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Original Article

Estimate the Hematological Characters in Patients With Autoimmune Hemolytic Anemia in private laboratories

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Abstract: Iron deficiency anemia is one of the major concern. The high rate incidence has been related to insufficient iron intake, accompanied by chronic intestinal blood loss due to parasitic and malarial infections. Iron deficiency anemia is the commonest type of anemia throughout the world. It is defined as a clinical condition, characterized by reduction in hemoglobin below the normal for the age, sex, physiological conditions and altitude above sea level in a patient. It is a global problem, mainly affecting poor people, pregnant, lactating females, growing children and elderly people. It has been reported to affect about 50-60% of young children and pregnant females as well as 20-30% of non-pregnant females in the developing countries. This high rate has been related to insufficient iron intake, high nutritional needs during childhood and pregnancy, poor bioavailability of iron, and an accompanying chronic intestinal blood loss due to parasitic and malarial infestations. The samples were collected from private laboratories .where 100 blood samples were obtained and the studied tests were performed to detection of CBC and ferritin levels.This study revealed that out of 100 anemic patients, 35% were that of AIHA. The most affected age group was 21-40 years with frequency 42.55%. AIHA was more common in females (42.85%) than in male (21.62%). Out of 100 anemic patients, microcytic hypochromic anemia was predominant in 47% followed by macrocytic anemia (31%) and then normocytic normochromic anemia (22%). Out of 47 microcytic hypochromic anemic patients, 12 had normal serum ferritin. There was a statistical significant difference in Hb (p=0.011), MCV (p=0.0001), MCH (p=0.0001), MCHC (p=0.0001) and serum ferritin (p=0.0001) among all types of anemia. There was a statistical significant positive correlation of ferritin with Hemoglobin (0.257, p=0.01), MCV (0.772, p=0.0001), MCH(0.741, p=0.0001) and MCHC (0.494, p=0.0001).

Keywords: Hematological Characters in Patients With Autoimmune

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Introduction:

Anemia is a disease in which the concentration of hemoglobin ,Hb Hemoglobin is the red blood cell protein molecule which transports oxygen from the lung to the tissues of the body and returns carbon dioxide from the tissues to the lungs. Hemoglobin consists of four protein molecules bound together (the globulin chains). Two alpha-globulin chains and two beta-globulin chains form the normal adult hemoglobin (Hgb or Hb abbreviated). and red blood cells (RBC) are below normal levels, which are inadequate to satisfy the physiological demands of the person affects approximately a third of the world's population. The name comes from ancient Greek: anemia ("lack of blood"), anemia ("not"). [1]

Anemia is linked to elevated female and infant morbidity and mortality, adverse birth outcomes in adult employment and reduced cognitive and behavioral growth in infants. "Anemia is highly common in developing countries and is regarded as a public health concern. It happens at all stages of life, especially in women and children who are pregnant, 1.62 billion people worldwide are anemic".[2]

Anemia is the second leading disease cause in the world and thus one of the most significant public health issues in the world". Anemia has multifactorial causes with complicated interactions among nutritional and other factors, which are a challenge to resolve effectively population determinants of anemia. Anemia has many causes including: "infectious diseases, for instance, malaria, hookworm and shistosomiasis. Micronutrient deficiencies including folate, vitamin B12 and vitamin A. Increased chance of having anemia is also seen in individuals with chronic diseases like kidney diseases, cancer, diabetes and associated conditions". There are different varieties of anemia classifications. [3].

Anemia is caused by a variety of red cell defects such as a production defect, a maturation defect , a hemoglobin synthetic defect or a genetic defect in the maturation of hemoglobin or the synthesis of anomalous hemoglobin or thalassemia. Many studies suggests that fetal/neonatal Iron deficiency discusses long-term risks to brain function . Early iron deficits (ED) not only affect the brain and function, but also have an after-treatment effect . Dopamine synthesis, myelination, composition and function modifications for the long run are part of the path. The brain does not normally function, as it has an iron deficiency. Deficiency in iron Headache, vertigo, delirium, restless leg syndrome are all linked with anemia. Anemia is today the main global risk factor for the wellbeing of adolescents and pregnant mothers.[4]

Establishing appropriate Hb thresholds to define anemia is essential for ensuring that anemia is correctly identified, and its negative effects prevented. As important, understanding the diverse and complex etiology of anemia is crucial for developing appropriate interventions that address the context-specific causes of anemia and for monitoring the success of anemia control programs.[5]

Young women are a vulnerable group of iron deficiency anemia due to the relatively high need for iron caused by growth, sexual maturity, and menstruation. In addition, the lack of iron intake from foods consumed daily also increases the chances of anemia. This is associated with consumption patterns due to dieting to manage the body.[6]

Anemia can be caused by blood loss, decreased red blood cell production, and increased red blood cell breakdown .Causes of bleeding include bleeding due to inflammation of the stomach or intestines, bleeding from surgery, serious injury, or blood donation .Causes of decreased production include iron deficiency, vitamin B12 deficiency, thalassemia and a number of bone marrow tumors .Causes of increased breakdown include genetic disorders such as sickle cell anemia, infections such as malaria, and certain autoimmune diseases .Anemia can also be classified based on the size of the red blood cells and amount of hemoglobin in each cell. If the cells are small, it is called microcytic anemia; if they are large, it is called macrocytic anemia; and if they are normal sized, it is called normocytic anemia.[7]

Symptoms of anemia include :Tiredness, lethargy, feeling faint and becoming breathless easily, headaches, irregular heartbeats (palpitations), altered taste, sore mouth and ringing in the ears (tinnitus). Anemia in pregnancy increases the risk of complications in both mother and baby such as low birth weight baby, preterm (premature) delivery and postnatal depression. Low iron reserves in the baby may also lead to anemia in the newborn baby . A large number of patients diagnosed with anemia of chronic disease present with no active inflammation or dietary issues. These include many with reduced limb loading, such as spinal cord injured patients, astronauts, elderly people with limited mobility,

bed-bound and experimental bed-rest subjects.[8]

Certain groups of individuals, such as pregnant women, benefit from the use of iron pills for prevention. Dietary supplementation, without determining the specific cause, is not recommended. The use of blood transfusions is typically based on a person's signs and symptoms. In those without symptoms, they are not recommended unless hemoglobin levels are less than 60 to 80 g/L (6 to 8 g / dL).[18] These recommendations may also apply to some people with acute bleeding .Erythropoiesis-stimulating agents are only recommended in those with severe anemia

In most cases, it is possible to add anemia prevent -ion or control activities to an already existing health or health-related program without large investments of time or resources. Raising awareness of anemia prevention and control, promoting behavior change in the community, advocating for increased funding for national anemia programming, and training to build capacity among health workers are activities that can be implemented by any and all sectors, and across sectors. They are most effective when approached in a coordinated and targeted manner.[9]

Health professionals, governments, donors, nongovernmental organizations, the commercial sector, and civil society all have roles to play in achieving worldwide anemia prevention and control. Effectively implementing interventions requires an integrated approach of financial, technical, and political commitment and support. Partnerships and collaboration among these various players should be built at the national, provincial/ state, district, and local levels from the outset of anemia programming. Input from and coordination among all potential parties is most critical in the key initial phase of planning an anemia strategy.[10]

2.1. Pathophysiology

Blood is composed of water-based plasma (54%), white blood cells and platelets (1%), and red blood cells (45%).5 Hemoglobin, the primary protein of the red blood cell, binds oxygen from the lungs and transports it to the rest of the body. Oxygen is then ex- changed for carbon dioxide, which is car- red back to the lungs to be exhaled. Hemoglobin is made up of four globin chains, each containing an iron ion held in a porphyrin ring known as a hem group.5 When the body detects low tissue oxygen, the endothelial cells in the kidneys secrete the hormone erythropoietin (EPO), which stimulates the bone marrow to increase red cell production. This feedback loop can be interrupted by renal failure or chronic disease.

In addition, bone marrow can- not produce enough red blood cells if there are insufficient levels of iron, amino acids, protein, carbohydrates, lipids, folate , and vitamin B12.5 Toxins (eg, lead), some types of cancer (eg, lymphoma), or even common infections (eg, pneumonia) can suppress the bone marrow, causing anemia. The more severe the anemia, the more likely oxygen transport will be compromised and organ failure will ensue.

Mutations affecting the genes that en- code the globin chains within hemoglobin can cause one of the more than 600 known hemoglobinopathies (genetic defects of hemoglobin structure), such as sickle cell disease and thalassemia. 5,9 While it is important to identify and treat patients with hemoglobinopathies, most anemia have other causes, such as iron deficiency, chronic disease, bone marrow defects, B12 deficiency, renal failure, medications, alcoholism, pregnancy, nutritional intake prob-lems, gastrointestinal malabsorption , and active or recent history of blood loss. [28]

2.2.Diagnosis

Iron Deficiency: Diagnosis

1. RBC indices are of little diagnostic value unless the MCV is below 70f which is only seen in iron

2.deficiency and thalassemia. Serum iron can be decreased in a variety of states including iron deficiency, inflammation, and stress. The serum iron level varies tremendously from morning to evening and from day to day. The minuscule amount of iron in a multivitamin can falsely elevate the serum iron for up to 24 hours.

3. The total iron binding capacity is very specific for iron deficiency (near 100%) but has poor sensitivity (less than 30%).

4. The iron saturation (Fe/TIBC x 100) can be decreased below 16 percent in both anemia of chronic disease and iron deficiency and is of little help in distinguishing between the two.

5. In the normal patient the serum ferritin is directly correlated with iron stores. This relationship holds true even in inflammatory states although the curve is "shifted to the left. That is, for a given level of storage iron in a patient with an inflammatory state the serum ferritin is higher. A ferritin level of greater than 100ng/ml rules out iron deficiency anemia in most patients. One good rule of thumb is that in patients more than 65 years of age a ferritin below 50 ng/ml is associated with iron deficiency. The measurement of the serum ferritin is the most useful and cost-effective test of iron stores.[29]

The most efficient approach to detecting iron deficiency is to perform a serum ferritin. If it is more than 100 ng/dl, this eliminates iron deficiency. Very low values are diagnostic of iron deficiency. Although laboratories will often state that ferritins of over 12-36 ng/dl are in the "normal range" it is important to remember that many older patients may be iron deficiency with ferritin in the 50-80 ng/dl range.[28]

Microcytic Anemia: Differential Diagnosis

1. Iron Deficiency. The lack of iron results in decreased hemoglobin available to the developing red cell. Thus, the erythrocytes produced are under hemoglobinized which result in smaller cells. The earliest sign of iron deficiency is decreased iron stores. This stage has a normal CBC and indices, although one can see microcytic/hypochromic cells on the smear.

The anemia gradually evolves into the classic microcytic- hypochromic anemia. Diagnosis is made by showing decreased iron stores on bone marrow examination. Biochemically the diagnosis is established by a high TIBC or a low ferritin. The major diagnostic difficulty is distinguishing iron deficiency from anemia of chronic disease.[30]

2. Anemia of Chronic Disease, (anemia of defective iron utilization). In patients with inflammatory states iron is sequestered in the RE system and is unavailable for use by the developing red cell (defective iron utilization). Thus at the erythrocyte level the defect is identical to iron deficiency and therefore results in production of under hemoglobinized red cells. This can result in a microcytic/hypochromic anemia. Increase production of the protein hepcidin is response for the multiple changes in iron metabolism seen in inflammation. Additional factors including shorten red cell survival and decreased levels of erythropoietin add to the hypoproliferative state. The inflammatory state also leads to a decreased serum iron and decreased TIBC. Recently the spectrum of diseases associated with anemia of chronic disease has expanded. Besides the classic association of temporal arteritis (may be a presenting sign), rheumatoid arthritis, cancer etc., anemia of chronic disease has been found in patients with non-inflammatory medical conditions such as congestive heart failure, COPD and diabetes. Patients with anemia of chronic disease can have hemoglobin decreased into the lower 20% range and many (20-30%) will have red cell indices in the microcytic range. Diagnosis is made by proving ample bone marrow iron stores with decrease sidero blasts (iron containing red cell precursors). Biochemically anemia of chronic disease remains a clinical diagnosis of exclusion with the key test is to rule out iron deficiency. The serum erythropoietin level is inappropriately low vales when compared with the hematocrit. The serum iron is decreased in both conditions and the TIBC is low in states where iron deficiency and chronic disease co-exists thus rendering these tests useless. The finding of an elevated ferritin over 100ng/ml is an adequate demonstration of good iron stores. In the older patient or one with back pain, one should also rule-out the presence of multiple myeloma by performing a serum protein electrophoresis. In difficult cases one can resort to assessing bone marrow stores of iron. In the future assays of hepcidin levels will be helpful as this protein is the chemical mediator of the anemia of chronic disease.[31]

3. Thalassemia. In this disorder it is the defective production of hemoglobin that leads to microcytosis . The main types are the beta-thalassemia, alpha-thalassemia and Hemoglobin E. Patients who are heterozygotes for beta-thalassemia have microcytic indices with mild (30ish)anemia. Homozygotes have very severe anemia. Peripheral smears in heterozygotes reveal microcyte and target cells. Diagnosis is established in by hemoglobin electrophoresis that shows an increased HbA. Beta-thalassemia occurs in a belt ranging from Mediterranean countries, the Middle East, India, and Pakistan to Southeast Asia. Patients with beta-thalassemia traits who are of child bearing age need to have their spouse screened for beta-thalassemia and Hemoglobin E. Alpha-thalassemia also presents with microcytosis. Patients with alpha-thalassemia will have normal hemoglobin electrophoresis. The diagnosis of alpha-thalassemia is made by excluding other causes of microcytosis, a positive family history of microcytic anemia, and a life-long history of a microcytic anemia. Exact diagnosis requires DNA analysis. Alpha-thalassemia is distributed is a

similar pattern to beta-thalassemia except it very high frequency in Africa (up to 40%). In patients of African descent the finding of alpha-thalassemia requires no further evaluation. In patients from Asia of child bearing age the spouse should be screened (if need be with DNA analysis) to assess the risk of bearing a child with severe thalassemia. Hemoglobin E is a beta-hemoglobin chain defect that presents in a similar fashion to the thalassemia. Hemoglobin E occurs in Southeast Asia, especially in Cambodia, Laos and Thailand. Patients who are heterozygotes are not anemic but are microcytic. Patients who are homozygotes are mildly anemic with microcytosis and target cells. The importance of Hemoglobin E lies in the fact that patients with both genes for Hemoglobin E and beta-thalassemia have severe anemia and behave in a similar fashion to patients with homozygote beta-thalassemia.[32,33]

Thalassemia	**MCV**	**Hgb**	**Electrophoresis**	**Other Features**
Beta-Thalassemia				
Major	50-75	< 7	Raised HbA2	Severe anemia,
Intermedia	50-75	< 9	Raised HbA2	Target cells on smear
Trait	65-75	9-10	Raised HbA2	Target cells on smear
Alpha Thalassemia				
Trait-1 (α α/α-)	80-85	NI	Normal	
Trait-2 (α/α-) or (a a/--)	65-75	12-13	Normal	
Hemoglobin H (a-/--)	60's	9-8	HgbH	Hemolysis, splenomegaly
Hemoglobin Barts(- -/--)	-	-	HgbH,Hbg Barts	Hydrops fetalis
Hemoglobin E				
Heterozygous	80-85	12	HgbE present	Rare target cells on smear
Homozygous	70's	11-12	HgbE predominant	Target cells on smear

4. Sideroblastic Anemia. Defective production of the hem molecule is the basis of this disorder. The deficit of hem leads to the under hemoglobinazation of the erythroid precursors and microcytosis. Sideroblastic anemia can be congenital, can be due to toxins such as alcohol, lead, INH, or can be an acquired bone marrow disorder. The peripheral smear may show basophilic stippling in lead poisoned patients, a dimorphic (macrocytic and intensely microcytic red cells) in patient with acquired sideroblastic anemia, or stigmata of a myelodysplastic syndrome. Diagnosis is made by the finding of ringed sideroblasts on the bone marrow iron stain. Iron studies in patients with sideroblastic anemia usually show signs of iron-overload.[34]

5.Hemolytic Anemias

The laboratory signs of hemolytic anemia include:

1. Increased LDH (LDH1) -sensitive but not specific.

2. Increased indirect bilirubin-sensitive but not specific.

3. Increased reticulocyte count-specific but not sensitive

4. Decreased haptoglobin-specific but not sensitive.

5. Urine hemosiderin-presence of any is specific but not sensitive.

The indirect bilirubin is proportional to the hematocrit, so with a hematocrit of 45% the upper limit of normal is 1.00 mg/dl and with a hematocrit of 22.5% the upper limit of normal for the indirect bilirubin is 0.5mg/dl. Since tests for hemolysis suffer from a lack of sensitivity and specificity, one needs a high index of suspicion for this type of anemia.

6.Autoimmune hemolytic anemia (AIHA)

Are due to red cell destruction by autoantibodies. AIHA may be idiopathic or associated with malignancies, drugs or other autoimmune disorders. The major subgroups are Warm antibody hemolytic anemia (IgG), Cold antibody hemolytic anemia (IgM) and Drug induced. In patients with AIHA one usually sees microspherocytes on the peripheral smear and splenomegaly may be present on exam. The diagnosis is established by the finding of a positive direct antibody test (direct Coombs). Not all patients with a positive direct antibody test will have AIHA. The direct antibody test will detect IgG and occasionally complement in patients with warm antibody disease. Cold antibody disease will only demonstrate complement and not IgG.[35]

Patients with warm antibody disease should be started on prednisone 60 mg/day and rituximab 1000 mg in Twice days for fortien days. Rituximab has been shown to improve the durability of steroid remissions. In those who do not respond or require high doses of prednisone splenectomy may induce remission in 50%. Many patients will require further immunosuppression with agents such as mycophenolate. Treatment of cold antibody disease is difficult as these patients will not respond to steroid or splenectomy. Rituximab has been reported to be effective and should be the initial therapy for symptomatic patients. Drug induced hemolytic anemia requires stopping the implicated drug. All patients with hemolysis can become folate deficiency so folate replacement should be given to all. Microangiopathic hemolytic anemia are due to mechanical destruction of red cells. The most common associated diseases are disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, valvular disease, or the presence of an artificial heart valve. One sees schistocytes in the peripheral smear and an elevated LDH. The exact cause of the microangiopathic hemolytic anemia is determined by the history and laboratory testing.[36]

Paroxysmal nocturnal hemoglobinuria is an acquired hemolytic anemia that is due to a clonal proliferation of erythrocytes abnormally sensitive to the action of compliment. Hemolysis may be more conspicuous at night leading to the characteristic hemoglobinuria. Patients with paroxysmal nocturnal hemoglobinuria demonstrate the routine lab abnormalities of hemolysis such as a very high LDH. The diagnosis is made by performing flow cytometry to demonstrate the lack of glycosyl phosphatidylinositol-anchored proteins. Patients are often pancytopenic and can present with aplastic anemia. Patients with PNH also have a high incidence of thrombosis including visceral vein thrombosis. Treatment consists of giving the C5 complement inhibitor eculizumab or the longer activing ravulizumab.[37]

7.Congenital hemolytic anemia

Three fundamental processes lead to congenital hemolytic anemia: 1) membrane defects, 2) hemoglobin defects, or 3) enzyme defects.

One of the most common congenital causes of hemolysis is hereditary spherocytosis. In this disease the red cell membrane is abnormal leading to increased splenic destruction. Patients often have a family history of gallstones. Another rare cause of hereditary hemolysis due to membrane defects includes hereditary elliptocytosis. The diagnosis of hereditary spherocytosis is made by finding that spherocytes are present on the peripheral smear and splenomegaly is present on exam. The laboratory values are consistent with hemolysis and the MCHC is elevated. The diagnosis is established by the finding of increased osmotic fragility or demonstrating Band 3 defects. The most common hemoglobin defect is sickle cell anemia. In this disease the abnormal hemoglobin leads to destruction of the red cell. Diagnosis is established by hemoglobin electorphoreisis. Patients may also have chronic hemolysis due to unstable hemoglobins. These patients will often have "Heinz" bodies present on a specially stain blood smear and may have an abnormal hemoglobin electrophoresis. Enzyme deficiencies such as glucose-6-phosphate dehydrogenase deficiency are also important causes of hereditary hemolytic syndromes. The same population at risk for thalassemia is also at risk for G-6-PD deficiency. It is sex linked and thus only affects males. This defect is in the hexose monophosphate shunt and renders the RBC to be unable to withstand oxAIHAtive stress. Most people with this disease have hemolysis only with such stressors as infections and intake of oxAIHAtive drugs. There are two main subtypes-African (A-) and Mediterranean that tends to be more severe. Such drugs as dapsone, pymethroprine, pyridium, and sulfamethoxazole may provoke severe hemolysis in these patients[38].

Macro cytosis

An increased MCV can be due to many reasons but careful review of the patient's history and blood smear can narrow the diagnostic possibilities. The differential can be divided into two broad categories based on RBC morphology.

Round macrocytosis-due to abnormal lipid composition of the erythrocyte membrane.

Diagnosing Vitamin B12 and/or Folate Deficiency

When a patient is believed to have a megaloblastic anemia or a process consistent with vitamin B12 deficiency, one should draw a serum methylmalonic acid, a serum homocysteine level (a more sensitive indicator of tissue stores than serum or red cell folate) and since up to 30% of patients with megaloblastic anemia have concurrent iron deficiency, a serum ferritin.

Recent data suggests that B:

2 levels may not be accurate especially in older patients. Many patients will have low B:2 levels but not tissue deficiency. Up to 15% of patients with normal B12 levels will have tissue deficiencies. Measuring serum levels of methymalonic acid, a metabolic precursor that is increased in B12 deficiency, is now the preferred method of diagnosis especially in older patients. Many patients who are B12 deficiency will also have elevated serum homocysteine levels. Patients with high homocysteine levels should have a methylmalonic acid drawn to insure that they do not have B12 deficiency.

Folate deficiency

Like B12 levels, serum folate levels do not reflect body stores of folate. Serum homocysteine levels are more accurate.[3]

3. Material and Methods

In this cross sectional study one hundred clinically anemic patients of either sex, with age more than 10 years and hemoglobin less than or equal to 10 gm/dl were selected from Private laboratories Detailed history, including history of present illness, past illness, socioeconomic status, dietary habits and alcohol consumption was taken from each patient. In all patients, preliminary blood tests including full blood counts and red cell indices were performed on 5 ml venous blood. Leishman stained blood film was made for every patients and examined. Further, serum ferritin estimation was performed to confirm the diagnosis. Serum ferritin estimation was done by competitive ELISA method. Serum ferritin <30 ng/ml was considered diagnostic in males and <15 ng/ml was considered diagnostic in females. Those patients not giving consent to the study and those receiving treatment for malignancies and liver diseases were excluded from the study.

Data were entered in excel worksheet and analyzed in SPSS 20, IBM, Inc., Chicago, IL software. Mean and frequencies of continuous variables were calculated. The analysis of variance (ANOVA) and Pearson's correlation was applied to assess significance differences. A p-value <0.05 was considered statistically significant.

Equipments and Materials

Device	Processing company and origin
Centrifuge	**Sigma (Germany)**
Vacutainer Tubes	**Biotic (USA)**
EDTA tubes	**Nantong (China)**
Ethanol alcohol	**BDH (England)**
Syringes 5ml	**Nantong (China)**
Pipettes	**Biotic (USA)**
Control Positive	**Nantong (China)**
Control Negative	**Nantong (China)**
Tourniquet	**Unolok (England)**
CBC Device	**Nantong (China)**
A calculator, whether it is ((laptop)) or ((desktop))	**Unolok (England)**
Cotton	**Unolok (England)**
Gel tube	**Nantong (China)**

METHODS

Complete Blood Count

Sample collection methods

The sample is collected by drawing blood into a tube containing an anticoagulant-typically EDTA-to stop its natural clotting. The blood is usually taken from a vein, but when this is difficult it may be collected from capillaries by a finger stick, or by a help rick in babies.

Working methods

Testing is typically performed on an automated analyzer, but manual techniques such as a blood smear examination or manual hematocrit test can be used to investigate abnormal results. [47] Cell counts and hemoglobin measurements are performed manually in laboratories lacking access to automated instruments.

Iron screening steps

The blood iron test is performed like any normal blood test by drawing a blood sample from a vein in the arm after cleansing the skin in the area, placing a plastic band on the top of the arm to facilitate the vein's appearance and drawing blood using a syringe. The drawing process will not take more than 5 minutes, then the sample. will be sent to the laboratory for examination.

How to perform an iron test

Iron levels in the blood have changed depending on the patient's recent diet, and therefore the patient is asked to perform the examination early in the morning or after fasting for 12 hours before the examination is performed, and he is allowed to drink only water during that period. The doctor must be informed of all medications and nutritional supplements that the patient is taking, as some medications may affect the result of the examination.

Coombs Test

Specimen Collection

The collection of a blood sample for anti globulin testing requires a tube that is anti- coagulated with ethylenediaminetetraacetic acid (EDTA); in standard practice, this collection tube traditionally has a lavender, red, or pink top. EDTA is used to chelate serum calcium to prevent in vitro fixation of complement factor C3 that would otherwise lead to a falsely negative result.

Procedures

The AGT is routinely used to screen human sera for anti-human RBC antibodies. The procedure is as follows:

-Use a gel microtube (it contains anti-IgG)

-Add 25 microliters of serum to the microtube

-Add 50 microliters of low ionic strength solution (LISS) - suspended red blood cells at a 0.8% concentration to the reaction chamber of the microtube

-Incubate at 37 degrees Celcius for 15 minutes

-Spin for 10 minutes in a centrifuge at approximately 70 x g.

- After centrifugation, the positive reaction gets graded from 0 to 4+-

-Negative reactions have RBC pellets on the bottom of the microtube with no agglutination

-One + reaction is indicated by erythrocyte agglutination at the lower half of the gel column

-Two + reaction has erythrocytes dispersed throughout the microtube

-Three + reaction contains erythrocytes displayed in the upper half of the gel column

-A four + reaction is indicated by a solid band of erythrocyte on the top of the micro tube chamber.

4. Results

The samples were collected from private laboratories, where 100 blood samples were obtained and the studied tests were performed . As it was a cross sectional study, one hundred consecutive samples diagnosed as anemia based on hematological tests.

Table 1. Distribution of AIHA based on demographic features in study subjects (n=100)

Features	Total anemia patients	Patients with AIHA	Frequency of AIHA (%)
Non -Nutritional supplements	41	17	41.46
Nutritional supplements	59	18	30.50
Recipient blood	35	11	31.42
Non- Recipient blood	65	24	36.92

(Table 1) AIHA was predominant in Nutritional (41.46%) than non Nutritional (30.50) AIHA was more common in non-exercising subjects (36.92%) as compared to exercising subjects(31.42).

Table 2. Age distribution of AIHA patients in study subjects (n=100)

Age groups (years)	Total Anemic patients	Patients with AIHA	AIHA (%)
1-20	10	3	30
21-40	47	20	42-55
41-60	27	7	25.92
61-80	16	5	31.25
Total	100	35	35

The mostly affected age group (Table 2) in anemic patients was 21-40 years (42.55%) followed by 61-80 years (31.25%).

Table 3. Sex wise distribution of AIHA in anemic patients (n=100)

Sex	Total anemic patients	Patient with AIHA	Frequency of AIHA	Patients with other types of anemia	Other types of anemia(%)
Male	37	8	21.62%	29	78.37
Female	63	27	42.85%	36	57.14
Total	100	35	35%	65	65

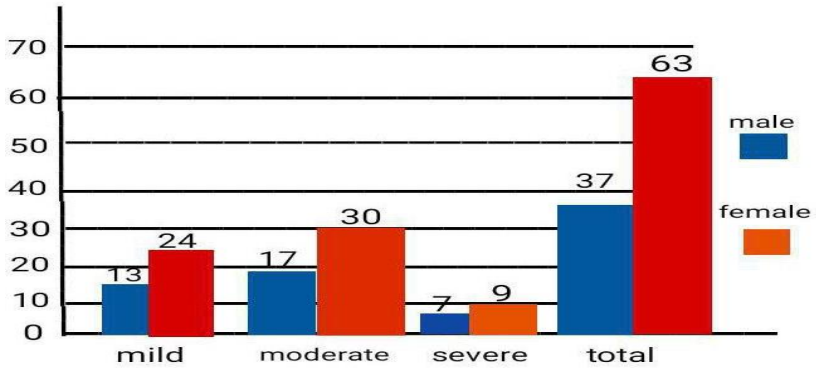

Figure2. Distribution of anemia [mild anemia (Hb-9.1- 10.5 g/dl), moderate anemia (Hb-6-9 g/dl) and severe anemia (Hb<6.0 g/dl)] based on hemoglobin level (WHO criteria) (n=100)

Figure 2 shows that moderate anemia was predominant in 17% of male and 30% of female followed by mild anemia, 13% in male and 24% in female and severe anemia 7% in male and 9% in female. Thus, mild moderate and severe anemia was predominant in female patients than in male patients.

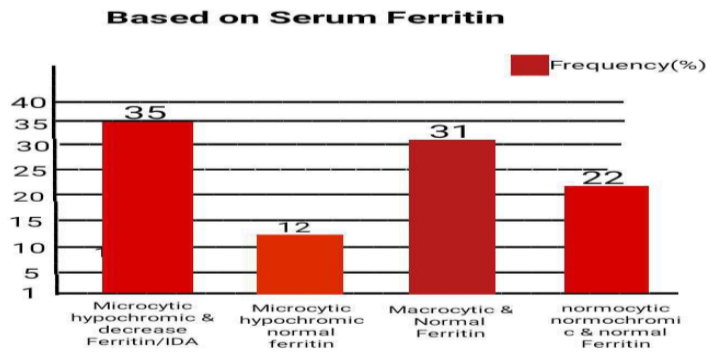

Figure 3. Distribution of patients based on peripheral smear (PS) and serum ferritin

Figure 3 shows that patients with AIHA 35% have microcytic hypochromic anemia and decrease ferritin. 31%. Patients with macrocytic anemia have normal ferritin and 22% of patients with normocytic normochromic anemia have normal ferritin. Out of 47 microcytic hypochromic patients, 12% had normal serum ferritin.

Table 4. Distribution of serum ferritin in anemic patients (n=100)

Ferritin (ng/ml)	(%)Male	Ferritin (ng/ml)	(%)Female
≤ 30	8(21.62)%	≤ 15	**27(42.85)%**
≥ 29	29(78.38)%	≥ 14	**36(57.15)%**
Total	37	Total	**63**

The decrease in serum ferritin in anemic patients was42.85% (Table4) in female as compared to 21.62% in male patients

Table 5. Hematological parameters in types of anemia (n=100)

Variables	Anemia	N	Mean ± SD	p-value
Hb (gm/dl)	AIHA	35	1.94±7.43	**0.05<p**
	MIHC	12	8.38±1.23	
	MAC	30	1.77 ± 7.91	
	NNC	23	1.29 ± 8.94	
RBC (million/µl)	AIHA	35	99 ± 493.	**0.05<p**
	MIHC	12	1.87±3.87	
	MAC	30	81±3.22	
	NNC	23	64.±3.17	
(%)PCV	AIHA	35	8.17±26.28	**0.05<p**
	MIHC	12	4.45±27.90	
	MAC	30	5.85±27.99	
	NNC	23	3.83±28.77	
MCV (FL)	AIHA	35	8.62±67.76	**0.05>p**
	MIHC	12	8.05±69.91	
	MAC	30	5.4±105.30	
	NNC	23	4.56±87.73	
MCH (pg/dl)	AIHA	35	4.13±21.62	**0.05>p**
	MIHC	12	3.52±21.78	
	MAC	30	2.29±33.83	
	NNC	23	2.39±29.44	
MCHC (g/dl)	AIHA	35	1.74±30.28	**0.05>p**
	MIHC	12	1.64±30.88	
	MAC	30	1.27±32.73	
	NNC	23	1.22±31.83	
Ferritin (ng/ml)	AIHA	35	6.53±13.76	**0.05>p**
	MIHC	12	40.77±55.74	
	MAC	30	21.69+ 134.84	
	NNC	23	23.45±138.75	

There was a significant difference among types of anemia (Table 5) viz a viz AIHA (Microcytic hypochromic and decreased ferritin), MIHC (Microcytic hypochromic and normal ferritin), MAC (Macrocytic and normal ferritin) and NNC (Normocytic normochromic and normal ferritin) with Hb (p=0.011), MCV (p=0.0001), MCH (p=0.0001), MCHC (p=0.0001) and serum ferritin (p=0.0001) respectively.

Table 6. Pearson's correlation of variables in study subjects (n=100)

Variables	Ferritin (r)	p-value
Age	0.170	**0.09**
Hb	0.257	**0.01**
RBCS	0.166	**0.098**
MCV	0.772	**0.0001**
MCH	0.741	**0.0001**
MCHC	0.494	**0.0001**
PCV	0.167	**0.096**

There was a statistical significant positive correlation (Table 6) of ferritin with Hb (r=0.257, p=0.01), MCV (r=0.772, p= 0.0001), MCH (r=0.741, p=0.0001) and MCHC (r=0.494, p=0.0001).

5-DISCUSSION

The method used for serum ferritin estimation is non-invasive method used to assess the storage of iron in the body. Based on serum ferritin concentrations, most of the patients were found to suffer from AIHA (53%). Females were affected more than males and most affected age group was between 21-30 years." Ozdemir Netal have shown that iron level and TIBC fluctuate with age and dietary status of the patients. Hence, we justify the investigation to be made for peripheral smear and serum ferritin estimation in anemic patients and correlation were made on those parameters to find out AIHA[48].

In our study, the overall prevalence of iron deficiency anemia in anemic patients was 35%, which was more than that of study conducted by Sinha AK et al" in Biratnagar, Nepal. Their study revealed that 25.57% patients were suffering from AIHA but Lamsal KS et al (TUTH Kathmandu)" showed that 41.35% patients were having AIHA which was more than that of our study. The reason may include increased demand of iron by body in pregnancy, increased loss of iron due to parasitic infestation or due to chronic blood loss, low iron intake, and may be due to decreased absorption of iron.[49]

Our study revealed that the majority of the patients with AIHA were in age between 21-40 years (42.55%) followed by age group between 61-80 years (31.55%). This is similar to that of study conducted by Sinha AK etal "and PatelS etal ". The age group 21-40 includes the females of reproductive age group, increased demand of iron during pregnancy; menstrual blood loss and excessive bleeding during labor period might be the 18-20 possible reasons. In our study, we also have shown distribution of the AIHA with dietary habits of patients. The prevalence of AIHA was more in vegetarian patients (41.46%) followed by non vegetarian patients (30.50%). This is because the iron-rich foods such as beans present low bio availability due to the presence of phytates and fibers. On the other hand, meats have much more iron contents with high bio availability and absorption. Hem iron in meat is easily absorbed from mucosa of duodenum in comparison to non hem iron in vegetables[50]

The categorization of anemic patients based on the peripheral smear examination revealed that there was predominance of microcytic hypochromic anemia (47%) followed by macrocytic anemia (31%) then normocytic normochromic anemia (22%). Out of 47 microcytic hypochromic anemic patients, 35 patients were having AIHA which was confirmed by measuring serum ferritin level of the patients, the remaining 12 patients having microcytic hypochromic blood picture had normal serum ferritin. Serum ferritin was also performed in macrocytic and normocytic normochromic individuals and results were found within normal range. This was similar result as that of WHO/CDC which had shown that AIHA was one of the major concerns in health in developing 8,12 countries like Nepal. However, large number of samples with multi centric approach is required to valAIHAte the limitation behind present study.[51,52].

Conclusion

We conclude from the study we conducted: Iraq is considered one of the Arab countries whose population is most

exposed to anemia of all types. The age group (21-40) is most susceptible to anemia. Women are considered the group most affected by anemia because they are exposed to many physiological conditions, including pregnancy, miscarriage, and menstruation.

Recommendations

This study recommends that no anemic patients should be treated blindly just seeing the hemoglobin level. They must be investigated to find out the cause and type of anemia before starting the treatment. The peripheral smear in conjunction with serum ferritin estimation needs to be included for susceptible individuals to screen the AIHA and other types of anemia. Anemia Effects on the Hb in age group , pcv ,RBCs

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