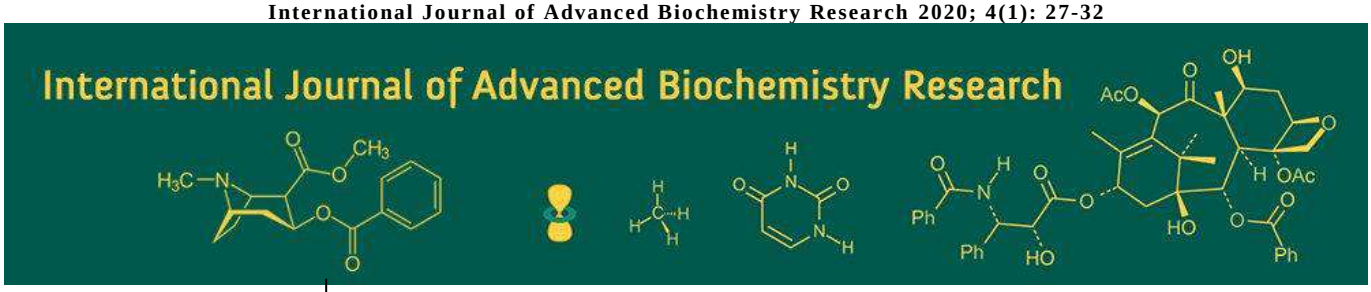

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Vitamin d and parathyroid hormone levels and their association with insulin resistance in type 2 diabetes subjects

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Abstract
Vitamin D deficiency is associated with metabolic diseases like Type-2 diabetes mellitus, obesity and insulin resistance. Previous studies have evaluated the effects of vitamin D and parathyroid hormone (PTH) separately in isolation rather than studying the combined effects of both hormones together as a reflection of the status of the PTH-Vitamin D axis. Low vitamin D coupled with increased PTH may be a better indicator, which is the novelty of the present study.
A total of 151 Type 2 diabetic subjects attending the outpatient clinics of endocrinology department of MMC&RI, Mysore, were chosen for the study. Vitamin D, PTH, calcitonin and Insulin levels were analysed using chemiluminescence Immuno assay techniques. Comparison was made between normal (vitamin D >20ng/ml) and vitamin D deficient (vitamin D <20ng/ml) groups. Significant increase in FBS, HbA1c, insulin levels and HOMA-IR value was seen in vitamin D deficient group ($p<0.05$). Vitamin D was negatively correlated with PTH, FBS, PPBS and HbA1c at $p<0.01$.
Significant vitamin D deficiency and increased PTH levels are seen in Type 2 diabetic subjects. Insulin resistance was more common among diabetic subjects having both vitamin D deficiency and elevated PTH when compared to those diabetics with isolated vitamin D or elevated PTH.

Keywords: Vitamin D, PTH, diabetes, insulin resistance

1.1 Introduction
It has been estimated that 1 billion people worldwide have Vitamin D deficiency or insufficiency [1, 2]. In 2000, a study by All India Institute of Medical Sciences, New Delhi showed that up to 90% of apparently healthy subjects were having hypovitaminosis D [3]. This comes as a surprise since we are living in a tropical country and have ample sunlight throughout the year.
Vitamin D deficiency is associated with metabolic diseases like obesity, higher fasting glycemia and insulin resistance [4]. However, there are conflicting reports stating that Vitamin D supplementation has not consistently benefited in reversing hyperglycemia despite achieving normal Vitamin D range [5].
Previous studies have evaluated the effects of Vitamin D and PTH with insulin resistance (IR) and beta cell function separately rather than studying the combined effects of both hormones together as a reflection of the status of the PTH-Vitamin D axis [6, 7]. For the comprehensive assessment of any hormone it is very important to study the effect of functional regulators of the hormone in conjunction with each other. There is a paucity of literature on the combined assessment of Vitamin D and PTH and their effects on insulin sensitivity and glycemic status in Type 2 diabetic (T2DM) subjects [8].
Studies have shown that the active form of vitamin D, calcitriol, does not impact insulin release when pancreatic islets are under normal conditions, instead requiring a stressed environment such as exposure to pathologic cytokines or vitamin D deficiency for the detection of its effect [8]. Thus it would appear that low vitamin D coupled with increased PTH may be a better indicator of reflecting the state of vitamin D deficiency/insufficiency that leads to dysregulation of glucose homeostasis, which is the novelty of the present study.

Previous studies have shown conflicting relationship between low vitamin D and impaired glucose homeostasis. Therefore, in the current study we aimed at analyzing the impact of vitamin D in combination with PTH in evaluating the effect of vitamin D on glucose homeostasis and insulin resistance in diabetic subjects.

1.2 Objectives

- Estimating vitamin D, PTH, FBS, HbA1C levels and the ratio of Vitamin D/PTH, Insulin, calcium and phosphorous levels in Type 2 diabetic subjects
- Estimating the markers of Insulin resistance HOMA-IR, HOMA beta cell and QUICKI (Quantitative Insulin sensitivity check index) in the above subjects

1.3 Methodology

Study population and study design

It is a cross sectional study. Participants for the study were selected from the type 2 diabetic subjects who visited endocrinology OPD of K.R. Hospital, Mysuru, during July 2016 to March 2018. The study enrolled 151 T2DM subjects of 18 to 60 years age. Participants on supplementation of vitamin D and calcium and those having thyroid diseases were excluded.

The study was approved by institutional ethics committee at Mysore medical college & research institute, Mysuru. A written informed consent was taken from all the participants before enrolment.

Basic information was collected as a questionnaire, which included age, gender, occupation, food habit, information about presence of metabolic disorders like Diabetes, Obesity, Osteoporosis, any treatments they underwent, etc. was collected as elicited by the patients.

Around 5 ml of blood was collected from participants and processed for whole blood and serum and stored at -80 ºC till further use. Fasting (FBS) and Post Prandial Blood Sugar (PPBS), Calcium, Phosphorus in serum and HbA1C in EDTA-blood were analysed using Cobas C311 fully automated chemistry analyser (Roche diagnostics) using spectrophotometric principles.

Vitamin D, PTH, calcitonin and insulin levels were analysed using Cobas E411 fully automated immunoassay analyser (Roche diagnostics) using chemiluminescence immuno assay technique.

Insulin sensitivity and Insulin resistance parameters were calculated as follows:

Homeostasis model assessment insulin resistance [HOMA-IR] was analyzed using formula: [9]

$$\text{HOMA-IR} = [\text{Fasting insulin } (\mu U/ml) \times \text{Fasting glucose } (mg/dl)]/405$$

The quantitative insulin sensitivity check index [QUICKI] is calculated as

$$\text{QUICKI} = 1/\log(\text{Fasting insulin}) + \log(\text{Fasting glucose}).$$

Homeostasis model assessment β cell function (HOMAβ-cell function) was calculated as

$$\text{HOMA } \beta \text{ cell\%} = \text{Fasting insulin } (\mu U/ml) \times 360 / \text{Fasting glucose } (mg/dl) - 63.$$

Diabetes was defined as Fasting blood glucose ≥126 mg/dL, Post prandial blood glucose ≥200 mg/dl, HbA1c ≥6.5% or use of oral anti-diabetic agents (after diagnosis of T2DM). HOMA-IR score ≥3 is considered as insulin resistant.

Based on vitamin D concentration subjects were categorized as deficient (<20ng/ml), insufficient (20 to 29ng/ml) and sufficient (≥30ng/ml). Comparison of biochemical parameters was made between vitamin D groups of <20 ng/ml and >20ng/ml. Tertiles of PTH were defined as 1sttertile (PTH ≤34.6pgm/ml), 2ndtertile (PTH 34.6 to <43.8 pgm/ml) and 3rd tertile (PTH ≥43.8 pgm/ml).

Statistical methods

Data are represented as mean ± standard deviation or % prevalence. The difference in mean values between groups was tested by independent samples t-test. Pearson correlation coefficient was calculated for vitamin D with other parameters. Difference in prevalence between groups was analysed by z-test. Statistical tests were performed using SPSS statistical software.

1.4 Results

The study population consisted of 151 T2DM subjects which included 53 male and 98 female participants with mean age of 51±7 years (Table 1).

Study participants were grouped into vitamin D deficient (<20ng/ml) and normal (which includes both vitamin D sufficient and insufficient, >20ng/ml) subgroups. Majority of females (63%) were vitamin D deficient when compared to males (40%). Overall, 55% of the participants were vitamin D deficient, 23% were insufficient (20-30ng/ml) and 22% were vitamin D sufficient (>30ng/ml) as shown in Fig 1. The prevalence of obesity, central obesity and IR were not significantly different between deficient and normal vitamin D groups (Table 2). Whereas, the prevalence of hyperinsulinemia was significantly higher in vitamin D deficient group compared to normal.

Comparison between vitamin D deficient and normal groups as indicated in Table 3 showed significant increase in FBS, HbA1c and fasting insulin in vitamin D deficient group.

The evaluation of the implications of vitamin D status on glucose homeostasis and IR showed significant increase in FBS, HbA1c, insulin and IR in those diabetic individuals with vitamin D deficiency when compared to that of vitamin D normal group (Table 3). A significant rise in PTH and reduction in vitamin D: PTH ratio was observed in vitamin D deficient group (Table 3). However, there was no significant difference in calcitonin, calcium and phosphorus was observed between vitamin D groups.

Further, tertiles of PTH, based on its concentration were made to evaluate the combined effect of vitamin D and PTH status on IR in diabetic subjects. The data as indicated in figure 2, showed that vitamin D deficiency with higher level of PTH (3rd tertile) showed clinically meaningful increase in the prevalence of IR though the statistical significance was not found.

The correlation data indicated that (Table 4) vitamin D showed significant negative correlation with PTH and glycemic status at $p<0.01$.

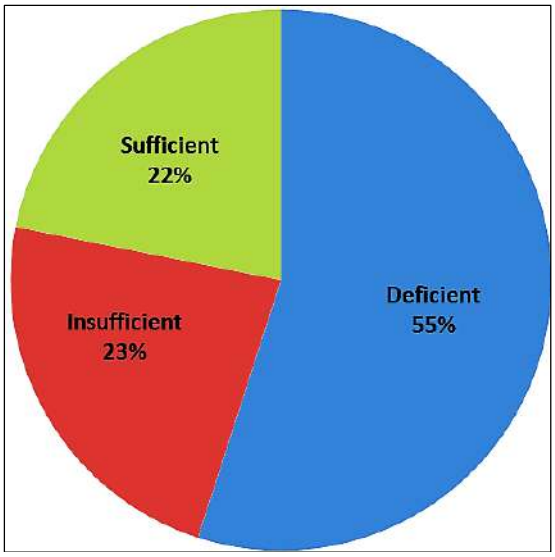

Fig 1: Vitamin D status in T2DM subjects Deficient <20ng/ml, Insufficient 20-30ng/ml, Sufficient >30ng/ml.

Table 1: Anthropometric and biochemical parameters of the study subjects

Anthropometric data	Mean ±SD (n=151)	95% CI
Gender (Count)		
Male	53	
Female	98	
Age (years)	51±7	49.33-51.74
BMI (kg/m2)		
Male	25.04±2.92	24.24-25.85
Female	27.99±4.32	27.12-28.86
Waist circumference (cm)		
Male	94.45±8.83	92.01-96.88
Female	91.58±10.01	89.57-93.58
Waist: Hip ratio		
Male	0.98±0.05	0.96-0.99
Female	0.89±0.05	0.88-0.90
Biochemical Parameters		
FBS (mg/dl)	164.63±66.09	153.63-175.20
PPBS(mg/dl)	254.96±87.92	240.00-268.78
HbA1C (%)	8.68±2.13	8.31-9.01
Fasting Insulin (µU/ml)	18.46±16.68	15.74-21.16
HOMA-IR (%)	7.94±12.19	5.954-9.91
Beta Cell Function (%)	84.64±102.40	68.00-101.27
QUICKI	0.30±0.03	0.29-0.30
Calcium (mg/dl)	9.22±0.43	9.15-9.29
Phosphorous(mg/dl)	3.59±0.53	3.50-3.67
Vitamin D (ng/ml)	21.10±11.61	19.23-23.03
PTH (pg/ml)	43.24±21.63	39.79-46.86
Calcitonin (pg/ml)	3.84±5.89	2.83-4.72
Vit D : PTH ratio	618.53±472.72	542.59-697.51

Table 2: Comparison of prevalence of risk factors of insulin resistance between vitamin D groups

Parameters	Vitamin D Deficient (Vit D<20ng/ml) (n= 83)	Vitamin D Normal (Vit D ≥20ng/ml) (n=68)	p-value
Gender (count):			
Male	21 (40%)	32(60%)	0.014
Female	62 (63%)	36 (37%)	
Obesity	57 (69%)	39 (57%)	0.151
Central obesity	73 (88%)	53 (78%)	0.099
Hyper insulinemia	31 (37%)	9 (13%)	<0.001
Insulin resistance	68 (82%)	55 (81%)	0.87

Values are count (%). Significant difference between groups was tested by z-test.

Table 3: Comparison between vitamin D deficient and normal groups

Parameters	Vitamin D Deficient (Vit D<20ng/ml) (n= 83)	Vitamin D Normal (Vit D ≥20ng/ml) (n=68)
Gender (count):		
Male	21	32
Female	62	36
Age (years)	50.12±7.42	51.42±7.51
BMI:		
Male	25.32±3.124	24.85±2.81
Female	28.17±4.26	27.69±4.4
Waist circumference (cm):		
Male	95.19±9.7	93.96±8.33
Female	92.5±10.35	90±9.34
Waist/Hip ratio:		
Male	0.98±0.05	0.98±0.04
Female	0.90±0.06	0.89±0.04
FBS (mg/dl)	173.96±71.97	153.23±56.56*
PPBS(mg/dl)	267.62±92.19	239.50±80.37
HbA1C (%)	9.012±2.31	8.27±1.82*
Insulin (µU/ml)	20.98±19.87	15.49±10.72*
HOMA IR (%)	9.808±15.7	5.734±4.33*
Beta cell (%)	82.72±123.64	86.67±67.8
QUICKI	0.296±0.03	0.3052±0.021*
Calcium (mg/dl)	9.162±0.45	9.299±0.3827
Phosphorous(mg/dl)	3.58±0.55	3.59±0.50
Vitamin D	12.85±4.35	31.16±9.55**
PTH	49.1±25.55	36.08±12.41**
Calcitonin (pg/ml)	3.003±3.35	4.86±7.86
Vit D: PTH ratio	325.0±180.4	976.79±472.7**

Values are Mean± SD. Independent samples t-test was performed to analyze the significant difference in mean values between groups.
p<0.05*, P<0.001**

Table 4: Correlation of vitamin D with other biochemical parameters

Parameter	Pearson Correlation	*P* value
Vit D vs. PTH	-.351	<0.01
Vit D vs. calcitonin	0.092	0.263
Vit D vs. Insulin	-0.103	0.213
Vit D vs. FBS	-.256	0.002
Vit D vs. PPBS	-.227	0.005
Vit D vs. HbA1c	-.235	0.004
Vit D vs. HOMA-IR	-0.153	0.064
Vit D vs. HOMA β	0.058	0.481
Vit D vs. QUICKI	0.139	0.089
Vit D vs. Calcium	0.124	0.129
Vit D vs. Phosphorous	0.011	0.896

Vitamin D showed significant negative correlation with PTH and glycemic status at *p*<0.01.

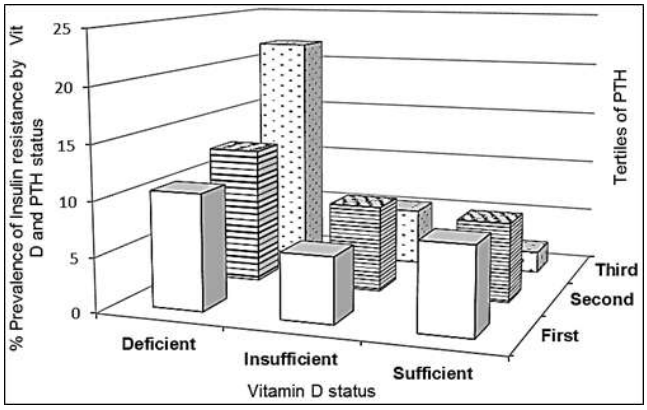

Fig 2: Prevalence of insulin resistance by vitamin D and PTH status in T2DM subjects

Among 151 T2DM subjects, 128 (81.5%) were having insulin resistance. Further, prevalence of IR stratified was by vitamin D and PTH status. There was no statistically significant difference between groups, however, vitamin D deficient with higher level of PTH subjects showed clinically meaningful increase in the prevalence of IR.

1.5 Discussion

The present study has shown that out of 151 Diabetic subjects, 78% of the participants were vitamin D deficient/insufficient with 55% having vitamin D deficiency and 23% having vitamin D insufficiency. This observation is supported by the studies by Goswami *et al.* [10], Sowjanya Bachali *et al.* [11], and Kirubhakaran Kanakaraju *et al.* [12] and a study by Daga *et al.* [13] from North India. Vitamin D deficiency seen in the subjects, despite India being a tropical country with plenty of sunlight has been attributed to use of sunscreen lotions which prevent vitamin D synthesis, modern lifestyle which limits the outdoor activity, old age, consumption of foods not fortified with vitamin D and in females who follow purdah system [12].

T2DM is found to have low grade inflammation due to the increase in circulating cytokine such as TNF and IL-6 which also contributes to the development of insulin resistance. Vitamin D being an immunosuppressant, has been shown to down-regulate the transcription of various proinflammatory cytokine genes like Interleukin-2, Interleukin-12, and TNF-α. Studies have shown that Vitamin D3 has a protective role on β cell mass and prevents it from apoptosis. Decreased vitamin D levels seen in the current study subjects further potentiates the role of vitamin D in Pathogenesis of T2DM [12].

Vitamin D is known to have a direct effect on β-cell function mediated by binding of the circulating active form, 1,25-dihydroxy vitamin D [1,25(OH)2D], to the vitamin D receptor, which is expressed in pancreatic β-cells [14]. Mice lacking a functional vitamin D receptor show impaired glucose-stimulated insulin secretion, due to a reduction in

insulin biosynthesis. Importantly, the enzyme 25-hydroxyvitamin D-1a-hydroxylase (CYP27B1),which is expressed in β-cells causes activation of vitamin D within the β-cell thereby allowing for a paracrine effect of circulating 25(OH)D [14]. The calcitriol directly activates the transcription of human insulin receptor gene, activates peroxisome proliferator activator receptor (PPAR). Vitamin D stimulates the expression of insulin receptor and enhances insulin mediated glucose transport *in vitro* [11]. Significant increase in FBS, HbA1C and fasting insulin seen vitamin D deficient T2DM group in the present study is consistent with the above findings. Regulation of insulin secretion by vitamin D appears to be independent of calcium concentration, however, because insulin secretion is a calcium dependent process, the effects of vitamin D may be indirectly mediated via regulation of calcium flux through the β-cell [14].

The present study also shows significant insulin resistance (as seen by increased HOMA-IR and decreased QUICKI-quantitative insulin sensitivity check index) however the inverse association of 25(OH)D with HOMA-IR and insulin were not statistically significant in the present study. While some studies have shown significant inverse association of 25(OH)D with HOMA-IR [15, 16] others have shown inconsistent findings [14].

Data on observational studies and clinical trials involving vitamin D supplementation in T2DM are inconsistent. Many studies have reported inverse associations between 25(OH)D levels and the effectiveness of glycemic control while others have found no such associations. Moreover, the effect of PTH, a known mediator of vitamin D action, was not considered [17]. Studies have shown that increased PTH concentration is associated with lower insulin sensitivity and higher prevalence of metabolic syndrome independently of vitamin D status. Furthermore, studies which did not show significant association between HbA1c and 25(OH)D levels became significant after adjusting for PTH levels. Previous studies have explained the relationship between vitamin D and PTH in the context of hypovitaminosis D and secondary hyperparathyroidism, without considering the independent effects of PTH on the glycemic status and insulin resistance. Recent studies have also suggested that rather than individual association, hypovitaminosis D combined with high parathyroid hormone concentrations are more significantly associated with glycaemic dysregulation as shown in a study by Karras *et al.* [18], in elderly patients with prediabetes. Present study also shows significant negative correlation of vitamin D and PTH, very significantly decreased vitD/PTH ratio in vitamin D deficient type 2 DM group, increased prevalence of IR in a strata of subjects who were vitamin D deficient with higher level of PTH which are all consistent with the above explanation. The precise mechanisms of how increased PTH is associated with increased IR are not clear. Data on the independent and combined effects of vitamin D and PTH on the glycemic status of general populations and also in T2DM involving vitamin D supplementation are lacking. Hence large population based and cross sectional studies are recommended to validate these findings.

1.6 Conclusion

Thus the present study concludes that significant vitamin D deficiency/insufficiency was observed in type 2 diabetes subjects. The insulin resistance markers were higher in type 2 diabetes subjects with vitamin D deficiency/insufficiency. Increased prevalence of insulin resistance in a strata of type 2 DM subjects who were vitamin D deficient with higher level of PTH. Large population based studies are required to validate the findings.

1.7 Acknowledgement

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