

**Do Vitamin D Levels Correlate To Body Mass Index and Insulin Resistance in Urban Indian Females**

<sup>1</sup>Hatolkar V.S., Professor, Department of Biochemistry, JIIUs IIMSR, Warudi, Badnapur

<sup>2</sup>Shaikh Shaista Parveen, Associate Professor, Department of Biochemistry, JIIUs IIMSR, Warudi, Badnapur

<sup>3</sup>Afshan Kausar, Associate Professor, Department of Physiology, JIIUs IIMSR, Warudi, Badnapur

<sup>4</sup>Hazari Nirmala, Associate Professor, Department of Biochemistry, GMC, Aurangabad

**Corresponding Author:** Hazari Nirmala, Associate Professor, Department of Biochemistry, GMC, Aurangabad

**Citation this Article:** Hatolkar V.S., Shaikh Shaista Parveen, Afshan Kausar, Hazari Nirmala, “Do Vitamin D Levels Correlate To Body Mass Index and Insulin Resistance in Urban Indian Females”, IJMSIR- August - 2022, Vol – 7, Issue - 4, P. No. 128 – 135.

**Type of Publication:** Original Research Article

**Conflicts of Interest:** Nil

**Abstract**

**Background:** Vitamin D deficiency found rampant all throughout the Indian subcontinent; has been linked with various disorders like hypertension, diabetes, obesity and high triglyceride levels ultimately leading to increased risk of cardiovascular mortality.

**Objective:** To examine the concentration of 25-hydroxyvitamin D (25[OH]D) in apparently healthy females of age group 40-60 years and its association with BMI, blood sugar levels, serum insulin and insulin resistance.

**Methodology:** This was an observational cross sectional study. About 250 apparently healthy females were evaluated for anthropometric (height, weight, B.M.I.) and biochemical (fasting insulin and glucose, 25[OH]D, and HOMA-IR) parameters. Appropriate statistical analysis was done to find the vitamin D, obesity status amongst study participants and comparison of biochemical parameters between obese and non-obese groups. Correlation analysis was done to find relationship of vitamin D with B.M.I, blood sugar, serum insulin and

HOMA-IR. Results: Our study demonstrated that 11% participants were found to be vitamin D sufficient, 73% were insufficient and 16 % were deficient. About 64.6% were found to be obese. Vitamin D status comparison between obese and non-obese group by Chi square test showed a significant difference ( $p < 0.0001$ ). 25(OH)D levels showed significant negative correlation with BMI ( $r = -0.658$ ), BSL ( $-0.432$ ), Insulin ( $-0.24483$ ) and HOMA IR ( $-0.276$ ). B.M.I., vitamin D, serum insulin and HOMA- IR showed significant difference between obese and non-obese groups.

**Conclusions:** Vitamin D deficiency is widespread among urban Indian females and it is significantly associated with obesity, fasting blood sugar levels and insulin resistance

**Keywords:** Anthropometric parameter, Insulin resistance, Vitamin D, obesity.

**Introduction**

Vitamin D deficiency has been documented as one of the major health problems worldwide. India, located between latitude of 8.40 -N and 37.60 N, receives ample sunlight

and ultraviolet B radiation in all seasons. Due to this reason, it has been assumed that levels of vitamin D are adequate in Indians.<sup>1</sup> But on contrary vitamin D deficiency is found rampant all throughout the Indian subcontinent, prevalence in the general population being 70%–100%.<sup>2</sup> The social taboos and cultures affect lifestyle of the people in India. Most of them are vegetarians while the others are poor and deprived, affecting the nutritional status thus leading to deficiency vitamins and other nutrients. In addition, most Indians are unaware about the available vitamin D supplements and their requisite intake.<sup>3</sup> Especially, Indian women are more likely to be vitamin D deficient as majority of them are confined to household work. Also they have high coverage of body parts with clothing, thus limiting their exposure to adequate sunlight.<sup>4</sup>

Serum levels of 25-hydroxyvitamin D [25(OH)D] are revealed as the best markers of total vitamin D stores in body, reflecting exposure to ultraviolet radiation and the total dietary intake.<sup>5</sup> Limiting values have been suggested for evaluation of vitamin D status. If vitamin D is  $\leq 20$  ng/ml, it is referred as “deficiency”, if it is in range of 21-29 ng/ml “insufficiency”, and if the level  $\geq 30$  ng/ml it is “sufficiency”. If 25-OH vitamin D level exceeds 150 ng/ml, it is known as intoxication.<sup>6</sup> Vitamin D is the undiagnosed and untreated nutritional deficiency in India. Vitamin D deficiency has been linked with various disorders like hypertension, diabetes, obesity and high triglyceride level ultimately leading to increased risk of cardiovascular mortality.<sup>7</sup>

A number of studies have revealed an inverse correlation between vitamin D levels and body mass index (BMI)<sup>8,9</sup>. Abdominal obesity which is enormously prevalent in Indians is alleged to be a significant factor

leading to insulin resistance and T2DM. Industrialization and urbanization have caused significant changes in lifestyles of people. The abundance, cheapness, and easy accessibility of food ingredients has caused excessive calorie intake. On the other hand decrease in physical activity results in less energy expenditure.<sup>10</sup> The mechanisms causing decreased vitamin D levels in obese are not fully understood, but probably being a fat soluble vitamin, it gets sequestered in fat compartments decreasing its bioavailability.<sup>11</sup> Another reason is that obese individuals due to sedentary life have reduced amount of sun exposure.<sup>12</sup>

Obesity and insulin resistance are most important and modifiable risk factors for diabetes. Moreover, a novel association between IR and vitamin D deficiency has been proposed. The prevalence of type 2 diabetes mellitus (type 2 DM) is rising at an alarming rate in India. Defects in pancreatic  $\beta$ -cell function, insulin sensitivity, and systemic inflammation all contribute to the development of type 2 DM. Vitamin D has been implicated with in vivo and in vitro effects on insulin sensitivity.<sup>13</sup> Several studies have proposed an inverse association of vitamin D levels with insulin resistance.<sup>14</sup> A possible explanation is that  $\beta$  cells of pancreas express vitamin D receptors which can indirectly influence calcium entry and thus insulin secretion. So vitamin D level plays an important role in glucose metabolism and regulation.<sup>15</sup> Low levels of serum 25(OH)D, and raised parathyroid hormone (PTH) can raise calcium in adipocytes which can stimulate lipogenesis predisposing a patient for obesity and increasing the risk of diabetes.<sup>16</sup> The question remains as to what the health consequences of these reduced 25D levels are, especially in obesity with dysfunctional adipose tissue and insulin resistance.

In growing pandemic of metabolic syndrome, identifying the exact prevalence of Vitamin D deficiency in females can be one of the modifiable risk factors in them before the progression to obesity and diabetes mellitus. In the above context, this cross-sectional study was undertaken with the objective to estimate Vitamin D status and correlate it with anthropometric measure of obesity (BMI), fasting blood sugar, serum insulin and insulin resistance (HOMA IR) among apparently healthy females (40-60 years age group) of non-manual employment group (confined to house hold work) from Aurangabad, Maharashtra.

#### Material And Methods

The study participants comprised of 250 apparently healthy females from Aurangabad, Maharashtra in the age group of 40-60 years. All the study participants were housewives, involved in indoor house hold work with minimal sun exposure. This cross-sectional study was conducted from January 2019 to May 2019. The participants of study were informed about the protocol and written consent was taken. Females with chronic diseases, known case of diabetes mellitus, pregnant and lactating women, those taking drugs for obesity, Vitamin D or calcium supplementations for last 6 months, were excluded. A proper history was taken and inclusion-exclusion criteria precisely looked over.

**Anthropometric Measurements:** Weight was measured with an accuracy of 0.1 kg using a weighing machine. Height was measured using a wall stadiometer to the nearest 1 mm without shoes. The formula for calculation of BMI is, weight (in kg)/height (in m<sup>2</sup>). Those with BMI  $\geq 25$  kg/m<sup>2</sup> were categorized as obese while  $< 25$  as non-obese.<sup>17</sup>

**Biochemical Parameters:** Following overnight fasting of minimum 8 hours venous blood was collected from all participants in plain bulb (2 ml) and fluoride bulb (1 ml).

Serum was separated for analyzing insulin, and 25(OH) D levels and glucose levels. The serum level of 25(OH) D was measured by fully automated Chemo Luminescent Immuno Assay (CLIA). Vitamin D deficiency was defined as 25(OH)D  $\leq 20$  ng/ml and level of 21-29 ng/ml was insufficient while  $\geq 30$  ng/ml was considered optimal. Plasma glucose was analyzed by glucose oxidase-peroxidase method on fully auto analyzer. Serum insulin was measured using Chemo Luminescent Immuno Assay. Homeostatic model assessment (HOMA-IR) was calculated as (Fasting Glucose  $\times$  Insulin)/405 (Insulin in  $\mu$ IU/ml and glucose in mg/dl).<sup>18</sup>

#### Statistical Analysis

Vitamin D and obesity status was assessed amongst study participants as percentiles. Quantitative variables were expressed as mean  $\pm$  standard deviation. To study whether there is any significant difference in average levels of the various parameters of study between obese and non-obese group, we used student t-test. Pearson correlation coefficient of vitamin D with BMI, Insulin, HOMA & BSL was computed. A significant level of 95% was taken for all tests ( $p < 0.05$ ).

#### Observation And Results

A total of 250 females were enrolled in the study after reviewing inclusion-exclusion criteria. Based on vitamin D status, the individuals were divided into three groups, viz.: (1) Vitamin D sufficiency: (25(OH) D  $\geq 30$  ng/ml), (2) vitamin D insufficiency: (25(OH) D 21-29 ng/ml), (3) vitamin D deficiency: (25(OH) D  $\leq 20$  ng/ml). Amongst the study participants approximately 11% (n=27) were found to be vitamin D sufficient, 73% (n=183) were found to be vitamin D insufficient and 16% (n=40) were vitamin D deficient. (figure1). Amongst the study participants, 86 (34.4%) were found to be non-obese

(BMI < 25 kg/m<sup>2</sup>) and remaining 164 (65.6%) were obese with BMI ≥ 25 kg/m<sup>2</sup>. (figure 2)

Vitamin D status was compared between obese and non-obese group and Chi square test showed a significant difference (p < 0.0001). In obese group 32 females were vitamin D deficient, 131 were insufficient and only 1 was found to be sufficient. While in non-obese group 8, 51 and 27 females were deficient, insufficient and sufficient respectively. (Table 1, figure 3).

The participants were in the age group of 40-60 years and the mean age was 49.10 ± 10.01 years. The mean and standard deviation of quantitative variables is shown in table 2. Analysis of the data by Pearson correlation revealed that serum level of 25(OH)D was having significant negative correlation with BMI (r = -0.658), BSL (-0.432), Insulin (-0.24483) and HOMA IR (-0.276) as shown in table 3

Table 4 shows comparison of various parameters between obese and non-obese groups. There was no significant difference in age between the two groups. BMI and Vitamin D levels showed significant difference between obese and non-obese (P-value < 0.0001). Significant difference was found in serum insulin levels and HOMA-IR but blood sugar levels did not vary significantly between the two groups.

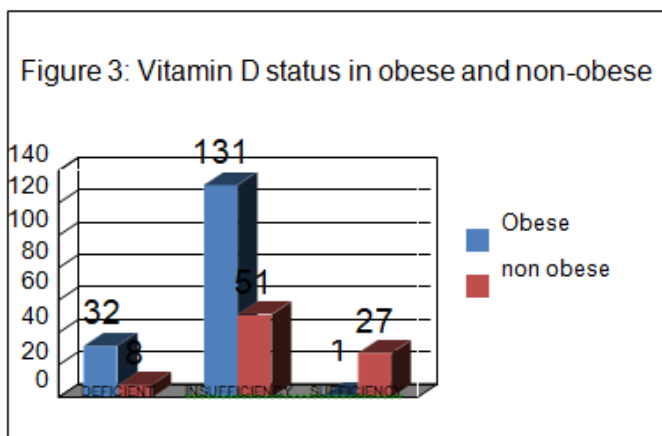
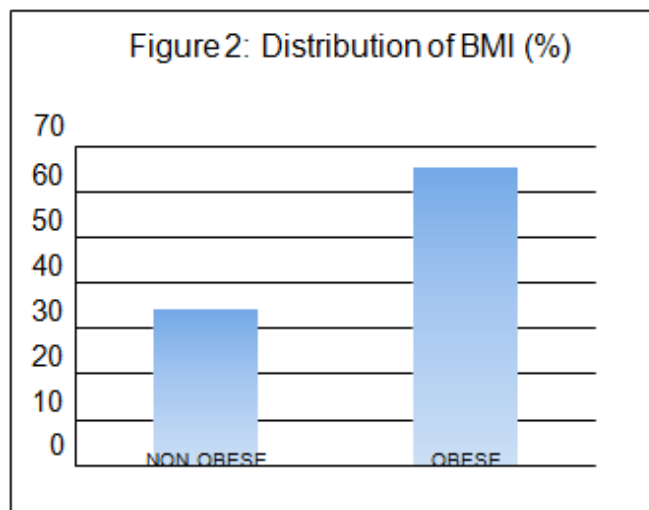


Table 1: Chi square test of vitamin D status among obese and non-obese

Vitamin D status	Obese	Non obese	P value
DEFICIENT	32	8	0.0001
INSUFFICIENCY	131	51	
SUFFICIENCY	1	27	

Table 2 : Mean and standard deviations of quantitative variables

Parameters	MEAN	Standard deviation SD
Serum vitamin D (ng/ml)	29.655	6.82358
BMI (kg/m <sup>2</sup> )	23.325	4.065864
Blood sugar (mg/dl)	86.5	23.33452
Serum insulin (μIU/ml)	16.44	6.272037
HOMA-IR	3.69	2.286504

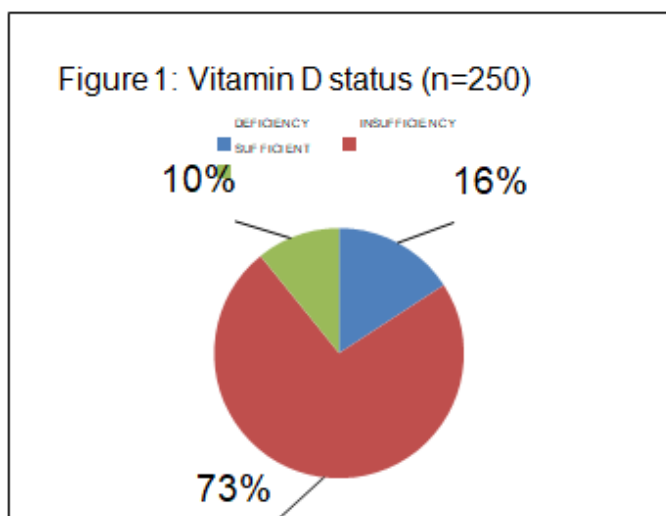


Table 3: Pearson correlation of vitamin D with BMI BSL Insulin, HOMA.

Parameter	R value
BMI	-0.658
BSL	-0.432
Insulin	-0.24483
HOMA	-0.276

Table 4 : Comparison of anthropometric and biochemical variables between obese and non-obese group

Characteristics	Non-obese (n=86)	Obese (n=164)	P-value
Age	48.01 ± 12.44	46.19 ± 9.05	0.18
BMI	23.14 ± 1.48	30.06 ± 3.20	<0.0001*
Vitamin D	29.65 ± 6.62	24.65 ± 4.53	<0.0001*
Insulin	14.59 ± 3.04	16.45 ± 7.55	0.02*
BSL	96.0 ± 9.26	100.80 ± 28.29	0.2
HOMA-IR	3.06 ± 0.94	5.48 ± 2.80	<0.0001*

**Discussion**

Vitamin D insufficiency is increasingly being recognized in India as well as globally, affecting majority of the individuals regardless of their gender, age, race, and geography. Owing to its diverse effects on health, the extensive vitamin D deficiency in India will considerably add to the huge burden on the healthcare system.<sup>19</sup> In the present study, approximately 11% (n=27) females were found to be vitamin D sufficient, 73% (n=183) were found to be vitamin D insufficient and 16 % (n=40) were vitamin D deficient. The very high prevalence of vitamin D deficiency and insufficiency (89%) is consistent with previous studies. Sahu et al. reported Vitamin D deficiency in 88.6% adolescent female in rural area of North India, they stated that urban females are more prone as compared to their rural counterparts to Vitamin D deficiency, probably because of less exposure to sunrays as a result of urbanization, pollution and less outdoor activities.<sup>20</sup> Kumar et al found that prevalence of Indian women with vitamin D deficiency was 65.24%, 32.86% were insufficient and only 1.90% were vitamin D sufficient.<sup>21</sup> Goswami et al showed that in

spite of plentiful sunlight, healthy people of Delhi were vitamin D deficient. Apart from inadequate direct sunlight, low-calcium, high-phytate diets, skin pigmentation of Indians, pregnancy in females, and climatic variations may affect vitamin D levels.<sup>22</sup> In our study population about 34.4% females were non obese and 65.6% were obese. We found significant difference in vitamin D levels among obese and non – obese groups. Obesity is associated with alterations in the vitamin D physiology as seen from our and some previous studies demonstrating a negative correlation of vitamin D levels with BMI. Vitamin D being fat soluble gets accumulated in the adipose tissue and its bio availability is reduced for action at other sites.<sup>23,24</sup> Fat cells are endocrinologically active and have the vitamin D receptor (DVR). Hence, fat tissue is among the target tissues for active vitamin D. Also sufficient Vitamin D levels cause inhibition of adipogenesis.<sup>25</sup> We also found statistical significant difference in blood sugar levels, serum insulin and HOMA IR between the obese and non-obese groups. Vitamin D levels negatively correlated with blood sugar, insulin and HOMA IR. Thus obese individuals are more prone for vitamin D deficiency and insulin resistance leading to raised blood sugar levels. Same has been demonstrated from previous studies. Bhatt et al demonstrated that lower vitamin D levels are linked with higher blood glucose values in Indian women who are pre diabetic. The prevalence (%) of vitamin D deficiency, insufficiency and sufficiency was 68.6, 25.9 and 5.5, respectively and obesity was 61.7% in their study.<sup>26</sup> In a study by Sahasrabudhe et al 73% participants had vitamin D deficiency and individuals with severe vitamin D deficiency had maximum IR.<sup>27</sup> Another study on conducted Indian postmenopausal

women by Agarwal et al. showed that only 5.6% subjects had normal 25-OHD level and 7.0% had vitamin D insufficiency while 87.3% were vitamin D deficient, with mean serum 25-OHD levels being  $12.73 \pm 7.63$  ng/ml; 81% were obese and 25-OHD levels inversely correlated with BMI and HOMA-IR. <sup>28</sup>Vitamin D deficiency influences insulin resistance and the pathogenesis of type 2 diabetes, through effects on both cell function and insulin sensitivity. Several roles of vitamin D deficiency are reported affecting insulin resistance through various mechanisms including increasing related pro inflammatory cytokines and acute phase reactants, as found in vitamin D deficiency, mediating low-grade inflammation. <sup>29</sup>Abbasi et al showed that subjects with pre-diabetes and low circulating 25(OH)D levels were most insulin resistant and had impaired  $\beta$  cell function as compared with normal fasting glucose-vitamin deficient and normal fasting glucose-vitamin D sufficient subgroups. <sup>30</sup> Dutta et al found that individuals with the lowest 25(OH)D levels had the highest level of insulin resistance. Vitamin D supplementation has been found to decrease progression to diabetes and helped in achieving normal blood glucose levels. Thus further trials were warranted with vitamin D supplementation in individuals with pre-diabetes. <sup>31</sup> High prevalence of MS as well as vitamin D insufficiency and deficiency existed among postmenopausal women of Singur block, West Bengal, India. 25(OH)D had significant inverse and direct relationship with fasting BSL and waist circumference. <sup>32</sup>

### Conclusion

Vitamin D deficiency is widespread among urban Indian females and it is significantly associated with obesity, fasting blood sugar levels and insulin resistance. Low vitamin D levels in serum may accelerate the progress of

diabetes in obese persons. Vitamin D levels should be assessed in obese individuals that may be helpful in treatment of obesity and prevention of diabetes. There is urgent need for accessibility of quality-regulated and affordable vitamin D supplements and vitamin D fortified foods to the Indian population. The results of this study indicated that vitamin D determination can be used for the prognosis and early detection of females at risk for metabolic syndrome.

### Limitations

Being cross sectional study, no cause or effect of 25(OH)D levels on B.M.I and diabetes mellitus could be studied. The seasonal variation and amount of sunlight exposure and nutritional status of participants, comparison of urban vs rural population need to be considered in future studies.

### References

1. Akhtar S, Sofi NY, Jain M. Vitamin D status in South Asian populations - risks and opportunities. *Crit Rev Food Sci Nutr* 2016;56:1925–40.
2. Ritu G, Gupta A, Vitamin D Deficiency in India: Prevalence, Causalities & Interventions. *Nutrients* 2014; 6:729-775.
3. Garg R, Agrawal P, Malhotra N. Prevalence of vitamin D deficiency in Indian women. *Int J Reprod Contracept Obstet Gynecol.* 2018 ;Jun;7(6):2222-2225
4. Sofi NY, Jain M, Kapil U, et al. Status of serum vitamin D and calcium levels in women of reproductive age in national capital territory of India. *Indian J Endocrinol Metab* 2017;21:731–3.
5. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-81
6. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporos Int* 2005; 16: 713-716.

7. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, et al. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: Data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med.* 2007;167:1159–65.
8. Parikh SJ, Edelman M, Uwaifo GI, et al. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* 2004;89:1196-9
9. Wortsman J, Matsuoka LY, Chen TC, et al. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;72:690-3
10. Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. *J Clin Endocrinol Metab* 2008; 93(11 Suppl 1):S9–S30
11. Bhatt SP, Misra A, Sharma M, et al. Vitamin D insufficiency is associated with abdominal obesity in urban Asian Indians without diabetes in North India. *Diabetes Technol Ther* 2014;16:392–7.
12. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004;80(6 Suppl):1678S-88S.
13. Sung CC, Liao MT, Lu KC, Wu CC. Role of vitamin D in insulin resistance. *J Biomed Biotechnol.*2012;2012:634195
14. Chiu KC, Chu A, Go VL, et al. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004;79:820-
15. Pittas AG, Lau J, Hu FB, et al. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2007;92:2017-2
16. Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G. Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among Type 2 diabetic patients. *Diabetes Care.* 2006;29(1):722-4.
17. Misra A, Chowbey P, Makkar BM, Vikram NK, Wasir JS, Chadha D, Joshi Sr, Sadikot S, Gupta R, Gulati S, Munjal YP: Consensus Group: Consensus statement for diagnosis of obesity, abdominal obesity and the metabolic syndrome for Asian Indians and recommendations for physical activity, medical and surgical management. *J Assoc Physicians India* 2009;57:163–170.
18. Singh Y, Garg MK, Tandon N, Marwaha RK. A study of insulin resistance by HOMA-IR and its cut-off value to identify metabolic syndrome in urban Indian adolescents. *J Clin Res Pediatr Endocrinol.* 2013;5(4):245-51.
19. Agarwal N, Mithal A, Dhingra V, Kaur P, Godbole MM, Shukla M. Effect of two different doses of oral cholecalciferol supplementation on serum 25-hydroxy-vitamin D levels in healthy Indian postmenopausal women: A randomized controlled trial. *Indian J Endocrinol Metab.* 2013;17(5):883-9.
20. Sahu M, Bhatia V, Aggarwal A, Rawat V, Saxena P, Pandey A, et al. Vitamin D deficiency in rural girls and pregnant women despite abundant sunshine in northern India. *Clin Endocrinol (Oxf).* 2009;70(5):680-4.
21. Kumar R , Kunwar K, Kumar A , Agrawal N. An Observational study to assess the prevalence of Vitamin D deficiency in Indian women. *International Journal of Health and Clinical Research,* 2020; 3(12):290-294
22. Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon N, Kochupillai N, et al. Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. *Am J*

- Clin Nutr 2000; 72 : 472-5.
23. Taheri E, Saedisomeolia A, Djalali M, et al. The relationship between serum 25-hydroxy vitamin D concentration and obesity in type 2 diabetic patients and healthy subjects. *J Diabetes Metab Disord* 2012; 11:16. doi:10.1186/2251-6581-11-16 32.
24. Gandhe M , V Kuzhandai Velu , S R Sree Shyamini , Subiman Saha , R Ramesh , M Sathish Babu.225(OH) Vitamin D3 status in healthy adolescents.Vol 3 | Issue 2 | Apr - Jun 2016 *Indian J Child Health*
25. Ozkan B. Nutritional rickets. *J Clin Res Pediatr Endocrinol* 2010; 4: 137-143
26. Bhatt S, Misra A, Gulati S, Singh N, Pandey R. Lower vitamin D levels are associated with higher blood glucose levels in Asian Indian women with pre-diabetes: a population-based cross-sectional study in North India *MJ Open Diab Res Care* 2018;6:e000501. doi:10.1136/bmjdr-2017-000501
27. Sahasrabudhe A. V , Pitale S , Gupta M , Chari S , Sagdeo M. Study of vitamin D levels and its correlation with insulin resistance. *National Journal of Physiology, Pharmacy and Pharmacology* | Vol 7 | Issue 9
28. Agarwal N, Mithal A, Kaur P, Dhingra V, Godbole MM, Shukla M. Vitamin D and insulin resistance in postmenopausal Indian women. *Indian J Endocrinol Metab.* 2014;18(1):89-93.
29. A. Deleskog, A. Hilding, K. Brismar, A. Hamsten, S. Efendic, and C.-G. Ostenson, "Low serum 25-hydroxyvitamin D level " predicts progression to type 2 diabetes in individuals with prediabetes but not with normal glucose tolerance," *Diabetologia*, vol. 55, no. 6, pp. 1668–1678, 2012
30. Abbasi F, Blasey C, Feldman D, et al. Low circulating 25-hydroxyvitamin D concentrations are associated with defects in insulin action and insulin secretion in persons with prediabetes. *J Nutr* 2015;145:714–9.
31. Dutta D, Maisnam I, Shrivastava A, et al. Serum vitamin-D predicts insulin resistance in individuals with prediabetes. *Indian J Med Res* 2013;138:853–60.
32. Srimani S, Saha I, Chaudhuri D. Prevalence and association of metabolic syndrome and vitamin D deficiency among postmenopausal women in a rural block of West Bengal, India \**PLOS ONE* | <https://doi.org/10.1371/journal.pone.0188331> November 30, 2017