

Iron Deficiency Anemia and Vitamin D Deficiency in Female patients presenting to OPD in Sialkot

KHUDIJA BIBI¹, ASIFA ASHRAF², BUKHTAWAR AKBER³, MOMIN ALI BABER⁴, BARKAT ALI BABAR⁵, SALEH EHSAN⁶

Department of Biochemistry, Govt. Kh. Safdar Medical College, Sialkot

Correspondence to Dr. Asifa Ashraf, Email: asifaashraf690@gmail.com

ABSTRACT

Aim: To determine the relation of iron deficiency anemia and level of vitamin D in healthy females and diseased group

Methods: A cross sectional study with convenient sampling was done having iron deficiency anemia and low level of Vitamin D in females. Total subjects were n= 90 group A has iron deficiency anemia and low level of vitamin D and Group B is control group comprised normal female.

Results: We collected blood from all subjects from antecubital vein. Blood is analyzed for Hb and Hematocrit percentage.

Serum was separated to determine the level of Vitamin D & serum ferritin.

Conclusion: It is observed that hemoglobin, hematocrits, serum Ferritin and vitamin D levels were lower in the diseased group when compared with the healthy females with P value of < 0.001.

Keywords: Iron deficiency, anaemia, Vit. D deficiency

INTRODUCTION

Hemoglobin is globular protein it is composed of heme and globin protein. Heme is composed of protoporphyrin IX and ferrous iron (Fe²⁺)¹.

It is essential for erythropoiesis, cellular immunity & oxidative metabolism. It plays crucial roles in oxygen transport, energy system catalysis, and the creation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein. Cell metabolism causes the formation of free radicals & reactive oxygen species².

Antioxidant system neutralizes free radicals and reactive oxygen species. Iron deficiency anemia (IDA) cause increase in oxidative stress due to an increase in oxidant levels and a decrease in antioxidant enzyme capability³.

In iron deficiency anemia iron containing protein for example peroxidase, cytochrome, catalase and myoglobin are affected. Tissue are more exposed to Oxidative stress in iron deficiency anemia¹. Because enzyme which prevent tissue from oxidative stress their activity is impaired⁴. Ferritin is one of the acute phase reactant⁵.

Vitamin D is fat soluble vitamin, it plays important role in bone metabolism and immunity. Vitamin D is a sterol that have hormone like function's active form of vitamin D is 1,25-dihydrocholecalciferol⁶. The most prominent actions of 1,25 diOH-D3 are to regulate calcium and phosphorus level in plasma⁷.

MATERIAL & METHODS

This study was a cross sectional study that was done at private setting in Sialkot. Convenient sampling was done. Ninety participants are included in this study n= 90. We divided then into two groups in which Group A is (diseased) n=50 included female with low Hb i.e., less than 12mg/dl (normal level 12mg/dl to 15mg/dl) Hematocrit less 45% (above 45%) Serum ferritin less 50 (above 50micro gram to 200) and Vitamin D i.e., 25-hydroxy vitamin D is less than 20g/ml (above 20micro g/ml to 50microgram/ml. In group B (control) included normal healthy females. Permission was granted by IRB.

Blood sample collection:

EDTA: Venous blood sample 2mL taken from anti-cubital vein forms each participant and collected in EDTA anticoagulant vial for Hemoglobin (Hb), hematocrit.

Serum: Venous blood sample 2-3mL taken from anti-cubital vein form each participant and collected in gel clot vial. After blood clotting the serum collected by centrifugation for measurement of Serum Ferritin and Vitamin D i.e., 25-hydroxyvitamin.

Received on 14-11-2021

Accepted on 23-05-2022

Kits / Chemicals:

- CBC was tested on commercially available fully automatic 5 parts differential hematology analyzer.
- Serum Ferritin performed on automated quantified by State-of-the-art Instrument i.e., MiniVidas® using the Enzyme Linked Fluorescent Assay Technique.
- iii. 25-hydroxy vitamin D levels were done by Enzyme Linked Immunosorbent Assay.

Statistical analysis: Statistical analysis was done by Graph pad prism version 6. T-test was applied to compare the quantitative variable between the two groups. P value of <0.05 was considered statistically significant.

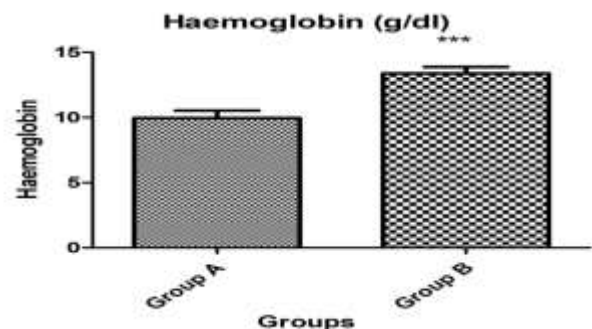
RESULTS

Blood Hb & Hematocrit level: It was observed that in complete blood count level of Hb & Hematocrit were significantly lower which causes Iron deficiency anemia in diseased group i.e., Group A compared to control group i.e., group B. The P value was <0.01 that was very statistically significant.

Table 1: Mean + SEM for the Hemoglobin

	Group A Mean± SEM	Group B Mean±SEM	P-value
Blood Hemoglobin(gm/dl)	9.963±0.5655***	13.38±0.503	<0.001

Figure 1: Graphical presentation of blood Hemoglobin levels ***P value <0.001.

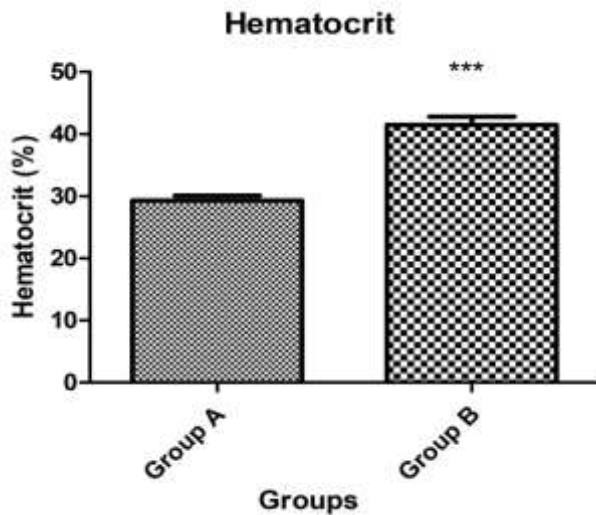


Blood Hematocrit percentage: Blood Hematocrits level significantly low less the 45% in group A as compared to Group B. The difference was statistically significant with the P value <0.001.

Table 2: Mean ± SEM of Blood Hematocrit %

	Group A MEAN±SEM	Group B Mean±SEM	P-Value
Blood Hematocrit (%)	29.30±0.815	41.46±1.362	<0.001

Figure 2: Graphical presentation of blood Hematocrit (%) levels *** P value < 0.001.

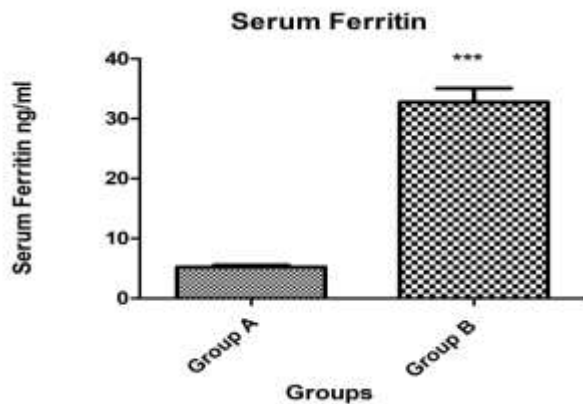


Serum Ferritin Levels: Serum ferritin level are higher in group b i.e. Normal control group as compared to Group A i.e., diseased group. The difference was statistically significant with P value of <0.001.

Table 3: Mean±SEM for Serum Ferritin

	Group A MEAN±SEM	Group B MEAN±SEM	P-value
Serum Ferritin(n/g)	5.225±3.19***	32.74±2.34	<0.001

Figure 3: Graphical presentation of Serum Ferritin ***P value <0.001.

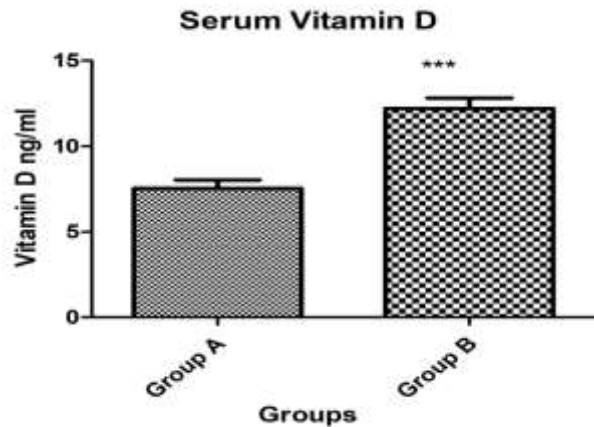


Serum Level of Vitamin D i.e., (25-Hydroxy vitamin): It was observed that the serum 25- hydroxyvitamin D levels were significantly lower in the diseased group i.e. A as compared to normal control group i.e. Group B. P value was < 0.001 that was very statistically significant.

Table 4: Mean ±SEM for the serum 25-hydroxyvitamin D

	Group A Mean± SEM	Group B Mean ±SEM	P-value
Serum 1,25-dihydroxy vitamin level (ng/ml)	7.538±0.489	12.21±0.6063	<0.001

Figure 4: Graphical presentation of serum 1,25- dihydroxy vitamin D levels *** P value <0.001.



DISCUSSION

Iron deficiency anemia is the most common nutritional disorder in the underdeveloped countries. It occurs when there is imbalance in the iron intake, iron stores and loss of iron from the body. Iron deficiency does not cause death, but it has significant worse effects on human body health. IDA is major health issue in under developed countries. IDA along with low level Vitamin D had more severe effects on human health².

Vitamin D is a fat soluble. It is a group of sterol which acts like hormone. 1,25-dihydroxy cholecalciferol or calcitriol (1,25-diOH-D)³ is the active form of vitamin D. It is the biomarker for vitamin D status in the blood⁹.

Recommended dietary allowance for adult is 200IU at the age of 50 and 400 -600 IU after 50 years. It is mainly present in the fish, liver and egg yolk. Endogenous vitamin D is formed from 7-dehydrocholesterol an intermediate of cholesterol, which is converted to cholecalciferol in the dermis when exposed to sunlight.

The main function of Vitamin D to maintain adequate level of calcium & phosphate in the plasma by decreasing its loss from the body & increase absorption of calcium from the intestine⁸.

Calcitriol i.e. 1,25-diOH-D3 stimulate the release of phosphate and calcium from bone. Which result in the increase in plasma calcium and phosphate.

Iron is essential for physical growth, cellular functioning, neurological development and synthesis of important hormone in human body. Heme iron is formed when iron in form of ferrous (Fe++) incorporated with protoporphyrin IX⁹. Most of the iron i.e. 3 to 4 grams of iron is present in the hemoglobin. Other is stores in the form of ferritin or hemosiderin in the spleen, bone marrow & liver and in the myoglobin in muscle tissue. Iron is present in almost all cells. Blood contains 14.5g of Hb per 100ml. About 75% of total iron is in Hemoglobin and 5% is in myoglobin and 15% in ferritin¹⁰.

In our study we observed that blood level of Hb, Hematocrit, Serum Ferritin causes the IDA. The IDA patients had low level vitamin D in their serum. Our study also supported by the result of healthy group. Appropriate amount of iron and vitamin D is necessary for the treatment of IDA along with Vitamin D deficiency.

Iron is mainly given in as ferrous fumarate, ferrous sulfate, and ferrous gluconate forms⁴. After taking iron level rises in few weeks. Vitamin D is given in the form of Cholecalciferol & ergocalciferol⁵. Adequate supplementation with iron and vitamin D decreases the adverse effects of IDA and vitamin D deficiency.

Due to less resources & budget we are unable to include other parameter like red blood indices i.e. TRBC, MCV, MCHC, RDW-CV, Peripheral smear of blood, serum phosphate level, serum calcium level etc.

CONCLUSION

In our study we found that females with IDA also have low level of vitamin D when compared with the healthy female control group. The result is statistically significant with p value < 0.001.

Conflict of interest: Nil

REFERENCES

1. Cullis JO, Fitzsimons EJ, Griffiths WJ, Tsochatzis E, Thomas DW, British Society for Haematology. Investigation and management of a raised serum ferritin. *British journal of haematology*. 2018 May;181(3):331-40.
2. Carrascoza F, Silaghi-Dumitrescu R. The dynamics of hemoglobin-haptoglobin complexes. Relevance for oxidative stress. *Journal of Molecular Structure*. 2022 Feb 15;1250:131703.
3. Fujii J, Homma T, Kobayashi S, Warang P, Madkaikar M, Mukherjee MB. Erythrocytes as a preferential target of oxidative stress in blood. *Free Radical Research*. 2021 Aug 3;55(8):781-99.
4. Balakirev NA, Maksimov VI, Deltsov AA. Development and application of iron supplements, and principles of iron deficiency anemia therapy in fur farming. *InIOP Conference Series: Earth and Environmental Science 2021 Sep 1 (Vol. 848, No. 1, p. 012215)*. IOP Publishing.
5. Saffarionpour S, Diosady LL. Multiple emulsions for enhanced delivery of vitamins and iron micronutrients and their application for food fortification. *Food and Bioprocess Technology*. 2021 Apr;14(4):587-625.
6. Chu C, Tsuprykov O, Chen X, Elitok S, Krämer BK, Hoche B. Relationship Between Vitamin D and Hormones Important for Human Fertility in Reproductive-Aged Women. *Frontiers in endocrinology*. 2021 Apr 14;12:362.
7. Xu J, Chen K, Zhao F, Huang D, Zhang H, Fu Z, Xu J, Wu Y, Lin H, Zhou Y, Lu W. Association between vitamin D/calcium intake and 25-hydroxyvitamin D and risk of ovarian cancer: a dose-response relationship meta-analysis. *European Journal of Clinical Nutrition*. 2021 Mar;75(3):417-29.
8. Dai Z, McKenzie JE, McDonald S, Baram L, Page MJ, Allman-Farinelli M, Raubenheimer D, Bero LA. Assessment of the methods used to develop vitamin d and calcium recommendations—a systematic review of bone health guidelines. *Nutrients*. 2021 Jul;13(7):2423.
9. Dai Z, McKenzie JE, McDonald S, Baram L, Page MJ, Allman-Farinelli M, Raubenheimer D, Bero LA. Assessment of the methods used to develop vitamin d and calcium recommendations—a systematic review of bone health guidelines. *Nutrients*. 2021 Jul;13(7):2423.
10. Mohammed MA, Abed MT, Shweash M, Fawzi HA, Hendi MQ, Durajj AT, Salih GM. Relationship between vitamin D deficiency and serum ferritin level in healthy women.