

1.1 General Introduction

Hemoglobin and Packed cell volume regarded as one of the important hematological parameters which used in the diagnosis and follow up of anemia and polycythemia.⁽¹⁾ Hemoglobin is life giving substance of every Red blood cells, The oxygen carrying component of the red cells, major organ of the human body depend on oxygenation for growth and function. This process is under control of Hemoglobin. Since hemoglobin is the carrier of oxygen to the tissues (the primary function of red cells), the hemoglobin and Hematocrit estimations represent a more functional assessment than does the red cells count.⁽²⁾ Hemoglobin and PCV are used in conjunction with total red blood cell count in calculating the red cell indices which includes mean cell volume, mean cell hemoglobin and Mean Cell Hemoglobin Concentration. Hb was estimated by a method depending on optical density and was expressed as mass/volume, or even as a percentage in relation to a rather arbitrary 'normal' that represented 100%. PCV was a measurement of the proportion of a column of centrifuged blood that was occupied by red cells. Now expressed as a decimal fraction representing volume/volume, it was initially expressed as a percentage⁽³⁾. Hemoglobin and mean cell hemoglobin concentration, which are used in morphological classification of anemia. Environmental, nutritional, genetics, ethnic, cultural diversity all of these factors affects on the result obtained for both tests.

1.2 Literature review

1.2.1 Hemoglobin

Hemoglobin is the molecule responsible for transport of oxygen under physiological condition with a molecular weight of 64.500 and consists of four poly peptide chains each carrying a heme group.

Heme consist of ring of carbon –hydrogen-nitrogen atoms called protoporphyrin IX with an atom of ferrous ion, heme component bind with one molecule of oxygen or carbon dioxide.

Globin is the protein portion of hemoglobin chain consists of α and β globins' chains 2alpha chains with 141 amino acid and two beta chains with 146 amino acid.

1.2.1.1 Heme Synthesis

Hemoglobin consists of two components : a protein chain (**globin**) and heme molecule. Heme consists of a protoporphyrin ring into which a ferrous iron atom has been inserted . The initial reaction in the heme synthesis pathway is the combination of glycine and succinyl coenzyme A (CoA) to form δ - amino levulinic acid (ALA), which is catalyzed by the enzyme aminolevulinic acid synthetase (**ALA synthetase**) . Pyridoxal 5'-phosphate (derived from pyridoxine, or vitamin B6), is an essential cofactor in the reaction. The final step in the pathway is insertion of the ferrous iron atom into protoporphyrin IX , 'Housekeeping' ALAS (ALAS1 is coded by a gene on chromosome 3, but, in erythroid cells erythroid –specific ALAS2 predominates and is coded on the X chromosome. ALA can be utilized for the formation of both purines and haem. Four molecules of porphobilinogen condense under the influence of porphobilinogen deaminase (PBGD) and uroporphyrinogen co-synthase to form the tetrapyrrole ring compound uroporphyrinogen III. The latter is converted to protoporphyrin IX. Finally, iron in the the influence of the enzyme ferrochelatase. Iron in haem has six coordinating valencies: four link the iron to nitrogen atoms in each pyrrole ring, whereas the remaining two link haem to histidine residues in the globin chain, the distal bond being unstable and easily replaced by oxygen to form oxyhemoglobin. The mitochondria play a major role in haem synthesis as they contain ALAS, coproporphyrinogen oxidase and ferrochelatase, the enzyme sequence from ALA to coproporphyrinogen being situated in the cytoplasm. The mitochondria are also the site of the citric acid cycle, which supplies succinate. The

mature red cell, which lacks mitochondria, is therefore unable to synthesize haem. A number of porphyrins are formed by side reactions during the synthesis of protoporphyrin. In the porphyrias, many of these compounds accumulate in the major sites of sites of haem synthesis the liver and the red cells

1.2.1.2 Control mechanisms in haem synthesis

As well as the developmental regulation of heme formation during erythroid differentiation, synthesis of heme and globin is coordinated so that there is no significant excess of either. Moreover, the entry of iron into the cell and its incorporation into haem are regulated so that the normal cell obtains sufficient iron for its needs, but not more. ALAS2 is rate limiting in the erythroid–haem biosynthetic pathway. Its activity reaches a peak in the polychromatic normoblast and then diminishes so that no activity is present in the mature cell. Interleukin 3 and erythropoietin act as inducers for transcription of mRNA for ALAS2 and PBGD, and this may be most important in the developmental regulation of haem production during erythroid differentiation.

1.2.1.3 Pathological alterations in heme synthesis:-

1.2.1.3.1 Porphyrrias

These are a group of inherited or acquired diseases, each characterized by a partial defect in one of the enzymes of heme synthesis. Increased amounts of the intermediates of haem synthesis accumulate, the disorders being classified by whether the effects are predominantly in the liver or the erythron .

1.2.1.3.2 Congenital erythropoietic porphyria

This is a very rare autosomal recessive disorder that is due to reduced uroporphyrinogen III synthase activity. Most patients are heteroallelic for mutations in the uroporphyrinogen III synthase gene. Large amounts of porphyrinogens accumulate, and their conversion by spontaneous oxidation to photoactive porphyrins leads to severe, and disfiguring, cutaneous photosensitivity and dermatitis, as well as a hemolytic anaemia with splenomegaly. Increased amounts of uroporphyrin and coproporphyrin, mainly type I, are found in bone marrow, red cells, plasma, urine and faeces. Ring sideroblasts have been found in the marrow in some cases but rarely in large numbers. The age of onset and clinical severity of the disease are highly variable, ranging from non-immune

hydrops fetalis to a later onset in which there are only cutaneous lesions. Treatment, including avoidance of sunlight and splenectomy to improve red cell survival, is only partially effective. High-level blood transfusions to suppress erythropoiesis (combined with iron chelation therapy) have been used to reduce porphyrin production sufficiently to abolish the clinical symptoms. Allogeneic bone marrow transplantation has been successful.

1.2.1.3.3 Erythropoietic protoporphyria

This is the most common erythropoietic porphyria and is usually caused by an autosomal dominant inherited deficiency of results in increased free (not Zn) protoporphyrin concentrations in bone marrow, red cells, plasma and bile. Bone marrow reticulocytes are the primary source of the excess protoporphyrin. This leaks from cells and is excreted in the bile and faeces. Molecular analysis of the ferrochelatase gene has revealed a variety of missense, nonsense and slicing mutations as well as deletions and insertions. The onset of the disease is usually in childhood. Expression of the gene is variable, and photosensitivity and dermatitis range from mild or absent to moderate in degree. There is little hemolysis, but a mild hypochromic anaemia may occur, and accumulation of protoporphyrins can occasionally lead to severe liver disease. Treatment is by the avoidance of sunlight; β -carotene may also diminish photosensitivity. Iron deficiency should be avoided as this may increase the amount of free protoporphyrin.

1.2.1.3.4 Porphyria cutanea tarda

This is the most common of the hepatic porphyrias and occurs worldwide. The incidence in the UK has been estimated at 2–5 per million.

Type I or ‘sporadic’ porphyria cutanea tarda (PCT) accounts for 80% of cases of PCT. The underlying metabolic abnormality is decreased activity of uroporphyrinogen decarboxylase (UROD) in the liver.

Type II disease is an autosomal dominant disorder caused by mutations in the UROD gene.

Type III disease is a rare familial form and appears to result from unknown inherited defects that affect hepatic UROD activity. There is a marked increase in porphyrins in liver, plasma, urine and faeces. In the urine, uroporphyrin and heptacarboxyl porphyrin predominate with lesser amounts of coproporphyrin and penta- and hexacarboxyl porphyrin. The disease is characterized by photosensitivity and dermatitis. It is precipitated in middle or later life, more often in men than women, by factors such as liver disease, alcohol excess or oestrogen therapy. A modest increase in liver iron is a common feature. Either the homozygous or heterozygous presence of the *HFEC282Y* and *H63D* mutations

may predispose to the development of PCT. Prevalence of the C282Y mutation is increased in both sporadic (type I) and familial (type II) PCT. In the UK, only homozygosity for C282Y (found in about 25% of patients) is significantly more common than in the general population (0.7%). In southern Europe, where C282Y is much less common, the H63D mutation is associated with PCT. Iron is known to inhibit uroporphyrinogen decarboxylase. Removal of the iron by repeated phlebotomy is standard treatment usually leading to remission

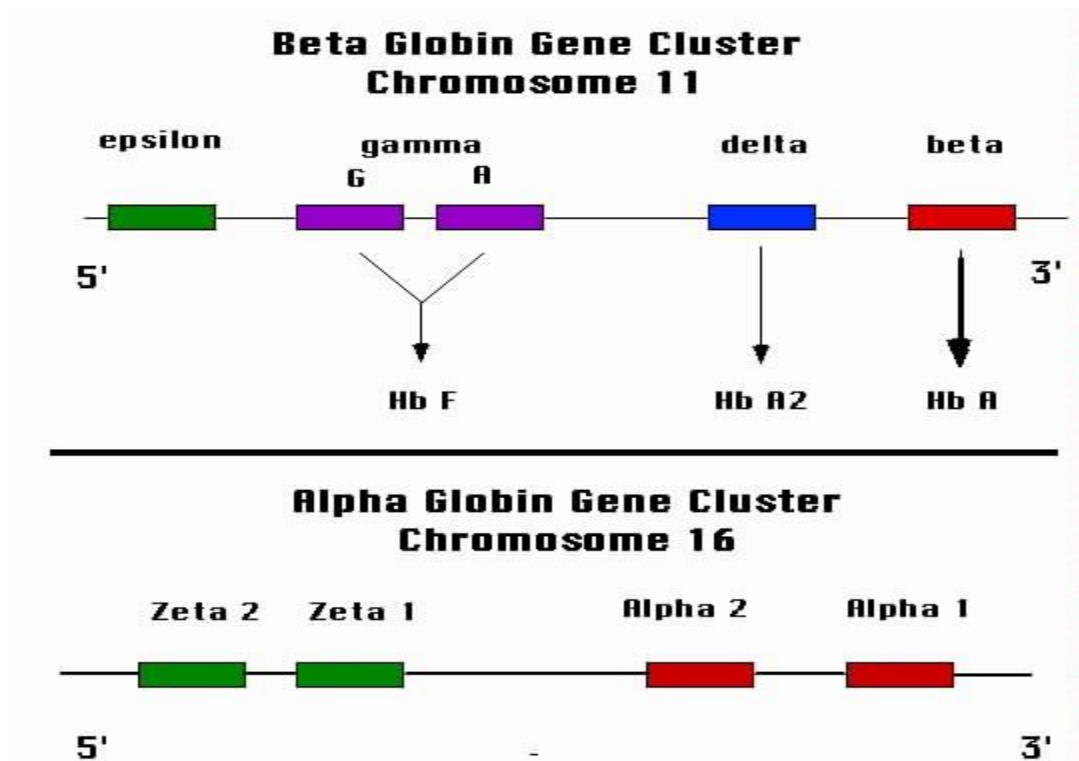
1.2.1.4 Globin synthesis:-

1.2.1.4.1 Transcription and processing

When a globin gene is transcribed, messenger RNA (mRNA) is synthesized from one of its strands by the action of RNA polymerase II. This process involves the interaction of a number of transcription factors, other proteins and, possibly, the LCR to form an initiation complex. The primary transcript is a large mRNA precursor that contains both introns and exons. While in the nucleus, it undergoes a number of modifications first, the introns are removed and the exons are spliced together. This too is a complex, multistep process involving several different proteins that constitute the spliceosome. The exon-intron junctions always have the sequence GT at their 5' end, and AG at their 3' end; if there is a mutation at these sites, normal splicing cannot occur. The mRNAs are modified at their 5' end by the addition of a CAP structure, and at their 3' end by the addition of a string of adenylic acid residues (poly-A). The processed mRNA now moves into the cytoplasm to act as a template for globin chain production.

1.2.1.4.2 Translation

Amino acids are transported to the mRNA template on carriers called transfer RNAs; there are specific transfer RNAs for each amino acid. The order of amino acids in a globin chain is determined by a triplet code, i.e. three bases (codons) code for a particular amino acid. The transfer RNAs also contain three bases, which are complementary to mRNA codons for particular amino acids. The transfer RNAs carry amino acids to the template, where they find the right position by codon-anticodon base pairing. The mRNA is translated from the 5' to the 3' end (left to right). The transfer RNAs are held in appropriate steric conformation with the mRNA by two subunits that make up the ribosomes. There are specific initiation (AUG) and termination (UAA, UAG, UGA) codons. When the ribosomes reach the termination codon translation ceases, the completed globin chain is released, and the ribosomal subunits fall apart and are recycled. Individual globin chains combine with haem, which is synthesized through a separate pathway, and with themselves to form definitive haemoglobin molecule



Chromosomal Inheritance of Beta ,AlphaGoblin Gene

Fig (1)

1.2.1.5 Function of Hemoglobin

Oxygen delivery is the principal purpose of the hemoglobin molecule. Additionally, it is a structure capable of pulling CO₂ away from the tissues, as well as keeping the blood in a balanced pH . The hemoglobin molecule loads oxygen on a one-to-one basis, one molecule of hemoglobin to one molecule of oxygen in the oxygen-rich environment of the alveoli of the lungs. Hemoglobin becomes saturated with oxygen, oxy hemoglobin, and has a high affinity for oxygen in this pulmonary environment, because the network of capillaries in the lungs makes the diffusion of oxygen a rapid process. As the molecule transits through the circulation, deoxy hemoglobin is able to transport oxygen and unload to the tissues in areas of low oxygen affinity. As hemoglobin goes through the loading and unloading process, changes appear in the molecule. These changes are termed **Allosteric** changes, a term that relates to the way hemoglobin is able to rotate on its axis, determine the action of salt bridges between the globin structures, and dictate the movement of 2,3-DPG. The hemoglobin molecule appears in a tense and a relaxed form. When tense ,hemoglobin in not oxygenated, 2,3-DPG is at the center of the molecule, and the salt bridges between the globin chains are in place.

When oxygenated, the relaxed form is in place; 2,3-DPG is expelled, salt bridges are broken, and the molecule is capable of fully loading oxygen.

1.2.1.6 Hemoglobin – Oxygen dissociation curve:

Oxyhemoglobin dissociation curve describes the relation between oxygen saturation, or content of hemoglobin and the oxygen tension at equilibrium.

Oxygen dissociation curve has a sigmoid shape under normal condition only upper part is used. Under influence of acidosis curve is shifted to the right and more oxygen is released.

Affinity of hemoglobin for oxygen and the de-oxygenation in the tissue is influenced by temperature, CO₂ concentration, 2,3- diphospho glycerate in the red cells.⁽⁴⁾

1.2.1.6.1 Oxygen supply to peripheral tissues is influenced by three mechanisms;-

- 1-The blood flow: which is controlled by heart beats volume and the constriction or dilatation of peripheral vessels.
- 2- Oxygen transport capacity which depends on the number of red blood cells and the hemoglobin concentration.
- 3- Oxygen affinity of hemoglobin.

In anemic patients, stroke volume of heart is increased heart beats faster in addition 2,3diphosphoglycerate concentration increase to facilitate the oxygen dissociation in tissues .compensation mechanisms can take different weeks.

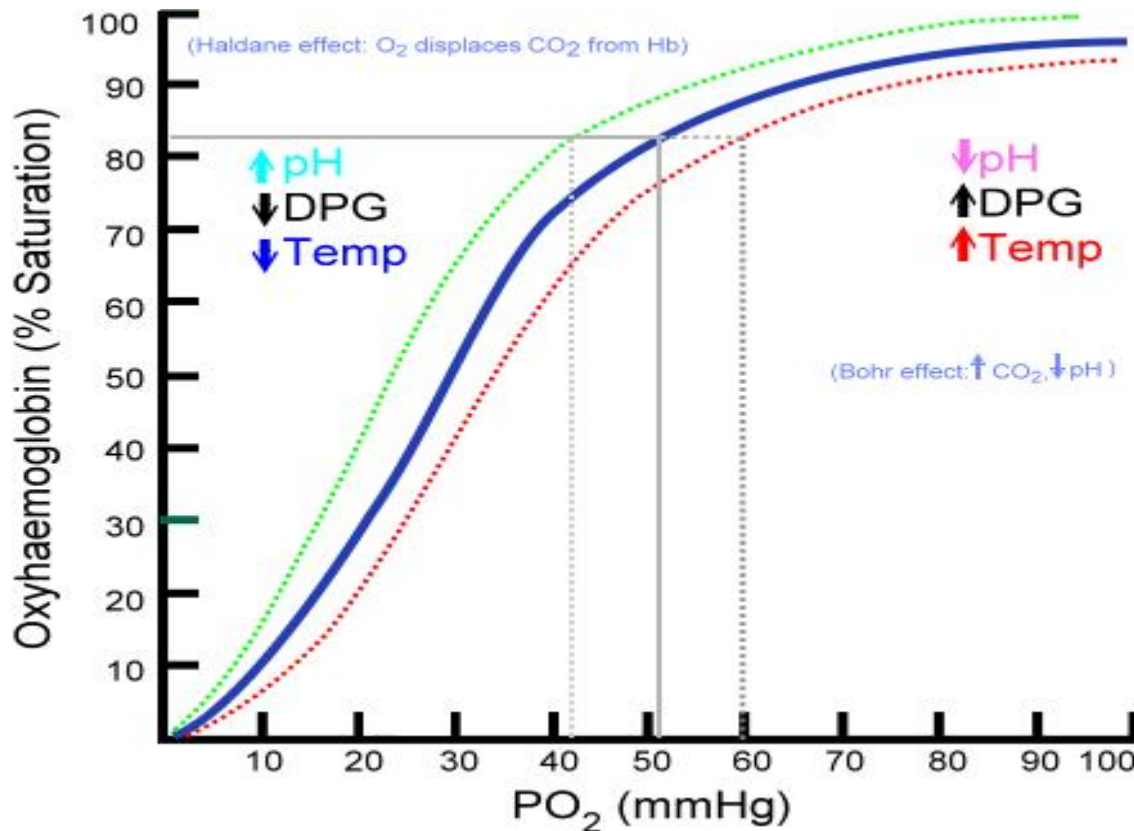


Fig (2): Hemoglobin ,Oxygen dissociation Curve

1.2. 1.7 Types of hemoglobin:-

1.2.1.7.1 Adult hemoglobin:-

Consist of two types:-

HB-A:- comprise 97% of adult hemoglobin consist of $\alpha_2\beta_2$ chains . Detected in the fetus with small amount at 8 weeks of life, during the first month of post natal life HB-A is completely replaced HB-F and small adult level is fully established by six month.

HB-A2 :- comprise minor of adult hemoglobin consist of alpha two- delta two chains .

Present in very small amount at birth and reach the adult level (1,5- 3,5) during first year.

Elevated in :- Thalassemia , Megaloblastic anemia .

Reduced in :- iron deficiency anemia.

1.2.1.7.2 Fetal hemoglobin;-

Consist of;-

HB-F :- in intra uterine life up to birth with structural formula $\alpha_2\gamma_2$. It is accounts about 70%- 90% of total hemoglobin.

Elevated in :- Hemoglobinopathies . thalassemia , a plastic anemia , sidroblastic anemia ,megaloblastic anemia , paroxysmal nocturnal hemoglobinuria .

Bart HB:- found in cord blood with formula γ_4 .Both HB Bart and Portland are increased in the cord blood of neonate s with α - Thalassemia .

1.2.1.7.3 Embryonic hemoglobin:-

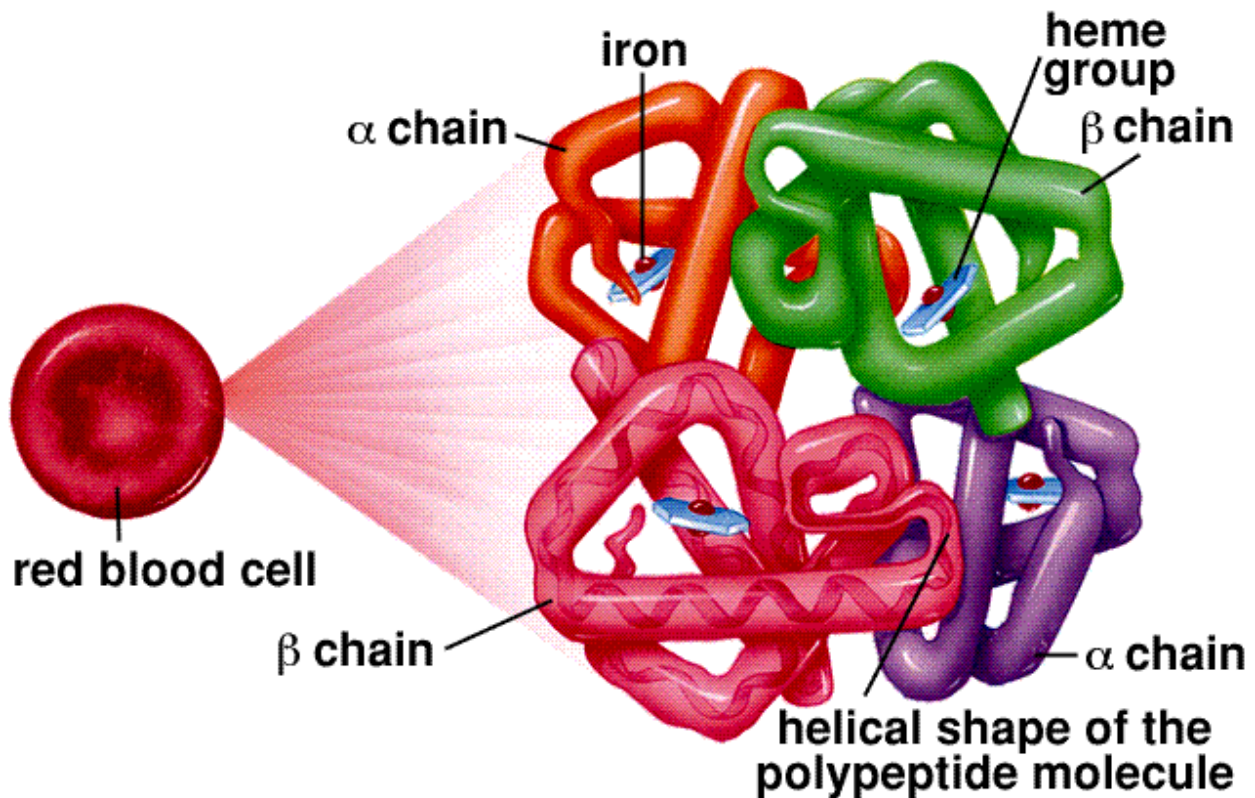
Consist of;-

Grower -1 ($\zeta_2\epsilon_2$);- two zeta two epsilon .

Grower -2($\alpha_2\epsilon_2$);- two alpha two epsilon.

HB Portland ($\zeta_2\gamma_2$): two zeta two gama.

Hemoglobin Molecule



Hemoglobin Structure fig (3)

1.2.1.7.4 Hemoglobin variants [pigments]

Normal hemoglobin is a highly stable protein, which can be converted to cyanmethemoglobin, a colored pigment. This conversion is the basis for most of the colorimetric procedures used to measure hemoglobin, and it depends on a versatile and viable hemoglobin compound. Hemoglobin that are physiologically abnormal have a higher oxygen affinity and produce conditions that are usually toxic to the human body. These abnormal hemoglobins include; methemoglobin, sulfhemoglobin, and carboxy hemoglobin. The amounts of any of these abnormal hemoglobins in the bloodstream can be potentially fatal. Often, the production of abnormal hemoglobins results from accidental or purposeful ingestion or absorption of substances, drugs, and so on that are harmful. At times, abnormal hemoglobins are produced as a result of inherited defects. In the abnormal hemoglobin methemoglobin, iron has been oxidized to the Fe^{3+} state,

which is no longer capable of binding oxygen. Methemoglobin builds up in the circulation and if the level is above 10%, individuals appear **cyanotic**, having a blue color, especially in the lips and fingers. Aniline drugs and some antimalarial treatments may induce a methemoglobinemia in individuals who are unable to reduce methemoglobin. Hemoglobin M, an inherited condition arising from an amino acid substitution, may also result in cyanotic conditions. Carboxyhemoglobin levels are increased in smokers and certain industrial workers. As a hemoglobin derivative, carboxyhemoglobin has an affinity for carbon monoxide that is 200 times greater than for oxygen; therefore, no oxygen is delivered to the tissues. For this reason, carbon monoxide poisoning, either deliberate or accidental, is efficient and relatively painless. Sulfhemoglobin can be formed on exposure to agents such as sulfonamides or sulfa containing drugs. The affinity of sulfhemoglobin for oxygen is 100 times lower than that of normal hemoglobin. It may be toxic at a very low level.

1.2.1.8 Defect in HB

Defect in HB result from ;-

- 1- Synthesis of abnormal hemoglobin .
- 2- Reduced the rate of synthesis of normal α or β globin chains.

Some group of syndrome which arise from synthesis of α or β chains with an amino acid substitutions , The clinically most important abnormality is sickle cells anemia HB-C .D,andE are also common .

Unstable hemoglobin is rare and cause chronic hemolytic anemia of varying severity with intravascular hemolysis . Abnormal HB may also cause familial polycythemia or congenital methemoglobinemia.

1.2.1.8.1Thalassemia ;-

This are a heterogeneous group of genetic disorders which result from a reduced the thalassaemias rate of synthesis of α or β chains .The thalassaemias are classified as α or β , depending on which pair of globin chains is synthesized Inefficiently⁽⁸⁾. into:

- α .thalassemia.
- B .thalassemia
- β .thalassemia.
- HB lepre⁽⁷⁾

1.2.1.8.1.1 Alpha thalassems;- There are usually caused by gene deletions. There are normally four copies of the α globin gene and the clinical severity can be classified according to the number of genes that are missing into:-

- Alpha Thalassemia major (one alpha gene deletion).
- Alpha thalassemia minor (two alpha gene deletions).
- b H (three alpha gene deletions).
- Hydrops Fetal (four alpha gene deletions).

1.2.1.8.1.2 Beta Thalassemia ;-

This condition occurs on average in one of four offspring if both parents are carriers of the beta thalassemia trait. Either no beta chain or small amounts are synthesized. Excess α chain precipitates in the erythroblast and immature red cell causing the severe ineffective erythropoiesis and hemolysis that is typical of the disease.

1.2.1.8.1.3 $\delta\beta$ Thalassemia ;-

This envelope failure of both δ and β chains.

1.2.1.8.1.4 Hb Lepore:-

This is abnormal hemoglobin caused by an unequal crossing over of the β and δ gene to produce a polypeptide chain consisting of δ chain at its amino end and β chain at its carboxyl end.

1.2.1.8.1.5 Hereditary persistence of fetal Hb:-

These are heterogeneous groups of gene conditions caused by deletion or cross overs affecting the production of β and γ chains or in non-deletion form by point mutations upstream from the γ globin gene.

1.2.1.8.2 Sickle cell anemia

Group of hemoglobin disorders in which sickle beta globin is inherited.

Homozygous sickle cell anemia is the most common (HbSS) whilst double heterozygous conditions (HbSC) and (HbSB) are also causing sickling disease.

HbS $\alpha_2\beta_2$ is in soluble form crystal when exposed to low oxygen tension.

The sickle beta globin results from substitution of valine for glutamic acid at position 6 in β chain.

1.2.1.8.2.1 HbC disease:-

caused by substitution of lysine for glutamic acid in the beta globin chain.

1.2.1.8.2.2 Hb D disease :-

substitution of glycine for glutamic acid.

1.2.1.8.2.3 Hb E disease :-

substitution of lysine for glutamic acid.

1.2.1.9 Pre analytical Variation in hemoglobin concentration :-

1.2.1.9.1 Variation IN THE Red BLOOD Cells Components':

There is considerable variation in the red blood cell count (RBC) and hemoglobin concentration (Hb) at different periods of life and there are also transient fluctuations, the significance of which is often difficult to assess. At birth and at any period subsequently, the RBC is high immediately after birth. Values for Hb >200 g/l, RBC higher than $6.0 \times 10^{12}/l$ and a haematocrit (Hct) over 0.65 are encountered frequently when cord clamping is delayed and blood from the placenta and umbilical artery re-enters the infant's circulation.

After the immediate postnatal period, the Hb falls fairly steeply to a minimum by about the 2nd month. The RBC and Hct also fall, although less steeply. Cells may become microcytic with the development of iron deficiency. -The Hb and RBC increase gradually through childhood to reach almost adult levels by puberty. However, in a health survey of apparently normal men and women in Britain, mean Hb values of 145 g/l for men and 128 g/l for women have been reported. The lower normal limits for Hb (i.e. 2SD below the mean) are usually taken as 130 and 120 g/l, respectively, but in some apparently normal men and women lower limits of 120 and 110 g/l, respectively, have been noted. Statistically, at least 1% of a normal population have levels more than 3SD below the mean, but in some studies there have been considerably larger numbers. It is possible that some have nutritional deficiencies, especially iron deficiency, without clinical effects. The levels in women tend to be significantly lower than those of men. Apart from a hormonal influence on hemopoiesis, iron deficiency is likely to be a factor influencing the difference; the extent to which menstrual blood loss is a significant factor is not clear because a loss of up to 100 ml of blood with each period may lead to iron depletion without causing anaemia. There may be ethnic differences in Hb. A major 6-year survey in the USA has shown that in socially comparable populations the Hb in Black Americans is 5–10 g/l lower than their White counterparts at all ages and as much as 20 g/l lower in the first 2 years of life.

1.2.1.9.2 Pregnancy:-

In normal pregnancy, there is an increase in erythropoietic activity. However, at the same time, an increase in plasma volume occurs, and this results in a progressive decrease in Hb, Hct and RBC. The level returns to normal about a week after delivery. There is a slight increase in MCV during the 2nd trimester. Serum ferritin decreases in early pregnancy and usually remains low throughout pregnancy even with supplementary iron is given.

1.2.1.9.3 The Eldery:-

In healthy men and women, Hb, RBC, Hct and other red cell indices remain remarkably constant until the 6th decade. Anemia becomes more common in those older than 70–75 years and is associated with poor clinical outcomes due to poorer cognitive status, increased frailty and an elevated risk of hospitalization and of complications during hospitalization. In the elderly, the difference in Hb between men and women is less than in younger subjects, so that a difference of 20 g/l in younger age groups is reduced to 10 g/l or less in the elderly. There is a concomitant increase in serum iron in women, although serum ferritin levels remain higher in men than in women. Factors which contribute to the lower Hb in the elderly include renal insufficiency, inflammation, testosterone deficiency, diminished erythropoiesis, stem cell proliferative decline and

myelodysplasia. Moderate or severe anemia should never be attributed to ageing *per se* until underlying disease has been excluded; however, a significant number of elderly subjects with anemia have no identifiable clinical or nutritional causes.

1.2.1.9.4 Posture :-

There is a small but significant alteration in the plasma volume with an increase in hemoglobin and Hct as the posture changes from lying to sitting, especially in women. Conversely, changing from walking to lying down results in a 5–10% decrease in the Hb and Hct.

Thus, subjects should rest for 5–10 min before their blood is collected. The difference in position of the arm during venous sampling, whether dependent or held at atrial level, can also affect the Hct. These aspects highlight the relevance of using a standardized method for blood collection, although this is not necessarily practicable in routine practice.

1.2.1.9.5 Diurnal and Seasonal Variation :-

Changes in Hb and RBC during the course of the day are usually slight, about 3%, with negligible changes in the MCV and MCH. However, variation of 20% occurs with reticulocytes. Studies of diurnal variation of serum erythropoietin have shown conflicting results. Pronounced, but variable, diurnal variations are seen in serum iron and ferritin and in patients taking iron-containing supplements.

-It has been suggested that minor seasonal variations also occur, but the evidence for this is conflicting.

1.2.1.9.6 Altitude:-

The effect of altitude is to reduce plasma

1.2.1.9.7 Exercise :-

Optimal athletic performance depends on proper function of many organs, including the blood. Several haematological parameters can affect or be influenced by physical activity including blood cells and coagulation mechanisms. For example endurance athletes may develop so-called 'sports anemia', which is thought to be the result of increased plasma volume. Increasing oxygen delivery by raising the haematocrit is a simple acute method to improve athletic performance. Legal means of raising the haematocrit include altitude training and hypoxic tents. Illegal means include blood doping and the administration of erythropoietin (EPO). Endurance athletes may also have decreased levels of serum iron and ferritin, possibly associated with loss of iron in sweat. Conversely, in sprinters who require a short burst of very strenuous muscular activity, there is a transient increase in RBC by $0.5 \times 10^{12}/l$ and in Hb by 15 g/l, largely because of reduction in plasma volume and to a lesser extent the re-entry into the circulation of cells previously sequestered in the spleen. The effects of exercise must be distinguished

from a form of hemolysis known as ‘runner’s anemia’ or ‘march hemoglobinuria’, which occurs as a result of pounding of the feet on the ground.

1.2.1.9.8 EFFECTS OF SMOKING:-

Both active and passive cigarette smoking have a significant effect on many hematological normal reference values. Some effects may be transient and their severity varies between individuals as well as by the number of cigarettes smoked. Smoking 10 cigarettes a day results in slightly higher Hb, Hct and MCV. This is probably at least in part a consequence of the accumulation of carboxy hemoglobin in the blood together with a decrease in plasma volume. After a single cigarette, the carboxyhemoglobin level increases by about 1%, and in heavy smokers the carboxy hemoglobin may constitute 4–5% of the total Hb. Smoking may be associated with polycythaemia. The leucocyte count increases, largely as a result of an increase in the neutrophils and neutrophil function may be affected. Smoking may also cause an increase in CD4-positive lymphocytes and total lymphocyte count. Smokers tend to have higher platelet counts than nonsmokers, but the counts decrease rapidly on cessation of smoking. Studies of platelet aggregation and adhesiveness have given equivocal results, but there appears to be a consistent increase in platelet turnover with decreased platelet survival and increased plasma β -thromboglobulin. Elevated fibrinogen concentration (with increased plasma viscosity) and reduced proteins have been reported, but smoking does not seem to have any consistent effects on the fibrinolytic system.⁽⁹⁾

1.2.1.10 Method of Hemoglobin estimation:

1.2.1.10.1 Hemoglobinometry

Hemoglobin concentration of a solution may be estimated by measurement of its color by its power of combining with oxygen or carbon monoxide or by its iron content, but again the method is impractical for routine purposes.

1.2.1.10.2 Spectrophotometer Method

Two methods are commonly used are:-

1.2.1.10.2.1 Hemoglobin cyanide (HICN; cyanomethhemoglobin method)

Major advantages of HICN method is availability of stable and reliable reference preparation. But the use of potassium cyanide has been viewed as potential hazard, alternative non hazardous reagent that have been proposed are sodium azide and sodium laurylsulphate which convert hemoglobin to hemoglobin azide and hemoglobin sulphate, respectively they are used in some automated systems.

1.2.1.10.2.2 Oxy hemoglobin (HbO₂) Method

Its simplest and quickest method for general use with a photometer, its disadvantages is that it is not possible to prepare stable HbO₂ standard, so the

calibration of this instrument should be checked regularly using HICN reference solution or standard of preserved blood or hemolysate. The reliability of the method is not affected by moderate increase in the plasma protein.

1.2.1.10 .3 Sahle's acid hematin Method

Which is less accurate because the color develops slowly, is unstable and begins to fade almost immediately after reaching its peak, the alkaline hematin method gives a true estimate if carboxy hemoglobin is present, plasma protein and lipid has little effect on the development of the color, although they cause turbidometry.

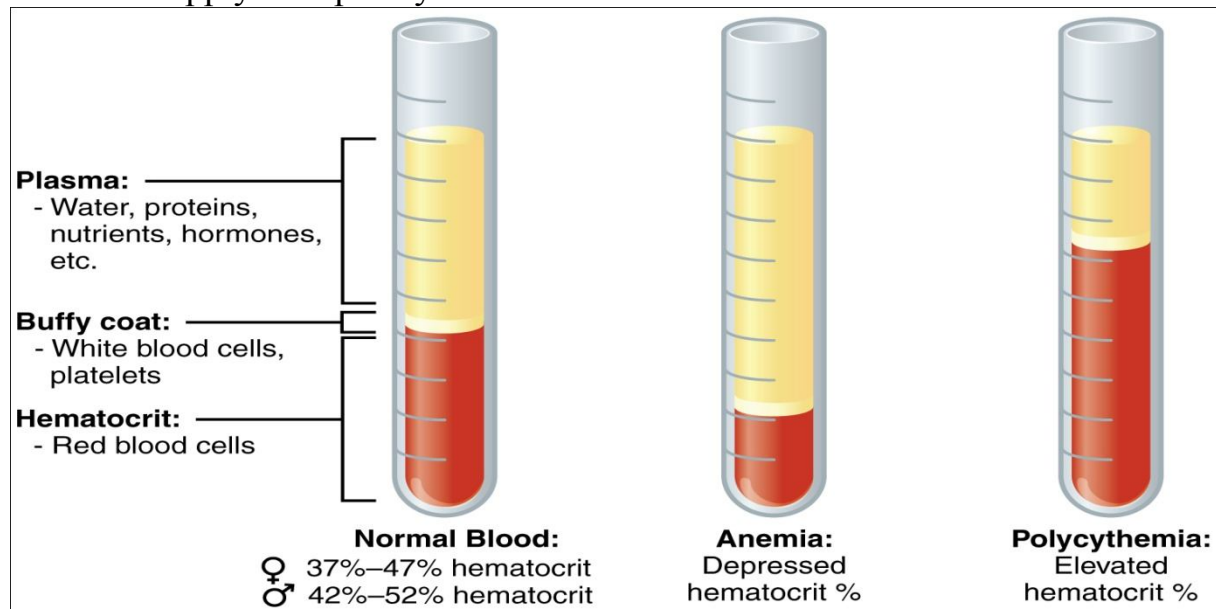
The original method was more cumbersome and less accurate than HICN or HbO₂ method, but a modified method has been developed in blood which is diluted in alkaline solution with non-ionic detergent and read in spectrophotometer at an absorbance of 575 nm against standard solution of chlorohematin.

1.3 Packed cell volume or Hematocrit:

1.3.1 Definition:-

-The packed cell volume (PCV) can be used as a simple screening test for anemia as a reference method for calibrating automated blood count systems and as a rough practical hematology.

-A guide to the accuracy of hemoglobin measurements. The PCV_1000 is about three times the Hb expressed in g/l. In conjunction with estimations of HB and RBCs it can be used in the calculation of red cells indices. However used in under-resourced laboratories may be limited by the need for a specialized centrifuge and a reliable supply of capillary tubes.



Comparison of PCV in normal blood and anemic, polycythemic blood
Fig (4)

1.3.2. Accuracy of Microhaematocrit

The microhematocrit method has an adequate level of accuracy and precision for clinical utility. However, attention must be paid to a number of factors that may produce an inaccurate result.

1.3.2.1 Anticoagulant

K2-EDTA is recommended, because K3-EDTA causes shrinking of the red cells, reducing the PCV by about 2%. Anticoagulant concentration in excess of 2.2 mg/ml may also cause a falsely low PCV as a result of cell shrinkage.

1.3.2.2 Blood Sample

Because the PCV gradually increases with storage, the test should be performed within 6 h of collecting the blood sample, but a delay of up to 24 h is acceptable if

the blood is kept at 4°C. Failure to mix the blood sample adequately will produce an inaccurate result. The degree of oxygenation of the blood also affects the result because the PCV of venous blood is 2% higher than that of fully aerated blood (which has lost CO₂ and taken up O₂). To ensure adequate oxygenation and sample mixing, the free air space above the sample should be more than 20% of the container volume. Capillary Tubes Variation of the bore of the tubes may cause serious errors if they are not within the narrow limits of defined specifications

that should be met by manufacturers: length 75 ± 0.5 mm; internal diameter 1.07–1.25 mm; wall thickness 0.18–0.23 mm; and bore taper not exceeding 2% of the internal diameter over the entire length of the tube.

1.3.2.3 Centrifuge

Centrifuges should be checked at intervals (at least annually) by a tachometer for speed and by a stopwatch for timer accuracy. Efficiency of packing should also be tested by centrifuging samples of normal and polycythaemic blood for varying times from 5 to 10 min to determine the minimum time for complete packing of the red cells.

1.3.2.4 Reading

The test should be read as soon as possible after centrifugation because the red cells begin to swell and the interface becomes progressively more indistinct. To avoid errors in reading with the special reading device, a magnifying glass should be used. White cells and platelets (the buffy coat) must be excluded as far as possible from the reading of the packed red cell volume. If a special reading device is not available, the ratio of red cell column to whole column can be calculated from measurements obtained by placing the tube against arithmetic graph paper or against a ruler.

1.3.2.5 Plasma Trapping

The amount of plasma trapped between red cells, especially in the lower end of the red cell column, and red cell dehydration during centrifugation generally counterbalance each other and the error caused by trapped plasma is usually not more than 0.01 PCV units. Thus, in routine practice, it is unnecessary to correct for trapped plasma, but if the PCV is required for calibrating a blood cell analyzer or for calculating blood volume, the observed PCV should be reduced by a 2% correction factor after it has been centrifuged for 5 min or for 10 min with polycythemic blood. It is, however, preferable to use the surrogate reference method. Plasma trapping is increased in macrocytic anemias, spherocytosis, thalassemia, hypochromic anemias and sickle cell anemia; it may be as high as 20% in sickle cell anemia if all the cells are sickle cells. Basic hematological techniques International Council for Standardization in Hematology⁽⁹⁾

1.4 Previous studies:-

The reference value of the same study in south Africa normal value of hemoglobin was (11.8 -16.8) g/dl ,PCV was(37-49%)

The reference value in the same study in Turkish normal value of hemoglobin 14-15 % g/dl and PCV 43-46%.

In previous study done in 1997 in Sudan In Khartoum state in hematological parameters find that Hb and PCV value of adult Sudanese are low than

European , Japan and South Africa .

In this study found that the Hb is 11.0 -16.0 g/dl in males and 10.1 - 15.5 g/dl in females . and PCV value is 30.0 – 48.4% in males and 28.4 – 47.1 % In females .

1.5 Rational

Environmental, gender, socioeconomic status and many other factors effect on hemoglobin and packed cell volume results. This research is aimed to study mean hemoglobin and packed cell volume among student of Sudan University of science and technology to see if there is a differences in the results of these two essential laboratory tests between males and female, in other hand, to compare with international reference values for the same age group.

1.6 Objectives

General Objective:-

To estimate of Hb and PCV among student of Sudan University of science and technology.

Specific Objectives:-

1. To estimate of hemoglobin level by HiCN method.
2. To measure of packed cell volume by micro hematocrit method.

Materials and Methods :-

2.1 Study design: - This is a descriptive cross sectional study carried out to determine mean value of hemoglobin and PCV among student in Sudan University for science and Technology.

2.2 Study area: - Conducted in Sudan University Western and Southern part.

2.3 Study population: - 500 student from Sudan university of science and technology who agree to participate in this research.

2.4 Tools and Data Collection: - EDTA anti-coagulated blood sample used to evaluate HB and PCV. Also questionnaire was used to obtain information about student.

2.5 Selection Criteria:-

2.5.1 Inclusion Criteria: - All students in Sudan University who agree to participate in this study and apparently look healthy.

2.5.2 Exclusion Criteria: - Student with history of anemia, medication uptake, Smokers, and a heavy menstruation Girls.

2.6 Ethical considerations:-Participant were informed in there simple language about the research study and its benefits and method of sample collection.

2.7 Data Analysis:- SPSS soft program used to obtain Mean ,Stander Deviation , P value by in dependent T-test , Person Correlation .

2.8 Methods

2.8.1 Method of Sample collection:-

2.8.1.1 Requirements:- K₂EDTA containers , Cotton , 70% alcohol , Syringes , Tourniquet , Capillary tubes , Drabkin's Reagent , Glass tubes , Color meter , Micro hematocrit centrifuge , Reading device , HB chart .

2.8.1.2 Procedure:- donor allow to sit on suitable chair , Then do disinfection for the puncture site by using 70 % alcohol, applying the tourniquet and collect 2.5 ml from superficial vein .

2.9 Method of hemoglobin estimation:-

2.9.1 HiCN method:

2.9.1.2 Principle ;-Whole blood is diluted 1 in 201 in drabkin solution which contain potassium ferric cyanide and potassium cyanide . Red blood cells hemolyzed and HB is oxidized by ferric cyanide into meth hemoglobin, this is converted to HICN by cyanide read by colorimeter at 540 nm.

2.9.1.3 Procedure:

- Prepare three test tubes [5ml] for blank .Test, Stander.
- Place 4, 8 ml of D.W in each test tubes .Then add two drops from drabkin's reagent in each tube.
- Add 20µm from the stander solution in the stander tubes.
- Add 20µm from the sample in the test tube

wait for 5minute then read absorbance of Test and Stander tubes against the blank. by using filter at 540 nm.

2.10 Estimation of PCV:

2.10.1 Microhaematocrit method:

2.10.1.1 Requirements:

Capillary tubes , Sealing agent , Micro Hematocrit centrifuge , Reading device Cotton .70% alcohol , sterile syringe .

2.10.1.2 principle

Blood sample is centrifuged in specific tubes , for minimum time to obtain maximum package .

Distance occupied by packed RBCs is measured and expressed as percentage against the whole blood in the tube .

2.10.1.3 procedure

The micro haematocrit method is carried out on blood contained in capillary tubes 75 mm in length and having an internal diameter of about 1 mm .The tubes may be Plain for use with anticoagulated blood samples or coated inside with 1 IU heparin for the direct collection of capillary blood. The centrifuge used for the capillary tubes provides a centrifugal force of c12 000 g and 5 min centrifugation results in a constant PCV . When the PCV is > 0.5 , it may be necessary to centrifuge for a further 5 min.

-Allow blood from a well-mixed specimen, or from a free flow of blood by skin puncture, to enter the tube by capillarity , leave at least 15 mm unfilled . Then seal the tube by a plastic seal (e.g. Cristaseal , Hawksley, Lancing , Sussex) . Sealing the tube by heating is not recommended because the seals tend to be tapered and there is the likelihood of lysis . After centrifugation for 5 min , measure the proportion of cells to the whole column (i.e. the PCV) using a reading device.

2.11 Data Analysis

2.11.1 Analysis of Frequency:-

out of 500 individuals included in this study 251 males and 249 females ,all of them are between the age of 17-25 years ,from Sudan University of Science & Technology students.

2.11.2 Data Processing:-

After analysis of samples under the study its performed the values were subjected to statistical treatment ,This treatment include partitioning of the

values into appropriate groups gender and calculating the mean , stander deviation for Hb and PCV for both males and females.

3.1 Demographic Data

Table-1: Distribution of study population according to genders

Gender	Frequency	Percentage %
Male(n =251)	251	50.2
Female (n=249)	249	49.8

Table-2: Mean of HB , PCV in males and females

Parameter		mean	S.D	Range	P.value
HB	Male	12.9	1.8	9.3- 16.5 g/dl	.0000
	Female	11.2	1.6	8- 14.4 g/dl	.0000
PCV	Male	46	4.5	37- 55 %	.000
	Female	40	4.6	30.8 – 49.2 %	.000

3.2 Hemoglobin Measurement:-

Table -3 : The mean of Hb according to the gender

Hb g/dl	Male (n=251)	Female (n=249)
Mean	12.9	11.2
SD	1.8	1.6

3.3 Hematocrit (Packet Cell Volume)

Table-4 :The Mean of Hematocrit (PCV)according to gender

Hct (PCV%)	Male(n=251)	Female (n=249)
Mean	46	40
S.D	4.5	4.6

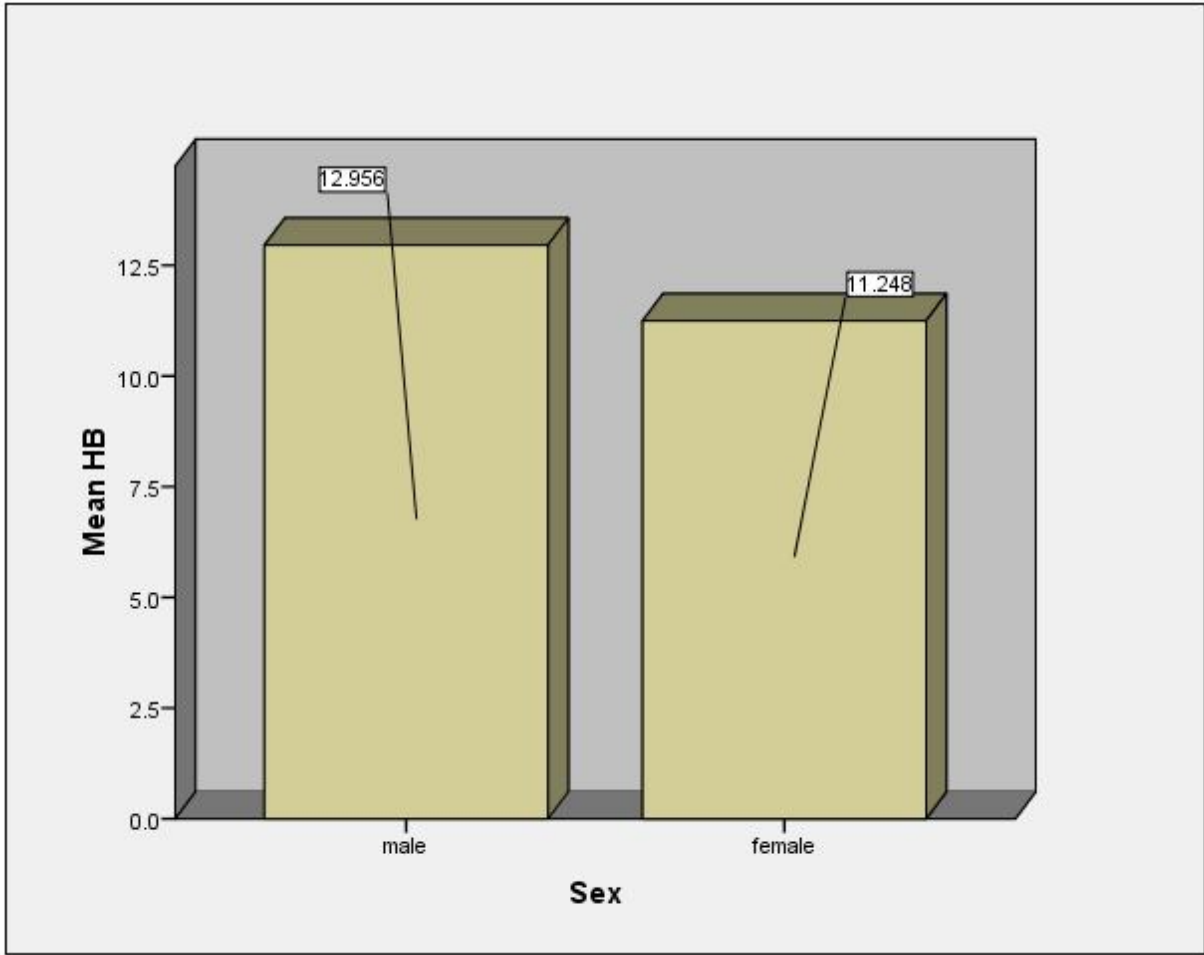


Fig (3-2) Hb in Study Population

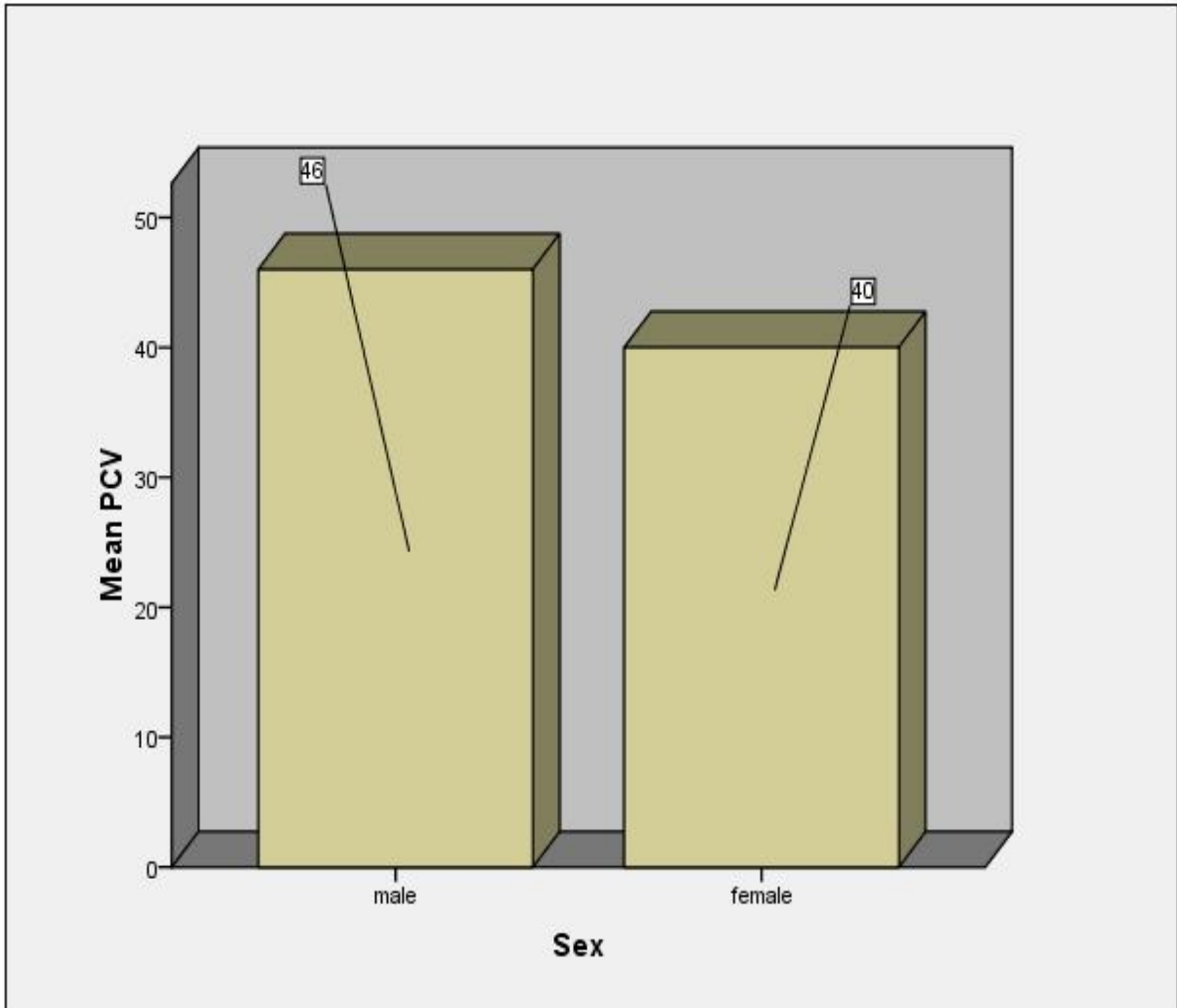


Fig (3-1) PCV in Study Population

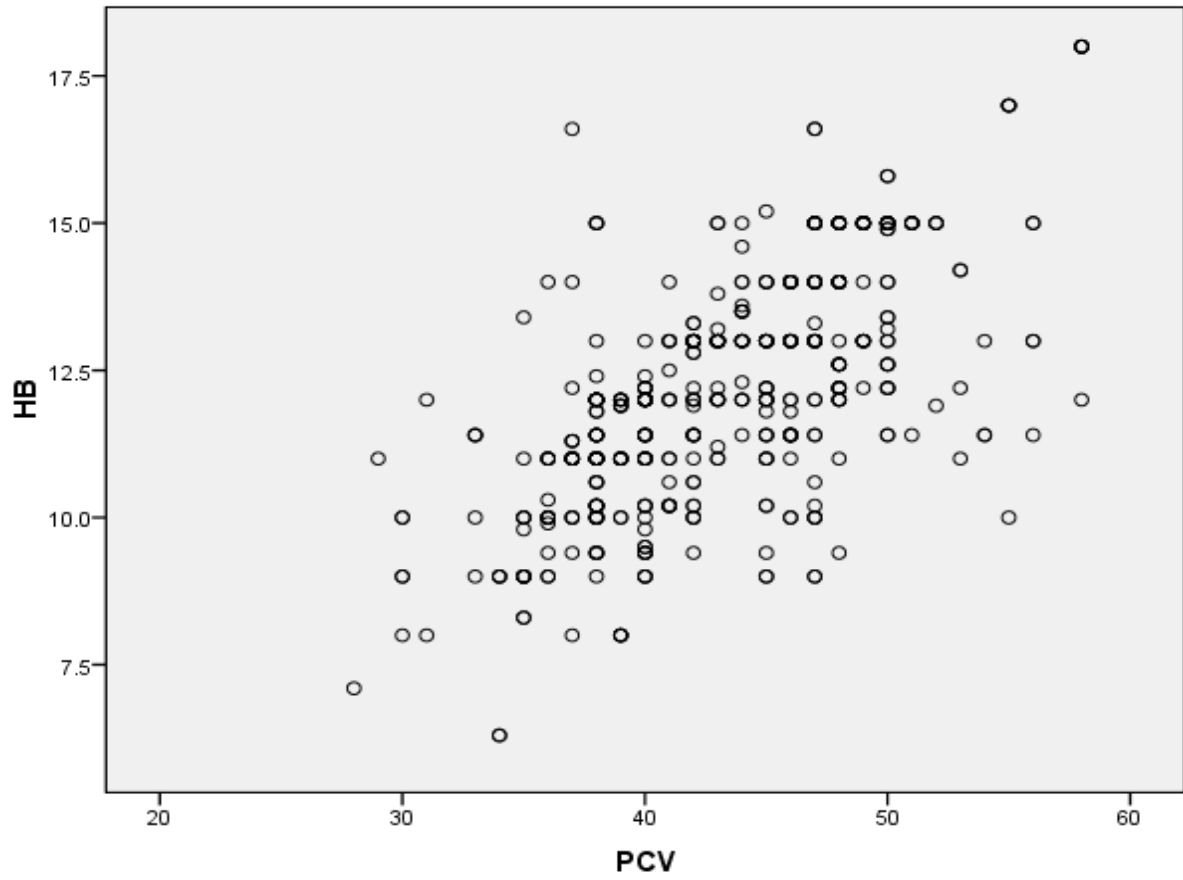


fig (3-3) Correlation between PCV and Hb

4. Discussion

4.1 Discussion

This study was carried out in Sudan university of science and technology , during the period from November 2013 to February2014. Five hundred student were participated in this study,(251) were males (51%) and (249) were females (49%) .

The result of this study show that mean of hemoglobin concentrations in males was 12.9 g/dl and in females it was 11.2 g/dl , with P. value 0.000

Indicating that there was a significant difference in mean Hb values between males and females .

Mean of PCV value was 46% - 40 % for males and females respectively , P.value 0.00 0 indicating that where a significant differences in mean PCV between males and females .

The result of this study is compared with other previous study done in Sudan in 1997 in Khartoum state which find that Hb and PCV values of adult Sudanese are low than European , Japan and south Africa. Our study is agree with this result .

Other study done in south Africa show that the normal value of hemoglobin was (11.8 -16.8) g/dl ,PCV was(37-49%). Also other study done in Turkish show that the normal value of hemoglobin 14-15 % g/dl and PCV 43-46%.

There is a difference between above study due to difference in climate , nutritional and ethnic factors.

4.2 Conclusion

This study showed that Hb and PCV values among Student in University of science and technology was significantly decreased in compare with international reference values for the same age group , this may be due to nutritional status , environmental , generics , ethnics, and cultural diversity factors .

4.3 Recommendations

Result of this study can be base line data for hematological parameter of Sudanese student.

This kind of study should be applied into larger area.

Increase awareness in society about importance of suitable diet.

Our research done from adult healthy population hence be suggest other may fellows to study from children.

Other studies done from different state university .

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Questionnaire

بسم الله الرحمن الرحيم

Sudan University for Science and Technology

College of Medical Laboratories Sciences

Measurement of Hemoglobin and Haematocrit among apparently healthy Student in Sudan University

Sample Number ()

Personal Data

Age

Sex

Residences

Medical History

Family history of disease

Cause of Disease

Duration of disease

Under treatment intake

- Iron supplement ()
- Folic acid ()

براءة أخلاقية

الاسم / الرقم-----

سوف يتم اخذ عينة دم من الوريد بواسطة حقنة طعن معقمة وذلك بعد تعقيم منطقة اخذ العينة بواسطة مطهر وجميع الأدوات المستخدمة معقمة ومتبع فيها جميع وسائل السلامة المعملية وليس هناك اثار جانبية للعملية

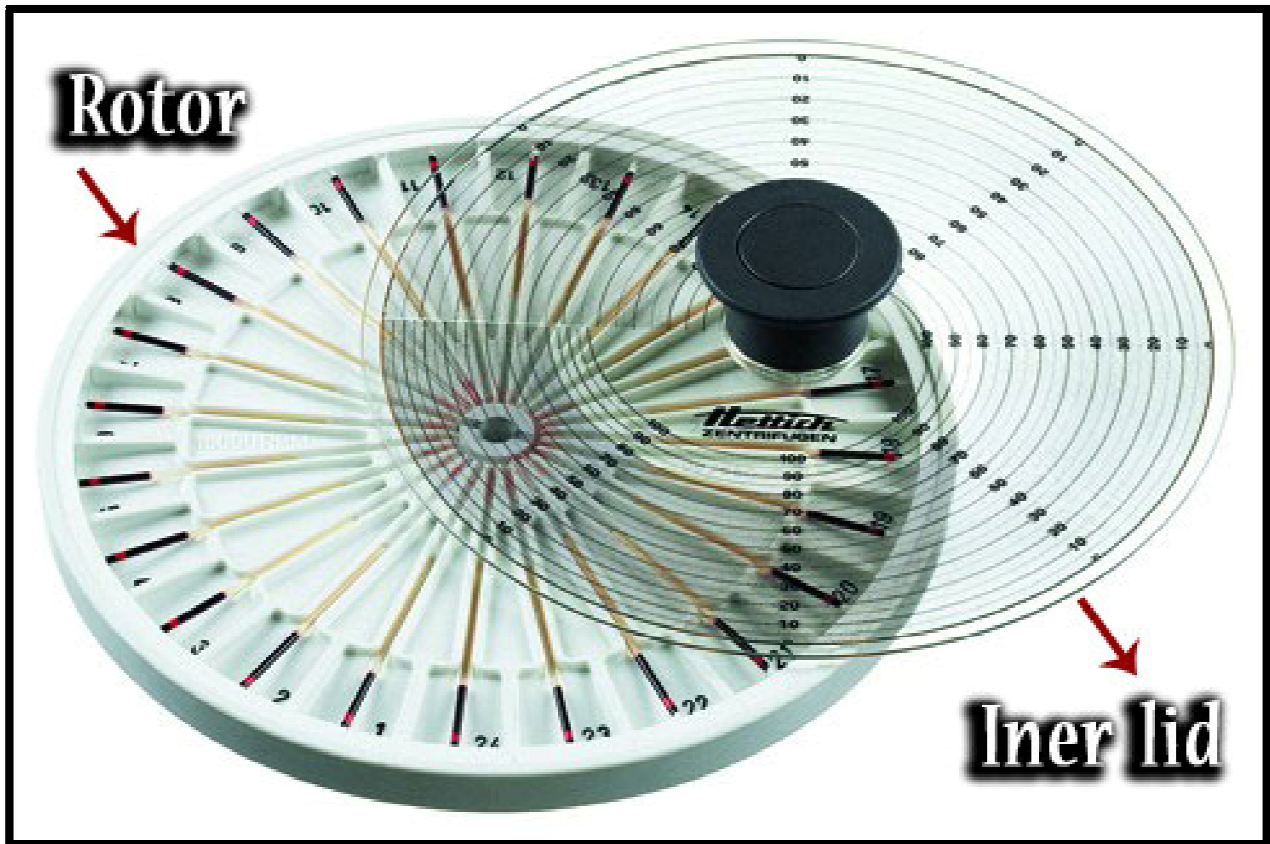
وقد يحدث تورم بسيط لمنطقة اخذ العينة وسوف يزول بعد فترة قصيرة الغرض من اخذ العينة هو البحث العلمى وسوف يستلم المريض نسخة من نتائج التحليل وسيتم الاحتفاظ بالنتائج فى سرية تامة

- أوافق أنا المذكور أعلاه على اخذ عينة دم لإجراء الدراسة

- التاريخ الإمضاء



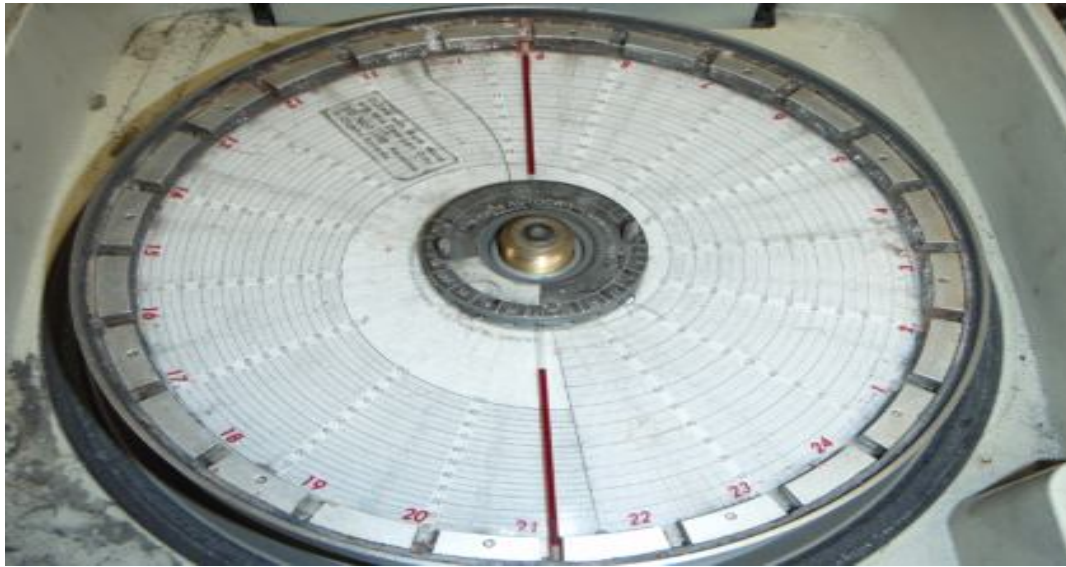
Reading Device



Micro hematocrite Centrifuge



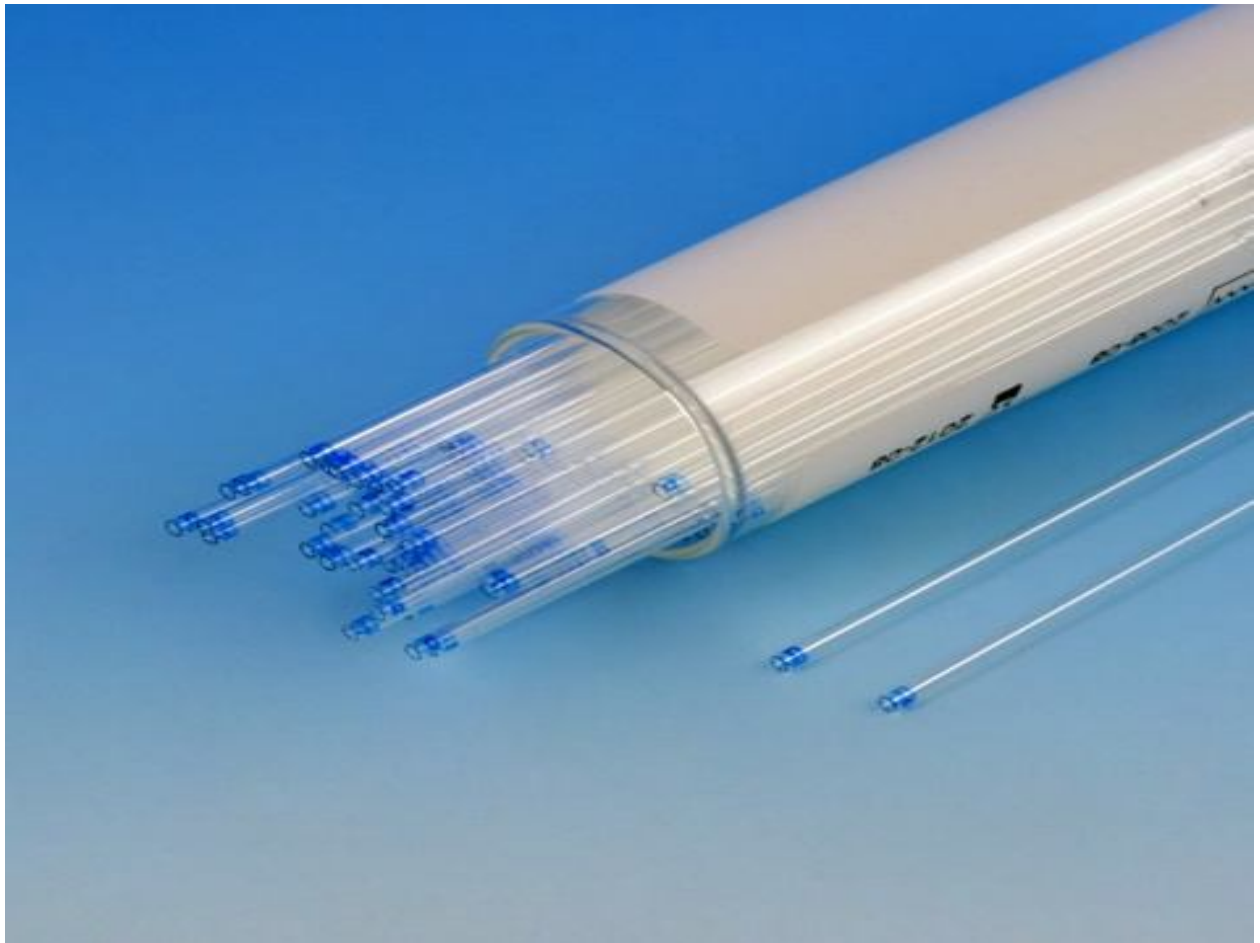
JENWAY 6051 Colorimeter



Balance in Microhematocrite Centrifuge



Sealing Agent



Capillary tubes.