



## Correlation of Vit D3 and serum ferritin levels in anemic patients in a hospital based study.

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**ABSTRACT... Objectives:** To determine the correlation of Vit D3 levels with serum ferritin in patients with anemia. **Study Design:** Cross Sectional study. **Setting:** Department of Pathology, Qazi Hussain Ahmed Medical Complex Nowshera. **Period:** 5<sup>th</sup> Jan 2019 to 31<sup>st</sup> Dec 2020. **Material & Methods:** Data entered in SPSS 25<sup>th</sup> version. Descriptive statistics was used for numerical variables. Pearson correlation was used for correlation of Vit D3 with serum ferritin levels. Normality of data was checked by Shapiro wilk test. Mann Whitney U test was used to show the difference of Vit D3 and ferritin levels in gender. **Results:** The total of 192 anemic patients with Hb<10g/dl as per definition of WHO<sup>1</sup> were referred for ferritin level estimation with 142(74%) females and 50(26%) males. Out of total, 47 were also advised with Vit D3 level estimation with 29(61.7%) females and 18(38.3%) males. Mean with standard deviation for age was 30+9.6 years. Mean with standard Error of mean of Vit D3 was (Mean-15.5ng/ml, SE 2.19). Mean with standard Error of mean of serum ferritin was (Mean-48.2ng/ml, SE 5.90). We observed 35(74.5%) cases out of 47, as Vit D3 deficient with a count less than 20 ng/ml. We observed that 118 (61.5%) were iron deficient with serum ferritin less than 15ng/ml. Person correlation showed a statistically significant correlation of Vit D3 with ferritin ( $p= 0.022$ ,  $r=0.7$ ). Spearman ranked correlation showed a statistically significant correlation between the categories of Vit D3 and ferritin ( $p=0.022$ ,  $r=0.7$ ). Mann Whitney U Test showed no significant difference in gender groups for both the variables ( $p= 0.86$  &  $p=0.33$  respectively) thus retain the null hypotheses. **Conclusion:** The frequency of deficiency Vit D3<20ng/ml in anemic patients was 74% while that of ferritin<15ng/ml in anemic patients was 61.5%. There is a strong statistically significant correlation of Vit D3 with serum ferritin in anemic patients with Hb<11g/dl.

**Key words:** Correlation Statistics, Serum Ferritin, Vit D3.

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### INTRODUCTION

Vitamin D3 also called sunshine vitamin, is a fat soluble vitamin than increase the absorption of calcium, magnesium and phosphate in the intestine. In human its variant D3 and D2 are existing as vital compounds.<sup>1</sup>

Vit D3 deficiency is prevalent worldwide affecting more than a billion population globally. Vit D deficiency can result in progression of Osteoporosis, rickets and osteomalacia.<sup>2</sup>

Beside the appreciable sun light in Pakistan still the burden of Vit D3 deficiency is highly alarming and devastating the nation both on health ground as well as a threat to economy.

So for so after searching the literature we failed to find the researchers to have assessed vitamin D deficiency across all age and gender groups.

Vitamin D deficiency in Pakistan is reportedly endemic in nature and is more commonly reported in children, women and elderly population.<sup>3</sup> The more dangerous than that is that Vit D3 deficiency is undermined, under-diagnosed and under-treated among the dietary insufficiencies worldwide<sup>4,5</sup> this includes Pakistan as well as a nation.<sup>6</sup>

In past its role in regard with calcium absorption was mainly on focus but in recent era its additional functions like its role in prevention of

cardiovascular disease and anemia are under discussion in literature.<sup>7</sup>

On the other hand Ferritin is a protein which stores iron, and indicates the iron storage / reservoir in the body. The depletion/exhaustion of these stores can lead to sever IDA. Ferritin is the main storage form of iron, therefore is the best indicator for assessment of iron deficiency.<sup>8</sup> The determination of serum ferritin by in-vitro is done to assess body iron stores. In adulthood ferritin concentration is directly related to the iron storage in the body. Serum ferritin concentration has the highest sensitivity and specificity to detect iron deficiency as reported in the literature.<sup>9</sup>

Studies have reported a strong correlation between the Vit D3 and serum ferritin levels and their contribution in co-morbidities associated with their deficiencies. A study reported that vitamin D was positively associated with serum ferritin levels with a (p= 0.041).<sup>10</sup> In terms of anemia, specially the Iron deficiency anemia, literature has reported that there is a positive correlation of Vit D3 with serum ferritin levels.<sup>11,12</sup>

Another study reported that there was a strong relationship of Vit D3 deficiency and decreased iron store as sole risk factors for chronic neck pain.<sup>13</sup> They further correlated it with female gender being more exposed to neck pain when presented with hypovitaminosis and decreased ferritin.

Therefore the present study was designed as to ascertain the correlation of Vit D3 levels with serum ferritin in anemic patients in our population.

## MATERIAL & METHODS

This cross sectional study was conducted in the department of Pathology, Qazi Hussain Ahmed Medical Complex Nowshera from 5<sup>th</sup> Jan 2019 to 31<sup>st</sup> Dec 2020.

Ethical committee Nowshera Medical College approval was taken before execution of study via notification no. 78/NMC/ERB, Dated: 07-01-2019.

Inclusion criteria were all anemic patients as

per WHO criteria<sup>1</sup> with HB<11g/dl, referred to pathology laboratory of Qazi Hussain Ahmed Medical Complex Nowshera for Vit D3 and serum ferritin levels irrespective of age and gender.

Exclusion criteria were patients with Vit D supplementation. Similarly all candidates taking oral or IV iron therapy for their already diagnosed IDA were also excluded. Patients with request of serum ferritin or Vit D3 with Hb>11g/dl were also excluded from the study.

About 2 ml blood was drawn from each individual, by veni-puncture under aseptic condition. Blood in Gel bottles was stored for serum ferritin and Vit D3 analysis. Then blood was centrifuged. Serum Vit D3 was measured by electro-chemiluminescence immunoassay using Roche Cobas E411 Chemistry Analyzer for which commercial kits of Roche diagnostics were used as per the instructions of the manufacturer.

Patients were categorized on the basis of Vit D3 levels as follow,

1. Vit D3 Deficient = Vit D3 (less than 20 ng/ml)
2. Vit D3 Insufficient=Vit D3 (20.1-29.9ng/ml)
3. Vit D3 Sufficient=Vit D3 (>30ng/ml)

We followed the laid down criteria of Hb is less than 11g/dl as per WHO recommendations.<sup>14</sup> We received 90%patients form gynecology OPD and indoor patients.

We categorized our patients in three categories on the basis of serum ferritin levels. Analysis of serum ferritin levels based on the recommendations of the WHO.<sup>15</sup>

1. IDA: less than 15 ng/ml
2. Normal or otherwise: >150ng/ml

Data entered in SPSS 25<sup>th</sup> version. Descriptive statistics was used for numerical variables like age, vit D3 level, ferritin levels and gender. Normality of data check by Shapiro wilk test.

Pearson correlation was used for correlation of Vit D3 with serum ferritin levels. Spearman

ranked correlation test was performed to see the correlation of categories of Vit D3 with Categories of Serum ferritin. Man Whitney U test was used for skewed data to show the difference of Vit D3 and serum ferritin levels in gender groups.

## RESULTS

The total no of patients under trial were 192 who were referred for ferritin level estimation with 142(74%) females and 50(26%) males. Out of 192, 47 were also advised the Vit D3 level estimation with 29(61.7%) females and 18(38.3%) males. (Table-I).

The descriptive statistics of age with Mean plus standard deviation was 30+9.6 years. Minimum age was 18 months to a maximum of 70 years. Mean with standard Error of mean of Vit D3 was (Mean-15.5ng/ml, SE 2.19) with minimum of 3ng/ml to maximum of 70ng/ml. Mean with standard Error of mean of serum ferritin was (Mean-48.2ng/ml, SE 5.90), with minimum of 1.72ng/ml to maximum of 438.6ng/ml as recorded in our study population. (Table-II)

We categorized patients referred for Vit D3 estimation into three categories.<sup>17</sup>

1. Vit D3 Deficient =Vit D3 (less than 20 ng/ml)
2. Vit D3 Insufficient=Vit D3 (20.1-29.9ng/ml)
3. Vit D3 Sufficient=Vit D3 (>30ng/ml)

It was noticed that 35(74.5%) were Vit D3 deficient with less than 20 ng/ml and 7(14.9%) as Vit D3 insufficient with Vit D3 in range of 20.1-29.9ng/ml. Only 5(10.6%) were having normal Vit D3 levels. (Table-III)

We categorized our patients in three categories on the basis of serum ferritin levels. Analysis of serum ferritin levels based on the recommendations of the WHO.<sup>10</sup>

Iron Deficiency Anemia: less than 15 ng/ml

We observed that 118 (61.5%) were iron deficient (serum ferritin less than 15 ng/ml). (Table-IV)

Person correlation test was performed to see the correlation of Vit D3 levels with Serum ferritin levels and it was observed that a statistically significant strong uphill positive correlation was seen between the levels of Vit D3 with Serum ferritin with (p= 0.022, r-0.7). (Table-V)

To dig out further Spearman ranked correlation test was performed to see the correlation of categories of Vit D3 with Categories of Serum ferritin as mentioned in table3 &4, and it was observed that a statistically significant strong uphill positive correlation was seen between the categories of Vit D3 with Categories of Serum ferritin with (p= 0.022, r-0.7). (Table-VI)

Using Shapiro Wilk test to see the distribution of data. It was observed that data was distributed in skewed manner in both Vit D3and ferritin variables with significant p= 0.001. (Table-VIIa)

Mann Whitney U Test was used to determine the difference in median of Vit D3 with Median of Serum ferritin in gender groups, and it was observed that no statistically significant difference was observed in gender groups for both the numerical variables under study, thus retain the null hypotheses. (Table-VII b)

Gender		Ferritin		Vit D3	
		Frequency	Percent	Frequency	Percent
Valid	Male	50	26.0	18	38.3
	Females	142	74.0	29	61.7
	Total	192	100.0	47.0	100

Table-I. Gender wise distribution of patients.

		Ferritin Level	Vit D3 Level	age
N	Valid	192	47	192
	Missing	0	145	0
Mean		45.67	15.51	30
Std. Error of Mean		5.31	2.19	.70
Median		11.93	11.10	30.00
Mode		5.60	3.00	30.00
Std. Deviation		73.59	15.07	9.69
Range		436.88	67.00	68.40
Minimum		1.72	3.00	1.60
Maximum		438.60	70.00	70.00

Table-II. Descriptive statistics of Vit D3, Ferritin levels and age.

	Frequency	Percent	Cumulative Percent
Vit D3 <20ng/ml	35	18.2	74.5
Vit D3 (20.1-29.9)ng/ml	7	3.6	89.4
Vit D3 >30ng/ml	5	2.6	100.0
Total	47	24.5	
System	145	75.5	
Total	192	100.0	

Table-III. VIT D3 Categorization.

		Frequency	Percent	Cumulative Percent
Valid	Ferritin level less than 15ng/ml	118	61.5	61.5
	Ferritin > 15ng/ml	74	38.5	100.0
	Total	192	100.0	

Table-IV. Ferritin categorization for Iron deficiency anemia as per WHO Guidelines.

		VIT D3 Level	Ferritin Level
Vit D3 Level	Pearson Correlation	1	.822**
	Sig. (2-tailed)		.022
	N	47	47
Ferritin Level	Pearson Correlation	.822**	1
	Sig. (2-tailed)	.022	
	N	47	192

\*\* . Correlation is significant at the 0.01 level (2-tailed).

Table-V. Pearson Correlations of VIT D3 with ferritin levels.

		Vit D3 Catagories	Ferritin Catagories
Spearman's rho	Vit D3 Catagories	Correlation Coefficient	1.000
		Sig. (2-tailed)	.744**
		N	47
	Ferritin Catagories	Correlation Coefficient	.744**
		Sig. (2-tailed)	.022
		N	47

\*\* . Correlation is significant at the 0.01 level (2-tailed).

Table-VI. Spearman Correlations of VIT D Categories with Ferritin Categories.

## Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Vit D3 Level is the same across categories of gender.	Independent-Samples Mann-Whitney U Test	.860	Retain the null hypothesis.
2	The distribution of Ferritin Level is the same across categories of gender.	Independent-Samples Mann-Whitney U Test	.339	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

**Table-VII. Mann Whitney U Test for non-parametric variables.**

## DISCUSSION

Having read the importance of the both nutrients we are sure to have probably recorded the higher rate of Vit D3 deficiency and insufficiency in our target population i.e. 74.5% with Vit D3 level less than 20 ng/ml. A study from Karachi reported that 76.2% of their study sampling<sup>16</sup> had had deficiency that was matching our findings. Similarly it was higher than recorded in a study from Germany (58%)<sup>17</sup>, however it was nearly similar to what have been reported from Scottish population (78%).<sup>18</sup> On the other hand the frequency of depleted or deficient than the required iron stores was recorded in our population as 61.5%. Abuaisha M et al<sup>16</sup> reported the prevalence of iron deficiency in 57.5% females at 95% confidence interval, and 7.6% in males that closely matching our findings.<sup>19</sup>

Globally the researchers are working on the association of Vit D3 with serum ferritin levels.<sup>20</sup>

Andıran and colleagues<sup>21</sup> have reported a positive strong liner correlation of 25-Hydroxyvitamin D and serum ferritin levels in Korea and the US, population respectively. But Monlezun and colleagues<sup>22</sup> reported that the serum 25(OH) D was not having any statistical relation in population of US and Portugal, respectively.

In present study using Person correlation test it was observed that a statistically significant strong uphill positive correlation exists between the levels of Vit D3 with Serum ferritin with ( $p=0.022$ ,  $r=0.7$ ). Studies have reported that vitamin D is inversely associated occurrence of IDA in women. Lee et al<sup>23</sup>, observed that the probability for iron

deficiency anemia based on ferritin levels less than 12ng/ml, in patients with vitamin D deficiency [25(OH)D <15 ng/ml] was higher with an odds ratio of 1.86, that further increases to 2.59 (95% CI, 1.11–6.07) if we were able to control the other risk factors in healthy women of Korea, “what the authors claimed”.<sup>23</sup> Another study has reported that Vit D3 level were remarkably lower in patients with Iron deficiency anemia as compared to non-anemic patient based on serum ferritin level less than 12ng/ml with a significant p-value of 0.001.<sup>24</sup>

In present study when we observed the same relation in the categories of Vit D3 & Serum ferritin designed as per WHO guidelines<sup>16</sup> we observed using spearman ranked correlation test, that a statistically significant strong uphill positive correlation was seen between the categories of Vit D3 with Categories of Serum ferritin with ( $p=0.022$ ,  $r=0.7$ ).

Researchers have studied that there was a strong statistically positive correlation between the levels of ferritin and Vit (44.05 ± 25. with iron deficit disorders with vitamin D (13.1 ± 5.66 vs. 32.9 ± 9.12 ng/ml) and serum ferritin of (15.76 ± 18.06 vs. 98.2 ± 52.37 ng/ml).<sup>25</sup> Arabi SM et al also reported that supplementation with vitamin D had significant effect on hemoglobin and ferritin levels along with effects on transferrin saturation.<sup>26</sup> We also observed using Mann Whitney U Test on our skewed data to determine the difference in median of Vit D3 with Median of Serum ferritin in gender groups, and it was observed that no statistically significant difference was observed in gender groups for both the numerical variables under study, thus retain the null hypotheses. But Yoon H et al from the Korea have reported that serum vit D3 level had a negative association/ relation with the serum ferritin levels in male gender, but was positively related with serum ferritin levels in female gender.<sup>10</sup> Munasinghe LL et al finding matched our results that Age, gender, income, ethnicity, blood pressure status, and serum total cholesterol were associated with serum ferritin and vit D3 levels but this association was not statistically significant.<sup>27</sup>

Regarding Vit D3 levels in gender groups Similarly



Muscogiuri G<sup>28</sup> have reported a higher rate of vitamin D deficiency in male gender as compared to female gender (56% vs. 47%;  $p < 0.001$ ). From these discussions we can admit a gender role in acquiring Vit D3 deficiency as apparently evident from our findings but not statistically significant at 95% confidence interval. Some researcher have not find any gender preference like in present study and some have reported its high prevalence in female gender<sup>1</sup> and some have reported its higher rate in male gender.<sup>28</sup>

Regarding ferritin we observed that 118 (61.5%) were iron deficient (serum ferritin less than 15 ng/ml). Abuaisha M et al<sup>29</sup> reported the prevalence of iron deficiency in 57.5% females at 95%. Muscogiuri G<sup>28</sup> published in *Reprod Health* reported that iron deficiency anemia (IDA) prevalent was 41% in antenatal women.<sup>28</sup>

## CONCLUSION

It is concluded that the frequency of deficiency Vit D3 < 20 ng/ml in anemic patients was 74%. The frequency of serum ferritin < 15 ng/ml in anemic patients was 61.5%. There is a strong statistically significant correlation of Vit D3 with serum ferritin in anemic patients with Hb < 11 g/dl. Therefore it is recommended that as IDA is more common in female gender therefore all the females attending the antenatal care services in tertiary and secondary care hospital with Hb less than 11 g/dl must be screened for serum ferritin levels and Vit D3 levels and remedial action may be taken well in time.

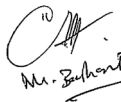
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### AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Hamzullah Khan	Paper design, concept, data collection, analysis, writing the manuscript.	
2	Mohammad Basharat	Data collection, critical review.	