## **Original Research Article**

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# Factors responsible for low serum vitamin-D levels in type-2 diabetes mellitus females: a cross-sectional study

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#### **ABSTRACT**

**Background:** Diabetes mellitus are a group of disorders as a result of reduced insulin secretion, insulin resistance, and increased glucose production and many more unknown etiologies. As compared to diabetic non-obese, diabetic obese individuals have an increased chance of 13.5 % in developing diabetic complications. Many diabetic patients are found to be vitamin-D deficient and its role in insulin regulation is being studied in many studies. Thus, diabetic complications and vitamin-D deficiency are likely to be developed in obese diabetic females.

**Methods:** Type-2 diabetic females from 30 years to 60 years, attending in medicine as out-patient and female type-2 diabetes patients admitted in medical ward of RRMCH were assessed clinically. Parameters such as anthropometric measurements, weight, height, waist-hip ratio, tested for serum levels vitamin-D including other relevant investigations related to diabetes were done. Totally, 156 patients were assessed.

**Results:** Assessment of 156 patients are as follows, 48.47±9.56years was the mean age of this study group. SD: 5.10±4.36 years is the mean diabetic duration of this study population. 0.98 was the mean waist-hip ratio. 98.93 cm was the mean waist circumference. 24.97 was the mean BMI. Mean fasting blood sugar (FBS) and post-prandial glucose test (PPBS) were: 202.73 mg/dl, 280.99 mg/dl respectively, 9.33% was the mean HbA1C. Majority of the females with type-2 diabetes (92.5%) had low levels of serum vitamin-D. 16.19 ng/ml was the mean serum vitamin-D levels. lower levels of serum vitamin-D were significantly associated with diabetic duration (p=0.082+), poor glycaemic control (p<0.001\*\*) and increased BMI (p=0.011\*).

**Conclusions:** Majority of the females with type-2 diabetes mellitus were with waist-hip ratio more than 0.8 and Waist circumference more than 80 cm. Most of the study population had poor diabetic control. Lower levels of serum vitamin-D were found in almost all females with type-2 diabetes mellitus (92.5 %) and the most likely risk factor being obesity and poor glycaemic control as a conclusion of this study.

Keywords: Female type-2 diabetes, FBS, PPBS, Serum vitamin-D

#### INTRODUCTION

Diabetes mellitus are a group of disorders as a result of reduced insulin secretion, insulin resistance, and increased glucose production and many more unknown etiologies. Diabetes mellitus stands as the commonest of all chronic diseases in the world and number of diabetic patients is raising day by day. Globally as of 2017, 425 million individuals were found to affected by diabetes. Globally, the adult population had a prevalence of diabetes at 8.8%.

Worldwide, 204 million women were the estimated number type-2 diabetes as on 2017.<sup>2</sup> As on 2015, America had a total of 14.9 million type-2 diabetic women. As on 2017 in India, 72.9 million was the total number of diabetes patients, prevalence of diabetes mellitus in India was 10.1%.<sup>3</sup> In India, the prevalence of type-2 diabetic female is about 37.87%. As on 2017, 3.87% was the prevalence of women with type-2 diabetes in India.<sup>4</sup> Type-2 diabetes now a days are incident in age group as early in their 20s. And risk of occurrence of type-2 diabetes goes

up as age advances, subsequently if the risk factors of diabetes such as higher weight and low physical activities are not changed. Compared to diabetic men, diabetic women are at greater risk of developing visual defect due to cataract, retinopathy and cardiovascular disease especially after menopause.

NFHS-3 (National family health survey) data indicates, In India 22% of women are either overweight or obese.<sup>5</sup> As compared to diabetic non-obese, diabetic obese individuals have an increased chance of 13.5% in developing diabetic complications.<sup>6</sup> This indicates there might be higher prevalence of complications obesity is associated with diabetes. Many diabetic patients are found to be vitamin D deficient and its role in insulin regulation is being studied in many studies.<sup>7,8</sup> Thus diabetic complications and vitamin-D deficiency are likely to be developed in obese diabetic females.

With this idea, the following study is done to estimate the serum levels of vitamin-D in females with type-2 diabetes and its association to various clinical and biochemical factors.

#### Objectives of study

Objectives of the study were to study the clinical, biochemical profile of type-2 diabetes in women and to study the various clinical and biochemical factors influencing the level of vitamin-D in type-2 diabetes women.

#### **METHODS**

#### Study design

This study was an observational cross-sectional study.

#### Study place

The study carried out at Rajarajeshwari Medical College and Hospitals, Bengaluru, India

#### Study period

The study performed for 1 year from January 2015 to January 2016

#### Sources of data

Type-2 diabetic females from 30 years to 60 years, attending in medicine as out-patient and female type-2 diabetes patients admitted in medical ward of RRMCH.

#### Inclusion criteria

Type-2 diabetic female patients aged from 30 to 60 years, who attend as outpatient in department of general medicine as well as admitted in the female medical wards of RRMCH were included in the study.

#### Exclusion criteria

Patients who are other types of diabetes except type-2 such as, gestational diabetes mellitus, type-1, MODY, LADA. Patients with known renal disease, Crohn's disease, cystic fibrosis, celiac disease, malabsorption syndrome. Patients who are case of any carcinoma (including breast, colon). Patients who have Patients with history of steroid treatment and intake of oral contraceptive pills for more than 6 months. Patients who are on supplementation of oral/parenteral calcium and vitamin D, were excluded from the study.

#### Ethical approval

The ethical approval was obtained from the institution's IEC committee.

#### Data collection methods

All female patients who have been diagnosed to have type-2 diabetes mellitus are first taken an informed consent for participation in our study. Then, the complete history regarding onset of diabetes mellitus and the details of respective treatment for the same are taken. Patient's anthropometric details are measured, such as weight, height, waist-hip ratio, and a detailed clinical examination is done as per proforma.

Investigations such as: Fasting and post-prondial blood sugars, HbA1C and serum vitamin-D.

Serum vitamin D is processed by 'ARCHIT ECT25-OH vitamin D assay kit'. The data collected were analysed by statistician to find the association of vitamin-D with various clinical and laboratory parameters in female patients with type-2 diabetes by using student T test using SPSS software.

#### Statistical methods

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean±SD (min-max) and results on categorical measurements are presented in number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made, Assumptions were like-1. Dependent variables should be normally distributed, 2. Samples drawn from the population should be random, cases of the samples should be independent

Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients, Chi-square/Fisher exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Pearson correlation between study variables is performed to find the degree of relationship.

#### Statistical software

The Statistical software namely SAS 9.2, SPSS 15.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate tables etc

#### **RESULTS**

#### Age distribution among study population

Among the assessed 156 patients, 71 patients were belonging to more than 50 years. 85 patients were on the range of 30 to 50 years. The mean±SD was 48.47±9.56.

#### Duration of DM in years

Out of 156 patients, 76 patients were having duration of diabetes less than 5 years. 80 patients were having duration of diabetes >5 years. The mean±SD was 5.10±4.36.

#### BMI $(kg/m^2)$ distribution in patients studied

Among the 156-study population, 89 patients were having normal BMI i.e., (18.5-24.9). 65 patients were with >25.2 patients were lean diabetic and the mean±SD was 24.97+4.16

Table 1: Waist circumference (cm) distribution in patients studied.

Hip and waist circumference	Waist circumference No. of patients	Waist circumference (%)	Hip circumference No. of patients	Hip circumference (%)
<80	9	5.8	10	6.4
80-90	36	23	11	7.1
90-100	35	22.4	57	36.5
100-110	59	37.8	53	34
>110	17	10.9	25	16
Total	156	100	156	100

Mean±SD: 98.93±12.18

111 patients were having waist circumference >90 cm. 36 patients were having waist circumference in range of 80-90 cm. 9 patients were having waist circumference <80 cm. 135 patients were having hip circumference >90 cm. 11 patients were having hip circumference in range of 80-90 cm. 10 patients were having hip circumference <80 cm.

#### Waist hip ratio distribution in patients studied

Out of the assessed 156 patients, waist-hip ratio in 130 patients was more than 0.9, waist-hip ratio in 26 patients was in range of 0.8-0.9 and mean±SD was 0.98±0.08.

#### Distribution of sugar parameters in patients studied

Among the 156 patients, 140 patients were having fasting blood sugar (FBS)>126 mg/dl, 202.73±81.73, 120 patients were having post-prandial glucose test (PPBS)>200 mg/dl and mean±SD was 280.99±94.14

#### HbA1c levels in patients studied

Out of the 156-study population, 142 patients were having HbA1C>7 indicating poor control of diabetes. Fourteen patients were with good control of blood sugar with HbA1C<7 and mean±SD was 9.33±1.83.

Ninety-one patients were having diabetes <5 years of duration. Sixty-five patients were having diabetes >5 years of duration. Among them forty-one were >50 years of age.

Table 2: Age distribution of patients studied in relation of duration DM.

Age	Duration o	Duration of DM (year) (%)		
(years)	≤5	>5	Total (%)	
30-40	37 (40.7)	3 (4.6)	40 (25.6)	
41-50	24 (26.4)	21 (32.3)	45 (28.8)	
51-60	30 (33)	41 (63.1)	71 (45.5)	
Total	91 (100)	65 (100)	156 (100)	

P<0.001\*\*, Significant, Chi-Square test

40 patients with BMI >25 was having duration of diabetes less than 5 years. 27 patients with BMI>25 was having duration of diabetes more than 5 years.

80 patients with duration of DM<5 years were with HbA1C>7 (poor control of sugar). 62 patients with>5 years of DM were with poor control of blood sugar.

Duration of DM does not have any relation with BMI and HbA1C (Table 2 to 4).

Table 3: Duration of DM in relation to BMI.

<b>Duration of</b>	BMI (kg/n	n <sup>2</sup> ) (%)					Total (0/)
DM (years)	<18.5	18.5-24.9	25-29.9	30-34.9	35-39.9	40 and above	Total (%)
≤5	1 (50)	50 (56.2)	26 (59.1)	13 (76.5)	1 (33.3)	0 (0)	91 (58.3)
>5	1 (50)	39 (43.8)	18 (40.9)	4 (23.5)	2 (66.7)	1 (100)	65 (41.7)
Total	2 (100)	89 (100)	44 (100)	17 (100)	3 (100)	1 (100)	156 (100)

P=0.409, not significant, Fisher exact test

Table 4: Duration of DM in relation to levels of HbA1c.

Duration	HbA1c (%)		
of DM	Good	Poor	Total (%)
(years)	control<7	control>7	
≤5	11 (78.6)	80 (56.3)	91 (58.3)
>5	3 (21.4)	62 (43.7)	65 (41.7)
Total	14 (100)	142 (100)	156 (100)

P=0.107, Not significant, Chi-Square test

#### Vitamin D (ng/ml) levels in patients studied

Among the 156-study population, 121 patients were having vitamin-D levels <20 ng/ml (i.e., deficiency) 8 patients were having normal vitamin-D levels (30-100 ng/ml) (i.e., normal). 27 patients were having vitamin-D levels ranging from 20-30 ng/ml (i.e., insufficiency) and Mean±SD: 16.19±8.97

61 patients with <5 years diabetes duration have vitamin-D levels<20 ng/ml. Sixty patients with >5 years diabetes

duration have vitamin-D levels <20 ng/ml.

Vitamin-D levels are inversely proportional to duration of diabetes.

61 patients were having normal BMI with vitamin-D less than 20 ng/ml. 60 patients with BMI>25 have vitamin-D levels <20 ng/ml. 22 patients with normal BMI have vitamin-D levels in the range of 20-30 ng/ml. 5 patients with BMI>25 have vitamin-D levels in the range of 20-30 ng/ml. 8 patients with normal BMI had normal range of vitamin-D (30-100 ng/ml).

Vitamin-D levels are inversely proportional to BMI.

88 patients with waist circumference >90 cm have <20 ng/ml of vitamin-D levels. 25 patients with waist circumference of 80-90 cm have <20 ng/ml of vitamin-D levels. 8 patients with waist circumference of <80 cm have <20 ng/ml of vitamin-D levels.

Significant association of vitamin-D levels with waist circumference demonstrated.

Table 5: Comparison of clinical variables according to vitamin-D levels of patients studied.

Variables	Vitamin D (ng/dl)			Total	n volue
	<20	20-30	30-100	Total	p value
Age (years)	48.11±9.72	49.19±8.76	51.50±10.10	48.47±9.56	0.571
Duration	4.91±4.20	$4.81\pm3.08$	8.88±8.13	5.10±4.36	0.041*
BMI (kg/m²)	25.43±4.05	23.75±4.64	22.18±1.60	24.97±4.16	0.024*
Waist circumference	99.12±12.63	$100.22\pm9.38$	91.75±12.52	98.93±12.18	0.212
Hip circumference	$101.58\pm12.64$	98.56±5.42	91.5±11.51	$100.54 \pm 11.85$	0.041*
Waist hip ratio	$0.98\pm0.08$	1.01±0.09	$0.98\pm0.09$	$0.98\pm0.08$	0.125

<sup>\*</sup>ANOVA test

Table 6: Duration of DM in years in relation to vitamin-D levels of patients studied.

<b>Duration of DM</b>	Vitamin D (ng/dl	Total (0/ )		
(years)	<20	20-30	30-100	Total (%)
<5	61 (50.4)	13 (48.1)	2 (25)	76 (48.7)
5-10	53 (43.8)	13 (48.1)	3 (37.5)	69 (44.2)
11-15	4 (3.3)	1 (3.7)	1 (12.5)	6 (3.8)
>15	3 (2.5)	0 (0)	2 (25)	5 (3.2)
Total	121 (100)	27 (100)	8 (100)	156 (100)

P=0.082+, significant, Fisher exact test

Table 7: Vitamin D (ng/ml) levels in relation to BMI distribution of patients studied.

Vitamin D	BMI (kg/r						
(ng/ml)	<18.5	18.5-24.9	25-29.9	30-34.9	35-39.9	40 and above	Total (%)
<20	2 (100)	59 (66.3)	41 (93.2)	16 (94.1)	3 (100)	0 (0)	121 (77.6)
20-30	0 (0)	22 (24.7)	3 (6.8)	1 (5.9)	0 (0)	1 (100)	27 (17.3)
30-100	0 (0)	8 (9)	0 (0)	0 (0)	0 (0)	0 (0)	8 (5.1)
Total	2 (100)	89 (100)	44 (100)	17 (100)	3 (100)	1 (100)	156 (100)

P=0.011\*, significant, Fisher exact test

Table 8: Waist circumference in relation to vitamin D levels of patients studied in relation to vitamin D levels.

Waist circumference	Vitamin D (ng/dl) (%	■ Total		
waist circumference	<20	20-30	30-100	10tai
<80	8 (6.6)	0 (0)	1 (12.5)	9 (5.8)
80-90	25 (20.7)	7 (25.9)	4 (50)	36 (23.1)
90-100	29 (24)	6 (22.2)	0 (0)	35 (22.4)
100-110	45 (37.2)	11 (40.7)	3 (37.5)	59 (37.8)
>110	14 (11.6)	3 (11.1)	0 (0)	17 (10.9)
Total	121 (100)	27 (100)	8 (100)	156 (100)

P=0.434, Not Significant, Fisher Exact test

Table 9: Vitamin D (ng/ml) levels in relation to HbA1c distribution of patients studied.

Vitamin D (ng/dl)	HbA1c (%)	HbA1c (%)		
Vitanini D (lig/til)	Good control<7	ood control<7 Poor control>7		
<20	5 (35.7)	116 (81.7)	121 (77.6)	
20-30	4 (28.6)	23 (16.2)	27 (17.3)	
30-100	5 (35.7)	3 (2.1)	8 (5.1)	
Total	14 (100)	142 (100)	156 (100)	

P<0.001\*\*, Significant, Chi-Square test

Among 121 patients have vitamin-D deficiency (<20 ng/ml), 116 patients had a poor diabetic control (HbA1C>7).

Among 27 patients have vitamin-D insufficiency (20-30 ng/ml), 23 patients had poor control of diabetes (HbA1C>7). 8 patients have sufficient levels of vitamin-D (30-100 ng/ml).

Vitamin-D levels are inversely proportional to HbA1C levels.

Table 10: Pearson correlation between vitamin-D levels and clinical variables.

D	or and land	
Pearson correlation	r value	p value
Vitamin D (ng/ml) vs age in years	0.245	0.002**
Vitamin D (ng/ml) vs BMI (kg/m²)	-0.304	<0.001**
Vitamin D (ng/ml) vs waist circumference	-0.251	0.002**
Vitamin D (ng/ml) vs hip circumference	-0.337	<0.001**
Vitamin D (ng/ml) vs waist hip ratio	0.017	0.836

As per Pearson correlation, vitamin-D levels have significant correlation to age, BMI, waist circumference, hip circumference. But, vitamin-D levels are not in correlation with waist-hip ratio.

# Pearson correlation between vitamin-D levels and sugar parameters variables

Vitamin D (ng/ml) vs FBS (mg/dl): The r value was -0.336 and respective p<0.001\*\*.

Vitamin D (ng/ml) vs PPBS (mg/dl): The r value was - 0.365and respective p<0.001\*\*\*

Vitamin D (ng/ml) vs HbA1c: The r value was -0.390 and respective p<0.001\*\*

Vitamin-D levels have significant Pearson correlation to sugar control.

#### DISCUSSION

We studied 156 females with diabetes who were admitted in medicine ward. 74.3% patients were of age >40 years. In 1987 "The Eluru survey", a government run survey of the Indian rural population showed results similarly such that known case of diabetes had a prevalence of 6.1% in both genders aged 40 or over and increased to 13.3% in 50-59 age group.

The number of diabetic patients were more with the progression of age (Table 2). Clinical significance of p<0.001\*, Chi-square test has been demonstrated in our study. In. Ramachandran et al, demonstrated that Asians from age 40 to 64 years had 5 times more prevalence of diabetes compared to the Europeans. <sup>10</sup>

Majority of the patients had normal BMI (57.1%) and rest of them had BMI >25 (41.6%). Kamath et al, showed that 23.3% of diabetic patients was within normal BMI and 59.9% of them had BMI>25.<sup>11</sup>

Kamath et al shows that 86.6% of diabetic patients had a waist circumference of>80 cm and chamukuttan et al, showed that the Asian Indians have higher upper body adipose content and higher visceral fat for their BMI when the western population is compared.<sup>3,12</sup> Body mass index of an urban Indian was 23 kg/m<sup>2</sup>, and average measurement of women waist circumference was 80 cm.

and waist-hip ratio was 0.81.<sup>13</sup> Most of patients in the study in the Table 1 (94.2%) have waist circumference of more than 80 cm. 93.6 % had a hip circumference as shown in the Table 1 of more than 80 cm. All patients (100%) had the waist-hip ratio>0.8.

Among the total patients, 91% of them had poor control of diabetes. Where as in Gopinath et al, showed that 73.5% of patients had poor control of diabetes. <sup>14</sup> Grineva et al, demonstrated 78% of diabetic females were either overweight or obese, with 83.6% of them with their Waist circumference >80 cm. <sup>15</sup>

Patient's diabetic duration was not significantly related to BMI in the observed patients (Table 1). Whereas on the contrary, Kamath et al, showed as 59.9 % diabetic females had increased BMI.<sup>12</sup>

In this study, diabetic duration was not related to glycemic control (Table 4). Almost similar results were demonstrated in Gopinath et al showed though a greater number of patients with inadequate glycemic control were with longer duration of diabetes, and the same was not significantly (p=0.142) correlated with poor glycemic status.<sup>14</sup>

From the evaluated patients, 94.9 % (n=148) of them had vitamin-D insufficiency and 77.6 % (n=121) of them had vitamin-D deficiency (<20 ng/ml). Alhumaidi et al, showed vitamin-D deficiency <20 ng/mL in 76.6% of diabetic patients. Daga et al in North India showed that 91.1% of the assessed patients with diabetes had vitamin-D insufficiency.

Comparision of vitamin-D and clinical variables such as duration of diabetes, BMI, hip circumference showed moderate significance according to p value in this study (Table 5). Similar results as regards to BMI have also been demonstrated by Alhumaidi et al.<sup>16</sup>

The relation of diabetic duration with serum levels of vitamin-D had significant p value of 0.082 as mentioned in the Table 3. On the contrary, diabetic duration need not be in relation to serum levels of vitamin-D in a given patient. Hence, the complications of diabetes are not only related to duration of the diabetes, but also to the maintenance of good glycemic control. Similar results have also been showed by the Shaikh et al, age, diabetic duration did not show any impact on vitamin D deficiency or insufficiency.<sup>18</sup>

Serum levels of vitamin-D in relation to the patient's BMI were significant (p=0.011) (Table 7), inferring that BMI in patients and levels of vitamin-D are inversely correlated. Serum levels of vitamin-D related to waist circumference (Table 8) in patients were not of clinical significance in this study (p=0.434 by fisher exact test). Similar findings have been showed in McGill et al, showing that there were reasonable but significant inverse relationships of levels of vitamin-D with respect to weight (p=0.0009), BMI

(p=0.005) and waist circumference (p=0.03) even though no relationship could be shown with the percentage of fat.<sup>19</sup>

Serum levels of vitamin-D associated with glycemic status of a patient (Table 9) were of good clinical significance (p=0.001). This signifies that glycemic control of diabetic patient is one of the most important factors in serum vitamin-D level determination. The stricter the glycemic control, the more the vitamin-D levels in a given patient. Or, it could be also interpreted that the patients having poor glycemic control would need supplementation of vitamin-D due to more probability of vitamin-D deficiency in them. Similar findings have also been showed by Athanassiou et al. Vitamin-D levels were found to be related inversely with HbA1c levels in the group of type 2 diabetes patients (p=0.008, r=0.058).<sup>20</sup>

In accordance to correlation of Pearson's test (Table 10), almost all of the clinical variables except that of waist-hip ratio were of highly significant (p<0.001) with serum levels of vitamin-D. The glycemic variables were highly correlated with serum levels of vitamin-D. Similarly, McGill et al in their study demonstrated, low serum vitamin D3 levels was inversely associated to weight and BMI, large waist and raised HbA1c.<sup>19</sup>

Observation from Wortsman et al showed serum vitamin D3 levels were high in non-obese and obese individuals subsequently following exposure to an identical duration and intensity of UV-B radiation. More vitamin-D3 were expected among obese individuals as they had more body surface area and thereby more exposure to UV-B radiation. But on the contrary, the raise in serum vitamin D3 levels was 57% lower in the obese than in the non-obese individuals after 24 hours of exposure.<sup>21</sup>

Blum et al, showed that the mean serum levels of vitamin D3 in individuals, the subcutaneous fat tissue samples were 102.8 nmol/kg. The mean level of vitamin D3 in serum was 7.78 nmol/l. So, the fat tissue concentrations of vitamin-D3 and serum were correlated positively (r=0.68, p=0.003).

Hurst et al, studied that Insulin sensitivity improved in the insulin-resistant women after supplementation of 100 mcg (4000 IU) vitamin D3. Despite substantial increase in serum 25(OH)D concentration at 3 months, nil significant changes were seen in insulin sensitivity until serum 25(OH)D concentrations reached the levels above 80 nmol/l.<sup>23</sup>

Talaei et al studied that, there was marked improvements in serum levels of FPG, insulin and in HOMA-IR (Hemeostatic model assessment-insulin resistance) after supplementation with vitamin D, suggested that supplementation of vitamin D could decrease insulin resistance in T2DM.<sup>24</sup>

#### Limitations

If a greater number of study population and time had been there, this study could have had more broader interpretation and thus could have had wider implication on estimation, there by supplementation of vitamin D to necessary patients.

#### **CONCLUSION**

Diabetes mellitus is still one of the top global burden prevalent in 387 million individuals as of 2014. It is predicted to be increased to 205 million by 2035. Diabetes has been proven to have numerous complications with the advancement of age. New complications, new modes of management are being found lately and this process is rapidly updated. Serum levels of vitamin-D are always one of the interesting topics for many experts in medical field and we need more time, research to understand it. Vitamin-D deficiency is prevalent in most of the diabetic females and the most common cause for the same in this study being obesity and poor sugar control. Hence, healthy life style practices, good sugar control and early supplementation vitamin-D is recommended for all diabetic patient.

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