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# Evaluation of vitamin D level and iron profile among anemic and apparently healthy Sudanese individual in Khartoum State in 2022- 2023.

**Dalia A H Alhashmi[1], Wala Eldin Osman Elradi[1], Elharam IbrahimAbdallah[1], Ranya Mohammed El Magzoub[2], Dalia Mohammed Osman Khalfallah[2], Mervat Mohammed Mazhar[3], Manal S. A. Elseid [3], Sara A B Mahala[2], Safa A. Wdidi[4], Huziefa Saad Elfaki[5]& Marwa Mohamed A. A[6].**

**1-Faculty of Medical Laboratory Science-Hematology Department-Alzaeım Alazhary unıversıty-Sudan**

**2- Faculty of Applied Medical Sciences, Department of Medical Laboratory Technology, Northern Border University, Arar, KSA.**

**3- Faculty of science – Math department- Northern Boarder University-Arar- KSA.**

**4-Omdurman friendship Hospital-Hematology Department-Sudan.**

**5-Alneelain University – Medical laboratory sciences- Hematology Department -Sudan.**

**6- Alneelain University – Medical laboratory sciences- microbiology Department-Sudan.**

**Corresponding Author**: Wala Eldin Osman Elradi, Associate Professor, Department of Hematology and Blood Transfusion, Faculty of Medical Laboratory Sciences, Alzaeim Alazhari University, Khartoum, Sudan. Email add: mamoma125elradi@gmail.com

**Abstract:** Vitamin D deficiency and anemia are important public health problems and are common in both acute and chronic illness. so this study aimed to determine vitamin D and iron profile levels among anemic and apparently healthy Sudanese individual in Khartoum State. This descriptive cross sectional study was conducted to assess vitamin D and iron profile among 60 patients with anemia, they were 20 (33.3%) males and 40 (66.7%) females and 30 apparently healthy individual they were 14 (46.7%) males and 16 (53.3%) females. in this study There were 34 patients with iron deficiency anemia (79% of whom had vitamin D deficient), while 21patients with anemia of chronic disease (71% had vitamin D deficient), also there were 5 patients with megaloblastic anemia (60% had vitamin D deficient) and 30apparently healthy individual (70% had vitamin D deficient). The vitamin D level should no significant difference between anemic patients and apparently healthy individual (P V > 0.05), so vitamin D deficiency and insufficiency was prevalent, in all the groups. This study concluded that there was very low level of vitamin d among anemic and apparently healthy individual. Regarding iron profile also there was different variance in the results that indicate apparently healthy individuals have some types of pre-latent and latent iron deficiency anemia. As for the relationship of vitamin D with iron profile, there was no correlation between iron profile and vitamin D level among anemic and apparently healthy individual. with regard to the level of vitamin D between both sexes, there was a significant decrease in the level of vitamin D in females compared to meals.

**Keyword: Hepcidin, vitamin D, Anemia**

## Introduction

With over 2 billion cases worldwide, anemia is the most prevalent blood disorder and a major health burden [(1)]. The hemoglobin (Hb) levels in the blood fall below normal ranges in this condition, and the count of red blood cells (RBCs) also decreases. As much as one-third of the global population suffers from anemia, making it a very common condition. It often has no symptoms, is mild, and doesn't need to be treated. It is a symptom of an underlying medical condition rather than an illness. Typically, it is categorized based on the biological mechanism [(2)]. Anemia affects people of all ages and is a common issue in both developed and developing nations. Anemia is defined by the World Health Organization (WHO) as a hemoglobin (Hb) level that is less than 13. g/dl for men and less than 12.0 g/dl for women. However, the distribution of normal hemoglobin differs depending on race, sex, and physiological state. According to age, sex, and race, new lower limits for normal Hb levels have been proposed. Anemia is typically not an isolated occurrence but rather a complex phenomenon. Hematological parameters, underlying pathological mechanisms, and medical history should all be taken into account for diagnosis and classification. Anemia is becoming more common in older adults due to population aging, particularly in Western nations. Given that anemia in this population raises morbidity and mortality, it is crucial to comprehend its pathophysiology. Iron, folic acid, or vitamin B12 deficiencies were the cause of anemia in one-third of the patients; chronic disease-related anemia accounted for another third of cases. However, one-third of patients have anemia for which there is no known underlying cause or specific pathological process; this condition is known as "anemia of unknown etiology." Chronic subclinical pro-inflammatory conditions and increasing resistance of bone marrow erythroid progenitors to erythropoietin may be the cause of unexplained anemia [(3)]. There is evidence that anemia, particularly in older adults, may be linked to poor outcomes in a variety of diseases. Nearly 10% of men and women over 65 who participated in a recent large-scale population survey based on WHO criteria (NHANES-III) were anemic. These percentages increased to 26% for men and 20% for women over the age of 85. It is unclear if the androgen-dependent age-related difference in the lower limit should continue past the age of 65. Many of these individuals seemed well, and most of the time, clinical examinations did not identify a particular cause of anemia. These findings imply that cutoffs that are marginally below "normal" can be applied to older adults. But it is far too simple to assume that mild anemia in the elderly is a physiological anemia, which runs the risk of neglecting the underlying illness. The lower hemoglobin threshold that should be used to diagnose anemia in the general population—especially in the elderly—is a topic of debate. Hemoglobin was determined using standardized automated methods using two distinct, relatively new large databases (Scripps-Kaiser and NHANES-III). Good agreement was obtained, and new lower limits were proposed. These cut-off values (5% of the normal distribution) seem to be 137 g/L for white men aged 20 to 59 and 132 g/L for men over 60; the corresponding values for women, irrespective of age, are 122 g/L. These limits are lower for African-Americans: 129 g/l for young men and 127 g/l for men over 60, compared to 115 g/l for women of all ages. Although this simplification isn't always accurate, many practical methods assume that a decrease in hematocrit (HCT) corresponds to a decrease in

hemoglobin concentration. When analyzing RBCs with a high mean hemoglobin concentration (MCHC), all impedance-based hematology analyzers incorrectly overestimate Hct, while underestimating Hct in hypochromic red blood cells. In the last example, diagnosing anemia in people who are iron deficient may be overestimated if Hct is used instead of the more precise measured Hb. Furthermore, when analyzing crescent cells that cannot be spheroidized, optical-based instruments with iso-volumic spheroidization may produce falsely elevated PCT. The presence of RBC agglutinates is a common artifact of the separation between Hb results—which are typically accurate—and Hct results—which are typically underestimated—in all automated analyzers. Because the analyzer only counts cells up to 200–300 fL as red blood cells, large clumps of red blood cells are not counted as red blood cells. Low Hct and erroneously low RBC counts may result from this. On the other hand, agglutinins had no effect on Hb, which was measured subsequent to RBC lysis. MCHC is therefore unusually high, typically more than 360 g/L. Moreover, excessive turbidity can lead to an overestimation of hemoglobin (Hb) in patients with severe hypertriglyceridemia, intravenous lipid emulsion treatment, or elevated white blood cell counts. However, this is not a common occurrence [(4)].

**Etiology**

The immediate treatment of anemia depends on the underlying cause. An etiology-based approach is important for the prevention, diagnosis, and treatment of anemia. While acute anemia is primarily due to acute blood loss or acute hemolysis, chronic anemia is more common and secondary to various causes. Using a kinetic approach, anemia is classified according to the associated pathophysiological mechanisms, including decreased erythropoiesis, increased erythrocyte destruction, and blood loss. The reticulocyte count is used to estimate the extent of effective erythropoiesis, and the corrected reticulocyte count is < 2% in hypo proliferative anemia and > 2% in hyper proliferative anemia [(2)]. The etiology of anemia depends on whether the anemia is hypo proliferative (corrected reticulocyte count < 2%) or hyper proliferative (corrected reticulocyte count > 2%). Hypo proliferative anemia is further subdivided based on mean corpuscular volume into microcytic (MCV < 80 fl), normocytic (MCV 80-100 fl), and macrocytic (MCV > 100 fl) [(5-8)].

**I\ Types of anemia according peripheral appearance**

1) Hypoproliferative Microcytic Anemia (MCV<80 fl) Includes: Lead poisoning, thalassemia, iron deficiency anemia, anemia of chronic disease (AOCD), and sideroblastic anemia (which can also be linked to elevated MCV and a dimorphic cell population).

**2) Hypoproliferative Normocytic Anemia (MCV 80-100 fL)** These include: Renal failure, aplastic anemia, red blood cell aplasia, myelofibrosis or myeloproliferative processes, anemia of chronic disease (AOCD), and multiple myeloma. Both hemolysis and hypoproliferative disorders are possible causes of macrocytic anemia. Therefore, when evaluating patients with macrocytic anemia, it is crucial to determine the corrected

reticulocyte count. The MCV exceeds 100 fl and the adjusted reticulocyte count is less than 2% in hypoproliferative macrocytic anemia. However, if the reticulocyte count is greater than 2%, hemolytic anemia should be suspected.

**3) Hypoproliferative Macrocytic Anemia (MCV>100 fL)** Causes: Refractory anemia (RA), refractory anemia with ringed sideroblasts (RA-RS), refractory anemia with excess blasts (RA-EB), refractory anemia with excess blasts in transformation, hypothyroidism, alcohol, myelodysplastic syndrome (MDS), folic acid and vitamin B12 deficiency, and chronic myelomonocytic leukemia (CMML). drug-induced by antiretrovirals, antibacterials, hypoglycemics, chemotherapeutics, diuretics, and anticonvulsants.

**4) Hemolytic anemia (HA)** is divided into extravascular and intravascular causes.

I/ Extravascular hemolysis: The liver and spleen remove red blood cells from the bloodstream too soon. This accounts for most cases of hemoglobinopathies (thalassemia, sickle cell disease), enzyme deficiencies (pyruvate kinase deficiency, G6PD deficiency), membrane abnormalities (hereditary spherocytosis, hereditary elliptocytosis syndrome), and drug-induced hemoglobinopathies. II/ Intravascular hemolysis: Less common causes of RBC lysis in the circulation include PNH, AIHA, transfusion reactions, MAHA, DIC, infection, and venom/snakebite [(5-8)].

**II\ Types of anemia not genetic caused**

**a) Iron deficiency anemia**

Iron deficiency anemia is a common comorbidity of multiple diseases and a global health concern affecting women, children, and the elderly. The etiology varies, can be attributed to several risk factors that either increase iron demand and losses or decrease iron intake and absorption, and frequently coexist in a single patient. There is mounting evidence that iron deficiency anemia negatively impacts the clinical outcome of numerous diseases, despite the fact that symptoms may not be specific. Early detection and treatment of iron deficiency anemia can be aided by increased knowledge of the condition's prevalence and effects. Serum ferritin and hemoglobin levels can be easily measured to make a diagnosis; however, chronic inflammatory. There are oral and intravenous iron preparations available; however, before choosing a course of treatment, a number of patient- and disease-related factors need to be taken into account. Current recommendations and updates on the diagnosis and treatment of iron deficiency anemia in various clinical contexts are provided in this review [(9)].

**b) Macrocytic anemia**

A macrocytosis (mean corpuscular volume (MCV) greater than 100 fL) is the definition of macrocytotic anemia. Hemoglobin less than 13 g/dL in either gender, or PCT less than 41%. There are two types of it: nonmegaloblastic and megaloblastic (multilobular neutrophils). The non-megaloblastic form is caused by a combination of factors, while the megaloblastic form is caused by reduced DNA synthesis as a result of folic acid and/or vitamin B12 deficiency. Macrocytic anemia is a blood disorder with reduced

hemoglobin and an increased mean corpuscular volume (> 100 fL). Its causes range from easily treatable to fatal [10-11]

### c) Anemia of chronic disease

Chronic disease-related anemia is a disorder where there is a specific underlying disease and a decrease in hemoglobin, hematocrit, and red blood cell counts due to a complicated process that is frequently brought on by proinflammatory cytokines, hepcidin, and cellular immune mechanisms. After iron deficiency anemia, this is the second most common type of anemia in the world. Usually, their severity corresponds with the underlying disease. The illness is frequently linked to kidney failure, cancer, autoimmune diseases, and chronic inflammation. Prior to starting treatment, a complete diagnosis should be made, taking into account the severity of the underlying disease as well as the assessment of biochemical parameters and complete blood counts. The primary basis for the differential diagnosis of anemia associated with chronic illness is the rule out of other anemia types, particularly iron deficiency anemia. Low iron and transferrin concentrations but high ferritin concentrations, mildly to moderately reduced hemoglobin levels, and a decreased percent reticulocyte count are important characteristics of anemia of chronic disease. The expanding understanding of the pathological mechanisms underlying cancer biology and chronic disease has led to the inclusion of new biochemical markers in the diagnosis of this anemia. These include: quantities of other hematopoietic factors, including erythropoietin, creatinine, hepcidin, folate, and vitamin B12. Treatment for anemia of chronic disease still primarily consists of iron, folic acid, and vitamin B12 supplements combined with a diet high in the hematopoietic factors mentioned above. The benefits and potential side effects of each administration route—oral, intramuscular, or intravenous—must be carefully considered. The patient's clinical condition must also be evaluated. Hope is being sparked by a novel strategy for treating anemia and underlying diseases. Novel strategies have been connected to both complementing deficits and drug delivery that molecularly targets particular proteins or receptors implicated in the onset of chronic disease-related anemia [12].

## Epidemiology

Central Asia, South Asia, and West Africa have all been found to have high rates of anemia in children under the age of five; in some of these nations, the prevalence of anemia in the general population is reported to be higher than 70%. According to estimates, 59% of Tanzanian children under the age of five suffer from anemia, with boys being more likely than girls to be affected. Anemia increases a woman's risk of both negative perinatal and maternal health outcomes, including intrauterine growth retardation, preterm birth, stillbirth, impaired physical and cognitive development, and growth retardation Add about children. Negative maternal outcomes include antepartum and postpartum hemorrhage [13].

## Vitamin D

It is a fat-soluble vitamin, vitamin D. Dermal synthesis after exposure to ultraviolet B (UVB) radiation remains because few foods naturally contain vitamin D (oily fish, such as sardines, herring, tuna, mackerel, salmon and cod liver oil, egg yolks, shiitake mushrooms, liver or organ meats). Additionally, it makes up 90% of vitamin D supplements, making it the primary method of obtaining the vitamin. Animals provide ergocalciferol (vitamin D2), while plants provide cholecalciferol (vitamin D3). After exposure to UVB irradiation (wavelength 290-315 nm), cholesterol-like precursors (7-dehydrocholesterol) in skin epidermal cells can be converted to provitamin D, which is further isomerized to vitamin D3. Vitamins D2 and D3 are not biologically active. To get the active form, they must undergo additional enzymatic conversion It first goes through 25-hydroxylation in the liver to become 25(OH)D, or calcifediol, which is the primary form of vitamin D that circulates and has a two to three weeks' half-life. It then undergoes 1-alpha-hydroxylation in the kidney, where it is transformed into its most active form, 1,25(OH)2D (calcitriol), which has a half-life of 4-6 hours. Growth hormone, hypophosphatemia, and parathyroid hormone (PTH) are some of the mediators that drive this process. It is possible that 1,25(OH)2D has an autocrine-paracrine effect because 1-α-hydroxylation also occurs in non-renal sites like alveolar macrophages, osteoblasts, lymph nodes, placenta, colon, mammary gland, and keratinocytes. The vitamin D receptor (VDR), which is widely expressed in nucleated cells, is how it works. Its primary biological function is to support intestinal calcium absorption and cell differentiation, which in turn supports calcium homeostasis. A lack of vitamin D causes inadequate 25(OH)D circulation, which lowers the synthesis of 1,25(OH)2D and the absorption of calcium while raising PTH levels. Given that VDR is found in the colon, osteoblasts, activated T and B lymphocytes, monocytes, pancreatic β-cells, and the small intestine, [(14)].

**Vitamin D and anemia**

As a fat-soluble vitamin, vitamin D can be produced by exposure to direct sunlight (cholecalciferol) or obtained through diet (ergocalciferol from phytosterols). This vitamin is hydroxylated in the liver to produce 25-hydroxyvitamin D (25OHD), which is subsequently transformed in kidney cells into calcitriol (1,25(OH)2D). It appears that vitamin D plays a role in immune modulation, cell growth regulation, differentiation, and inducing erythropoiesis in bone marrow cells, in addition to preventing chronic disease. Adult anemia and vitamin D levels are inversely correlated, according to a number of observational studies. The stimulation of erythroid progenitor cell receptors by calcitriol (1,25-hydroxyvitamin D) can aid in the development and growth of these cells. Hepcidin levels' mRNA expression has been reported to be downregulated by the anti-inflammatory effects of vitamin D. It is believed that antimicrobial hepcidin peptides participate in iron uptake and release through the inhibition and activation of cellular iron exporters, or iron transporters. As a result, variations in the prevalence of anemia and iron status are anticipated (15). Because vitamin D deficiency is linked to many chronic illnesses and diseases, including osteoporosis, cancer, and metabolic syndrome, it is also a significant public health concern. Moreover, vitamin D signaling alterations as well as

vitamin D deficiency have been linked, particularly in individuals with renal illness. Dietary consumption or prolonged skin exposure to UV radiation are two ways to meet your needs for vitamin D. Growing research in recent years has connected vitamin D deficiency to a higher risk of anemia, a common disorder that affects up to 20% of children. Large-scale research, however, has not shown a connection between vitamin D and hemoglobin levels in the general population [(16)].

**Lab diagnosis of anemia not related to genetic issues**

**I/ Complete blood count**

Anemia is a condition marked by a lack of red blood cells or by blood hemoglobin levels that fall below a range that is estimated for a given sex and age [(17)]. Although automated red blood cell counts (RBCs) have been recommended as a more accurate way to diagnose anemia, not all medical facilities around the world have access to this technology. Therefore, this technology is not appropriate for point-of-care testing, particularly in nations with lower and middle incomes. Multiple RBC indices can be collected simultaneously by automated RBCs, which can be used to evaluate anemia. [(18)]. Peripheral blood smears (PBSs) can also be used to diagnose anemia. A microscopic analysis of PBSs can reveal important details regarding alterations in the size and form of red blood cells as well as the presence of inclusion cells. Hematologists use RBC morphology as a crucial tool to determine the best test for a conclusive diagnosis and to suggest appropriate clinical and laboratory follow-up. Clinical parameters and RBC morphology can be used to analyze anemia. A trained laboratory technician will place a tiny drop of blood on a glass slide, stain it with Giemsa, Leishman, or Wright-Giemsa, and then examine it under a microscope to perform a morphological analysis of the blood smears. Red blood cells, platelets, and white blood cells (WBC) are the various cell types seen in a blood smear. PBS pictures showcasing different blood cells. Red blood cells are more common than white blood cells and platelets, as can be seen. A pathologist assesses the size, shape, and color of red and white blood cells during a swab. They estimate the quantity of platelets as well. RBC indices serve to define RBC mass, and any deviation in the size, volume, or shape of RBCs indicates an abnormality in RBCs [(17)].

**II/ Iron profile**

**A**: Iron circulation, intestinal absorption, and dietary intake are the primary mechanisms regulating the essential element iron [(19)]. The amount of iron in blood plasma that is bound to transferrin is measured by serum iron. At any given time, very little of the body's iron is bound to transferrin. Because iron is rapidly bound by transferrin and is influenced by food intake, blood iron levels can vary greatly both within and between days. As a result, serum iron measurement by itself doesn't yield much clinically relevant data [(20)].

**B: Ferritin:** Is primarily found in cells and is the body's most significant iron storage protein. On the other hand, a testable soluble form can be detected in the blood. Males have higher levels than females during adolescence, and this trend continues into late

adulthood. Its concentrations vary with age and sex. Ferritin levels in women rise after menopause, when they stay comparatively low. Ferritin starts to rise in both sexes around the age of 70. An adult's ferritin level of less than 15 μg/L indicates iron deficiency. Although elevated ferritin is an acute phase protein and is therefore elevated in many diseases and infections, elevated ferritin may indicate iron overload. [(20)].

**C: Total iron-binding capacity/transferrin:** The total number of transferrin binding sites per unit volume of plasma or serum is measured using the TIBC assay, which also determines the amount of iron that can bind to unsaturated transferrin. A stand-in for transferrin is TIBC. Since levels do not fluctuate until stores of iron are exhausted, TIBC, in contrast to serum iron, does not fluctuate quickly in plasma concentrations and is not a helpful indicator of an early iron deficiency. Immunological techniques can be used to measure the concentration of transferrin, an iron transporter. In inflammatory and iron overload disorders, transferrin and TIBC are both reduced, while in iron deficiency disorders, they are both elevated [(21-22)].

**Specific objectives of this study**

-To measure serum iron, ferritin, TIBC and Transferrin saturation beside 25-hydroxy vitamin D among subjects having anemia and apparently healthy individual.
-To compare data of anemic and apparently healthy individual with reference ranges of measured parameters.
-To compare data between genders.
-To compare data between different types of anemia.
- To find frequency of pre-latent and latent iron deficiency anemia among apparently healthy individual.
-To compare vitamin D according to different classification among apparently healthy individual
-To compare iron profile of anemic and apparently healthy individual to their vitamin D status (Deficient. Insufficient and Sufficient**)**
-To compare vitamin D status with different types of anemic and apparently healthy individual.
-To compare vitamin D and iron profile between anemic subjects and apparently healthy individual.
-To find correlation of CBC. iron profile with vitamin D.
-To find correlation of CBC, iron profile and vitamin D with age.

**2-Materials and methods**

This study was conducted as descriptive cross sectional study. General population involved in this study included subjects having anemia and apparently healthy individual determined according to hemoglobin concentration, all apparently healthy individual was selected according to hemoglobin levels that were within the normal range. All cases of anemia were selected according to hemoglobin levels that were less than normal range. Patients with iron deficiency and anemia of chronic disease were classified according to their iron profile While it was already known that patients with megaloblastic anemia had

a low level of vitamin B12. Area selected was Omdurman friendship Hospital-Omdurman City-Khartoum state. Inclusion and Exclusion criteria included I/ Anemic patients with types not related to genetic causes (IDA, Macrocytic anemia and anemia of chronic disease) and II/ Apparently healthy individuals with normal Hb concentration. This study was approved by the Department of Hematology, Alzaeim Alazhary University, as well as the hospital administration and patients as well. Patients and healthy subjects were also contacted and given results for vitamin D and iron profile. Data collected by asking patients general queries, such as age and gender. Whole blood samples were collected from 60 patients with different types of anemia and 30 healthy subjects. for CBC EDTA added blood samples were collected and for iron profile and vitamin D heparin added containers were used.

Whole blood samples for CBC were assessed via automated hematology analyzer (Mindray BC3000)-Chinses trade mark with suitable reagents provided. Serum iron profile parameters were assessed by chemical automation analyzer BS200. Vitamin D was assessed by Enzyme Linked Immunosorbent Assay (ELISA) via BTS 350+ Biosystem $^{TM}$. vitamin D deficiency was defined as 25(OH)D $\leq$ 20ng/dl, vitamin D insufficiency as 25 (OH)D of 20-30 ng/dl, and normal 25 vitamin D sufficiency as 25(OH)D > 30 ng/dl. Serum iron was defined for women 65 – 175 mg/dl and for men 50-170. Serum ferritin was defined for women 15-150mg/dl and for men 25 – 380 mg/dl. TIBC was defined 250-425 mg/dl. % saturation calculated by dividing the iron concentration by the total iron binding capacity and defined 20-50%.

**Data analysis**

Data was analyzed by statistical package of social science (SPSS) version 23 to obtained descriptive statistic, means, standard deviation and frequencies .one sample T test used to compare data with normal range and independent T test (tow sample T test) to determines whether there is a statistically significant difference between the means in two unrelated groups. Anova to determines whether there is a statistically significant difference between the means of three unrelated groups and chi square test to determine whether there is a relationship between two categorical variables (vitamin D status and type of anemia) beside personal correlation.

## 3-Result

This descriptive cross sectional study was conducted to assess iron profile and vitamin D among 60 patients with anemia, they were 20 (33.3%) males and 40 (66.7%) females as in figure 3-1 and 30 apparently healthy individual they were 14 (46.7%) males and 16 (53.3%) females as in figure 3-2.

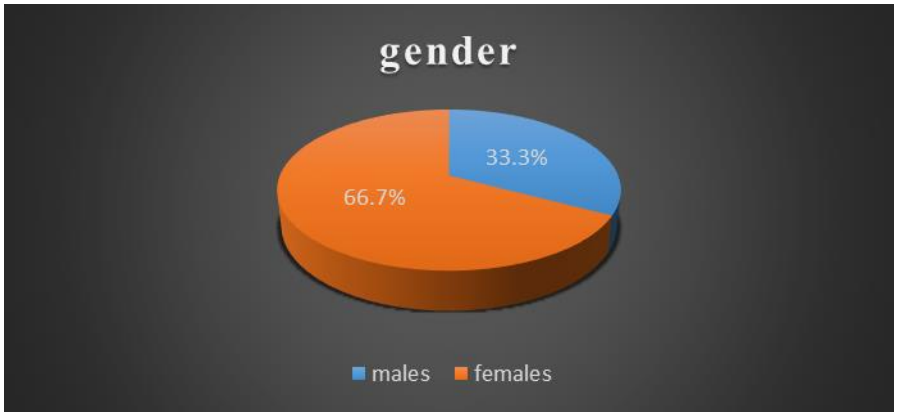


Figure 3-1: gender distribution of anemic subjects

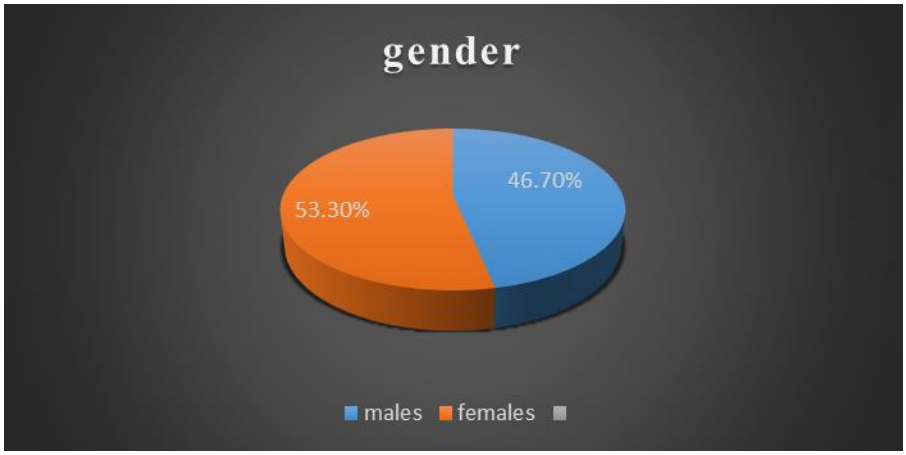


Figure 3-2: gender distribution of apparently healthy individual subjects

Complete blood count (CBC) was analyzed for patients presented anemia and according to parameters were measured patients sorted to 3 types of anemia, IDA, anemia of chronic disease (ACD) and macrocytic anemia. Lower level of Hb assessed was 6.2g/dl, while high level approached 12g/dl. Serum iron lower level was 4 µg /dl and high level 500 µg /dl, ferritin low level assessed 3.0 and high 978, TIBC decreased level was 100 µg /dl and increased 1071 µg /dl .%saturation decreased level was 1.5 % and increased 95 % . Vitamin D low one was 11.2ng/ml and high was 34 ng/ml as in table 3-1.

Complete blood count (CBC) was analyzed for apparently healthy individual and according to iron profile result classified to 3 types true non anemic, prelatent and latent. Lower level of Hb assessed was 13 g/dl, while high level approached 15.9g/dl. Serum iron lower level was 4 µg /dl and high level 925 µg /dl, ferritin low level assessed 2.5 and high 500, TIBC decreased level was 94 µg /dl and increased 1080 µg /dl. % saturation decreased level was 0.3 % and increased 101 %. Vitamin D low one was 2.0 ng/ml and high was 38 ng/ml as in table 3-2.

**Table 3-1: measured parameters among anemic patients**

| **CBC** | | | | |
|---|---|---|---|---|
| **Parameters** | No | **Minimum** | **maximum** | **Mean ± SD** |
| **Hb (g/dl)** | 60 | 6.2 | 12.0 | 9.8 ± 1.6 |
| **PCV (%)** | | 21 | 42 | 30.0 ± 4.3 |
| **MCV (fl)** | | 54 | 113 | 74.7 ± 11.2 |
| **MCH (pg)** | | 15 | 34 | 23.9 ± 3.9 |
| **MCHC (g/dl)** | | 23 | 39 | 31.9 ± 2.0 |
| **RBC ($\times 10^{12}$/l)** | | 2.6 | 5.6 | 4.2 ± 0.7 |
| **RDW-CV** | | 10.7 | 37.0 | 17.1 ± 3.6 |
| **WBCs ($\times 10^{9}$/l)** | | 1.1 | 20.8 | 7.4 ± 2.9 |
| **Neutrophils (%)** | | 28 | 87 | 56.5 ± 14.4 |
| **Lymphocyte (%)** | | 9 | 60 | 32.5 ± 13.0 |
| **Monocyte (%)** | | 3 | 19 | 10.5 ± 3.4 |
| **Platelet ($\times 10^{9}$/l)** | | 63 | 979 | 348.0 ± 143.6 |
| **Iron profile** | | | | |
| Serum Iron µg /dl) NR M:(50-170) F:( 65-175) | 60 | 4 | 500 | 128.4 ± 17.6 |
| Ferritin µg /dl F: (15-150) M:(25 – 380) | | 3.0 | 978.0 | 133.9 ± 27.0 |
| TIBC µg /dl (250-425) | | 100 | 1071 | 357.1 ± 190.2 |
| % saturation 20-50%. | | 1.5 | 95.0 | 32.8 ± 24.2 |
| **Vitamin D** | | | | |
| Vitamin D ng/ml NR (> 30 ng/dl) | 60 | 11.2 | 34.0 | 17.4 ± 5.0 |

**Table 3-2: measured parameters among apparently healthy individual**

| **CBC** | | | | |
|---|---|---|---|---|
| **Parameters** | No | **Minimum** | **maximum** | **Mean ± SD** |
| **Hb (g/dl)** | 30 | 13.0 | 15.9 | 13.8 ± 0.8 |
| **PCV (%)** | | 38 | 46 | 41.0 ± 2.1 |
| **MCV (fl)** | | 80 | 98 | 87.0 ± 4.6 |
| **MCH (pg)** | | 27 | 36 | 31.9 ± 2.3 |
| **MCHC (g/dl)** | | 31 | 39 | 35.5 ± 2.2 |
| **RBC ($\times 10^{12}$/l)** | | 3.4 | 5.5 | 4.5 ± 0.4 |
| **RDW-CV** | | 12.5 | 17.0 | 13.7 ± 0.9 |
| **WBCs ($\times 10^{9}$/l)** | | 3.2 | 14.0 | 7.5 ± 2.6 |
| **Neutrophils (%)** | | 41 | 79 | 61.2 ± 11.4 |
| **Lymphocyte (%)** | | 15 | 45 | 29.3 ± 9.8 |
| **Monocyte (%)** | | 4 | 16 | 10.2 ± 3.6 |
| **Platelet ($\times 10^{9}$/l)** | | 144 | 512 | 280.2 ± 101.2 |
| **Iron profile** | | | | |
| **Serum Iron µg /dl)** | 30 | 4 | 925 | 82.9 ± 31.4 |
| **Ferritin µg /dl** | | 2.5 | 500.0 | 155.1 ± 27.3 |
| **TIBC µg /dl** | | 94 | 1080 | 775.2 ± 282.7 |
| **% saturation** | | .3 | 101.0 | 14.9 ± 4.5 |
| **Vitamin D** | | | | |
| **Vitamin D ng/ml** | 30 | 2.0 | 38.3 | 19.3 ± 9.7 |

Sorting anemic patients according to hemoglobin and indices to IDA (56.7%), anemia of chronic disease 35% and megaloblastic anemia 8.3% as in and figure 3-3.

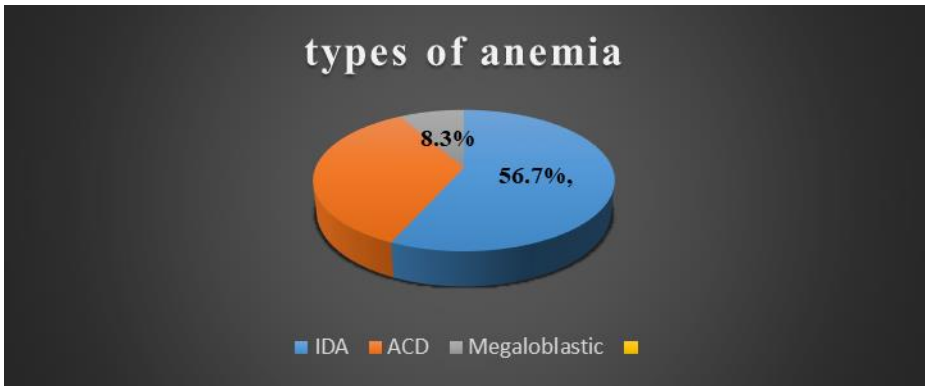


**Figure 3-3: Distribution of anemia diagnosed**

Sorting (apparently healthy individual) according to iron profile result to true non anemic (80%), pre-latent 6.7% and latent 13.3 % as in and figure 3-4.

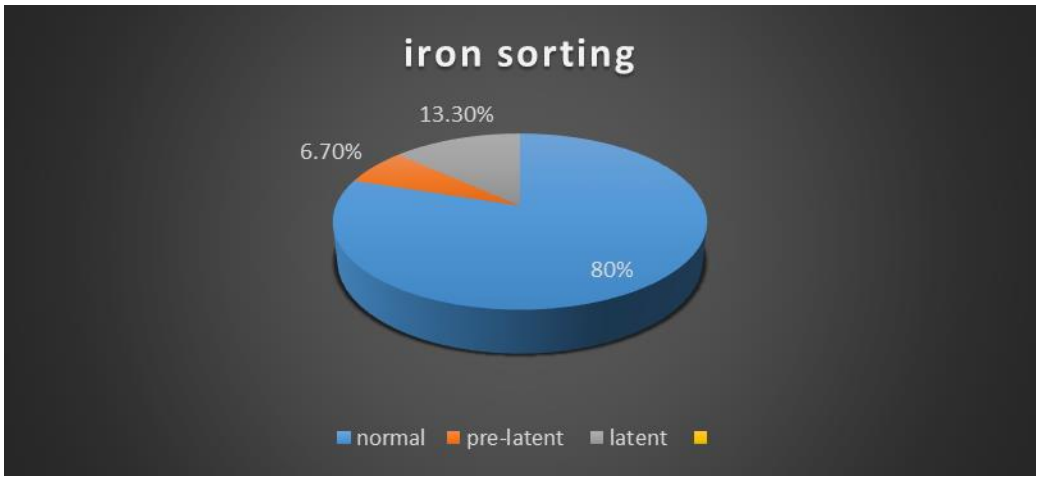


**Figure 3-4: apparently healthy individual sorting according to iron status.**

Comparing anemic patients' CBC with normal range showed that significant difference obtained comparison of Hb, indices, RBC (were decreased among case group than normal rang) platelet, RDW-CV, WBC and monocyte (were increased in case group) as p value for each was <0.05, as well as, neutrophil and lymphocyte had no significant difference as p value for each was >0.05 as in table 3-5.

**Table 3-5: Comparison of CBC of anemic group and reference range**

| Parameter | Study population | | P. value |
|---|---|---|---|
| | case | N.R | |
| **Hb (g/dl)** | 9.8 ± 1.6 | 13.5 | 0.000* |
| **PCV (%)** | 30.0 ± 4.3 | 40.5 | 0.000* |
| **MCV (fl)** | 74.7 ± 11.2 | 88 | 0.000* |
| **MCH (pg)** | 23.9 ± 3.9 | 31 | 0.000* |
| **MCHC (g/dl)** | 31.9 ± 2.0 | 35.5 | 0.000* |

| | | | |
|---|---|---|---|
| **RBC ($\times 10^{12}$/l)** | 4.2 ± 0.8 | 4.7 | 0.000* |
| **RDW-CV** | 17.1 ± 3.6 | 15 | 0.000* |
| **WBCs ($\times 10^{9}$/l)** | 7.4 ± 2.9 | 6.3 | 0.005* |
| **Neutrophils (%)** | 56.5 ± 14.4 | 60 | 0.062 |
| **Lymphocyte (%)** | 32.5 ± 13.0 | 32.5 | 0.984 |
| **Monocyte (%)** | 10.5 ± 3.4 | 8 | 0.000* |
| **Platelet ($\times 10^{9}$/l)** | 348.0 ± 143.6 | 300 | 0.013* |

Comparing (apparently healthy individual) CBC with normal range showed that significant difference obtained comparison of Hb, RBC, RDW-CV, WBC and monocyte (as p value for each was <0.05 as well as, indices, neutrophil, lymphocyte and platelet had no significant difference as p value for each was >0.05 as in table 3-6.

**Table 3-6: Comparison of CBC of apparently healthy individual and reference range**

| **Parameter** | **Study population** | | **P. value** |
|---|---|---|---|
| | **Non anemic** | **N.R** | |
| **Hb (g/dl)** | 13.8 ± 0.8 | 13.5 | 0.047* |
| **PCV (%)** | 41.0 ± 2.1 | 40.5 | 0.203 |
| **MCV (fl)** | 87.0 ± 4.6 | 88 | 0.245 |
| **MCH (pg)** | 31.9 ± 2.3 | 31 | 0.052 |
| **MCHC (g/dl)** | 35.5 ± 2.2 | 35.5 | 0.934 |
| **RBC ($\times 10^{12}$/l)** | 4.5 ± 0.4 | 4.7 | 0.004* |
| **RDW-CV** | 13.7 ± 0.9 | 15 | 0.000* |
| **WBCs ($\times 10^{9}$/l)** | 7.5 ± 2.6 | 6.3 | 0.022* |
| **Neutrophils (%)** | 61.2 ± 11.4 | 60 | 0.559 |
| **Lymphocyte (%)** | 29.3 ± 9.8 | 32.5 | 0.081 |
| **Monocyte (%)** | 10.2 ± 3.6 | 8 | 0.002* |
| **Platelet ($\times 10^{9}$/l)** | 280.2 ± 101.2 | 300 | 0.292 |

Comparing iron profile and vitamin D of anemic subjects with reference ranges of such parameters, significant difference obtained by serum ferritin and vitamin D (were decreased among case group than normal rang) as for each p value <0.05 and serum iron, TIBC and % saturation have no significant difference as in table 3-7.

**Table 3-7: Comparison of iron profile and vitamin D of anemic and reference range**

| **Parameter** | **Study population** | | **P. value** |
|---|---|---|---|
| | **Case** | **N R** | |
| **Serum Iron** | 128.4 ± 17.6 | 112 | 0.354 |
| **Ferritin** | 133.9 ± 27.0 | 197 | 0.023* |

| | | | |
|---|---|---|---|
| **TIBC** | 357.1 ± 24.6 | 337 | 0.416 |
| **% Saturation** | 32.8 ± 24.2 | 33 | 0.957 |
| **Vitamin D Ng/ml** | 17.4 ± 5.0 | 45 | 0.000* |

Comparing iron profile and vitamin D of apparently healthy individual subjects with reference ranges of such parameters, significant difference obtained by serum TIBC, saturation% and vitamin D as for each p value <0.05 and serum iron and ferritin have no significant difference as in table 3-8.

**Table 3-8: Comparison of iron profile and vit D of apparently healthy individual and N R**

| **Parameter** | **Study population** | | **P. value** |
|---|---|---|---|
| | **Non anemic** | **N R** | |
| **Serum Iron** | 82.9 ± 31.4 | 112 | 0.362 |
| **Ferritin** | 155.1 ± 149.7 | 197 | 0.136 |
| **TIBC** | 775.2 ± 282.7 | 337 | 0.000* |
| **% Saturation** | 14.9 ± 4.5 | 33 | 0.000* |
| **Vitamin D Ng/ml** | 19.3 ± 9.7 | 45 | 0.000* |

Considering gender, comparing CBC parameters of anemic patient between males and females showed that there was no significant difference for none of measured hemograms' parameters, as p value for each was >0.05, as well as for iron profile and significant difference obtained by vitamin D as p value < 0.05 as in table 3-9 and table 3-10 respectively.

**Table 3-9: Comparison of CBC of anemic patients' gender**

| **Parameter** | **Gender** | | **P. value** |
|---|---|---|---|
| | **Male (n=19)** | **Female (n=41)** | |
| **Hb (g/dl)** | 9.8 ± 1.9 | 9.8 ± 1.4 | 0.991 |
| **PCV (%)** | 30.1 ± 5.2 | 29.9 ± 3.9 | 0.949 |
| **MCV (fl)** | 72.3 ± 8.2 | 75.9 ± 12.2 | 0.250 |
| **MCH (pg)** | 23.1 ± 3.2 | 24.2 ± 4.3 | 0.307 |
| **MCHC (g/dl)** | 31.8 ± 2.4 | 32.1 ± 1.9 | 0.715 |
| **RBC ($\times 10^{12}$/l)** | 4.4 ± 0.8 | 4.1 ± 0.7 | 0.112 |
| **RDW-CV** | 16.3 ± 2.3 | 17.5 ± 4.0 | 0.222 |
| **WBCs ($\times 10^{9}$/l)** | 8.5 ± 3.8 | 6.9 ± 2.4 | 0.063 |

| | | | |
|---|---|---|---|
| **Neutrophils (%)** | 55.5 ± 17.8 | 56.9 ± 12.9 | 0.734 |
| **Lymphocyte (%)** | 32.9 ± 16.1 | 32.3 ± 11.5 | 0.868 |
| **Monocyte (%)** | 11.3 ± 2.7 | 10.1 ± 3.7 | 0.235 |
| **Platelet ($\times 10^9$/l)** | 373.8 ± 196.9 | 335.8 ± 110.8 | 0.346 |

**Table 3-10: Comparison of iron profile and vitamin D among anemic's genders**

| **Parameter** | **Gender** | | **P. value** |
|---|---|---|---|
| | **Male (n=19)** | **Female (n=41)** | |
| **Serum Iron** | 122.9 ± 114.4 | 130.9 ± 146.4 | 0.835 |
| **Ferritin** | 152.6 ± 51.8 | 125.3 ± 31.8 | 0.642 |
| **TIBC** | 360.6 ± 162.8 | 355.5 ± 203.5 | 0.925 |
| **% saturation** | 32.1 ± 24.6 | 33.2 ± 24.4 | 0.872 |
| **Vitamin D** | 19.6 ± 5.7 | 16.5 ± 4.4 | 0.024* |

Considering gender, comparing CBC parameters of apparently healthy individual between males and females showed that there was significant difference obtained by Hb, MCV, RBC, WBC and monocyte for each p value <0.05 and PCV, MCH, MCHC, RDW, Neutrophils, lymphocyte, platelets, iron profile have no significant difference as p value for each was >0.05 and significant difference obtained by vitamin D as p value < 0.05 as in table 3-11 and table 3-12 respectively.

**Table 3-11: Comparison of CBC of apparently healthy individual according to gender**

| **Parameter** | **Gender** | | **P. value** |
|---|---|---|---|
| | **Male (n=14)** | **Female (n=16)** | |
| **Hb (g/dl)** | 14.1 ± 0.9 | 13.5 ± 0.5 | 0.045* |
| **PCV (%)** | 41.4 ± 2.3 | 40.7 ± 1.9 | 0.393 |
| **MCV (fl)** | 89.1 ± 4.9 | 85.3 ± 3.4 | 0.019* |
| **MCH (pg)** | 31.7 ± 2.5 | 32.0 ± 2.3 | 0.746 |
| **MCHC (g/dl)** | 35.2 ± 2.3 | 35.8 ± 2.1 | 0.466 |
| **RBC ($\times 10^{12}$/l)** | 4.7 ± 0.5 | 4.3 ± 0.3 | 0.004* |
| **RDW-CV** | 13.8 ± 0.7 | 13.7 ± 0.9 | 0.891 |

| | | | |
|---|---|---|---|
| **WBCs ($\times 10^9$/l)** | 6.3 ± 2.1 | 8.5 ± 2.6 | 0.016* |
| **Neutrophils (%)** | 61.1 ± 10.1 | 61.4 ± 12.8 | 0.944 |
| **Lymphocyte (%)** | 27.9 ± 8.5 | 30.4 ± 10.9 | 0.494 |
| **Monocyte (%)** | 12.4 ± 2.4 | 8.3 ± 3.3 | 0.001* |
| **Platelet ($\times 10^9$/l)** | 288.0 ± 96.8 | 273.3 ± 107.6 | 0.699 |

**Table 3-12: Comparison of iron profile and vitamin D of apparently healthy individual gender**

| **Parameter** | **Gender** | | **P. value** |
|---|---|---|---|
| | **Male (n=14)** | **Female (n=16)** | |
| **Serum Iron** | 117.4 ± 64.9 | 52.8 ± 15.7 | 0.313 |
| **Ferritin** | 144.7 ± 47.6 | 164.3 ± 125.2 | 0.728 |
| **TIBC** | 783.7 ± 291.5 | 767.8 ± 284.2 | 0.881 |
| **% saturation** | 18.8 ± 8.2 | 11.6 ± 4.5 | 0.435 |
| **Vitamin D** | 24.2 ± 10.3 | 15.1 ± 6.8 | 0.007* |

Regarding to type of anemia diagnosed (IDA, ACD and megaloblastic anemia), Measured CBC parameters were compared, Hb, PCV and MCHC were low among the 3 types and no significant differences obtained (p value >0.05). MCV and MCH was increased among megaloblastic anemia than the other types and significant difference obtained (p value=0.000). RDW-cv was increased among IDA and no significant difference obtained. RBC count was decreased among megaloblastic anemia than other types and significant differences (p value<0.05). the rest of CBC parameters didn't bring significant as in table 3-13.

**Table 3-13: Comparison of CBC according to type of anemia among anemic group**

| **Parameters** | **Diagnosis** | | | **P. value** |
|---|---|---|---|---|
| | **IDA (n=34)** | **ACD (n=21)** | **Megaloblastic (n=5)** | |
| **Hb (g/dl)** | 9.8 ± 1.5 | 9.9 ± 1.7 | 9.5 ± 1.2 | 0.892 |
| **PCV (%)** | 30.4 ± 4.0 | 29.9 ± 4.9 | 27.6 ± 3.2 | 0.402 |
| **MCV (fl)** | 70.3 ± 6.6 | 75.5 ± 8.5 | 101.2 ± 8.2 | 0.000* |
| **MCH (pg)** | 22.5 ± 3.0 | 24.4 ± 3.6 | 31.2 ± 2.9 | 0.000* |
| **MCHC (g/dl)** | 31.7 ± 1.6 | 32.1 ± 2.3 | 33.8 ± 3.0 | 0.085 |
| **RBC ($\times 10^{12}$/l)** | 4.4 ± 0.6 | 4.1 ± 0.8 | 2.9 ± 0.4 | 0.000* |
| **RDW-CV** | 18.1 ± 4.0 | 15.9 ± 1.8 | 16.0 ± 4.8 | 0.059 |

| | | | | |
|---|---|---|---|---|
| **WBCs ($\times 10^9$/l)** | 7.0 ± 2.4 | 8.1 ± 3.7 | 7.0 ± 3.5 | 0.412 |
| **Neutrophils (%)** | 56.0 ± 13.2 | 56.7 ± 17.4 | 58.4 ± 10.0 | 0.940 |
| **Lymphocyte (%)** | 32.6 ± 11.7 | 32.2 ± 16.1 | 33.2 ± 9.0 | 0.988 |
| **Monocyte (%)** | 10.3 ± 3.6 | 11.4 ± 3.1 | 7.6 ± 2.4 | 0.073 |
| **Platelet ($\times 10^9$/l)** | 342.7 ± 113.7 | 379.5 ± 184.7 | 251.2 ± 88.7 | 0.191 |

Among the 3 types of anemia, ferritin was decreased more among IDA then both other anemia and significant difference obtained (p value=0.015). serum iron was slightly increased among ACD than megaloblastic anemia and less in IDA. TIBC was Increased among ACD than other anemias and no significant difference obtained. Saturation% and vitamin D was decreased more among IDA and no significant difference obtained as in table 3-14.

**Table 3-14: Comparison of iron profile and vitamin D according to diagnosis**

| **Parameters** | **Diagnosis** | | | **P. value** |
|---|---|---|---|---|
| | **IDA (n=34)** | **ACD (n=21)** | **Megaloblastic (n=5)** | |
| **Serum Iron** | 96.4 ± 22.3 | 171.3 ± 30.9 | 166.2 ± 51.7 | 0.113 |
| **Ferritin** | 52.9 ± 16.7 | 230.0 ± 61.9 | 282.3 ± 81.7 | 0.001* |
| **TIBC** | 340.8 ± 215.0 | 382.3 ± 161.8 | 362.4 ± 122.8 | 0.739 |
| **% Saturation** | 26.4 ± 23.1 | 41.2 ± 25.1 | 41.0 ± 17.9 | 0.063 |
| **Vitamin D** | 16.9 ± 5.1 | 17.7 ± 3.5 | 20.1 ± 9.0 | 0.412 |

Among the 3 types of apparently healthy individuals, vitamin D was decreased more among true non anemic and no significant difference obtained as in table 3-15

**Table 3-15: Comparison of vitamin D according to different classification among apparently healthy individual .**

| **Parameters** | **Diagnosis** | | | **P. value** |
|---|---|---|---|---|
| | **True non anemic (n=24)** | **Pre-latent (n=2)** | **latent (n=4)** | |
| **Vitamin D** | 17.8 ± 9.1 | 26.4 ± 16.2 | 25.4 ± 8.7 | 0.199 |

Anemic patients regarding to vitamin D level usually sorted to deficient, which less than 20 ng/ml were 45 and insufficient less than 30 ng/ml were 12 and sufficient more than 30 ng/ml were 3 as in table 3-16.

**Table 3-16: sorting anemic patients according to vitamin D levels**

| **Vitamin D** | **Frequency** | **Percent** |
|---|---|---|

| | | |
|---|---|---|
| **Deficient** | 45 | 75.0 % |
| **Insufficient** | 12 | 20.0 % |
| **Sufficient** | 3 | 5.0 % |
| **Total** | 60 | 100.0 |

Apparently healthy individual regarding to vitamin D level usually sorted to deficient, which less than 20 ng/ml were 19 and insufficient less than 30 ng/ml were 7 and sufficient more than 30 ng/ml were 4 as in table 3-17.

**Table 3-17: sorting apparently healthy individual according to vitamin D levels**

| Vitamin D | Frequency | Percent |
|---|---|---|
| **Deficient** | 19 | 63.3 % |
| **Insufficient** | 7 | 23.3 % |
| **Sufficient** | 4 | 13.3 % |
| **Total** | 30 | 100.0 |

Comparison of iron profile of anemic patient with the 3 subgroups of vitamin D level, (deficient, insufficient and sufficient groups) were no significant differences as in table 3-18

**Table 3-18: Comparisons of Iron profile of anemic patient according to Vitamin D level**

| Vitamin D | Deficient | Insufficient | Sufficient | P. value |
|---|---|---|---|---|
| **Serum Iron** | 123.2 ± 19.8 | 159.9 ± 45.1 | 81.7 ± 68.4 | 0.596 |
| **Ferritin** | 154.2 ± 33.9 | 71.3 ± 40.5 | 81.3 ± 50.4 | 0.437 |
| **TIBC** | 350.2 ± 191.1 | 376.8 ± 197.8 | 382.3 ± 210.0 | 0.890 |
| **%saturation** | 32.0 ± 23.8 | 39.3 ± 25.4 | 19.2 ± 15.9 | 0.405 |

Comparison of iron profile of apparently healthy individuals with the 3 subgroups of vitamin D level, (deficient, insufficient and sufficient groups) were no significant differences as in table 3-19

**Table 3-19: Comparisons of Iron profile of apparently healthy individual according to Vitamin D level**

| Vitamin D | Deficient | Insufficient | Sufficient | P. value |
|---|---|---|---|---|
| **Serum Iron** | 86.0 ± 47.0 | 100.4 ± 44.3 | 37.9 ± 25.5 | 0.848 |

| | | | | |
|---|---|---|---|---|
| **Ferritin** | 132.2 ± 121.9 | 156.8 ± 128.4 | 261.4 ± 137.9 | 0.302 |
| **TIBC** | 808.1 ± 272.2 | 698.1 ± 333.9 | 753.8 ± 289.4 | 0.685 |
| **%saturation** | 12.0 ± 5.3 | 25.8 ± 12.2 | 9.8 ± 8.4 | 0.424 |

Regarding to vitamin D, as it sorted to 3 phases among anemic and non-anemic subjects, comparing the levels among anemic showed that IDA has high frequency of deficient Vit D, then ACD and later megaloblastic anemia, insufficient among ACD were more, then IDA and sufficient vitamin found only 1 megaloblastic and 2 of IDA but no significant different obtained as p value was 0.3 as in table 3-20.

**Table 3-20: Comparisons of Vitamin D level with different type of anemia**

| **Vitamin D** | **Diagnosis** | | |
|---|---|---|---|
| | **IDA (n=34)** | **ACD (n=21)** | **Megaloblastic (n=5)** |
| **Deficient** | 27 (79%) | 15 (71.4%) | 3(60%) |
| **Insufficient** | 5 (14%) | 6 (28.6%) | 1(20%) |
| **Sufficient** | 2 (5.8%) | 0 | 1(20%) |
| **P. value** | 0.300 | | |

While apparently healthy individuals revealed that true non anemic subjects were sorted according to iron states, to pre-latent, latent and true non-anemic, deficient vit D was more among true non-anemic as they were most of subjects, and also insufficient were 5 and sufficient only2, no significant difference obtained p value >0.05 as in table 3-21.

**Table 3-21: Comparisons of Vitamin D level with different classification of apparently healthy individual**

| **Vitamin D** | **Diagnosis** | | |
|---|---|---|---|
| | **True non anemic (n=24)** | **Pre-latent (n=2)** | **latent(n=4)** |
| **Deficient** | 17 (70.8%) | 1 (50%) | 1(25%) |
| **Insufficient** | 5 (20.8%) | 0 | 2 (50%) |
| **Sufficient** | 2 (8.3) | 1 (50%) | 1 (25%) |
| **p. value** | 0.213 | | |

Comparing iron profile and vitamin D with anemic patients and apparently healty individuals were obtained significant differences by serum TIBC and % saturation as p

value <0.05 and serum iron, ferritin and vitamin d have no significant difference as in table 3-22

**Table 3-22: Comparison of iron profile and vitamin D between anemic and apparently healthy individual**

| Parameter | | | P. value |
|---|---|---|---|
| | anemic(n=60) | Non anemic (n=30) | |
| **Serum Iron** | 128.4 ± 17.6 | 82.9 ± 31.4 | 0.175 |
| **Ferritin** | 133.9 ± 27.0 | 155.1 ± 149.7 | 0.623 |
| **TIBC** | 357.1 ± 190.2 | 775.2 ± 282.7 | 0.000* |
| **% saturation** | 32.8 ± 24.2 | 14.9 ± 4.5 | 0.001* |
| **Vitamin D** | 17.4 ± 5.0 | 19.3 ± 9.7 | 0.222 |

Pearson's correlation of iron profile with vitamin D of anemic patients, there were in significant differences for serum iron, ferritin, TIBC and % saturation. Weak positive correlations obtained by serum iron, TIBC and% saturation. Weak negative correlations obtained with serum ferritin as in table 3-23

**Table 3-23: Correlations of iron profile with vitamin D of anemic patient**

| Parameters | Vitamin d | |
|---|---|---|
| | Pearson Correlation | P. value |
| **Serum Iron** | .097 | .461 |
| **Ferritin** | -.149 | .257 |
| **TIBC** | .051 | .702 |
| **% Saturation** | .049 | .709 |

Pearson's correlation of CBC measured with vitamin D of anemic patients, only positive correlation with RBC, WBC and lymphocyte percentages as in table 3-24

**Table 3-24Correlations of CBC with vitamin D of anemic patients**

| Parameters | Vitamin D | |
|---|---|---|
| | Pearson Correlation | P. value |
| **Hb** | -.117 | .375 |
| **PCV** | -.118 | .370 |
| **MCV** | -.083 | .527 |
| **MCH** | -.090 | .493 |
| **MCHC** | -.082 | .534 |
| **RBC** | .033 | .811 |
| **RDW-CV** | -.043 | .744 |
| **WBCs** | .109 | .409 |
| **Neutrophils** | -.053 | .688 |
| **Lymphocyte** | .060 | .649 |
| **Monocyte** | -.066 | .617 |
| **Platelet** | -.025 | .025 |

Pearson's correlation of CBC measured parameters with age of anemic patients, there were significant differences for MCV, MCH and neutrophil % with positive correlation and RBC and lymphocyte percentages with negative correlation for each of them. positive correlations obtained by Hb, MCV, MCH, and MCHC beside neutrophil % and negative correlations obtained with PCV, RBC, RDW-cv, WBC, lymphocyte%, Monocyte% and platelet count as in table 3-25.

**Table 3-25: Correlations of CBC with age of anemic patient**

| Parameters | Age (years) | |
|---|---|---|
| | Pearson Correlation | P. value |
| **Hb** | .073 | .585 |
| **PCV** | -.008 | .954 |
| **MCV** | .385 | .003* |
| **MCH** | .381 | .003* |
| **MCHC** | .140 | .292 |
| **RBC** | -.299 | .028* |
| **RDW-CV** | -.120 | .366 |
| **WBCs** | -.090 | .499 |
| **Neutrophils** | .407 | .001* |
| **Lymphocyte** | -.388 | .002* |
| **Monocyte** | -.241 | .066 |
| **Platelet** | -.232 | .080 |

Pearson's correlation of iron profile and vitamin D with age of anemic patients, there were significant differences for ferritin with weak positive correlation and vitamin D with weak negative. And no significant differences obtained by serum iron, TIBC with positive correlation and % saturation with negative as in table 3-26.

**Table 3-26: Correlations of iron profile and vitamin D with age of anemic patients**

| Parameters | Age (years) | |
|---|---|---|
| | Pearson Correlation | P. value |
| **Serum Iron** | .043 | .749 |

| | | |
|---|---|---|
| **Ferritin** | .281 | .031* |
| **TIBC** | .071 | .591 |
| **% Saturation** | -.017 | .898 |
| **Vitamin D** | -.264 | .043* |

Pearson's correlation of iron profile with vitamin D of apparently healthy individual. weak positive correlations obtained by serum iron, ferritin, and % saturation. weak negative correlations obtained with serum TIBC only (no significant differences obtained) as in table 3-27.

**Table 3-27: Correlations of iron profile with vit D of** apparently healthy individuals

| **Parameters** | **Vitamin d** | |
|---|---|---|
| | **Pearson Correlation** | **P. value** |
| **Serum Iron** | .015 | .938 |
| **Ferritin** | .210 | .264 |
| **TIBC** | -.171 | .365 |
| **% Saturation** | .172 | .362 |

Pearson's correlation of CBC measured with vitamin D of apparently healthy individuals only negative correlation with MCH, MCHC, WBC and lymphocyte percentage and Platelets (no significant differences obtained) in table 3-28.

**Table 3-28 Correlations of CBC with vitamin D of apparently healthy individuals**

| **Parameters** | **Vitamin d** | |
|---|---|---|
| | **Pearson Correlation** | **P. value** |
| **Hb** | .097 | .609 |
| **PCV** | .106 | .578 |
| **MCV** | .186 | .325 |
| **MCH** | -.239 | .204 |
| **MCHC** | -.245 | .191 |
| **RBC** | .115 | .543 |
| **RDW-CV** | .486 | .007 |
| **WBCs** | -.174 | .357 |
| **Neutrophils** | .037 | .846 |
| **Lymphocyte** | -.148 | .434 |

| | | |
|---|---|---|
| **Monocyte** | .265 | .157 |
| **Platelet** | -.207 | .273 |

Pearson's correlation of CBC measured parameters with age of apparently healthy individuals. weak positive correlations obtained by PCV, MCV, MCH, RBC, MCHC Monocyte%. and weak negative correlations obtained with Hb, RDW-cv, WBC, neutrophil % and lymphocyte%. And No liner correlation with platelet (no significant differences obtained) as in table 3-29.

**Table 3-29: Correlations of CBC with age of apparently healthy individuals**

| **Parameters** | **Age (years)** | |
|---|---|---|
| | **Pearson Correlation** | **P. value** |
| **Hb** | -.021 | .911 |
| **PCV** | .028 | .882 |
| **MCV** | .231 | .220 |
| **MCH** | .204 | .279 |
| **MCHC** | .162 | .392 |
| **RBC** | .216 | .253 |
| **RDW-CV** | -.124 | .513 |
| **WBCs** | -.289 | .121 |
| **Neutrophils** | -.044 | .818 |
| **Lymphocyte** | -.054 | .778 |
| **Monocyte** | .287 | .124 |
| **Platelet** | .000 | .997 |

Pearson's correlation of iron profile and vitamin D with age of apparently healthy individuals. weak positive correlations obtained by serum ferritin, TIBC and vitamin D. weak negative correlations obtained with serum iron and % saturation (no significant differences obtained) as in table 3-30**.**

**Table 3-30: Correlations of iron profile and vit D with age of apparently healthy individuals.**

| **Parameters** | **Age (years)** | |
|---|---|---|
| | **Pearson Correlation** | **P. value** |
| **Serum Iron** | -.148 | .434 |

| | | |
|---|---|---|
| **Ferritin** | .009 | .963 |
| **TIBC** | .092. | .628 |
| **% Saturation** | -.185 | .328 |
| **Vitamin D** | .050 | .792 |

## Discussion

Anemia and VDD are major health problems. Vitamin D has long regulated calcium, phosphorus and bone metabolism, but in recent years it has gained importance as a regulator of biological functions, including immune functions, cellular Proliferation and cardiovascular function. Vitamin D plays an important role in the pathogenesis of anemia. Today, the new pediatric guideline from the National Academy of Sciences covers all infants, children, and adolescents. Start early with at least 400 IU of vitamin D per day after birth and replaces the previous suggestion. Vitamin D supplementation of at least 200 IU per day, from the first 2 months of life until puberty. Basic mechanisms involved in the associations There is considerable interest between VDD and anemia nutritionists, scientists, clinicians and the general public. The correct mechanisms of the relationship between VDD and anemia remain unclear. Vitamin D has been found to stimulate red blood cell precursors and vitamin D receptors multiple target tissues other than kidney, including bone marrow. Vitamin D and its metabolites are found in many tissues and have receptor sites for the active form of vitamin D, calcitriol. Calcitriol plays a key role here regulates immune function by inhibiting the expression of pro-inflammatory cytokines produced by various immune cells, thus provides negative feedback to prevent excessive inflammation. This has been proven in in vivo and invitro studies. Calcitriol reduces the production of cytokines and thus reduces inflammatory environment and anemia. There is another explanation. VDD can stimulate the immune system Cells in the bone marrow microenvironment produce cytokines that induce impaired erythropoiesis. Vitamin D regulates the hepcidin-ferroportin axis Macrophages can promote iron utilization. Based on this, further mechanistic studies should be carried out. Assess whether this is a direct causal effect of the VDD[22].

**** In this study the percentage of IDA subjects was higher (79%) in the vitamin D deficiency group than in the 25(OH)D sufficient group (5.8%) and The percentage of Vitamin D deficiency subjects was higher (79%) in the IDA than in the normal iron status group (70.8%).this study was agreed with a study on healthy females athletes. Vitamin D deficiency was defined as a 25(OH)D concentration < 75 nmol/L. ID was classified based on ferritin, total iron binding capacity (TIBC) and blood morphology indices. The percentage of ID subjects was higher (32%) in the vitamin D deficiency group than in the 25(OH)D sufficient group (11%). The percentage of Vitamin D deficiency subjects was higher (75%) in the ID than in the normal iron status group (48%). [(23)].

****In this study There were 34 patients in the iron deficiency anemia (79% vitamin D deficient) and true non anemic 24 persons (70% vitamin D deficient). The vitamin D level was no significant difference between the groups($p>0.05$), vitamin D deficiency and

insufficiency was prevalent, in all the groups this study was disagreed with a study investigated the relationship between serum vitamin D level, hemogram parameters, and serum iron level in preschool children. The patients were divided into 3 groups (iron deficiency, iron deficiency anemia, and a control group). Vitamin D deficiency, insufficiency, and normal categories were also used based on assessment of the serum vitamin D level. There were 41 children in the iron deficiency group (9.8% vitamin d deficient)32 classified as iron deficiency anemia (25% vitamin d deficient) and 35 age- and sex mated controls (8.6% vitamin d deficient). The vitamin D level was statistically significant between the groups ($p<0.05$), so vitamin D deficiency and insufficiency were prevalent, especially in children with iron deficiency anemia **(24)**.

* In this study 63% of apparently healthy individuals had vitamin D deficiency (>20 ng/ml), 23.3% had insufficient levels (20-29.9 ng/ml) and only 13.3% had normal levels (>30 ng/ml) and female participants having lower levels (15.1 ± 6.8 ng/ml) than male (24.2 ± 10.3 ng/ml) and vitamin D significant difference with gender obtained this study was agreed with a study of apparently healthy individuals of various ages were randomly recruited from various public areas, and their blood samples were obtained. ~74.3% of the population had vitamin D deficiency (20 ng/ml), 21.1% had insufficient levels (20-29.9 ng/ml) and only 4.5% had normal levels (30-60 ng/ml). the mean 25(OH)D level was significantly lower in females (14.05±7.55 ng/ml) than in males (17.87±6.46 ng/ml). On the whole, vitamin D deficiency was highly prevalent among the studied population. **(25)**

* our study revealed no relationship between ferritin with vitamin D. no significant differences between vitamin d and ferritin indicating that vitamin D deficiency is not associated with anemia this study was disagreed with a study carried out in Ramadi city. The participants divided into two groups according to age: group I with age 20 – 35 years and group II with age 36 – 50 years. In the present study mean vitamin D3 in group II 11.8 ± 3.5 ng/dL was significantly lower than group I (35.3 ± 12.2 ng/dL) p-value < 0.001, a similar finding observed for ferritin (19.6 ± 13.9 vs 66.7 ± 52.1, p-value <0.001). There was a direct relationship between ferritin with vitamin D. However, this relationship was only significant in group II (p-value <0.01), while in group I it was statistically significant (p-value 0.07). In conclusion, low vitamin D levels associated with low ferritin, indicating that vitamin D deficiency is associated with anemia **(26)**.

*In this study no significant association between vitamin D and serum ferritin level among anemic but there is a significant association between anemia and serum ferritin showing p value $p<0.05$ this study was agreed with a total of 196 females of child bearing age 15-49 years who were not currently taking any vitamin D supplementation were selected through consecutive sampling. The study participants were interviewed by using a preformed questionnaire. For determining hemoglobin, Ferritin and vitamin D levels, blood samples were taken from all participants in aseptic environment. The data were analyzed by statistical method generated on the computer software SPSS 23. Study results revealed that there is no significant association between anemia and vitamin D

level but there is a significant association between anemia and serum ferritin showing p value $p<0.05$. It was concluded that there is not a significant association between anemia and vitamin D level but there is a significant association between anemia and serum ferritin showing p value $p=0.017$ Moreover no association between vitamin D and serum ferritin level among anemic females of child bearing age were seen[(27)]

**Conclusion**

- This study concluded that there was very low level of vitamin d among anemic and apparently healthy individual.

-Regarding iron profile also there was different variance in the results that indicate apparently healthy individuals have some types of pre-latent and latent iron deficiency anemia

-As for the relationship of vitamin D with iron profile, there was no correlation between iron profile and vitamin D level among anemic and apparently healthy individual.

-with regard to the level of vitamin D between both sexes, there was a significant decrease in the level of vitamin D in females compared to meals **.**

**4-3 Recommendation**

- Vit D level assessment could be Routinely checked for all age groups, as Sudanese have noted that it is lower than normal, even among healthy people.

- It It's crucial to community to pay attention to the importance of evaluating vitamin D and its supplements for anemia and healthy individuals, in addition to iron supplements among healthy individuals to prevent the spread of anemia caused by iron deficiency, especially in countries where unhealthy nutrition is prevalent.

- It was also recommended that apparently healthy individual must not used in researches as control groups unless complete hematological and biochemical finding is done because it may affect most finding.

**4-4Limitation**

There are some limitations to this study, including the small sample size, the season in which samples were collected, and the failure to exclude anemic patients undergoing treatment in the study

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