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STUDY OF FACTORS INFLUENCING SERUM VITAMIN-D DEFICIENCY IN WOMEN WITH TYPE-2 DIABETES MELLITUS.

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ABSTRACT

Type-2 Diabetes is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Diabetes mellitus is one of the most common chronic diseases across the world and number of diabetic patients is on rise. In 2017, there were 425 million people with diabetes globally. A prevalence of 8.8% among the gobal adult population. On 2017, it was estimated that almost 204 million women in the world have type-2 diabetes. Total of 14.9 million women have type-2 diabetes in America as on 2015. In India, the total number of diabetes patients are 72.9 million as on 2017. Prevalence of diabetes mellitus in India is 10.1 % as on 2017. Among Indian type-2 diabetic patients, female prevalence accounts for 37.87%. So, the prevalence of women with type-2 diabetes in India is 3.87% as on 2017. The prevalence of women with type-2 diabetes mellitus in Karnataka is 22.04 %. Many people are diagnosed type-2 diabetes in their teens or early 20s. And with advancing age, the risk of getting type-2 diabetes goes up too, especially if you don't change any of the diabetes risk factors like your weight and physical activity level. Women who get type-2 diabetes are at greater risk than men of cardiovascular disease and blindness.

INTRODUCTION

According to the recent National Family Health Survey (NFHS-3), 22% of women in India are either obese or over weight. Obese diabetic patients have 13.5 % more chances of developing diabetic complications compared to non –obese diabetic patients. This means obesity is associated with higher prevalence of complications. Vitamin D has found to be deficient in diabetic patient and it's role in insulin regulation. Obese females are more prone to develop diabetic complications and hypovitaminosis-D.

With the above idea I would like to do this study,by estimating vitamin D levels in serum of type-2 diabetic females and its correlation with various clinical and biochemical parameters.

OBJECTIVES

1. To Study the Clinical, Biochemical Profile of Type-2 Diabetes in Women.
2. To Study the Various Clinical and Biochemical Factors Influencing the Level of Vitamin-d in Type-2 Diabetes Women.

MATERIALS AND METHOD

Sources of Data

All female patients aged 30 to 60 years who are a case of type-2 diabetes mellitus, attending as outpatient in medicine opd, as well as admitted in all female medical wards of Rajarajeswari medical college and hospital.

Criteria for Sample Collect

Inclusion Criteria

All female patients aged 30 to 60 years who are a case of type-2 diabetes mellitus, attending as outpatient in medicine opd as well as admitted in all female medical wards of Rajarajeswari medical college and hospital.

Exclusion Criteria

1. Patients who are case of type-1 diabetes.
2. Patients who are case of any carcinoma (including breast, colon).
3. Patients with known renal disease, Crohn's disease, cystic fibrosis, celiac disease, malabsorption syndrome.
4. Patients who have gestational diabetes mellitus.
5. Patients with history of steroid intake and oral contraceptive pills for more than 6 months.
6. Patients who are on calcium and vitamin D supplements.

Methods of Data Collection
Study Type: A cross-sectional observational study.
Sample Size: 156
Study Period: 1 Year.

Study Methodology
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, a detailed history, especially regarding onset of diabetes mellitus and the treatment under going are taken. Patient's anthropometric measurements are done, namely height, weight, waist-hip ratio, and a detailed clinical examination is done according to proforma.

Investigations Such As
1. Complete Blood Count.
2. Urine Routine, Urine Microalbuminuria [Strip Method- Qualitative]
3. Fasting Blood Sugar, Post Prandial Blood Sugar, Glycosylated Hemoglobin.
4. Serum 25-Hydroxy Vitamin D :
5. Serum Calcium Level.
6. Thyroid Function Tests.
7. Lipid Profile.
8. Liver Function Tests.
9. Renal Function Tests.
10. Chest X-Ray.
11. Electrocardiography.
12. 2D-Echocardiography.
13. Fundoscopy.
14. Special Investigations Whenever Indicated

a. [Namely, Nerve Conduction Study, Serum Cortisol, Serum Parathyroid Hormone].

Serum Vitamin D is estimated by 'ARCHIT ECT25-OH Vitamin DAssay kit'. With the assistance of a statistician, the collected data will be analysed to find the correlation of vitamin-D with various parameters in type-2 diabetic female patients in their respective group will be found by using student –T test using SPSS software.

RESULTS

Study design: An observational clinical study.

Table 1: Age distribution of patients studied.

Age in years	No. of patients	%
30-40	40	25.6
41-50	45	28.8
51-60	71	45.5
Total	156	100.0

Mean ± SD: 48.47±9.56

Inference: 71 patients were belonging to more than 50 years.

85 patients were on the range of 30 to 50 years.

Table 2: Duration of DM in years.

Duration of DM in years	No. of patients	%
<5	76	48.7
5-10	69	44.2
11-15	6	3.8
>15	5	3.2
Total	156	100.0

Mean ± SD: 5.10±4.36

Inference: 76 patients were having duration of diabetes less than 5 years.
80 patients were having duration of diabetes more than 5 years.

Table 3: BMI (kg/m^2) distribution in patients studied.

BMI (kg/m^2)	No. of patients	%
<18.5	2	1.3
18.5-24.9	89	57.1
25-29.9	44	28.2
30.0-34.9	17	10.9
35-39.9	3	1.9
40 & above	1	0.6
Total	156	100.0

Mean ± SD: 24.97±4.16

Inference: 89 patients were having normal BMI .ie. (18.5 to 24.9).
65 patients were having BMI more than BMI > 25.
2 patients were lean diabetic.

Table 4: Waist Circumference (in cm) distribution in patients studied.

Hip and Waist Circumference	Waist circumference No. of patients	Waist circumference percentage	Hip circumference No. of patients	Hip circumference Percentage
<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

Inference: 111 patients were having waist circumference more than 90 cm.
36 patients were having waist circumference in range of 80-90 cm.
9 patients were having waist circumference less than 80 cm.

Inference: 135 patients were having hip circumference more than 90 cm.
11 patients were having hip circumference in the range of 80-90 cm.
10 patients were having hip circumference less 80 cm.

Table 5: Waist Hip Ratio distribution in patients studied

Waist Hip Ratio	No. of patients	%
<0.8	0	0.0
0.8-0.9	26	16.7
0.9-1	64	41.0
1-1.1	59	37.8
>1.1	7	4.5
Total	156	100.0

Mean ± SD: 0.98±0.08

Inference: 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

Table 6: Distribution of sugar parameters in patients studied.

Sugar parameters	No. of patients (n=156)	%	Mean ± SD
FBS (mg/dl)			
• <100	5	3.2	202.73±81.73
• 100-126	11	7.1	
• >126	140	89.7	
PPBS (mg/dl)			
• <140	2	1.3	280.99±94.14
• 140-200	34	21.8	
• >200	120	76.9	

Inference: 140 patients were having FBS more than 126 mg/dl.
120 patients were having PPBS more than 200 mg/dl.

Table 7: HbA1c levels in patients studied.

HbA1c	No. of patients	%
Poor Control>7	142	91.0
Good Control<7	14	9.0
Total	156	100.0

Mean ± SD: 9.33±1.83

Inference: 142 patients were having HbA1C more than 7 indicating poor control of diabetes.
Only 14 patients were underngood control of blood sugar with HbA1C less than 7.

Table 8.1: Age distribution of patients studied in relation of Duration DM.

Age in years	Duration of DM (yrs)		Total
	≤5	>5	
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.

Inference
91 patients were having diabetes less than 5 years of duration.
65 patients were having diabetes more than 5 years of duration. Among them 41 were more than 50 years of age.

Table 8.2: Duration of DM in relation to BMI.

Duration of DM (yrs)	BMI (kg/m^2)						Total
	<18.5	18.5-24.9	25-29.9	30-34.9	35-39.9	40 & above	
≤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

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

Table 8.3: Duration of DM in relation to levels of HbA1c.

Duration of DM (yrs)	HbA1c		Total
	Good control< 7	Poor 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

Inference
80 patients with duration of DM less than 5 years were having HbA1C > 7 (poor control of sugar). Whereas 62 patients with more than 5 years of DM were having poor control of blood sugar.

Inference: 121 patients were having Vitamin-D levels less than 20ng/ml. (ie. Deficiency) Only 8 patients were having normal Vitamin-D levels (30-100 ng/ml). (ie. Normal) 27 patients were having Vtamin-D levels ranging from 20-30 ng/ml. (ie. Insufficiency).

Inference of 8.1,8.2,8.3
Duration of DM does not have any relation with BMI and HbA1C.

Table 9: Vitamin D (ng/ml) levels in patients studied.

Vitamin D (ng/ml)	No. of patients	%
<20	121	77.6
20-30	27	17.3
30-100	8	5.1
Total	156	100.0

Mean ± SD: 16.19±8.97

Table 10: Comparison of clinical variables according to Vitamin-D levels of patients studied.

Variables	Vitamin D (ng/dl)			Total	P value
	<20	20-30	30-100		
Age in 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±3.08	8.88±8.13	5.10±4.36	0.041*
BMI (kg/m^2)	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±9.38	91.75±12.52	98.93±12.18	0.212
HIP Circumference	101.58±12.64	98.56±5.42	91.5±11.51	100.54±11.85	0.041*
Waist Hip Ratio	0.98±0.08	1.01±0.09	0.98±0.09	0.98±0.08	0.125

ANOVA test

Table 11: Duration of DM in years in relation to Vitamin-D levels of patients studied.

Duration of DM (yrs)	Vitamin D (ng/dl)			Total
	<20	20-30	30-100	
<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

Inference: 61 patients with less than 5 years diabetes duration have Vitamin-D levels less than 20 ng/ml. 60 patients with more than 5 years diabetes duration have Vitamin-D levels less than 20 ng/ml

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

Table 12: Vitamin D (ng/ml) levels in relation to BMI distribution of patients studied.

Vitamin D (ng/ml)	BMI (kg/m^2)						Total
	<18.5	18.5-24.9	25-29.9	30-34.9	35-39.9	40 & above	
<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

Inference: 61 patients were having normal BMI with Vitamin-D less than 20 ng/ml.
60 patients with BMI more than 25 have Vitamin-D levels less than 20 ng/ml.
22 patients with normal BMI have Vitamin-D levels in the range of 20-30 ng/ml.
5 patients with BMI more than 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.

Table 13: Waist Circumference in relation to Vit D levels of patients studied in relation to Vitamin D levels.

Waist Circumference	Vitamin D (ng/dl)			Total
	<20	20-30	30-100	
<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

Inference: 88 patients with waist circumference more than 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 less than 80 cm have < 20 ng/ml of Vitamin-D levels.

Vitamin-d levels are significantly associated with wasit circumference.

Table 14: Vitamin D (ng/ml) levels in relation to HbA1c distribution of patients studied.

Vitamin D (ng/dl)	HbA1c		Total
	Good 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

Inference

121 patients have Vitamin-D deficiency (< 20 ng/ml), among them 116 patients had poor control of diabetes (HbA1C > 7).

27 patients have Vitamin-D insufficiency (20-30 ng/ml), among them 23 patients had poor control of diabetes (HbA1C > 7).

Only 8 patients have sufficient levels of Vitamin-D (30-100 ng/ml).

Vitamin-d Levels are Inversely Proportional to HBA1C Levels

Table 15: Pearson Correlation between Vitamin-D levels and clinical variables.

Pearson Correlation	Rvalue	P value
Vitamin D (ng/ml) **vs** Age in years	0.245	0.002**
Vitamin D (ng/ml) **vs** BMI (kg/m^2)	-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

Pearson correlation

Inference
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.

Table 16: Pearson Correlation between Vitamin-D levels and Sugar parameters variables.

Pearson Correlation	r value	P value
Vitamin D (ng/ml) **vs**FBS (mg/dl)	-0.336	<0.001**
Vitamin D (ng/ml) **vs**PPBS (mg/dl)	-0.365	<0.001**
Vitamin D (ng/ml) **vs**HbA1c	-0.390	<0.001**

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

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: 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, Stata 10.1, MedCalc 9.0.1 Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

DISCUSSION

We studied 156 diabetic female patients admitted in medicine ward of our hospital. 74.3 % patients were in the age group more than 40 years. **" The Eluru survey"** in a rural Indian population on 1987 demonstrated similar result showing that the The prevalence of known diabetes was 6.1% in all subjects aged 40 or over and rose to 13.3% in the age group 50-59 years.[1]

The number of diabetic patients were more with the progression of age [Table 81]. Clinical significance of P<0.001*, Chi-Square test has been demonstrated in our study. In. **A. Ramachandran et al**, demonstrated that Asians aged 40-64 years had five times higher prevalence of diabetes as compared to the Europeans.[2]

Most of the patients [Table 3] had normal BMI (57.1 %) and rest of them had BMI more than 25 (41.6 %). **Ashwin Kamath** et al, showed that 23.3% of diabetic patients had normal BMI and 59.9% of them had BMI > 25.[3]

Majority of patients [Table 4] (94.2 %) have waist circumference of more than 80 cm. 93.6 % had a Hip circumference [Table 4] of more than 80 cm. All patients (100 %) had a Waist-Hip ratio [Table 5] >0.8. According to **Ashwin Kamath** et al,[3] 86.6% of diabetic patients had waist circumference of > 80 cm and **CHAMUKUTTAN et al,**[4] demonstrates that Asian Indians have higher upperbody adiposity and higher visceral fat for a given BMI when compared with the Western population. BMI for an urban Indian is 23 kg/m^2, and cut off values for Waist Circumference were 80 cm for women, and for Waist-Hip Ratio were 0.81 for women[4]. Similarly, **Grineva EN et al**, showed that 78% of diabetic females were overweight or obese, with WC more than 80 cm in 83.6%.[5]

Among the total patients [Table 7], 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.[6]

Duration of diabetes was not significantly related to BMI in the observed patients [Table 8.2]. Whereas in contrary, **Ashwin Kamath** et al, showed as 59.9% diabetic females had raised BMI.[3]

Duration of diabetes in our study was not related to glycemic control [Table 8.3]. Similar result has been demonstrated by **Gopinath et al showed** though a higher number of patients with poor glycemic control were falling in the longer duration of diabetes, duration of diabetes was not significantly (p=0.142) associated with poor glycemic status.[6]

Among the evaluated patients [Table 9], 94.9 % (n=148) of them had Vitamin-D insufficiency and 77.6 % (n=121) of them had Vitamin-D deficiency (< 20 ng/ml). Similarly, **In Mohammed ALHUMAIDI et al**, showed deficiency of Vitamin-D with levels less than 20 ng/mL in 76.6% of the patients with type-2 diabetes.[7] Other study that was performed by **Daga et al**. in the North of

India demonstrated that 91.1% of diabetic patients had vitamin-D insufficiency[8].

Distribution of patients in comparision with Vitamin-D and clinical variables such as duration of diabetes, BMI, Hip circumference showed moderate significance according to p value in this study [Table 10]. Similar result with respect to BMI have also been studied by **Mohammed Alhumaidi et al**, MAEDICA – a Journal of Clinical Medicine 2013; 8(3): 231-236.[7]

The duration of diabetes in relation to Vitamin-D levels shows, suggestive significance with p value = 0.082 [Table 11]. But, duration of diabetes does not necessarily be related to Vitamin-D levels of a patient, as the complications related to diabetes are not only influenced by duration of diabetes, but also by the compliance of treatment and apt glycemic control. The above result have also been demonstrated by **Imran Ali shaikhet et al** that In Multivariate analysis, age, duration of diabetes did not show any impact on Vitamin D deficiency or insufficiency.[9]

Vitamin-D in relation to patients distributed as per their BMI showed moderate significance (p= 0.011) in our study [Table 12]. This means as the BMI increases in patients, the corresponding Vitamin-D levels in them are low. Vitamin-D levels in relation to waist circumference [Table 13] in patients were not of clinical significance in this study (p = 0.434 by fisher exact test). Similar findings have been demonstrated by **Anne-Thea McGill et al**, showing that there were modest but significant inverse relationships of vitamin-D with weight (p = 0.0009), BMI (p = 0.005) and waist circumference (p = 0.03) but no relationship could be shown with fat %.[10]

Vitamin-D levels in relation to glycemic status of a given patient [Table 14] showed strong clinical significance (p = 0.001). This finding could infer that glycemic control in a given diabetic patients is one of the most important factor in determining Vitamin-D levels. Better the glycemic control, the more the Vitamin-D level in a given patient. Or, it could be also interpreted that the patients having poor glycemic control would need the addition of Vitamin-D supplements due to more probablity of Vitamin-D deficiency in them. Similar results have also been demonstrated by **I Kostoglou-Athanassiou, P Athanassiou et al**. ,showed Vitamin-D levels were found to be inversely associated with HbA1c levels in the group of diabetic type 2 patients (p = 0.008, r = 0.058, linear regression analysis).[11]

According to pearson's correlation [Table 15], almost all the clinical variables except Waist-Hip ratio were of strong clinical significance (p < 0.001) with Vitamin-D levels in this study. The sugar parameter variables [Table 16] were also of strong significance in correlation with Vitamin-D levels. Similar results were also demonstrated by **Anne-Thea McGill et al** in their study, low serum vitamin D3 was inversely related to weight and BMI, large waist and raised HbA1c.[10]

Wortsman et al, Observed that blood vitamin D3 concentrations increased in both the obese andnon-obese subjects after exposure to an identical amount of UV-B rradiation. Moreover, the obese subjects had a larger body surfacearea of exposure and therefore would be expected to produce morevitamin D3, resulting in higher blood vitamin D3 concentrations,than would the non-obese control subjects. However, the increasein blood vitamin D3 concentrations was **57%** less in the obese thanin the nonobese subjects 24 hour after the exposure.[12]

Miriam Blum et al, demonstrated that the mean vitamin D3 concentration in subjects' subcutaneousfat tissue samples was 102.8 ± 42.0 nmol/kg. The mean vitamin D3 concentration in serum was 7.78 ± 3.99 nmol/l.Vitamin D3 concentrations of fat tissue and serum werepositively correlated (r = 0.68, P = 0.003).[13]

P. R. von Hurst et al, studied that Insulin sensitivity did improve in these insulin-resistant women with vitamin D supplementation of 100 mcg (4000 IU) vitamin D3. No significant change was seen in insulin sensitivity until serum 25(OH)D concentrations reached levels above 80 nmol/l, and despite substantial increase in serum 25(OH)D concentration at 3 months.[14]

Talaei et al., in their study showed that, there is significant improvements in serum FPG, insulin and in HOMA-IR (Hemeostatic model assessment-Insulin resistanceafter treatment with vitamin D, suggested that vitamin D supplementation could reduce insulin resistance in T2DM.[15]

CONCLUSION

Type-2 diabetes mellitus places a huge burden over the world affecting 387 million people around the world as of 2014. It is expected to increase by 205 million people by 2035. Diabetes has many complications as the age progresses and in every year a new complication, new mode of treatment is being found and this process is constantly evolving. Vitamin-D is currently the topic of interest for many experts in the field of medicine and its various functions is being found in ongoing researches. Vitamin-D deficiency is found in almost all diabetic female patients and the most probable cause for it according to this study is poor glycemic control and obesity. So, life style modification, prompt glycemic control and early Vitamin-D supplementation is necessary for every diabetic patients.

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