

Association between Vitamin D and serum ferritin levels in anemic women of child bearing age in Malakand KPK

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ABSTRACT

Background: Vitamin D deficiency is a widespread issue around the world. Ferritin is a massive protein with 24 subunits and light and heavy chains. The body's intracellular iron storage is represented by ferritin. Vitamin D deficiency and iron deficiency are frequently found together. Mechanistic interactions between iron and vitamin D metabolism are now thought to be at the root of these correlations.

Objective:

1. To determine mean vitamin D and serum Ferritin in anaemic women of child bearing aged between 15-49 years.
2. Correlation between vitamin D level and serum Ferritin in anemic women of child bearing age.

Methodology: A Cross sectional survey was conducted from October 2020 to April 2020 at Malakand. 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 haemoglobin, 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.

Result: Study results revealed that there is no significant association between anaemia and vitamin D level but there is a significant association between anaemia and serum ferritin showing p value $p < 0.05$. Furthermore Mean age of the study participants was 30.2806 ± 9.176 , mean weight, Height, Hemoglobin, Vitamin D and serum Ferritin was 62.1684 ± 10.16062 , 159.7398 ± 32.84960 , 9.5592 ± 1.12275 , 25.701 ± 13.8711 and 12.1738 ± 12.32928 respectively.

Conclusion: It was concluded that there is not a significant association between anaemia and vitamin D level but there is a significant association between anaemia and serum ferritin showing p value $p = 0.017$ Moreover no association between vitamin D and serum ferritin level among anaemic females of child bearing age were seen .

Key Words: Anemia, Vitamin D, Serum Ferritin, Correlation

INTRODUCTION

Vitamin D3, commonly known as sunshine vitamin, is a fat-soluble vitamin that helps the gut absorb calcium, magnesium, and phosphate. Its variants D3 and D2 occur as essential chemicals in humans ¹. Vitamin D3 deficiency is a worldwide problem that affects more than a billion people. Osteoporosis, rickets, and osteomalacia can all be caused by a lack of vitamin D ². Ferritin, on the other hand, is a protein that accumulates iron and represents the body's iron storage / reservoir. These stockpiles can be depleted/exhausted, resulting in severe IDA. Because Ferritin is the most important storage form of iron, it is the greatest diagnostic of iron deficiency ³. Low serum Ferritin levels are linked to diseases like iron deficiency anaemia (IDA) and low bone mineral density, whereas high serum ferritin levels are linked to heart disease, insulin resistance, and metabolic syndrome.

Vitamin D deficiency and iron deficiency are frequently found together. Mechanistic interactions between iron and vitamin D metabolism are now thought to be at the root of these correlations ⁴. Vitamin D's main function was previously thought to be regulating calcium levels and bone metabolism through its role in calcium and phosphate absorption in the intestines. Additional roles of vitamin D have been studied, including its effects on the prevention of diseases including cardiovascular disease and anaemia ⁵. Ferritin is a protein found within cells that stores iron and releases it in a regulated manner ⁶. Vitamin D is involved in bone marrow stem cell proliferation and differentiation, and it may also play a role in red cell development. Vitamin D, by encouraging erythropoiesis and suppressing hepcidin expression, has the ability to affect circulating iron status ⁷. Because of the impact of menstruation and pregnancy, iron deficiency is the most common nutrient deficiency in the developing world, and women of childbearing age are at the greatest danger. Since the 1,25 hydroxyvitamin D hormone is involved in the proliferation and maturation of erythroid progenitor cells, a deficiency of this

hormone can impair erythropoiesis ⁸. Iron deficiency and its effects are particularly dangerous for women of reproductive age. The relationship between anaemia and ferritin varies depending on the type of anaemia. Ferritin levels fall in people with iron deficiency anaemia, but they may rise in people with chronic disease and inflammation anaemia. Vitamin D aids in the prevention of anaemia as well as the reduction of insulin resistance and inflammation. Vitamin D increases ferritin by down-regulating hepcidin in people with iron deficiency anaemia. However, vitamin D may be decreased ferritin for the improvement of inflammation status in subjects with chronic disease anaemia and inflammation ⁹. In various skeletal sites, vitamin D is a key regulator of osteoblast activity ¹⁰.

Vitamin D insufficiency is apparently pervasive in Pakistan, with children, women, and the elderly being the most affected ¹¹. Even more harmful is the fact that vitamin D insufficiency is one of the most under-diagnosed, under-treated dietary deficiencies in the world ¹². This number includes Pakistan as a country ¹³. Its involvement in calcium absorption was formerly the main emphasis, but in recent years, its additional activities, such as its participation in the prevention of cardiovascular disease and anaemia, have been discussed in the literature ¹⁴. Ferritin, on the other hand, is a protein that accumulates iron and serves as a marker for the body's iron storage/reservoir. These stockpiles can be depleted/exhausted, resulting in severe IDA. Because ferritin is the most important storage form of iron, it is the greatest diagnostic of iron insufficiency ³. In the case of anaemia, particularly iron deficiency anaemia, literature has shown that Vit D3 levels are positively correlated with serum ferritin levels ¹⁵. A low-cost screening test with excellent reliability and accuracy for detecting iron deficiency is required. The bone marrow aspiration is the gold standard for diagnosing iron deficiency (to assess the iron stores). However, for a highly frequent medical problem, especially in developing nations, the operation is intrusive, complex, and

expensive. Serum ferritin is an alternative to the bone marrow aspiration test, and it has been determined to be the best test for identifying those with ID from those who do not. Unfortunately, many basic health care clinics, particularly in underdeveloped countries, do not offer a free serum Ferritin estimate test. As a result, the purpose of this study was to determine the prevalence of iron deficiency and iron deficiency anaemia among healthy women of childbearing age, as well as to see if it could be detected using simple parameters obtained from a complete blood count, with serum Ferritin level as a reference standard.

Objectives:

1. To determine mean vitamin D and serum ferritin in anemic women of child bearing aged between 15-49 years.
2. Correlation between vitamin D level and serum ferritin in anemic women of child bearing age

MATERIALS AND METHODS

A Cross sectional survey was conducted from October 2020 to April 2020 at Malakand division. 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. Diabetic patients, Renal impairment, Liver disease, Pregnant and Lactating women and women with Malignant conditions were excluded . After taking approval from hospital ethical review board all the study participants were given an informed consent after that they allow the principle investigator for conducting their research. The study participants were interviewed by using a pre formed questionnaire containing questions related to their Demographic variables, haemoglobin level, vitamin D level and serum Ferritin level as outcome variables followed by Variables related to their diet and habits. For taking Blood samples to know haemoglobin, Ferritin and vitamin D levels participants were given aseptic environment. Their samples were then collected were collected by venepuncture in to a dry, clean and sterile white without anticoagulant substance until it makes a clot. Blood samples were

allowed to stand for 20-30mint for clot formation and then it is centrifuged and after letting the tubes stand for some time the serum was collected. The supernatants serum was taken and stored in Aliquot tube at – 20C. The collected sample were then analysed to see the haemoglobin, vitamin D and serum Ferritin level. The results were then compared to see any association between them. The data were analyzed by statistical method generated on the computer software SPSS 23. Quantitative variables including age, weight, Height, hemoglobin level, vitamin D level and serum ferritin level were presented as mean +/- standard deviation. Independent sample t test was used to see the association between quantitative attributes. Chi-Square test was used to see the association between qualitative attributes.

RESULTS

A total of 196 subjects participated in the study. Table 1 shows the mean age of the study participants was 30.2806±9.176, mean weight 62.1684±10.16062, Mean Height 159.7398±32.84960, mean Hemoglobin level 9.5592±1.12275, mean Vitamin D level 25.701±13.8711 and mean serum Ferritin level was 12.1738±12.32928. Table 2 shows Literacy rate amongst the sampled population was 57.1% (i.e. those who have educational levels from read and write to any higher qualifications). Furthermore out of total 196 participants 175(89.3%) were married. Table 3 shows frequency distribution of anemia, Vitamin D and Serum Ferritin and it was seen that 119(60.7%) of the participants had moderate anemia, 66(33.7%) had Vitamin D value between 15-25 and 156(79.6%) had Serum ferritin between 2-15. Table 5 shows that there is no significant association between anaemia and vitamin D level but there is a significant association between anaemia and serum ferritin showing p value p<0.05. Table 6 shows that there is not a significant association between vitamin D and serum ferritin level among anaemic females of child bearing age showing p-value p=0.255

Table 1: Mean ± Standard deviation Distribution of quantitative variables

Variables(n=196)	Age of the participants	Weight of the participants	Height of the participants	Hemoglobin level	Vitamin D	Serum Ferritin
Mean ±Std. Deviation	30.28±9.17	62.165±10.16	159.74±32.85	9.56±1.12	25.7±13.87	12.17±12.32

Table 2: Frequency distribution of demographic variables

Variable(n=196)		Frequency (n)	% age
Education status	Illiterate	84	42.9%
	Literate(can read/write)	24	12.2%
	Primary	36	18.4%
	Secondary	32	16.3%
	Intermediate	10	5.1%
	Graduation	6	3.1%
	Master	4	2%
Marital status	Married	175	89.3%
	Unmarried	21	10.7%
	Total	196	100%

Table 3: Frequency distribution of Outcome variables

Variable(n=196)		Frequency (n)	Percentage (%)
Classification of anemia	Mild Anemia	73	37.2%
	Moderate Anemia	119	60.7%
	Severe Anemia	4	2%
Vitamin D	2-15	48	24.5%
	15.1-25	66	33.7%
	25.1-35	32	16.3%
	35.1-45	34	17.3%
	45.1-55	12	6.1%
	55.1-65	4	2%
Serum ferritin	2-15	156	79.6%
	15.1-25	25	12.8%
	25.1-35	4	2%
	35.1-45	1	0.5%
	45.1-55	1	0.5%
	55.1-65	3	1.5%
	65 and above	6	3.1%
Total	196	100%	

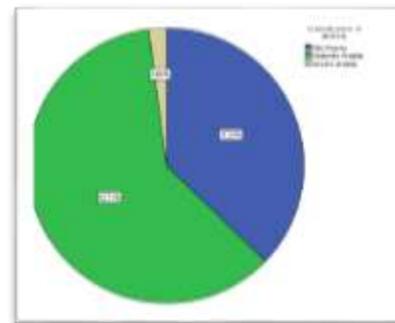


Figure 1: Frequency distribution of anemia

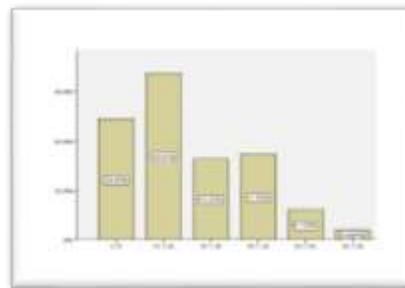


Figure 2: Frequency distribution of Vitamin D

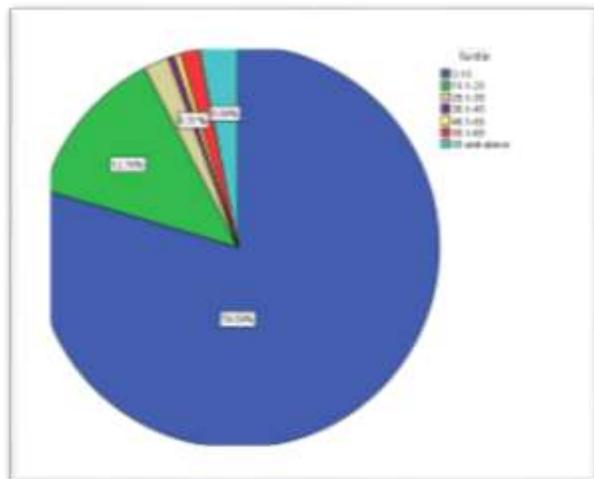


Figure 3: Frequency distribution of Serum Ferritin

Table 4: Mean distribution of vitamin D and serum ferritin levels among different levels of Anaemia among women of child bearing age

Aneamia status	n=196	Vitamin D levels		Serum Ferritin levels	
		Mean ±Std. Deviation	n=196	Mean ± Std. Deviation	n=196
Mild Anemia	73	25.86 ± 14.8815	73	16.9262 ± 17.26108	
Moderate Anemia	119	25.724 ± 13.235	119	9.4601 ± 6.83462	
Severe Anemia	4	22.1 ± 16.8434	4	6.175 ± 1.74428	
Total	196	25.701 ± 13.8711	196	12.1738 ± 12.32928	

Table 5: Association of Different categories of Anemia with vitamin D level and serum ferritin level

ANOVA		
		p-value
Vitamin D	Between Groups	0.871
	Within Groups	
	Total	
Serum ferritin	Between Groups	<0.05
	Within Groups	
	Total	

Table 6: Association of vitamin D level and serum ferritin level

Variables(n=196)	Serum ferritin	p-value
Vitamin D	Between Groups	0.255
	Within Groups	

DISCUSSION

Serum ferritin is a nutritional indicator of iron status that measures total iron storage in the body^{16, 17}. It is an acute-phase reactant that rises in the systemic response to inflammation^{18, 19}, making it an inflammatory marker²⁰. In the presence of inflammation, Serum Ferritin elevated can disguise deficient iron status¹⁷. Serum ferritin has been associated to diabetes²¹ and metabolic syndrome²². Vitamin D significance in bone and mineral metabolism is well recognized some studies have looked at the effect of vitamin D on inflammation²³ but there is no consistency across the few studies that looked at the association between vitamin D and Serum ferritin as an inflammation marker¹⁶ and none of these studies looked at the effect of vitamin D on greater Serum Ferritin concentrations indicative of inflammation.

In our study Mean Hemoglobin, Vitamin D and Serum Ferritin were 9.5g/dl, 25.7 and 12ng/ml respectively. Similar findings were reported in a previous study by Hamzullah Khan et al.²⁴, in 2020, they observed that 118 (61.5%) were iron deficient with serum ferritin less than 15ng/ml and whereas unlike our study Vitamin D3 was insufficient among 7(14.9%) only in ranges between 20.1-29.9ng/ml. Abuaisha M et al.²⁵, in 2019 reported the prevalence of iron deficiency in 57.5% females at 95%. Muscogiuri G et al.²⁶, in 2020 published in Reproductive Health reported that

iron deficiency anemia (IDA) prevalent was 41% in antenatal women.

The findings of our study revealed there is not a significant association between anaemia and vitamin D level and vitamin D and serum Ferritin level among anaemic females of child bearing age showing p value 0.255, dissimilar findings were reported by Hamzullah Khan et al.²⁴, In 2020 as they found significant association between vitamin D and serum Ferritin with p value 0.02 and they concluded that There is a strong statistically significant association of Vitamin D3 with serum Ferritin in anaemic patients with Hb<11g/dl. In present study a significant association between anaemia and serum ferritin showing p value p=<0.05. Researchers are looking into the link between vitamin D3 and serum ferritin levels all around the world²⁷. According to other previous studies there is a substantial link between Vit D3 and serum Ferritin levels and their contribution to co-morbidities linked with respective deficiencies. Vitamin D levels were found to be positively linked with serum ferritin levels in a research with a (p= 0.041)¹⁴. In the case of anaemia, particularly iron deficiency anaemia, literature has shown that Vit D3 levels are positively associated with serum Ferritin levels¹⁵.

CONCLUSION

Our study results concluded that there is not a significant association between anaemia and vitamin D level but there is a significant association between anaemia and serum Ferritin showing p value p=0.017 Moreover no association between vitamin D and serum Ferritin level among anaemic females of child bearing age were seen .

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REFERENCES

- Holick MF, editor High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc; 2006: Elsevier.
- Riaz H, Finlayson A, Bashir S, Hussain S, Mahmood S, Malik F, et al. Prevalence of Vitamin D deficiency in Pakistan and implications for the future. Expert Rev Clin Pharmacol. 2016;9(2):329-38.
- Babaei M, Shafiei S, Bijani A, Heidari B, Hosseini SR, Vakili Sadeghi M. Ability of serum ferritin to diagnose iron deficiency anemia in an

- elderly cohort. *Revista brasileira de hematologia e hemoterapia*. 2017;39:223-8.
4. Thomas CE, Guillet R, Queenan RA, Cooper EM, Kent TR, Pressman EK, et al. Vitamin D status is inversely associated with anemia and serum erythropoietin during pregnancy. *The American Journal of Clinical Nutrition*. 2015;102(5):1088-95.
 5. Munasinghe LL, Ekwaru JP, Mastroeni MF, Mastroeni SS, Veugelers PJ. The association of serum 25-hydroxyvitamin D concentrations with elevated serum ferritin levels in normal weight, overweight and obese Canadians. *PLoS One*. 2019;14(3):e0213260.
 6. Gombar S, Parihar K, Choudhary M, Gombar S. Comparative study of serum ferritin and vitamin D in thalassemia patients with healthy controls. *Int J Res Med Sci*. 2018;6(2):693-5.
 7. Masoud MS, Alokail MS, Yakout SM, Khattak MNK, AlRehaili MM, Wani K, et al. Vitamin D supplementation modestly reduces serum iron indices of healthy Arab Adolescents. *Nutrients*. 2018;10(12):1870.
 8. Lucisano S, Di Mauro E, Montalto G, Cernaro V, Buemi M, Santoro D. Vitamin D and anemia. *J Ren Nutr*. 2014;24(1):61-2.
 9. Madar AA, Stene LC, Meyer HE, Brekke M, Lagerlöv P, Knutsen KV. Effect of vitamin D3 supplementation on iron status: a randomized, double-blind, placebo-controlled trial among ethnic minorities living in Norway. *Nutr J*. 2015;15(1):1-10.
 10. Judistiani RTD, Madjid TH, Irianti S, Natalia YA, Indrati AR, Ghozali M, et al. Association of first trimester maternal vitamin D, ferritin and hemoglobin level with third trimester fetal biometry: result from cohort study on vitamin D status and its impact during pregnancy and childhood in Indonesia. *BMC Pregnancy Childbirth*. 2019;19(1):1-8.
 11. Kiani RA. Biochemical analysis and frequency of Vitamin D deficient people in different age and gender. *Journal of The Society of Obstetricians and Gynaecologists of Pakistan*. 2018;8(4):249-54.
 12. Gill TK, Hill CL, Shanahan EM, Taylor AW, Appleton SL, Grant JF, et al. Vitamin D levels in an Australian population. *BMC Public Health*. 2014;14(1):1-11.
 13. Humayun Q, Iqbal R, Azam I, Khan AH, Siddiqui AR, Baig-Ansari N. Development and validation of sunlight exposure measurement questionnaire (SEM-Q) for use in adult population residing in Pakistan. *BMC Public Health*. 2012;12(1):1-8.
 14. Yoon H, Bae NY, Gi MY, Park BY, Seong JM. The association between serum ferritin and 25-hydroxyvitamin D and metabolic syndrome in Korean women: the Korea National Health and Nutrition Examination Survey 2010–2012. *J Clin Biochem Nutr*. 2017;61(1):60-6.
 15. Jeong DW, Lee HW, Cho YH, Yi DW, Lee SY, Son SM, et al. Comparison of serum ferritin and vitamin d in association with the severity of nonalcoholic Fatty liver disease in Korean adults. *Endocrinology and Metabolism*. 2014;29(4):479-88.
 16. Malczewska-Lenczowska J, Sitkowski D, Surala O, Orysiak J, Szczepańska B, Witek K. The association between Iron and vitamin D status in female elite athletes. *Nutrients*. 2018;10(2):167.
 17. Huang Y-F, Tok T-S, Lu C-L, Ko H-C, Chen M-Y, Chen SC-C. Relationship between being overweight and iron deficiency in adolescents. *Pediatr Neonatol*. 2015;56(6):386-92.
 18. Langer AL, Ginzburg YZ. Role of hepcidin-ferroportin axis in the pathophysiology, diagnosis, and treatment of anemia of chronic inflammation. *Hemodialysis International*. 2017;21:S37-S46.
 19. Khan A, Khan WM, Ayub M, Humayun M, Haroon M. Ferritin is a marker of inflammation rather than iron deficiency in overweight and obese people. *J Obes*. 2016;2016.
 20. Alam F, Memon AS, Fatima SS. Increased body mass index may lead to hyperferritinemia irrespective of body iron stores. *Pakistan journal of medical sciences*. 2015;31(6):1521.
 21. Akter S, Nanri A, Kuwahara K, Matsushita Y, Nakagawa T, Konishi M, et al. Circulating ferritin concentrations and risk of type 2 diabetes in Japanese individuals. *Journal of diabetes investigation*. 2017;8(4):462-70.
 22. Lee JA, Hwang JS, Hwang IT, Kim DH, Seo J-H, Lim JS. Low vitamin D levels are associated with both iron deficiency and anemia in children and adolescents. *Pediatr Hematol Oncol*. 2015;32(2):99-108.
 23. Azizieh F, Alyahya KO, Raghupathy R. Association between levels of vitamin D and inflammatory markers in healthy women. *Journal of Inflammation Research*. 2016;9:51.
 24. Khan H, Basharat M. Correlation of Vit D3 and serum ferritin levels in anemic patients in a hospital based study. *The Professional Medical Journal*. 2020;27(11):2376-82.
 25. Abuaisa M, Itani H, El Masri R, Antoun J. Prevalence of iron deficiency (ID) without anemia in the general population presenting to primary care clinics: a cross-sectional study. *Postgrad Med*. 2020;132(3):282-7.
 26. Muscogiuri G, Barrea L, Somma CD, Laudisio D, Salzano C, Pugliese G, et al. Sex differences of vitamin D status across BMI classes: An observational prospective cohort study. *Nutrients*. 2019;11(12):3034.
 27. Tharwat RJ, Balilah S, Habib HM, Mahmoud NH, Beek FS, Almadani FK, et al. Ferritin and Vitamin D levels and its relation to bone diseases in thalassemic adults: A hospital-based retrospective cohort study. *Journal of Applied Hematology*. 2019;10(1):15.