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CASE-CONTROL STUDY ON VITAMIN D STATUS AND GLYCAEMIC CONTROL IN SELECTED NEWLY DETECTED FEMALE TYPE-2 DIABETICS

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ABSTRACT
Background: Vitamin D is essential for human health and a growing body of evidence links vitamin D status to health outcomes beyond those associated with the musculoskeletal system. **Objectives:** To find the relationship between vitamin D on Glycaemic control**. Materials and methods**: A case-control study was designed with the study protocol based on the ethics of clinical trials in India. All biochemical determinations were carried using biochemical kits by trained technicians. Fifty newly detected female T2DM subjects were recruited for the case control study. They were categorised into two groups of 25 subjects each equally matched for age, SE factors and initial glycaemic control. All subjects were treated for hyperglycaemia by the diabetologists. Dietary advice was given and all subjects attended a nutrition education session on dietary recommendations and physical activity. No OHA was given to both groups. Vitamin D supplementation was given to the deficient group initially up to a period of four months. **Results and Discussion:** The serum vitamin D increased from the four to eighth month by a mean of 15.46ng/ml. After eight months in the cases group of vitamin D deficiency subjects the administration of vitamin D supplements and OHA had achieved HbA1c reduction (7.4± 0.26%) and sufficient vitamin D status (23.82± 2.6ng/ml). **Conclusion:** Glycaemic control could be brought about in newly detected female diabetics with VDD after supplementation with therapeutic doses of vitamin D and OHA.

KEYWORDS: T2DM; vitamin D deficiency; glyceamic control; newly detected diabetes.

INTRODUCTION
Based on preclinical animal and laboratory research several kinds of clinical or health effects of vitamin D deficiency have been reported. Vitamin D is essential for human health and a growing body of evidence links vitamin D status to health outcomes beyond those associated with the musculoskeletal system.

Doddamani *et al.*, (2013) in a study showed that 70% (35 cases) of the newly detected T2DM patients were vitamin D deficient (< 20ng/ml). In 28 out of 35 cases, vitamin D levels were in the range of 10-20ng/ml. In 7 patients vitamin D levels were <10ng/ml. Optimal levels of vitamin D (20ng/ml) was found in 15 patients. They concluded that Vitamin D is a risk factor for development of T2DM. There might be potential beneficial role of vitamin D supplementation and improving glycaemic status in T2DM.

The initial link between vitamin D and T2DM began in 1970's. Several studies have looked at how much vitamin D people get and if they develop T2DM later in life. Also studies from different parts of India have shown widespread Vitamin D deficiency in all age groups. Women who consumed 800IU or more of Vitamin D per day had a 23 % lower risk for development of incident diabetes compared with women who consumed less than 200IU per day and those who consumed less than 400IU per day Vitamin D supplements compared with women who consumed less than 100IU per day had a 13% lower risk of diabetes (Scragg *et al.,* 2004) and suggested that Vitamin D deficiency might play a role in hyperglycaemia and involved in pathogenesis of T2DM.

A total of 21 prospective studies involving 76,220 participants and 4,996 incidentT2DM cases were included for meta-analysis. Comparing the highest to the lowest category of 25(OH)D levels, the summary relative risk for T2DM was 0.62 (95% CI0.54–0.70). A spline regression model showed that higher 25(OH)D levels were monotonically associated with a lower diabetes risk. This inverse association did not differ by sex, duration of follow-up, study sample size, diabetes

diagnostic criteria, or 25(OH)D assay method. A linear trend analysis showed that each 10nmol/L increment in 25(OH)D levels was associated with a4% lower risk of T2DM (95% CI 3–6; P for linear trend,0.0001) and concluded that the meta-analysis showed an inverse and significant association between circulating 25(OH)D levels and risk of T2DM across a broad range of blood 25(OH)D levels in diverse populations (Song *et al.*, 2013).

Parildar et al., (2013) investigated the impact of vitamin D supplementation on glucose metabolism in Vitamin D-deficient patients with pre-diabetes of 66 subjects with the mean ages 52.2±9.9 years were included in this prospective and a 6-month follow-up study between the years 2008-2010. Vitamin D deficient patients (<25ng/ml) were supplemented with oral Vitamin D. Vitamin D deficiency (<25ng/ml) was found in 93.9% of the patients. Post replacement Vitamin D levels increased significantly and insulin, HbA1c, and HOMA-IR decreased significantly following Vitamin D replacement and concluded that Vitamin D deficiency was very common in their study population.

MATERIALS AND METHODS

The case-control research design was adopted in this study. We identified the newly detected type 2 diabetics with vitamin D deficiency (cases) and otherwise similar subjects who do not have vitamin D deficiency (controls) and compared their glycaemic control. Ideally, the cases and controls were selected from the same population so that they could be representative of that population. Case-control studies are retrospective, meaning that the focus is on exposures that occurred in the past and on the ways in which these exposures may have affected an individual's present health.

The study was conducted at Diabetes Specialty Centers, Coimbatore which a private hospital. The team consisted of a well experienced diabetologists, physician assistants, dietician, sufficient nursing staff and pharmacist. The study protocol was approved by the Institutional Review Board and Independent Ethics Committees at the Research wing of the Centre where the study was conducted. All patients provided written informed consent prior to participation in the study.

By purposive sampling method, the investigator enlisted from October 2013 – December 2014 around 410 newly detected type 2 diabetics. The inclusion criteria were newly detected T2DM; FBG: >100 mg/dl; HbA1c >5.6 % and Oral Glucose Tolerance Test (OGTT): FBG: >126mg/dl, 2hr :> 200mg/dl. The exclusion criteria were Osteoporosis; Complicated cases of diabetes mellitus; Diagnosed cases of T2DM on treatment; Medication that affect vitamin D metabolism and its absorption (phenytoin, rifampin, isoniazid, ketoconazole).

Baseline data was collected through a interview schedule which had five sets of questions on socio-economic status; Anthropometric data; Dietary survey; Biochemical data and Physical activity. Biochemical parameters include fasting and post prandial blood glucose level, Lipid profiles (Total Cholesterol, Triglycerides, High Density Lipoprotein, and Low Density Lipoprotein), renal parameters (Urea, Creatinine), Micro albuminuria and HbA1c values were determined for the selected subjects using standard biochemical kits.

Only 103 subjects took the serum vitamin D test. From these subjects 50 female newly detected T2DM subjects were recruited for the case control study. They were separated into two groups of 25 subjects each. Among the selected subjects 25 vitamin D deficient subjects were the cases and 25 non-deficient vitamin D subjects were the controls taking care that they were equally matched for age, SE factors and glycaemic control.

Blood Glucose levels, HbA1c, and vitamin-D values were analysed and the comparative study on glycaemic control was conducted between vitamin D deficient & non deficient selected subjects for two months, four months and eight months. All biochemical determinations were carried out as given in the previous section.

Vitamin D Supplementation

On recruitment all selected subjects of the case-control study were not recommended OHA drugs. Initially, glycaemic control was recommended through diet and physical exercise for a period of four months. At the end of four months these selected subjects came for a review. At that time, those who had not achieved glycaemic control they were given OHA drugs and Vitamin D supplements by the medical officer. They were then followed up for the next four months.

In the case-control study all the selected vitamin D deficient female cases (n = 25) were administered Vitamin D drugs Ergocalciferol capsules and Alpha D3 capsules (Alfacalcidol 0.25mcg to 0.5 mcg) orally once a day for four months and oral hypoglycaemic drugs Biguanides (Metformin 250mg to 500mg) orally twice a day as recommended by the physicians. Individualised diet control advice was imparted to the selected female subjects to enable control of blood sugars. To know the vitamin D status and glycaemic control of the selected vitamin D deficient subjects serum vitamin D (n = 11) and blood sugars (n = 25)were analysed after four months of medication.

Hypothesis: We hypothesized that vitamin D deficiency may be prevalent in a population of T2DM patients and that vitamin D may be related to glucose control in this group of patients. Hence we state the null hypothesis as "there is no difference in the Glycaemic control of the

vitamin D deficient and non-deficient newly detected female diabetics."

RESULTS AND DISCUSSION

The serum Vitamin D status of the selected female and male subjects is presented in the following table. This data could be obtained for about 25% of the selected subjects only. This data was used to identify the subjects for the case-control study.

Table 1: Vitamin D Status of the Selected Subjects (n = 103).

Vitamin D status	Females (n=77)		Males (n=26)	
	Numbers	%	Numbers	%
Severe deficiency (<10ng/mL)	29	38	04	15
Deficiency (10-20 ng/mL)	11	14	06	23
Sufficiency (20-30 ng/mL)	09	12	05	19
Normal (>30 ng/mL)	28	36	11	43
Total	77	100	26	100

A normal level of vitamin D is defined as a 25(OH)D concentration greater than 30ng/mL (75nmol/L), Vitamin D insufficiency is 25(OH)D concentration of 20 to 30ng/mL (50 to75 nmol/L), Vitamin D deficiency is 25(OH)D level less than 20ng/mL (50nmol/L) and Vitamin D severe deficiency is 25(OH)D level less than 20ng/mL (50nmol/L) (Stroud *et al.*, 2008).

In a study conducted at Sree Balaji Medical College and Hospital, Chrompet, Chennai, Tamilnadu, India the mean vitamin D value was 18.492ng/mL ±3.49 among the 50 cases of type 2 diabetics with mean FBG 146.22mg/dl (Balasubramanian *et al.*, 2012). The role of vitamin D3 in T2DM is suggested by cross-sectional studies showing that low serum concentrations of 25- hydroxyvitamin D_3 [25(OH)D_3] have association with impaired glucose tolerance and diabetes. There are more recent accumulating evidences to suggest that altered vitamin D3 and calcium homeostasis may also play a role in the development of T2DM.

The null hypothesis: There is no difference in glycaemic control of the cases and controls before the supplementation study.

Vitamin D deficient and Vitamin D non-deficient female subjects who were equally matched with respect to age, physical activity status, nutritional status and glycaemic control were selected for the case control study. The table below represents the glycaemic control the fasting glucose, postprandial glucose and HbA1c levels of the selected 50 female respondents of categorized two groups as 25 Vitamin D deficient and 25 non-deficient cases.

The fasting glucose and postprandial of the selected Vitamin D deficient female cases was 127.6±15.85mg/dl and 216.8 ±18.2mg/dl respectively. For the selected Vitamin D non-deficient female cases the glycaemic control remains moreover same as Vitamin D deficient female cases. The HbA1c was 7.29± 0.39% for Vitamin D deficient and 7.23±0.35% for Vitamin D nondeficient female cases. Among female newly detected diabetics there was no statistically significant difference between vitamin D deficient and non-deficient subjects with respect to FBG, PPBG and HbA1c $t(48) = 1.96$; $p \geq .05$; CI.95 . Therefore, we fail to reject the null hypothesis that there is no difference in glycaemic control of the cases and controls before the supplementation study. Therefore the two groups are equally matched.

Table-2: Initial Glycaemic Parameters and Vitamin D in the Selected Vitamin D Deficient and Non-Deficient Female Diabetics.

Parameters (means)	Cases	Controls	t value	df	p	95% CI	S/NS
FBG (mg/dl)	127.6±15.85	124.3±13.57	0.840	48	$p \geq .05$	1.96	NS
PPBG (mg/dl)	216.8 ±18.2	214.70±16.51	0.429	48	$p \geq .05$	1.96	NS
HbA1c (%)	7.29± 0.39	7.23±0.35	0.550	48	$p \geq .05$	1.96	NS
Vitamin D ng/mL	8.36 ± 0.8	35.12 ± 2.4	-49.33	48	$p \leq .05$	1.96	S

Cases -Vitamin D deficient (Serum level<10µg/mL), n = 25
Controls - Vitamin D non-deficient (Serum level >30µg/mL), n = 25

In the present study the glycaemic control of Vitamin D deficient cases was poor when compared to Vitamin D non-deficient cases. The mean HbA1c increased by 0.6% among Vitamin D deficient subjects; also fasting glucose increased by 12mg/dl and postprandial glucose by 14.2mg/dl. Among the Vitamin D non-deficient subjectsHbA1c stayed at good control, fasting glucose reduced with a difference of 13.3mg/dl and postprandial glucose came down by 13.9mg/dl. Poor glycaemic control was noticed in vitamin D deficient subjects when compared to vitamin D non-deficient subjects. Initially the HbA1c levels were almost the same in both the cases (7.29 ± 0.39 %) and control (7.23 ±0.35 %).patients, and they were well correlated ($r2 = 0.841$) and there is no difference between the case and control groups ($p \leq 0.001$).

In the cases group 25(OH)D_3 levels were lower than in the control group, 25(OH)D_3 levels being 8.36 ± 0.8ng/ml and 35.12 ± 2.42ng/ml respectively and the difference was significant ($p \leq 0.001$). In the vitamin D deficient group the HbA1c increased in the second month (7.32% ± 0.2) and fourth month (7.92% ± 0.3), whereas in the control group (non-deficient) the HbA1c values started reducing in the second month (7.04% ± 0.33) and fourth month (6.82% ± 0.2) showing good glycaemic control. At the end of four months of the case control study the hypothesized difference is taken as zero, the paired t test for the data analysis, the output was that the t value found to be greater when compared with one-sided critical value. Further the p value is small to believe that the difference has occurred due to chance. It shows that the difference is significant for fasting, postprandial blood sugars and HbA1c percentage among vitamin D deficient and non deficient subjects.

Table-3: Glycaemic Control in Vitamin D Deficient (cases) and Non-Deficient (controls) Female Diabetics.

Glycaemic Parameters	Time Period					
	Initial		After 2 months		After 4 months	
	D Def	Non-def	D Def	Non-def	D Def	Non-def
FBG(mg/dl)	127.6±15.85	124.3±13.57	135.8±12.6	115.9±12.63	140.4±11.7	111±12.3
PPBG(mg/dl)	216.8±18.2	214.70±16.51	222.4±18.22	209.5±19.4	231±15.97	200.8±23.5
HbA1c (%)	7.29± 0.39	7.23±0.35	7.32±0.22	7.04±0.33	7.92±0.33	6.82±0.2

The null hypothesis is rejected, that the glycaemic control in newly detected female diabetics with vitamin D deficiency and those without vitamin D deficiency is different**.** Talaei *et al.,* (2013) stated in a study of 100 participants including 70 women (70%) and 30 men (30%) took part in the study. All results were presented as Mean±SD, or medians of non-normally distributed. Around 24% of the participants were Vitamin D deficient {serum 25(OH)D ≤ 20ng/ml (50nmol/l)}. Mean serum 25(OH)D concentration was 43.03±19.28ng/ml (107.5±48.2nmol/l). The results at baseline and at the end, for FPG were 138.48±36.74mg/dl and 131.02±39mg/dl (P=0.05), for insulin, 10.76±9.46µIu/ml and 8.6±8.25µIu/ml (P=0.028) and for HOMA-IR, 3.57±3.18 and 2.89±3.28 (P=0.008) respectively and concluded that the data showed significant improvements in serum FPG, insulin and in HOMA-IR after treatment with vitamin D, suggested that vitamin D supplementation could reduce insulin resistance in T2DM.

Vitamin D Supplementation

From the above quoted studies we understand that vitamin D is required for insulin secretion and sensitivity. In our study the selected subjects were newly detected diabetics and we wanted to see the glycaemic outcome in the deficient and non-deficient cases. All subjects were treated for hyperglycaemia by the diabetologists. Dietary advice was given and all subjects attended a nutrition education session on dietary recommendations and physical activity. No OHA was given to both groups. Vitamin D supplementation was given to the deficient group initially up to a period of four months. After the fourth month all the selected vitamin D deficient female cases (n = 25) were administered therapeutic supplementation of vitamin D Ergocalciferol capsules and Alpha D3 capsules (Alfacalcidol 0.25mcg to 0.5 mcg) orally once a day for three months and oral hypoglycaemic drugs Biguanides (Metformin 250mg to 500mg) orally twice a day as recommended by the physicians because they could not achieve glycaemic control.

The mean vitamin D status of the control group of selected vitamin D deficient subjects is presented in the Figure-1. After supplementation of vitamin D from the fourth month of diagnosis, for the selected vitamin D deficient subjects, the serum vitamin D increased in the next four months by 15.46ng/mL. So the subjects responded well to the treatment and their serum vitamin D levels improved well. In one study lower 25(OH)D3 levels were observed in female diabetes mellitus type 2 patients with vitamin D deficiency than in controls (non-deficient female T2DM).

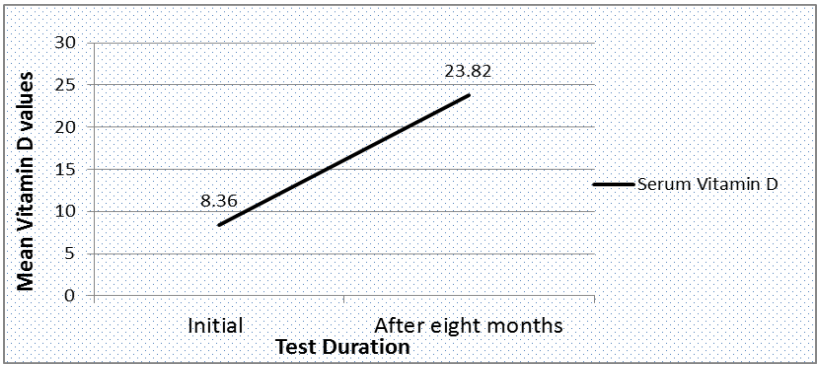

Fig. 1: Mean Vitamin D status of the Control Group Subjects (n = 11) after Supplementation at Fourth to Eighth Month.

Test Duration Since we could follow only eleven vitamin D deficient subjects after the fourth month when OHA and vitamin D supplementation was given the table below represents the glycaemic status of the cases group (vitamin D deficient subjects n=11).

Table-4: Glycaemic status of the Selected vitamin D Deficient Subjects (n=11).

Glycaemic parameters	On recruitment	After four months without OHA	After eight months with OHA + Vitamin D	Difference Between fourth and eighth month	"t" value
FBG mg/dl	124.09± 17.72	142.65± 13.44	114.73 ± 5.55	-27.92	8.612*
PPBG mg/dl	213.09± 21.13	224.12± 16.46	171.18 ±9.84	-53.94	14.442*
HbA1c %	7.17± 0.47	7.95 ± 0.28	7.4 ± 0.26	-0.55	13.987*

*Significant; $p \leq 0.05$

From the table it is clear that Vitamin D supplementation along with OHA has been a means of bringing about glycaemic control among those with vitamin D deficiency. For the selected vitamin D deficient subjects, FBG reduced by 27.92mg/dl (t= 8.612), PPBG reduced by 53.94mg/dl (t= 14.442) and HbA1c also reduced by 0.55% (t= 13.987). On recruitment HbA1c levels were almost the same in the cases and the control group, HbA1c levels being 7.29 ± 0.39% and 7.23 ±0.35%, respectively ($p < 0.001$). In the cases group of diabetes mellitus type 2 patients, 25(OH)D3 levels were lower than in the control group, 25(OH)D3 levels being 8.36 ± 0.8ng/ml and 35.12 ± 2.42ng/ml in the patients, respectively ($p < 0.001$). At the second month group subjects HbA1c was 7.32 ± 0.2% and by fourth month 7.92 ± 0.3%, whereas the control groups second month HbA1c (7.04 ± 0.33%) and fourth month HbA1c (6.82 ± 0.2%) remained good control. After eight months in the trial group T2DM vitamin D deficiency patients with administration of vitamin D supplements and OHA, had achieved the HbA1c control (7.4 ± 0.26%) and sufficient vitamin D status (23.82 ± 2.6ng/ml).

In one study, Vitamin D levels were found to be negatively correlated with HbA1c levels. This was in agreement with other studies (Cimbek, *et al.,* 2013). HbA1c is considered an indicator of average blood glucose concentrations during the preceding 2–3 months and thus a long-term marker of glucose homeostasis. There was a negative correlation between Vitamin D levels and PP, but no correlation between fasting glycemia and 25(OH)D was found, although that, Vitamin D levels being related to glycemic control where HbA1c reflects glucose control over time and it is more accurate than FBS and PP. At fourth month the vitamin D deficient group was administered vitamin D supplements and OHA, had achieved the HbA1c control (7.4 ± 0.26 %) and sufficient vitamin D status (23.82 ± 2.6ng/ml).

CONCLUSION

Vitamin D status is important in the management of individual glycaemia was independently associated with HbA1c. In the present study the glycaemic control of Vitamin D deficient cases was poor when compared to Vitamin D non-deficient cases as indicated by fasting blood glucose, postprandial blood glucose and HbA1c values. We have been able to prove this in the eight months of the follow-up. Regular laboratory tests are needed to assess vitamin D levels can be useful in in the management of T2DM.

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