Assessment of iron status in Sudanese patients with low red cell indices irrespective of their Hb values in Soba Hospital

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وابنزلنا الحديد فيه بأأس شديد ومنافع للناس وليعلم الله من ينصره بالغيب إن الله قوي عزيز

(صَدِقُ اللهِ العَظِيمِ)

(سورة الحديد 25)
Dedication to my family
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Abstract

This is a prospective study which was conducted at Soba University Hospital (University of Khartoum) in the period from October 2002 to April 2003.

The aim of this study was to assess the iron status in patients with low red cell indices regardless of their haemoglobin values.

80 patients and 20 controls were included in this study. All controls had normal Hb and red cell indices. All studied patients had reduction of one or more red cell indices namely MCH, MCV, and MCHC, 58 of them had low Hb values as well, while 22 had normal Hb values.

Iron status were assessed in all patients and controls. Results showed that: of the 58 anaemic patients with low indices, 32.8 % were suffering from iron deficiency anaemia, 48.2 % were suffering from anaemia of chronic disease while 19 % had normal iron profile.

50 % of the 22 non-anaemic patients with low indices show evidence of iron deficiency, 13.6 % showed abnormal iron profile due to chronic conditions while 36.6 % had normal iron profile.

Of the 20 controls; 18 (90 %) had normal iron profile while 2 (10%) showed evidence of subclinical iron deficiency.

It was concluded that 81% of anaemic patients with low indices showed abnormal iron status either due to obvious iron deficiency or chronic diseases while only 19 % had normal iron profiles. These may need further investigation to exclude other causes of hypochromic anaemia.
63.6% of non-anaemic patients showed abnormal iron status either due to iron deficiency or associated chronic diseases but a significant number 36.6% had unexplained reduction of red cell indices despite of normal Hb levels and normal iron status. It is suggested that this can be either due to mild haemoglobinopathies or most probably a machine error.
بسم الله الرحمن الرحيم

ملخص الدراسة


وكان الهدف من الدراسة هو تقييم حالة الحديد (Iron status) في المرضى الذين لديهم نقص (عوز) في دالات كريات الدم الحمراء (low red cell indices) في الدم . بغض النظر عن مستوى الهيموغلوبين (Haemoglobin).

وقد شملت هذه الدراسة 80 مريضا و 20 شخصا يمثلون المجموعة المحكمة (Controls).

كل المرضى الذين شملتهم الدراسة كان لديهم نقص في واحد أو أكثر من دالات كريات الدم الحمراء والتي تشمل متوسط حجم الخيلية، متوسط الهيموغلوبين في الخيلية و متوسط تركيز الهيموغلوبين في الخيلية.

58 مريضا كان لديهم نقص في مستوى الهيموغلوبين في الدم (فقر دم) بينما 22 مريضا كانت نسبة الهيموغلوبين في الحدود الطبيعية . وقد تم فحص حالة الحديد لدى المرضى والمجموعة المحكمة على حد سواء. النتائج : دلت النتائج على أن 32.8 % من المرضى الذين لديهم نقص في مستوى الهيموغلوبين في الدم مع وجود نقص في دالات كريات الدم الحمراء كانوا يعانون من فقر الدم بسبب نقص عنصر الحديد ، 48.2 % كانوا يعانون من فقر الدم بسبب الأمراض المزمنة بينما 19% كانت حالة الحديد سوية.

50% من المرضى الذين كانت لديهم نسبة الهيموغلوبين طبيعية ولديهم نقص في دالات كريات الدم الحمراء كانوا يعانون من نقص عنصر الحديد ، 13.6% كانت حالة الحديد غير سوية بسبب الأمراض المزمنة المزمنة بينما 36.6% كانت حالة الحديد لديهم بشكل سوي.

(Controls) كانت حالة الحديد سوية بينما 90% من المجموعة المحكمة.

10% كان لديهم نقص في مخزون الحديد.
الخلاصة: أظهرت النتائج أن 81% من المرضى الذين شملتهم هذه الدراسة والذين يعانون من فقر الدم مع وجود نقص في دالات كريات الدم الحمراء (low red cell indices) كانت حالة الحديد لديهم غير سوية إما بسبب نقص عنصر الحديد أو بسبب الأمراض المزمنة بينما 19% كانت حالة الحديد لديهم سوية. وقد يتطلب في مثل هذه الحالات إجراء المزيد من الفحوصات المخبرية لاستبعاد الاحتمالات الأخرى المسببة لفقر الدم المصاحب بنقص في صباغ الخلية (Hypochromic anaemia).

4.6% من المرضى الذين ليس لديهم فقر دم مع وجود نقص في دالات كريات الدم الحمراء كانت حالة الحديد لديهم غير سوية إما بسبب نقص عنصر الحديد أو بسبب الأمراض المزمنة الملازمة بينما 36.6% كان لديهم نقص في دالات كريات الدم الحمراء على الرغم من عدم وجود نقص في مستوى الهيموجلوبين أو تغير في حالة الحديد. قد يعود السبب في ذلك للاختلاز إختصاصي بسيط (mild haemoglobinopathies) والاحتمال الأكبر أن يكون بسبب خطأ من جهاز الفحص (a machine error).
Chapter one: introduction & literature review

1-1: Normal iron metabolism
1-1-1: Introduction
1-1-2: Distribution of iron in tissue
1-1-3: Iron absorption
1-1-4: Iron transport in biological fluids
1-1-5: Transferrin receptors
1-1-6: Iron storage
1-1-6-1: Ferritin
1-1-6-2: Haemosiderin
1-1-7: Cellular iron homeostasis
1-1-8: Internal iron exchange
1-1-9: Normal iron balance
1-2: Diagnostic methods for assessment of body iron status
1-2-1: Assessment of iron storage
1-2-1-1: Serum ferritin
1-2-1-2: Tissue biopsy
1-2-2: Assessment of iron transport compartment
1-2-2-1: Serum iron---------------------------------------------------------------(13)
1-2-2-2: Total iron binding capacity (TIBC)--------------------------------------(14)
1-2-2-3: Transferrin receptors -------------------------------------------------(14)
1-2-2-4: Red cell protoporphyrin-----------------------------------------------(15)
1-2-2-5: Red cell ferritin--------------------------------------------------------(16)
1-2-2-6: Percentage of hypochromic red cells------------------------------(17)
1-2-3: Assessment of functional compartment-----------------------------------(17)
1-3: Red cell indices------------------------------------------------------------- (18)
1-3-1: Definition and measurement of red cell indices ------------------------(18)
1-3-1-1: Mean cell volume (MCV)-----------------------------------------------(18)
1-3-1-2: Mean cell haemoglobin (MCH)------------------------------------------(19)
1-3-1-3: Mean cell haemoglobin concentration (MCHC)------------------------(19)
1-3-1-4: Red cell distribution width (RDW)------------------------------------(19)
1-3-2: Clinical importance of red cell indices -------------------------------(19)
1-3-3: Factors that affects interpretation of red cell indices-----------------(21)
1-4: Classification of anaemia--------------------------------------------------(22)
1-4-1: Classification of anaemia according to underlying mechanism----(22)
1-4-2: Classification of anaemia based on RBC morphology-----------------(23)
1-5: Evaluation of microcytic anaemia------------------------------------------(23)
1-5-1: Iron deficiency anaemia---------------------------------------------------(24)
1-5-2: Thalassaemia-----------------------------------------------------------(26)
1-5-3: Sideroblastic anaemia----------------------------------------------------- (28)
1-5-4: Anaemia of chronic disease---------------------------------------------(30)
1-6: Justification---------------------------------------------------------------(33)
1-7: Objectives-----------------------------------------------------------------(33)

Chapter two: Material & methods
2-1: Subjects---------------------------------------------------------------(34)
2-2: Samples---------------------------------------------------------------(34)
2-3: Instruments-----------------------------------------------------------(35)
Chapter three: Results

3-1: General consideration

3-2: Group 1 (Children)

3-2-1: age distribution

3-2-2: Haemoglobin values

3-2-3: values of Red cell indices

3-2-4: Biochemical findings

3-2-4-1: S. iron levels in studied children

3-2-4-2: TIBC values in studied children

3-2-4-3: S. ferritin levels in studied children

3-3: Group 2 (Females)

3-3-1: age distribution

3-3-2: Haemoglobin values

3-3-3: Females with normal Hb and low cell indices

3-3-3-1: values of Red cell indices

3-3-3-2: Biochemical findings

3-3-3-2-1: S. iron levels in 17 studied females with normal Hb

3-3-3-2-2: TIBC values in 17 studied females with normal Hb

3-3-3-3: S. ferritin levels in 17 studied females with normal Hb

3-3-4: Females with low Hb and low cell indices

3-3-4-1: values of Red cell indices

3-3-4-2: Biochemical findings

3-3-4-2-1: S. iron levels in 22 studied females with low Hb
3-3-4-2-2: TIBC values in 22 studied females with low Hb-------------(51)
3-3-4-2-3: S. ferritin levels in 22 studied females with low Hb---------(51)

3-4 : Group 3 (Males ) -------------------------------------------(54)
3-4-1: age distribution-------------------------------------------(54)
3-4-2: Haemoglobin values------------------------------------------(54)
3-4-3 : Males with normal Hb and low cell indices-----------------(55)
3-4-3-1: values of Red cell indices ------------------------------(55)
3-4-3-2: Biochemical findings--------------------------------------(56)
3-4-3-2-1: S. iron levels in 5 studied males with normal Hb------(56)
3-4-3-2-2: TIBC values in 5 studied males with normal Hb-------(56)
3-4-3-3: S. ferritin levels in 5 studied males with normal Hb----(56)
3-4-4 : Male with low Hb and low cell indices--------------------- (58)
3-4-4-1: values of Red cell indices ------------------------------(58)
3-4-4-2: Biochemical findings--------------------------------------(59)
3-4-4-2-1: S. iron levels in 25 studied males with low Hb--------(59)
3-4-4-2-2: TIBC values in 25 studied males with low Hb--------- (59)
3-4-4-2-3: S. ferritin levels in 25 studied males with low Hb----(60)
3-5 : Controls----------------------------------------------------- (63)
3-5-1: biochemical findings----------------------------------------(63)
3-6 : Biochemical findings in all studied patients-----------------(63)
3-6-1: Biochemical findings in studied patients with normal Hb and
low red cell indices --------------------------------------------- (63)
3-6-2: Biochemical findings in studied patients with low Hb and
low red cell indices --------------------------------------------- (65)

Chapter four : Discussion & conclusions
Discussion---------------------------------------------------------(68)
Conclusions--------------------------------------------------------(76)
Table (1) : Summarizes the results of the red cell indices in the studied children

Table (2) : Relationship between S. iron, TIBC, and transferrin saturation to S. ferritin levels in the studied children

Table (3) : Summarizes the results of the red cell indices in studied females with normal Hb

Table (4) : Relationship between S. iron, TIBC, and transferrin saturation to S. ferritin levels in the studied females with normal Hb and low indices

Table (5) : Summarizes the results of the red cell indices in studied females with low Hb

Table (6) : Relationship between S. iron, TIBC, and transferrin saturation to S. ferritin levels in the studied females with low Hb and low indices

Table (7) : Summarizes the results of the red cell indices in studied males with normal Hb

Table (8) : Relationship between S. iron, TIBC, and transferrin saturation to S. ferritin levels in the studied males with normal Hb and low indices

Table (9) : Summarizes the results of the red cell indices in studied males with low Hb

Table (10) : Relationship between S. iron, TIBC, and transferrin saturation to S. ferritin levels in the studied males with low Hb and low indices

Table (11) : Results of S. Ferritin in controls

Table (12) : Summarizes the results of biochemical values in all studied patients with normal Hb and low indices
Table (13) : Summarizes the results of biochemical values in all studied patients with low Hb and low indices-------------------------(66)
List of figures:

Figure (1): MCV values in the studied children-------------------------(39)
Figure (2): MCHC values in the studied children------------------------(39)
Figure (3): S. iron levels values in the studied children--------------(40)
Figure (4): TIBC values in the studied children------------------------(41)
Figure (5): S. ferritin values in the studied children------------------(42)
Figure (6): Hb values in studied females with low indices-------------(43)
Figure (7): MCV values in studied females with normal HB-------------(45)
Figure (8): MCHC values in studied females with normal HB-------------(45)
Figure (9): S. iron values in studied females with normal HB----------(46)
Figure (10): TIBC values in studied females with normal HB----------(47)
Figure (11): S. ferritin values in studied females with normal HB------(47)
Figure (12): MCV values in studied females with low HB--------------(49)
Figure (13): MCHC values in studied females with low HB--------------(50)
Figure (14): S. iron values in studied females with low HB-----------(50)
Figure (15): TIBC values in studied females with low HB-------------(51)
Figure (16): S. ferritin values in studied females with low HB--------(52)
Figure (17): Hb values in 30 studied males with low indices----------(54)
Figure (18): MCV values in studied males with normal HB-------------(55)
Figure (19): MCHC values in studied males with normal HB------------(56)
Figure (20): S. iron values in studied males with normal HB--------- (57)
Figure (21): MCV values in studied males with low HB-----------------(58)
Figure (22): MCHC values in studied males with low HB----------------(59)
Figure (23): S. iron values in studied males with low HB------------- (59)
Figure (24): TIBC values in studied males with low HB---------------(60)
Figure (25): S. ferritin values in studied males with low HB--------- (61)
Figure (26): S. ferritin values in all studied patients with normal HB and low indices--------------------------------------------- (64)
Figure (27): S. iron, TIBC and TS in relation to S. ferritin in studied patients with normal HB and low indices

Figure (28): S. ferritin values in all studied patients with low HB and low indices

Figure (29): S. iron, TIBC and TS in relation to S. ferritin in studied patients with low HB and low indices
**Abbreviations**

DMT1 : Divalent metal transport 1  
RES    : Reticulo-endothelial system  
IRP    : Iron regulatory proteins  
IREs   : Iron-response elements  
eALA    : erythroid aminolevolinic acid synthesase  
TfRs    : Transferrin receptors  
TIBC   : Total iron binding capacity  
TS     : Transferrin saturation  
Fe     : Serum iron  
ZPP    : Zinc protoporphyrin  
FEP    : Free erythrocyte protoporphyrin  
Hb     : Haemoglobin  
Hct    : Haematocrit  
RBC    : Red blood cells  
WBC    : White blood cells  
MCV    : Mean cell volume  
MCH    : Mean cell haemoglobin  
MCHC   : Mean cell haemoglobin concentration  
pg     : picogram  
fl     : femtoliter  
RDW    : Red cell distribution width  
ACD    : Anaemia of chronic disease  
IFN    : Interferon  
IL     : Interleukin  
EDTA   : Ethylenediamine tetra-acetic acid  
ELISA  : enzyme-linked immunoassay
Introduction & literature review

1-1: Normal iron metabolism:

1-1-1: Introduction: -

Iron is an essential element for all living cells and plays an important role in many metabolic pathways\(^{(1)}\). All mammalian cells have an absolute requirement for iron, more likely because iron is abundant, comprising 5% of the earth's crust, and versatile, existing in two interconvertible redox states\(^{(2)}\). Iron has the capacity to accept and donate electrons readily, interconverting between ferric and ferrous forms. This capability makes it a useful component of cytochromes, oxygen-binding molecules (i.e., hemoglobin and myoglobin), and many enzymes. However, iron can also damage tissues by catalyzing the conversion of hydrogen peroxide to free-radical ions that attack cellular membranes, proteins, and DNA. Proteins sequester iron to reduce this threat\(^{(3)}\).

1-1-2: Distribution of iron in tissue:

The amount of total body iron is around 3 to 4 grams\(^{(4)}\), about 50 mg/kg in males and 40 mg/kg in females\(^{(5)}\). More than two thirds of the body’s iron content is incorporated into hemoglobin in developing erythroid precursors and mature red cells\(^{(3)}\), with 450 ml (1 unit) of whole blood containing about 200 mg of iron\(^{(5)}\). Approximately 10 to 15% are present in muscle fibers (in myoglobin) and other tissues (in enzymes and cytochromes)\(^{(3)}\). Most of the remainder is contained in the storage proteins; ferritin and haemosiderin. These are found mainly in the reticulo-endothelial cells of the liver, spleen and bone marrow (which gain iron from breaking down red cells), and in parenchymal liver cells (which normally gain most of their iron from the plasma iron-transporting protein, transferrin)\(^{(5)}\).
1-1-3: Iron absorption:

Iron absorption depends not only on the amount of iron in diet, but also and more importantly, on the bioavailability of that iron, as well as the body's needs for iron⁵. Dietary iron exist in two forms; haem and non-haem iron⁶, much of the dietary iron is non-haem iron derived from cereals, with lesser component of haem-iron derived from haemoglobin or myoglobin in red or organ meats. Haem iron is more readily absorbed than non-haem iron, being less subjected to influence by other dietary constituent's⁵. Absorption of non-haem iron is increased by simultaneous ingestion of foods high in ascorbic and citric acid such as citrus fruits, and of meat. Foods high in tannates, such as; tea, phytates, and polyphenols inhibit the absorption of non-haem iron⁶.

Iron is released from protein complexes by acid and proteolytic enzymes in the stomach and small intestine, and haem is liberated from haemoglobin and myoglobin⁵.

Dietary iron absorption occurs in the proximal duodenum in the presence of an acidic pH. Ferric iron is exposed to stomach acid along with ferric reductase activity in the duodenal brush border to produce ferrous iron, which is absorbed⁴. The entrocyte is a highly specialized cell of the duodenal epithelium that coordinates iron uptake and transport into the body⁷. Regulation of iron uptake into the body occurs at two interfaces of the intestinal epithelium:

(i) the apical membrane of the differentiated entocyte, which is specialized for transport of haem and ferrous iron into the cell. The most extensively characterized uptake pathway is via the divalent metal transport, DMT1 (previously called Nramp2 and DCT1). Its expression is regulated by body iron stores, but it may also be susceptible to regulation by dietary iron, or through recently observed post translation mechanism.
(ii) the basolateral membrane mediates the transfer of the iron, which transported into the intestinal epithelial cells, to the rest of the body. Iron that is not exported into the plasma is lost with exfoliation of the intestinal epithelium\(^7\).

Recently, gene coding for a protein, termed ferroprotein, which affects basolateral iron transport in zebra fish, was reported. A second gene and its product, hephaestin, has also been isolated from mice\(^4\). Hephaestin is similar to plasma Ceruloplasmin and is presumed to function as a ferroxidase\(^3\).

The absorption of intestinal iron is regulated in several ways: first, it can be modulated by the amount of iron recently consumed in the diet; mechanism referred to as the dietary regulator. For several days after a dietary iron bolus, absorptive enteroctyes are resistant to acquiring additional iron. This phenomenon has previously been called "mucosal block". A second regulatory mechanism also senses iron levels but responds to total body iron rather than dietary iron. The mechanism has been termed the store regulator. The third regulatory mechanism, known as the erythropoietic regulator, does not respond to iron level at all. Rather, it modulates iron absorption in response to the requirements for erythropoiesis\(^3\). The erythropoietic regulator has a greater capacity to increase iron absorption than the stores regulator\(^8\).

1-1-4: Iron transport in biological fluids:

Once entered into circulation iron is complexed to and transported by transferrin to the different tissues. Transferrin is characterized by the ability to bind to ferric irons with very high affinity together with two synergistic carbonate (CO\(_3\)\(^2-\)) ions. In addition to iron, transferrin might be involved in the transport of a number of other metals such as aluminum, manganese, copper and cadmium. Affinity of transferrin for iron is pH–dependent\(^9\). Transferrin is a single-chain glycoprotein, mol.wt 79,500,
which is present in plasma and extra cellular spaces and has plasma half-life of 8-11 days. The transferrin gene is on chromosome 3 and the protein is synthesized predominantly by the liver. Synthesis being inversely related to iron stores. Normal plasma contains 1.8-2.6 g/l, two atoms of ferric iron may be attached to each molecule\(^5\).

Transferrin displays three main functions: (i) it solubilizes ferric iron, otherwise largely insoluble at neutral pH, ii) it binds iron with high affinity avoiding that this metal in its free state could generate free radicals, iii) it functions as an iron supplier for the cells\(^9\).

At any given time, approximately 0.1% (3 mg) of total body iron circulates in an exchangeable plasma pool. In normal individuals, essentially all circulating plasma iron is bound to transferrin (Tf) the mechanism (s) by which transferrin acquired iron from intestinal absorptive cells and reticulo-endothelial system (RES) cells is unknown\(^4\). In addition to transferrin, another molecule, lactoferrin, is able to bind iron with high affinity, its main function is related not to transport of iron but to the sequestration of this metal in peculiar tissue compartment. However, the main physiological role of this protein seems to be related to its role in host defense against bacterial infections\(^9\).

**1-1-5: Transferrin receptors (TfRs):**

Cellular iron uptake is mediated by transferrin receptors (TfRs), which are present on virtually all mammalian cells\(^10\). The transferrin receptor is a transmembrane, homodimeric glycoprotein with a molecular mass of 180 kDa. The main function of TfR is delivering iron into cells via endocytosis of transferrin\(^11\).

Transferrin interacts with two membrane receptors, the transferrin receptor 1 and 2. TfR1 is a typical type membrane receptor expressed in virtually all cell types, with exception of mature erythrocyte. Human TfR1
is encoded by a single gene present on chromosome 3, some tissues express particularly high levels of TfR1 such as erythroid cells, hepatocytes, placental tissue and rapidly dividing cells. TfR1 is also expressed at the level of endothelial capillaries. Recently, a second transferrin receptor (TfR2) was identified, whose pattern of expression and regulation is different from that reported for TfR1, analysis of TfR2 mRNA expression in various murine tissue showed that only the hepatic tissue express high levels of this mRNA species. The gene encoding TfR2 is located on chromosome 22. A peculiarity of TfR2 with respect to TfR1 is related to the pattern of tissue distribution, which is apparently restricted in the case of TfR2 to hepatocyte and erythroid cells. Similarly, the regulation of the expression of TfR1 and TfR2 show several important differences in that the latter one is modulated by the cell growth rate, but not by the iron level\(^9\).

A small amount of TfR circulates normally in plasma. It originates from the extracellular chain of TfR presents in the membrane of every cell. The erythroid precursors in the bone marrow are the major determinants of these serum concentrations. In conditions with increased erythroid marrow mass there is an elevation in TfR concentration, whereas in case of erythroid hypoplasia or aplasia, serum TfR concentration falls. The concentration of serum TfR also depends on the adequacy of iron availability to tissues, when iron stores are exhausted and iron availability is compromised, an early and progressive rise in serum TfR concentration occurs\(^{12}\).

Once bound, transferrin receptor-transferrin-iron complex is internalized into calthrin-coated endosomes. Here an ATP-dependent proton pump acidifies the endosome until the pH is < 6. At that pH, transferrin loses its iron and becomes more tightly bound to the transferrin receptor, iron egress through the endosomal membrane in a process using DMT1 and an endosomal oxido-reductase. The transferrin molecule
recycles to the cell surface where it releases back to the circulation. Although the mechanism of intracellular iron movement is unknown, the iron atom’s ultimate fate either incorporates into enzymes or storage into ferritin\(^4\).

1-1-6: Iron storage:

The level of body iron stores is affected both by dietary intake and by the physiological need of iron for erythropoiesis\(^{13}\). Iron is primarily stored in tissue as ferritin or haemosiderin\(^{14}\).

1-1-6-1: Ferritin:

Ferritin protein is an iron-containing spherical rhombic dodecahedron protein of 24 repeating subunits with molecular weight of approximately 460,000, with iron core of ferric-oxide phosphate and, when fully saturated, may be over 20% iron by weight\(^{15}\). Ferritin is multimeric protein which concentrates and stores the excessive cytosolic iron and subsequently, donates the stored for iron cellular needs. Each ferritin molecule accumulates up to 4500 iron atoms\(^{15}\).

Mammalian ferritin is composed of two chains; H and L. These subunits show a similar three-dimensional structure and 50% of sequence similarity. The H and L subunits have different functions in that, the H subunit exhibits ferroxidase activity and catalyses the first step in iron storage, i.e. the oxidation of Fe\(^+2\) to Fe\(^+3\), the L subunit dose not possess ferroxidase activity and seems to be involved in the core iron formation in the cavity of ferritin molecule. A third subunit, the G subunit, is also found in the serum, but is thought to derive from the L- subunit through glyceration during the secretion process. The mechanism of serum ferritin production is poorly known.

A new type of ferritin subunit in human cells was recently discovered. This ferritin was called mitochondrial ferritin, and was
expressed as a precursor target to mitochondria where its processed into functional protein exhibiting structural and functional properties similar to those observed in cytoplasmic ferritin\(^{(9)}\).

Human erythroblasts contain much more ferritin than the cells of other haematopoietic lineage and mature erythrocytes. It has been shown recently for human erythroid precursor that the iron which accumulated in ferritin before hemoglobin (Hb) synthesis was gradually removed from the ferritin and transferred to Hb, i.e. ferritin can serve as source of iron for Hb production\(^{(16)}\).

A small fraction of ferritin in equilibrium with stores circulates in the plasma, plasma ferritin protein is elevated in the presence of excess stores and is decreased with iron deficiency\(^{(15)}\).

**1-1-6-2: Haemosiderin:**

It is a water insoluble non-crystalline protein-iron complex, visible by light microscope when stained by Prussian blue (pearl's reaction). It has an amorphous structure, with higher iron to protein ratio (up to 37\%) and is probably formed by partial digestion of ferritin aggregate by lysosomal enzyme. In normal subjects, the majority of storage iron is present as ferritin, and haemosiderin is predominantly found in the macrophages rather than hepatocytes\(^{(5)}\). Body iron is stored within molecules of ferritin directly related to body iron stores of haemosiderin and appears to be in equilibrium with them\(^{(15)}\).

**1-1-7: Cellular iron homeostasis:**

Iron appears to exert self-regulatory control over erythroblast iron uptake, iron storage and its incorporation into haem\(^{(17)}\). This system of regulation mechanisms operates through the interaction of two cytoplasmic proteins, the iron regulatory proteins (IRP-1 and IRP-2) with specific
sequences called iron response elements (IREs) present either in the 3' or 5' untranslated regions of the mRNA encoding gene involved in iron metabolism\(^9\). The mRNA regulation of iron uptake proteins, such as transferrin receptor, DMT1, and ferritin expression is a central event in intracellular iron and oxygen homeostasis, and demonstrate the key regulatory steps for many proteins of iron metabolism\(^4\).

Iron regulatory proteins (IRPs) 1 and 2 bind with equally high affinity to iron-responsive element (IREs) and thereby, post-translationally regulate several gene of iron metabolism\(^{18}\).

IRPs bind with the IREs when intracellular iron concentrations are low. When IRPs bind to IREs on 5' end of mRNA, such as ferritin, synthesis of the protein from mRNA is blocked. When IRPs bind to IREs located on the 3' end of mRNA, such as in transferrin receptor, the mRNA message is protected from nucleases, allowing synthesis of the receptor, this result in more messages being translated to protein. Therefore, when intracellular iron is high, less transferrin receptor is produced for iron uptake and more of storage protein ferritin is made. Conversely, when intracellular iron is low, more protein for the transferrin receptor is made and less ferritin for iron storage is made. Beside the mRNA for ferritin H and L subunits and transferrin receptor, the mRNAs of DMT1, the erythroid specific form of aminolevulinc acid synthase, mitochondrial aconitase and ferroprotein have IRE like elements.

Mitochondrial aconites, an enzyme evolved in the citric acid cycle of cellular respiration, has an IREs on the 5' end, functionally, like ferritin mRNA, Mitochondrial respiratory activity is down regulated (5' IREs block mRNA translation) under conditions of low iron concentration. In the developing red cells, the pathway seems to be similar, with roughly 80% of the circulating iron being utilized for hemoglobin synthesis. The rate limiting enzyme in haem synthesis, mitochondrial erythroid aminolevulinc acid synthesase (eALA) has an IRE element on its 5' end\(^{4}\).

1-1-8: Internal iron exchange:

The major pathway of internal iron exchange is a unidirectional flow from plasma transferrin to the erythron (defined as the totality of erythroid elements at all stages of developments), to macrophage and back to plasma transferrin. About four-fifth of iron passing through the transferrin compartment each day is flowing to and from the erythron.

Physiologically, immature erythroid cells acquire iron from transferrin through a specific transferrin receptor located on the surface membrane, senescent erythrocytes are phagocytized by specialized macrophage in the spleen, bone marrow, and liver. Within macrophages, the inducible haem oxygenase 1 catabolizes haem, releasing ferrous iron to a Fe-ATPase iron transporter, which seems to be responsible for intracellular transmembrane iron transport. The exact means of iron exits from the macrophage is uncertain but may involve ferroprotein 1. Ceruloplasmin may be required for the mobilization of iron from macrophages and other tissues and for its oxidation and incorporation into ferric transferrin.

The phagocytosis of aged erythrocyte and flawed immature red cells accounts for almost all of the storage iron found in the macrophages of the liver, bone marrow and spleen, by contrast, the parenchymal cells of the liver may either take iron from or give iron to plasma transferrin.

The remaining one-fifth of iron movement to and from transferrin consists mostly of iron shifted between the plasma and extracellular
transferrin compartments, exchanged between extravascular transferrin and parenchymal tissue, or moved to and from hepatocytes\(^{19}\).

1-1-9: Normal iron balance:

In case of humans, the amount of iron that is absorbed rather than the amount that is excreted is controlled\(^{7}\). There's no mechanism for excretion of the iron\(^{6}\), inappropriate responses or lack of response lead to anaemia or iron overload\(^{7}\). In individuals with normal body iron, the store regulator is responsible for meeting normal iron loss as well as the additional requirement for growth and demonstration\(^{8}\).

Requirements are higher in menstruating women and during period of rapid growth in infancy and adolescence\(^{5}\). In women of reproductive age average daily need about 1.2—2 mg /day, owing to monthly loss of 30-80 ml of menstrual blood. During pregnancy the net need is about 500 mg or more. Lactation adds small increase over basal needs. So females of reproductive age and adolescents of both sexes are in more precarious states, many diet do not meet their daily iron requirement and iron deficiency will ensue\(^{6}\).

The world health organization (WHO) dietary allowance for iron, based on current knowledge of normal iron homeostasis, is 5—10 mg /day. The amount of iron absorbed from the diet from \(< 1\% — > 80\%\), is regulated by GIT and depends on the adequacy of iron status. The absorption rate is about 6% for men and 18% for non pregnant women during child bearing years, reflecting lower iron stores in women resulting from menstruation and pregnancy\(^{14}\).
1-2: Diagnostic methods for assessment of body iron status:

In order to assess body iron status, the three main compartments of iron; storage iron, transport iron, and functional iron compartment, particularly haemoglobin in red cells, should be considered\(^5\).

1-2-1: Assessment of iron storage:

1-2-1-1: Serum ferritin:

In normal people, the serum ferritin concentration correlates with hepatic and macrophage iron stores, assessed by quantitative phlebotomy or tissue biopsy\(^5\). It has been estimated that 1 ug/l plasma ferritin in normal adult is equal to 8-10 mg tissue iron stores\(^8\).

In most normal adults, concentration range is from 15 – 300 ug/l, but these vary with age and sex; serum ferritin level is higher in men than women of child bearing age, levels in postmenopausal women are close to those found in men\(^20\). In a recent study, the conventional reference range for ferritin was found to be considerably lower than the optimal decision limit for iron deficiency, these values have generally been calculated from a population that has been determined to be non anaemic on the bases of haemoglobin values only, this is, however, an inadequate measure to exclude the effect of latent iron deficiency on the reference value of ferritin, because anaemia is known to develop as a late manifestation of iron deficiency\(^21\). Measurement of serum ferritin level is generally accepted as the best non-invasive means to determine body iron stores, but only if serum level of ferritin and serum level of iron ran the same direction\(^13\).

Quantitated serum ferritin measured using antibody to ferritin protein does not reflect iron content of the ferritin, serum ferritin is an acute
phase reactant, and a proferritin (a ferritin protein with almost no iron in it, and not in equilibrium with body stores), is elevated in any inflammatory state such as; infection, rheumatoid arthritis, hepatitis and cancer, due in part to interleukin1 enhancing the translation of apoprotein mRNA\(^{(15)}\). Elevation of serum ferritin levels may occur without elevation of iron stores. Serum ferritin level >400 ug/l defines iron overload in most clinical laboratories, but in fact, such interpretation require confirmation by finding high percentage of saturation of iron binding capacity, thus, high serum ferritin accompanied by high percentage of saturation of serum transferrin usually indicate iron overload. However, high serum ferritin accompanied by a percentage saturation of transferrin <45 usually indicate that inflammation caused the high ferritin\(^{(15)}\).

Serum ferritin <12 ug/l is diagnostic of iron deficiency, but its sensitivity is compromised in the presence of the inflammation\(^{(15)}\), further investigation such as bone marrow examination or determination of the response to iron treatment may be needed\(^{(22)}\). The specificity of low serum ferritin for absent marrow iron is 98%, indicating the importance of determination of serum ferritin cut off, it has been noted that when lower limit for serum ferritin is set at less than 12 ug /l, the sensitivity is 61% and the specificity is 98%\(^{(14)}\).

Although serum ferritin has high specificity for iron deficiency especially when combined with other markers such as Hb, the test is expensive and has limited availability in a clinic setting. Therefore it is not used commonly for screening, in addition to that it can be elevated in the setting of inflammation, chronic infection and other diseases\(^{(23)}\).

1-2-1-2: Tissue biopsy:
Liver biopsy (in diagnosis of iron overload) or bone marrow biopsy (in the differential diagnosis of iron deficiency) allows the direct examination of iron stores by histological staining for iron. Stainable marrow is the reference standard for iron deficiency, although the absence of iron is not absolute proof of iron deficiency, the presence of stainable marrow iron reliably excludes iron deficiency. Bone marrow examination shows normal iron despite actual iron deficiency when megaloblastic anaemia is present. However, examination of stained aspirate of bone marrow for haemosiderin is still considered the gold standard method for evaluation of iron status. This technique is invasive and not suitable for screening purposes.

Liver biopsy is the gold standard for quantifying iron in patient with iron overload. The hepatic iron concentration exceeds 80 umol per gram of liver (dry weight) resulting in hepatic iron index of more than 1.9 mmol per kilogram per year (the hepatic iron index is the ratio of the hepatic iron concentration to the age of the patient in year).

1-2-2: Assessment of iron transport compartment:

1-2-2-1: Serum iron:

Serum iron concentration can be measured directly, and generally decreases as iron stores are depleted. However, serum iron may not reflect iron stores accurately because it’s influenced by several factors including; iron absorption from meals, infection, inflammation and diurnal variation. Morning levels are generally assumed to be higher than afternoon or evening levels. Circulating iron, bound to transferrin, comprises only a very small amount (0.01%) of the total body iron, and has very high turnover rate of 10-20 times per day in normal subjects, these factors contribute to the variability of measurement encountered and
severely limit the diagnostic usefulness of an individual serum iron measurement\textsuperscript{(20)}.

1-2-2-2: Total iron binding capacity (TIBC):

TIBC indirectly measures transferrin levels, which increase as serum iron concentrations and stored iron decrease\textsuperscript{(23)}. It is a more stable measurement than the serum iron\textsuperscript{(20)}. Unfortunately, this test is also affected by factors other than iron status, for example; TIBC is decreased with malnutrition, inflammation, chronic infection and cancer\textsuperscript{(23)}. Since TIBC and transferrin are increased in iron deficiency and decreased in inflammation, measurement may be within normal range when both conditions co-exist\textsuperscript{(24)}.

Measurement of both, serum iron and TIBC, provide more information than individual measurement\textsuperscript{(20)}. Calculation of the percentage transferrin saturation from serum iron and TIBC (serum iron /TIBC 100) reflects the transport compartment, a saturation levels <16\% or less reflects inadequate transport iron to sustain erythropoiesis\textsuperscript{(20)}.

1-2-2-3: Transferrin Receptors (TfRs):

In the human body, TfRs can be found in two forms: the cell surface bound form; which can be detected by flow cytometry using monoclonal antibody against CD71, and the circulating form in serum, which can be detected by immunoassay\textsuperscript{(1)}.

The serum TfRs concentration, in contrast to serum ferritin, provides direct information about any deficit in the adequacy of iron supply to erythropoiesis\textsuperscript{(26)}. Decreased concentration of serum TfRs was found in patients with erythroid hypoplasia and increased numbers in those with erythroid hyperplasia. Consequently, serum TfRs measurement was introduced as a useful indicator of bone marrow erythropoiesis. Recent
studies have shown that the measurement of serum TfRs is a dependable
test for the diagnosis of iron deficiency and is useful for differentiation of
iron deficiency anaemia from the anaemia of chronic disease and
inflammation\(^\mathrm{(1)}\). Unlike conventional laboratory tests of iron status, serum
TfRs are unaffected by underlining acute or chronic inflammation.
Therefore, serum TfRs measurement is particularly promising for
evaluation of iron status when iron deficiency is simultaneously present
with overt or subclinical infection or inflammations, it is also promising for
assessment of iron status in pregnancy because it is not confounded by
gestational effects\(^\mathrm{(27)}\). Furthermore, serum TfRs is elevated earlier than
erthrocyte protoporphyrin or reduction in MCV\(^\mathrm{(24)}\).

With the exception of the conditions associated with markedly
enhanced erythropoiesis, which can increase TfRs independently (e.g.
megaloblastic anaemia, thalassaemia), the serum TfRs determination is
a specific test of iron status. Serum TfRs measurement is also reliable and
a single, small amount of blood sample is adequate for its accurate
determination. These sensitivity, specificity and reliability characteristics
of serum TfRs measurement enable it to enhance conditions in iron status
assessment in complex situations\(^\mathrm{(27)}\).

The measurement of TfRs has become a widely used tool in
assessing iron status, but its use has mainly been restricted to academic and
scientific research\(^\mathrm{(10)}\). It is limited use is because of limitations in
methodology and definition (standardization) of reference range\(^\mathrm{(26)}\).

The combination of serum TfRs and serum ferritin provide
complete information about storage and functional iron compartment, using
this combination along with haemoglobin concentration, it is possible to
define the iron nutritional status completely\(^\mathrm{(26)}\).

**1-2-2-4: Red cell protoporphyrin:**
Zinc protoporphyrin (ZPP) is synthesized by developing erythrocytes, when there is insufficient iron available to ferrochelatase for haem production\textsuperscript{(20)}. In iron deficiency and lead poisoning, the enzyme ferrochelatase catalyzes the incorporation of zinc instead of iron into protoporphyrin IX, resulting in the formation of ZPP\textsuperscript{(28)}.

Values are standardized by expressing the result as a ratio to heme\textsuperscript{(20)}. ZPP/heme reflect iron status during haemoglobin synthesis and detect iron deficiency before the onset of anaemia\textsuperscript{(23)}.

ZPP is not the same as free erythrocyte protoporphyrin (FEP), which is created in the laboratory when zinc is stripped from ZPP\textsuperscript{(23)}. Elevation of ZPP is noted several weeks after the onset of iron deficiency.

ZPP can be measured easily and cheaply using haemoflurometry, it has been used in several studies to assess iron status, with the usual laboratory cut off point of 1.2 mol/l, specificity was only 4.1%. This is, therefore, an exceptional poor test of iron status; over diagnosis of iron deficiency using this measurement is common\textsuperscript{(24)}. Also both ZPP and FEP are elevated with lead poisoning and chronic disease making them less useful for the diagnosis of anaemia\textsuperscript{(23)}.

ZPP is indicative of both absolute and functional iron deficiency and may substitute for percentage hypochromia when the instruments required for this analysis are unavailable\textsuperscript{(20)}.

1-2-2-5: Red cell ferritin:

Red cell ferritin was measured by means of radio immunoassay with antibody to liver (basic) and heart (acidic) ferritin, the normal means values for basic and acidic ferritin are 8.9 and 22.7 altogram (ag)/cell, respectively.

The red cell ferritin content reflects changes occurring in tissues both in iron deficiency and iron overload. Basic ferritin is more closely related to the body iron status than acidic ferritin, and acidic/basic ferritin
ratio is increased in iron deficiency and decreased in iron overload. It can be used for evaluating the adequacy of iron supply to erythroid marrow, particularly in patients with increased red cell turnover. Moreover, it may be useful in evaluating the body iron status in the patient with haemochromatosis and liver disease(28).

The major factor determining the red cell ferritin content appears to be the transferrin saturation, that is the distribution of iron between monoferric and diferric transferrin(28). Red cell ferritin is increased in disorders of haemoglobin synthesis including; iron loading anaemia such as the thalassemia disorders, which limits it use and it remains a research tool(5).

1-2-2-6: Percentage of hypochromic red cells:

As iron supply to the erythron diminishes, the new red cells produced are increasingly hypochromatic. Assessment of the haemoglobin content of individual red cells, which is possible using some automated cell counter (e.g. the Bayer H series) allows measurement of the percentage of hypochromic cells. Value of >10% may help in the early identification of impaired iron supply(5).

1-2-3: Assessment of functional compartment:

Functional iron is present in red blood cells as haemoglobin, available for oxygen storage in tissues as myoglobin, and for cell aerobic metabolism in cytochromes(14).

Red cell haemoglobin:

Reduced amounts of haemoglobin accompany an overall reduction in body iron in iron deficiency anaemia or acute blood loss. In other anaemias such as the megaloblastic anaemia, iron is redistributed from red cells to macrophage iron stores, with a corresponding increase in marrow stainable iron and serum ferritin level(5).
Anaemia is diagnosed when there is reduction of haemoglobin in grams per 100 ml to at least two standard deviation below the means, adjusted for age, sex and altitude of residence. Normal values increase in proportion to the elevation of residence above sea level and cigarette smoking\(^{(14)}\). Based on the world health organization (WHO) definition, anaemia is considered when haemoglobin is less than 11 g/dl for children aged 0-4 years and pregnant women, haemoglobin is less than 12 g/dl for children aged 5-12 years and non-pregnant women and haemoglobin is less than 13 g/dl for men\(^{(6)}\).

Measurement of haemoglobin is a more sensitive and direct test for anaemia than measurement of haematocrit, both are inexpensive and readily available tests for anaemia and are used most commonly for screening of iron deficiency. However, haemoglobin and haematocrit are late markers of iron deficiency\(^{(23)}\).

1-3: Red cell indices:

From the measured haemoglobin (Hb), haematocrit (Hct) and red blood cells (RBC), it is possible to derive other values, which reflect the red cell volume and haemoglobin content and concentration. These values are commonly referred to as the red cell indices. They are; the mean cell volume (MCV), the mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC)\(^{(30)}\).

The automated blood cell counter will provide an accurate measurement of red cell indices including the red cell distribution width (RDW)\(^{(31)}\). MCV is the only red cell index directly measured by the electronic counter, whereas the other indices are calculated\(^{(32)}\).

1-3-1: Definition and measurement of red cell indices:

1-3-1-1: Mean cell volume (MCV):
It is the average volume of red blood cell expressed in femtoliter (cubic micrometer)\(^{(33)}\). The measurement of the MCV uses the effect of the average RBC size in the Hct. It is calculated by dividing the Hct value by the RBC count \(^{(34)}\), the normal MCV value is 80-95 fl\(^{(35)}\).

1-3-1-2: Mean cell haemoglobin (MCH):

It is the average content of haemoglobin per red blood cells expressed in picogram / cell\(^{(33)}\). MCH is based on estimates of the quantity (weight) of Hb in the average RBC. It is calculated by dividing the blood Hb level by the RBC count. Reference values are 27-31 pg by manual methods and 26-34 pg by coulter counter\(^{(34)}\).

1-3-1-3: Mean cell haemoglobin concentration (MCHC):

It is the average concentration of haemoglobin in a given volume of packed red blood cells, expressed in gram per deciliter\(^{(33)}\). MCHC estimates the average concentration of Hb in the average RBC, it depends on the relationship of the amount of Hb to the volume of RBC, and thus the MCHC doesn’t depend on cell size alone, a macrocyte with normal amount of Hb has normal MCHC.

The MCHC is calculated by dividing the Hb value by the Hct value, reference values are 32%-36%(32-36 g/dl) by manual methods or 31-37% by coulter counter\(^{(34)}\).

1-3-1-4: Red cell distribution width (RDW):

RDW, which is derived from the red cell histogram that accompanies automated red cell sizing, is an index of the variation in the size of red cell, and can be used to detect subtle degree of anisocytosis\(^{(36)}\). It is a quantitative estimation of anisocytosis and is typically computed as the coefficient of variation of red cell size distribution\(^{(37)}\).

1-3-2: Clinical importance of red cell indices:
• Abnormal red cell indices may suggest an underlying abnormality before anaemia develops e.g. macrocytosis with early vit. B12, folate deficiency or alcoholic toxicity.

• Abnormal indices may also point to important disorder in which anaemia may not occur e.g. some cases of the thalassaemia trait in which the red cell are microcytic but because of their increased numbers, the haemoglobin concentration in blood is normal$^{(38)}$.

• The MCV is the most useful index. It enables the classification of anaemia by red cell size as microcytic, normocytic or macrocytic$^{(32)}$, it is useful as first step in approaching the etiologic diagnosis of an anaemia$^{(35)}$.

• In one study MCV was quite specific in identifying patients who had low ferritin levels, specificity was 83%, however, sensitivity was only 48% . MCV was also specific (88%) in identifying patients who had low ferritin with elevated TIBC, but the sensitivity was only 43$^{(39)}$.

• In another study, in severe deficiency of iron stores, the MCH, with abnormal values showed a minor specificity (0.62), while MCV specificity was (0.88) . With serum iron <20 ng/ml, specificity of MCV was (0.92). Simultaneous alteration in MCH and MCV has similar specificity (0.92) and predictive positive value$^{(40)}$ . MCH usually is parallel to MCV$^{(31)}$.

• Low MCH (<27 pg) was found to be superior to low MCV (<77 fl) in predicting low serum ferritin values . The occurrence of deficiency of ferritin was 90% when MCH was very low (MCH<23 pg)$^{(41)}$.

• MCHC is most useful as method for detecting erythrocyte cellular dehydration$^{(42)}$.

• MCHC is more frequently reduced in iron deficiency anaemia than with other causes of microcytosis, but the drop in MCHC occurs
relatively late and the diagnostic utility of this parameter is poor. Elevation of MCHC is associated with hereditary spherocytosis, but this finding is not always present\(^{(37)}\), it is increased in approximately 50% of cases. Patient with sickle cell anaemia also may have a population of erythrocytes with elevated MCHC because of cellular dehydration\(^{(42)}\).

- RDW provides information about cell size variability and is a sensitive means of detecting abnormal population of erythrocytes that otherwise may go undetected, even with careful visual examination of blood smear\(^{(42)}\).

- RDW is used frequently in conjunction with MCV to differentiate among various causes of anaemia e.g. RDW is high in iron deficiency anaemia but low in thalassaemia minor. In one study in adults, high RDW >15% was 71% to 100% sensitive and 50% specific in diagnosis iron deficiency. Another study of 12 month old infants found that high RDW >14% was 100% sensitive and 82% specific in iron deficiency anaemia\(^{(23)}\).

- MCV and RDW may provide classification of erythrocytes based on their size and size distribution\(^{(42)}\).

**1-3-3: Factors that affects interpretations of red cell indices:**

- MCV and MCH gradually increase with age both in male and female and MCHC remains constant in all age populations of both sexes\(^{(43)}\).

  - MCV, MCH, and MCHC are affected only by average cell measurement either of size or quantity of haemoglobin.
  - Marked hyperlipidemia may increase Hb, MCH and MCHC values.
  - Higher titer of cold agglutinins may decrease Hct and RBC count and increase MCV, MCH and MCHC values.
  - Marked erythrocytosis may falsely decrease the RBC count and the Hct value from true levels and falsely elevate MCH and MCHC values\(^{(34)}\).

**1-4: Classification of anaemia:**
Anaemia may be classified according to underlying mechanism (pathogenesis) or based on the morphology of RBC:

1-4-1: Classification of anaemia according to underlying mechanisms:

Three mechanisms may be responsible:

1. Deficiency of vital haemopoietic raw material (factor deficiency anaemia). The most common causes are; iron deficiency, deficiency of vit B12, folic acid or both.

2. Failure of blood forming organ to produce or to deliver mature RBC to the peripheral blood (production defect anaemia). This may be due to:
   - i) replacement of marrow by fibrosis or by neoplasm (primary or metastatic),
   - ii) hypoplasia of bone marrow, most commonly produced by certain chemicals or toxics suppression of marrow production or delivery without actual marrow hypoplasia, found to be of variable extent in some patients with certain systemic disease, the most common of these disease are: sever infection, chronic renal disease, wide spread malignancy (without extensive marrow replacement), collagen disease and hypothyroidism.

3) RBC loss from peripheral blood (depletion anaemia):
   - This is commonly due to:
     - i) haemorrhage, acute or chronic,
     - ii) haemolytic anaemia (RBC destroyed or RBC survival time shortened) or
     - iii) hypersplenism (splenic sequestration)\(^{34}\).

1-4-2: Classification of anaemia based on RBC morphology:

This classification depends on the appearance of the RBC on the peripheral blood smear, red cell indices or both.

Depending on MCV, anaemia is classified as microcytic, normocytic or macrocytic. These may be further subdivided according to the average amount of RBC haemoglobin (MCH) into hypochromic or normochromic\(^{34}\). So three groups of anaemia are distinguished;

* Microcytic, hypochromic,

* Normocytic, normochromic and

* Macrocytic anaemia\(^{30}\).

1-5: Evaluation of microcytic anaemia:
Microcytic anaemia generally results from inadequate haemoglobin synthesis. The failure of haemoglobin production may be caused by hereditary defects in globins synthesis as in thalassaemia, by defect in haem synthesis as in sidroblastic anaemia or by inadequate iron incorporation into the haem moiety because of either iron deficiency or failure of iron mobilization as seen in the anaemia of chronic disease.

The laboratory evaluation of microcytic anaemia should include determination of iron indices, namely serum iron, TIBC, and serum ferritin. Examination of peripheral blood smear for evidence of thalassaemia trait, or course basophilic stippling which suggests sidroblastic anaemia, and haemoglobin electrophoresis to assist in defining potential thalassaemia trait. The definitive diagnosis may require bone marrow aspiration to assess both iron stores and the effectiveness of iron incorporation into developing red cell precursors (35).

1-5-1: Iron deficiency anaemia:

Iron deficiency is by far the most common cause of anaemia in general and microcytic anaemia in particular (35). Iron deficiency anaemia develops as the end result of a series of steps that begin by depletion of stored iron (36).

The staging of iron deficiency by Oski et al (1983) is a useful concept and various measurements can be used to define the stages.

Stage I: storage iron depletion, identified by marrow examination or serum ferritin.

Stage II: iron deficient erythropoiesis, identified by FEP, transferrin receptor, abnormal RBC morphology (microcytosis <80 fl, anisocytosis RDW>15%) and transferrin saturation <10%.

Stage III: iron deficiency anaemia, identified by the above plus haemoglobin level below the lower limit of normal as result of progressive depletion of the functional iron compartment (44,45).

There is no evidence that iron stores depletion or iron deficient erythropoiesis alone have any adverse clinical effects, whereas iron deficiency anaemia is associated with alteration of immunological, GIT and mental functions (45). Low MCV and elevated RDW suggest iron deficiency (46). But also remember that early iron deficiency is generally a normocytic anaemia, the Hct usually falling to <30 before the MCV decline to <80 (35).
The peripheral blood smear in chronic iron deficiency anaemia typically shows RBC hypochromia and microcytosis, but lesser degrees of microcytosis are more difficult to recognize than hypochromia. Some of the peripheral blood changes may appear before actual anaemia\(^{(47)}\). In iron deficiency anaemia, the smear shows marked anisocytosis and the severity of anaemia is proportional to the decline in the MCV\(^{(32)}\).

An array of tests can be used for evaluating anaemia, the gold standard for identifying iron deficiency is a direct test; bone marrow examination with Prussian blue staining, however, bone marrow aspiration is too invasive for routine use, so indirect assays generally are used\(^{(23)}\).

The diagnosis of moderate to severe iron deficiency anaemia is easy. It is characterized by: reduced serum ferritin, reduced serum iron level, increased TIBC, increase red cell protoporphyrin level and increase in the haemoglobin concentration after the institution of iron therapy\(^{(36)}\).

Low serum ferritin is the best single laboratory parameter for the diagnosis of iron deficiency. Serum iron, TIBC and Hb electrophoresis, if necessary, can help to differentiate the type of microcytic anaemia in patient with normal or elevated levels of serum ferritin\(^{(48)}\).

Serum ferritin<12 ug/l is diagnostic of iron deficiency. However, values between 12 and 100 ug/l are difficult to interpret because inflammation, even in the presence of iron deficiency, causes elevation of serum ferritin\(^{(22)}\). The sTfRs offers an advantage in assessing iron status because of its indicated ability to distinguish iron deficiency depletion from anaemia of chronic disease and can identify iron depletion and functional iron deficiency in patient with concurrent inflammation\(^{(19)}\).

Evaluation of sTfRs is five times more expensive than ferritin and should be reserved for patient in whom marrow iron assessment is being considered\(^{(49)}\). The diagnosis of mild forms of iron deficiency anaemia may present a greater challenge, the laboratory tests may be less reliable and
values of iron deficient and iron sufficient persons may overlap considerably\(^{(36)}\).

**1-5-2: Thalassaemia:**

The thalassaemias, a group of inherited disorders of haemoglobin synthesis, are the most common single gene disease worldwide\(^{(50)}\). The frequency of the thalassaemia is dependent on the ethnic origin\(^{(51)}\).

Thalassaemia is caused by defective or absent synthesis of either of two chains alpha or beta, of adult haemoglobin tetramer as result of gene deletion or mutation. At cellular level, this results in reduced amount of normal functioning haemoglobin and an imbalance between globin types. The unaffected chain is continually produced, whereas the affected chain production is decreased or absent. The precipitation of the remaining globin chains alter red cell in the intramedullary space causing ineffective erythropoiesis. The mature red cells that make their way out from the marrow have shortened survival because of increased haemolysis resulting in severe anaemia.

Ineffective erythropoiesis is a feature of beta-thalassaemia as the relative excess of unstable alpha-chain precipitate and disintegrate causing oxidative damage to red cell membrane. Conversely, the excess of beta-chain in alpha thalassemia is more stable. Denatured globin chains aggregate resulting in less intramedullary membrane damage, which accounts for the clinical difference in disease severity\(^{(50)}\). Three different clinical and haematological conditions are recognized; thalassaemia minor, thalassaemia intermedia, and thalassaemia major.

Thalassaemia major is characterized by severe microcytic anaemia, spleen and liver enlargement and characteristic skeletal abnormalities. Haemoglobin electrophoresis demonstrates absent to minimal haemoglobin
A (2%-5%) and variable haemoglobin A2 (0-3%), the percentage of haemoglobin F dominating (70-95%) (52).

Thalassaemia intermedia is used to describe patients, with clinical picture of thalassaemia which (although not transfusion dependent) is associated with a more severe degree of anaemia than is found in carrier state of thalassaemia. The clinical features are extremely variable (5). Although it is associated with profound microcytosis, thalassaemia intermedia and thalassaemia major do not present a diagnostic problem in this setting as they are both associated with slightly elevated (2–7%) reticulocytes count and are characterized by physical stigmata of massive bone marrow expansion (36).

Thalassaemia minor, however, may go unrecognized into adulthood until a routine laboratory evaluation or an intercurrent problem prompts medical attention. These patients have mild to minor anaemia with hypochromic and microcytic red cells. Spleen enlargement may also be present.

Available clues to diagnosis of either beta or alpha-thalassaemia minor can be the presence of moderate peripheral blood smear abnormalities despite insignificant anaemia (51). Microcytic hypochromic cell are seen in both iron deficiency and thalassaemia, however, for same level of anaemia, there is often greater poikilocytosis as well as target cells and basophilic stippling in patient with thalassaemia trait, the morphological aberrations are out of proportion to the mildness of anaemia (32). By using cytometry plots, the proportion of hypochromatic cells is greater than the proportion of microcytic cells in iron deficiency whereas the reverse is true in thalassaemia.

In thalassaemia trait, iron level is usually normal to high, TIBC is normal and ferritin level is normal to high (35). Beta-thalassaemia trait is
easily confirmed by elevated haemoglobin A2 level (>3.5%)\(^{(51)}\), and there is inconstant minor increase in fetal haemoglobin (2–5\%)\(^{(52)}\).

Although the definitive diagnosis of beta-thalassaemia trait requires the demonstration of abnormal Hb A2 level or reduced beta chain synthesis, serum ferritin and MCV measurement are useful screening procedures for the initial diagnosis of beta- thalassaemia and iron deficiency\(^{(53)}\).

Mentzer index was developed to help in distinguishing thalassaemia from iron deficiency anaemia. It is calculated by dividing the RBC count by the MCV, when the quotient is less than 13 thalassaemia is likely, and if the quotient is greater than 13, iron deficiency is more likely\(^{(46)}\).

Alpha thalassaemia trait (deletion or mutation of two of the four alpha chains), usually causes microcytosis with or without mild anemia. Clinically the patients are asymptomatic. Haemoglobin electrophoresis may reveal Hb Bart’s (gamma 4 homotetramer) in the neonatal period but may subsequently be normal in older patient. Diagnosis involves family history, elimination of beta-thalassaemia as diagnosed by Hb electrophoresis and subsequent confirmation of alpha-globins loci mutation by molecular approach. Individuals with single alpha-globins deletion are silent carriers and asymptomatic\(^{(32)}\).

The importance of diagnosing thalassaemia minor lies in genetic counseling and in the recognition that these disorders require little or no therapy, it is unusual for affected patients to need transfusion or marrow stimulators such as erythropoietin. At times laboratory data may suggest iron deficiency, but iron supplementation is usually not needed unless the patient has excessive blood loss and confirmed iron deficiency. Unnecessary iron therapy is harmful because of iron overload and potential organ damage\(^{(51)}\).
1-5-3: Sidroblastic anaemia:

The sidroblastic anaemias are a heterogeneous group of disorders, it has two distinctive features which are ring sidroblasts in bone marrow (abnormal erythroblast with excessive iron accumulation in the mitochondria) and impaired haem synthesis. Most commonly, the sidroblastic anaemias are classified as hereditary or acquired conditions.

The hereditary forms are primary X-linked, although some families display autosomal dominant or recessive modes of transmission.

Acquired sidroblastic anaemia, which is far more common than hereditary varieties is usually caused by drugs and toxins. The next large subgroup is refractory anaemia with ring sidroblasts, which is a subset of the myelodysplastic syndromes.

The exact mechanism by which disturbed haem metabolism produces sidroblastic anaemia remains elusive, the signal feature is mitochondrial iron deposition. Ring sidroblasts comprise between 15% and 50% of erythroblasts in most of the patients.

The bone marrow of many patients with sidroblastic anaemia displays erythroid hyperplasia consistent with the concept of ineffective erythropoiesis in these conditions. Ineffective erythropoiesis increases iron absorption from the GIT. Therefore patients with even mild sidroblastic anaemia can develop substantial iron overload.

Sidroblastic anaemia tends to be a moderate to severe condition with Hb ranging usually from 4-10 g/dl. Blood smears sometimes reveal basophilic stippling, hypochromia and microcytosis, although normocytic and macrocytic pictures are possible, particularly in myelodysplastic syndromes.

Dimorphic red cells are characteristic of female carriers of the hereditary condition. Patient with pure sidroblastic anaemia or refractory anaemia with ring sidroblasts can also manifest a dimorphic red cells population. Coexistence of normal and sidroblastic maturation is the probable basis of this phenomenon. Electronic blood analyzers display these anomalies as elevated RDW\(^{(54)}\).

In sidroblastic anaemia iron level is usually normal to high, TIBC is normal and ferritin level normal to high. It is confirmed by the presence of ring sidroblasts in bone marrow aspiration\(^{(35)}\).

1-5-4: Anaemia of chronic disease:

Anaemia of chronic disease, the most frequent anaemia among hospitalized patients, develops in chronic inflammatory disorders such as; chronic infection, cancer or autoimmune disease\(^{(55)}\). Because these conditions are very common, the anaemia of chronic disease is one of the most frequent anaemias encountered and is only second in incidence to iron deficiency anaemia\(^{(56)}\). A number of different pathways contribute to
anaemia of chronic disease such as; diversion of iron traffic, diminished erythropoiesis, a blunted response to erythropoietin and bone marrow invasion by tumor cells and pathogens. The underlying diversion of iron traffic leads to withdrawal of metal from the site of erythropoiesis and its circulation to the storage compartment in the reticulo-endothelial system resulting in hypoferremia and hyperferritinemia.

Pro-inflammatory and anti-inflammatory cytokines, acute phase proteins, and free radicals are prominently involved in causing this disturbance of iron homeostasis. Pro-inflammatory cytokines; IL1 and TNF, stimulate ferritin synthesis in macrophages and hepatocytes via both transcriptional and translational pathways.

Anti-inflammatory cytokines; IL4, IL10 and IL3, modulate iron metabolism by two different pathways. First, by opposing interferon (IFN)-mediated activation of iron regulatory proteins (IRP) thereby increasing ferritin translation, and second, by augmentating transferrin receptor mRNA expression most likely by reversing the inhibitory effect of interferon (IFN) on transferrin receptor transcription. Thus, anti-inflammatory cytokines are able to increase iron retention in activated macrophages and may also contribute to the development of anaemia. Part of the action of these cytokines is indirect via the activation or deactivation process involved in the generation of nitric oxide, hydrogen peroxide or super oxide anion. The latter substances affect iron homeostasis via their influence on posttranscriptional regulation of ferritin and transferrin receptor expression.

Iron strongly affects cell mediated immune function. In addition to the role of iron in proliferation and differentiation of lymphocyte subsets, iron directly inhibits the activity of interferon (INF). This pro-inflammatory cytokine is centrally involved in the co-ordination of cell-mediated immune effectors mechanisms against invading pathogens. Therefore, withdrawal of metabolically active iron from circulation and storage of the metal, as occur in the anaemia of chronic disease, may act to strengthen the immune response via stimulatory effect of INF – mediated immune effectors mechanism.

Overall, anaemia of chronic disease seems to be the product of activated immune system using defense strategy of withholding iron, an essential growth factor for invading pathogen, while increasing the efficiency of cell mediated immunity.

Plasma erythropoietin concentration in patients with anaemia of chronic disease is normal or even increased when compared to healthy subjects.

Anaemia of chronic disease is primarily an anaemia due to underproduction of red cells, with low reticulocytes production, and is most
often a normocytic normochromic anaemia. However in 30-50% of patients, the red cells are hypochromic microcytic\(^\text{(56)}\).

No definitive test will confirm a diagnosis of anaemia of chronic disease. Patients with this anaemia classically have low serum iron level, low TIBC, elevated serum ferritin and saturation of transferrin \(>10\%\). The diagnosis is primarily one of exclusion. Diagnostic studies may involve bone marrow aspirate to confirm adequate iron stores with poor incorporation into siderocytes\(^\text{(35)}\).

The measurement of serum TfRs may now provide an alternative, which unlike ferritin, doesn’t increase with infection or inflammation.

The most sensitive means available to distinguish iron deficiency and anaemia of chronic disease is a combination of plasma transferrin receptor and plasma ferritin concentration i.e. the transferrin receptor-ferritin index, which is the transferrin receptor concentration divided by the plasma ferritin concentration or in some studies the log of plasma ferritin concentration\(^\text{(19)}\). Values of both sTfRs and sTfRs-ferritin index are raised in iron deficiency even in the presence of chronic disease but normal or slightly raised in anaemia of chronic disease\(^\text{(49)}\).

The value of diagnostic tests will depend on the planned investigation. In an area with high prevalence of iron deficiency, it may be more important to have very sensitive screening tests so that iron supplements are given to all that appear to be deficient. Alternatively, a more specific test may be chosen to target the most at risk population for more intensive management. A sequential approach may be used, e.g. test with high sensitivity such as serum ferritin may be followed by test with high specificity such as serum transferrin receptor /ferritin ratio\(^\text{(24)}\).

Diseases of iron metabolism are likely to be both more frequent than expected, and to exhibit a wider range of clinical severity and effects.
Review of blood indices and serum iron and ferritin markers may alert the clinician to most disorders\(^{(4)}\).

### 1-6: Justification:

After the introduction of Sysmex Kx-21 as a service in routine of haematology laboratories in Sudan, it has been noticed that a significant number of results showed low red cell indices in presence of normal Hb.

This study is designed to assess iron status in such individuals as well as in individuals with anaemia and low red cell indices in order to compare the findings and to try to find an explanation for the low indices in presence of a normal Hb values.

### 1-7: Objectives:

1. To measure S. iron, TIBC and S. ferritin in all patients showing low red cell indices irrespective of Hb levels.
2. To compare the results of above mentioned tests in patients with normal Hb and low red cell indices with those with low Hb and low red cell indices.
3. To assess the significance of low red cell indices in individuals with normal Hb.
4. To suggest possible explanation of low indices in those with normal Hb and normal iron status.
Materials and methods

2-1: Subjects:

This study was conducted at Soba University Teaching Hospital (university of Khartoum) in the period from October 2002 –April 2003.

80 in-patient & out-patients with low red cell indices (MCV≤ 76 fl and/or MCH ≤ 26 pg and/or MCHC ≤ 31 g/l) irrespective of haemoglobin levels were studied.

20 healthy individuals, matched for age and sex, with normal red cell indices were taken as controls.

Those patients who received blood transfusion during the last 4 months or who have taken iron supplements during the last week were excluded.

2-2: Samples:

1. 2.5 ml venous blood in EDTA were collected for measurement of hematological values.

2. 4 ml where collected as clotted blood to obtain 2 ml of serum, which was stored in cryo-tubes at –20 degree centigrade for measuring the biochemical values.

For the target population & controls the following investigations were done:

1. Haemoglobin (Hb).
2. Haematocrit (Hct).
3. Red cell indices (MCV, MCH and MCHC).
4. Peripheral blood film.
5. Serum iron.
6. Total iron binding capacity (TIBC).
7. Serum ferritin.
Hb, Hct and red cell indices were measured by automatic blood counter (Sysmex Kx –21), at the same time peripheral blood film was made as a control.

Serum iron and TIBC were measured by an automatic instrument (BTS- 370 plus), which performs spectrometric measurements.

Serum ferritin was estimated by ELIZA technique.

2-3: Instruments:

2-3-1: Sysmex Kx-21:

This is an automated multi-parameter blood cell counter for in vitro diagnostic use in clinical laboratories.

The Sysmex Kx-21 processes approximately 60 samples an hour and display on the LCD screen the particle distribution curve of WBC, RBC and platelets, along with data of 18 parameters as analytical results.

The Sysmex Kx-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detecting method. The RBC count and platelets are taken by the RBC detector block also using the DC detection method. The Hb detector block measures the Hb concentration using a non-cyanide hemoglobin method.

MCV is measured directly, while MCH and MCHC are obtained by calculation. MCH is calculated by HB/RBC, and MCHC is calculated by Hb/Hct.

2-3-2: BTS 370 plus (biosystem):

This is a computer controlled, bench-top instrument designed to perform spectrometric measurements at predetermined wavelengths of analyte concentration and enzyme activity using various reagents.

Any combination of tests can be performed, on up to 60 samples per work list. The analyzer automatically performs all reagent and sample pipetting, incubations, photometric measurements and calculations.
2-4: Principles of methods:

2-4-1: Serum iron:

Iron – ferrozine, spectrophotometric (Biosystem)

Principle of method:

Transferrin bound ferric iron in the sample is released by guanidinium and reduced to ferrous by means of hydroxylamine. Ferrous iron reacts with ferrozine forming a colour complex that can be measured by spectrophotometry.

2-4-2: Iron binding capacity:

Precipitation/spectrophotometric (Biosystem).

Principle of method:

An excess ferric iron is added to the sample to saturate serum transferrin. Uncomplexed ferric ion is precipitated with magnesium hydroxide carbonate and the iron bound to protein in the supernatant is then spectrophotometrically measured.

2-4-3: Serum ferritin:

Pathozyme–ferritin (Omega): pathozyme–ferritin is an enzyme immunoassay (EIA) for the quantitative determination of ferritin in human serum.

Principle of the test:

Specific anti-ferritin antibodies are coated on to micro-titration wells. Test sera are applied, then monoclonal anti-ferritin labeled with horse radish peroxidase enzyme (conjugate) is added. If human ferritin is present in the sample, it will combine with antibody on the well and the enzyme conjugate, resulting in the ferritin molecules being sandwiched between the solid phase and the enzyme linked antibodies.
After 45 minutes incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. On addition of substrate (TMB), a colour will develop only in those wells in which enzyme is present, indicating the presence of ferritin.

The reaction is stopped by addition of dilute hypochloric acid and the absorbance is then measured at 450 nm.

The concentration of ferritin is directly proportional to the colour intensity of the test sample.
Results

3-1: General consideration:

80 patients with low red cell indices irrespective of haemoglobin values were studied. They were divided into three groups; i) children, ii) females and iii) males.

20 healthy individuals with normal haemoglobin and normal red cell indices were taken as controls.

3-2: Group 1 (Children):

3-2-1: Age distribution:

11 children were included, minimum age was 6 years and maximum was 12 years (mean 9 yrs).

3-2-2: Haemoglobin (Hb) values:

All children showed low haemoglobin levels (100%).

3-2–3: Values of red cell indices (Table 1):

Of the 11 children, 7 patients (63.6%) had low MCV while 4 (36.4 %) had normal MCV (Fig.1).

All children (100%) showed low MCH.

8 children (72.7%) had low MCHC while 3 (27.3%) had normal MCHC (Fig. 2).

Table (1): Summary of the results of the red cell indices in the studied children:

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Low</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td>4 (36.6%)</td>
<td>7 (63.6%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>MCH</td>
<td>0</td>
<td>11 (100%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>MCHC</td>
<td>3 (27.3%)</td>
<td>8 (72.7%)</td>
<td>11 (100%)</td>
</tr>
</tbody>
</table>

Fig. (1)
3-2-4: Biochemical finding:
3-2-4-1: S. Iron levels in studied children:

4 children (36.4%) had normal S. iron while 7 (63.6%) had low S. iron levels (Fig. 3).

![Fig. (3)](image)

S. iron levels in the studied children

3-2-4-2: TIBC values in the studied children:

4 children (36.4%) had high TIBC, 2 (18.2%) had normal TIBC and 5 (45.5%) had low TIBC levels (Fig. 4).

3-2-4-3: S. ferritin values in the studied children:

7 children (63.6%) had normal ferritin levels while 4 (36.4%) had low S. ferritin levels (Fig. 5).

All children with low serum ferritin had; low S. iron, normal or high TIBC and low transferrin saturation.

Children with normal S. ferritin levels showed the following results:
1 patient had normal S. iron, normal TIBC and normal transferrin saturation,
3 patients had normal S. iron and low TIBC, 2 of them showed calculated normal transferrin saturation and one had high transferrin saturation,
**1 patient had low S. iron, high TIBC and low transferrin saturation, and**
2 patients had low S. iron, low TIBC and normal transferrin saturation.
(See table 2).
**Fig. (5)**

**S. ferritin in studied children**

![Pie chart showing the distribution of low and normal ferritin levels among children.](image)

**Table (2):** Relationship between S. iron, TIBC and transferrin saturation to S. ferritin levels in the studied children:

<table>
<thead>
<tr>
<th></th>
<th>Fe=n TIBC=n</th>
<th>Fe=n TIBC=↓</th>
<th>Fe=n TIBC=↓</th>
<th>Fe=↓ TIBC=n,↑</th>
<th>Fe=↓ TIBC=↓</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ferritin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4 (36.4%)</td>
<td>-</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td>Normal ferritin</td>
<td>1 (9.1%)</td>
<td>2 (18.2%)</td>
<td>1 (9.1%)</td>
<td>1 (9.1%)</td>
<td>2 (18.2%)</td>
<td>7 (63.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>1 (9.1%)</td>
<td>2 (18.2%)</td>
<td>1 (9.1%)</td>
<td>5 (45.5%)</td>
<td>2 (18.2%)</td>
<td>11 (100%)</td>
</tr>
</tbody>
</table>

(Fe = Serum iron, TIBC = total iron binding capacity, TS = transferrin saturation)
3-3: Group 2 (females):

3-3-1: Age distribution:

39 patients were included with minimum age 19 years and maximum age 75 years (mean 36.11 yrs).

3-3-2: Haemoglobin values:

17 patients (43.6 %) showed normal Hb while 22 patients (56.4 %) showed low Hb levels (Fig. 6)

Fig.(6)
Hb values in 39 studied females with low red cell indices
3-3-3: females with normal Hb and low red cell indices (17 patients):

3-3-3-1: Values of red cell indices (Table 3):

16 patients (94%) had normal MCV while 1 patient (6%) had low MCV (Fig. 7).

All patients with normal Hb showed low MCH.

11 patients (65%) had normal MCHC while 6 patients (35%) had low MCHC (Fig. 8).

Table (3): Summary of the result of the red cell in 17 studied females with normal HB:

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Low</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td>16 (94%)</td>
<td>1 (6%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>MCH</td>
<td>0</td>
<td>17 (100%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>MCHC</td>
<td>11 (65%)</td>
<td>6 (35%)</td>
<td>17 (100%)</td>
</tr>
</tbody>
</table>

Fig.(7):
MCV values in 17 studied females with normal Hb

Fig. (8)

MCHC values in 17 studied females with normal Hb

3-3-3-2: biochemical finding:

3-3-3-2-1: S. iron levels in 17 females with normal Hb:
1 patient (5.9%) had high S. iron level,
5 patients (29.4%) had normal S. iron level and,
11 patients (64.7%) had low S. iron level (Fig. 9).

**Fig. (9)**
S. iron values in 17 studied females with normal Hb

3-3-3-2-2: TIBC in 17 studied females with normal Hb:
4 patients (24%) had high TIBC,
8 patients (47%) had normal TIBC, and
5 patients (29%) had low TIBC (Fig. 10)

3-3-3-2-3: S. ferritin levels in 17 studied females with normal Hb:
9 patients (53%) had normal S. ferritin levels.
8 patients (47%) had low S. ferritin levels (Fig. 11).

**Fig. (10)**
TIBC values in 17 studied females with normal Hb
Fig. (11)
S. ferritin values in 17 studied females with normal Hb:

Studied females with normal Hb and low ferritin showed the following results:
1 patient had high S. iron, normal TIBC and high transferrin saturation,
1 patient had normal S. iron, normal TIBC and normal transferrin saturation,
6 patients had low S. iron, normal or high TIBC and low transferrin saturation.

**Studied females with normal Hb and normal ferritin showed the following results:**
1 patient had normal S. iron, normal TIBC and normal transferrin saturation,
3 patients had normal S. iron, low TIBC and normal transferrin saturation,
3 patients had low S. iron, normal or high TIBC and low transferrin saturation,
2 patients had low S. iron and low TIBC, one of them showed normal transferrin saturation while the other had low transferrin saturation.

**Table (4):** Relationship between S. iron, TIBC and TS to S. ferritin levels in the studied females with normal Hb and low red cell indices:

<table>
<thead>
<tr>
<th></th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Fe =↓</th>
<th>Fe =↓</th>
<th>Fe =↓</th>
<th>Fe =↑</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TIBC=n</td>
<td>TIBC=↓</td>
<td>TIBC n,↑</td>
<td>TIBC=↓</td>
<td>TIBC=↓</td>
<td>TIBC=↑</td>
<td></td>
</tr>
<tr>
<td>Low S. ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (5.9%)</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Normal S. ferritin</td>
<td>1 (5.9%)</td>
<td>3 (17.6%)</td>
<td>3 (17.6%)</td>
<td>1 (5.9%)</td>
<td>0</td>
<td>9 (53 %)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2 (11.8%)</td>
<td>3 (17.6%)</td>
<td>9 (52.9%)</td>
<td>1 (5.9%)</td>
<td>1</td>
<td>17 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

(Fe= Serum iron, TIBC= total iron binding capacity, TS= transferrin saturation)

**3-3-4: Females with low red cell indices and low Hb (22 patients):**

**3-3-4-1: Values of red cell indices (Table 5):**
12 patients (54.5%) had normal MCV while 10 patients (45.5%) had low MCV (Fig. 12).

All the patients had low MCH (100%).

6 patients (27.3%) had normal MCHC while 16 patients (72.7%) had low MCHC (Fig. 13).

Table (5): summary of the results of red cell indices in studied females with low Hb:

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Low</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td>12 (54.5%)</td>
<td>10 (45.5%)</td>
<td>22 (100%)</td>
</tr>
<tr>
<td>MCH</td>
<td>0</td>
<td>22 (100%)</td>
<td>22 (100%)</td>
</tr>
<tr>
<td>MCHC</td>
<td>6 (27.3%)</td>
<td>16 (72.7%)</td>
<td>22 (100%)</td>
</tr>
</tbody>
</table>

Fig.(12)
MCV values in 22 studied females with low Hb

Fig.(13)
MCHC values in 22 studied females with low Hb
3-3-4-2: biochemical findings:

3-3-4-2-1: S. iron levels in 22 females with low Hb:

4 patients (18.2 %) had normal S. iron while 18 patients (81.8 %) had low S. iron (Fig. 14).

---

**Fig. (14)**
S. iron values in 22 studied females with low Hb

---

3-3-4-2-2: TIBC levels in 22 females with low Hb:
5 patients (22.7%) had high TIBC, 4 patients (18.2%) had normal TIBC, and 13 patients (59.1%) had low TIBC (Fig. 15).

3-3-4-2-3: S. ferritin levels in 22 females with low Hb:
2 patients (9.1%) had high S. ferritin levels,
12 patients (54.5%) had normal S. ferritin levels and
8 patients (36.4%) had high S. ferritin levels (Fig. 16).
Females with low Hb and low S. ferritin showed the following results:
6 patients had low S. iron, normal or high TIBC and low transferrin saturation,
2 patients had low S. iron and low TIBC, one of them showed calculated low transferrin saturation and one had normal transferrin saturation.

Females with low Hb and normal S. ferritin showed the following results:
1 patient had normal S. iron, normal TIBC and normal transferrin saturation,
3 patients had normal S. iron, low TIBC and normal transferrin saturation,
2 patients had low S. iron, high TIBC and low transferrin saturation,
6 patients had low S. iron and low TIBC, 4 of them showed calculated normal transferrin saturation and 2 patients had low transferrin saturation.

Females with low Hb and high S. ferritin showed the following results:
2 patients had low S. iron and low TIBC, one of them showed low transferrin saturation and one had normal transferrin saturation.

Table (6) : Relationship between S. iron, TIBC and TS to S. ferritin levels in the studied females with low Hb and low red cell indices :
<table>
<thead>
<tr>
<th></th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Fe = ↓</th>
<th>Fe =↓</th>
<th>Fe =↓</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TIBC n</td>
<td>TIBC↓</td>
<td>TIBC n,↑</td>
<td>TIBC↓</td>
<td>TIBC↓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TS = n</td>
<td>TS =↓</td>
<td>TS =↓</td>
<td>TS =↓</td>
<td>TS = n</td>
<td></td>
</tr>
<tr>
<td>Low S. ferritin</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27.3%</td>
<td>4.5%</td>
<td>4.5%</td>
<td>36.4%</td>
</tr>
<tr>
<td>Normal S. ferritin</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>12</td>
</tr>
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<td></td>
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<td>13.6%</td>
<td>9.1%</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>4.5%</td>
<td>4.5%</td>
<td>9%</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>4</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.6%</td>
<td>31.8%</td>
<td>18.2%</td>
<td>100%</td>
</tr>
</tbody>
</table>

(Fe= Serum iron, TIBC= total iron binding capacity, TS= transferrin saturation)

3-4: Group 3 (Males):

3-4-1: Age distribution:
30 patients were included with minimum age 16 years and maximum age 73 years (mean 39.8 yrs).

3-4-2: Haemoglobin values:
5 patients (16.7%) showed normal Hb while 25 patients (83.3 %) showed low Hb levels (Fig. 17).

Fig.(17)

Hb values in 30 studied males with low red cell indices

3-4-3: Males with low red cell indices and normal Hb:
3-4-3-1: Values of red cell indices (Table 7) :
4 patients (20 %) had normal MCV while 1 patient (80 %) had low MCV (Fig. 18).

All patients with normal Hb showed low MCH.
4 patients (80%) had normal MCHC while only one patient (20%) had low MCHC (Fig. 19).

**Table (7)**: Summary of the results of red cell indices in studied males with normal HB:

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Low</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td>4 (80%)</td>
<td>1 (20%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>MCH</td>
<td>0</td>
<td>5 (100%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>MCHC</td>
<td>4 (80%)</td>
<td>1 (20%)</td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

*Fig.(18)*

*MCV values in 5 studied males with normal Hb*

*Fig.(19)*


3-4-3-2: biochemical finding:

3-4-3-2-1: S. iron levels in 5 studied males with normal Hb:

4 patients (80%) had normal S. iron level, and 1 (20%) had low S. iron level (Fig. 20).

3-4-3-2-2: TIBC values in 5 studied males with normal Hb:

All patients with normal Hb showed low TIBC.

3-4-3-2-3: S. ferritin values in 5 studied males with normal Hb:

All patients with normal Hb showed normal ferritin.
S. iron values in 5 studied males with normal Hb

Males with normal Hb and normal S. ferritin showed the following results (Table 8):

4 patients (80%) had normal S. iron and low TIBC, 3 of them showed normal transferrin saturation while one had high transferrin saturation, 1 patient (20%) had low S. iron, low TIBC and low transferrin saturation.

**Table (8):** Relationship between S. iron, TIBC and TS to S. ferritin levels in the studied males with normal Hb and low red cell indices:

<table>
<thead>
<tr>
<th></th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Fe =↓</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TIBC=↓</td>
<td>TIBC=↓</td>
<td>TIBC=↓</td>
<td></td>
</tr>
<tr>
<td>TS = n</td>
<td>TS=↑</td>
<td>TS=↑</td>
<td>TS=↑</td>
<td></td>
</tr>
<tr>
<td>Normal S.ferritin</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>(60 %)</td>
<td>(20 %)</td>
<td>(20%)</td>
<td>(100%)</td>
<td></td>
</tr>
</tbody>
</table>

3-4-4: Males with low red cell indices and low Hb (25 patients):

3-4-4-1: Values of red cell indices (Table 9):
18 patients (72 %) had normal MCV while 7 patients (18 %) had low MCV (Fig. 21).
All the patients had low MCH (100%).
12 patients (48 %) had normal MCHC while 13 patients (52 %) had low MCHC (Fig. 22).

Table (9) : Summary of the results of red cell indices in studied males with low Hb:

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Low</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCV</td>
<td>18 (72 %)</td>
<td>7 (28 %)</td>
<td>25 (100 %)</td>
</tr>
<tr>
<td>MCH</td>
<td>0</td>
<td>25 (100 %)</td>
<td>25 (100 %)</td>
</tr>
<tr>
<td>MCHC</td>
<td>12 (48 %)</td>
<td>13 (52 %)</td>
<td>25 (100 %)</td>
</tr>
</tbody>
</table>

Fig.(21)
*MCV values in 25 studied males with low Hb*

Fig.(22)
3-4-4-2: biochemical findings:

3-4-4-2-1: S. iron levels in 25 studied males with low Hb:

7 patients (28%) had normal S. iron while 18 patients (73%) had low S. iron (Fig. 23).

Fig. (23)
S. iron levels in 25 studied males with low Hb

3-4-4-2-2: S. TIBC levels in 25 studied males with low Hb:
2 patients (8%) had high TIBC, 2 patients (8%) had normal TIBC, and 21 patients (84%) had low TIBC (Fig. 24).

3-4-4-2-3: S. ferritin levels in 25 studied males with low Hb:
13 patients (52%) had high S. ferritin levels,
11 patients (44%) had normal S. ferritin levels and
1 patients (4%) had low S. ferritin levels (Fig. 25).
Males with low Hb and high ferritin showed the following results:
4 patients had normal S. iron and low TIBC, 2 of them showed normal transferrin saturation and 2 patients had high transferrin saturation,
7 patients had low S. iron and low TIBC, 5 of them showed normal transferrin saturation and 2 patients had low transferrin saturation,
2 patients had low S. iron, high TIBC and low transferrin saturation,

Males with low Hb and normal ferritin showed the following results:
3 patients had normal S. iron, low TIBC and normal transferrin saturation
7 patients had low S. iron and low TIBC, 5 of them showed normal transferrin saturation and 2 patients had low transferrin saturation.
1 patient had low S. iron, normal TIBC and low transferrin saturation,

Males with low Hb and low ferritin showed the following results:
1 patient had low S. iron, normal TIBC and low transferrin saturation.

Table (10) : Relationship between S. iron, TIBC and TS to S. ferritin levels in the studied males with low Hb and low red cell indices:
<table>
<thead>
<tr>
<th>Fe = n</th>
<th>TIBC n</th>
<th>TIBC ↓</th>
<th>TS = n</th>
<th>TS = ↑</th>
<th>Fe = ↓</th>
<th>TIBC = n</th>
<th>TS = ↓</th>
<th>TS = ↑</th>
<th>Fe = ↓</th>
<th>TIBC ↓</th>
<th>TS = n</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. ferritin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4 %)</td>
<td></td>
<td>(4 %)</td>
<td></td>
<td>(4 %)</td>
<td>(4 %)</td>
<td>(4 %)</td>
<td>(4 %)</td>
<td>(4 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. ferritin</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(12 %)</td>
<td></td>
<td>(4 %)</td>
<td></td>
<td>(8 %)</td>
<td>(20 %)</td>
<td>(44 %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. ferritin</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8 %)</td>
<td>(8 %)</td>
<td>(8 %)</td>
<td>(8 %)</td>
<td>(8 %)</td>
<td>(20 %)</td>
<td>(52 %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(20 %)</td>
<td>(8 %)</td>
<td>(8 %)</td>
<td>(16 %)</td>
<td>(16 %)</td>
<td>(40 %)</td>
<td>(100 %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Fe = Serum iron, TIBC = total iron binding capacity, TS = transferrin saturation)

3-5: controls (20):
20 healthy individuals, 8 females and 12 males, with normal Hb values and normal red cell indices were taken as controls.

3-5-1: Biochemical findings:

All studied controls had normal S. iron, normal TIBC and normal transferrin saturation. Only 2 (10%) had low S. ferritin with normal S. iron, normal TIBC and normal transferrin (Table 11).

Table (11): Result of S. ferritin levels in controls:

<table>
<thead>
<tr>
<th></th>
<th>normal</th>
<th>low</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>6 (30%)</td>
<td>2 (10%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Males</td>
<td>12 (60%)</td>
<td>0</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Total</td>
<td>18 (90%)</td>
<td>2 (10%)</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>

3-6: Biochemical findings in all studied patients:

3-6-1: Biochemical findings in studied patients with normal Hb and low red cell indices:

Of the 22 patients with normal Hb and low red cell indices; 8 (36.4%) patients showed low S. ferritin while 14 (63.6%) had normal S. ferritin (Fig. 26).

Fig.(26)
S. ferritin levels in all studied patient with normal Hb and low red cell indices
**Table (12):** Summary of the results of biochemical values in all studied patients with normal Hb and low red cell indices:

<table>
<thead>
<tr>
<th></th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Fe = n</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TIBC</td>
<td>TIBC</td>
<td>TIBC</td>
<td>TIBC</td>
<td>TIBC</td>
<td>TIBC</td>
<td>TIBC</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>TS = n</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Low ferritin</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>4.5%</td>
<td></td>
<td></td>
<td>27.3%</td>
<td></td>
<td></td>
<td>4.5%</td>
<td>36.4%</td>
</tr>
<tr>
<td>Normal ferritin</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4.5%</td>
<td>27.3%</td>
<td>4.5%</td>
<td>13.6%</td>
<td>9.1%</td>
<td>4.5%</td>
<td></td>
<td>63.6%</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>9.1%</td>
<td>27.3%</td>
<td>4.5%</td>
<td>40.9%</td>
<td>9.1%</td>
<td>4.5%</td>
<td>4.5%</td>
<td>100%</td>
</tr>
</tbody>
</table>

(Fe=Serum iron, TIBC = total iron binding capacity, TS = transferrin saturation)

**Fig.(27)**

S. iron, TIBC, and TS in relation to S. ferritin in all studied patients with normal Hb and low red cell indices:
3-6-2: Biochemical findings in all studied patients with low Hb and low red cell indices:

Of the 58 patients with low Hb and low red cell indices, 13 (22.4%) patients showed low S. ferritin, 30 (51.7%) had normal S. ferritin and 15 (25.9%) had high S. ferritin (Fig. 28).

Fig.(28)

S. ferritin levels in all studied patient with low Hb and low red cell indices
Table (13): Summary of the results of biochemical values in all studied patients with low Hb and low red cell indices:

<table>
<thead>
<tr>
<th></th>
<th>Fe=n TIBC=n</th>
<th>Fe=n TIBC=↓</th>
<th>Fe=n TIBC=↑</th>
<th>Fe=↓ TIBC=n↑</th>
<th>Fe=↓ TIBC=↓</th>
<th>Fe=↓ TIBC=↑</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low ferritin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(19%)</td>
<td>(1.7%)</td>
<td>(1.7%)</td>
<td>(22.4%)</td>
</tr>
<tr>
<td>Normal ferritin</td>
<td>2 (3.4%)</td>
<td>8 (13.8%)</td>
<td>1 (1.7%)</td>
<td>4 (6.9%)</td>
<td>4</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6.9%)</td>
<td>(19%)</td>
<td>(19%)</td>
<td>(51.7%)</td>
</tr>
<tr>
<td>High ferritin</td>
<td>0</td>
<td>2 (3.4%)</td>
<td>2 (3.4%)</td>
<td>3 (5.2%)</td>
<td>6</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3.4%)</td>
<td>(10.3%)</td>
<td>(25.9%)</td>
<td>(25.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (3.4%)</td>
<td>10 (17.2%)</td>
<td>3 (5.2%)</td>
<td>16 (27.6%)</td>
<td>8</td>
<td>18</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5.2%)</td>
<td>(13.8%)</td>
<td>(31%)</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

(Fe= Serum iron, TIBC = total iron binding capacity, TS= transferring saturation)

Fig.(29)

S. iron, TIBC, and TS in relation to S. ferritin in all studied patients with low Hb and low red cell indices:
Discussion

Since iron deficiency is the commonest cause of low red cell indices, this study was designed to assess iron status in some patients showing low red blood cell indices regardless of their haemoglobin values.

80 patients were included in this study of whom 11 were children, 39 were females, and 30 were males. 58 (72.5%) of the studied patients were anaemic; of these 11 were children, 25 were males and 22 were females, while 22 (27.5%) had normal Hb values of whom 5 were males and 17 were females.

In all the studied patients the MCH values were low, despite the fact that 22 of them were non-anaemic. However, the reduction in MCV and MCHC were variable, where only 26 patients showed low MCV of whom only 2 were non-anaemic, and 48 had low MCHC of whom 9 were non-anaemic. In fact all of the 22 non-anaemic patients (27.5%) showed reduction in one or more of their red blood cell indices. This proves our observation that quite a significant number of results issued by the Sysmex Kx-21 machine show low red cell indices in presence of normal Hb.

The MCH is considered by many researchers as the most sensitive indicator of iron deficiency as far as red blood cell indices are concerned. A previous study reported that low MCH (< 27 pg) was found to be superior to low MCV (< 77 fl) in predicting low S. ferritin values. The occurrence of deficiency of S. ferritin was 90% when MCH was very low (MCH < 23 pg) (Broin SD, et al 1990)\(^{(41)}\). In another study it was reported that at mean MCH > 30 pg the probability of low transferrin saturation was 0.04 for hospitalized anaemic patients and 0.14 for ambulatory anaemic patients while for MCH < 27 pg the corresponding probabilities were much higher being 0.52 and 0.67 respectively (Griner PF et al 1978)\(^{(58)}\). Another study in children reported that, MCH and transferrin saturation (TS) were parameters that showed the highest sensitivity and lowest non-specificity where 97% of the anaemic and non-anaemic children were identified as iron deficient by MCH less than 25 pg and / or TS less than 17.5%, and only 0 to 6% of the children without iron deficiency showed MCH and TS below these values. Also MCH, S. ferritin and TS in a group of mothers were tested with high sensitivity (97%, 88%, and 79% respectively) and it was concluded that MCH seems to be a sensible and specific screening test for detecting possible cases of iron deficiency in both maternal and infant population (Piedras J, et al 1981)\(^{(59)}\).

However other workers claimed that its specificity is much lower being
around 62% as reported by (Baptista–Gnozalez HA et al, 1990)\(^{(40)}\). In our study 26 patients had low MCH values alone of whom 13 were non-anaemic, of these; 18 patients had abnormal iron status while only 8 had normal iron status. So MCH showed 100% sensitivity but only 58% specificity, this is in keeping with the previously mentioned low specificity of MCH.

In contrast to MCH, MCV is considered to be a more specific but less sensitive indicator of iron deficiency as reported by Sedward S J, et al (1990) that MCV was quite specific in identifying patients who had low ferritin levels, specificity was 83% but sensitivity was only 48%. In the same study MCV was also specific (88%) in identifying patients who had low ferritin with elevated TIBC, but the sensitivity was only 43%\(^{(39)}\). In our study no patient had low MCV alone and so we were not able to calculate its sensitivity and specificity.

Combined reduction of red blood cell indices is thought to be more specific. In a previous study it was reported that in severe deficiency of iron stores, the MCH alone showed a minor specificity (0.62) while MCV specificity was (0.88). Simultaneous alteration in MCH and MCV has specificity (0.92) and a positive predictive value (Baptista–Gnozalez HA et al, 1990)\(^{(40)}\). In another study it was reported that the sensitivity of MCV values < 70 fl in iron deficiency anaemia was 90%; specificity was 53.8%. The sensitivity of RDW values ≥ 15% was 83.3% and specificity was 57.7%. The positive predictive value could be increased to 97.8% by combining both and by combining the haemoglobin with MCV and RDW in screening for iron deficiency, the diagnostic accuracy of iron deficiency anaemia can be increased (Kim SK et al, 1996)\(^{(60)}\).

In our patients combined reductions of MCH and MCV were seen in 8 patients of whom only 2 were non-anaemic, of these; 5 patients had abnormal iron status while 3 had normal iron status. The sensitivity was only 8.2% but the specificity was 84.2%. Reduction of MCH and MCHC were seen in 28 of patients of whom 7 were non-anaemic, of these; 21 had abnormal iron status while 7 had normal iron status. The sensitivity was 34.4% but the specificity was 63.1%. The reduction of the three indices were seen in 18 patients all of them were anaemic of these; 17 patients had abnormal iron status but only one patient had normal iron status making its specificity very high being 94.7% but sensitivity was only 27.8% in this group.

MCHC is more frequently reduced in iron deficiency than with other causes of microcytosis, but the drop in MCHC occurs late and the diagnostic utility of this parameter is therefore rather poor (37).
However, previous study has reported that mean values of both MCHC and CHCM (the Technicon H*1 analyzer provides a direct MCHC measurement termed the CHCM) were significantly lower in patients with iron deficiency than in patients with other causes of microcytic anaemia. It was also observed that MCHC was significantly greater than CHCM in patients with iron deficiency anaemia, but not in patients with other causes of microcytic anaemia. Both MCHC and the difference between MCHC and CHCM showed potential value as parameters for the differential diagnosis of iron deficiency anaemia from other causes of microcytic anaemia (Bentley SA, et al 1989) (61).

Iron status assessment in these 80 patients with low red cell indices gave very unexpected results. Only 13 patients (16%) showed classical findings of iron deficiency anaemia in that they had low Hb, low red cell indices, low S. ferritin, low S. iron and low TS but TIBC values however were not elevated in all of them, 4 (31%) had normal TIBC, 7 (54%) had high TIBC and 2 patients (15%) had low TIBC. This is in keeping with the literature reports that TIBC is not elevated above reference limits in 30–40% of patients with chronic iron deficiency anaemia. In another study, 69% of iron deficiency anaemia with low serum iron levels had an elevated TIBC, 11% had a TIBC within reference limits and an additional 21% had decreased TIBC values (47). The figure of 16% is unexpectedly low since all studied patients had at least a low MCH value and some had low MCV and/or low MCHC as well, MCH as mentioned before is a very sensitive marker of iron deficiency.

11 of our studied patients (13.8%), whose Hb values were normal, gave results suggestive of subclinical iron deficiency. 9 of them had a classical findings, namely low S. iron, normal or high TIBC and low TS but S. ferritin being normal in three of them which may be caused by inflammatory conditions in association with their illness. Of the remaining two; one had normal iron status parameters except the low S. ferritin level (deficient store) while the latter had high S. iron and TS values despite of low S. ferritin and normal TIBC, one possibility that this patient may have been taking vitamins containing iron elements which will raise the S. iron and transferrin saturation values or this could be due to technical error in the measurement of S. iron level. It is of course acceptable to find deficient stores and low circulatory iron levels in patients who have not yet developed overt iron deficiency and so these 11 patients will fall in this category. However these patients had low MCH values and so one will have to find an explanation for that. Iron deficiency in polycythaemic patients undergoing repeated venesections can cause such findings but none of these 11 patients was known to be polycythaemic. Another possibility would be that these patients have higher than expected red blood cell count and so the Hb value is not much affected despite the deficient status and
low MCH. This will happen if for example the patient is suffering from bleeding when the bone marrow will try to compensate for the lost red blood cell but not for the lost iron. A third possibility is that the initial Hb of these patients was higher than the present and so at the time of study they had lower Hb values than their normal. The last possibility is that this low MCH is a machine error and so this will mean that the error was actually in either the Hb or the RBC count or in both of them.

RBC count by manual technique is known to be quite inaccurate and it is supposed to be more accurate by automated machines. However still some factors may lead to erroneously high RBC count by automated machine such as recirculation of cells which have already been counted or counting of bubbles, lipid droplets, microorganisms and extraneous particles as cells.

6 (7.5 %) of our anaemic patients showed results of iron profile consistent with iron deficiency except for normal or high S. ferritin levels. We think that these patients are likely to be suffering from inflammatory conditions in association with their iron deficiency. Ferritin is a known acute phase reactant which can be elevated in inflammatory conditions despite iron deficiency.

So if we add up these three groups of patients, the number of patients with iron deficiency will rise to 30 (37.5 %), this figure in our opinion is still low since the commonest cause of low red cell indices is iron deficiency.

In our study, anaemia of chronic disease (ACD) seems to be quite common than iron deficiency, 28 patients (35 %) showed evidence from iron studies of possible ACD in that they had low Hb, low red cell indices, low S. iron, low TIBC, low or normal TS and normal or high S. ferritin values. Only 4 of them had normal S. iron which may be due to lack of reliability of S. iron measurements which are affected by many factors or due to technical errors. The clinical diagnoses of these patients was in keeping with the laboratory findings. A further 3 patients (3.75 %) had normal Hb values despite iron parameters supportive of ACD which poses again a problem of how to justify their normal Hb values and again we will have to use our previously mentioned trials at explaining such a problem. If we add these patients to those with ACD the number of patients with ACD will rise to 31 (38.8 %).

11 of our patients (13.8 %) with low Hb and low red cell indices showed normal iron status except for a low TIBC in 9 of them which may be due to malnutrition or protein deficiency associated with their illness. In fact two of these patients were children diagnosed as a nephrotic syndrome which will explain the low TIBC but in the others we could not prove this from the clinical data available. This group of patients needs further investigation to exclude other causes of microcytic anaemia apart from iron deficiency and ACD. In fact one patient in this group was a child with very
mild anaemia and normal iron status, but interestingly enough he had very low red cell indices which where disproportional to his mild anaemia. Such results are suggestive of a haemoglobinopathy e.g. B-thalassaemia minor. So this child needs further investigation like Hb electrophoresis and Hb A2 estimation.

The real problem we faced was how to explain low indices in presence of normal Hb and normal iron status, this occurred in 8 (10 %) of our studied patients. Mild haemoglobinopathies can cause such findings but they have to be investigated, These findings correspond to what was reported in literature that abnormal indices may point to important disorders in which anaemia may not occur e.g. some cases of the thalassemia trait in which the red cell are microcytic but because of their increased numbers, the haemoglobin concentration in blood is normal(38). Another explanation that should be entertained is the technical machine error. Again in this group of the patients, TIBC was low in 7 of them which may be explained e.g. by protein deficiency or malnutrition associated with their illness although we could not prove this. Another possibility is that the reference values taken in this study ( 250 – 350 ug/dl for women and 300 – 400 ug/dl for men according to the kit from the biosystem company ) may be higher than the truth in our population. Moreover, a lot of reference books reported a reference values less than these e.g. 250 – 350 ug/l for both sex ( William’s textbook of haematology) or 200 – 400 ug/l for both ( essential haematology ). So if we applied these values in our patients, most of them if not all will become within normal ranges. The last possibility is that this low TIBC is a technical error.

Peripheral blood film showed a variable degree of hypochromia with or without microcytosis in all patients ( anaemic & non-anaemic) with abnormal iron status and low red cell indices. Also most of the anaemic patients with normal iron status and low indices showed some degree of hypochromia with or with out microcytosis , only two of them had normal blood picture . However, 6 ( 75%) of non-anaemic patients with normal iron status and low indices showed normal blood picture while only 2 (25%) showed mild hypochromia. So of the 80 studied ( anaemic & non-anaemic) patients; 61 (76.25%) showed abnormal iron status while 19 (23.25 %) patients showed normal iron status .

Of the 58 anaemic patients with low red cell indices, 47 (81 %) showed abnormal iron status. Of these; 19 (32.8 %) showed results consistent with iron deficiency either as obvious iron deficiency anaemia or in association with concurrent inflammation, 28 (48.2 %) showed results suggestive of anaemia of chronic diseases while 11 patients (19 %) showed normal iron status. Some of them may need further investigation to exclude
other causes of hypochromic anaemia apart from iron deficiency and ACD. These findings correspond to what was reported in literature that anaemia of chronic disease is the most frequent anaemia among hospitalized patients (55).

Of the 22 non-anaemic patients with low red cell indices, 14 patients (63.6%) had abnormal iron status, of whom 11 (50%) showed results suggestive of subclinical iron deficiency either as obvious iron deficiency or in association with concurrent inflammation. In 3 patients (13.6%) the findings of iron profile were like the anaemia of chronic diseases, while a significant number 8 patients (36.6%) had normal iron status despite their low indices, a phenomena which needs further investigations before we can blame the machine for it.
Conclusions:

1) Most of the low indices associated with low Hb in our study were due to anaemia of chronic disease.
2) Iron deficiency comes second as a cause of anaemia and low indices in this study.
3) The MCH is more sensitive but less specific indicator of abnormal iron status.
4) Combined reduction of red cell indices is more specific in detection of abnormal iron status.
5) Concurrent inflammation can give erroneous S.ferritin values.
6) Low red cell indices in presence of normal Hb must be carefully interpreted, haemoglobinopathies and polycythaemia should be excluded in such cases.
Recommendations:

7) A study involving a large number of patients is needed.
8) Detection of haemoglobinopathies must be included in future studies.
9) Manual checks of low indices in presence of normal Hb should be done.
10) RDW should be included in future studies.
11) Reference values of iron status parameters, in regarding to age and sex, in our population should be studied in future researches.
12) Reference values of red cell indices in Sudanese population as well as normal values of red cell indices by automation should be established.
13) Proper calibrations of automated cell counter using reference material as well as intra-laboratory control should be encouraging.
14) Peripheral blood film still remains most important part in determinant the type of anaemia.
References


Appendix

Questionnaire

Sample No: __________  Date: __________
Lab. No: __________
Name of patient: _______________
Age: _______  Sex: _______
Tribe: __________  Occupation: _______
Clinical finding:

Haematological finding:

<table>
<thead>
<tr>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
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Blood film:
RBC:

WBC:

Platelets:

Biochemical finding:

<table>
<thead>
<tr>
<th>S. Ferritin (ng/ml)</th>
<th>S. Iron (ug/l)</th>
<th>TIBC (ug/l)</th>
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