

Original research article

A Observational Study of Anaemia in Adult Age Group at a Tertiary Centre in Uttar Pradesh

Dr. Sandeep Kumar¹, Dr. Neeta Kapoor², Dr. K. Manu³,

¹ Assistant Professor, Dept. of Pathology, Prasad Institute of Medical Sciences, Lucknow.

² Associate Professor, Dept. of Pathology, Prasad Institute of Medical Sciences, Lucknow.

³ Assistant Professor, Dept. of Pathology, Prasad Institute of Medical Sciences, Lucknow.

Corresponding Author: Dr. K. Manu

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, Prasad Institute of Medical Sciences, Lucknow (February 2022 to May 2022), Uttar Pradesh. It is 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 (around 43%)^[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].

Materials and Methods

Study Design:

The study was carried out at Department of Pathology, Prasad Institute of Medical Sciences, Lucknow (February 2022 to May 2022), Uttar Pradesh. It is 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

Those on iron or vitamin B-complex supplements.

1. Chronic liver failure as these patients have low vitamin B12 levels.
2. Patients on anti-convulsants and anti-cancer drugs as these patients have low folate levels.
3. 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.

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 Sysmex 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 ERBA fully automated analyser. These tests were done to rule out patients of chronic liver failure and chronic renal failure.

Special investigations: These included:

Serum vitamin B12: These parameters were measured by Calbiotech ELISA method. Measurement of vitamin B12 is based on the principle of based on the principal of delayed competitive binding assay. ^[11] Streptavidin coated wells are incubated with extracted Vitamin B12 standards, controls, samples, and Intrinsic Factor-Biotin conjugate at room temperature for 45 minutes.

During the incubation, the biotin-labeled intrinsic factor will bind to the Vitamin B12 in the sample, standard, or quality control serum. After the 45 minute incubation, Vitamin B12-Enzyme conjugate is added which competes with the Vitamin B12 of the sample, standard, or quality control serum for remaining sites on the Intrinsic Factor for an additional 30 minutes. All unbound conjugates are then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 15 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The color intensity is inversely proportional to the amount of Vitamin B12 in the sample. The total assay procedure run time is 1.5 hours. 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 (rvalue) 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: showing the 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: showing the distribution of patients according to Age group above 18 years to 61years

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 61 years.

Table 3: showing the Correlation between 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: showing 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: Showing 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 .

{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 61 years. 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 - 100fL). 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 needs and 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 inconcordance 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 Vancouver 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. The high proportion of microcytic anaemia and the fact that gender differences were only seen after the menarche period in women indicate that iron deficiency was the main cause of anaemia. In a study of children aged 12–23 months in two rural districts in India, 72% of children with anaemia had low ferritin levels^[29]. Effective public health programmes aimed at reducing iron deficiency among young women could have a major impact in reducing maternal and infant mortality^[30]. The WHO organization recommends introducing solid and semisolid food at the age of six months because breastfeeding does not suffice to maintain optimal growth after this age. However, at age 6–8 months only 45% of children receiving breastfeeding are given solid or semisolid food^[31]. In this study, we observed an increased prevalence of anaemia with age. Interestingly, the proportion of normocytic anaemia was highest in older adults, suggesting that other causes than iron deficiency might have contributed to the high prevalence of anaemia in this group. Recent studies have shown the poor bioavailability of vitamin B12 in the typical Indian vegetarian diet^[32]. Anaemia is a late indicator of iron deficiency, so it is estimated that the prevalence of iron deficiency is 2.5 times that of anaemia [1, 2]. The estimated prevalence of anaemia in developing countries is 39% in children <5 years, 48% in children 5–14 years, 42% in women 15–59 years, 30% in men 15–59 years, and 45% in adults >60 years^[33]. In our

study, we did not observe an increase in haemoglobin concentrations during the study period suggesting that the programme has not achieved a reduction in the prevalence of anaemia in our setting our results are in agreement with other studies in India^[34]. Indian Government recommends iron and folic acid supplementations to younger children^[35]. However, the programme implementation has been poor due to lack of logistic planning and accountability^[35].

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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