Survey of Hemoglobin levels among Adolescent girls, Hindu College, Guntur

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ABSTRACT:
AIM: To survey the hemoglobin levels among adolescent girls.

BACKGROUND INFORMATION: Hemoglobin is a conjugated protein. It consists of a protein combined with an iron containing pigment. The protein part is globin and the iron containing pigment is heme. Hemoglobin carries the respiratory gases, oxygen and carbon dioxide.

MATERIALS & METHODS: Red blood cells are converted to acid hematin which is a dark brown coloured compound. The colour of the formed acid hematin complex corresponds to the Haemoglobin concentration in the blood and is matched with the standard which is a reference brown glass given in the Sahli's apparatus by diluting with N/10 hydrochloric acid or distilled water until the colour of acid hematin complex match with the colour of the standard.

RESULITS & CONCLUSION : In our study , it was observed that the haemoglobin levels of most of the girls was in the range of 7-9 g/dl(32 %) followed by 11-13 g/dl (32 %).From the results it was observed that most of the girls are suffering with anaemia.

Keywords: Haemoglobin, Red blood cells, Hematin, Sahli apparatus

INTRODUTION

Red blood cells (RBCs) are the non-nucleated formed elements in the blood. These are produced in bone marrow. Red blood cells are also known as erythrocytes (Erythros = red). Reticulocytes are the precursors of red blood cells. Erythrocytes exhibit a diameter of 7-8 µm. They are biconcave wherein their periphery is thicker than their central portion (so that the cell membrane is maximized enabling exchange of gases and their transport). Red colour of the red blood cell is due to the presence of the colouring pigment called haemoglobin. These are significant component of blood. RBCs play a vital role in transport of respiratory gases. Haemoglobin (Hb) is the iron containing colouring matter of red blood cell (RBC). It is a chromo protein forming 95% of dry weight of RBC. Molecular weight of haemoglobin is 68,000.

NORMAL VALUES OF HAEMOGLOBIN

- Average hemoglobin (Hb) content in blood is 14 to 16 g/dl. However, the value varies depending upon the age and sex of the individual.

Age
At birth : 25 g/dL
After 3rd month : 20 g/dL
After 1 year : 17 g/dL
From puberty onwards : 14 to 16 g/dL
At the time of birth, hemoglobin content is very high because of increased number of RBCs
Gender
In adult males : 15 g/dL
In adult females : 14.5 g/dL

STRUCTURE OF HAEMOGLOBIN

Haemoglobin is a conjugated protein. It consists of a protein combined with an iron containing pigment. The protein part is globin and the iron containing pigment is **heme.**

- ★ **IRON -** Normally, it is present in ferrous (Fe^{2+}) form. It is in unstable or loose form. In some abnormal conditions, the iron is converted into ferric (Fe^{3+}) state, which is a stable form.
- ★ **PORPHYRIN -** The pigment part of heme is called porphyrin. It is formed by four pyrrole rings (tetrapyrrole) called, I, II, III and IV. The **pyrrole rings** are attached to one another by methane (CH_4) bridges. The iron is attached to 'N' of each pyrrole ring and 'N' of globin molecule.
- ★ **GLOBIN -** Globin contains four polypeptide chains. Among the four polypeptide chains, two are β- chains and two are α-chains.

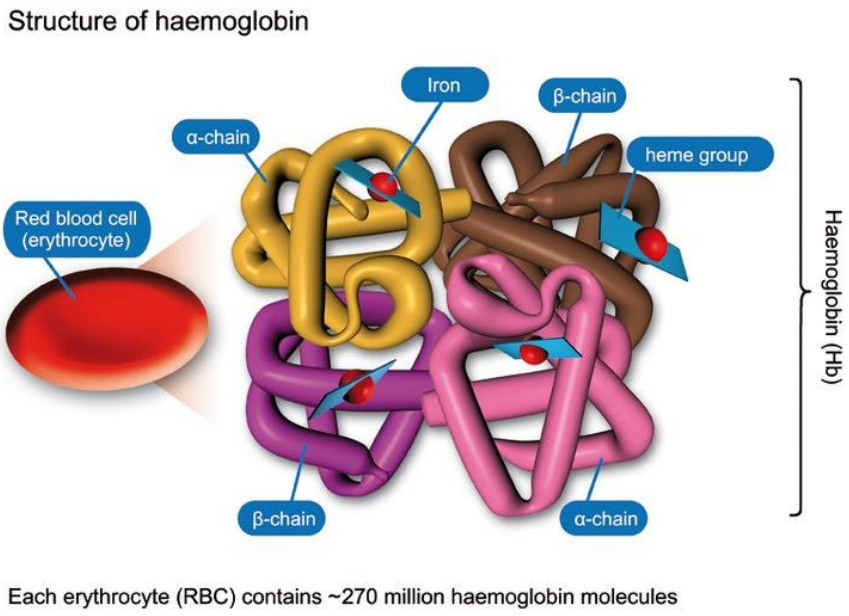

Figure – 1 : Structure of haemoglobin

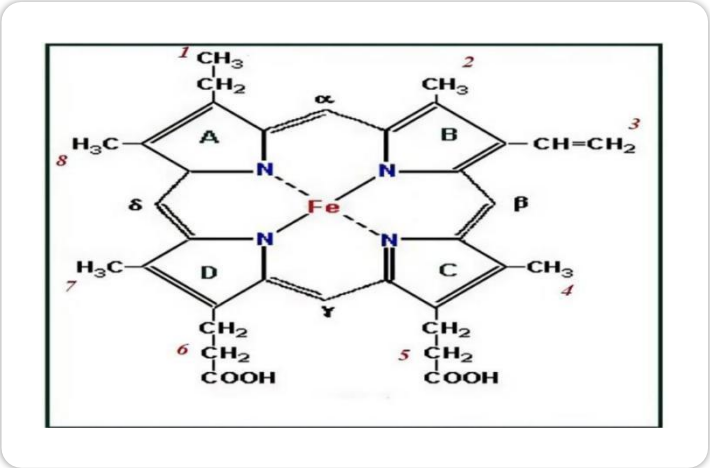

Figure – 2 : Chemical structure of haemoglobin

FUNCTIONS OF HAEMOGLOBIN

A. **TRANSPORT OF RESPIRATORY GASES**

Main function of haemoglobin is the transport of respiratory gases:

1. Oxygen from the lungs to tissues.
2. Carbon dioxide from tissues to lungs.

1. Transport of Oxygen

When oxygen binds with haemoglobin, a physical process called **oxygenation** occurs, resulting in the formation of oxyhaemoglobin.The iron remains in ferrous state in this compound. Oxyhaemoglobin is an unstable compound and the combination is reversible, i.e., when more oxygen is available, it combines with haemoglobin and whenever oxygen is required, haemoglobin can release oxygen readily.When oxygen is released from oxyhaemoglobin, it is called reduced haemoglobin or ferro haemoglobin.

2. Transport of Carbon Dioxide

When carbon dioxide binds with haemoglobin, carbo haemoglobin is formed. It is also an unstable compound and the combination is reversible, i.e., the carbon dioxide can be released from this compound. The affinity of haemoglobin for carbon dioxide is 20 times more than that for oxygen.

Other functions of haemoglobin include the following

- Haemoglobin is a 'heme' protein that is only found in the cytoplasm of the RBC (erythrocytes). It imparts red colour to the blood.
- Haemoglobin binds reversibly and loosely to oxygen to form oxyhaemoglobin

  Haemoglobin + oxygen ⇒ **oxyhaemoglobin**
- Oxygen is released in the tissues where O_2 level is low. Thus haemoglobin helps to carry CO_2 in the reverse direction.

B. It buffers the blood pH and maintains it tolerable limits.

C. Haemoglobin maintains the shape of the red blood cells.

D. Haemoglobin is the source of physiologically (example Bilirubin active catabolites).

ABNORMAL HAEMOGLOBIN

Abnormal types of haemoglobin or haemoglobin variants are the pathologic mutant forms of haemoglobin. These variants are produced because of structural changes in the polypeptide chains caused by mutation in the genes of the globin chains. Most of the mutations do not produce any serious problem. Occasionally, few mutations result in some disorders.

There are two categories of abnormal haemoglobin:

1. Haemoglobinopathies
2. Haemoglobin in thalassemia and related disorders.

SYNTHESIS OF HAEMOGLOBIN

Synthesis of haemoglobin actually starts in Proerythroblast stage However, haemoglobin appears in the intermediate normoblastic stage only. Production of haemoglobin is continued until the stage of reticulocyte. Heme portion of haemoglobin is synthesized in mitochondria. And the protein part, globin is synthesized in ribosomes.

SYNTHESIS OF HEME

Heme is synthesized from succinyl CoA and the glycine. The sequence of events in synthesis of haemoglobin.

FORMATION OF GLOBIN

Polypeptide chains of globin are produced in the ribosomes. There are four types of polypeptide chains namely, alpha, beta, gamma and delta chains. Each of these chains differs from others by the amino acid sequence. Each globin molecule is formed by the combination of 2 pairs of chains and each chain is made of 141 to 146 amino acids. Adult haemoglobin contains two alpha chains and two beta chains. Foetal haemoglobin contains two alpha chains and two gamma chains.

Each polypeptide chain combines with one heme molecule. Thus, after the complete configuration, each haemoglobin molecule contains 4 polypeptide chains and 4 heme molecules.

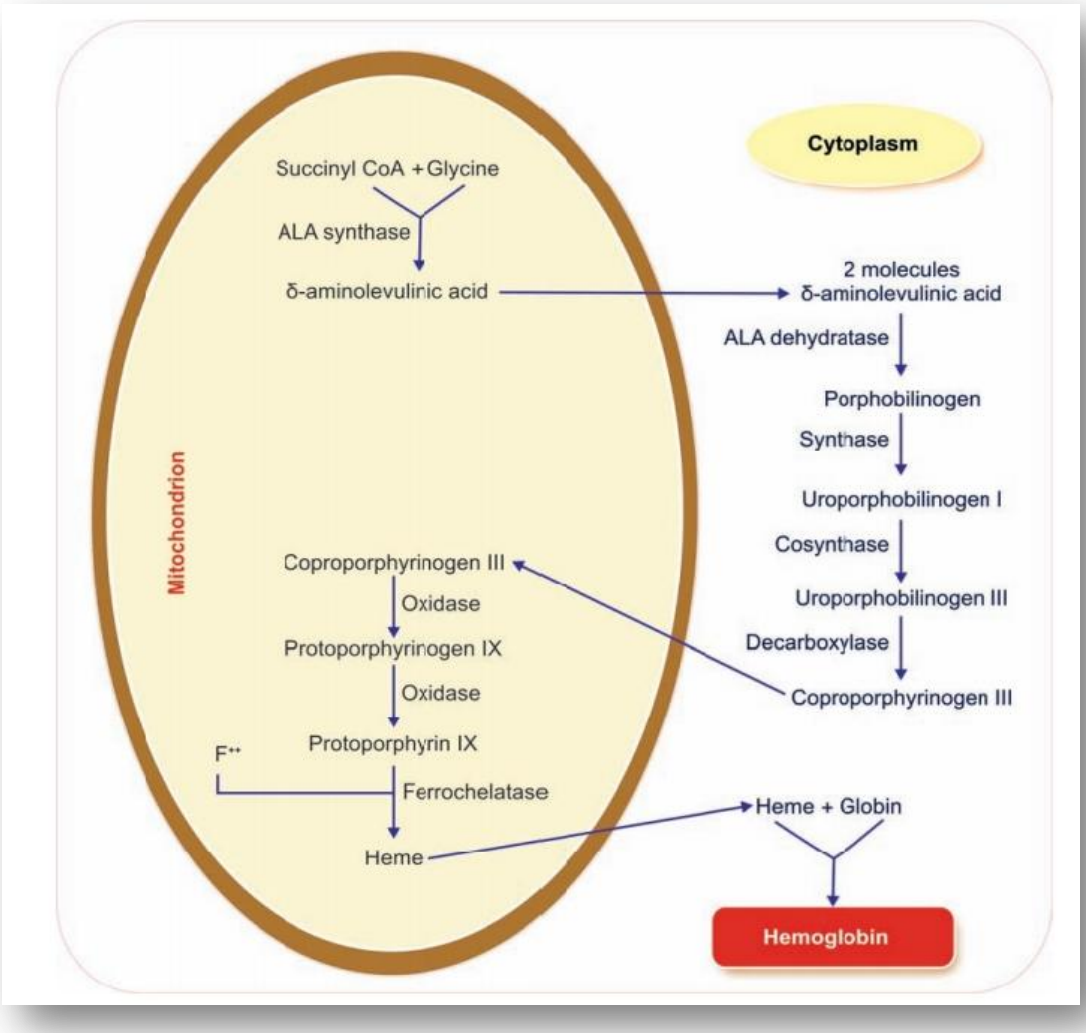

Figure – 3 : Synthesis of haemoglobin

DESTRUCTION OF HAEMOGLOBIN

After the lifespan of 120 days, the RBC is destroyed in the reticuloendothelial system, particularly in spleen and the haemoglobin is released into plasma. Soon, the haemoglobin is degraded in the reticuloendothelial cells and split into globin and heme. Globin is utilized for the resynthesis of haemoglobin. Heme is degraded into iron and porphyrin. Iron is stored in the body as ferritin and hemosiderin, which are reutilized for the synthesis of new haemoglobin. Porphyrin is converted into a green pigment called biliverdin. In human being, most of the biliverdin is converted into a yellow pigment called bilirubin. Bilirubin and biliverdin are together called the bile pigments.

IRON METABOLISM - IMPORTANCE OF IRON : Iron is an essential mineral and an important component of proteins, involved in oxygen transport. So, human body needs iron for oxygen transport. Iron is important for the formation of haemoglobin and myoglobin.

NORMAL VALUE AND DISTRIBUTION OF IRON IN THE BODY

Total quantity of iron in the body is about 4 g. Ap proximate distribution of iron in the body is as follows:

In the haemoglobin : 65% to 68%
In the muscle as myoglobin : 4%
As intracellular oxidative heme compound : 1%
In the plasma as transferrin : 0.1%
Stored in the reticuloendothelial system : 25% to 30%

DIETARY IRON

Dietary iron is available in two forms called heme and non-heme.

Heme Iron - Heme iron is present in fish, meat and chicken. Iron in these sources is found in the form of heme. Heme iron is absorbed easily from intestine.

Non-heme Iron -Iron in the form of non-heme is available in vegetables, grains and cereals. Non-heme iron is not absorbed easily as heme iron. Cereals, flours and products of grains which are enriched or fortified (strengthened) with iron become good dietary sources of non-heme iron particularly for children and women.

ABSORPTION OF IRON

Iron is absorbed mainly from the small intestine. It is absorbed through the intestinal cells (enterocytes) by pinocytosis and transported into the blood.

Iron is present mostly in ferric (Fe^{3+}) form. It is converted into ferrous form (Fe^{2+}) which is absorbed into the blood. Hydrochloric acid from gastric juice makes the ferrous iron soluble so that it could be converted into ferric iron by the enzyme ferric reductase from enterocytes. From enterocytes, ferric iron is transported into blood by a protein called ferroprotein. In the blood, ferric iron is converted into ferrous iron and transported.

TRANSPORT OF IRON : Immediately after absorption into blood, iron combines with a β-globulin called Apo transferrin (secreted by liver through bile) resulting in the formation of transferrin. And iron is transported in blood in the form of transferrin. Iron combines loosely with globin and can be released easily at any region of the body.

STORAGE OF IRON

Iron is stored in large quantities in reticuloendothelial cells and liver hepatocytes. In other cells also it is stored in small quantities. In the cytoplasm of the cell, iron is stored as ferritin in large amount. Small quantity of iron is also stored as hemosiderin.

DAILY LOSS OF IRON

- In males, about 1 mg of iron is excreted everyday through faeces.
- In females, the amount of iron loss is very much high. This is because of the menstruation. One gram of haemoglobin contains 3.34 mg of iron. Normally, 100 mL of blood contains 15 gm of haemoglobin and about 50 mg of iron (3.34 × 15). So, if 100 mL of blood is lost from the body, there is a loss of about 50 mg of iron. In females, during every menstrual cycle, about 50 mL of blood is lost by which 25 mg of iron is lost. This is why the iron content is always less in females than in males.
- Iron is lost during haemorrhage and blood donation also. If 450 ml of blood is donated, about 225 mg of iron is lost.

MATERIALS AND METHODS

This is the prospective observational study which does not involve in any other inversive techniques. This estimation of haemoglobin was carried out in girls between the age group of 16 - 20 years who are willing to participate in the study in the laboratory of Human Anatomy and Physiology, Department of Biomedical sciences, Hindu college, Guntur.

Inclusion criteria

- Girls who are willing for the study within the age group of 16- 20 years.
- Only Females were included in the study

Exclusion criteria

- Girls below 16 years age
- Girls above 20 years age
- Males were excluded from the study

ESTIMATION OF HAEMOGLOBIN BY SAHLI'S METHOD

Figure – 4 : Sahli's haemometer

PRINCIPLE OF SAHLI's METHOD / ACID HEMATIN METHOD

The principle of Sahli's Method or Acid hematin method is quite easy that when the blood is added to N/10 Hydrochloric acid (HCl), the haemoglobin present in RBCs is converted to acid hematin which is a dark brown coloured compound. The colour of the formed acid hematin complex corresponds to the Haemoglobin concentration in the blood and is matched with the standard which is a reference brown glass given in the Sahli's apparatus by diluting with N/10 hydrochloric acid or distilled water until the colour of acid hematin complex match with the colour of the standard.

REAGENTS REQUIRED FOR SAHLI's METHOD / ACID HEMATIN METHOD

- N/10 hydrochloric acid (It is prepared by diluting concentrated hydrochloric acid 0.98 ml in distilled water and volume is made up 100 ml).
- Distilled water.

APPARATUS & EQUIPMENTS REQUIRED FOR SAHLI'S METHOD / ACID HEMATIN METHOD

- Sahli's Apparatus
  - Haemoglobin pipette (0.02 ml or 20 µl capacity)
  - Sahli's graduated Haemoglobin tube
  - Thin glass rod Stirrer for Haemoglobin Tube
  - Sahli's Comparator box with brown glass standard
- Spirit swab
- Blood Lancet
- Dry cotton swab
- Pasteur pipette

PROCEDURE OF SAHLI's METHOD / ACID HEMATIN METHOD

- N/10 Hydrochloric acid(HCL) was taken in Haemoglobin tube (has two graduations – one side gm/dl, and other side shows the Hb %age), up to the mark 2.0 – the lowest marking
- Venous or Capillary blood is drawn up to 20µl mark of haemoglobin pipette exactly. For capillary blood draw, the tip of the middle or ring finger was boldly pricked with the help of Blood lancet or pricking needle. Wiped out the first drop of blood and sucked the blood from the second drop into Hb pipette up to the mark of 20 µl. Filled the Haemoglobin pipette by capillary action.

Figure 5 : Adding 0.1 N HCL in Haemoglobin tube

Figure – 6 : Sucking blood into micro lpipette (blood collection)

- Wiped out the surface of the pipette with the help of tissue paper/ cotton so that excess blood may not be added to the Hb tube.
- Dispensed the blood into N/10 hydrochloric acid taken in the haemoglobin tube, rinsed the pipette with the same solution and mixed properly with the help of stirrer.
- Placed the tube at room temperature for 10 minutes for complete conversion of haemoglobin into acid hematin.
- After the reaction completes, placed the Hemoglobin tube in the column in Sahli's Comparator box and started diluting the dark brown coloured compound (Acid Hematin) formed in the Haemoglobin tube using the N/10 HCl or distilled water by adding drop by drop of it into the solution and mixed with the help of stirrer after each addition.
- This process is done until the endpoint comes matching the colour of standard with the colour of the test.
- Once the colour is matched with the standard brown glass, lifted the stirrer and noted the reading in Sahli's Haemoglobin tube by taking the lower meniscus in consideration.
- Now added one more drop of distilled water and mixed it properly with the help of stirrer. If colour is still matching with the standard another drop was added till it matches with the standard and noted the reading and, if it gets lighter after adding the first extra drop, it shows reading taken before dilution was correct. Noted down that reading as the final result.
- Reading of this method is expressed in Haemoglobin gm/dl (gram/100 ml) of blood.

Figure – 7 : Filling of blood into pipette

ADVANTAGES OF SAHLI'S METHOD / ACID HEMATIN METHOD

- This method is simple and easy to perform.
- It is portable and test can be done any place.
- It is cost effective and less time consuming.

- This method gives best possible result.

DISADVANTAGES OF SAHLI'S METHOD / ACID HEMATIN METHOD

- Visual intensity may be different for different individuals by this method, we are not able to measure the inactive haemoglobin.
- The endpoint disappears soon so it is difficult to know the actual endpoint and also the Proper stable standard is not available.

CLINICAL SIGNIFICANCE OF HAEMOGLOBIN ESTIMATION

Haemoglobin estimation gives a brief idea of the pathological conditions to the physician so that your physician can easily understand the cause of pathology and prescribe an effective treatment for it.

Raised Haemoglobin Content -

- Polycythaemia Vera
- Congenital Heart disease
- High Altitudes
- Elevated erythropoietin levels (Tumours of Kidney, Liver, CNS, Ovary, Renal Diseases etc.)
- Adrenal hypercorticism
- Therapeutic androgens

Reduced Haemoglobin Content - Haemoglobin may reduce in the following conditions

- Leukaemia
- Tuberculosis
- Iron deficiency anaemia
- Parasitic infections severely in hookworm infection
- Sickle cell anaemia
- Thalassemia
- Aplastic anaemia
- Haemolytic anaemia
- Loss of blood because of trauma
- Pernicious anaemia

RESULTS

The haemoglobin percentage was measured from 40 individuals and the results are as follow. Out of 40 girls, three of the girls contain very poor levels of haemoglobin content i.e., 5 - 7 grams. Majority of the girls (13 members) contains low content of haemoglobin which is between 7 – 9 grams. Eleven of the contain 9 – 11 grams of haemoglobin content and thirteen of them contain 11 – 13 grams of haemoglobin content. Where only one has haemoglobin content between 13 – 15 grams. The results are shown graphically in the below graph for better understanding. The overall study showed that most girls between the age group of 16 – 20 years are having reduced content of haemoglobin. According to our questionnaire the subject's nutritional study showed inadequate intake of iron rich foods (green leafy vegetables, red meat) and citrus foods (lemons, limes, grape fruits etc.,) are rich in vitamin C which increase iron absorption. Also consumption of excessive fast foods is observed in girls diet which may impact on haemoglobin content.

S.no	Haemoglobin content in grams	No. of girls
1.	5 – 7 grams	3
2.	7 – 9 grams	13
3.	9 – 11 grams	11
4.	11 – 13 grams	13
5.	13 – 15 grams	1

Table : Haemoglobin content in adolescent girls

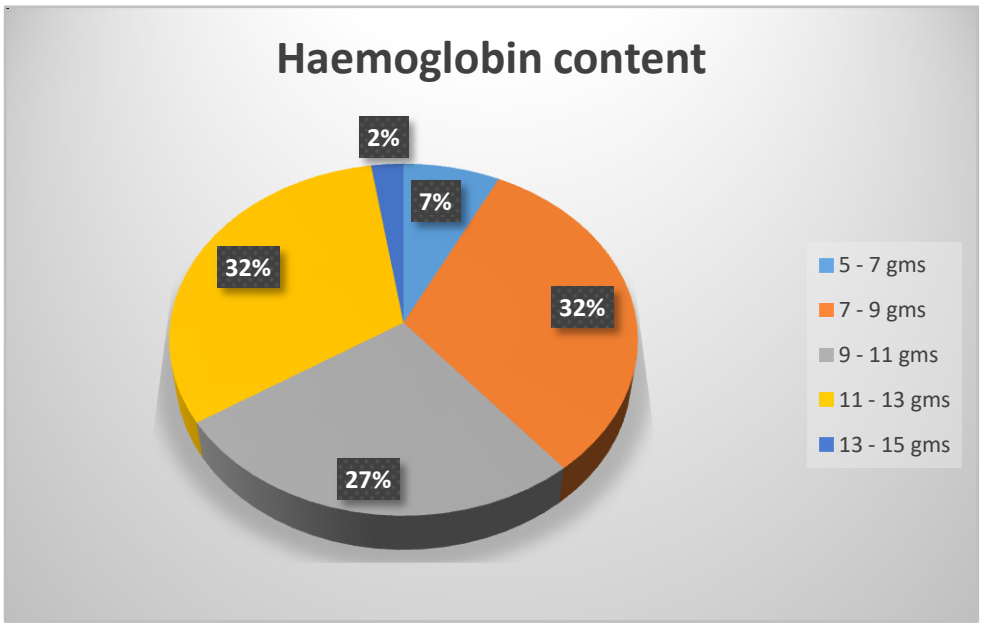

Figure 08 : Haemoglobin Content

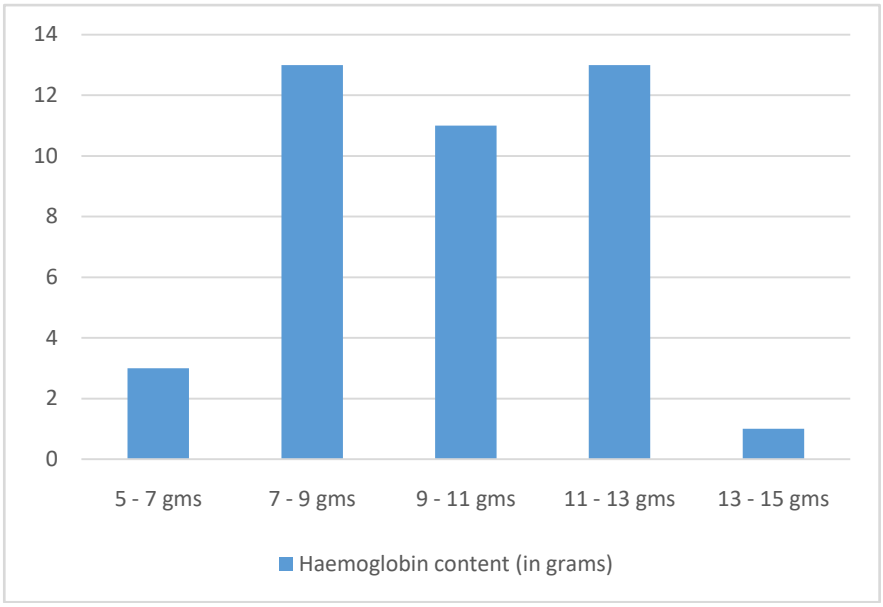

Figure 09 : Haemoglobin content

CONCLUSION :

In our study , it was observed that the haemoglobin levels of most of the girls was in the range of 7-9 g/dl(32 %) followed by 11-13 g/dl (32 %).From the results it was observed that most of the girls are suffering with anaemia.

RECOMMENDATIONS :

Haemoglobin percentage can be improved and anaemia can be treated by implementing some of the remedies.

1. Increasing iron intake : - A person with reduced levels of haemoglobin may benefit from eating more iron-rich foods. Iron works to boost the production of haemoglobin, which also helps to form more red blood cells.

Iron-rich foods include meat and fish, peanuts, soy products, including tofu, dried fruits, such as dates and figs, broccoli, green leafy vegetables, such as kale and spinach, green beans, nuts and seeds.

2. Increasing folate intake :- Folate is a type of vitamin B that plays an essential part in haemoglobin production. The body uses folate to produce heme, a component of haemoglobin that helps to carry oxygen.

If a person does not get enough folate their red blood cells will not be able to mature, which could lead to folate-deficiency anaemia and low haemoglobin levels. Good sources of folate include beef, spinach, rice, peanuts, black-eyed peas, kidney beans, avocadoes, lettuce

3. Maximizing iron absorption :- Consuming iron in foods or supplements is important, but a person should also help their body to absorb that iron. Foods rich in vitamin C, such as citrus fruits, strawberries, and leafy green vegetables, can boost the amount of iron absorbed. Taking a vitamin C supplement may also help. Vitamin A and beta-carotene can aid the body in absorbing and using iron. Foods rich in vitamin A include fish, liver, squash, sweet potatoes, kale and collards

Foods high in beta-carotene include yellow, red, and orange fruits and vegetables, such as carrots, sweet potatoes, squash, cantaloupes, mangoes.

4. Taking iron supplements :- Taking iron supplements is only recommended for individuals who are severely anaemic and also during pregnancy.

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