Prevalence of Anaemia among Khalawi Students in Wad Magboul Village, Eastern Gezira Locality, Gezira State, Sudan
(2012 – 2013)

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Dedication

I would like to dedicate this work to:

My parents....
My wife and kids....
My lovely family....
Acknowledgement

My deep gratitude to Almighty God Allah for giving me the ability to conduct and complete this study.

I am very grateful to my supervisor, professor Awad El Seed Mustafa, for his fatherly guidance and patience during the course of this study. His invaluable comments, advice and encouragement were extending beyond the limit of this work.

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بسم الله الرحمن الرحيم

انتشار فقر الدم وسط طلاب خلاوي ود المقبول – منطقة رفاعة الريفية – الجزيرة – وسط السودان في الفترة ما بين ديسمبر 2012 إلى يوليو 2013

محمد سعيد السمنى الشيخ الطيب

أجرت هذه الدراسة التحليلية القطعية، التحليلية المجتمعية خلاوي قريه ود المقبول مدينة 15كلم في الفترة ما بين 17/12/2012 إلى 20/07/2013 واستهدفت الدراسة 180 للمشاركين، تم أخذ 4.5 من الدم من كل مشارك حيث وضعت 2.5 مل من الكمية المأخوذة في أتربة بوا مادة منع للتجلط ثم حلت بواسطة جهاز فحص الدم الكامل لمعرفة معدلات مكونات الدم (كريات الدم الحمراء، البيضاء و الصفائح الدموية). وضع المنتقم من الكمية المأخوذة (2مل) في أتربة ليس بها مواد منع للتجلط لقياس نسبة الفيبرين. تم إجراء فحص البول والبراز العمومي. حللت الدراسة إحصائيا بإيجاد مربع كاي وكان متوسط مستوي الهيموغلوبين للفئة المستهدفة 11.75 السيلينتر و قورنت النتائج مع متوسط قيمة مستوي الهيموغلوبين المرجعية (13.5 السيلينتر) حيث كانت القيمة الإحصائية (0.000) مما يدل على أن هناك فرق معنوي ذو دالة إحصائية. أيضاً قامت مقارنة مستوي الفيبرين مع نوع الغذاء المتناول وأيضاً كانت القيمة الإحصائية (0.000) مما يدل أيضا على أن هناك فرق معنوي ذو دالة إحصائية. لوحظ انخفاض واضح في نسبة الفيبرين إلى درجة إعدادها في بعض العينات مما يعكس حالة عزز الحديد في هذه العينات. و طبقاً لعينات التي لوحظ فيها انخفاض نسبة الفيبرين، فإنه عند إجراء الفحص المجهري لمسحات الشرايين المطابقة لها، قد لوحظ انخفاض تركيز خضاب الدم (الهيموغلوبين)، الحجم النووي المتوسط والهيموغلوبين النووي المتوسط لكريات الدم الحمراء مصحوباً بإرتفاع الصفائح الدموية وهذا ربما يكون نتيجة لفقر الدم بسبب نقص الحديد الذي يصاحب عادة ارتفاع في عدد الصفائح الدموية.

عدد الكريات (الخلايا) الشبكية في عدد من العينات (38 عينة)، فقد يكون بسبب الإصابة بالملاريا، البلهارسيا وبعض أنواع فقر الدم التحليلي. خلصت الدراسة إلى ارتفاع نسبة الإصابة بفقر الدم بسبب عوز الحديد نتيجة لفقر مستوي الحديد في الغذاء المتناول. وكذلك نتيجة للإصابة ببعض الأمراض الطفيلية مثل الملاريا البلهارسيا. أوصت الدراسة بتحسين نوعية الغذاء للفئة المستهدفة حيث الإكتاز من الأطعمة الغنية بالحديد.

أيضاً أوصت الدراسة بإجراء دراسات أخرى تضمن عدد من الخلاوي.
Prevalence of Anaemia among Khalawi Students in Wad Magboul Village, Eastern Gezira Locality, Gezira State, Sudan

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ABSTRACT

This is prospective cross – sectional analytical community based study. It was carried out with the aim of evaluating the prevalence of anaemia among quranic schoolchildren in khalawi Wad EL Magboul village, rural Rufaa, Gezira State, central Sudan. Venous blood samples weretaken from 180 male participants to measure the hematological parameters (white blood cells, red blood cells, hemoglobin concentration, packed cell volume, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, red cell distribution width and platelets) using an automated cell counter (sysmex KN21), accompanied by peripheral blood films, for blood cell morphology and reticulocyte counts were assessed to detect any haematological abnormalities. Serum ferritin was determined, blood films for malaria, urine and stool analysis were done. The study was statistically analyzed using SPSS. The mean Hb value was 11.75g/dl and there was significant correlation when compared with the mean Hb reference value (13.5g/dl) P value 0,000. 95% confidence interval of the difference. Also there was significant correlation between type of diet and low serum ferritin levels, Chi square test was 1.57, P value = 0.005. In this study it was found that a considerable numbers of participants were anaemic, few of them were leucopenic and very few were thrombocytopenic (may be due to malaria parasite). A considerable numbers with thrombocytosis accompanied by low MCV, MCH and low reticulocyte counts, most probably due to iron deficiency anaemia. High eosinophil percentage in a number of cases also were (due to schistosomiasis). High reticulocyte count was detected in some cases which might be due to malaria, bilharzia and haemolytic anaemias. The study concluded that the majority of the study group subjects were prone to iron deficiency anaemia, followed by haemolytic, then macrocytic and sickle cell anaemia. The study recommend better diet with high iron content, and more studies to be carried and more interference by health education and. other interventional methods. Supportive donation from philanthropists and Islamic endowments are highly recommended.
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List of Abbreviation

ALAS: Aminolaviolenic acid synthase.
ATP: Adenosine triphosphate.
ACD: Anaemia of chronic disease.
AIHA: Autoimmune haemolytic anaemia.
BFU- E: Burst forming unit – erythroid.
BMPs: Bone morphogenic proteins.
CBCs: Red blood cells.

CFU – GEMM: Colony forming unit- granulocyte erythroid, monocyte and megakaryocyte.

CFU- E: Colony forming unit- erythroid.
CD: Cluster differentiation.
CFU- E/Mk: Colony forming unit- erythroid/ megakaryocyte.
CBC: Complete blood count.
DNA: Deoxyribonucleic acid.
DMT-1: Divalent metal transporter-1.
DCytb1: Duodenal cytochrome b.
EPO: Erythropoietin.
EKLF: Erythroid Kruppel-like factor.
EPOR: Erythropoietin receptor.
FOG: Friend of GATA.
F$^{+2}$: Ferrous iron.
F$^{+3}$: Ferric iron.
Fl: Fimtolitre.
GATA: Guanine, Adenine, Thiamine.
G-CSF: Granulocyte colony stimulating factor.
G6PD: Glucose – 6 phosphate dehydrogenase.
GDF: Growth differentiation factor.
HSCs: Haematopoietic stem cells.
HIF: Hypoxia inducible factor.
HRI: Haem regulated inhibitor.
Hb: Haemoglobin.
HJV: Hemojovulin.
IRP: Iron regulatory proteins.
IREs: Iron response elements.
IDA: Iron deficiency anaemia.
JAK: Janus associated kinase.
mRNA: Messenger RNA.
MCV: Mean cell volume.
MCH: Mean cell haemoglobin.
MCHC: Mean cell haemoglobin concentration.
L: Miceo-litre.
NADH: Nicotinamide adenine dinucleotide.
NADPH: Nicotinamide adenine dinucleotide phosphate.
PAGE: Polyacrylamide gel electrophoresis.
PNH: Paroxysmal nocturnal haemoglobinuria.
PCV: Packed cell volume.
Plts: Platelets.
Pg: Picogram.
RNA: Ribonucleic acid.
RDW: Red cell distribution.
SCA: Sickle cell anaemia.
SH: Sulphhydryl groups.
SPSS: Statistical software package of social science.
STAT: Signal transducer and activator of transcription.
TF: Transferrin.
TFR: Transferrin receptor.
TGF: Transforming growth factor.
TIBC: Total iron binding capacity.
TMPRSS6: Matriptase.
TNF: Tumor necrosis factor.
2-3 DPG: 2-3 diphosphoglycerate.
TWSG-1: Twisted gastrulation protein.
VEGF: Vascular endothelial growth factor.
WBCs: White blood cells.
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Chapter One

Introduction and Literature Review

1.1. Introduction:

Khalawi are traditional religious schools, they are very popular in Sudan. The Khalawi (Quran Schools) and Maseeds (Mosques) played and are still playing great roles in designing the consciences of the people of Sudan. They were the available institutes for teaching the people the teachings of Islam. So they were the places where the people got their education. Most of them were established by clergy and charitable persons. There is another characteristic distinguishing those institutes. Most of them were established in remote and isolated spots of land where no one wanted to live. The idea behind that choice was that those pious men wanted quiet and calm areas so that they could devote most of their time for worshiping and learning. Now cities have grown up around those remote and holy places. (1)

There is no fixed period or duration for the study in khalwa, although the period usually ranges between six and eight years, since the enrollment of the child. Children as young as five years may be accepted. (2)

Khalawi funding depends mainly on Islamic endowments. Also they receive donations from philanthropists who believe in the role of being alone in maintaining the book of God in the hearts of the people of Sudan. The students or heiran are involved mainly in the preparation of food from the grain flour making a porridge-like preparation by boiling named Assida. Then a navigator is made from lentis and beans. (2)

Heiran are also involved in agriculture and preparing the fields, harvest, and storage of the harvest. Students live in hostels or Khalawi attached to almasid, they are usually living separately from young adults.
The duration of the stay student ranges from seven to fifteen years. He has to study every day from dawn until noon, and sometimes continue to study the evening. On finishing his studies, he is given a certificate, and sometimes allowed to open a retreat in which he helps students in the same approach taken by his teachers who taught in the same Khalawi. There is some sort of corporal punishment for neglectant students. (2)
1.2. Literature review.

1.2.1. Hematopoiesis and the Bone Marrow:

Hematopoiesis is the process of making blood cells. The term comes from the Greek haima (blood) and poiein (to make). For the average adult, the bone marrow produces \( \sim 5 \times 10^{11} \) cells per day. Production of blood cells is highly regulated and balanced. \(^{(3)}\)

1.2.2. Haematopoiesis of embryology:

In the first few weeks of gestation the yolk sac is the main site of haemopoiesis. However, definitive haemopoiesis derives from a population of stem cells first observed on the dorsal aorta termed the AGM (aorta-gonads-mesonephros) region. These common precursors of endothelial and haemopoietic cells (haemangioblasts) are believed to seed the liver, spleen and bone marrow and from 6 weeks, the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth. The bone marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses and mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation. \(^{(4)}\)

1.2.3. Postnatal haematopoiesis:

In infancy all the bone marrow is haemopoietic (intramedullary haemopoiesis) but during childhood there is progressive fatty replacement of marrow throughout the long bones so that in adult life haemopoietic marrow is confined to the central skeleton and proximal ends of the femurs and humeri. Even in these haemopoietic areas, approximately 50% of the marrow consists of fat. The remaining fatty marrow is capable of reversion to haemopoiesis and in many diseases there is also expansion of haemopoiesis down the long bones. Moreover, the liver and spleen can resume their fetal haemopoietic role ('extramedullary haemopoiesis'). \(^{(4)}\)
During infancy and childhood, there is active hematopoiesis in the medullary cavity of virtually every bone. With age, the hematopoietically active marrow (red marrow) is gradually replaced by inactive marrow (yellow marrow), which consists predominantly of adipose tissue.\(^{(4)}\)

In adults, hematopoiesis is restricted to the proximal long bones and the axial skeleton (skull, vertebral bodies, ribs, sternum, and pelvis). The yellow marrow can resume active hematopoiesis under conditions of chronic hematologic stress (chronic bleeding or hemolytic anemia).\(^{(3)}\)

### 1.2.4. Production of specific cell lines:

- **Erythrocyte Production (Erythropoiesis):**

  The process of erythropoiesis includes all steps of haemopoiesis, starting with the initial specification of haemopoietic stem cells (HSCs) from mesoderm during embryogenesis.

  HSCs either undergo self-renewal or, through the process of lineage specification, differentiate and proliferate to form committed erythroid progenitors.\(^{(5)}\)

  Many other cytokines, growth factors and hormones also influence erythroid proliferation, differentiation and maturation. Finally, they undergo terminal differentiation through a series of erythroblastic maturation stages to develop into red blood cells.\(^{(5)}\)

### 1.2.5. Differentiation of HSCs to form erythroid progenitors:

In human adult bone marrow, approximately 1 per \(10^4 – 10^6\) nucleated cells are long-lived, multipotent HSCs that can be enriched on the basis of their cell-surface markers (e.g. CD33\(^+\) and CD34\(^+\)) and lack of lineage-specific markers, but such markers do not exclusively select stem cells. The pathway of differentiation from HSCs to committed erythroid progenitors is still the topic of some debate. One model (proposed by Weissman) posits a common myeloid progenitor from which the granulocyte/monocyte, erythroid and megakaryocyte
lineages develop. In a second model (Jacobsen) erythroid/megakaryocytic progenitors split before the separation of lymphoid and granulocyte/monocyte lineages. (5)

Erythroid cells can be found in multilineage colonies (CFU -GEMM), which include granulocytes, macrophages and megakaryocytes, and in bipotential colonies with megakaryocytes (CFU - E/Mk). (5)

Erythroid differentiation and maturation within the adult bone marrow in vivo is dependent on the microenvironment provided by the stromal cells (fibroblasts, fat cells, endothelial cells, macrophages and smooth muscle cells). There are also immunoregulatory cells (monocytes, macrophages and lymphocytes) that contribute to local cytokine production.

Erythroblasts are not randomly distributed in the bone marrow but are organized into erythroblastic islands containing one or two central macrophages, surrounded by layers of erythroblasts at different stages of maturation. (5)

1.2.6. The transcription factor programme underlying erythropoiesis:

At present, the key transcription factors known to be involved in specifying HSCs as they develop during embryogenesis and in maintaining them throughout life include Runx1 (AML - 1), SCL (tal - 1), LMO2 (rhombotin), Tel (ETV6), MLL and GATA -2.

Once progenitor cells have been committed to become erythroid cells, the most important transcription factors that enable them to proceed through terminal differentiation are GATA – 1 and its cofactor FOG - 1 (friend of GATA - 1). GATA -1 was first identified by its ability to bind functionally important regulatory sequences in the globin genes. Since then, GATA – binding motifs have been found in the promoters and/or enhancers of virtually all erythroid - specific genes studied, including haem biosynthetic enzymes, red cell membrane proteins
(including blood group antigens) and erythroid transcription factors such as erythroid Kruppel-like factor (EKLF) and GATA-1 itself.\(^5\)

GATA-1 may protect mature erythroblasts from apoptosis by directly or indirectly inducing expression of the anti–apoptotic protein Bcl-XL. GATA-1 almost certainly regulates gene expression working as part of multiprotein complexes interacting, for example, with FOG-1, LMO2, SCL and a variety of ubiquitously expressed transcription factors. FOG-1 is a protein containing multiple zinc fingers, four of which interact with GATA-1.\(^5\)

**1.2.7. Terminal maturation of committed erythroid cells:**

After the erythroid programme has been specified, the final phase of erythropoiesis involves the maturation of committed erythroid progenitors to fully differentiated red cells.\(^5\)

Erythroid precursors are derived from the CFU-GEMM. The earliest progenitor committed exclusively to erythroid lineage is the burst-forming unit–erythroid (BFU-E); this is followed by the colony-forming unit–erythroid (CFU-E). The earliest recognizable RBC precursor is the proerythroblast, which is characterized by fine nuclear chromatin and intensely blue cytoplasm.\(^3\)

The last nucleated RBC precursor is the orthochromatophilic erythroblast, which is characterized by well-hemoglobinized cytoplasm; the nucleus is then lost, producing the reticulocyte. Reticulocytes contain ribonucleic acid (RNA) for 4 days; normally, the first 3 days are spent in the marrow and fourth in the blood. However, under intense stimulation by erythropoietin, reticulocytes may be released into the blood early where they may contain RNA for 2.0 to 2.5 days (shift reticulocytes).\(^3\)

It has been estimated that, on average, four divisions occur within the morphologically recognizable proliferating precursor pool, so that each newly formed pronormoblast develops into 16 red cells. As a small amount of cell death
(ineffective erythropoiesis) normally occurs, the average amplification is slightly less than 16 - fold. (5)

1.2.8. The regulation of erythropoiesis by signaling pathways:

As committed erythroid cells become late BFU - E and CFU - E, they upregulate expression of the receptor for erythropoietin (EpoR). It is estimated that in the steady state, with low levels of circulating Epo, a high proportion of erythroid cells die through apoptosis. This provides a reserve that can be rescued by the increase in Epo levels that accompany anaemia. Signalling through EpoR not only prevents apoptosis but also stimulates proliferation. It is at the late progenitor/early precursor stages (CFU - E/pronormoblast) that there is considerable proliferative potential for expanding the overall level of erythropoiesis. Soon after reaching the CFU - E stage, erythroid cells enter the phase of terminal differentiation, after which there is only limited potential for further expansion. The two major components regulating erythropoiesis include sensing hypoxia (through hypoxia inducible factors HIF) and regulating the supply of erythroid precursors, via the Epo – EpoR signaling pathway. (5)

1.2.9. Haemoglobin:

Hemoglobin is a protein that serves as a carrier of oxygen from the lungs to the tissues. To work properly, the hemoglobin has to hold on to oxygen molecules with just the right amount of force. If the hemoglobin molecule binds the oxygen molecules too loosely, then it will not be capable of picking them up at the lungs. If it binds the oxygen too tightly, then when it gets out to the tissues it will not release the oxygen to the tissues that need it. (3) Normal adult blood contains three types of haemoglobin. The major component is haemoglobin A with the molecular structure $\alpha_2\beta_2$. The minor haemoglobins contain $\gamma$ (fetal Hb or HbF) or $\delta$ (Hb A$_2$) globin chains instead of $\beta$ chains. In the embryo and fetus, Gower 1, Portland, Gower 2 and fetal Hb dominate at different stages. The genes for the globin chains occur in two clusters: $\varepsilon, \gamma, \delta$ and $\beta$ on chromosome 11 and $\zeta$ and $\alpha$ on chromosome 16. Two types of $\gamma$ chain occur, G $\gamma$ and A $\gamma$ which differ by a glycine or alanine amino acid
at position 136 in the polypeptide chain. The α-chain gene is duplicated and both α genes (α1, and α2) on each chromosome are active. (4)

All the globin genes have three exons (coding regions) and two introns (non-coding regions whose DNA is not represented in the finished protein). The initial RNA is transcribed from both introns and exons, and from this transcript the RNA derived from introns is removed by a process known as splicing. (Figure 1.1). (4)

The introns always begin with a G-T dinucleotide and end with an A-G dinucleotide. The splicing machinery recognizes these sequences as well as neighbouring conserved sequences. The RNA in the nucleus is also 'capped' by addition of a structure at the 5’ end which contains a seven methylguanosine group. The cap structure may be important for attachment of the mRNA to ribosomes. The newly formed mRNA is also polyadenylated at the 3’ end, this stabilizes it. Thalassaemia may arise from mutations or deletions of any of these sequences. (4)
Figure (1.1): The expression of a human globin gene from transcription, excision of introns, splicing of exons and translation to ribosomes. (A.V. Hoffbrand. Essential haematology)

A number of other conserved sequences are important in globin synthesis and mutations at these sites may also give rise to thalassaemia. These sequences influence gene transcription, ensure its fidelity, specify sites for the initiation and termination of translation, and ensure the stability of newly synthesized mRNA. Promoters are found 5’ of the gene, either close to the initiation site or more distally. They are the sites where RNA polymerases bind and catalyse gene transcription. Enhancers occur either 5’ or 3’ to the gene. Enhancers are important in the tissue-specific regulation of globin gene expression and in regulation of the synthesis of the various globin chains during fetal and postnatal life. The locus control region (LCR) is a genetic regulatory element, situated a long way upstream of the β-globin cluster, that controls the genetic activity of each domain, probably
by physically interacting with the promoter region and opening up the chromatin to allow transcription factors to bind. The a-globin gene cluster also contains an LCR-like region termed HS40. GATA-1, FOG and NF-E2 transcription factors, expressed mainly in erythroid precursors, are important in determining the expression of globin genes in erythroid cells. Globin mRNA enters the cytoplasm and attaches to ribosomes (translation) where the synthesis of globin chains takes place. \(^{(4)}\)

**1.2.9.1. Switch from fetal to adult haemoglobin:**

The globin genes are arranged on chromosomes 11 and 16 in the order in which they are expressed. Certain embryonic haemoglobins are usually only expressed in yolk sac erythroblasts. The β-globin gene is expressed at a low level in early fetal life, but the main switch to adult haemoglobin occurs 3-6 months after birth when synthesis of the γ chain is largely replaced by the β chain. How this switch comes about is largely unknown. However, it is clear that the methylation state of the gene (expressed genes tend to be hypomethylated, non-expressed hypermethylated), the state of the chromosome packaging and various enhancer sequences all play a part in determining whether a particular gene will be transcribed. \(^{(4)}\)

The main purpose of erythropoiesis is to synthesize large amounts of haemoglobin. Globin mRNA sequences are first expressed in pronormoblasts and early basophilic erythroblasts. Globin chain synthesis parallels accumulation of globin mRNA, increasing at the polychromatic and orthochromatic stages. \(^{(3)}\)

The individual components of the haemoglobin synthetic pathway (iron, free porphyrins, haem and monomeric globin chains) are all extremely toxic to the cell, and consequently many positive and negative feedback loops have evolved and been incorporated into this process. The synthesis of globin must be very accurately matched with the synthesis of haem in which some steps occur in the cytoplasm and others in the mitochondria. \(^{(3)}\)
The discovery of hepcidin, which controls the uptake of iron from the gut, iron transport across the placenta and iron release from macrophages, adds another level of control to this complex system. With no DNA-containing nucleus, the erythrocyte cannot reproduce itself or program itself to adapt to various challenges by synthesizing new proteins. With no mitochondria, it cannot generate the large amount of energy enjoyed by almost all other cells of the body. (3)

1.2.10. The red cell:

In order to carry haemoglobin into close contact with the tissues and for successful gaseous exchange, the red cell, 8 μm in diameter, must be able: to pass repeatedly through the microcirculation whose minimum diameter is 3.5 μm, to maintain haemoglobin in a reduced (ferrous) state and to maintain osmotic equilibrium despite the high concentration of protein (haemoglobin) in the cell. (4)

Its total journey throughout its 120-day lifespan has been estimated to be 480 km (300 miles). To fulfill these functions, the cell is a flexible biconcave disc with an ability to generate energy as adenosine triphosphate (ATP) by the anaerobic glycolytic (Embden-Meyerhof) pathway and to generate reducing power as NADH by this pathway and as reduced nicotinamide adenine dinucleotide phosphate (NADPH) by the hexose monophosphate shunt. (4)

1.2.10.1. Red cell metabolism: Embden-Meyerhof pathway:

In this series of biochemical reactions, glucose that enters the red cell from plasma by facilitated transfer is metabolized to lactate. For each molecule of glucose used, two molecules of ATP thus two high-energy phosphate bonds are generated. This ATP provides energy for maintenance of red cell volume, shape and flexibility. The red cell has an osmotic pressure five times that of plasma and an inherent weakness of the membrane results in continual Na⁺ and K⁺ movement. (4)

A membrane ATPase sodium pump is needed, and this uses one molecule of ATP to move three sodium ions out and two potassium ions into the cell. The Embden-Meyerhof pathway also generates NADH which is needed by the enzyme
methaemoglobin reductase to reduce functionally dead methaemoglobin (oxidized haemoglobin) containing ferric iron (produced by oxidation of approximately 3% of haemoglobin each day) to functionally active, reduced haemoglobin. \(^{(4)}\)

The Luebering Rapoport shunt, or side arm, of this pathway generates 2,3-DPG which forms a 1:1 complex with haemoglobin and, as mentioned above, is important in the regulation of haemoglobin's oxygen affinity. \(^{(4)}\)

### 1.2.10.2. Hexose monophosphate (pentose phosphate) Pathway:

Approximately 10% of glycolysis occurs by this oxidative pathway in which glucose-6-phosphate is converted to 6-phosphogluconate and so to ribulose-5-phosphate. NADPH is generated and is linked with glutathione which maintains sulphhydril (SH) groups intact in the cell including those in haemoglobin and the red cell membrane. NADPH is also used by another methaemoglobin reductase to maintain haemoglobin iron in the functionally active Fe\(^{2+}\) state. \(^{(4)}\)

In one of the most common inherited abnormalities of red cells is glucose-6-phosphate dehydrogenase (G6PD) deficiency, the red cells are extremely susceptible to oxidant stress. \(^{(4)}\)

### 1.2.11. Red cell membrane:

The red cell membrane comprises a lipid bilayer, integral membrane proteins and a membrane skeleton. Approximately 50% of the membrane is protein, 20% phospholipids, 20% cholesterol molecules and up to 10% is carbohydrate. Carbohydrates occur only on the external surface while proteins are either peripheral or integral, penetrating the lipid bilayer. Several red cell proteins have been numbered accordingly to their mobility on polyacrylamide gel electrophoresis (PAGE), e.g. Band 3, proteins 4.1, 4.2. The membrane skeleton is formed by structural proteins that include α and β spectrin, ankyrin, protein 4.1 and actin. These proteins form a horizontal lattice on the internal side of the red cell membrane and are important in maintaining the biconcave shape. Spectrin is the most abundant and consists of two chains, α and β, wound around each other to
form heterodimers which then self-associate head to head to form tetramers. These tetramers are linked at the tail end to actin and are attached to protein band 4.1. At the head end, the β spectrin chains attach to ankyrin which connects to band 3, the trans membrane protein that acts as an anion channel (‘vertical connections’). Protein 4.2 enhances this interaction, figure (1.2). (4)

**Figure (1.2): The structure of the red cell membrane.** (A.V Hoffbrand. Essential haematology)

Defects of the proteins may explain some of the abnormalities of shape of the red cell membrane (e.g. hereditary spherocytosis and elliptocytosis) while alterations in lipid composition because of congenital or acquired abnormalities in plasma cholesterol or phospholipid may be associated with other membrane abnormalities. For instance, an increase in cholesterol and phospholipid has been suggested as one cause of target cells whereas a large selective increase in cholesterol may cause acanthocyte formation. (4)
1.2.12. Anaemia:-

1.2.12.1. Definition:-

Anaemia is defined as a reduction in the haemoglobin concentration of the blood below normal for age and sex. It is the most common disorder of the blood. However, it can include decreased oxygen binding ability of each haemoglobin molecule due to deformity or lack in numerical development as in some other types of haemoglobin deficiency. (4)

Because haemoglobin normally carries oxygen from the lungs to the tissues, anaemia leads to hypoxia in organs. Since all human cells depend on oxygen for survival, varying degrees of anaemia can have a wide range of clinical consequences. (4)

Anemia is a common hematologic problem in adults and children. A systematic approach to the patient with anemia can rapidly lead to the appropriate diagnosis of the most common entities with minimal diagnostic testing. It also facilitates the specialized investigation necessary to identify some of the less commonly encountered congenital and acquired anemias. (6)

The word "anemia" is composed of two Greek roots that together mean "without blood," but to use this literal translation as a definition would be a gross exaggeration. Still, the modern definition is simple: anemia is any condition characterized by an abnormal decrease in the body's total red blood cell mass. (3)

1.2.12.2. How the body adapts to anaemia:-

In anemia, the cardiac output increases, and that allows more hemoglobin to be exposed to the peripheral tissues, making up for the decreased hemoglobin concentration. Accordingly, the heart rate increases, which gives us one of the cardinal clinical manifestations of anemia, tachycardia, or fast heart rate. (3)

The various organs of the body are quite capable of cutting deals among themselves when times are bad (redistribution of blood flow). In the case of
anemia, all the organs conjoin to protect the two most oxygen-demanding organs in the body, the brain and the heart. If these organs don't get enough oxygen, the rest of the body is in real trouble. Fortunately, the skin can get by without nearly as much blood as it normally enjoy in good times, and as a response to anemia, small blood vessels in the skin contract, causing a greater resistance to the flow of blood than is present in more vital organs. The diversion of blood flow from the skin causes one of the cardinal clinical features of anemia--pallor. \(^{(3)}\)

There is a simple organic acid, called 2,3-diphosphoglycerate (2,3-DPG) that is elaborated within the red cell under anemic conditions, it causes hemoglobin to bind oxygen less avidly (decrease of hemoglobin-oxygen affinity), and to give it up as much to the starved tissues as possible. \(^{(3)}\)

### 1.2.12.3 Anaemia as a public health problem:–

Anaemia is a public health problem that affects populations in both developing and developed countries. Its primary cause is iron deficiency, but a number of other conditions, such as malaria, parasitic infection, other nutritional deficiencies, and haemoglobinopathies are also responsible, often in combination. It occurs with major consequences for human health as well as social and economic development, and at all stages of the life cycle. The WHO global database on anaemia can be used to describe the nutritional status of populations and to identify the needs for interventions to prevent and control anaemia. The indicator used is haemoglobin concentration. \(^{(7)}\)

### 1.2.12.4 Assessing anaemia:–

Anaemia is an indicator of both poor nutrition and poor health. Hb concentration is the most reliable indicator of anaemia at the population level, as opposed to clinical measures which are subjective and therefore have more room for error. Measuring Hb is frequently used as a proxy indicator of iron deficiency. In addition, in populations, where the prevalence of inherited haemoglobinopathies is high, the mean level of Hb is lower.
The main objective for assessing anaemia is to inform decision-makers on the type of measures to be taken to prevent and control anaemia.\(^7\)

### 1.2.12.5. Mechanisms of Anaemia causation:

A general understanding of the different mechanisms of anemia and representative diagnostic entities facilitates appropriate diagnosis. In the setting of acute or chronic blood loss, increased red blood cell production occurs in response to erythropoietin, provided that adequate nutrients are present for the formation of new cells.\(^6\)

Aside from hemorrhage, anemias can be categorized as either hypoproliferative or hemolytic (hyperproliferative). Hypoproliferative anemias are due to impaired red blood cell production and often result from acquired nutritional deficiencies or systemic diseases. Hemolytic anemias may be either congenital or acquired, the former being more common in children and the latter in adults, and are a consequence of the premature destruction of erythrocytes.\(^6\)

### 1.2.12.6. Classification of Anaemia:

Anemia can be approached from five perspectives: morphologic, pathophysiologic (also called functional or kinetic), heme synthesis defect, globin synthesis defect and sideroblastic defect.\(^10\)

#### 1.2.12.6.1. Morphologic Approach:

The initial distinction is based on the red cell size; anaemias are classified as:

- Microcytic (iron deficiency anaemia, thalassemia, sideroblastic anaemia and anaemia of chronic disease-severe cases).
- Normocytic (anaemia of chronic disease-most cases, anaemia of renal disease, combined nutritional deficiency: iron plus folate or cobalamin, marrow failure and hypothyroidism).
Macrocytic (megaloblastic anaemia-folate or cobalamin deficiency, haemolytic anaemia-reticulocytosis, liver disease, hypothyroidism, myelodysplasia). \(^{10}\)

1.2.12.6.2. **Pathophysiologic (Functional or Kinetic) Approach:**

The pathophysiologic approach is based primarily on the reticulocyte count. Anaemias are classified into three broad categories:

- **Hypoproliferative anemias:** (anaemia of chronic disease), marrow damage (stem cell damage by chemotherapy, drugs, chemicals, infections and aplastic anaemia), structural damage {radiation, metastatic malignancies, myelofibrosis).
- **Maturation defects:** nuclear: {megaloblastic anemia (B12 or folate deficiency), intrinsic marrow disease: myelodysplasia }, and cytoplasmic (severe iron deficiency, thalassemia and sideroblastic anaemia).
- **Hyperproliferative anaeimias:** (acute blood loss, acute hemolysis {intra- and extravascular}, chronic hemolysis {environmental disorders, membrane defects, metabolic defects, haemoglobinopathies, paroxysmal nocturnal haemoglobinuria-PNH}). \(^{10}\)

1.2.12.6.3. **Heme synthesis defect:**

- Iron deficiency anaemia.
- Anaemia of chronic disease (more commonly presenting as normocytic Anaemia).

1.2.12.6.4. **Globin synthesis defect:**

- Alpha -, and beta- thalassemia.
- Hb S syndrome.
- Hb E syndrome.
- Hb C syndrome.
- And various other unstable haemoglobin diseases.
1.2.12.6.5. Sideroblastic defect:-

- Hereditary Sideroblastic anaemia.
- Acquired Sideroblastic anaemia including lead toxicity.
- Reversible Sideroblastic anaemia. (9)

1.2.13. Hypochromic anaemias:

Hypochromic anemia is the commonest type of anemia encountered in family practice. Although iron deficiency is by far the most common cause, it cannot be readily distinguished from hypochromic anemia due to other causes (thalassemia, secondary anemia and sideroblastic anemia) without knowing the state of the tissue iron stores. (8)

1.2.14. Iron deficiency:

For the plant disorder also known as "lime-induced chlorosis", Iron deficiency (sideropenia or hypoferremia) is one of the most common forms of the nutritional deficiencies. Iron is present in all cells in the human body, and has several vital functions. Examples include as a carrier of oxygen to the tissues from the lungs in the form of hemoglobin, as a transport medium for electrons within the cells in the form of cytochromes, and as an integral part of enzyme reactions in various tissues. Too little iron can interfere with these vital functions and lead to morbidity and death. The eventual consequence of iron deficiency is iron deficiency anemia where the body's stores of iron have been depleted and the body is unable to maintain levels of haemoglobin in the blood. Children and pre-menopausal women are the groups most prone to the disease. (3)

Total body iron averages approximately 3.8 g in men and 2.3 g in women. In blood plasma, iron is carried tightly bound to the protein transferrin. There are several mechanisms that control human iron metabolism and safeguard against iron deficiency. The main regulatory mechanism is situated in the gastrointestinal tract. When loss of iron is not sufficiently compensated by adequate intake of iron from
the diet, a state of iron deficiency develops over time. When this state is uncorrected, it leads to iron deficiency anemia. (3)

Iron (atomic weight 55.85) is essential for many metabolic processes. It shares with other transition metals two properties of particular importance in biology: the ability to exist in more than one relatively stable oxidation state and the ability to form many complexes. Its ability to exist in both ferric and ferrous states underlies its role in critical enzyme reactions concerned with oxygen and electron transport and the cellular production of energy. (5)

1.2.14.1. Distribution of body iron:

The concentration of iron in the adult human body is normally about 50 mg/kg in males and 40 mg/kg in females. The largest component is circulating haemoglobin, with 450 mL (1 unit) of whole blood containing about 200 mg of iron. Much of the remainder is contained in the storage proteins ferritin and haemosiderin. These are found mainly in the reticuloendothelial 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)

1.2.14.2. Proteins important in iron metabolism:

A- Haemoglobin & Myoglobin.
B- Mitochondrial haem and non - haem iron proteins.
C- Ferritin and haemosiderin.
E- Transferrin and transferrin receptor.
F- Divalent metal transporter 1.
G- Ferroportin (SLC40A1).
H- Growth differentiation factor and twisted gastrulation protein.
I- Hepcidin.
J- Matriptase - 2 (TMPRSS6).

1.2.14.3. Intracellular iron homeostasis:

Synthesis of several of the proteins involved in iron metabolism is regulated at the level of RNA translation by two cytoplasmic iron-dependent proteins, namely iron regulatory protein (IRP1) and IRP2. These are capable of binding to mRNAs that contain a sequence forming a stem-loop structure called an iron-responsive element or IRE. (5)

The levels of ferritin and TfR1 are linked to iron status so that iron overload causes a rise in tissue ferritin and a fall in TfR1, whereas in iron deficiency ferritin is low and TfR1 increased. This linkage arises through the binding of an iron regulatory protein (IRP) to iron response elements (IREs) on the ferritin and TfR1 mRNA molecules. Iron deficiency increases the ability of IRP to bind to the IREs whereas iron overload reduces the binding. (4)

1.2.14.4. Normal iron balance:

The amount of iron in the body at birth depends on the blood volume and haemoglobin concentration, the birth weight (which determines blood volume) being particularly important. Delay in clamping the cord leads to an increased red cell mass by placental transfusion. The level of maternal iron stores has little effect on fetal iron. The newborn contains about 80mg/kg at full term. Neonatal iron reserves are utilized for growth, and from 6 months to 2 years virtually no iron stores are present. Thereafter, iron stores gradually accumulate during childhood to around 5mg/kg. In men, there is a further increase between 15 and 30 years to about 10 – 12 mg/kg (total up to approximately 1 g), whereas iron stores remain lower in women (average 300 mg) until the menopause. It would take 4 years or more for a man to deplete body iron stores and start developing iron deficiency anaemia solely due to lack of dietary intake or malabsorption. Requirements are higher in menstruating women and during periods of rapid growth in infancy and adolescence. (5)
1.2.14.5. Iron absorption:

Iron absorption depends not only on the amount of iron in the diet but also, and more importantly, on the bioavailability of that iron, as well as the body’s needs for iron.

1.2.14.5.1. Dietary and luminal factors:

Much of dietary iron is non-haem iron derived from cereals, with a lesser component of haem iron from meat and fish. Iron is better absorbed from animal than vegetable sources. Iron is released from protein complexes by acid and proteolytic enzymes in the stomach and small intestine, and haem is liberated from haemoglobin and myoglobin. Iron is maximally absorbed from the duodenum and less well from the jejunum, probably because the increasingly alkaline environment leads to the formation of insoluble ferric hydroxide complexes. Acid pH, vitamin C and some low-molecular-weight chelates (e.g. sugars, amino acids) enhance absorption. Therapeutic ferrous iron salts are well absorbed on an empty stomach, but when taken with a meal absorption is reduced as a result of the same ligand-binding processes that affect dietary non-haem iron; phytates, tannates in tea and bran inhibit absorption. (5)

1.2.14.5.2. Mucosal factors:-

Non-haem iron is released from food as Fe$^{3+}$ and reduced by duodenal cytochrome b1 (DCyt b) to Fe$^{2+}$. This is transported across the brush border membrane by DMT1, which is upregulated in iron deficiency. Haem iron is initially bound by haem receptors at the brush border membrane and released intracellularly by haem oxygenase before entering the labile iron pool and following a common pathway with iron of non-haem origin. Iron absorption is regulated both at the stage of mucosal uptake and at the stage of transfer to the blood. DMT1 levels increase when intracellular iron is low and ferroportin concentration is also high due to low plasma hepcidin levels.
The amount of iron transported to the plasma through ferroportin is hepcidin – dependent, figure (1.3). \(^{(5)}\)

**Figure (1.3): The regulation of iron absorption.** (A.V. Hoffbrand. Essential haematology)

**1.2.14.6. Storage iron:**

In normal subjects, the majority of storage iron is present as ferritin, and haemosiderin is predominantly found in macrophages rather than hepatocytes. Normal concentrations of serum ferritin range from about 15 to 300 µg/L, and are higher in men (median about 90 µg/L) than in premenopausal women (median 30 µg/L). \(^{(5)}\)
1.2.14.6.1. Direct transfer of storage iron from macrophages to erythroblasts:

Some 80 – 90% of iron taken into developing erythroblasts is converted to haem within 1 hour. Any iron taken up in excess of the requirement for haem synthesis is incorporated in ferritin. The red cell ferritin content is therefore increased when haemoglobin synthesis is impaired, as in thalassaemia syndromes or sideroblastic anaemia. Excess iron may be seen in the cytoplasm of mature red cells as one or more siderotic granules. The spleen removes these granules by its pitting action. (5)

1.2.14.7. Iron supply to the tissues:

The serum iron and, more particularly, the saturation of the total iron - binding capacity of transferrin (TIBC) give a measure of the iron supply to the tissues. A serum transferrin saturation less than 15% is insufficient to support normal erythropoiesis. A rise in TIBC is characteristic of iron deficiency. A reduced serum iron concentration with a normal or reduced TIBC is a characteristic response to inflammation. Plasma concentrations of transferrin receptors reflect both the number of erythroid precursors and iron supply to the bone marrow. (5)

1.2.15. Iron deficiency anemia (IDA):

Iron-deficiency anemia is a common anemia caused by insufficient dietary intake and absorption of iron, and/or iron loss from bleeding which can be from a variety of sources such as intestinal, uterine or from the urinary tract. (11)

Globally, iron deficiency anaemia (IDA) is the most common type of anaemia and the most significant contributor to the onset of anaemia. So that IDA and anaemia are often used synonymously, and the prevalence of anaemia has often been used as proxy for IDA. (11, 12)

The most significant cause of iron-deficiency anemia in third world children is parasitic worms: hookworms, whipworms, and roundworms which cause intestinal
bleeding, which is not always noticeable in faeces, and is especially damaging to growing children. Malaria, schistosomiasis also contribute to iron deficiency. (3)

In men and postmenopausal women, the most common cause of iron-deficiency anemia is chronic gastrointestinal bleeding from nonparasitic causes, such as gastric ulcers, duodenal ulcers or gastrointestinal cancers. (4)

1.2.15.1. Signs and symptoms:
- General symptoms and signs of anaemia.
- Painless glossitis, angular cheilitis and koilonychia.
- Poor appetite, pruritus, dysphagia due to formation of esophageal webs (Plummer-Vinson syndrome).
- Unusual obsessive food cravings, known as pica, may develop.
- In severe cases, dyspnea can occur.
- In children iron deficiency is particularly significant as it can cause irritability, poor cognitive function and decline in psychomotor development.
- Palpitations, hair loss, fainting or feeling faint.
- Iron deficiency during development can lead to reduced myelination of the spinal cord, as well as a change in myelin composition.
- Iron-deficiency anemia has a negative effect on physical growth. (7, 9)

1.2.15.2. Diagnosis:
Anemia may be diagnosed from symptoms and signs, but when it is mild, it may not be diagnosed from mild nonspecific symptoms. Pica, an abnormal craving for dirt, ice, or other "odd" foods occurs variably in iron and zinc deficiency, but is neither sensitive nor specific to the problem, so is of little diagnostic help. Especially in adults over the age of 50, iron deficiency is often a sign of other
disease in the gastrointestinal tract, such as chronic bleeding from any cause (for example, a colon cancer) that causes loss of blood in the stool. Such loss is often undetectable, except with special testing (stool for occult blood). (11)

A diagnosis of iron-deficiency anemia then requires further investigation as to its cause. It can be caused by increased iron demand or decreased iron intake, and can occur in both children and adults. (9)

1.2.15.3. Laboratory findings:

- Low hemoglobin (Hb) and hematocrit values by definition makes the diagnosis of anemia.

- High red distribution width (RDW) as the body's iron stores begin to be depleted reflecting an increased variability in the size of red blood cells.

- A low mean corpuscular volume (MCV) and low mean corpuscular hemoglobin (MCH) and/or mean corpuscular hemoglobin concentration (MCHC).

- RBCs morphology shows microcytic hypochromic appearance.

- Target cells, and pencil-shaped poikilocytes.

- Very commonly, the platelet count is slightly above the high limit of normal in iron deficiency anemia.

- Low serum ferritin and low serum iron levels.

- Elevated serum transferrin and high total iron binding capacity. A low serum ferritin is the most sensitive lab test for iron deficiency anemia (Table 1.1). (12, 13)
**Table 1.1:**

<table>
<thead>
<tr>
<th>Change</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease</td>
<td>ferritin, hemoglobin, MCV</td>
</tr>
<tr>
<td>Increase</td>
<td>TIBC, transferrin, RDW</td>
</tr>
</tbody>
</table>

Iron-deficiency anemia and thalassemia minor present with many of the same lab results. It is very important not to treat a patient with thalassemia with an iron supplement, as this can lead to hemochromatosis (accumulation of iron in various organs, especially the liver). A hemoglobin electrophoresis provides useful evidence for distinguishing these two conditions, along with iron studies. (16)

**1.2.16. Anaemia of chronic disease (ACD):**

The observation that anemia is commonly associated with systemic illness led to the concept of an anemia of chronic disease (ACD). The pathogenesis of this anaemia appears to be related to decreased release of iron from macrophages to plasma, reduced red cell lifespan and an inadequate erythropoietin response to anaemia caused by the effects of cytokines such as IL-1 and tumour necrosis factor (TNF) on erythropoiesis. (6)

Hepcidin, released by the liver in response to inflammation, inhibits macrophage release of iron as well as iron absorption. The anaemia is corrected by successful treatment of the underlying disease and does not respond to iron therapy. Recombinant erythropoietin improves the anaemia in some cases. In many conditions this anaemia is complicated by anaemia resulting from other causes (e.g. iron, vitamin B12 or folate deficiency, renal failure, bone marrow failure, hypersplenism, endocrine abnormality, leucoerythroblastic anaemia). (4)
1.2.16.1. The characteristic features are:

1. Normochromic, normocytic or mildly hypochromic (MCV rarely <75 fL) indices and red cell morphology.

2. Mild and non-progressive anaemia (haemoglobin rarely <9.0 g/ dL) - the severity being related to the severity of the disease.

3. Both the serum iron and TIBC are reduced; sTfR levels are normal or low.

4. The serum ferritin is normal or raised.

5. Bone marrow storage (reticuloendothelial) iron is normal but erythroblast iron is reduced.

1.2.17. Megaloblastic anaemia:

The most common cause of macrocytic anaemia, is due to a deficiency of either Vit B12, Folic acid or both, and which can be due either to inadequate intake or insufficient absorption. A common biochemical feature is a defect in DNA synthesis, with lesser alterations in RNA and protein synthesis, leading to a state of unbalanced cell growth and impaired cell division. Most megaloblastic cells are not resting but vainly engaged in attempting to double their DNA, with frequent arrest in the S phase and lesser degrees of arrest in other phases of the cell cycle. An increased percentage of these cells have DNA values between 2 N (N = amount of DNA in the haploid genome) and 4 N because of delayed cell division. This increased DNA content in megaloblastic cells is morphologically expressed as larger than normal "immature" nuclei with finely particulate chromatin, whereas the relatively unimpaired RNA and protein synthesis results in large cells with greater "mature" cytoplasm and cell volume. The net result of megaloblastosis is a cell whose nuclear maturation is arrested (immature) while its cytoplasmic maturation proceeds normally independently of the nuclear events. The microscopic appearance of this nuclear-cytoplasmic asynchrony (or dissociation) is morphologically described as megaloblastic. Each cell lineage has a limited but unique repertoire of expression of defective DNA synthesis. This is significantly
influenced by the normal patterns of maturation of the affected cell line. Additional variables that affect RNA and protein synthesis can lead to the attenuation or modification of megaloblastic expression. \(^{(14)}\)

Megaloblastic hematopoiesis commonly manifests as anemia, but this feature is only a manifestation of a more global defect in DNA synthesis that affects all proliferating cells. The peripheral blood picture is characteristic and reflective of megaloblastic hematopoiesis within the bone marrow. The diagnosis is therefore usually straightforward, but because any condition that specifically disturbs DNA synthesis may lead to megaloblastosis, determination of the precise cause is necessary before institution of therapy. \(^{(14)}\)

Inappropriate therapy with vit. B12 can lead to disastrous consequences for the patient. In addition to the non-specific symptoms of anaemia, specific features of vitamin B12 deficiency include peripheral neuropathy, other features may include smooth, red tongue and glossitis. \(^{(14)}\)

### 1.2.18. Haemolytic anaemia:

Haemolytic anaemias are defined as those anaemias that result from an increase in the rate of red cell destruction. Because of erythropoietic hyperplasia and anatomical extension of bone marrow, red cell destruction may be increased several-fold before the patient becomes anaemic-compensated haemolytic disease. The normal adult marrow, after full expansion, is able to produce red cells at 6-8 times the normal rate provided this is 'effective'. It leads to a marked reticulocytosis, particularly in the more anaemic cases. Therefore, haemolytic anaemia may not be seen until the red cell lifespan is less than 30 days. \(^{(4)}\)

Haemolytic anaemia is brought on by the accelerated damage of red blood cells, which is when they are broken up before completion of their 120 days lifespan. When red blood cells become damaged this in turn means that the body is unable to produce a sufficient amount to substitute those destroyed.
This is despite increased cell production by the bone marrow, the body can also produce irregular red blood cells that are broken down. (4)

There are two core classes of haemolytic anaemia: acquired and inherited. Inherited haemolytic anaemia is passed on to child from parent, while acquired haemolytic anaemia is brought on by other factors, such as infection or an abnormal response of the immune system. Haemolytic anaemia can develop gradually or happen suddenly. It affects people in different ways being that there are mild, moderate and severe forms of the condition. Hereditary haemolytic anaemias are the result of 'intrinsic' red cell defects whereas acquired haemolytic anaemias are usually the result of an 'extracorpuscular' or 'environmental' change. Paroxysmal nocturnal haemoglobinuria (PNH) is the exception because although it is an acquired disorder the PNH red cells have an intrinsic defect. (12)

1.2.18.1. Inherited haemolytic anaemias:

Where inherited haemolytic anaemia is apparent one or more genes which manage red blood cell manufacture are defective, and this causes abnormal cell manufacture in the bone marrow. They can be:

I. **Membrane defect:** Hereditary spherocytosis, hereditary elliptocytosis.

II. **Defective red cell metabolism:** G6PD deficiency, pyruvate kinase deficiency.

III. **Haemoglobin:** Genetic abnormalities (Hb S, Hb C, unstable). (4)

1.2.18.1.1. Hereditary spherocytosis:

This is where the membrane of the red blood cells is abnormal, causing the cells to become spherical in shape which have a shorter lifespan than typical red blood cells, and are extracted from the bloodstream too early. Hereditary spherocytosis is mainly found amongst people of Northern European heritage. Hereditary spherocytosis (HS) is usually caused by defects in the proteins involved in the vertical interactions between the membrane skeleton and the lipid bilayer of the red cell. (4)
The marrow produces red cells of normal biconcave shape but these lose membrane and become increasingly spherical (loss of surface area relative to volume) as they circulate through the spleen and the rest of the RE system. Ultimately, the spherocytes are unable to pass through the splenic microcirculation where they die prematurely.

The inheritance is autosomal dominant with variable expression; rarely it may be autosomal recessive. The anaemia can present at any age from infancy to old age. Jaundice is typically fluctuating and is particularly marked if the haemolytic anaemia is associated with Gilbert's disease (a defect of hepatic conjugation of bilirubin); splenomegaly occurs in most patients. Pigment gallstones are frequent; aplastic crises, usually precipitated by parvovirus infection, may cause a sudden increase in severity of anaemia. (4)

1.2.18.1.2. Hereditary elliptocytosis:

This has similar clinical and laboratory features to HS except to the appearance of the blood film, but it is usually a clinically milder disorder. It is usually discovered by chance on a blood film and there may be no evidence of haemolysis. Occasional patients require splenectomy. The basic defect is a failure of spectrin heterodimers to self-associate into heterotetramers. A number of genetic mutations affecting horizontal interactions have been detected.

Patients with homozygous or doubly heterozygous elliptocytosis present with a severe haemolytic anaemia with microspherocytes, poikilocytes and splenomegaly (hereditary pyropoikilocytosis). (4)

1.2.18.1.3. Glucose-6 phosphate dehydrogenase deficiency (G6PD):

Glucose-6-phosphate dehydrogenase (G6PD) functions to reduce nicotinamide adenine dinucleotide phosphate (NADP) while oxidizing glucose-6-phosphate. It is the only source of NADP in red cells and as NADP is needed for the production
of reduced glutathione and a deficiency renders the red cell susceptible to oxidant stress.

In such cases red blood cells do not have a vital enzyme recognised as G6PD. This means that when the blood cells encounter specific substances (such as medication) in the blood, they can rupture and break down. This class of haemolytic anaemia is most prevalent amongst African American and Mediterranean men. (4)

1.2.1.8.1.4. Pyruvate kinase deficiency:

This class of haemolytic anaemia happens if red blood cells are absent of an enzyme identified as pyruvate kinase. Without pyruvate kinase the cells are more inclined to break up and die prematurely. (4)

This is inherited as an autosomal recessive, the affected patients being homozygous or doubly heterozygous. Over 100 different mutations have been described. The red cells become rigid as a result of reduced adenosine triphosphate (ATP) formation. The severity of the anaemia varies widely (haemoglobin 4-10 g/dL) and causes relatively mild symptoms because of a shift to the right in the oxygen (O₂) dissociation curve caused by a rise in intracellular 2,3 diphosphoglycerate (2,3-DPG). Clinically, jaundice is usual and gallstones frequent. Frontal bossing may be present. The blood film shows poikilocytosis and distorted 'prickle' cells, particularly post-splenectomy. Laboratory tests show that autohaemolysis is increased but, in contrast to HS, it is not corrected by glucose; direct enzyme assay is needed to make the diagnosis. Splenectomy may alleviate the anaemia but does not cure it and is indicated in those patients who need frequent transfusions. (4)

1.2.18.1.5. Haemoglobin abnormalities:

These result from the following:

2. Reduced rate of synthesis of normal α- or β-globin chains (the α- and β-thalassaemias). (4)

In many cases, however, the abnormality is completely silent. The clinically most important abnormality is sickle cell anaemia. Haemoglobin (Hb) C, D and E are also common and, like Hb S, are substitutions in the β chain. Unstable haemoglobins are rare and cause a chronic haemolytic anaemia of varying severity with intravascular haemolysis. Abnormal haemoglobins may also cause (familial) polycythaemia or congenital methaemoglobinemia. (4)

The genetic defects of haemoglobin are the most common genetic disorders worldwide. They occur in tropical and subtropical areas and most appear to have been selected because the carrier state affords some protection against malaria. β Thalassaemia is more common in the Mediterranean region while α-thalassaemia is more common in the Far East. (4)

1.2.18.1.5.1. Thalassaemias:

These are a heterogeneous group of genetic disorders that result from a reduced rate of synthesis of α or β chains. Thalassaemia is an umbrella term for a collection of anaemic conditions. These occur when the body does not produce sufficient kinds of haemoglobin and causes irregular red blood cells to be produced, die prematurely and be taken out of the body. Thalassaemia is mainly prevalent amongst individuals of Mediterranean, Asian and African ancestry. (4)

1.2.18.1.5.1.1. Clinical features

1. Severe anaemia becomes apparent at 3-6 months after birth when the switch from γ to β-chain production should take place.

2. Enlargement of the liver and spleen occurs as a result of excessive red cell destruction, extramedullary haemopoiesis and later because of iron overload. The large spleen increases blood requirements by increasing red cell destruction and pooling, and by causing expansion of the plasma volume.
3. Expansion of bones caused by intense marrow hyperplasia leads to a thalassaemic facies and to thinning of the cortex of many bones with a tendency to fractures and bossing of the skull with a 'hair-on-end' appearance on X-ray. (4)

4. The patient can be sustained by blood transfusions but iron overload caused by repeated transfusions is inevitable unless chelation therapy is given.

5. Infections can occur for a variety of reasons. In infancy, without adequate transfusion, the anaemic child is prone to bacterial infections. (4)

1.2.18.1.5.2. Sickle cell anaemia:

Sickle cell disease is a group of haemoglobin disorders in which the sickle-β globin gene is inherited. Homozygous sickle cell anaemia (Hb SS) is the most common while the doubly heterozygote conditions of Hb SC and Hb Sβthal also cause sickling disease. Hb S (Hb α2β2S) is insoluble and forms crystals when exposed to low oxygen tension.

Deoxygenated sickle haemoglobin polymerizes into long fibres, each consisting of seven intertwined double strands with cross-linking. The red cells sickle and may block different areas of the microcirculation or large vessels causing infarcts of various organs. The sickle β-globin abnormality is caused by substitution of valine for glutamic acid in position 6 in the β chain. It is very widespread and is found in up to one in four West Africans, maintained at this level because of the protection against malaria that is afforded by the carrier state. (4)

1.2.18.1.5.2.1. Clinical features:

Clinical features are of a severe haemolytic anaemia punctuated by crises. The symptoms of anaemia are often mild in relation to the severity of the anaemia because Hb S gives up oxygen (O2) to tissues relatively easily compared with Hb A, its O2 dissociation curve being shifted to the right.

The clinical expression of Hb SS is very variable, some patients having an almost normal life, free of crises but others develop severe crises even as infants and
may die in early childhood or as young adults. Crises may be vaso-occlusive, visceral, aplastic or haemolytic. \(^{(4)}\)

1.2.18.2. **Acquired categories of haemolytic anaemia:**

This is when red blood cells are sometimes broken down even if they are healthy. This may be caused by a range of factors, including infection or disease. In most cases the damage occurs in the bloodstream or the spleen (this is more common). Three main forms of acquired haemolytic anaemia exist, which are alloimmune, autoimmune and drug-induced. \(^{(12)}\)

Autoimmune haemolytic anaemia (AIHA): people who have autoimmune haemolytic anaemia produce antibodies in opposition to their own red blood cells. This category of haemolytic anaemia affects around half of those with the condition and tends to be found in those over the age of forty. It can be serious if not properly treated. \(^{(12)}\)

1.2.18.2.1. **Paroxysmal nocturnal haemoglobinuria (PNH):**

Otherwise known as PNH this is an acquired genetic condition, which leads to the red blood cells becoming irregular owing to a deficiency of specific types of protein. The body breaks these red blood cells down quicker than usual, and the condition can remain constant at a minor level or flare up suddenly. Individuals with PNH have a higher risk of blood clots and decreased levels of platelets and white blood cells. \(^{(12)}\)

1.2.19. **Other sources of harm to red blood cells:**

Infections can harm red blood cells as can toxic chemicals and external agents, including snake venom, tick-borne diseases and malaria. \(^{(12)}\)

1.2.20. **Aplastic anaemia:**

Aplastic anemia is a syndrome of bone marrow failure characterized by peripheral pancytopenia and marrow hypoplasia. Mild macrocytosis is observed in association
with stress erythropoiesis and elevated fetal hemoglobin levels. Paul Ehrlich introduced the concept of aplastic anemia in 1888 when he studied the case of a pregnant woman who died of bone marrow failure. However, it was not until 1904 that Anatole Chauffard named this disorder aplastic anemia. (12)

Although it is not a cancer, aplastic anemia may be associated with certain cancers or cancer treatments.

Aplastic anemia is a rare, potentially fatal disease in which the bone marrow doesn't make enough blood cells. People with aplastic anemia have low levels of all three types of blood cells:

- Red blood cells, which carry oxygen.
- White blood cells, which fight infection.
- Platelets, which help blood to clot.

Aplastic anemia is a problem with the bone marrow stem cells. In aplastic anemia, something either destroys the stem cells or drastically changes the environment of the bone marrow so that the stem cells can't develop properly. Several factors can cause this problem, including:

- Exposure to radiation (radiation sickness).
- Chemotherapy.
- Environmental toxins (insecticides, benzene, nitrogen mustards).
- Many different medications, including chloramphenicol (Chloromycetin), phenylbutazone (Butazolidin), sulfonamides (Gantanol and others), anticonvulsants, cimetidine (Tagamet) and others. (12)
- Certain viral infections, including viral hepatitis, parvovirus B19, human immunodeficiency virus (HIV) and infectious mononucleosis (Epstein-Barr viral infection). (12)
• Autoimmune disease, where the body inappropriately attacks its own blood stem cells.

Some people are more likely to develop aplastic anemia because of their genetic (inherited) make up. Fanconi’s anemia is an inherited condition that causes aplastic anemia and also physical abnormalities. Some women develop a mild form of aplastic anemia during pregnancy, but it tends to disappear after delivery. In 50-65% of patients with aplastic anemia, the cause of the illness is not clear. \(^{(12)}\)

### 1.2.21. Systematic Approach to the Evaluation of Anaemia:

The correct diagnosis of anemia can often be determined by combining a thorough history and physical examination with thoughtful reviews of the complete blood cell count, reticulocyte count, and peripheral blood smear. Such an approach minimizes cost and the time to accurate diagnosis. \(^{(6)}\)

#### 1.2.21.1. History and Physical Examination:

Because anemia can be a primary disorder or secondary to other systemic processes, a careful history and physical examination will provide valuable insight into the potential cause. \(^{(6)}\)

#### 1.2.21.2. Reticulocyte Count:

As a marker of red blood cell rate of production, the reticulocyte count provides important information in directing the initial investigation of anemia. However, when significant numbers of nucleated red blood cells or nuclear debris are present in the peripheral blood, this diagnostic accuracy declines, and manual counting methods are generally preferable. \(^{(6)}\)
1.2.21.3. Examination of the peripheral blood smear:

Review of a well-made peripheral blood smear remains one of the most informative and rewarding diagnostic procedures. It offers the chance to confirm the findings of the automated complete blood cell count, which can be inaccurate in the presence of nucleated red blood cells or rouleaux formation. Review of the blood smear also allows for evaluation of other cell lineages, which might suggest a primary marrow or infiltrative disease. The finding of hypersegmented neutrophils suggests a megaloblastic process, and this morphologic abnormality can be seen in the blood smear before there are significant changes in the hemoglobin or MCV. Also, only the blood smear reveals the unique morphologic changes occurring with various hemolytic disorders. (6)

1.2.22. Control of Anaemia:-

In order to effectively combat it, the contributing factor must be identified and addressed. In settings where iron deficiency is the most frequent cause, additional iron intake is usually provided through iron supplements to vulnerable groups. Food based approaches to increase iron intake through food fortification and dietary diversification are important. Strategies should be include addressing other causes of anaemia, and should be built in the primary health care system and existing programmes. (15)
Chapter Two

Rationale and Objectives

2.1. Rationale:-

Khalawi students are a heterogeneous group with different ethnic and sociocultural background. Such studies will open a venue for other studies in many fields of interest e.g. communicable diseases, nutritional and sociocultural. There are many such khalawi in Sudan which might benefit from this study.

2.2. Objectives:-

2.2.1. General Objective:-

To assess the prevalence of anaemia among quranic school students in Wad El Magboul Khalawa in the period from December 2012- July 2013.

2.2.2. Specific objectives:-

2.2.2.1. To identify the common type of anaemia using complete blood count (CBC), morphology and reticulocyte count.

2.2.2.2. To identify the mean Hb level among the quranic school students in Wad EL Magboul setting.

2.2.2.3. To identify the possible common cause(s) of anaemia among the study group.
Chapter Three

Methodology

3.1. Study Type:

A prospective cross – sectional analytical community based study was conducted in khalawi Wad EL Magboul village, rural Rufaa, Gezira State central Sudan.

3.2. Study Area:

The study was carried out in Gezira state, Wad EL Magboul village, quranic schools (Khalawi). Wad EL Magboul is a small village about 15 km to the south east Rufaa. Wad EL Magboul Khalawi are considered as one of the famous Khalawi not only in Gezira state but also in Sudan because the students or (Heiran) came from different places of Sudan. The Khalawi consist of 15 rooms, each room contains from 10 -15 students being from the same places or regions. From 1925 to 1960 these Khalawi were built in Al dweneeb village where several small Khalawi were branched from there and since 1960 the Khalawi were transferred to Wad EL Magboul in Rufaa rural locality where the current Khalawi now exist. The total number of students (Heiran) studying in these Khalawi are about 180 students, from different places and of different ages. Two diets are served, breakfast and dinner which are made mainly from dura porridge (Assida) with a curry made from lentis or beans.

3.3. Study Population:-

Quranic school students (Heiran) studying in Khalawi Wad El Magboul living at least for the last one year.

3.4. Study Setting:-

Department of haematology, Medical laboratory, Faculty of Medicine, University of Geziera, Wad Medani.
3.5. Inclusion criteria:-

- Any student in Khalawi Wad El Magboul living at least for one year.
- Age between 8 to 18 years.

3.6. Exclusion criteria:-

Students living at home but studying in khalawi Wad El Magboul or those who are receiving treatment to any type of anaemia or taking hematinics as prophylaxis. Any student more than 18 years.

3.7. Methods:

3.7.1. Sampling and Tests:

A comprehensive convenient sampling method (total coverage) was adopted and a total of 180 quranic male students (Heiran) who are living in khalawi Wad EL Magboul as a study group were screened for complete blood count (CBC); peripheral blood smears and reticulocyte count. They also were screened for serum ferritin, BFFM, urine and stool analysis. This analysis was conducted at the medical laboratory, faculty of medicine, university of Gezira, departments of haematology and microbiology. 5 ml of venous blood sample were collected from an antecubital vein by a 5ml syringe from each student and divided in EDTA and plain tubes to perform CBC, peripheral blood smears, reticulocyte count, thick film for malaria and serum ferritin level. The site of collection was cleaned using 70% alcohol and left to dry. An elastic tourniquet was applied to the arm for a period not exceeding one minute to avoid haemoconcentration. 2.5 ml of blood was taken into a container with 0.05ml (K2 EDTA) as an anticoagulant with a concentration of 1.5- 2.2 mg/ml and then the sample gently mixed. 2 ml of blood was delivered in a plain tube, after clot, and then centrifuged, the serum was taken to another plain tube to perform serum ferritin level. Urine and stool samples were also taken in appropriate plastic containers to perform urine and stool analysis.
The blood samples were tested within 2 hours of sample collection using an automated blood cell counters (sysmex KN21 analyzer) with a flow cytometry using a laser light to perform full blood count: white blood cell counts (WBCs), red blood cell counts (RBCs), haemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume(MCV) , mean corpuscular haemoglobin (MCH) , mean corpuscular haemoglobin concentration (MCHC), and platelet counts (PLTs). It is calibrated by a standardized commercially prepared calibrators. The World Health Organization (WHO) has suggested levels of haemoglobin below which anaemia is said to be present. These levels are < 11g/dL (110 g/L) in children aged 1-2 years and < 11.2g/dL (112 g/L) in children aged 3-5 years and less than 13.5g/dl in children aged 6-12 years.\textsuperscript{(20)}

3.7.2. Making a blood film:

Preparation of thin blood films was performed. The frosted glass slides were clean and free of grease. A drop of blood was placed near frosted end of the slide and spreader was applied at an angle of 45, infront of the drop of blood making a thin blood film using a cover glass as spreader and was then allowed to dry.

Then they were labeled with the participant’s number and date of sample collection. The films were then fixed in absolute methanol for 10 - 20 minutes. The films were placed horizontally on the staining rack and flooded with Leishman’s stain and left for 5 minutes. A double volume ofbuffer 7.2 was added with gentle blowing over the surface without touching the film surface. The films were left for another 8 minutes and then washed off with buffered 7.2. The back of the slide was cleaned using cotton dipped in alcohol and then left to dry. The same method of preparing the blood film for red cell morphology is used for preparing films for reticulocyte count but the stain used here was methylene blue: 2 or 3 drops of the dye solution were delivered into a 75 x 10 mm plastic tube by means of a plastic Pasteur pipette. 2–4 volumes of the patient’s EDTA-anticoagulated blood were added to the dye solution and mixed. The mixture was kept at 37°C for 15–20 min. The red cells were resuspended by gentle
mixing and films were spread on glass slides in the usual way, then they were examined when dried without fixing or counterstaining. An area of film was chosen for the count where the cells are undistorted and where the staining is good. The x100 oil-immersion objective lens was used to count the cells. At least 100 reticulocytes had been counted and at least 10 fields were examined to determine the average number of red cells per field.

3.7.3 Examination of the blood films:

The identification of the specimen was checked and matched with the corresponding full blood count (FBC) form. The films were examined macroscopically to confirm adequate spreading followed by microscopic examination. A low power field (10 objective) was used to assess the quality of the stain and a (40 objective) to determine the suitable area for blood film examination. The differential white blood counts were performed manually using the oil emersion lens. At least one 100 cells were examined. The manual differential white blood cells were compared with the automated differential white blood cells. The morphology of the red cells regarding the staining character, shape, size of the cells and the presence of nucleated red blood cells was recorded. The platelets were examined and estimation of their number, size, morphology and presence of aggregates were commented on.

3.7.4 Determination of serum ferritin:

Serum ferritin was determined manually using colorimeter by adding 2 volumes (2 ml) of reagent A to 1 volume (1ml) of reagent B (working reagent). Then the working reagent and the instrument were brought to 37 °C. Zeroing of the instrument by distilled water was done. Then 1‘ml from the working reagent to 30 1 of the sample were pipetted into a cuvette which was mixed and inserted into the instrument and the stopwatch was started. Then the absorbance at 540 nm after 10 seconds (A₁) and after 5 minutes (A₂) was recorded.
3.7.5. Thick films for malaria:

Then thick blood films for malaria were also done and with Giemsa stain were stained, and microscopically examined.

3.7.6. Urine analysis:

Explanation to the patient for the need to collect the urine was done. The containers were labeled with the date, the name and number of the patient, and the time of collection. As soon as possible, the specimen was delivered. Urine for sugar and acetone was examined by the dipstick method, then about 5 ml of well mixed urine aseptically was transferred to a labeled conical tube and was centrifuged at 500–1000 g for 5 minutes. The supernatant fluid (by completely inverting the tube) was poured into a second container not the original one. Then, a wet preparation from the remaining sediment was done and microscopically was examined using the x10 and x 40 objective.

3.7.7. Stool analysis:

Stool analysis was done and the appearance of the specimen has been described (colour of the specimen, whether it is formed, semifomed, unformed or fluid, the presence of blood, mucus or pus, the presence of worms, e.g. (Enterobius vermicularis, Ascaris lumbricoides, or tapeworm segment, e.g. Taenia species). Then a drop of fresh physiological saline on one end of a slide was placed, and a small amount of fresh specimen was mixed with the drop of saline. Then the preparation was covered with a cover glass and microscopically was examined using the x10 and x 40 objectives with the condenser iris closed sufficiently to give good contrast. The specimen was examined for motile E. histolytica trophozoites containing red cells, motile G. lamblia trophozoites, motile Strongyloides larvae, and the eggs and cysts of parasitic pathogens.
3.8. Statistical analysis:

The results were analyzed using statistical software package of social sciences (SPSS) version 16 and descriptive data were expressed as means and percentages. with P value 0.05. 95 % confidence interval of the difference Chi square test = 1.57.

3.9. Ethical clearance:

Ethical approval was obtained from the University of Gezira ethical committee, the medical laboratory and khalawi Wad EL Magboul authority. Target population was informed about the study objectives and was consented prior filling questionnaires. Confidentiality and privacy of target population was guaranteed.
Chapter Four

Results

4.1. The participants’ characteristics:

4.1.1. Age distribution

A total of 180 subjects, age between 8 – 18 years with a mean age of 12.31 years (SD +/− 2.26). Table (4.1).

4.1.2. Tribal distribution:

The study group subjects were mainly from Hausa (67.8%), next to that was Gaaleen (10%), Tama (7.8%), Misseria (6.7%), Habbania (3.3%), Fur (1.7%), Massalit (1.7%) and Rizegat (1.1%), table (4.2), figure (4.1).

4.1.3. Other participants’ characteristics:

All the students were from the rural areas. Of them 141 students (84.6%) were found to be from poor families, while 39 students (15.4%) belonged to families of moderate socioeconomic status. About 136 students (75.56%) were coming from families consisting of 5 – 10 members, while 36 students (20%) came from families consisting of more than 10 members and only 8 students (4.44%) of families consisting of less than 5 members. The type of diet for all the students didn’t contain meat or vegetables, because they eat the same type of food every day (twice a day) which contains mainly dura and beans.

The monthly pocket money for 150 students (83.34 %) was found to be irregular, while only 30 students (16.66 %) had their adequate money.

Regarding period of stay in the khalwa up to the time of the study, 88 (49.28%) for one year, 54 (30.24%) for 2 years, 22 (12.32%) for 3 years and 16 (8.96%) for more than 3 years. During this period, the frequency of home visits was variable. The home visit for 161 students (89.16%) was once a year, and they stay there for
an average of one month. While the home visit for 15 students (8.4%) was 3 monthly during which they stayed for 1 – 2 weeks. Only 4 students (2.24%) had monthly visits to their home and most of them stay for about one week. The knowledge about anaemia among the students was varying. Some students 23/180 (12.88%) had some knowledge about anaemia as they having anaemia before. About 49 students (27.44%) know about but didn’t have anaemia before, while 108 students (60.48%) had no knowledge about anaemia at all. The majority of the students 177/180 (98.2%) had no history of chronic disease, while 3 students (1.8%) were found to have a positive history of recurrent mild epistaxis.

The majority of the students, (161, 89.44%) were not admitted to hospital at all, while 19 students (10.56%) were hospitalized with different diseases: malaria, 10 students (5.6%), 2 students (1.11%) had a history of hospitalization due to acute appendicitis and 3 students (1.67%) due to gastroenteritis, while 4 students (2.22%) due to Schistosomiasis.

4.1.3.1. Clinical examination:

Clinical examinations revealed pallor in 77 students (42.78%). None of the students had organomegaly or jaundice. Also there were no hair, eye, mouth or nail changes detected among the participants.

4.1.3.2. Investigations:

Blood films for malaria showed that 49 students (27.2%) had a positive blood films for falciparum malaria. Urine analysis revealed that 164 students (91.1%) had clear urine, 14 students (7.8%) were found to have haematuria and ova of S. haematobium, while 2 students (1.1%) were found to have pyuria. In 169 students (93.4%) stool examination was negative, while 11 students (6.6%) had intestinal worms (Enterobius vermicularis), table (4.3).
4.1.4: The White cell count and morphology:

The mean white blood cell counts value was found to be \(5.19 \times 10^3\) / l +/- 1.52 standard deviation, with a minimum value of \(1.60 \times 10^3\) / l and maximum value \(14.30 \times 10^3\) / l. 93.9% (169 cases) had normal WBCs between 3 – 9x10^3 (table 4.4).

Regarding WBCs morphology 152 cases (84.12%) had normal morphology, 14 cases (7.84%) had eosinophilia, 5 cases (2.8%) had hypersegmented neutrophils. 8 cases (5.04%) had reactive and atypical lymphocytes, and one case (0.56%) showed few lymphoblasts, but no evidence of marrow failure or organomegaly. A later CBC was done and no blasts were detected so it could be atypical lymphocytes due to viral infection. No myeloblast, promyelocytes, myelocytes, metamyelocytes were detected in the blood films. Table (4.5).

4.1.5: Red blood cell count & morphology:

The mean RBCs value was found to be \(4.54 \times 10^6\) / l +/- 0.560 standard deviation, with a minimum value of \(3.10 \times 10^6\) / l and maximum value of \(6.45 \times 10^6\) / l, table (4.4).

RBCs morphology: Normochromic normocytic red cells were found in 49 cases (27.4%), dimorphic blood films in 19 cases (10.6%), anisocytosis, with predominant microcytic hypochromic cells in 103 cases (57.68%), macrocytes in 7 cases (3.9%), sickle cells in 5 cases (2.8%), target cells in 9 cases (5.04%), polychromatic cells in 16 cases (8.96%) nucleated red blood cells in 14 cases (7.84). Marked rouleaux formation was detected in 9 cases (5.04). No autoagglutination was detected in the smears. Table (4.6).

4.1.6. Haemoglobin level:

The mean Hb value was found to be 11.75 g/dl +/- 1.87 standard deviation, with a minimum value of 7.50 g/dl and maximum value of 15.40 g/dl, table (4.4).
4.1.7. Packed cell volume (PCV):

The mean PCV value was found to be 36.59 % +/- 3.51 % standard deviation with a minimum value of 24.90 % and maximum value of 45.40 %, table (4.4).

4.1.8: Mean cell volume (MCV):

The mean MCV value was found to be 77.83 fl +/- 8.11 standard deviation with a minimum value of 58.60 fl and maximum value of 101.00 fl. 104 cases (58.24%) had MCV in the range of 78 – 86 fl. Figure (4.3).

4.1.9. Mean cell haemoglobin (MCH):

The MCH mean value was found to be 25.58 pg +/- 3.55 standard deviation with a minimum value of 17.70 pg and maximum value of 34.10 pg. 125 cases (69.4%) had MCV in the range of 25 – 30 pg (table 4.4), figure (4.4).

4.1.10: Mean cell haemoglobin concentration (MCHC):

The MCHC mean value was found to be 32.96 g/dl +/- 2.22 standard deviation with a minimum value of 22.10 g/dl and maximum value of 36.30 g/dl, table (4.3).

4.1.11: Platelets:

The platelets mean value was found to be $317 \times 10^3 / \mu l$ +/- 120.60 standard deviation with a minimum value of $25.00 \times 10^3 / \mu l$ and maximum value of $723.00 \times 10^3 / \mu l$ (table 4.4). Most of the students had normal platelet counts, but 5 cases (2.8 %), had thrombocytopenia. 25 cases (13.9 %) had platelets more than $450 \times 10^3 / \mu l$. Aggregates were found in 52 cases (29.5 %). Giant forms were found in 13 cases (7.2 %).
4.1.12: Reticulocyte count:

The reticulocyte count mean value was found to be 1.55 % ± 1.32 % standard deviation with a minimum value of 0.20 % and maximum value of 7 %, table (4.3). 38 cases (21.1%) had high reticulocyte counts (due to malaria, schistosomiasis and few other types of haemolytic anaemias, table(4.8).

4.2. The Serum ferritin:

The Serum ferritin mean value was found to be 50.31 μg/l ± 49.58 standard deviation, with a minimum value 0.00 μg/l and maximum value 300.00 μg/l. 101 cases (56.11%) had low ferritin, while none had ferritin > 300 μg/l, figure (4.6).

According to CBC findings combined with peripheral blood morphology and reticulocyte count, causes and types of anaemia were thought to be iron deficiency anaemia (IDA) in 134 cases (75.04%), macrocytic in 7 cases (3.9%), sickle cell anaemia in 5 cases (2.8%) and other types of haemolytic anaemias in 11 cases (6.2%), figure (4.7). The criteria used to diagnose IDA were low RBCs indices, microcytic hypochromic picture, low reticulocyte counts and low serum ferritin. All cases diagnosed as SCA were from Hausa (3 cases) and Misseria (2 cases) tribes. The presence of polychromasias, NRBCs and high reticulocyte counts without obvious sickle cells made the diagnosis of other haemolytic anaemias more likely, but no further investigations were done.

The relationship between mean Hb levels and different types of anaemia found, malaria and S.haematobium infection shown in table (4.9).
Table (4.1): Mean, standard deviation (Std.), minimum and maximum for the age in study group subjects (n = 180)

<table>
<thead>
<tr>
<th>Age/years</th>
<th>No</th>
<th>Mean</th>
<th>Std.</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/years</td>
<td>180</td>
<td>12.31</td>
<td>2.26</td>
<td>8</td>
<td>18</td>
</tr>
</tbody>
</table>

Table (4.2): Tribal distribution in study group subjects (n = 180)

<table>
<thead>
<tr>
<th>Tribe</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hausa</td>
<td>122</td>
<td>67.8</td>
</tr>
<tr>
<td>Gaaleen</td>
<td>18</td>
<td>10.0</td>
</tr>
<tr>
<td>Tama</td>
<td>14</td>
<td>7.8</td>
</tr>
<tr>
<td>Misseria</td>
<td>12</td>
<td>6.7</td>
</tr>
<tr>
<td>Habbania</td>
<td>6</td>
<td>3.3</td>
</tr>
<tr>
<td>Massalit</td>
<td>3</td>
<td>1.7</td>
</tr>
<tr>
<td>Fur</td>
<td>3</td>
<td>1.7</td>
</tr>
<tr>
<td>Rizegat</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table (4.3): BFFM, Urine & Stool analysis for study group subjects (n = 180)

<table>
<thead>
<tr>
<th>Type of investigation</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFFM (+)ve</td>
<td>49</td>
<td>27.2</td>
</tr>
<tr>
<td>BFFM (-) ve</td>
<td>131</td>
<td>78.8</td>
</tr>
<tr>
<td>Urine analysis clear</td>
<td>164</td>
<td>91.1</td>
</tr>
<tr>
<td>Haematuria &amp; Ova S.haematobium</td>
<td>14</td>
<td>7.8</td>
</tr>
<tr>
<td>Pyuria</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>Stool analysis clear</td>
<td>169</td>
<td>93.4</td>
</tr>
<tr>
<td>Worms</td>
<td>11</td>
<td>6.6</td>
</tr>
</tbody>
</table>
Table (4.4): Descriptive Statistics of CBC Parameters compared with the normal reference values:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Normal reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC counts</td>
<td>5.1918</td>
<td>1.52508</td>
<td>1.60</td>
<td>14.30</td>
<td>4.4 – 10.7 x 10^3 /l</td>
</tr>
<tr>
<td>RBC counts</td>
<td>4.5441</td>
<td>.56028</td>
<td>3.10</td>
<td>6.45</td>
<td>4.5 – 5.5 x 10^6 /l</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>11.7572</td>
<td>1.87932</td>
<td>6.00</td>
<td>15.40</td>
<td>11.5 – 15.5 g/dl</td>
</tr>
<tr>
<td>PCV</td>
<td>36.5967</td>
<td>3.51979</td>
<td>24.90</td>
<td>45.40</td>
<td>35 – 45 %</td>
</tr>
<tr>
<td>M C V</td>
<td>77.8356</td>
<td>8.11169</td>
<td>58.60</td>
<td>101.00</td>
<td>77 – 93 fl</td>
</tr>
<tr>
<td>MCH</td>
<td>25.5856</td>
<td>3.55822</td>
<td>17.70</td>
<td>34.10</td>
<td>26 – 34 pg</td>
</tr>
<tr>
<td>MCHC</td>
<td>32.9617</td>
<td>2.22566</td>
<td>22.10</td>
<td>36.30</td>
<td>31 – 37 g/dl</td>
</tr>
<tr>
<td>Platelets</td>
<td>317.2167</td>
<td>120.60</td>
<td>25.00</td>
<td>723.00</td>
<td>150 – 450 x 10^3 /l</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>1.5544</td>
<td>1.32679</td>
<td>.20</td>
<td>7.00</td>
<td>0.5 – 2.5 %</td>
</tr>
</tbody>
</table>
### Table (4.5): White blood cell morphology in the study group subjects (n = 180)

<table>
<thead>
<tr>
<th>White blood cell morphology</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal morphology</td>
<td>152</td>
<td>84.12</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>14</td>
<td>7.84</td>
</tr>
<tr>
<td>Reactive and a typical lymphocytes</td>
<td>9</td>
<td>5.04</td>
</tr>
<tr>
<td>Hypersegmented neutrophils</td>
<td>5</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>180</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

### Table (4 – 6): RBCs morphology in study group subjects (n = 180)

<table>
<thead>
<tr>
<th>RBCs morphology</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisocytosis, microcytic hypochromic</td>
<td>103</td>
<td>57.68</td>
</tr>
<tr>
<td>Normochromic normocytic</td>
<td>49</td>
<td>27.4</td>
</tr>
<tr>
<td>Dimorphic blood films</td>
<td>19</td>
<td>10.6</td>
</tr>
<tr>
<td>Polychromatic cells</td>
<td>16</td>
<td>8.6</td>
</tr>
<tr>
<td>Nucleated red blood cells</td>
<td>14</td>
<td>7.84</td>
</tr>
<tr>
<td>Rouleaux formation</td>
<td>9</td>
<td>5.04</td>
</tr>
<tr>
<td>Target cells</td>
<td>9</td>
<td>5.04</td>
</tr>
<tr>
<td>Macrocytes</td>
<td>7</td>
<td>3.9</td>
</tr>
<tr>
<td>Sickle cells</td>
<td>5</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Table (4.7): Haemoglobin level in study group subjects (n = 180)

<table>
<thead>
<tr>
<th>Haemoglobin values</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9 g/dl Severe anaemia</td>
<td>21</td>
<td>11.7</td>
</tr>
<tr>
<td>9-13.5 g/dl Mild anaemia</td>
<td>132</td>
<td>73.3</td>
</tr>
<tr>
<td>More than 13.5 g/dl</td>
<td>27</td>
<td>15.0</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (4.8): The reticulocyte count in study group subjects (n = 180)

<table>
<thead>
<tr>
<th>Reticulocyte counts</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 0.5%</td>
<td>72</td>
<td>40.0</td>
</tr>
<tr>
<td>0.5 - 2.5%</td>
<td>70</td>
<td>38.9</td>
</tr>
<tr>
<td>More than 2.5%</td>
<td>38</td>
<td>21.1</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table (4.9): Relationship between mean Hb levels and different types of anaemia found, malaria and S. haematobium infection

<table>
<thead>
<tr>
<th>Type of anaemia or parasitic infection</th>
<th>No.</th>
<th>Mean Hb level g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency anaemia</td>
<td>134</td>
<td>11.66</td>
</tr>
<tr>
<td>Macrocytic anaemia</td>
<td>7</td>
<td>12.45</td>
</tr>
<tr>
<td>Sickle cell anaemia</td>
<td>5</td>
<td>12.00</td>
</tr>
<tr>
<td>Other haemolytic anaemias</td>
<td>11</td>
<td>12.70</td>
</tr>
<tr>
<td>Malaria</td>
<td>49</td>
<td>11.98</td>
</tr>
<tr>
<td>S.haematobium infection</td>
<td>14</td>
<td>12.27</td>
</tr>
</tbody>
</table>

Figure (4.1): Tribal distribution (n= 180)
Figure (4.2): Mean cell volume (n = 180)
Figure (4.3): Mean cell haemoglobin (n = 180)

Figure (4.4): Serum ferritin values (n = 180)
Figure (4.5): Types of anaemia found in 153 students out of 180 study group subjects
Chapter Five

5.1. Discussion:

Anaemia is a common health problem that affects populations in both developing and developed countries. Its primary cause is iron deficiency, but a number of other conditions, such as malaria, parasitic infection, other nutritional deficiencies, and haemoglobinopathies are also responsible, often in combination. The WHO Global Database on Anaemia can be used to describe the nutritional status of populations and to identify the needs for interventions to prevent and control anaemia. (7)

This is probably the first study in this area. All the study group subjects came from rural areas, the majority of them belonged to certain tribes (Misseria, Hausa) who are also known to have congenital anaemias (sickle cell anaemia), and all of them eat the same type of poor diet which consist of dura and beans, so it showed that anaemia should be considered as a major health problem in quranic school (khalawi) children.

In the present study, the prevalence of anaemia in Khalawi Wad El Magboul was 88.33 %, and the prevalence was as high as 47.11 % in children aged 11 – 14 years, 38.15 % in children aged between 7 – 10 years and 3.07 % in children aged between 15 – 18 years.

This result is in agreement with one study carried out in Nepal about the prevalence of anaemia amongst adolescents. It was a cross sectional community based study carried out in Morang district to determine the prevalence and distribution of anaemia in terms of age, sex and locations (urban and rural) among adolescent population. Sahli method was used to determine the haemoglobin level. Three hundred and eight adolescents (127 urban, 181 rural in terms of location and 151 male, 157 female in terms of sex) participated in the study. The study found that the overall prevalence of iron deficiency anemia among adolescent population was 65.6% with the distribution of rural 62.4%, urban 70.0%, male 52.3% and female 78.3%. (19)
The present study is not in agreement with a study done in Urban Delhi in four primary schools, in which the prevalence of anaemia was 41.8%. This disagreement might be due to differences in two study areas and the difference socioeconomic status of the two study populations.

In Sudan, another parallel study was done about prevalence of anaemia among school children in eastern Sudan, done by Shams E. Musa et al who found that the prevalence of anaemia in a total of 401 children selected randomly from four primary schools in Kassala randomly was 93%. The prevalence of anaemia was estimated, clinically and by measuring haemoglobin concentration. The results was that clinical examination revealed anaemia in 373 of the students and haemoglobin estimations proved anaemia in 93% of the students enrolled in the study (Hb. less than 13.5 g/dl).

Iron deficiency anaemia was found to be the commonest in the present study and this agreed with another study conducted in departments of paediatrics, pathology & pharmacology, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi, India, about the Prevalence & etiology of nutritional anaemia among schoolchildren of urban slums. (21)

There was significant relation with the mean Hb value of the screened subjects which was found to be 11.75 g/dl (Std. 1.87, minimum value 7.5 g/dl, maximum 15.40g/dl) when compared with the mean Hb reference value (13.5 g/dl) (P value 0.000. 95 % confidence interval of the difference). Also there was significant correlation between the low serum ferritin when cross tabulated with the type of diet, (Chi square test = 1.57, P value = 0.005).

7 cases (3.9%) presented with white blood count less than $3 \times 10^3$ /L (leucopenia with neutropenia, associated with reactive lymphocytes) most probably due to chronic or viral infection. White blood count more than $9 \times 10^3$ /L was observed in 4 children (2.2%) dominated by neutrophils which was suggestive of pyogenic infection. Hypersegmented neutrophils were observed in 5 cases (2.8 %), suggestive of mixed deficiency. The eosinophils percentage of more than 6 % was
observed in 14 subjects (7.84 %), this eosinophilia probably reflects parasitism (e.g. Schistosomiasis).

5 children (2.8%) presented with platelets counts less than 150 x10^3 /l(thrombocytopenia), may be due to asymptomatic malaria parasitaemia. Aggregates were found in 52 cases (29.5 %) and part of this may be due to the presence of inappropriate clots.

Thrombocytosis with platelet counts more than 450 occurred in 25 subjects (13.9%) accompanied by low MCV and low MCH (suggestive of iron deficiency) which is one of the causes of thrombocytosis. Very commonly, the platelet count is slightly above the high limits of normal in iron deficiency anemia. This effect was classically postulated to be due to high erythropoietin levels in the body as a result of anemia, cross-reacting to activate thrombopoietin receptors in the precursor cells that make platelets. Such slightly increased platelet counts present no danger, but remain valuable as an indicator of iron deficiency.

The likely causes of anaemia in the study may include:

- Nutritional deficiency – major cause.
- Parasitic infestation (malaria, bilharzia).
- Rarely hereditary causes.
5.2. Conclusion:

1- The higher prevalence of iron deficiency anaemia, as noticed in the present study, may be attributed to inadequate food intake, poor stores and other nutritional deficiencies among these children.

2- Childhood anaemia continues to be a significant public health problem in school children and iron deficiency either alone or in combination is the commonest nutritional cause of anaemia.

3- Pure macrocytic anaemia in quranic schoolchildren aged 8 – 11 years due to vitamin B12 or folate deficiency is not detected, but there is a possibility of mixed deficiency in this age.

4- Causes of sickle cell anaemia were diagnosed on morphological basis.
5.3. Recommendations:

1- This high prevalence of anaemia among quranic schoolchildren in Khalawi Wad El Magboul highlights the need to develop pragmatic intervention programmes incorporating various strategies to improve dietary intake and bioavailability of iron; nutritional supplementation of iron and folic acid tablets and fortification of edible dietary items with iron.

2- More studies should be done in this area and the health authorities may interfere by health education and other interventional methods.

3- The most appropriate strategies would be integrated community and school based approach to reach adolescent population for prevention and control of iron deficiency anemia in quranic schoolchildren (Khalawi).

4- Encouragement of health status including health centers construction, and the support of Islamic endowments and the unlimited donation from philanthropists is highly recommended.
References


20. GJHS. Vol. 6 (1) June 2010; 9-13. Shams Musa & et al . Prevalence of Anaemia Among Schoolchildren in Eastern Sudan. Corresponding Author: Mamoun Magzoub Faculty of Medicine, University of Kassala, Kassala, Sudan.

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Appendix A

Some khalawi students waiting for pray
Khalawi rooms
Some khalawi students with their sheikh
Khalawi students reading session
Khalawi students preparing their woody plates for writing
Writing on their plates
Bathrooms used by students
Collecting plates in rest period
Clay containers (zeers) for drinking water
Praying and reading area
Appendix B

QUESTIONNAIR

Serial NO. (  )

1. Name………………………………… 2- Age…………………………
3- Residence…………………………… 4- Tribe………………………………
5. Number of family members………………………………………
7- Does the type of diet contains meat and vegetables? : Contains ( ). not containing ( ).
9- How do you meet your needs in case of inadequacy? …………………
12- Duration of staying at home each visit? One week ( ). More ( ).
13- Do you know about anaemia? 1. Yes ( ) 2. No ( )
14- What do you do if you have anaemia?
15- Have you got any chronic disease? 1. Yes ( ) 2. No ( )
16- Have you been hospitalized before? 1. Yes ( ) 2. No ( ).

Clinical examination:
1) Pallor: 1. Yes ( ) 2. No ( ).
3) Splenomegaly: 1. Yes ( ) 2. No ( ).