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Association of 25 Hydroxy Vitamin D and Microalbuminuria in Patients with Diabetic Nephropathy

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Abstract: Vitamin D, inspite of having role in maintaining the good functioning of bone metabolism, has anti-proliferative effect in cellular differentiation, immunomodulation and inhibition of the Renin - Angiotensin System (RAS). The aim was to assess the levels of serum 25-hydroxy vitamin D in patients with Diabetic nephropathy and to find the association between 25(OH)D levels and microalbuminuria in patients with Diabetic nephropathy. This case control study conducted after Ethical committee clearance comprised 50 Type 2 Diabetic nephropathy subjects & 20 age matched healthy individuals. ELISA-for serum 25- hydroxy vitamin D, GOD- POD for fasting glucose, Creatinine by Modified Jaffe's method, Arsenazo III method for calcium, Ammonium molybdate method for phosphorus, Creatinine by Modified Jaffe's method were studied. Urine Microalbumin was measured by Immunoturbidimetry method and Albumin Creatinine ratio was estimated. The mean 25 Hydroxy vitamin D concentration in patients with Diabetic nephropathy was 5.089±2.33ng/ml and in healthy individuals was 15.8805±3.91ng/mL, with highly significant p value (*p*= 0.0001). A negative linear correlation was seen between 25 hydroxy vitamin D levels and microalbumin (r = -0.703). Serum 25 hydroxy vitamin D was decreased in Diabetic nephropathy (Vitamin D deficiency), reduced in healthy individuals (Vitamin D insufficiency). Cautious supplementation with vitamin D may improve glycemic control and microalbuminuria in Type 2 DM.
Keywords: 25(OH)D - 25 Hydroxy vitamin D , BMI- Body Mass Index, RAS- Renin Angiotensin system, ELISA – Enzyme Linked Immunosorbent Assay, GOD- POD - Glucose oxidase peroxidase, DN -Diabetic Nephropathy.

INTRODUCTION

Diabetes Mellitus is the most common metabolic disorder, characterised by Chronic hyperglycemia which is associated with disturbance in metabolism of carbohydrate, fat and protein. According to World Health Organisation (WHO), approximately 250 million people currently have diabetes worldwide and this number will reach 380 million by the year 2030. India is considered as the Diabetes capital of the world. In India, approximately 40 million people found to have diabetes and this will reach 70 million by the year 2030[1].

This impending epidemic is also expected to trigger a steep increase in the complications associated with diabetes, such as nephropathy, ischemic heart disease, neuropathy, stroke and retinopathy[2]. Out of 100 diabetic people, nearly 33% of them gradually acquire Diabetic Nephropathy. Diabetic nephropathy is identified clinically at the earliest by microalbuminuria. The factor that may impact the differential development of diabetic nephropathy is vitamin D. Vitamin D, despite having role in maintaining the good functioning of bone metabolism, has anti –proliferative effect in cellular differentiation, immunomodulation and inhibition of the renin - angiotensin system (RAS) [3]. Vitamin D deficiency and insufficiency have an active role in the progression of kidney disease[4].Inhibition of the renin-angiotensin system by the vitamin D metabolite has been demonstrated in vitro; animal studies suggest that receptor-mediated vitamin D actions have a renoprotective role in diabetic nephropathy[5].

AIMS AND OBJECTIVES

To evaluate 25 hydroxy vitamin D levels and to correlate 25 hydroxy vitamin D & microalbuminuria in Diabetic Nephropathy patients.

MATERIALS AND METHODS

This case control study was done after obtaining the approval from institutional ethical committee.

This case control study conducted after Ethical committee clearance. The Study comprised

Cases - 50 Diabetic Nephropathy patients
Controls - 20 Age matched healthy individuals

Inclusion criteria

Diabetics were diagnosed using ADA (American Diabetes Association) criteria. Duration of diabetes less than 5 yrs.

Exclusion criteria

Liver failure, vitamin D deficiency patients, Type 1 Diabetes Mellitus without complication, Obstructive uropathy, chronic glomerulonephritis, malabsorption syndrome, patients taking drugs like barbiturates, phenytoin, Rifampicin, calcium, vitamin D, Pregnant and lactating mothers.

METHODS

Fasting venous blood sample was collected with strict aseptic precautions. Early morning Mid-stream urine specimen was collected in plastic sterile containers

- Serum 25 hydroxy vitaminD levels were determined by ELISA
- Plasma glucose by Glucose oxidase peroxidase method
- serumCreatinine by Modified Jaffe's method
- Serum calcium by Arsenazo III method
- Serum phosphorus by Ammonium molybdate method
- Urine Microalbumin was measured by Immunoturbidimetry method and Albumin Creatinine ratio was estimated.

Table-1: Serum 25(OH) D levels & its nutritional status

Serum 25(OH)D	Vitamin D nutritional status
>50 nmol/L (>20 ng/mL)	Sufficiency
30–50 nmol/L (12–20 ng/mL)	Insufficiency
12–30 nmol/L (5–12 ng/mL)	Deficiency
<12 nmol/L (<5 ng/mL)	Severe deficiency

Units: conventional units (ng/mL) or international system (SI) units (nmol/L). The conversion factor to SI units is: 1 ng/mL = 2.496 nmol/L[6].

STATISTICAL ANALYSIS

Data were analysed by SPSS software 16 version. Statistical analysis was performed using student's t-Test to detect the association between the selected variables. Pearson coefficient correlation was done on the selected variables in order to find the linear relationship in both cases & control groups.

RESULTS

There was highly significant difference between cases and controls with respect to fasting glucose, serum creatinine. There was significant difference between cases and controls with respect to BMI, while there was no significant difference among cases and controls with respect to smoking, alcohol intake, and the presence of hypertension, serum calcium, and serum phosphorus.

Table-2: Characteristics of patients in the study population

VARIABLES		**CONTROL**	**CASE**	**p VALUE**
AGE		56.71± 7.11	58.82± 5.11	.11 –NS
GENDER	MALE	12(48.88%)	28(53.33%)	.83 – NS
	FEMALE	08(51.11%)	22(46.66%)	
HYPERTENSION		16(35%)	20(44.44%)	.51 –NS
SMOKING		5(11.11%)	7(15.55%)	.75 – NS
ALCOHOLISM		4(8.88%)	4(8.88%)	1.00 – NS
BMI		27.21± 1.78	28.79± 2.47	0.01 – S
UACR		2.56± .64	124.89± 67.29	.000- HS
CREATININE		.62 ± .06	.68± .08	.000- HS
FASTING GLUCOSE		127.78 ± 29.72	188.44± 70.86	.000- HS
CALCIUM		9.63± .37	9.63± .37	1.000- NS
PHOSPHORUS		3.04 ± .33	3.08± .37	.540- NS

Table-3: 25 Hydroxy vitamin D levels among cases and controls

	CASES		CONTROLS		Student t test
25 Hydroxy vitamin D	Mean	SD	Mean	SD	.000
	5.6309	2.34	15.6062	3.46	

The mean and standard deviation of the 25 Hydroxy vitamin D levels among cases and controls were presented in Table 3 .The mean 25 Hydroxy vitamin D concentration in cases (patients with diabetic

nephropathy) was 5.63± 2.34 ng/ml, while in controls(healthy individuals) it was 15.60±3.46ng/ml (figure 1).. The difference in 25 Hydroxy vitamin D values between cases and controls were highly significant (p= 0.000).

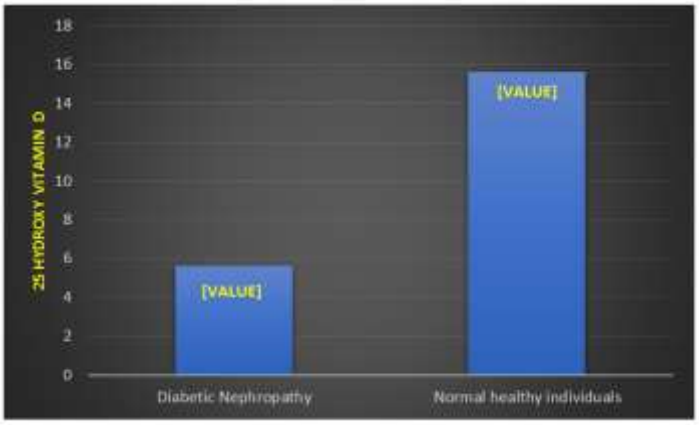

Fig-1: Bar diagram showing 25 Hydroxy vitamin D levels among cases and controls

	CASES	CONTROLS
Pearson correlation	-.115	.060
p value	.451	.699

Pearson coefficient correlation was done on variables like UACR and vitamin D in order to find the linear relationship in both the groups. It was observed that the concentration of UACR increases, the concentration of vitamin D decreases with a weaker negative linear relationship with r=-0.115 in cases

	CASES	CONTROLS
Pearson correlation	-.022	.051
p value	.884	.737

Pearson coefficient correlation was done on variables like vitamin D and fasting glucose in order to find the linear relationship in both the groups. It was observed that the concentration of fasting glucose increases, the concentration of vitamin D decreases with a weaker negative linear relationship with r=-0.022 in cases

DISCUSSION

DN is the most common complication of DM, often leads to End Stage Renal Disease (ESRD) with high mortality rate[7]. DN is associated with high rate of cardiovascular mortality, whose risk is two or three times increasing, when associated with proteinuria[8]. RAS has been implicated as a major mediator of progressive renal injury in DN. Hyperglycaemia stimulates the production of cytokines, including the angiotensin II (Ang II). This is a vasoactive peptide with glomerular hemodynamic actions that contributes to the onset of proteinuria. Hyperglycaemia causes the intrarenal production of factors by downregulating Vitamin D Receptor (VDR) and 1α –hydroxylase in proximal tubule cells, resulting in decrease in 1,25dihydroxyvitamin D3 reabsorption with increased levels of protein urinary excretion[9].The combination of hyperglycemia and the absence of Vitamin D Receptor (VDR) results in an intrarenal increase of RAAS activation, and simultaneously there is evidence that deficits in the active metabolite of 1,25 dihydroxyvitamin D3 indirectly stimulate the activation of TGF -1β[10]. In animal models, 25 (OH) vitamin D suppresses the RAS, and lower 25 (OH) vitamin D levels are particularly detrimental in the setting of RAS activation and hyperfiltration, which are characteristic of DN[11]. The use of vitamin D analogs to block RAS activation exerts a therapeutic effect by increasing the action of RAS blockers. 1,25 dihydroxyvitamin D3 and its analogues reduce proteinuria, a biomarker of kidney involvement. Thus, 1,25 dihydroxyvitamin D3 has protective functions by promoting the reduction of proteinuria. Angiotensin II has a pro -fibrotic, pro –angiogenic and pro -inflammatory actions. It is the main mediator of TGF β1 and connective tissue growth factor (CTGF) production at the level of mesangial and tubular cells, leading to an increased production of extracellular matrix and contributing to the development and progression of glomerulosclerosis and tubulointerstitial fibrosis, typical features of DN[12]. It regulates mesangial cell growth, by promoting glomerular proliferation or hypertrophy, and also promotes an increased expression and synthesis of extracellular matrix proteins, such as fibronectin, laminin, and collagen IV[13]. In its pro -angiogenic action, hypoxia

triggers increased expression and synthesis of VEGF, contributing to the progression of renal lesion[14].

In the present study, the mean of 25(OH) D was 5.6309±2.34 (vitamin D deficiency)and was observed to be very much decreased in DN, , the mean of 25(OH) D was 15.6062±3.46(vitamin D insufficiency) and was observed to be reduced in normal healthy individuals. The difference in 25(OH) D values between cases and controls were highly significant (p= 0.000). Similar result was also obtained by Vanessa A. Diaz et al. Studies demonstrating a benefit to vitamin D supplementation to prevent the progression of renal disease suggest that this may be a strategy to consider in future studies.

CONCLUSION

25hydroxy vitamin D was decreased in Diabetic Nephropathy patients (Vitamin D deficiency), reduced in healthy individuals (Vitamin D insufficiency. When a diabetic patient progresses from norm albuminuria to microalbuminuria, vitamin D levels decreases significantly. Cautious supplementation with vitamin D may improve glycemic control and microalbuminuria.

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