

# Biochemical Analysis and Frequency of Vitamin D Deficient People in Different Age and Gender

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## Abstract

**Objective:** To analyze different biochemical Parameters in Vitamin D deficient population and to estimate prevalence of vitamin D deficiency in different age and gender.

**Methodology:** This cross sectional study was conducted at Islamabad Diagnostic Centre (IDC), Islamabad from January 2017 to March 2018. In this study a total of 721 participants were recruited. Vitamin D3, total calcium, phosphorus, urea, creatinine and uric acid were analyzed on Architect 1000. Statistical analysis was analyzed by SPSS version 19.0.

**Results:** Out of 721 recruited patients, there were 374 (51.8%) male and 347 (48%) were female. The mean values of vitamin D, total calcium, phosphorus, urea, creatinine and uric acid were 23.4 ng/ml ( $\pm$  18.40 SD), 9.1 mg/dl ( $\pm$  0.66 SD), 3.4 mg/dl ( $\pm$  0.60 SD), 30.2 mg/dl ( $\pm$  1.4 SD), 0.94 mg/dl ( $\pm$  0.68 SD) and 5.6 mg/dl ( $\pm$  1.5 SD) respectively. The overall vitamin D deficiency (Deficient + Insufficient) was seen in 558 (77.4%) study participants while 160 (22.1%) participants having sufficient level. Vitamin D toxicity was seen in only 03 (0.41) patients. It was observed that there is statistical significant difference ( $p < 0.05$ ) in the mean value of all parameters.

**Conclusion:** Vitamin D deficiency in Pakistan is becoming endemic and it is much predominant in elder and female population. Female are more deficient due to social and religious limitations. They need more care of vitamin D blood levels due to less exposure to sun light in cities. Based on our finding renal function tests, serum calcium and phosphorus levels are not the conjecturer of vitamin D deficiency.

**Keywords:** Vitamin D, Sun light, Calcium, Phosphorus

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## Introduction

Vitamin D or the “sunshine” vitamin is soluble in fat and ability of this vitamin is to enhance absorption of calcium, magnesium, and phosphate in intestine. In humans, Vitamin D3 (cholecalciferol) and D2 (ergocalciferol) are the vital compounds.<sup>1</sup> The history of vitamin D and some other vitamin deficiencies are known for centuries. Deficiency of this vitamin causes Rickets, also known as “English Disease” due to

sudden eruption in 17<sup>th</sup> century and was described in detail by F. Glisson in 1650.<sup>2</sup> The biologically inactive vitamin D, which is from skin and diet required enzymatic conversion in kidney and liver for activation. Quantification of 25-hydroxyvitamin D or 25(OH) D in serum confirms individual vitamin D status.<sup>3-4</sup> In the absence of vitamin D, only about 60% of phosphorus and 10–15% of dietary calcium absorb.

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While the adequate serum levels of Vitamin D raise calcium by 30-40% and phosphorus absorption by 80%.<sup>5-6</sup>

Vitamin D2 and D3 can be taken from the diet and supplements. The sources of Vitamin D3 & D2 are different. The richest sources of vitamin D3 are egg yolk, butter, liver, fish oil and dietary supplements whereas plant sources like UV light grown mushrooms, fortified foods & dietary supplements contain vitamin D2.<sup>4-7</sup> Vitamin D3 increase blood levels of vitamin D more effectively than vitamin D2. Many studies revealed that inadequate solar exposure lead to vitamin D deficiency. Insufficient sunlight exposure is primary cause of vitamin D deficiency in adults and in children.<sup>7-9</sup>

Vitamin D plays important role by facilitating the gut to absorb calcium, maintain phosphate and calcium levels at right concentration and support & maintain healthy bone growth. Due to inadequate body levels of vitamin D, bones become brittle & weak and also associated with wide range of other conditions like cardiovascular diseases and malignancy.<sup>10-11</sup> There is protective relationship between low risk of malignancies and adequate serum vitamin D levels. Vitamin D receptors are present in many cell types that can be activated by 1, 25(OH) D. It regulates cell growth and promotes differentiation in normal functioning cells and impedes tumor growth, its invasion and spread to other sites.<sup>9-11</sup>

Vitamin D deficiencies are commonly expressed in all age-groups, geographic regions, and seasons.<sup>12-13</sup> Vitamin D deficiency is common in obese peoples, since their fat cells readily absorb and trap vitamin D due to its fat solubility nature.<sup>14</sup> Children with deficiency of vitamin D suffer from retarded growth and bone disease and in adults, vitamin D deficiency will trigger and worsen osteopenia and osteoporosis and lead to greater the risk of fracture. Patients suffering from frequent bone discomfort along with pains in joints and muscles<sup>15-18</sup>. These patients may be incorrectly diagnosed with musculoskeletal pain, fatigue, and tenderness in localized areas, depression, degenerative joint disease, joint stiffness, and other diseases.<sup>19-20</sup> It is well known that skeletal muscles perform their activity at their peak in the presence of sufficient levels of vitamin D in the body due to presence of vitamin D receptors on skeletal muscle tissues.<sup>21-22</sup>

The short wave ultraviolet rays of sunlight, when reaches the earth surface has striking consequence .During winters and in the early morning and late afternoon, scanty vitamin D<sub>3</sub> synthesis occurs therefore appropriate dietary supplements of vitamin recommended.<sup>23, 24</sup> There is also increase risk of developing vitamin D deficiency / insufficiency in some cultures, where females use the clothing that covers the whole body, including the face , they do not get sufficient sunlight, and is common in adults and children both.<sup>25, 26</sup> In aged people when compare to young adults, there is very low ability to make vitamin D3 due to low D3 precursor 7-dehydrocholesterol, in the aged skin.<sup>27</sup>

The significant role of vitamin D in our body should not be ignored. Since vitamin D deficiency is increasing on wide scale, it is difficult to get sufficient levels only from natural sources so it has to be supplemented through diet.<sup>28</sup>

## Methodology

This cross-sectional study was conducted at Islamabad Diagnostic Centre (IDC), Islamabad from January 2017 to March 2018. Study was approved from the ethical committee of IDC and a total of 721 patients were selected in this study. All these patients were walked in cases and they had no major illness. Blood samples were collected from recruited patients after taking written inform consent in serum separating tubes. Vitamin D3, total calcium, phosphorus, urea, creatinine and uric acid were analyzed on Architect 1000. Vitamin D level was reported as deficient if it was <20 ng/ml, insufficient if it was between 20-30 ng/ml and requiring treatment. Desirable level is >30 ng/ml and levels >110 ng/ml reported as toxic levels. Statistical analysis was analyzed by SPSS version 19.0. Descriptive statistic, ANOVA and bivariate correlation were performed and for this a p-value less than 0.05 was considered as statistically significant.

## Results

Out of 721 recruited patients, there were 374 (51.8%) male and 347 (48%) were female. The mean values of vitamin D, total calcium, phosphorus, urea, creatinine and uric acid were 23.4 ng/ml ( $\pm$  18.40 SD), 9.1 mg/dl ( $\pm$  0.66 SD), 3.4 mg/dl ( $\pm$  0.60 SD), 30.2 mg/dl ( $\pm$  1.4 SD), 0.94 mg/dl ( $\pm$  0.68 SD) and 5.6 mg/dl ( $\pm$  1.5 SD) respectively (Table I).

| Parameter     | Mean | Minimum | Maximum | Standard Deviation(±) |
|---------------|------|---------|---------|-----------------------|
| Vitamin D     | 23.4 | 1.3     | 151.2   | 18.4                  |
| Total Calcium | 9.1  | 7.1     | 11.7    | 0.6                   |
| Phosphorus    | 3.4  | 1.8     | 5.9     | 0.60                  |
| Urea          | 30.2 | 12.0    | 183.0   | 1.4                   |
| Creatinine    | 0.94 | 0.4     | 10.6    | 0.68                  |
| Uric Acid     | 5.6  | 1.7     | 16.1    | 1.5                   |

The overall vitamin D deficiency (Deficient + Insufficient) was seen in 558 (77.4%) study participants while 160 (22.1%) participants having sufficient level. Vitamin D toxicity was seen in only 03 (0.41%) patients (Table 2). The collected data was further categorized on the basis of age groups and vitamin D status, the most common age group was 46-65 year followed by 26-45 and then 66-85 with the frequency of 315 (43.7%), 240 (33.3%) and 125 (17.4%) respectively (Table 2). The vitamin D deficiency was most common in the age group >85 years followed by age group 66-85 year with the prevalence of 8 (100%) and 109 (87.2%) individually (Table II).

The study participants were further categorized into two main groups on the basis of gender, there were 347 (48.1%) male and 374(51.9%) were female patients. It was observed that vitamin D deficiency was more common in female population as compared to male, as shown in table 3 that Vitamin D was deficient in 210 (56.1%) females while 150 (43.2%) male were having deficient vitamin D levels.

Hypervitaminoses noted in only 3 (0.4%) patients and were all males (Table III).

In this study we have Analysis of variance (ANOVA) was done to check whether there is statistical difference between the mean values of vitamin D and age, serum phosphorus, serum total calcium, serum urea, serum creatinine and serum uric acid. It was observed that there is statistical significant difference ( $p < 0.05$ ) in the mean value of all parameters (Table IV).

We also assessed other laboratory parameters (Serum Urea, Creatinine, Uric acid, Total calcium (Ca) and phosphorus ( $PO_4$ ) in blood samples of recruited patients along with vitamin D status, out of 721 patients, 351 (97.5%) cases with normal calcium level had vitamin D deficiency, 194 (98.0%) patients had insufficient levels of vitamin D, and 154 (96.2%) patients had desirable levels, whereas 3 (100%) cases showed toxic levels of vitamin D in their serum. Overall total Ca was normal in 702 (97.4%) cases while only 19 (2.6%) had low serum Ca levels. Whereas, serum phosphate ( $PO_4$ ) levels was found normal in 338 (93.9%) cases with severe vitamin D deficiency, 194 (98.0%) patients with normal levels of  $PO_4$  had insufficient vitamin D, 152 (95.0%) patients with desirable and 3 (100.0%) cases with toxic levels of vitamin D had normal  $PO_4$  levels. Serum  $PO_4$  levels were on lower side only in 24 (3.3%) patients out of 721 (Table V).

Renal function tests (Urea, creatinine and uric acid) were also determined in serum of studied patients. Out of 721 there were only 51 (7.2%) patients had

| Age Groups | Deficient (%) <20.0 | Insufficient (%) 20.1-30 | Sufficient (%) 30.1-110 | Toxic (%) >110 | Total (%)  |
|------------|---------------------|--------------------------|-------------------------|----------------|------------|
| 0-15       | 7 (46.7)            | 4 (26.7)                 | 3 (20.0)                | 1 (6.7)        | 15 (2.0)   |
| 16-25      | 8 (44.4)            | 6 (33.3)                 | 4 (22.2)                | 0 (0.0)        | 18 (2.5)   |
| 26-45      | 110 (45.8)          | 69 (28.8)                | 61 (25.4)               | 0 (0.0)        | 240 (33.3) |
| 46-65      | 139 (44.1)          | 98 (31.1)                | 77 (24.4)               | 1 (0.3)        | 315 (43.7) |
| 66-85      | 88 (70.4)           | 21 (16.8)                | 15 (12.0)               | 1 (0.8)        | 125 (17.4) |
| >85        | 8 (100.0)           | -                        | -                       | -              | 08 (1.1)   |
| Total      | 360 (49.9)          | 198 (27.4)               | 160 (22.1)              | 3 (0.41)       | 721 (100)  |

| Gender | Deficient (%) <20.0 | Insufficient (%) 20.1-30 | Sufficient (%) 30.1-110 | Toxic (%) >110 | Total (%)  |
|--------|---------------------|--------------------------|-------------------------|----------------|------------|
| Male   | 150 (43.2)          | 81 (23.3)                | 113 (32.6)              | 3 (0.9)        | 347 (48.1) |
| Female | 210 (56.1)          | 117 (31.3)               | 47 (12.6)               | 0 (0.0)        | 374 (51.8) |
| Total  | 360 (49.9)          | 198 (27.4)               | 160 (22.1)              | 3 (0.41)       | 721 (100)  |

**Table IV: ANOVA Table**

|               |                | Sum of Squares | Df  | Mean Square | F     | Sig. |
|---------------|----------------|----------------|-----|-------------|-------|------|
| Age           | Between Groups | 111210.187     | 280 | 397.179     | 2.539 | .000 |
|               | Within Groups  | 68821.399      | 440 | 156.412     |       |      |
|               | Total          | 180031.587     | 720 |             |       |      |
| Phosphorus    | Between Groups | 151.051        | 280 | .539        | 2.099 | .000 |
|               | Within Groups  | 113.074        | 440 | .257        |       |      |
|               | Total          | 264.125        | 720 |             |       |      |
| Total Calcium | Between Groups | 221.886        | 280 | .792        | 3.684 | .000 |
|               | Within Groups  | 94.649         | 440 | .215        |       |      |
|               | Total          | 316.535        | 720 |             |       |      |
| Urea          | Between Groups | 83834.289      | 280 | 299.408     | 1.930 | .000 |
|               | Within Groups  | 68273.113      | 440 | 155.166     |       |      |
|               | Total          | 152107.401     | 720 |             |       |      |
| Creatinine    | Between Groups | 161.174        | 280 | .576        | 1.470 | .000 |
|               | Within Groups  | 172.258        | 440 | .391        |       |      |
|               | Total          | 333.432        | 720 |             |       |      |
| Uric Acid     | Between Groups | 993.889        | 280 | 3.550       | 2.542 | .000 |
|               | Within Groups  | 614.371        | 440 | 1.396       |       |      |
|               | Total          | 1608.260       | 720 |             |       |      |

| Vitamin D Status         | Calcium <8.5 mg/dl Low n(%) | Calcium 8.6- 10.5 mg/dl Normal n(%) | PO <sub>4</sub> <2.5 mg/dl Low n(%) | PO <sub>4</sub> 2.5-4.5 mg/dl Normal n(%) | PO <sub>4</sub> >4.6 mg/dl High n(%) | Urea <45.0 mg/dl Normal n(%) | Urea >46.0 mg/dl High n(%) | Creatinine <1.1 mg/dl Normal n(%) | Creatinine >1.2 mg/dl High n(%) | Uric acid < 7.0 mg/dl Normal n(%) | Uric acid > 7.1 mg/dl High n(%) |
|--------------------------|-----------------------------|-------------------------------------|-------------------------------------|---|--------------------------------------|------------------------------|----------------------------|-----------------------------------|---------------------------------|-----------------------------------|---------------------------------|
| Deficient <20 ng/ml      | 09(2.5)                     | 351(97.5)                           | 14 (3.7)                            | 338(93.9)                                 | 08 (2.2)                             | 329(91.4)                    | 31 (8.6)                   | 338 (93.9)                        | 22(6.1)                         | 305(84.7)                         | 55(15.3)                        |
| Insufficient 21-30 ng/ml | 4 (2.0)                     | 194 (98.0)                          | 9(4.5)                              | 184(92.9)                                 | 5(2.5)                               | 188(94.9)                    | 10(5.1)                    | 195 (98.5)                        | 03(1.5)                         | 174 (87.9)                        | 24(12.1)                        |
| Sufficient 31-110 ng/ml  | 6(3.8)                      | 154 (96.2)                          | 01(0.6)                             | 152(95.0)                                 | 07 (4.4)                             | 150(93.8)                    | 10(6.2)                    | 153 (95.6)                        | 07 (4.4)                        | 141 (88.1)                        | 19(11.9)                        |
| Toxic >110 ng/ml         | --                          | 3 (100.0)                           | -                                   | 3 (100.0)                                 | -                                    | 03(100)                      | -                          | 03(100)                           | -                               | 2(66.7)                           | 01(33.3)                        |
| Total (%)                | 19(2.6)                     | 702 (97.4)                          | 24 (3.3)                            | 677(93.9)                                 | 20 (2.8)                             | 670 (93.0)                   | 51 (7.0)                   | 689 (95.6)                        | 32 (4.4)                        | 622 (86.3)                        | 99 (13.7)                       |
| P Value                  | NS                          |                                     | NS                                  |   |                                      | NS                           |                            | NS                                |                                 | NS                                |                                 |

NS= Non Significant

high urea levels, 32 (4.4%) with high serum creatinine levels while high uric acid was seen in 99 (13.7%) patients. Raised urea level was observed in total 31 (8.6%) and 10 (5.1%) cases with vitamin D deficiency and insufficiency status respectively. Serum creatinine was found elevated in only 22 (6.1%) vitamin D deficient people. Uric acid was elevated in 55 (15.3%) study participants with vitamin D deficient status (Table V). We also tried to find out correlation with vitamin D status and estimated laboratory parameters and it was found that there is no significant relationship ( $p < 0.05$ ) of laboratory parameters with any status of vitamin D. (Table V)

## Discussion

Vitamin D plays important role in calcium homeostasis, also significantly involve in regulation of cellular differentiation and proliferation, immune functions, reproductive health and lowering the risk of large numbers of chronic illnesses like numerous infectious diseases, cardiovascular disorders, various malignancies and autoimmune diseases<sup>29-30</sup>. It was reported by many studies from all over the world that vitamin D deficiency is very common in all age groups and becoming one of the major public health concerns<sup>30</sup>. It is therefore, the assessment of vitamin D status in different age groups and gender is needed.

In present study, the status of vitamin D, calcium, phosphorus, urea, creatinine and uric acid were

measured in 721 blood samples of both genders. It was observed in our population that vitamin D was deficient in 49.9% (n=360) population while the 27.4% (n=198) having insufficient vitamin D levels. Comparatively vitamin D deficiency was more commonly observed in elder population and in females as seen in table 2 and 3. In our test population, Vitamin D deficiency was seen in 56.1% (n=210) females while 31.3% (n=117) females were having insufficient vitamin D levels. This showed that females are more deficient to vitamin D than males. However there is no statistical difference was seen in vitamin D deficiency between men and women. Hypervitaminoses noted in total 3 (1.0%) patients and were all males. Sun light is one of the main sources of vitamin D. This study was conducted in twin cities (Islamabad/ Rawalpindi), Islamabad is capital city of Pakistan, although twin cities are shiny cities of Pakistan but direct exposure to sun light is limited especially in female due to wearing of Hijab/ Burkah, full body covered clothes and use of sun blocks due to fear of skin cancer, while the male population

especially working in government and multinational companies are also use to wear long sleeves shirts. Trend of living in full covered houses and in apartments is also increasing. Due to above mentioned reasons vitamin D deficiency is more common in our population.

Our reported results are comparable with previous studies conducted in different cities and neighboring countries of Pakistan. Riaz *et al* conducted a larger study of 4830 participants and reported similar findings that vitamin D was deficient in 53.5% population while 31.20% had insufficient vitamin D levels. Our results are also related to past studies reported from same geographical region<sup>31</sup>. Chaudhary B *et al* gathered the data of 5693 adult patients belongs to Rawalpindi Islamabad and they had reported that 56.5% patients had vitamin D deficient status while 18.2% had insufficient level<sup>32</sup>. Khan H *et al* also reported vitamin D deficiency in 56.2% individuals<sup>33</sup>. Another national study showed 53.5% had Vitamin D deficiency, 31.2% had insufficient Vitamin D and only 15.3% normal Vitamin D<sup>31</sup>. Similar findings were also reported from a study conducted on 1,111 healthy individuals in our neighbor country Iran, they reported vitamin D

deficiency and insufficiency 50.8% and 19.6% individually<sup>34</sup>.

Our age and gender based results are also comparable with previous studies. In present study, vitamin D deficiency was most common in elder age group while many previous studies also reported same findings<sup>35-37</sup>. According to our reported results vitamin D deficiency and insufficiency were 56.1% and 31.3% respectively while Vitamin D deficiency and insufficiency prevalence reported by Riaz H *et al* was 58.9% and 30.5% respectively<sup>31</sup>, Naqvi KZ *et al* reported 69.6% deficiency in pregnant women<sup>38</sup>.

In this study we evaluated renal function tests (Urea, creatinine and uric acid), serum calcium and serum phosphorus along with vitamin D in studied patients and tried to find out their relationship with age and gender. It was observed that there is no significant relationship ( $p>0.05$ ) of vitamin D status with age and gender. Different national and international studies also reported that there was no significant correlation of serum calcium and phosphorus with any status of vitamin D<sup>39, 40</sup>. We found that renal function tests, serum calcium and phosphorus was normal in vitamin D deficient people (both male and female) of all age group.

## Conclusion

Vitamin D deficiency in Pakistan is becoming endemic and it is much predominant in elder and female population. Based on our finding renal function tests, serum calcium and phosphorus levels are not the conjecturer of vitamin D deficiency. There is a general agreement that ideal serum vitamin D concentrations should be greater than 40 ng/mL. Judicious exposure to sunlight appears to be the best way to increase the vitamin D concentrations. But when solar exposure is not possible particularly in winter season, intake of 1000-5000 IU/D vitamin D3 is good alternative. For the maintenance of adequate serum vitamin D levels, regular and sufficient amount of foods rich in vitamin D should be taken and spend some time in the sun. But vitamin D3 is probably the best choice if someone takes supplements.

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