

## ORIGINAL ARTICLE

## VITAMIN D STATUS AMONG VEGETARIANS AND NON-VEGETARIANS

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**Background:** Vitamin D helps in maintaining bone and muscle health. Vitamin D deficiency is a global problem, with the prevalence of deficiency being widespread in Pakistan. The present study was planned to associate serum vitamin D, Parathyroid Hormone (PTH), calcium and phosphate concentrations with dietary habits of individuals living in urban/rural areas of Sindh, Pakistan. **Methods:** This was a cross-sectional study at two locations, urban (Karachi) and rural (Haji Goth of Shadadpur city). The study period was May–October 2012 on 176 healthy subjects, aged 20–80 years. Venous blood was collected for analysis of vitamin D, PTH, calcium and phosphorus. Statistical analysis was done using SPSS-18. Statistical differences between variables were determined by student's *t*-test and  $p < 0.05$  was considered significant. **Results:** The 176 subjects were divided into non-vegetarians (93, 52.84%) and vegetarians (83, 47.16%) with each group subdivided into urban and rural. The BMI of non-vegetarians vs vegetarians was high ( $p < 0.001$ ). Vitamin D in non-vegetarians vs vegetarians was low ( $p < 0.001$ ). The vegetarians of urban compared to rural had low vitamin D ( $p < 0.05$ ). The PTH of non-vegetarians vs vegetarians was significantly high ( $p < 0.001$ ). Serum calcium was significantly low ( $p < 0.05$ ) in urban and rural subjects on either diet. **Conclusion:** Non-vegetarians had severe vitamin D deficiency, while vegetarians had vitamin D insufficiency irrespective of belonging to urban or rural area.

**Keywords:** Vitamin D, deficiency, insufficiency, non-vegetarians, vegetarians

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## INTRODUCTION

Vitamin D is a fat soluble vitamin that occurs in 2 forms: vitamin D<sub>2</sub> (ergocalciferol) and vitamin D<sub>3</sub> (cholecalciferol). Vitamin D<sub>3</sub> is obtained from food of animal origin and from ultraviolet light-stimulated conversion of 7-dehydrocholesterol in the skin.<sup>1</sup> In plants vitamin D<sub>2</sub> is the product of ultraviolet B (UVB) (290–315 nm) irradiation of ergosterol.<sup>2</sup> Sea foods (fishes like mackerel, salmon, and herring) contain a good amount of vitamin D.<sup>1</sup> Vitamin D can be consumed by humans as a supplement or in fortified foods like milk, soya, and cereals available in the market to fulfil vitamin D daily requirement.

Cholecalciferol is converted in the liver by the enzyme 25  $\alpha$ -hydroxylase to 25 (OH) D, which is the major circulating metabolite of vitamin D. In the kidney 25 (OH) D is converted by 1 $\alpha$ -hydroxylase to its active form, 1, 25 dihydroxy vitamin D [1, 25 (OH)<sub>2</sub> D] which is known as calcitriol or Vitamin D<sub>3</sub>.<sup>3</sup> Vitamin D plays a vital role in maintaining bone and muscle health by regulating calcium metabolism.<sup>4</sup> Deficiency of vitamin D causes an increase in parathyroid hormone.<sup>5</sup> Vitamin D with Parathyroid Hormone (PTH) regulates serum calcium levels by increasing gastrointestinal calcium absorption and bone resorption.<sup>4</sup> Vitamin D deficiency is a highly prevalent condition, present in approximately 30–50% of the general population. The most common cause of vitamin D deficiencies could be due to inadequate dietary intake and/or sun exposure.<sup>1</sup>

Vitamin D deficiency in children will cause growth retardation and classic sign and symptoms of

rickets.<sup>6</sup> In adults its deficiency causes skeletal mineralization defect associated with proximal muscle weakness, increase in body sway with an increased risk of falling and fracture due to osteopenia and osteoporosis.<sup>7</sup> Vitamin D is stored in fatty tissues and the large storage capacity in obese people leads to low circulating level of vitamin D.<sup>8</sup> Metabolism, storage and action of vitamin D is all influenced by body fat. Obesity is therefore associated with vitamin D deficiency.<sup>9</sup>

As reported by Hovsepian *et al*, 30–50% of both children and adults in United States suffer from vitamin D deficiency and also the rate of vitamin D deficiency is high in the tropical countries.<sup>10</sup> Vitamin D deficiency is also widespread in Pakistan; in Sindh alone 90.5% women are vitamin D deficient.<sup>11</sup> National Nutrition Survey (NNS) in 2011 reported that hypocalcaemia is epidemic in Pakistan, 51.9% of urban and 50.8% of rural population is affected.<sup>12</sup> Following modern human culture there is an increase in indoor lifestyle strategies which causes a decrease in cutaneous production of vitamin D.<sup>13</sup> Monitoring serum 25-hydroxyvitamin D levels and treating vitamin D deficiency is required for maintenance of musculoskeletal and general health.<sup>5</sup>

The present study was planned to associate serum vitamin D, PTH, calcium, and phosphate concentrations with the dietary habits of individuals living in urban and rural areas of Sindh, Pakistan.

## MATERIAL AND METHODS

A randomised cross-sectional study on two locations; urban area (Karachi) and rural area (Haji Goth of

Shadadpur) was selected. Apparently healthy subjects (176) consisted of males (n=89) and females (n=87) were included in the study with age range 20–80 years. The study period was from May–October 2012. Non-probability purposive sampling technique was used for data collection. A standardised questionnaire was used to collect demographical data such as age, sex, height and body weight were measured while wearing light weight clothing, but not shoes. The body mass index (BMI) was calculated as weight (Kg) divided by height (m) squared.<sup>14</sup> Dietary pattern was recorded as vegetarian or non-vegetarian. Patient with renal disease, diabetes mellitus, metabolic bone disease, mal-absorption, pregnancy immobility for more than one week, and any medication affecting bone metabolism were excluded.

Intravenous Blood samples were collected at the spot in vacutainers without anti-clotting agents and centrifuged within one hour of collection. The serum was collected in sample bottles and stored at -20 °C till analysis. Serum levels of calcium, phosphate, 25-Hydroxy vitamin D [25(OH) D] and parathyroid hormone (PTH) were measured.

Data were analysed using SPSS-18. Data were presented as Mean±SD for quantitative variables. The statistical difference between variables was determined using student's *t*-test. Differences were considered statistically significant with *p*<0.05.

## RESULTS

In study population of 176 subjects, there were 89 (50.57%) males and 87 (49.43%) females. Table-1 shows percentage of Total vegetarians and non-vegetarians with percentages of male and female vegetarians and non-vegetarians residing in urban and rural areas of Sind. BMI, Vit D, PTH, Calcium, and Phosphorus were compared in Vegetarians and Non vegetarians with respect to the samples taken from Urban and Rural areas using student *t*-test and *p*≤0.05, was considered as significant.

Table-2 shows comparison of vegetarians and non vegetarians for BMI, Vitamin D, PTH, Calcium, and Phosphorus and a significant mean difference was observed in BMI, Vitamin D and PTH in the two groups (*t* = -2.23, *df*=17; *t* = -9.77, *df*=17; and *t* = -6.74, *df*=17 respectively) with *p*<0.05 and *p*<0.001. Calcium and Phosphorus had no significant mean differences between two groups.

Table-3 shows comparison between vegetarians belonging to urban and rural areas for BMI, Calcium, Vitamin D, and Phosphorus (*t*=2.09, *df*=81; *t*= -2.44, *df*=81; *t*= -2.71, *df*=81; and *t*= -3.25, *df*=81) with *p*<0.05 and *p*<0.01 respectively. PTH shows no significant differences between the two groups.

Table-4 shows comparison among urban and rural non-vegetarians the differences in BMI, PTH and

Calcium (*t* = -6.69, *df*=91; *t* = -4.52, *df*=91; and *t*=4.37, *df*=91 respectively) with *p*<0.001 were significant. Differences for Vit D (*t*=2.48, *df*=91) with *p*<0.05 were significant. Serum Phosphorus level had no significant difference between the two groups.

**Table-1: Vegetarians and non-vegetarians from urban and rural areas of Sindh [n (%)]**

Diet	Area	Male	Female	Total
Vegetarian [83 (47.16%)]	Urban	24 (13.63)	16 (9.09)	40 (22.07)
	Rural	24 (13.63)	19 (10.07)	43 (24.04)
Non-vegetarian [93 (52.84%)]	Urban	16 (9.09)	32 (18.18)	48 (27.02)
	Rural	30 (17.04)	15 (8.52)	45 (25.05)

**Table-2: Comparison of variables in vegetarians and non-vegetarians**

Parameters	Vegetarians (n=83)	Non-Vegetarians (n=93)	<i>p</i>
BMI	20.43±2.72	21.24±2.03	0.027
25 (OH)D (ng/ml)	13.78±3.48	9.39±2.45	0.000
PTH (pg/ml)	84.39±26.27	113.09±29.61	0.000
Calcium (mg/dl)	6.36±1.42	6.15±1.47	0.329
Phosphorus (mg/dl)	5.58±1.51	5.74±1.06	0.400

**Table-3: Comparison of variables in urban and rural vegetarians**

Parameters	Rural (n=43)	Urban (n=40)	<i>p</i>
BMI	19.81±2.38	21.05±2.95	0.039
25 (OH)D (ng/ml)	14.58±3.08	12.62±3.49	0.008
PTH (pg/ml)	82.42±26.96	87.60±26.16	0.378
Calcium (mg/dl)	6.72±1.28	5.98±1.48	0.017
Phosphorus (mg/dl)	6.07±1.82	5.05±0.84	0.002

**Table-4: Comparison of variables in urban and rural non-vegetarians**

Parameters	Rural (n=45)	Urban (n=48)	<i>p</i>
BMI	19.80±1.88	22.19±.55	0.000
25 (OH)D (ng/ml)	10.02±2.79	8.79±1.93	0.015
PTH (pg/ml)	100.07±27.42	125.29±26.41	0.000
Calcium (mg/dl)	6.78±1.57	5.56±1.09	0.000
Phosphorus (mg/dl)	5.53±1.16	5.94±0.93	0.066

## DISCUSSION

Vitamin D deficiency or insufficiency is becoming more common in developed countries. In developing countries the prevalence of vitamin D deficiency is on increase.<sup>15</sup> Prolonged Vitamin D deficiency leads to osteomalacia, while insufficiency is associated with non-specific symptoms.<sup>16</sup>

Is body weight or body mass index (BMI) related to serum vitamin D levels? A study reported that high BMI leads to low vitamin D, while low vitamin D causing an increase in BMI is not possible.<sup>17</sup> In the present study comparing BMI in non-vegetarians population vs vegetarians *p*<0.05 was observed. It was observed that non-vegetarians BMI belonging to either urban or rural had comparatively higher mean values compared to vegetarians (*p*<0.001). This could mean that BMI depends upon dietary habits and life style of an individual. When we compared BMI with serum vitamin D levels no statistical significant correlations

was found, which is in agreement with an Iranian study.<sup>18</sup> Consumption of fast food reported by non-vegetarians may be a cause of the increase in BMI. Based on the values given for BMI<sup>19</sup>, the mean values calculated for vegetarians and non-vegetarians (urban and rural) in our study were within the normal range.

Vitamin D values 15–29 ng/ml suggest insufficiency, <15 ng/ml suggest deficiency, below 10 ng/ml indicate severe deficiency, while values at or above 30 ng/ml indicate sufficient vitamin D.<sup>20</sup> The present study utilised these values as markers of optimal levels of vitamin D.

In non-vegetarians a significantly low  $p < 0.001$  vitamin D level was observed compared to vegetarians. Similar results were observed in urban non-vegetarians having low vitamin D levels compared to rural non-vegetarians ( $p < 0.05$ ). Among urban vegetarians a significantly low vitamin D was seen compared to rural one ( $p < 0.001$ ). The urban vegetarian population had lower mean values of vitamin D compared to rural; the findings could be due to dietary variation of urban population having more fried food with less intake of milk, and less exposure to sunlight. The present results show that in urban population, vitamin D was severely deficient in non-vegetarians while deficient in vegetarians. Vitamin D insufficiency was prevailing in vegetarians, and deficiency existed in non-vegetarians irrespective of region.

Though vitamin D is fat soluble and dietary fat enhances its absorption our results suggest that the fat or meat in the diet creates hindrance in its absorption. Red meat is a valuable source of vitamin D, and the vitamin is in absorbable form, but according to Mawer and Davies, meat causes mal-absorption of vitamin D by some mechanism so far unknown.<sup>21</sup> The role of meat in the diet needs exploration.

Malabsorption of vitamin D impairs its utilisation and in some malabsorptive states, calcium malabsorption is the cause of hyperparathyroidism.<sup>22</sup> Vitamin D deficiency causes secondary hyperparathyroidism and bone loss.<sup>22</sup> Normal serum level of PTH is 16–46 pg/ml.<sup>23</sup> Our study observed that PTH levels among non-vegetarians were higher than vegetarians with significant differences ( $p < 0.001$ ). When comparing PTH levels between non-vegetarians of urban population vs rural a significant difference ( $p < 0.001$ ) existed. Our results show low vitamin D with high PTH in all non-vegetarians and vegetarians, irrespective of whether the subject is residing in urban or rural areas. The present study is in agreement with previous studies that have shown an inverse relationship between vitamin D and parathyroid hormone.<sup>22</sup>

Vitamin D increases the level of calcium in blood by 3 routes: Increasing the absorption of calcium from gut, increasing renal tubular re-absorption of calcium, and increasing release of calcium from bone.<sup>24</sup>

Normal serum calcium levels are 9–11 mg/dl, and normal phosphorus level is 2.7–4.5 mg/dl.<sup>27</sup>

The urban vegetarians had low mean values of calcium and phosphorus as compared to rural. A significant difference ( $p < 0.05$ ) for calcium and ( $p < 0.05$ ) for phosphorus was observed. Among the non-vegetarians of urban vs rural a significant difference ( $p < 0.001$ ) was observed. Serum calcium levels were low in both urban and rural population on either diet (vegetarian and non-vegetarian). Our study results could be attributed to fewer intakes of milk and dairy products among non-vegetarians.

A study carried out in South Indian vegetarians reported inadequate dietary calcium intake associated with high dietary phytate/calcium ratio which reduces the bioavailability of calcium in the gut. Thus high intake of phytate leads to low serum calcium and vitamin D levels.<sup>26</sup> Phytic acid is not lost on cooking and causes chelation of calcium and also inhibits intestinal absorption of calcium and vitamin D.<sup>27</sup>

We also observed deficiency of vitamin D and calcium in vegetarians, which could be due to high content of phytic acid in beans, nuts, fibre, and whole-grain foods, wheat, bran, rice bran, corn, rye, oats, brown rice, potatoes, and green leafy vegetables. Also there could be more consumption of potatoes in any form. Potatoes baked, boiled, micro-waved or fried retain virtually all of their phytate content which hinders calcium absorption.<sup>28</sup> Phytic acid can only be lost if beans are soaked and cooked for a long period of time.<sup>29</sup> Our subjects did not go through the lengthy process of soaking and cooking for very long period of time. Our study suggests that serum calcium and phosphorus do not exactly predict vitamin D deficiency and therefore serum calcium and phosphorus are poor markers of vitamin D levels while serum 25 (OH) D and PTH are better markers.

## CONCLUSION

Non-vegetarians had vitamin D deficiency, while vegetarians had vitamin D insufficiency irrespective of area (urban or rural). Large scale studies are needed to evaluate vitamin D status depending on dietary habits in our urban and rural population.

## RECOMMENDATIONS

We suggest that foods should be fortified with vitamin D and calcium according to recommended daily dietary allowance (RDA) as per Pakistan Council of Medical Research (PCMR).

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