Original Research Article

A prospective study of anaemia in adult age group in Kalaburagi district region

Abdul Hakeem Attar[1], Ayesha Farheen[2]

[1]Associate Professor, Department of Pathology, ESIC Medical College, Kalaburagi, Karnataka, India
[2]Associate Professor, Department of Pathology, ESIC Medical College, Kalaburagi, Karnataka, India

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Abstract

Introduction: Anaemia is a condition in which haemoglobin (Hb) concentration and/or red blood cell (RBC) numbers are lower than normal and insufficient to meet an individual's physiological needs of affects roughly one-third of the world's population. Vitamin B-12, also called cobalamin, has the biggest and most intricate chemical structure of all the essential vitamins. Without vitamin B-12, cannot make enough heme to produce functional red blood cells. People with low vitamin B-12 levels develop vitamin B-12 deficiency anemia, which is characterized by blood cells poor in hemoglobin**Materials and Methods:Study Design-** The study was carried out at Department of Pathology, ESIC Medical College & Hospital (January 2020 to may 2021), Kalaburagi. It is Prospective and observational study with 100 persons with the age group of 18 to 65 years were studied for estimation of Hb, vitamin B12 and Mean corpuscular volume (MCV). Detailed history and complete physical examination was recorded.**Result:**In our study 100 subjects were enrolled, 51% were males and 49% were females. The maximum number of patients were between 41-60 years of age group and least were more than 61years. The seroprevalence of vitamin B12, the results reveal that vitamin B12 deficiency would increase MCV. Out of 37 patients who were vitamin B12 deficient 8.1% had high MCV (>100 fL), 16.2% had low MCV (<80 fL) and 75.6% were with normal values of MCV (80 - 100 fL). Out of 63 patients with normal vitamin B12 level 28.5% had low MCV and 3.17% had high MCV and 68.2% were with normal value of MCV.**Conclusion:**There is no correlation between vitamin B12 levels and MCV in majority of the cases. MCV should not be the only criteria for ordering vitamin B12 for patients with anemia under evaluation.

Keywords: Vitamin B12 levels, Haemoglobin, Mean corpuscular volume.

Introduction

Vitamin B-12, also called cobalamin, has the biggest and most intricate chemical structure of all the essential vitamins. [1] It plays an essential role in health -- the vitamin keeps nerves functioning properly and also helps cells regulate gene activity. Cobalamin has an effect on body by helping produce haemoglobin, a protein essential for red blood cell function[2].Hemoglobin is the main protein of red blood cells use to transport oxygen. Every hemoglobin molecule contains four heme groups. Each of these heme groups contains an iron atom, and this iron is responsible for hemoglobin's ability to carry oxygen. [3] In oxygen-rich environments, such as in the blood vessels around the lungs, oxygen binds to the iron in hemoglobin. As blood travels, away from the lungs into tissues that are less oxygen-rich, the iron releases that oxygen so it can enter cells. As a need of constant supply of oxygen to maintain cellular metabolism for energy production [4].Vitamin B-12 contributes to hemoglobin synthesis by activating succinyl CoA, a chemical required to make heme. Succinyl CoA serves as a precursor for heme, and it undergoes several chemical modifications to eventually form an active hemoglobin protein. [5] Without vitamin B-12, cannot make enough heme to produce functional red blood cells. People with low vitamin B-12 levels develop vitamin B-12 deficiency anemia, which is characterized by blood cells poor in hemoglobin [6].Vitamin B-12 deficiency anemia affects several tissues. Might notice tongue becomes swollen and red, gums begin bleeding and loss of appetite. The anemia also affects energy levels and cognitive functioning, leaving of fatigued, irritable and unable to concentrate. [7] Vitamin B-12 deficiency anemia also causes a pale complexion and leads to digestive issues, which can include constipation or diarrhea. If already suffer from anemia, it might actually make vitamin B-12 deficiency worse -- pernicious anemia prevents from body from absorbing vitamin B-12 in the digestive tract, lowering the body's B-12 levels [8].Maintain a healthy vitamin B-12 level by getting recommended daily intake of cobalamin -- 2.4 micrograms. Dairy products, meats and eggs all contain vitamin B-12. Strict vegetarians may get cobalamin from fortified cereals or supplements. [9] Vitamin B-12 anaemia can serve as a warning sign for the potential development of more serious conditions. If Vitamin B-12 levels remain low for a long time, person can suffer permanent nerve damage, for example. If developed any symptoms of vitamin B-12 anaemia, visit doctor to receive the appropriate treatment. [10]

Anemia is characterized by hemoglobin (Hb) concentration being lower than a specific threshold, and thus creating an impairment in meeting the oxygen demands of tissues [26]

It is a major public health problem with around 1,620 million people worldwide diagnosed with anemia. Generally, a quarter of the world's population is considered anemic but the prevalence of anemia varies considerably between high-income countries (around 9%) and low-income countries(around43%)[27] . Anemia during childhood has been linked to growth delay, high risk of infections, and poor cognitive and motor development, which may lead to loss of work productivity later in life[28].

Types and causes :Anemia has three main causes: blood loss, lack of red blood cell production, or high rates of red blood cell destruction. These causes might be the result of diseases, conditions, or other factors. Anemia can be classified based on the size of RBC and amount of hemoglobin in each cell. If the size is small then

**Correspondence*
Dr. Ayesha Farheen
Associate Professor, Department of Pathology, ESIC Medical College, Kalaburagi, Karnataka, India.
E-mail: attar.hakeem@gmail.com

microcytic anemia, if it is large then macrocytic anemia, and if normal then normocytic anemia. Normocytic anemia is the most frequently encountered type of anemia. It is found in 6 percent of adult patients. Severe or long-lasting anemia can damage your heart, brain, and other organs in your body. Very severe anemia may even cause death .

These three main types of anemia are caused due to:

i. **Blood loss:** The causes of blood loss can be due to trauma or gastrointestinal bleeding.
ii. **Decreased RBC production:** The causes of decreased RBC production can be iron deficiency, lack of Vitamin B12, thalassemia and a number of neoplasms of the bone marrow.
iii. **Increased RBC breakdown:** The causes of increased breakdown include sickle cell anemia, malaria, or any other autoimmune disease.

Iron deficiency anemia: This is the most common type of anemia. It is caused by a lesser amount of iron in the human body. The human body's bone marrow needs iron to make hemoglobin. Without enough iron, the body cannot make enough hemoglobin for red blood cells. It occurs in many pregnant women. It is also caused by blood loss.

Vitamin deficiency anemia: The body also needs folate and vitamin B-12 to produce red blood cells. A lack of both folate and vitamin B-12 can cause decreased red blood cell production. Another way vitamin deficiency anemia occurs is if the body is not able to process vitamin B-12. This can also lead to vitamin deficiency anemia.

Anemia of chronic disease: There are many chronic diseases such as cancer, HIV/AIDS, rheumatoid arthritis, kidney disease, Crohn's disease, and other chronic inflammatory diseases that cause a decrease in the production of red blood cells.

Aplastic anemia: This happens when your body doesn't produce enough red blood cells. It is very rare and dangerous. It is caused by infections, medicines, autoimmune diseases, and exposure to toxic chemicals.

Anemias associated with bone marrow disease: There are many diseases such as leukemia and myelofibrosis that cause anemia by decreasing blood production in bone marrow.

Hemolytic anemia: This group of anemia happens when red blood cells are destroyed faster than bone marrow can replace them.

Sickle cell anemia: This is an inherited hemolytic anemia. It is caused by a defective form of hemoglobin that forces red blood cells in a sickle shape. These irregular blood cells die prematurely, resulting in a chronic shortage of red blood cells.

Thalassemia: Thalassemia is an inherited blood disorder in which the body makes an abnormal form of hemoglobin. The disorder results in excessive destruction of red blood cells, which leads to anemia. Thalassemia is inherited, meaning that at least one of your parents must be a carrier of the disease. It's caused by either a genetic mutation or a deletion of certain key gene fragments.

Symptoms

Because a low red blood cell count decreases oxygen delivery to every tissue in the body, anemia can cause a variety of signs and symptoms. At first anemia can be so mild that it goes unnoticed. According to the American Society of Hematology (ASH), most people don't realize they have mild anemia until they have a routine blood test. But symptoms worsen as anemia worsens. Feeling tired, weakness, shortness of breath after exertion, poor concentration or a poor ability to exercise, strange cravings to eat items that aren't food, such as dirt, ice, or clay, tongue swelling or soreness, cold hands and feet, On a greater scale: Confusion, feeling of death, loss of consciousness, increased thirst, and the patient may become pale.

a. Being pale or having yellow "sallow" skin
b. Unexplained fatigue or lack of energy
c. Shortness of breath or chest pain, especially with activity
d. Unexplained generalized weakness
e. Rapid heartbeat
f. Pounding or "whooshing" in the ears
g. Headache, especially with activity
h. Picophagia
i. Sore or smooth tongue
j. Brittle nails or hair loss

Diagnosis

The diagnosis of anemia at home is difficult unless bleeding is obvious. Specialists can, without much of a stretch, recognize anemia by drawing a blood test for a complete blood count (CBC). The CBC might be done piece of a standard general registration or in light of the nearness of signs and side effects suggestive of paleness. Physical examination and medicinal history likewise assume a significant part in diagnosing reasons for frailty. While performing a complete physical examination, the physician may particularly focus on general appearance, jaundice, paleness of the nail beds, heart sounds, and lymph nodes. Since anemia is just a side effect of another illness, specialists will need to figure out what condition is causing it. A few people may require numerous extra tests, and others may require not as many. For instance, a weak individual with known stomach ulcers commonly would not require various blood tests, but rather may need his or her stomach outwardly assessed and have the ulcers treated. Doctors also take into consideration the severity of the anemia when deciding what tests to order. When a person has severe anemia, the cause must be determined rapidly so that it can be treated appropriately.

The following tests are/can be carried out to diagnose anemia:

1. Complete blood count (CBC)
2. Stool hemoglobin test
3. Peripheral blood smear
4. Iron level
5. Transferrin level
6. Ferritin
7. Folate
8. Vitamin B12
9. Bilirubin
10. Lead level
11. Hemoglobin electrophoresis
12. Reticulocyte count
13. Liver function tests
14. Kidney function test
15. Bone marrow biopsy

Note: The CBC determines the severity and type of anemia and is typically the first test recommended. Information about other blood cells such as white blood cells and platelets is also included in this report. Hemoglobin and hematocrit measurements in a complete blood count test are commonly used to diagnose anemia. Specialists measure the amount of hemoglobin, which is an accurate reflection of RBC quantity in the blood.

A serum iron level may tell the specialist whether anemia might be identified with iron insufficiency or not. This test is generally joined by different tests that measure the body's iron stockpiling limit, for example, transferrin level and ferritin level.

Treatment

Since iron anemia is ordinarily intimation to another basic illness, it should be completely assessed by a specialist, and appropriate testing should be attempted to determine the cause. In this manner, if signs and indications of iron deficiency are available, one should contact his or her doctor immediately for assessment. Therapeutic treatment of anemia changes generally and relies on upon the cause and the seriousness of iron deficiency. If anemia is related with sudden blood loss from an injury or a quickly draining stomach ulcer, at that point hospitalization and transfusion of red blood cells might be required to calm the indications and supplant the lost blood. Additionally measures to control the draining may happen in the meantime to stop additionally blood misfortune. Blood transfusion may be required in other less critical circumstances as well. For instance, a person who is accepting chemotherapy for a cancer might be normal by the treating doctor to have bone marrow issues identified with the

chemotherapy. In this way, the specialist may check blood tallies routinely, and if the levels get to a sufficiently low level, he or she may arrange a RBC transfusion to help with the side effects of iron deficiency.Iron might be taken amid pregnancy and when the body iron levels are low. Vitamin supplements may supplant folate and vitamin B12 in individuals with poor dietary patterns. In individuals with vindictive pallor who can't ingest adequate measures of vitamin B12, month to month infusions of vitamin B12 are normally used to loaded the vitamin B 12 levels and correct the anemia. Epoetinalfa is a medicine that can be given as an infusion to build RBC generation in individuals with kidney issues. Next to no should be possible to self-treat anemia and therapeutic treatment is for the most part required. It is critical to keep on taking any medicine that is endorsed for other perpetual restorative issues. In the event that the explanation behind anemia is known, at that point measures to monitor it are essential. For instance, if frailty is caused by a stomach ulcer, at that point medicines, for example, aspirin or ibuprofen ought to be maintained a strategic distance from, unless generally coordinated by a specialist.

Materials and Methods

Study Design- The study was carried out at Department of Pathology, ESIC Medical College & Hospital(January 2020 to may 2021), Kalaburagi. It is Prospective and observational study with 100 persons with the age group of 18 to 65 years were studied for estimation of Hb, vitamin B12 and MCV. Detailed history and complete physical examination was recorded. Informed written consent was taken from all the study subjects.

Exclusion criteria

1. Those on iron or vitamin B-complex supplements.
2. Chronic liver failure as these patients has low vitamin B12 levels.
3. Patients on anti-consultants and anti-cancer drugs as these patients have low folate levels.
4. Patients with acute infections and acute inflammatory diseases, as these patients show false high ferritin levels. This is because ferritin is an acute phase reactant.
5. Chronic renal failure.

Blood sample collection: About 10 ml of peripheral venous blood was collected from all the study subjects.

Investigations

Routine investigations: These included:

1. Haemoglobin levels and complete blood count. These were done on Beckman Coulter cell counter. Peripheral blood smear was studied for red blood cell morphology.
2. Biochemical investigations included fasting blood glucose, serum electrolytes, liver function tests (SGOT, SGPT, ALP, serum bilirubin) and renal function tests (Blood urea and serum creatinine). The tests were done on Beckman coulter AU-480 fully automated analyser. These tests were done to rule out patients of chronic liver failure and chronic renal failure.

Special investigations: These included:

1. Serum vitamin B12: These parameters were measured on Access-2 chemiluminescence machine. Measurement of vitamin B12 is based on the principle of competitive binding immunoenzymatic assay. [11] Vitamin B12 is the sample binds to the conjugate, preventing the conjugate from binding to the solid phase antibody. After incubation in a reaction vessel, the material bound to the solid phase is held in a magnetic field while the unbound material is washed off. Then the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The photon (light) production is inversely proportional to the concentration of vitamin B12 or folic acid in the sample. Normal value of vitamin B12 is 180-971 pg/ml. [12]

Statistical analysis: Statistical analysis of results was done using SPSS 20 version software. Baseline characteristics of the study subjects were presented as mean + standard deviation. ANOVA (Analysis of Variance) was used for multiple comparisons of parameters between the two groups. p value was calculated to know the significance of difference in the individual variables among the two groups. p value < 0.05 was considered to be statistically significant, while p value < 0.001 was considered to be highly significant. Pearson's correlation coefficient (r value) was calculated between the values of serum ferritin and serum vitamin B12, and also r value was calculated between the values of serum ferritin and folic acid.

Result

In our study 100 subjects were enrolled.

Table 1: Distribution of patients according to Gender

Gender	Patients
Male	51 (51 %)
Female	49 (49 %)
Total	100 (100 %)

In table 1, total 100 patients were there, 51% were males and 49% were females.

Table 2: Distribution of Patients according to Age group

Age-group	Patients
18-40	34
41-60	47
>61	19
Total	100 (100 %)

In table 2, maximum number of patients were between 41-60 years of age group and least were more than 61years.

Table 3: Correlation of vitamin B12 and MCV with distribution of patients

Vit. B12 (pg/mL)	% of Patients	Mean MCV (fL)
500 - 1000 pg/mL	25	86.9
240 - 499 pg/mL	38	87.2
100 - 239 pg/mL	35	88.1
<100 pg/mL	2	104.6

The seroprevalence of vitamin B12, the results reveal that vitamin B12 deficiency would increase MCV in table 3.

Table 4:MCV distribution in patients with low vitamin B12 levels (n = 37)

No. of Patients		
Low MCV (<80 fL)	Normal MCV (80 - 100 fL)	High MCV (>100 fL).
6 (16.2%)	28 (75.6%)	3 (8.1%)

Out of 37 patients who were vitamin B12 deficient 8.1% had high MCV (>100 fL), 16.2% had low MCV (<80 fL) and 75.6% were with normal values of MCV (80 - 100 fL) in table 4

Table 5: MCV distribution in patients with normal vitamin B12 levels (n = 63)

No. of Patients		
Low MCV	**Normal MCV**	**High MCV**
18	43	2

Out of 63 patients with normal vitamin B12 level 28.5% had low MCV and 3.17% had high MCV and 68.2% were with normal value of MCV

Discussion

Anaemia is a condition in which haemoglobin (Hb) concentration and/or red blood cell (RBC) numbers are lower than normal and insufficient to meet an individual's physiological needsand affects roughly one-third of the world's population. [13] Anaemia is associated with increased morbidity and mortality in women and children,poor birth outcomes,decreased work productivity in adults,and impaired cognitive and behavioural development in children.Preschool children (PSC) and women of reproductive age (WRA) are particularly affected [14]

One of the most notable finding is every third person is Vitamin B12 deficient in this region in concordance with study by Ganji V *et al*. in 2006. [15] The population of the Indian subcontinent is >1 billion, most of whom consume a diet low in Cobalamin. Isolated reports suggest that Cobalamin deficiency in India is common; however, this problem has received little attention. The national strategies for improving micronutrient intake do not include Cobalamin. [16] In 1934 Wintrobe published synthesis of Red Cell Measurement for diagnosis and classification of Anaemia. This classification was based on derived Red Cell Indices. Erythrocyte Indices have been used in initial evaluation of Anaemia patients, High MCV values is traditional criteria for B12 and folate deficiency. [17] Pfeiffer CM *et al*. suggested that vitamin B12 should be determined in Anaemia patients when MCV > 100 fL.[18] Our study also proves inverse relationship of MCV with vitamin B12 deficiency as Rock CL *et al*. [19]. In our study, several interesting observations during our study in which traditional criteria of vitamin B12 and MCV association was not followed in half of the cases. Among 100 patients only 5% had macrocytosis, 19% had microcytosis and 74% were normocytic. These findings suggest that concomitant iron or other nutritional deficiencies or hemoglobinopathies may have been responsible for the normal or even low MCV values in some of our anaemic patients; such anaemias and hemoglobinopathies have been seen commonly in Southeast Asians. [20] Moreover, the combination in anaemia has been reported in the literature. [21] Archer SL *et al.* reported that 82% of patients with low B12 levels seen at Bellevue Hospital, New York, had MCV values below 95 fL; they suggested that further evaluation of the suspected B12 deficiency should not be deterred by a normal MCV value which is one of the important observation in our study. [22] In addition, one study from Vancuover AIDS conference (1996) suggested that MCV does not always get high even if vitamin B12 is low so a normal value does not necessarily mean that B12 levels is normal, which correlates with our findings. Thus, physicians should not consider elevated MCV as diagnostic criteria for Vitamin B12 deficiency. [23]Honein MA *et al*. analysed the diagnostic value of an elevated MCV for B12 deficiency where the sensitivity was only 17% - 30%, and up to 84% of the deficiency would be missed. [24] Any screening criteria selected would miss a significant number of B12 deficient patients, so there may be a case for universal B12 screening [25] . Genetic factors are responsible for hemoglobinopathies, such as sickle cell anemia and thalassemia, while in some settings infectious diseases like malaria, soil-transmitted helminths and schistosomiasis are major contributors to anemia. Nutritional anemia results from insufficient nutrients that are needed during Hb synthesis and erythropoiesis.

Conclusion

In this study we have noticed that every third person is vitamin B12 deficient in the region. There is no correlation between vitamin B12 levels and MCV in majority of the cases. MCV should not be the only criteria for ordering vitamin B12 for patients with anaemia under evaluation.

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