Disinterest in work	46	92.0
Dyspnoea	20	40.0
Worms in stools	6	12.0
NSAID Use	16	32.0



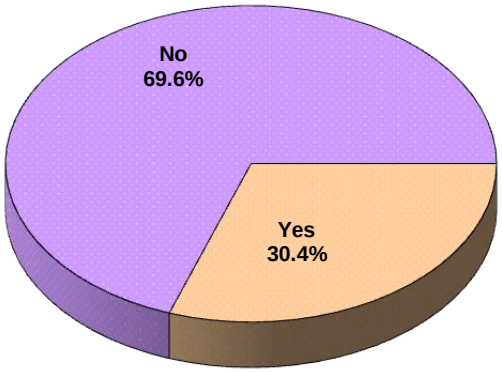
Weakness as a symptom was present in 49(98%) patients, malaise in 23 (46%) patients and disinterest in work in 46 (92%) patients. Dyspnoea was present in 20 (40%) patients. 6 (12%) patients gave history of passage of worms in stools. 16 (32%) patients had history of NSAID ingestion over a long time for different reasons.

Table 4: Bleeding complications

Bleeding complications	Number of patients (n=50)	%
Yes	12	24
No	38	76

Table 4a: Menstrual complaints

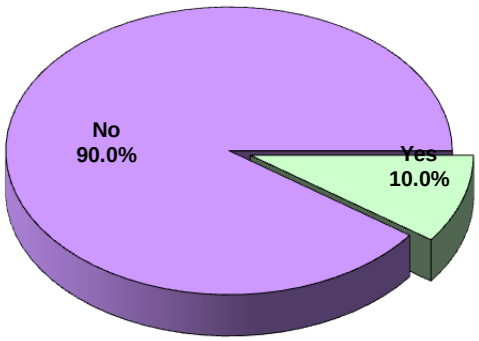
Menstrual complaints	Number of female patients (n=23)	%
Yes	7	30.4
No	16	69.6



Menstraul Complaints

Table 4b Bleeding other than PV

Bleeding other than PV	Number of patients (n=50	%
Yes	5	10.0
No	45	90.0

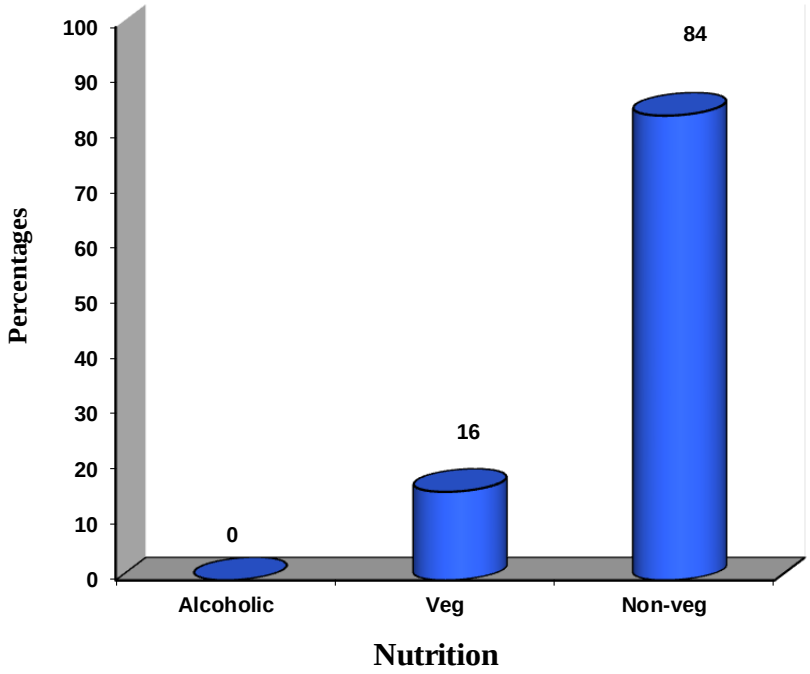


Bleeding other than PV

12 (24%) patients had bleeding complaints.7 (32%) out of 23 female patients had menstrual complaints. 5(10%) out of 50 patients had bleeding complaints other than Bleeding pervaginum.

Table 5: Nutrition

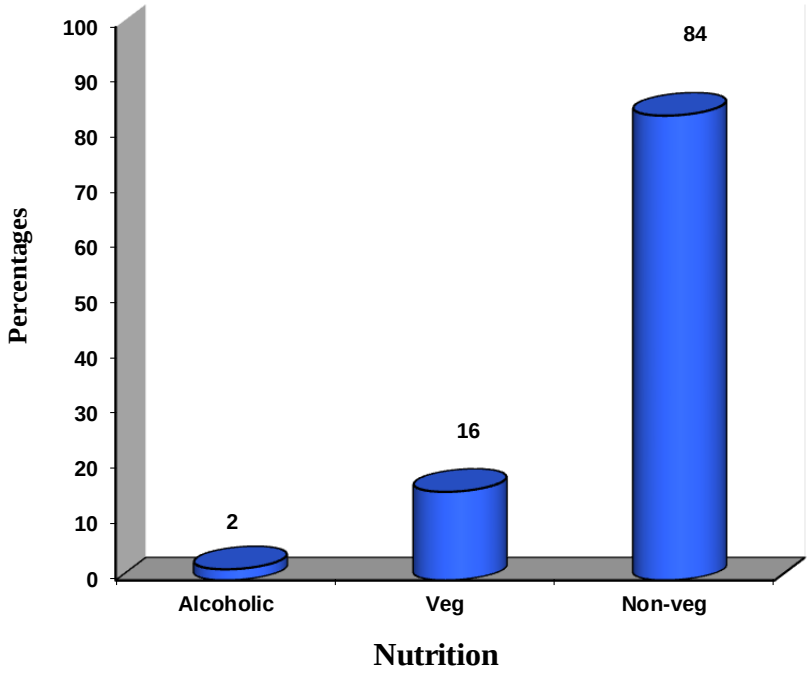
Nutrition	Number of patients (n=50)	%
Alcoholic	0	0.0
Veg	8	16.0
Non-veg	42	84.0



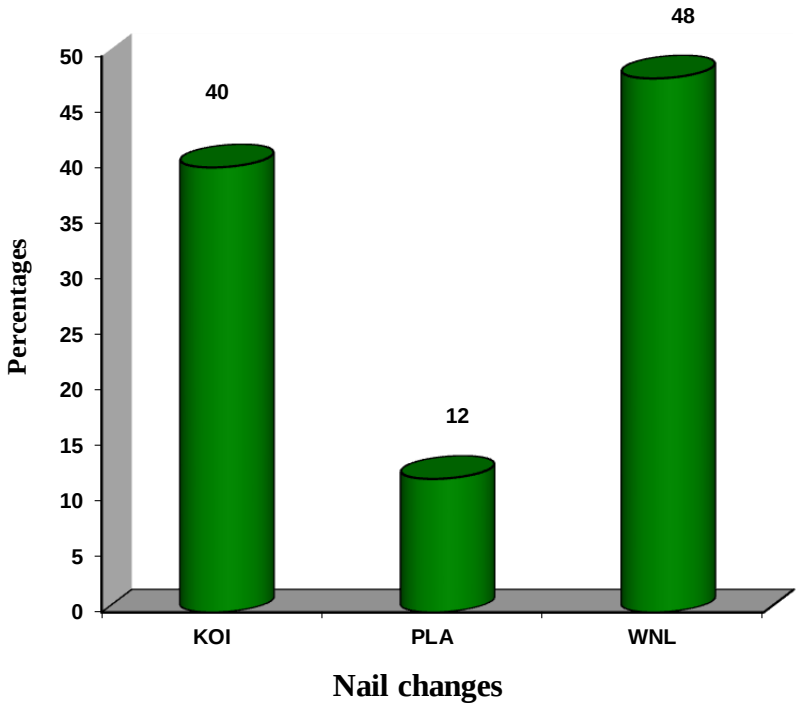
None of the patients included in the study were alcoholic.8 (16%) patients to be vegetarians and the remaining 42(84%) to be non vegetarians.

Table 6: Clinical signs

Clinical signs	Number of patients (n=50)	%
Pallor	50	100.0
Iceterus	0	0.0
LAP	0	0.0
Nail changes		
KOI	20	40.0
PLA	6	12.0
WNL	24	48.0



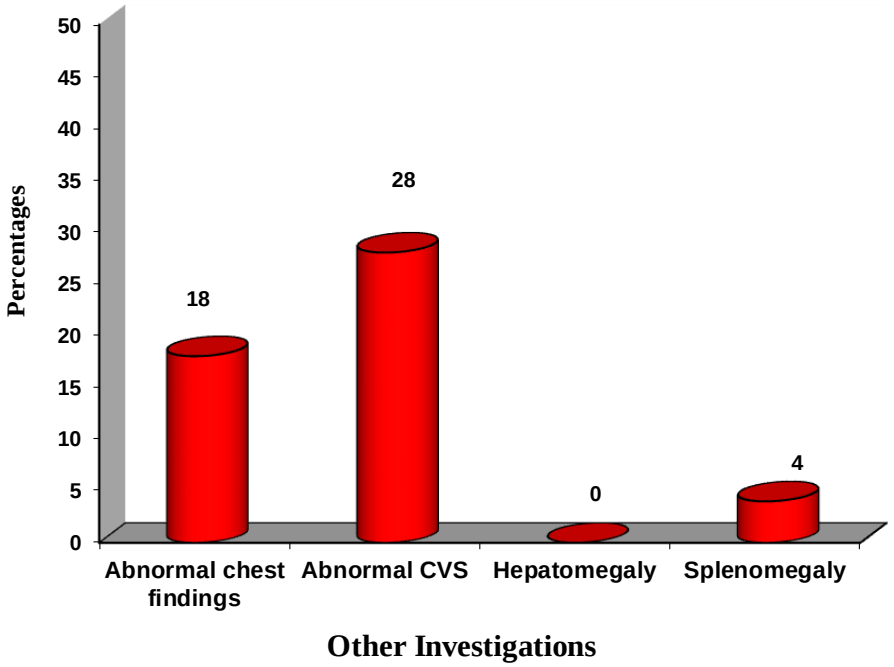
Pallor as a sign was present in all the patients. Icterus and Lymphadenopathy (LAP)was seen in none of the patient.



Nail changes were seen in 26 (48%) patients out of which 6 (12%) had platynychia(PLA) and 20 (40%) had koilonychia(KOI).

Table 7: Other Investigations

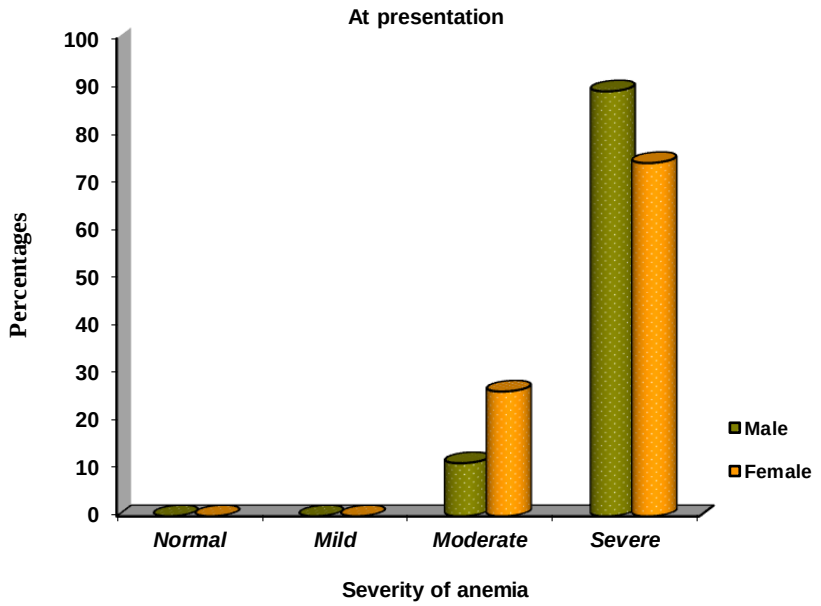
Other Investigations	Number of patients (n=50)	%
Abnormal chest findings	9	18.0
Abnormal CVS	14	28.0
Hepatomegaly	0	0.0
Splenomegaly	2	4.0



Abnormal chest findings(bilateral basal crepts) were present in 9 (18%)patients. Abnormal CVS findings were found in 14 patients (28%),Hepatomegaly was not seen in study population. Splenomegaly was seen in 2 (4%) patients.

Table 8: Severity of anemia

Severity of anemia	Male		Female		Total	
	No	%	No	%	No	%
At presentation						
• Normal	0	0.0	0	0.0	0	0.0
• Mild	0	0.0	0	0.0	0	0.0
• Moderate	3	11.1	6	26.1	9	18.0
• Severe	24	88.9	17	73.9	41	82.0
At week1						
• Normal	0	0.0	0	0.0	0	0.0
• Mild	0	0.0	0	0.0	0	0.0
• Moderate	6	22.2	6	26.1	12	24.0
• Severe	21	77.8	17	73.9	38	76.0
At 1 month						
• Normal	0	0.0	1	4.3	1	2.0
• Mild	5	18.5	2	8.7	7	14.0
• Moderate	14	51.9	17	73.9	31	62.0
• Severe	8	29.6	3	13.1	11	22.0
At 2 month						
• Normal	10	37.1	15	65.2	25	50.0
• Mild	12	44.4	7	30.4	19	38.0
• Moderate	5	18.5	1	4.3	6	12.0
• Severe	0	0.0	0	0.0	0	0.0
Total	**27**	**100.0**	**23**	**100.0**	**50**	**100.0**


At presentation
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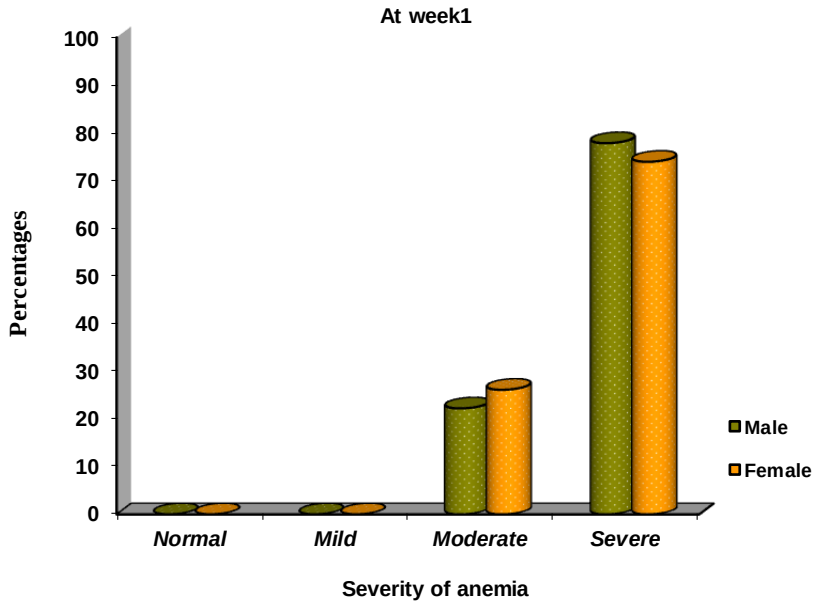
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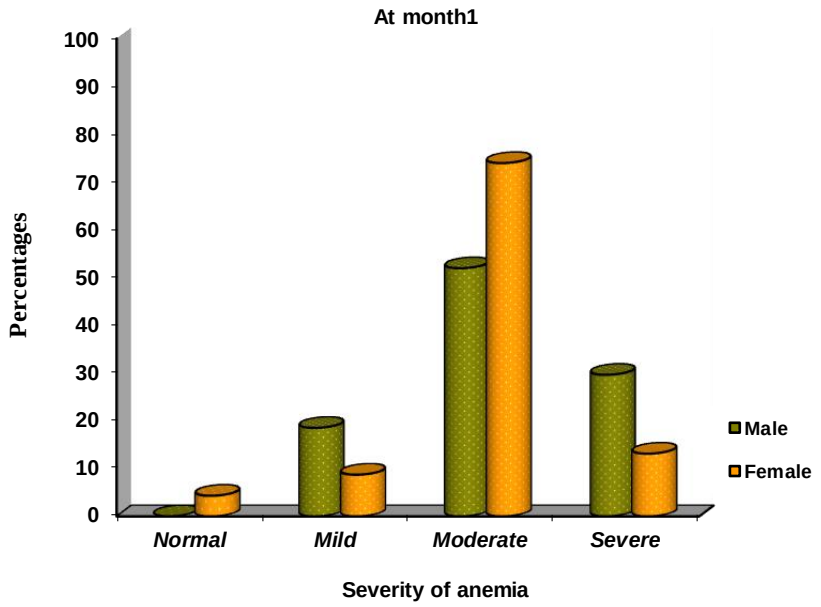
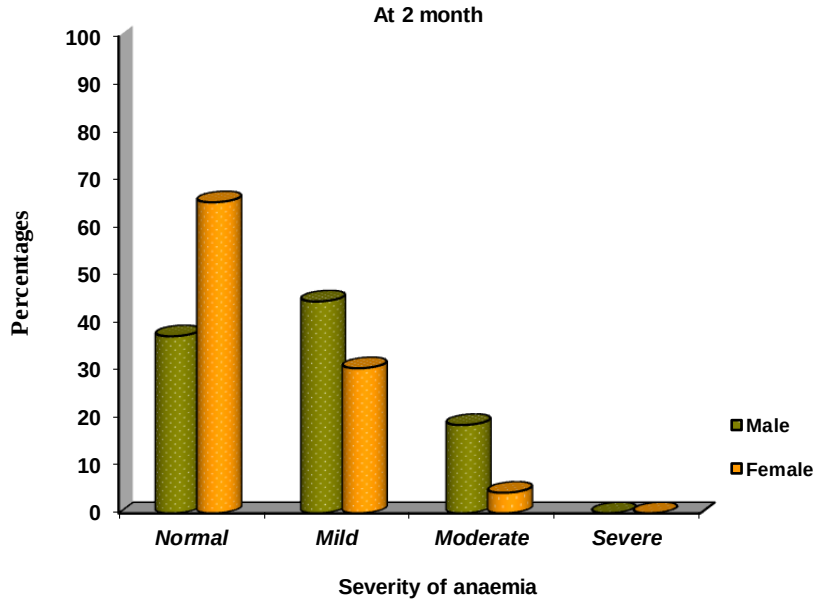
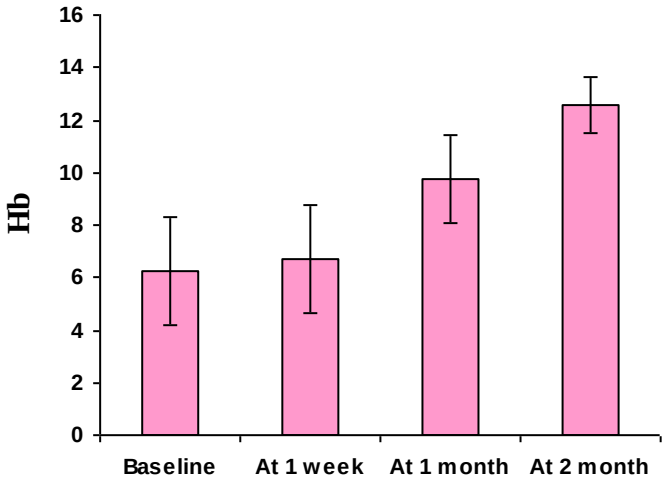




41(82%) patients had severe anemia and 9(18%) patients had moderate anemia. At 1 month 11 (22%)patients had severe anemia ,7(14 %)had mild anaemia. At 2 month 25(50%) patients were normal, 19(38%) had mild anemia while 6(12%) were still moderately anemic.

Table 9: Evaluation of hematological parameters at baseline, 1 week, 1 month and 2 month

	Baseline	At 1 week	At 1 month	At 2 month	P value
Hb	6.25±2.09	6.7±2.09	9.73±1.68	12.57±1.09	<0.001**
HCT	19.35±6.59	20.7±6.37	29.83±5.19	37.52±3.29	<0.001**
MCV	67.48±4.87	69.19±5.69	87.19±2.73	92.53±3.3	<0.001**
MCH	21.97±2.88	22.73±2.99	28.83±1.6	31.26±1.54	<0.001**
MCHC	31.66±3.45	32.62±3.55	32.68±1.8	33.59±1.64	0.002**
TLC	7936±2694.34	7686±1654.8	7528±1381.96	7454±1141.11	<0.001**
ESR	13.76±5.13	11.42±2.06	11.18±2.32	9.42±2.3	<0.001**
RETIC	4.36±1.33	7.39±1.14	-	-	<0.001**
S.Ferritn	7.55±2.47	-	156.29±54.32	280.79±82.24	<0.001**


Hb
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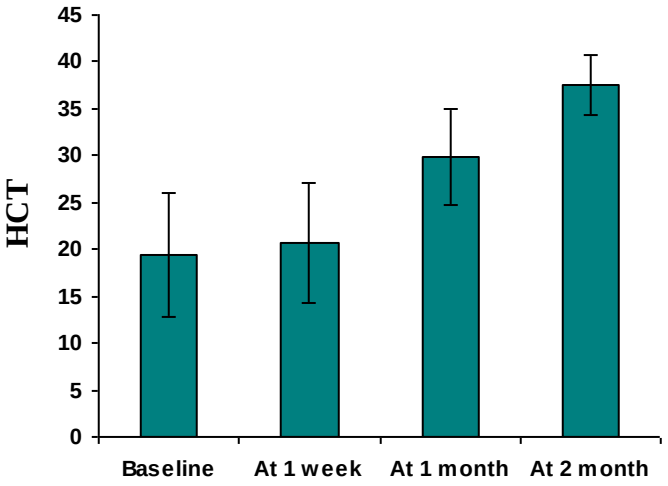
Baseline
At 1 week
At 1 month
At 2 month


HCT
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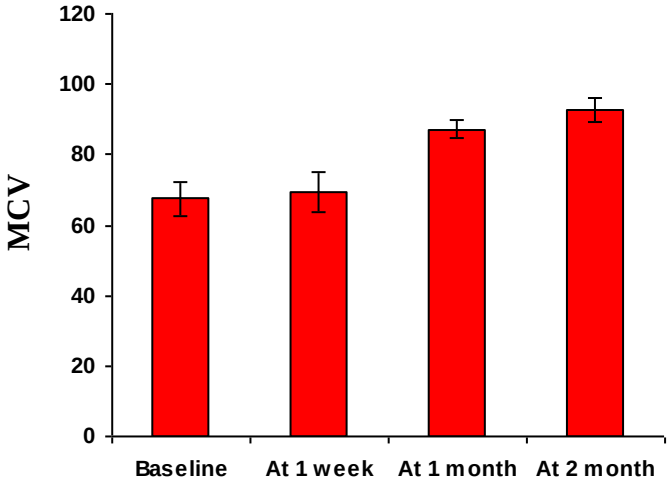
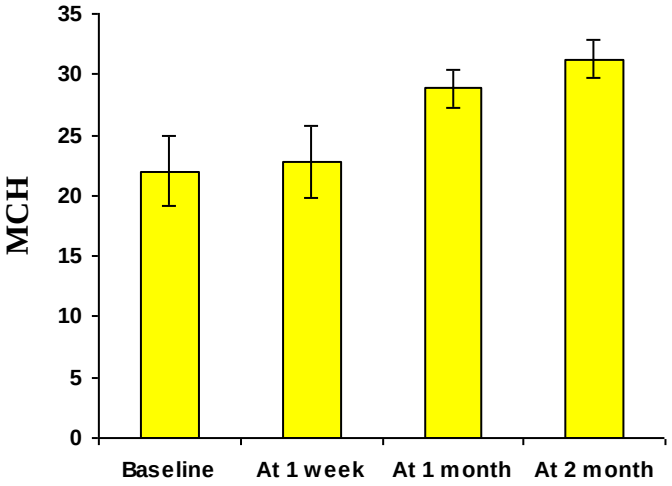
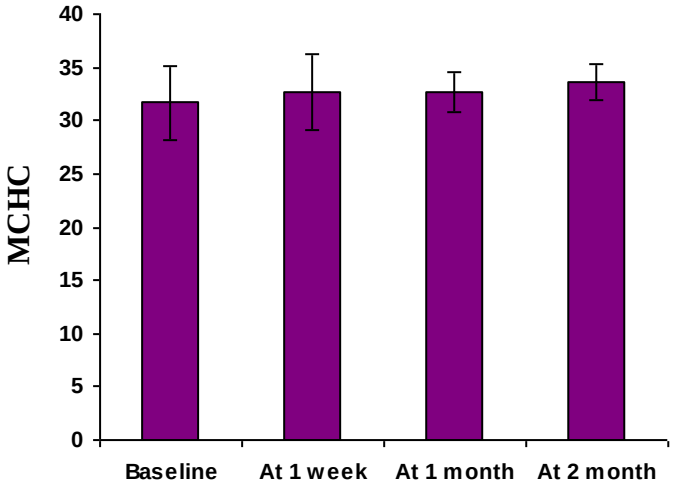
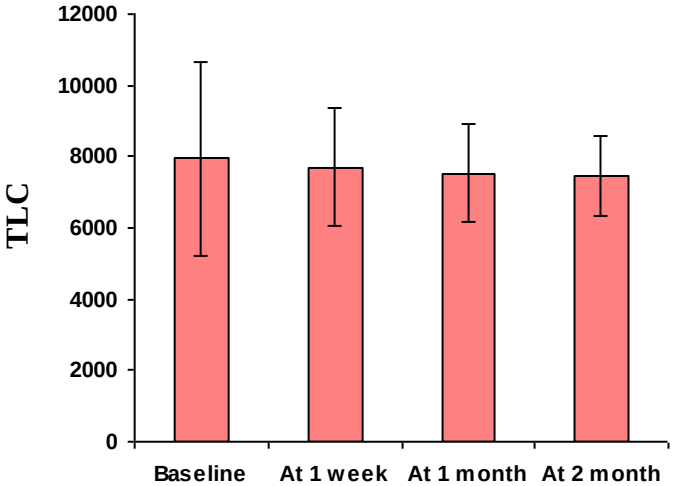
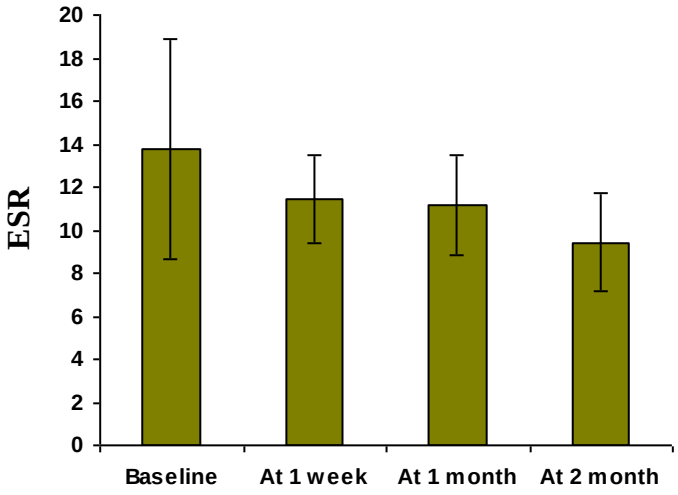
Baseline
At 1 week
At 1 month
At 2 month

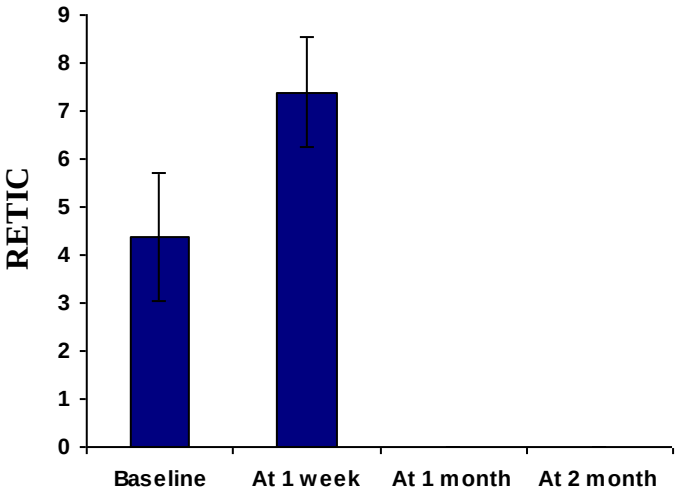

MCV
120
100
80
60
40
20
0
Baseline
At 1 week
At 1 month
At 2 month


MCH
35
30
25
20
15
10
5
0
Baseline
At 1 week
At 1 month
At 2 month

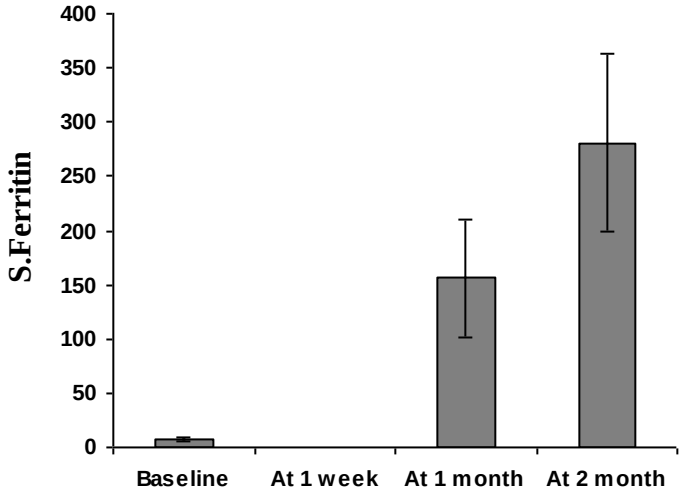

MCHC
40
35
30
25
20
15
10
5
0
Baseline
At 1 week
At 1 month
At 2 month


TLC
12000
10000
8000
6000
4000
2000
0
Baseline
At 1 week
At 1 month
At 2 month





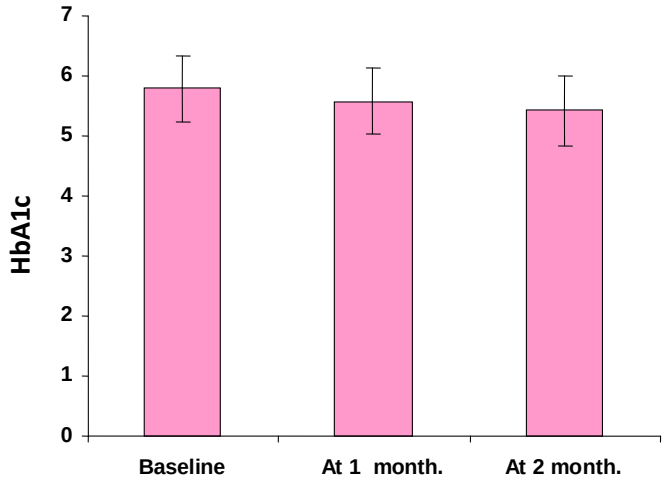
The mean hemoglobin value (g/dl) of patients at baseline was 6.25 (SD= 2.09) which rose to 12.57 (SD= 1.09) after 2 months of treatment.

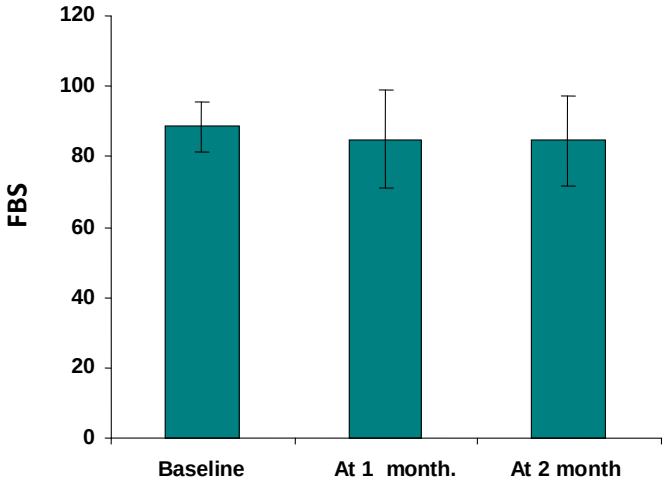


The mean serum ferritin values in patients at baseline was 7.55 (SD= 2.47) ng/ml which rose to 280.79 (82.24) at 2 months .

Table 10: Evaluation of Blood sugar parameters at baseline, 1 week, 1 month and 2 month

	Baseline	At 1 month	At 2 month	P value
HbA1c	5.79±0.55	5.57±0.55	5.42±0.59	<0.001**
FBS	88.44±7.05	85±13.76	84.53±12.82	0.084+





Mean fasting blood glucose value (mg/dl) in patients at baseline was 88.44 (SD=7.05) and the value at 2 month was 84.53 (SD= 12.82). The mean HbAlc value (%) in patients at baseline was 5.79(SD= 7.05). The mean HbAlc value (%) at 1 month in

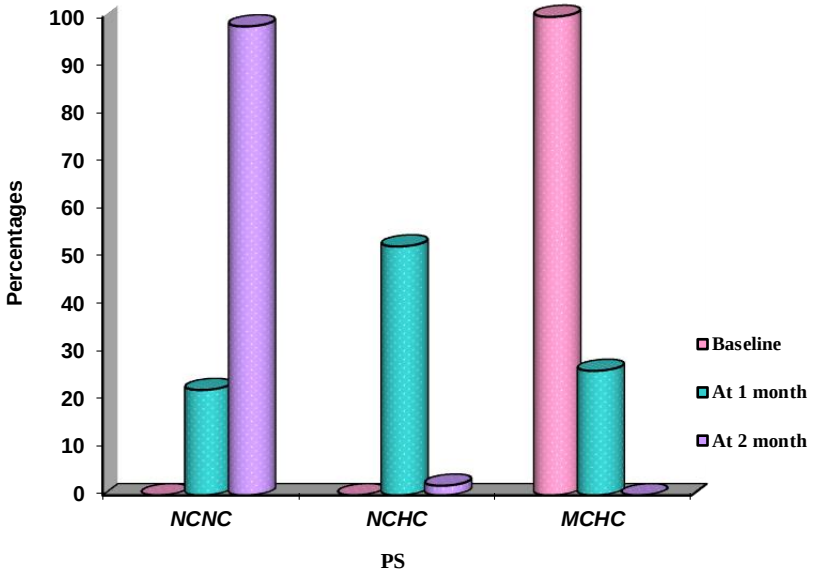
patients was 5.57(SD= 0.55). At 2 month, the mean HbAlc(%) value was 5.42(SD=0.59).

Table 11: Other Blood Investigations

	Min-Max	Mean ± SD
Blood Urea	14-50	27.32±7.01
Serum creatinine	0.4-1.5	0.85±0.26
Total cholesterol	77-195	168.26±20.45
LDL	62-130	82.14±15.51
HDL	33-55	43.36±5.83
Triglycerides	42-145	84.54±27.83

Table 12: Evaluation of Peripheral smear

PS	Baseline	At 1 month	At 2 month
NCNC	0	11(22.0%)	49(98.0%)
NCHC	0	26(52.0%)	1(2.0%)
MCHC	50(100.0%)	13(26.0%)	0
Total	50(100.0%)	50(100.0%)	50(100.0%)



At baseline peripheral smear of all 50 (100%)patients showed microcytic hypochromic picture. At 1 month microcytic hypochromic RBC'S were seen in 13 patients (26%),normocytic hypochromic picture was seen in 26 (52%) patients and normocytic normochromic blood picture was seen in 11 (22%) patients. At 2 months 49(98%) patients had normocytic normochromic blood picture in peripheral smear.

DISCUSSION

Iron deficiency anemia is most common form of anemia. HbAlc is one of the glycated hemoglobins which is used to assess the glycemic status of an individual over last 2-3 months and is mostly being used in diabetics and in those with impaired glucose tolerance. Certain studies have been done which show that HbAlc levels are affected in hemolytic anemias. In one of these studies [44] it was shown that HbAlc is decreased due to the reason that the life span of the RBC's is reduced. So, from these studies it became evident that HbAlc should be taken as a measure of glycemic control only if such disorders are ruled out. However, interest further arose as to what happens to HbAlc levels in more commonly encountered anemias like iron deficiency anemia. In one of the earliest studies [60], which studied the effect of iron deficiency anemia on HbAlc levels revealed that, HbAlc levels were higher in iron deficiency anemia patients. One further study [63] revealed that HbAlc levels are not elevated in iron deficiency anemia patients before and after treatment. Another study [65] showed that HbAlc levels were not different from that of controls at baseline but decreased after treatment. Further studies [66, 67]that were done revealed that HbAlc values were higher in iron deficiency anemia patients as compared to controls at baseline and decreased after treatment. Moreover, till date there is no valid reason available to prove that why HbAlc is higher in iron deficiency anemia patients or even if it is normal, why it decreases after treatment. The reasons given in studies quoted above in support of their findings are just speculations. The variations noticed in various studies and no valid reason to prove them initiated massive interest and prompted to undertake this study.

50 patients after applying the inclusion and exclusion criteria were analyzed during 2 months period and results analyzed using appropriate statistical methods.

Age and sex distribution in patients

There were 23 females (46%)and 27 (54%)males among the patients. The mean age of patients was 51.66 . This suggests that iron deficiency was almost equally prevalent

among both sexes in the study population. Probable reason for this observation could be decrease in menstrual blood loss in females after 45 as they attain menopause and mean age of the females included in the study population was 48.52 .The minimum age in patients was 30 years and maximum age was 60 years.

Symptoms and signs in iron deficiency anemia

Weakness as a symptom was present in 49(98%) patients, malaise in 23 (46%) patients and disinterest in work in 46 (92%) patients. Dyspnoea was present in 20 (40%) patients. 6 (12%) patients gave history of passage of worms in stools. These figures suggest that weakness and disinterest in work are highly common in iron deficiency anemia patients. Dyspnoea is less prevalent than the other two symptoms. 16 (32%) patients had history of NSAID ingestion over a long time for different reasons. Asking questions on menstrual bleeding revealed that 7 (32%) out of 23 female patients had menstrual complaints (post menopausal bleed). Asking about bleeding from sites other than per vaginum revealed that 5 (10%) out of 50 patients had such a bleed (piles) . None of the patients included in the study were alcoholic nor were on maintenance hemodialysis. Dietary pattern in iron deficiency anemia patients revealed 8 (16%) patients to be vegetarians and the remaining 42(84%) to be non vegetarians.

Pallor as a symptom was present in all the patients. Icterus and Lymphadenopathy was seen in no patient. Nail changes were seen in 26 (48%) patients out of which 6 (12%) had platynychia and 20 (40%) had koilonychia. This suggests that koilonychia is more frequently seen than platynychia in patients of iron deficiency anemia .

Abnormal chest findings(bilateral basal crepts) were present in 9 (18%)patients secondary to congestive cardiac failure due to anemia. Abnormal CVS findings were found in 14 patients (28%),all of them had ejection systolic murmur in different cardiac areas probably related to the hyperdynamic state seen in anemia patients. Hepatomegaly was not seen in study population. Splenomegaly was seen in 2 (4%) patients.

Hemoglobin values

The mean hemoglobin value (g/dl) of patients at baseline was 6.25 (SD= 2.09) which was significantly low. The mean hemoglobin in patients rose to 12.57 (SD= 1.09) after 2 months of treatment. The rise in hemoglobin value from baseline to the 2 month value was highly significant ($p<0.001$). This goes to suggest that patients responded well to the treatment.

Serum ferritin values

The mean serum ferritin values in patients at baseline was 7.55 (SD= 2.47) ng/ml. This goes on to suggest that iron stores in patients were nil at the time of enrollment [51]. At 2 months the mean ferritin value in patients rose to 280.79 (82.24) . The rise in serum ferritin was highly significant ($p<0.001$). The rise in serum ferritin on treatment was as expected [58] indicating increased availability of iron and increasing iron stores.

Fasting blood glucose values

Mean fasting blood glucose value (mg/dl) in patients at baseline was 88.44 (SD= 7.05) and the value at 2 month was 84.53 (SD= 12.82). The difference observed was not significant ($p=.084$).

HbAlc values

The mean HbAlc value (%) in patients at baseline was 5.79(SD= 7.05). The mean HbAlc value (%) at 1 month in patients was 5.57(SD= 0.55). At 2 month, the mean HbAlc(%) value was 5.42(SD=0.59). There was a significant decrease in the mean HbAlc (%) value after correction of iron deficiency anaemia from the baseline value ($p<0.001$).

Brooks et al [60] showed that HbAlc levels were higher in patients of iron deficiency anemia at baseline and decreased on treatment. The reason speculated by them was that the quaternary structure of hemoglobin gets altered and that, glycation of beta globin chain occurs more readily in the relative absence of iron. Sluiter et al [61] later gave a different reason to explain the findings of Brooks et al. They were of the view that the formation of glycated hemoglobin is an irreversible process and hence, the concentration of HbAl in one erythrocyte will increase linearly with the cell's age. In patients with normal blood glucose values but with red cells that are younger than

usual, as after treatment of iron deficiency anemia, HbAl concentration falls. However, if the iron deficiency has been persisting for a long time, the red cell production rate falls, leading not only to anemia but also to a higher than normal average age of circulating erythrocytes and, therefore, of increased HbAl. Mitchell et al [62] commented on the study done by Brooks et al and also on the reasoning of Sluiter et al. From the values available in Brooks study, they rather than taking HbAlc (%), calculated mean corpuscular hemoglobin HbAlc i.e. amount of HbAlc per cell and observed that though the percentage of HbAlc decreased during treatment, MCHbAlc remained relatively constant and there was no significant difference noted between baseline and post treatment values. They were also of the view that red cell age as proposed by Sluiter et al, was unlikely to explain the changes observed in HbAlc (%) in the Brooks study. Heyningen et al [63] demonstrated that there was no difference seen in HbAlc (%) values at baseline and after treatment in iron deficiency anemia patients and speculated that the differences observed previously could be due to different methods used in calculating HbAlc. But Rai et al [64], reported that there was no significant difference in HbAlc values calculated by colorimetry, ion exchange chromatography and affinity chromatography. Hansen et al [65] showed that there was no difference in HbAlc values at baseline between iron deficiency anemia patients and controls but demonstrated a fall in HbAlc levels after treatment which they explained by stating that it was due to increase in the number of immature erythrocytes. Further studies [66, 67] also demonstrated a baseline higher HbA1c in patients and fall after treatment but the reason speculated was different from the one given by Brooks et al and Sluiter et al. In these studies, the probable explanation of elevated HbAlc in iron deficiency anemia at baseline is that, if serum glucose is accepted to remain constant, a decrease in the hemoglobin concentration might lead to an increase in the glycated fraction but the exact mechanism still remains elusive.

Our observation of higher HbAlc levels at baseline and its subsequent decrease on iron supplementation also matches with those mentioned in various studies in the literature. We used accepted methodology (ion-exchange chromatography) in estimating HbAlc and the analysis was validated in our laboratory. A strict quality control was ensured.

Severity of anemia in patients

Anemia was graded as mild, moderate and severe according to the criteria described previously. 41(82%) patients had severe anemia and 9 (18%) patients had moderate anemia. The severe iron deficiency anemia was more common than that of moderate anemia in our study group. At 1 month 11 (22%)patients had severe anemia ,7(14 %)had mild anaemia. At 2 month 25

(50%) patients were normal,19(38 %)had mild anaemia while 6(12%)were still moderately anemic. These values tell that patients responded well to treatment.

Peripheral smear

At baseline peripheral smear of all 50 (100%)patients showed microcytic hypochromic picture,at 1 month microcytic hypochromic RBC'S were seen in 13 patients (26%),normocytic hypochromic picture was seen in 26 (52%) patients and normocytic normochromic blood picture was seen in 11 (22%) patients. At 2 months 49(98%) patients had normocytic normochromic blood picture in peripheral smear .These findings show the changes in the blood picture which come on the correcting the iron deficiency anaemia and also confirms that patients responded well to treatment

CONCLUSION

1. In our study there was a significant decrease in the mean HbAlc (%) value after correction of iron deficiency anaemia from the baseline value ($p<0.001$).

2. We recommend Iron deficiency should be corrected before any diagnostic or therapeutic decision is made based on HbA1c.

SUMMARY

A Prospective clinical non-controlled prognostic study with 50 Iron deficiency anemia patients who were nondiabetics was undertaken from November 2008 to July 2010 in R.L.JALAPPA Hospital. Patients were followed up for a period of 2 months. All Cases were managed as per standard treatment protocol [70]and were treated with iron replacement therapy/blood transfusion as required.

Result: The mean hemoglobin value (g/dl) of patients at baseline was 6.25 (SD= 2.09) which rose to 9.73(SD=1.68) at the end of 1month of treatment and to 12.57 (SD= 1.09) at the end 2 months of treatment. The mean serum ferritin values in patients at baseline was 7.55 (SD= 2.47) ng/ml which rose to 280.79 (82.24) at 2 months .The mean HbAlc value (%) in patients at baseline was 5.79(SD= 7.05), decreased to 5.57(SD= 0.55) and 5.42 (SD=0.59) at the end of 1 month and 2 month respectively. There was a significant decrease in the mean HbAlc (%) value after correction of iron deficiency anemia from the baseline value ($p<0.001$).

Conclusion: Iron deficiency should be corrected before any diagnostic or therapeutic decision is made based on HbA1c.

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PROFORMA

NAME CR.NOOPDNO

AGE DATE

Dob

SEX

ADDRESS

PRESENTING COMPLAINTS/OTHER SIGNIFICANT HISTORY AND DURATION

COMPLAINTS

Weakness

Malaise

Disinterest in work

Dyspnoea

Bleeding from any site

Menstrual complaints(in females)

Marriage(for females)

Age at child birth (in married females)

Passage of worms in stools

NSAID ingestion

Alcoholic

Hemodialysis

Vegetarian/Non-vegetarian

Examination

Oriented	
Temperature	°F
Pulse	/min
Blood Pressure	mm of Hg
Respiratory Rate	/min
Pallor	
Icteurs	
Cyanosis	
JVP	
Clubbing	
Pedal edema	
Lymphadenopathy	
Nails	
Chest	
CVS	
PA	
CNS	

Investigations

	BASELINE	1 WEEK	1MONTH	2 MONTH
HB				
PCV				
MCV				
MCH				
MCHC				
TLC				
DLC				
PLAT.				
ESR				
RET.COUNT				
S. FERRITIN				
FBS				
HBA1c				
B.UREA				
S. CR				
U.P.T				
PS				

ANNEXURE

KEY TO MASTERCHART

B/L	bilateral
B.Urea	blood urea
CVS	cardiovascular system
DLC	differential leucocyte count
ESM	ejection systolic murmur
ESR	erythrocyte sedimentation rate
F	female
FBC	fine basal crepts
FBS	fasting blood sugar
HB	hemoglobin
HbA1c	glycated hemoglobin
HCT	haematocrit
HDL	high density lipoprotein
Kl	koilonychia
LAP	lymphadenopathy
LDL	low density lipoproteins
M	male
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCHC	microcytic hypochromic
MCV	mean corpuscular volume
NA	not applicable
NCHC	normocytic hypochromic
NCNC	normocytic normochromic
NEO	neoaortic area

PA	platelets adequate
Pl	platelets
PL	platynychea
PLME	polymorphs,lymphocytes,monocytes,eosinophils
PMB	post menopausal bleeding
PS	peripheral smear
PUA	pulmonary area
Retic.	reticulocyte count
S.Cr	serum creatinine
S.Ferri-	serum ferritin
Sl	serial no
TA-	tricuspid area
TLC-	total leucocyte count
UPT-	urine pregnancy test
WNL -	within normal limits
0-	at baseline
1M-	at 1 month
1W-	at 1 week
2M-	at 2 month

MASTER CHART

Sl	Name	Age	Sex	Weakness	Malaise	Disinterest in work	Dyspnoea	Bleeding from any site Other than PV
1	AMRUTHA	52	F	YES	YES	YES	NO	NO
2	MARAPPA	59	M	YES	YES	YES	YES	YES(hemorrhoids)
3	CHINNAPIAH	54	M	YES	YES	YES	NO	NO
4	DEVAMMA	60	F	YES	NO	YES	NO	NO
5	MALATHI	53	F	YES	NO	YES	YES	NO
6	SHANKARANNA	54	M	YES	YES	YES	YES	NO
7	PANDURANGAPPA	60	M	YES	NO	YES	NO	NO
8	PARVATHI	52	F	YES	YES	YES	NO	NO
9	KANTHAMMA	28	F	YES	NO	YES	NO	NO
10	NAVNEETHA	53	F	YES	YES	YES	NO	NO
11	SARASAMMA	54	F	YES	NO	YES	NO	NO
12	OBALA REDDY	58	M	YES	NO	YES	NO	NO
13	MAMATHA	58	F	YES	NO	YES	NO	NO
14	NAGRAJ	52	M	YES	NO	YES	NO	NO
15	PANKAJAKSHI	57	M	YES	YES	YES	NO	NO
16	SHAUKADAMMA	54	F	YES	NO	YES	YES	NO
17	NOORAYESA	54	F	YES	YES	YES	NO	NO
18	NEELAVENI	53	F	YES	NO	YES	YES	NO
19	HAUSAVENI	58	F	NO	NO	NO	NO	NO
20	KANTHAMMA	58	F	YES	NO	YES	YES	NO
21	ANURADHA	51	F	YES	NO	YES	YES	NO
22	HEMALATHA	53	F	YES	YES	YES	NO	NO
23	MANJULA	54	F	YES	YES	YES	YES	NO
24	MARAKKA	56	F	YES	YES	YES	YES	NO
25	SIDDAMMA	58	F	YES	NO	YES	YES	NO
26	ANUSHA	53	F	YES	YES	NO	NO	NO
27	VINODAMMA	58	F	YES	YES	YES	YES	NO
28	LAKSHMI	53	F	YES	YES	YES	NO	NO
29	SIRISHS	58	F	YES	NO	NO	NO	NO
30	AMBIKA	54	F	yes	no	yes	NO	NO
31	GIRISH	53	M	YES	YES	YES	NO	NO
32	DAMODAR	54	M	YES	NO	YES	YES	YES(hemorrhoids)
33	NANJAPPA	54	M	YES	YES	YES	YES	NO
34	RAJGOPAL	53	M	YES	YES	YES	YES	YES (hemorrhoids)
35	SHANKAR REDDY	54	M	YES	YES	YES	YES	NO
36	AMARENDRA	53	M	YES	YES	YES	NO	NO
37	MAHESH	54	M	YES	NO	YES	YES	YES (hemorrhoids)
38	MAHADEVAPPA	54	M	YES	NO	YES	NO	NO
39	MAHESH	54	M	YES	NO	YES	YES	NO
40	KRISHNAMURTHY	50	M	YES	NO	YES	NO	NO
41	RAJESHKUMAR	35	M	YES	YES	YES	YES	NO
42	MUNIRAJU	54	M	YES	NO	YES	NO	NO
43	MANJUNATH	30	M	YES	NO	YES	NO	NO
44	MURUGESH	38	M	YES	YES	YES	YES	NO
45	SHYLESH	31	M	YES	NO	YES	NO	NO
46	SRINATH	31	M	YES	NO	NO	NO	NO
47	REDDAPPA	44	M	YES	YES	YES	NO	NO
48	BYRAPPA	50	M	YES	NO	YES	NO	YES (hemorrhoids)
49	NAWAZ SHARID	54	M	YES	YES	YES	YES	NO
50	MANIKYAM	54	M	YES	NO	YES	NO	NO

Sl	Menstrual complaints	Worms in stools	NSAID Ingestion	Alcoholic	Hemodialysis	Veg/ Non veg	pallor	Icterus
1	NO	NO	NO	NO	NO	NONVEG	YES	NO
2	NA	NO	NO	NO	NO	NONVEG	YES	NO
3	NA	NO	NO	NO	NO	NONVEG	YES	NO
4	NO	NO	YES	NO	NO	NONVEG	YES	NO
5	NO	NO	YES	NO	NO	VEG	YES	NO
6	NA	NO	NO	NO	NO	VEG	YES	NO
7	NA	NO	NO	NO	NO	NONVEG	YES	NO
8	NO	NO	NO	NO	NO	VEG	YES	NO
9	NO	NO	YES	NO	NO	NONVEG	YES	NO
10	Yes ,PMB	YES	NO	NO	NO	NONVEG	YES	NO
11	NO	NO	NO	NO	NO	NONVEG	YES	NO
12	NA	NO	NO	NO	NO	NONVEG	YES	NO
13	Yes ,PMB	NO	NO	NO	NO	NONVEG	YES	NO
14	NA	YES	NO	NO	NO	NONVEG	YES	NO
15	NA	NO	YES	NO	NO	NONVEG	YES	NO
16	NO	NO	YES	NO	NO	NONVEG	YES	NO
17	NO	NO	NO	NO	NO	NONVEG	YES	NO
18	NO	YES	NO	NO	NO	NONVEG	YES	NO
19	Yes ,PMB	NO	NO	NO	NO	VEG	YES	NO
20	NO	NO	YES	NO	NO	NONVEG	YES	NO
21	NO	NO	NO	NO	NO	NONVEG	YES	NO
22	NO	NO	YES	NO	NO	NONVEG	YES	NO
23	NO	NO	YES	NO	NO	NONVEG	YES	NO
24	YES,PMB	NO	NO	NO	NO	NONVEG	YES	NO
25	NO	NO	NO	NO	NO	NONVEG	YES	NO
26	NO	NO	YES	NO	NO	NONVEG	YES	NO
27	YES,PMB	NO	NO	NO	NO	NONVEG	YES	NO
28	YES,PMB	NO	NO	NO	NO	NONVEG	YES	NO
29	NO	NO	YES	NO	NO	NONVEG	YES	NO
30	YES,PMB	YES	NO	NO	NO	VEG	YES	NO
31	NA	NO	NO	NO	NO	VEG	YES	NO
32	NA	NO	NO	NO	NO	NONVEG	YES	NO
33	NA	NO	YES	NO	NO	NONVEG	YES	NO
34	NA	NO	NO	NO	NO	NONVEG	YES	NO
35	NA	NO	NO	NO	NO	NONVEG	YES	NO
36	NA	NO	YES	NO	NO	NONVEG	YES	NO
37	NA	NO	YES	NO	NO	NONVEG	YES	NO
38	NA	YES	NO	NO	NO	NONVEG	YES	NO
39	NA	NO	NO	NO	NO	VEG	YES	NO
40	NA	NO	YES	NO	NO	NONVEG	YES	NO
41	NA	NO	NO	NO	NO	NONVEG	YES	NO
42	NA	NO	YES	NO	NO	NONVEG	YES	NO
43	NA	NO	NO	NO	NO	NONVEG	YES	NO
44	NA	NO	NO	NO	NO	NONVEG	YES	NO
45	NA	YES	NO	NO	NO	NONVEG	YES	NO
46	NA	NO	NO	NO	NO	NONVEG	YES	NO
47	NA	NO	YES	NO	NO	NONVEG	YES	NO
48	NA	NO	NO	NO	NO	NONVEG	YES	NO
49	NA	NO	NO	NO	NO	NONVEG	YES	NO
50	NA	NO	NO	NO	NO	VEG	YES	NO

Sl	LAP	Nail changes	Chest	CVS	Hepatomegaly	Splenomegaly	Hb0	Hb1W	Hb1M	Hb2M
1	NO	NIL	WNL	WNL	NO	NO	7.5	7.6	10.7	11.8
2	NO	PL	WNL	ESM at PUA	NO	YES	4.9	5.5	8.6	11.3
3	NO	KL	WNL	WNL	NO	NO	4.7	4.4	8.4	12.0
4	NO	KL	WNL	ESM in PUA	NO	NO	4.6	5	9	11
5	NO	NIL	WNL	WNL	NO	NO	7.8	7.9	9.8	14
6	NO	NIL	WNL	WNL	NO	NO	3.5	4.5	10	12
7	NO	PL	WNL	WNL	NO	NO	4.3	5.0	7.0	12.0
8	NO	KL	WNL	ESM in PUA	NO	YES	8.1	8.4	11.8	12.2
9	NO	NIL	WNL	WNL	NO	NO	9	9.6	12.5	14
10	NO	NIL	WNL	WNL	NO	NO	4.2	4.5	6	11.8
11	NO	PL	WNL	WNL	NO	NO	6	6.6	10.6	11.4
12	NO	KL	WNL	WNL	NO	NO	6.5	7	9.5	11.5
13	NO	NIL	WNL	WNL	NO	NO	9.0	9.2	11.1	14.2
14	NO	KL	WNL	WNL	NO	NO	3.2	3.9	6.6	12
15	NO	NIL	WNL	WNL	NO	NO	5.3	6.3	8.3	11.8
16	NO	KL	WNL	ESM In PUA	NO	NO	3.5	4.5	6.8	10.9
17	NO	NIL	WNL	WNL	NO	NO	9.6	9.5	10.6	12.2
18	NO	NIL	FBC,B/L	ESM in PUA	NO	NO	6.5	6.8	10	12
19	NO	NIL	WNL	WNL	NO	NO	7	7.2	9.4	11.5
20	NO	KL	WNL	WNL	NO	NO	4.6	5.2	10	11.8
21	NO	KL	FBC,BL	ESM in PUA	NO	NO	3.6	4.2	8.9	11
22	NO	NIL	WNL	WNL	NO	NO	8.5	7.2	8.8	12.5
23	NO	PL	WNL	WNL	NO	NO	6.6	6.8	8.4	12.9
24	NO	KL	FBC,B/L	ESM in PUA	NO	NO	3.4	4.4	8.4	12.4
25	NO	NIL	FBC,B/L	ESM in PUA	NO	NO	7	8.6	10.8	13.2
26	NO	NIL	WNL	WNL	NO	NO	6	6.2	10.2	14.2
27	NO	KL	FBC,B/L	ESM in PUA	NO	NO	3.6	4.5	7.5	12.5
28	NO	NIL	WNL	WNL	NO	NO	6.8	7.2	10.4	12.5
29	NO	NIL	WNL	WNL	NO	NO	8.5	8.7	10.5	13.4
30	NO	KL	FBC,B/L	WNL	NO	NO	5.6	5.3	8.4	12.3
31	NO	NIL	WNL	WNL	NO	NO	6.3	6.4	9.3	12.2
32	NO	KL	FBC,B/L	ESM in NEO	NO	NO	2.3	2.5	6.2	10.1
33	NO	KL	WNL	WNL	NO	NO	6.1	6.6	9.3	13.2
34	NO	KL	WNL	WNL	NO	NO	3	3.4	8	12
35	NO	KL	WNL	ESM in NEO	NO	NO	3.7	4.1	8.2	12.2
36	NO	PL	WNL	WNL	NO	NO	5.9	5.9	9.9	12.7
37	NO	KL	WNL	WNL	NO	NO	6	6.3	9.5	12.5
38	NO	NIL	WNL	WNL	NO	NO	10	11.2	12.7	14.3
39	NO	KL	WNL	WNL	NO	NO	5.2	6.0	10.2	13.4
40	NO	NIL	WNL	WNL	NO	NO	8.3	9.4	12.4	14.4
41	NO	KL	FBC,B/L	ESM in PUA	NO	NO	3.9	4.4	9.8	12.2
42	NO	NIL	WNL	WNL	NO	NO	10.1	10.6	12	14.3
43	NO	NIL	WNL	WNL	NO	NO	8.6	9	11.4	14.2
44	NO	NIL	WNL	WNL	NO	NO	7	7.7	10.8	14
45	NO	KL	WNL	WNL	NO	NO	8	8.2	11.5	13.8
46	NO	NIL	WNL	WNL	NO	NO	9.5	9.7	12.6	14.5
47	NO	KL	FBC,B/L	ESM in TA	NO	NO	6.6	6.8	10.8	12.2
48	NO	WNL	WNL	WNL	NO	NO	8.1	10.4	12	13.7
49	NO	KL	WNL	ESM in TA	NO	NO	5.7	5.8	10.4	11.5
50	NO	NIL	WNL	WNL	NO	NO	8.7	8.8	10.7	12.6

Sl	HCT0	HCT1W	HCT1M	HCT2M	MCV0	MCV1W	MCV1M	MCV2M	MCH0	MCH1W	MCH1M
1	26.10	26.10	34.0	40.7	58.6	57.0	80.0	83.5	20.8	17.0	26.7
2	15.1	15.6	27.4	33.4	78.2	79.3	80.4	83.2	25.3	25.4	25.6
3	13.2	12.3	26.2	35	65.1	67.1	85.5	94.6	23.4	23.5	28.0
4	14.8	15	25	33	63.9	64	86	88	19.8	21	30
5	22.5	22.5	29	40	73.8	74	82	91	25.6	26	30
6	14	18	30	34	64	67.2	85	90	14	16.2	27.9
7	15.8	18	20.3	33.9	70.5	72	90	87.9	19.2	20	30.1
8	23.9	25.5	31.6	32.7	65.3	75.9	86.4	90.1	24.6	25.1	33.2
9	27	29.8	37.5	46.5	78.3	80.2	86.5	96	25.5	25.8	29
10	13.9	15.0	18.0	36	64	63.8	88	92.5	19.0	20	29
11	23.7	25.6	33.6	35	69	69.2	91	93	17.6	18	29
12	20.5	20.4	27.3	35.2	66.2	65.7	92	95	21.2	22.5	31.5
13	29	31	34.4	47	72	73	91.4	93.4	22.4	21.2	29.7
14	10	12	20.7	37.5	58.6	59	90	94	18.7	19.4	29.4
15	18.2	23	26	35	70	74.7	86	92	20.5	21.4	29.5
16	13	15.4	21.3	33	68.7	69.8	86.7	90.4	18.5	19.5	27.5
17	30.2	29.5	33.7	37.8	66.4	65.5	86.0	91.5	21.3	21.6	27.5
18	21.5	21.6	30.7	36.9	70.2	71.8	86.1	96.5	22.2	25	27.4
19	25	22	28	34.7	65.7	69	88.5	93.5	21	22	25
20	15	18	30.8	38	62	70	90	97	18.3	21	28.4
21	10.6	12.7	26.7	33.6	65.4	66.4	84.2	90	21.2	21.4	27.4
22	28.5	24	27	37.2	65.2	66.4	88	94.8	19.88	19.4	28.4
23	15	25.2	25.4	38.5	60	89	89.4	97.4	18.2	20.5	30.5
24	10.8	12.7	25	36	66.4	69.2	88.2	95	21.6	22.9	29.9
25	23.3	27.6	32.2	39	65.3	65.7	88.5	96.2	19.5	20.5	30
26	16.7	16.9	31.9	37.8	69.4	69.2	89.1	95	24.9	24.6	28.4
27	11.3	14.4	24.5	35.6	61.3	62.4	88.4	94.5	19.5	19.7	27.8
28	26.2	26.3	34.3	39.4	59.3	58.5	81.2	84.3	17	17	27
29	23.2	24.3	32.5	39.6	71.2	72.4	89.4	94.3	25.7	25.2	30.4
30	17.2	16.5	28.3	37.4	65.2	66.3	84.5	90.7	21.2	21.7	26.3
31	18.4	18.1	29.2	36.4	69.2	69.5	86.5	94.7	25.2	25.6	29.3
32	6.4	9.3	21.5	32.2	67.2	68.1	84.2	88.3	21.6	22.3	27.2
33	17.6	18.7	27.2	37.5	68.9	69.9	84.4	94.2	26	26.2	28.8
34	9	10.5	24.3	36.5	65.5	66.5	85.7	93.4	20.8	30	28
35	10.2	10.9	24.7	35.9	69	69.7	88.4	92.7	25.3	26.4	30.2
36	15.4	16.5	29	37	79.4	74.9	89.2	95	26.4	26.4	30.4
37	17.8	18	27.9	37.5	69.5	69.7	88.4	92.5	23.2	23.8	30
38	34	34.2	37.2	41.4	69.0	69.2	89.2	94.5	21.6	22.3	30.2
39	14.4	16	31	38	63	63.8	89.7	93.4	23	23.5	30
40	22.2	23.7	37.7	41.4	67	66.5	86.5	93	25.3	26.2	29.4
41	11	12.6	28.3	35.5	62.6	63.6	86.6	91.4	21.8	21.9	30
42	30.2	31	36.4	42.4	71	76.5	88.3	96.4	25	26	30.5
43	26.6	30	34.5	40.4	76	77	90.3	95.3	24.7	24.5	30.2
44	20	21	33	40	76	74	90	96	27	27.5	30.5
45	26.4	26.9	35	40.4	66.7	66.8	90	93.5	19.9	20.4	29.4
46	27	26.7	40	43.7	66.1	67.4	88.4	93.4	23.2	24.2	28.4
47	20	22	35	37.4	70	71	86.5	94	23.2	24.5	28.3
48	24.6	30.7	38.9	40	63.2	64.4	87.3	89.5	21.2	21.8	26.9
49	17.5	18.3	33.7	34.5	65.2	66.8	84.4	89	21.2	21.8	26
50	23.7	22.8	33.9	38.6	70.3	70.5	86.9	90.8	25.4	26.5	27.5

Sl	MCH2M	MCHC0	MCHC1W	MCHC1M	MCHC2M	TLC0	TLC1W	TLC1M	TLC2M	DLC0
1	28.7	35.5	29.5	32.7	33.1	14100	10200	8300	5500	P69L33M1E0
2	26.7	25.3	25.4	25.6	26.7	4200	4900	4600	9200	P63L31E3M3
3	32	35.5	35.8	35	33	5800	6000	7000	5000	P52L44M2E2
4	28	31	32	35	32	9200	9400	9600	9000	P77L19M2E2
5	32	34.7	35	34.4	35	9200	8200	8500	10000	P77L2M2E2
6	29.8	21.8	24	32.5	31.1	4800	4900	4800	5900	P62L36M2E2
7	32.5	27.2	28.0	34	36.5	4600	6500	8000	8300	P63L30M2E5
8	34.2	32.2	32.8	37.9	37.6	5000	7400	5300	7000	P62L36M1E1
9	30	32.5	32.0	30.2	31.0	10200	8300	8200	6200	P60L34M3E3
10	30	30	31.4	32.6	32.5	6700	6600	7500	8300	P72L24M1E3
11	29.7	25	25.8	31.6	31.7	6500	6800	6300	7000	P60L36M2E2
12	32.5	32	33.2	33.4	33.2	9800	8800	9600	7700	P76L20M2E2
13	28.4	32	33	32	31	11200	10300	9400	8500	P60L35M3E2
14	30.4	31.4	32.6	32.4	32.3	9500	8300	8500	7200	P54L41M3E2
15	30.8	28.6	30	31.5	33.7	6800	8700	9500	7800	P63L31M4E2
16	29.2	26.8	27.7	31.7	32.3	6300	6000	6900	6800	P56L42M2E0
17	30.5	32	33.2	31.6	33	7400	6800	6300	7000	P65L29M2E4
18	31.6	31.4	32.3	31.7	32.8	10600	7000	7600	6500	P70L28M1E1
19	32	33	32.4	34	34.5	12800	9000	8600	8400	P75L21M2E2
20	32	29.5	29.5	32	33.5	12300	8400	7600	8400	P78L20M1E1
21	30.4	32.4	32.2	32.6	33.7	8600	8300	7700	8400	P64L31M3E2
22	31.4	31	29.4	33.4	34	8700	8500	7500	8500	P65L31M2E2
23	31.9	30.6	32.9	33	34	8500	6500	6900	7200	P69L27M2E2
24	32.8	32.8	34.3	33.8	35.3	10700	6500	8700	7800	P68L28M2E2
25	32.8	30	31.4	33.4	35	6300	6700	6400	7400	P60L39M1E0
26	32.7	35.6	35.8	32	34.4	4700	5200	6300	6800	P60L33M4E2
27	32.9	31.2	31.5	31.7	34.2	4900	6700	5300	6200	P69L29M0E2
28	29	28	29	31	33.2	1400	10000	8200	5600	P69L29M2E0
29	32.3	25.7	25.2	30.4	32.3	8700	8600	7600	6700	P75L20M3E2
30	31.2	32.4	33.1	31.7	34.5	11000	10900	8000	6900	P72L28M0E0
31	32.5	36.2	37.5	33.2	34.5	11000	10000	9300	8600	P70L25M3E2
32	29.5	31.8	32.5	32.2	33.2	7500	8400	6700	7300	P69L30M0E2
33	32.3	28	38	34.9	35	9300	9500	9700	8800	P62L38M0E0
34	31.6	31.8	31.7	32.7	35	9300	9500	9700	8600	P62L38M0E0
35	32.8	26.6	37.9	34	35.3	5200	4900	6500	6800	P72L24M2E2
36	32.5	34.7	35.6	35	35	4800	4900	6300	5800	P65L33M2E0
37	31.2	33.4	34.2	33.8	33.9	6400	5900	6700	7700	P67L30M1E12
38	32.8	32	32.1	33.8	34.7	10800	8700	9100	8300	P72L27M0E1
39	32.5	36.5	37	33.4	34.7	9700	8700	7900	7700	P61L34M0E5
40	31.8	40	40.4	30.6	34	9000	7500	6900	7300	P70L25M1E4
41	31.8	34.8	34.6	34.5	34.2	4900	8000	8600	9600	P60L36M1E3
42	32.8	33	34	34.6	34	10800	9200	7600	6800	P70L26M3E1
43	33	32.6	31.7	32.9	34	10800	10000	7800	6800	P67L29M2E2
44	33	33.8	38	34	34	8900	8500	8700	7900	P64L32M3E1
45	31.8	29.8	30.4	32.8	34.2	5400	5700	6000	5700	P70L26M2E2
46	30.8	35	35.9	32	33.4	9700	8800	7900	9000	P60L38M1E1
47	30.4	33.2	34.7	31.7	32.6	4900	4700	4400	6700	P72L26M1E1
48	30.9	33.5	33.8	30.8	34.2	6300	7700	6400	7900	P72L24M2E3
49	30	32.5	32.7	30.8	34	5300	6300	6600	6000	P67L32M1E0
50	30.6	36.6	38	31.5	32.6	6300	7000	8900	8200	P67L32M1E0

Sl	DLC1W	DLC1M	DLC2M	ESR0	ESR1W	ESR1M	ESR2M	Retic.0	Retic1W
1	P66L33M1E0	P62L37M1	P69L26M2E3	21	13	11	3	2.5	5.7
2	P69L31	P66L33M1	P61L37E2	12	9	12	10	3.5	6
3	P64 L34M2	P65L33M1E1	P58L41E1	16	12	11	9	2.5	6
4	P59L36M3E2	P50L49M1	P65L35M0	14	11	12	7	3.0	5.9
5	P67L32M1	P71L29E0	P69L29M2	20	17	13	9	3.5	6.4
6	P50L40M1E1	P65L31M2E1	P64L30M1E2	11	9	13	7	6.3	7.5
7	P62L36M1E1	P60L36M1E3	P63L33M2E2	8	11	11	13	4	6.2
8	P69L31	P54L42M2E2	P65L33E2	12	12	10	10	6.5	8.5
9	P60L37M2E1	P61L35M1E3	P63L35M1E1	17	10	12	9	5.5	7.4
10	P68L27M3E2	P67L30M1E1	P69L39E1	10	9	12	14	6.6	9
11	P65L34M1E0	P68L27M4E1	P68L27M4E1	11	10	8	12	4.5	8
12	P66L32M1E1	P69L30M1	P59L36M1E2	4	10	12	10	5.5	9.4
13	P62L35M3	P69L30M0E1	P68L30M0E2	18	14	12	7	4.4	7.6
14	P60L37M2E1	P63L35M2E0	P70L25M3E2	14	16	12	10	5.5	6.2
15	P63L33M2E2	P59L40M1E0	P63L33M2E2	23	12	11	09	4.5	7.5
16	P60L36M2E2	P63L33M3E1	P56L42M1E1	6	12	11	10	4.4	7.2
17	P65L32M1E2	P63L33M2E2	P59L39M0E2	11	16	11	9	4.6	8.6
18	P65L32M1E2	P65L35	P66L32M3E2	20	12	8	7	4	7.2
19	P68L31M1E0	P69L30M1E0	P60L36M2E2	20	13	10	13	5.2	8.5
20	P65L30M2E1	P60L37M2E1	P68L32M0E0	12	14	12	9	3.6	7
21	P66L33M0E1	P60L40M0E0	P61L35M2E2	11	9	13	7	4.5	6.5
22	P68L32M0E0	P64L32M3E1	P60L37M2E1	11	8	6	7	5	7.2
23	P65L34M1E0	P64L33M2E1	P60L38M1E1	10	9	8	11	2.5	8
24	P66L32M2E0	P63L34M2E1	P58L41M1E0	20	11	9	9	3.6	5.6
25	P70L29M1E0	P65L34M1E0	P66L33M1E0	8	9	11	9	3.5	5.5
26	P59L37M2E2	P56L42M1E1	P56L42M1E1	17	11	9	12	3.2	6.6
27	P57L41M2E57	P57L36M2E5	P60L38M1E1	24	13	15	14	3.3	7
28	P67L32M1E0	P70L36M2E2	P61L39M0E0	20	15	14	6	3.7	7
29	P69L30M1E0	P69L20M1E0	P58L40M0E2	12	9	7	8	3.6	7.2
30	P68L32M1E0	P63L33M3E1	P70L28M1E1	20	13	11	13	2.6	7.8
31	P69L30M1E0	P60L37M0E3	P60L37M1E2	17	13	11	9	5.5	9
32	P62L37M0E1	P59L37M2E2	P60L36M4E1	11	13	11	9	2.6	7
33	P63L35M0E2	P60L36M2E2	P59L36M4E1	15	11	9	8	2.6	7.6
34	P67L32M0E1	P60L36M2E2	P60L35M4E1	11	10	9	11	2.6	6.7
35	P64L35M4E0	P70L30M0E0	P69L29M1E1	20	11	9	8	5.2	10
36	P61L35M2E2	P59L38M2E1	P59L38M2E1	14	11	13	9	3.1	5.6
37	P67L32M1E0	P67L31M1E1	P67L31M1E1	10	10	11	9	5.2	9
38	P69L30M0E1	P67L31M1E1	P56L41M2E1	11	13	9	9	6	8
39	P59L34M4E3	P60L36M1E2	P64L36M0E0	6	11	13	8	2.6	7
40	P67L32M1E0	P55L41M1E1	P56L40M2E2	7	11	13	8	4	8.6
41	P60L35M3E2	P68L38M0E0	P60L38M1E1	11	11	16	11	3	6
42	P64L35M0E1	P56L43M1E0	P51L45M3E1	11	9	9	12	5.50	9
43	P63L36M0E1	P60L40M0E0	P55L43M1E1	16	13	13	9	7	7.5
44	P70L30M1E0	P69L30M1E0	P67L29M2E2	6	11	13	9	5.6	9
45	P66L33M1E0	P67L32M1E0	P61L37M1E1	24	13	11	9	6.7	9
46	P59L37M2E3	P54L43M1E2	P60L37M1E2	10	9	12	8	6.2	7.6
47	P61L38M1E0	P59L37M1E2	P60L36M2E2	10	9	13	8	3.5	6
48	P63L36M0E1	P61L31M2E1	P60L38M1E1	12	12	9	8	3.6	7
49	P59L39M1E1	P61L36M3E3	P56L41M1E2	21	10	9	16	6.2	8.3
50	P54L44M1E1	P60L40M0E0	P64L32M2E2	12	11	19	10	5.6	7.5

Sl	PS0	PS1M	PS2M	S.Ferri0	S.Ferri1M	S.Ferri2M	HbA1c0	HbA1c1M	HbA1c2M
1	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	5.1	135.5	290.5	5.8	5.5	5.5
2	*MCHC*	*NCNC,PA*	*NCNC,PA*	10	135	255	6.0	5.5	5.4
3	*MCHC*	*MCHC,PA*	*NCHC,PA*	5.4	100	179.4	5.5	5.3	5.0
4	*MCHC*	*MCHC,PA*	*NCNC,PA*	12	160	239	6.1	5.9	5.6
5	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	12	131.8	313.6	5.1	5.0	5.0
6	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	5	155.6	249.1	6.0	5.8	5.8
7	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	10.7	152	250	5.5	5.3	5.0
8	*MCHC*	*NCHC*	*NCNC,PA*	5	164.6	217.3	6.6	6.4	6.4
9	*MCHC*	*NCNC*	*NCNC,PA*	10.1	165	201	6.1	5.9	5.8
1	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	5.2	110	185	5.1	4.9	4.9
11	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	11	105	185.2	5.9	5.8	5.8
12	*MCHC*	*NCHC,PA*	*NCNC,PA*	4	55	110	6.8	6.7	6.6
13	*MCHC,PA*	*NCHC,PA*	*NCNCPA*	11.4	98.4	148.7	6.6	6.6	6.4
14	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	9	77	167	5.4	5.3	5.1
15	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	6	138	239	4.4	4.3	4.0
16	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	5.5	103.8	178	6.7	6.5	6.4
17	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	10.3	121.7	388.6	6.5	6.6	6.5
18	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	6	178.7	233.5	5.1	4.9	4.8
19	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	5.2	85.5	178	5.8	5.6	5.6
20	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	10	202.9	313.4	5.7	5.5	5.5
21	*MCHC,PA*	*NCHC ,PA*	*NCNC,PA*	10.3	120	185	5.7	5.6	5.5
22	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	11.3	137.2	300	5.7	5.7	5.4
23	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	6	145	249	6.1	6.0	5.8
24	*MCHC,PA*	*NCHC ,PA*	*NCNC,PA*	6	110.8	270.2	5.7	5.6	5.6
25	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	7	110	380	5.5	5.7	5.6
26	*MCHC,PA*	*NCHC ,PA*	*NCNC,PA*	5.8	83.6	234.6	6.7	6.3	6.1
27	*MCHC,PA*	*NCHC ,PA*	*NCNC,PA*	8.5	240.3	306.8	5.5	5.3	5.1
28	*MCHC,PA*	*NCHC ,PA*	*NCNC,PA*	7.3	170.1	319.5	5.5	5.4	5.3
29	*MCHC,PA*	*NCHC ,PA*	*NCNC,PA*	8.7	234.8	319.7	6.6	6.5	6.4
30	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	5.7	100.3	300.4	5.5	5.3	5.3
31	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	5.4	147.5	180.3	5.7	5.5	5.4
32	*MCHC,PA*	*MCHC,PA*	*NCNC,PA*	5.7	244.6	340	4.6	4.6	4.5
33	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	8	190	334.3	5.7	5.5	4.9
34	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	7	256	382	5.9	5.6	5.3
35	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	5.8	115.2	321	5.4	5.3	5.1
36	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	5.2	272	414	5.7	5.7	5.2
37	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	6	172.3	233.9	5.9	5.7	5.5
38	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	5.2	132.4	320.3	4.8	4.7	4.4
39	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	10	218.5	414.8	5.5	5.2	5.1
40	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	6.6	178.2	344.5	5.8	5.4	5.3
41	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	9	160.8	342	4.9	4.5	4.1
42	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	5.3	208	320	6.4	5.2	5.1
43	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	11	264	420	5.7	5.4	5.3
44	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	5.2	272	439	6.8	6.6	6.6
45	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	5.6	138	298	5.7	5.2	5.2
46	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	6.5	160.4	250.6	5.7	5.5	5.3
47	*MCHC,PA*	*NCHC,PA*	*NCNC,PA*	6.4	130.5	235.6	6.4	5.9	5.9
48	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	11.8	195.9	431.6	6.1	5.6	5.3
49	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	11.5	112.3	235.4	5.6	5.3	5.1
50	*MCHC,PA*	*NCNC,PA*	*NCNC,PA*	5.0	218.4	395.6	5.8	5.5	5.3

Sl	FBS0	FBS1M	FBS2M	B.Urea	S.Cr	UPT(in F)	Pl0	Pl1W	Pl1M	Pl2M
1	93	95	75	34	1	NEG	1.36	1.67	2.18	2.37
2	93	98	88	34	1	NA	1.39	1.63	2.50	2.60
3	97	98	90	24	0.5	NA	1.83	1.95	3.06	2.13
4	98	86	94	24	1.1	NEG	1.43	2.13	1.72	1.86
5	78	93	96	41	1.3	NEG	1.85	1.94	1.82	2.00
6	88	90	82	26	1.0	NA	1.4	1.89	1.94	1.46
7	90	80	87	28	1.0	NA	1.79	1.90	1.80	1.65
8	86	76	80	28	0.9	NEG	1.38	2.06	3.30	3.16
9	80	82	92	48	1.2	NEG	3.27	3.65	2.35	3.15
10	98	84	84	28	0.7	NEG	2.08	2.78	1.66	2.18
11	100	100	80	28	0.6	NEG	1.68	1.98	3.12	2.65
12	90	70	80	20	0.8	NA	2.74	2.94	1.64	3.5
13	89	92	83	32	0.9	NEG	1.43	1.85	2.3	2.78
14	88	90	94	24	0.7	NA	4.5	3.96	3.12	2.95
15	90	95	91	20	0.7	NA	1.45	2.45	3.15	2.60
16	100	90	93	14	0.5	NEG	1.7	1.8	2.63	3.13
17	89	90	93	34	0.6	NA	3.56	3.42	2.60	3.15
18	84	94	90	22	0.6	NEG	1.5	2.5	3.5	2.6
19	99	5.2	5.4	30	0.5	NEG	3.7	2.97	1.99	2.90
20	85	95	90	29	0.8	NEG	3.71	2.78	3.15	2.90
21	77	87	85	25	1.2	NEG	2.7	2.6	2.65	2.74
22	100	80	80	23	0.6	NEG	1.9	2	1.99	2.15
23	85	75	85	28	1.2	NEG	1.64	2.14	3.14	2.90
24	80	90	80	30	1.2	NEG	1.5	2.5	3.14	2.68
25	100	90	84	50	0.9	NEG	3.5	2.5	2.6	2.7
26	80	70	86	22	0.8	NEG	1.26	2.1	2.14	2.38
27	90	84	86	20	0.6	NO	3.24	3.36	3.47	2.44
28	90	90	80	30	0.7	NEG	1.42	1.33	3.14	1.72
29	90	80	88	26	0.7	NEG	3.14	3.17	3.19	3.20
30	78	83	88	26	0.6	NEG	2.56	2.32	2.37	2.72
31	90	76	86	24	0.6	NA	3.15	3.23	3.27	3.28
32	93	82	78	30	0.4	NA	1.92	1.95	1.73	1.76
33	90	86	84	22	0.7	NA	1.91	1.97	1.77	1.77
34	80	86	82	27	0.7	NA	1.92	1.95	1.78	1.75
35	88	90	86	22	0.6	NA	1.68	2.20	1.97	2.34
36	95	97	87	34	1.2	NA	1.98	2.54	1.99	1.88
37	95	97	99	33	1.5	NA	1.64	1.79	1.89	1.57
38	84	96	88	25	0.7	NA	3.9	3.64	2.99	3.14
39	90	82	78	20	0.8	NA	4.1	3.48	3.70	3.15
40	90	88	86	22	0.6	NA	2.96	3.01	3.04	2.58
41	80	76	82	40	1.2	NA	1.62	1.98	1.99	2.51
42	90	80	92	26	1.0	NA	1.60	1.97	2.14	2.08
43	96	90	94	20	0.9	NA	2.94	2.92	2.94	2.60
44	80	86	90	32	0.9	NA	1.7	1.98	2.6	2.08
45	81	89	90	22	1.0	NA	1.94	2.18	2.98	2.56
46	82	84	87	22	0.7	NA	1.22	1.99	2.02	2.14
47	70	72	71	20	0.8	NA	1.63	1.97	2.06	1.97
48	91	94	89	21	0.9	NA	3.5	3.98	3.25	3.6
49	86	84	92	26	1.4	NA	2.69	2.29	3.04	2.56
50	86	83	76	30	1.2	NA	1.70	1.81	1.82	2.14

Sl	Total Cholesterol	LDL	HDL	Triglycerides
1	152	97	47	60
2	158	68	39	54
3	170	71	38	42
4	167	72	40	60
5	170	80	40	70
6	173	83	45	75
7	172	72	42	72
8	186	62	55	100
9	168	72	38	69
10	172	78	48	78
11	190	130	40	145
12	160	70	40	50
13	170	80	50	62
14	170	76	43	52
15	180	80	52	95
16	172	82	52	72
17	172	82	42	59
18	170	80	50	98
19	157	88	54	141
20	182	73	52	110
21	188	72	50	81
22	166	78	39	48
23	158	92	34	100
24	188	98	48	58
25	151	66	47	110
26	160	72	42	72
27	180	120	40	130
28	161	71	41	110
29	171	112	38	96
30	172	72	49	108
31	189	100	48	72
32	188	110	50	124
33	155	62	34	72
34	176	110	45	96
35	77	62	38	76
36	188	96	43	49
37	170	90	40	78
38	95	68	38	60
39	180	73	38	141
40	190	110	49	120
41	158	72	38	70
42	170	90	40	80
43	170	70	40	110
44	180	80	37	77
45	152	70	43	55
46	157	77	42	78
47	167	78	52	120
48	170	80	35	92
49	180	80	50	132
50	195	80	33	48

PRODUCT LITERATURE FOR FERRITIN KIT

04366522001V9

Ferritin

Ferritin

cobas®

REF 03737551 190 — 100 tests

• Indicates analyzers on which the kit can be used

Elecsys 1010	Elecsys 2010	MODULAR ANALYTICS E170	cobas e 411	cobas e 601
•	•	•	•	•

English

Intended use

Immunoassay for the in vitro quantitative determination of ferritin in human serum and plasma.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Ferritin is a macromolecule with a molecular weight of at least 440 kD (depending on the iron content) and consists of a protein shell (apoferritin) of 24 subunits and an iron core containing an average of approx. 2500 Fe^{3+} ions (in liver and spleen ferritin).[1]
Ferritin tends to form oligomers, and when it is present in excess in the cells of the storage organs there is a tendency for condensation to semicrystalline hemosiderin to occur in the lysosomes.
At least 20 isoferritins can be distinguished with the aid of isoelectric focusing.[2] This microheterogeneity is due to differences in the contents of the acidic H and weakly basic L subunits. The basic isoferritins are responsible for the long-term iron storage function, and are found mainly in the liver, spleen, and bone marrow.[1,3]
Acidic isoferritins are found mainly in the myocardium, placenta, and tumor tissue. They have a lower iron content and presumably function as intermediaries for the transfer of iron in various syntheses.[4,5,6]
The determination of ferritin is a suitable method for ascertaining the iron metabolism situation. Determination of ferritin at the beginning of therapy provides a representative measure of the body's iron reserves. A storage deficiency in the reticulo-endothelial system (RES) can be detected at a very early stage.[7]
Clinically, a threshold value of 20 µg/L (ng/mL) has proved useful in the detection of prelatent iron deficiency. This value provides a reliable indication of exhaustion of the iron reserves that can be mobilized for hemoglobin synthesis. Latent iron deficiency is defined as a fall below the 12 µg/L (ng/mL) ferritin threshold. These two values necessitate no further laboratory elucidation, even when the blood picture is still morphologically normal. If the depressed ferritin level is accompanied by hypochromic, microcytal anemia, then manifest iron deficiency is present.[1]
When the ferritin level is elevated and the possibility of a distribution disorder can be ruled out, this is a manifestation of iron overloading in the body. 400 µg/L (ng/mL) ferritin is used as the threshold value. Elevated ferritin values are also encountered with the following tumors: acute leukemia, Hodgkin's disease and carcinoma of the lung, colon, liver and prostate. The determination of ferritin has proved to be of value in liver metastasis. Studies indicate that 76 % of all patients with liver metastasis have ferritin values above 400 µg/L (ng/mL). Reasons for the elevated values could be cell necrosis, blocked erythropoiesis or increased synthesis in tumor tissue.
Two monoclonal mouse antibodies - M-4.184 and M-3.170 - are used to form the sandwich complex in the assay.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin-specific antibody labeled with a ruthenium complex[a] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1 Anti-Ferritin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-ferritin antibody (mouse) 3.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.
R2 Anti-ferritin-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-ferritin antibody (mouse) labeled with ruthenium complex 6.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.
Store the Elecsys Ferritin reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.
Stability:

unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on MODULAR ANALYTICS E170 and **cobas e** 601	6 weeks
on Elecsys 2010 and **cobas e** 411	6 weeks
on Elecsys 1010	4 weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20-25 °C; up to 20 hours opened in total)

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.
Serum collected using standard sampling tubes.
Li-, Na-heparin, K_3-EDTA, and sodium citrate plasma.
When sodium citrate is used, the results must be corrected by + 10 %.
Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.
Stable for 7 days at 2-8 °C, 12 months at -20 °C.[8]
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.
Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.
Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Ferritin

Ferritin

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Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- REF 03737586190, Ferritin CalSet, 4 x 1 mL
- REF 04415299190, PreciControl Anemia, for 2 x 2 mL each of PreciControl Anemia 1, 2 and 3 or REF 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2
- REF 11776452160, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2 (for USA)
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 1010/2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 1010/2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706829001, Elecsys 1010 AssayCup, 12 x 32 reaction vessels or REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 12135027190, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution
- REF 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
Resuspension of the microparticles takes place automatically before use.
Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25 °C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25 °C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8 °C after use.

Calibration

Traceability: The Elecsys Ferritin assay (REF 03737551) has been standardized against the Elecsys Ferritin assay (REF 11820982). The Elecsys Ferritin assay (REF 11820982) has been standardized against the Enzymun-Test Ferritin method. This in turn has been standardized against the 1st International Standard (IS) NIBSC (National Institute for Biological Standards and Control) "Reagent for Ferritin (human liver)" 80/602.
Recovery studies, including a published study[9], to assess traceability of the Elecsys Ferritin assay to more recent international standards (2nd IS 80/578 and 3rd IS 94/572) have been conducted, with results showing very good agreement.
Every Elecsys Ferritin reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Ferritin CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

Elecsys 1010 analyzer:

- with every reagent kit
- after 7 days (ambient temperature 20-25 °C)
- after 3 days (ambient temperature 25-32 °C)

For all analyzers:

- as required: e.g. quality control findings outside the specified limits

Quality control

For quality control, use Elecsys PreciControl Anemia 1, 2 and 3 or Elecsys PreciControl Tumor Marker 1 and 2.
Other suitable control material can be used in addition.
Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits.
Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in µg/L or ng/mL).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.31 mmol/L or < 0.5 g/dL), lipemia (Intralipid < 3300 mg/dL), and biotin < 205 nmol/L or < 50 ng/mL.
Criterion: Recovery within ± 10 % of initial value.
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 2500 IU/mL.
There is no high-dose hook effect at ferritin concentrations of up to 100000 µg/L (ng/mL).
In vitro tests were performed on 19 commonly used pharmaceuticals. No interference with the assay was found.
$Iron^{2+}$- and $iron^{3+}$-ions at therapeutic concentrations do not interfere with the Elecsys Ferritin assay.
In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.
For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

0.500-2000 µg/L (ng/mL) (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.500 µg/L (ng/mL). Values above the measuring range are reported as > 2000 µg/L (ng/mL) (or up to 100000 µg/L (ng/mL) for 50-fold diluted samples).

Dilution

Samples with ferritin concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:50 (either automatically by the MODULAR ANALYTICS E170, Elecsys 1010/2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 40 µg/L (ng/mL). After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the MODULAR ANALYTICS

Ferritin

Ferritin

cobas®

E170, Elecsys 1010/2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values[10]

Results of a study with the Enzymun-Test Ferritin method on samples from 224 healthy test subjects (104 women - mainly premenopausal - and 120 men) are given below. The values correspond to the 5th and 95th percentiles.

Men, 20-60 years: 30-400 µg/L (ng/mL)

Women, 17-60 years: 13-150 µg/L (ng/mL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 1010/2010 and **cobas e** 411 analyzers

		Repeatability[b]		Intermediate precision	
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/L (ng/mL)	CV %
Human serum 1	30.7	0.55	1.8	0.92	2.9
Human serum 2	232	4.26	1.8	7.52	3.2
Human serum 3	1482	30.2	2.0	37.8	2.6
PreciControl TM[c]1	22.2	0.48	2.1	0.71	3.2
PreciControl TM2	221	4.19	1.9	4.75	2.2

b) Repeatability = within-run precision
c) TM = Tumor Marker

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers

	Repeatability			Intermediate precision		
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %
Human serum 1	19.4	0.57	3.0	14.7	0.59	4.0
Human serum 2	234	7.31	3.1	361	15.8	4.4
Human serum 3	1446	51.4	3.6	1655	68.5	4.1
PreciControl TM1	22.2	0.63	2.9	23.8	1.03	4.3
PreciControl TM2	226	5.22	2.3	247	12.2	4.9

Analytical sensitivity (lower detection limit)

0.50 µg/L (ng/mL)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Method comparison

A comparison of the Elecsys Ferritin assay, REF 03737551 (y) with the Elecsys Ferritin assay, REF 11820982 (x) using clinical samples gave the following correlations:

Number of samples measured: 134

Passing/Bablok[11]	Linear regression
y = 1.00x + 0.72	y = 0.99x + 4.11
τ = 0.984	r = 0.999

The sample concentrations were between approx. 2.68 and 1891 µg/L (ng/mL).

Analytical specificity

Human liver ferritin 100 % recovery
Human spleen ferritin 85 % recovery
Human heart ferritin 1 % recovery

References

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information, and the package inserts of all necessary components.

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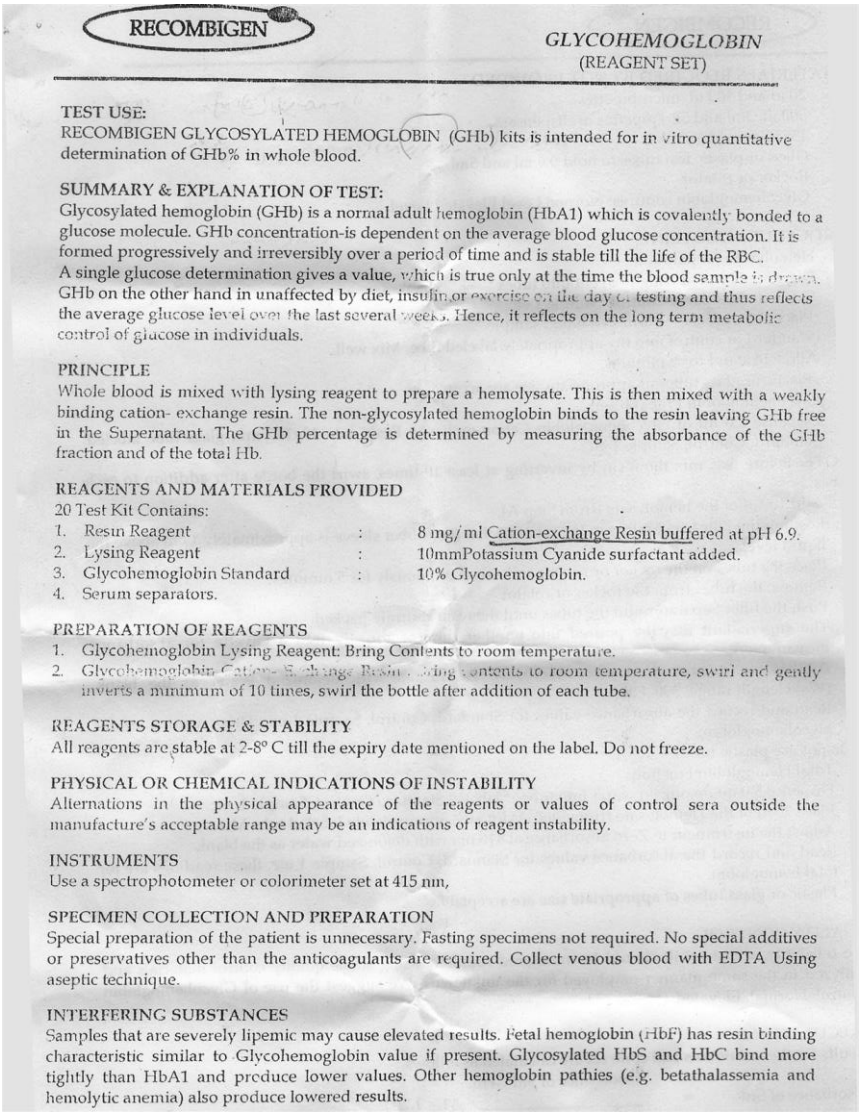
PRODUCT LITERATURE FOR GLYCATED HEMOGLOBIN KIT



RECOMBIGEN

GLYCOHEMOGLOBIN
(REAGENT SET)

TEST USE:
RECOMBIGEN GLYCOSYLATED HEMOGLOBIN (GHb) kits is intended for in vitro quantitative determination of GHb% in whole blood.

SUMMARY & EXPLANATION OF TEST:
Glycosylated hemoglobin (GHb) is a normal adult hemoglobin (HbA1) which is covalently bonded to a glucose molecule. GHb concentration-is dependent on the average blood glucose concentration. It is formed progressively and irreversibly over a period of time and is stable till the life of the RBC.
A single glucose determination gives a value, which is true only at the time the blood sample is drawn. GHb on the other hand in unaffected by diet, insulin or exercise on the day of testing and thus reflects the average glucose level over the last several weeks. Hence, it reflects on the long term metabolic control of glucose in individuals.

PRINCIPLE
Whole blood is mixed with lysing reagent to prepare a hemolysate. This is then mixed with a weakly binding cation- exchange resin. The non-glycosylated hemoglobin binds to the resin leaving GHb free in the Supernatant. The GHb percentage is determined by measuring the absorbance of the GHb fraction and of the total Hb.

REAGENTS AND MATERIALS PROVIDED
20 Test Kit Contains:

1.	Resin Reagent	:	8 mg/ml Cation-exchange Resin buffered at pH 6.9.
2.	Lysing Reagent	:	10mmPotassium Cyanide surfactant added.
3.	Glycohemoglobin Standard	:	10% Glycohemoglobin.
4.	Serum separators.		

PREPARATION OF REAGENTS
1. Glycohemoglobin Lysing Reagent: Bring Contents to room temperature.
2. Glycohemoglobin Cation Exchange Resin : Bring contents to room temperature, swirl and gently inverts a minimum of 10 times, swirl the bottle after addition of each tube.

REAGENTS STORAGE & STABILITY
All reagents are stable at 2-8° C till the expiry date mentioned on the label. Do not freeze.

PHYSICAL OR CHEMICAL INDICATIONS OF INSTABILITY
Alternations in the physical appearance of the reagents or values of control sera outside the manufacture's acceptable range may be an indications of reagent instability.

INSTRUMENTS
Use a spectrophotometer or colorimeter set at 415 nm,

SPECIMEN COLLECTION AND PREPARATION
Special preparation of the patient is unnecessary. Fasting specimens not required. No special additives or preservatives other than the anticoagulants are required. Collect venous blood with EDTA Using aseptic technique.

INTERFERING SUBSTANCES
Samples that are severely lipemic may cause elevated results. Fetal hemoglobin (HbF) has resin binding characteristic similar to Glycohemoglobin value if present. Glycosylated HbS and HbC bind more tightly than HbA1 and produce lower values. Other hemoglobin pathies (e.g. betathalassemia and hemolytic anemia) also produce lowered results.

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MATERIALS REQUIRED BY NOT PROVIDED

1. 20 µl and 100 ul micropipettes.
2. 500 µl, 3ml and 5ml pipettes or dispensers.
3. 13x100mm glass tubes.
4. Glass or plastic test tubes to hold 0.6 ml and 5ml.
5. Rocker or rotator.
6. Glycohemoglobin controls: Normal Level Elevated Level.

PROCEDURAL OUTLINE

A. Hemolysate Preparation:

- Dispense 500 µl Lysing Reagent into tubes *
  Labeled: Standard, Control, Sample 1 etc.
- Place 100 µl of the well-mixed blood sample
  Standard or control into the appropriately labeled tube. Mix well.
- Allow to stand for 5 minutes.

* Plastic or glass tubes of appropriate size are acceptable.

B. Glycohemoglobin Preparation:

1. Dispense 3.0 ml of Glycohemoglobin Cation-exchange Resin into 13x100 mm glass tube labeled: Standard. Control, Sample 1 etc.

NOTE: Before use, mix the resin by inverting at least 10 times, swirl the bottle after addition to each tubes.

2. Add 100 µl of the hemolysate (from Step A).
3. Position the Filter Separators in the tubes so that the rubber sleeve is approximately 1 cm above the liquid level.
4. Place the tubes on the rocker or rotator and mix continuously for 5 minutes.
5. Remove the tubes from the rocker or rotator.
6. Push the filter Separator into the tubes until the resin is firmly packed.
7. The supernatant may be poured into another tube or directly into a cuvette for absorbance measurement.
8. Adjust the instrument to zero absorbance at 415 nm with deionized Water as the blank. (Wavelength range: 390-420)
9. Read and record the absorbance values for Standard. Control, Sample 1 etc. These reading are for glycohemoglobin.

* Do not use plastic tubes.

C. Total Hemoglobin Fraction:

1. Dispense 5.0 ml deionized water into tubes * labeled Standard Control, Sample 1, etc.
2. Place 20 µl of the Hemolysate (from Step A) into the appropriately labeled tube Mix.
3. Adjust the instrument to Zero absorbance at 415 nm with deionized water as the blank.
4. Read and record the absorbance values for Standard, Control, Sample 1 etc. these readings are for total hemoglobin.

* Plastic or glass tubes of appropriate size are acceptable.

QUALITY CONTROL

The reliability of test result should be monitored routinely using stable quality control materials and analyzed in the same manner employed for the unknowns. We suggest the use of Glycohemoglobin Control: Normal, Elevated.

CALCULATIONS

Results for the unknowns and controls are calculated as follows.

$$\text{Absorbance of Std} = \frac{\text{Absorbance of Std GHb}}{\text{Absorbance of Std THb}} = A1 = 1.7(*)$$

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$$\text{Absorbance of sample} = \frac{\text{Absorbance of Sample GHb}}{\text{Absorbance of Sample THb}} = A2$$

$$\text{\% GHb in Sample} = \frac{A2}{A1} \times 10$$

10 = Std.Concentration

Run to Run: The inter run precision was established by assaying blood with normal and elevated glycohemoglobin levels for

Ten runs conducted over a five-day period.

Level	Mean	Std.Dev.	% CV
Normal	7.6	0.31	4.1
Elevated	13.0	0.60	4.6

LIMITATION OF PROCEDURE

Sample from patients with hemoglobinopathies or decreased erythorocytes survival times may show incorrect results. See section on " Specimen Collection".

EXPECTED VALUES

Non-Diabetic: 4.5% - 8.0%
Good Control: 8.0% - 9.0%
Fair Control: 9.0% - 10.0%
& Poor Control: 10.0% and above

Due to variations in inter-laboratory assay conditions, instrument and demographic variations it is recommended that each laboratory should establish its own normal range.

PERFORMANCE CHARACTERISTICS

Linearity:

The glycohemoglobin assay shows linearity for glycohemoglobin level in the range of 4.0-20.0% Blood samples with total hemoglobin grater than 18 g/dl should be diluted x 2 with deionized water before assay.

Precision:

Within Run: The intra assay precision was established by assaying bloods with normal and elevated glycohemoglobin levels.

Twenty times each

Level	Mean	Std.Dev.		% CV
Normal	7.6	0.21	2.7	
Elevated	13.4	0.23		1.0

CORRELATION

A comparative study of the glycohemoglobin procedure and another widely used commercial method showed correlation (r) of 0.96.

SENSITIVITY

This glycohemoglobin procedure has a sensitivity of 0.02% glycohemoglobin per 0.001 units of absorbance.

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* Estabilished in our Lab with known control values (10%)

RECOMBIGEN

CONVERSION CHART OF GLYCOSYLATED HEMOGLOBIN A1% AND GLYCOSYLATED HEMOGLOBIN A1c %

A1	A2
6.0	4.30
6.1	4.38
6.2	4.46
6.3	4.54
6.4	4.63
6.5	4.71
6.6	4.79
6.7	4.88
6.8	4.96
6.9	5.05
7.0	5.13
7.1	5.21
7.2	5.30
7.3	5.38
7.4	5.46
7.5	5.55
7.6	5.63
7.7	5.72
7.8	5.80
7.9	5.88
8.0	5.97
8.1	6.05
8.2	6.14
8.3	6.22
8.4	6.30
8.5	6.39
8.6	6.47
8.7	6.55
8.8	6.64
8.9	6.72
9.0	6.81
9.1	6.89
9.2	6.97
9.3	7.06
9.4	7.14
9.5	7.22
9.6	7.31
9.7	7.39
9.8	7.48
9.9	7.56
10.0	7.64

A1	A2
10.1	7.73
10.2	7.81
10.3	7.89
10.4	7.98
10.5	8.06
10.6	8.15
10.7	8.23
10.8	8.31
10.9	8.40
11.0	8.48
11.1	8.56
11.2	8.65
11.3	8.73
11.4	8.82
11.5	8.90
11.6	8.98
11.7	9.07
11.8	9.15
11.9	9.24
12.0	9.32
12.1	9.40
12.2	9.49
12.3	9.57
12.4	9.65
12.5	9.74
12.6	9.82
12.7	9.91
12.8	9.99
12.9	10.07
13.0	10.16
13.1	10.24
13.2	10.33
13.3	10.41
13.4	10.49
13.5	10.58
13.6	10.66
13.7	10.74

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