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RESEARCH ARTICLE

THE ASSESSMENT OF VITAMIN D STATUS IN THE PATIENTS OF DIABETIC NEPHROPATHY: A CASE CONTROL TERTIARY INSTITUTIONAL BASED STUDY

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ABSTRACT

Introduction: Diabetic nephropathy is an established risk factor for several cardiovascular diseases and the leading cause of chronic kidney disease in patients. Vitamin D promotes calcium metabolism and normal mineralization and formation of bone. Vitamin D has a great role in cell growth, proliferation, differentiation and apoptosis. It has important functions on neuromuscular and immune system.
Aims: To evaluate the status of vitamin D3 level in diabetic nephropathy patients and to find out any sort of relation of vitamin D3 level with diabetic nephropathy.
Materials and Methods: This observational, cross-sectional, case-control study was conducted in Hi-Tech Medical College & Hospital, in an urban area of Bhubaneswar, Odisha, over a period of two year (January 2013 to December 2014). Serum vitamin D3 levels were estimated in adult 200 cases of diabetic nephropathy as well as 50 age-sex matched normal individuals of same age group in the OPD of the Department of Medicine and Nephrology.
Results: Serum vitamin D3 level is remarkably decreased in diabetic nephropathy patients in comparison with that of the control group. And it is observed that in the older age group of diabetic nephropathy patients, the serum vitamin D3 levels are drastically reduced in contrast to that of the same age group of controls. It is also revealed that vitamin D3 levels are remarkably decreased with the progression of diabetic nephropathy.
Conclusion: From this study, we can draw a conclusion that the assessment of serum vitamin D3 in the cases of diabetic nephropathy is of prime importance and should start the treatment of vitamin D3 insufficiency and deficiency accordingly as soon as possible with the treatment of diabetic nephropathy.

INTRODUCTION

Vitamin D is associated with calcium metabolism and bone formation- it is our traditional way of thinking. It is very true that vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany. It is also needed for bone growth and bone remodelling by osteoblasts and osteoclasts. (Institute of Medicine, 2010; Cranney *et al.,* 2007) But now a day, the ideas are changing. Vitamin D serves several important roles in our body beyond this calcium regulation. Vitamin D is involved in regulation of neuromuscular & immune system and reduction of inflammation. (Institute of Medicine, 2010; Holick, 2006; Norman and Henry, 2006) This fat soluble vitamin also takes a great participation in modulation of cell growth. It plays an important role in cell proliferation, maturation and its differentiation. Vitamin D takes some responsibility in cell apoptosis (Institute of Medicine, 2010).

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In recent study, it is reported that vitamin D supplementation in infancy reduces the risk of development of type 1 diabetes. (Holick, 2004)

Vitamin D is converted to active metabolite form of 1, 25 $(OH)_2$ D_3 or calcitrol or vitamin D_3 by two consecutive steps of hydroxylation. The former step of hydroxylation occurs in liver while the later hydroxylation happens in kidney. The hydroxylation step is due to 25-hydroxylase; a hepatic enzyme, by which vitamin D is transformed into 25-(OH) vitamin D. In the next step, this 25-(OH) vitamin D is reduced to form calcitrol by 1 α-hydroxylase, a renal enzyme. After this vitamin D_3 binds with nuclear vitamin D receptor (VDR). It leads to VDR activation and heterodimerization with the Retinoid X receptor (RXR) and other concerned cofactors, to form VDR-RXR complex. This complex binds to the vitamin D respond elements of target genes to regulate gene transcription. This VDR is found in several organs like intestine, kidney, bone, different types of cells including the cells involved in immune system. Diabetic nephropathy is a risk factor for cardiovascular disease and the leading cause of chronic kidney disease in patients starting renal replacement therapy. (Gross *et al.,* 2005)

Aims and Objectives

The aim of this study was to explore the serum Vitamin D_3 levels amongst the patients of diabetic nephropathy and that result was compared with the levels of vitamin D_3 of control group.

MATERIALS AND METHODS

Study Details

Study Area

This study was conducted at Hi-Tech Medical College and Hospital, Bhubaneswar, India by the Department of Biochemistry in collaboration with the Department of Medicine and Department of Nephrology.

Study Population

Cases were recruited from the patients of diabetic nephropathy, attending outpatient department (OPD) of Department of Medicine and Department of Nephrology of Hi-Tech Medical College & Hospital for treatment. So, cases of diabetic nephropathy were screened and their stages of disease were measured by the Department of Medicine and Nephrology. Then these established cases were taken for further evaluation. Age-sex matched healthy controls were recruited from relatives and peers of the patients, persons attending OPD for routine health check-ups, and from staffs & faculties of Hi-Tech Medical College & Hospital.

Study Duration

The study was conducted by the Department of Biochemistry from January 2013 to December 2014.

Sample Size

The study was conducted on 200 cases and 50 healthy controls. Amongst 200 cases, 50 cases were recruited in each stages of CKD i.e. stage I, stage II, stage III, and stage IV.

Study Design

This was a hospital-based, observational, cross-sectional, case-control study. The study design included a prospective component as biochemical evaluations were carried out once, in a single hospital visit.

Definition of Diabetic Nephropathy

According to American Diabetes Association guidelines, levels of albumin and creatinine were measured in a random spot urine sample. (Molitch *et al.,* 2004) These measurements were used to calculate the urinary albumin-to-creatinine ratio. Values of <30 mg/g were characterized as being normal state, while values ≥30 mg/g describe either microalbuminuria or macroalbuminuria. (Molitch *et al.,* 2004) Thus, in this study; nephropathy was defined as a urinary albumin-to-creatinine ratio ≥30 mg/g.

Definition of Vitamin D_3 Insufficiency and Vitamin D_3 deficiency

Vitamin D_3 status in the serum is the predominant circulating form of vitamin D in the normal population. It is the most commonly used to determine vitamin D status. (Holick, 2007; National Kidney Foundation, 2003) Data suggests that levels ≥ 30 ng/ml is considered an indication of normal vitamin D_3 level. (Holick, 2007) Thus, individuals with vitamin D_3 levels 20 to 29 ng/ml were declared as vitamin D_3 insufficiency, whereas subjects with less than 20 ng/ml vitamin D_3 were regarded as vitamin D_3 deficiency. This is consistent with recommendations from the National Kidney Foundation. (National Kidney Foundation, 2003)

Data collection

A pre-designed, pre-tested, semi-structured questionnaire was used to collect various socio-demographic data like name, age, sex, address along with data about physical examination for anthropometric measurements and clinical history. Regarding anthropometric measurements, heights were measured in centimeter scale using a stadiometer; fraction values were approximated to the nearest centimeter. Weights were taken in kilograms using a calibrated weighing machine and fractions were approximated to its nearest kilogram. Body Mass Index (BMI) or the "Quetelet Index" was calculated as per the formula of Adolphe Quetelet. Regarding clinical history data, past and presently intake of medicines was recorded. History or persistence of two most important established risk factors of diabetic nephropathy; hypertension and dyslipidemia was recorded. (Gross *et al.,* 2005) Use of medicines regarding these two abnormalities were also noted. In biochemical parameter estimation, serum vitamin D_3 levels were determined by electro-chemiluminescence immunoassay using a Cobus auto-analyser (Roche Diagnostics).

Inclusion criteria for cases

Adult (18–80 years) patients diagnosed with diabetes (Type 1 and Type 2) and stage I-IVchronic kidney disease (CKD) [Glomerular Filtration Rate (GFR) 15–89 ml/min/1.73 m^2]. (Gross *et al.,* 2005)

Exclusion criteria for cases

- Patients with co-morbid conditions known to affect vitamin D metabolism including gastrointestinal, liver, rheumatoid or bone disorders (e.g. hyperthyroidism, untreated celiac disease, cancer, Paget's disease, sarcoidosis, malabsorption, etc.).
- Patients who receive drugs like oral glucocorticoids, cholestyramine, colestipol, Orlistat, digoxin, etc. which interfere with vitamin D regulation.
- Patients who take another form of active D metabolites like calcitriol, vitamin D_2.
- Patients with stage V CKD (GFR <15 ml/min/1.73 m^2), receiving dialysis or on a kidney transplant list.

Inclusion criteria for controls

- Apparently healthy subjects or persons attending OPD for routine health check-up with age-sex matching with case group.

- Patients with serum 25(OH)D <37.5 nmol/l at time of screening to control for correction of vitamin D deficiency. (Crocker *et al.,* 1997)

Exclusion criteria for controls

- Patients with pre-existing hypercalcemia (>2.75 mmol/l), hyperphosphatemia (>2.0 mmol/l), severe secondary hyperparathyroidism (PTH >66 pmol/l), and serum 25(OH)D>200 nmol/l.
- Patients undergoing strict heavy exercise for weight control and/or those who used sunscreen lotion on a regular basis.
- Pregnant women will be excluded from this study.

The whole procedures involved were transcription, preliminary data inspection, content analysis and interpretation.

RESULTS

The study was conducted at Hi-Tech Medical College and Hospital, Bhubaneswar under the Department of Biochemistry in association with the Department of Medicine and Nephrology. 200 adult patients (whose age were ≥ 18 yrs) suffering from diabetic nephropathy were enrolled as the cases in this study, after obtaining informed consent. Age and sex matched 50 healthy individuals were also included as controls for comparison purpose.

Table 1. Summarized data of socio-demographic, anthropometric and clinical history parameters of Case group

Parameters		Stage I (n=50)	Stage II (n=50)	Stage III (n=50)	Stage IV (n=50)
Age	18-45 yrs	12	08	05	03
	> 45 yrs	58	42	45	47
Sex	Male	21	23	22	19
	Female	29	27	28	31
BMI	< 30 kg/m^2	20	18	22	24
	≥ 30 kg/m^2	30	32	28	26
Diagnosed Hypertension		45	42	46	48
Diagnosed high Cholesterol		39	43	47	49

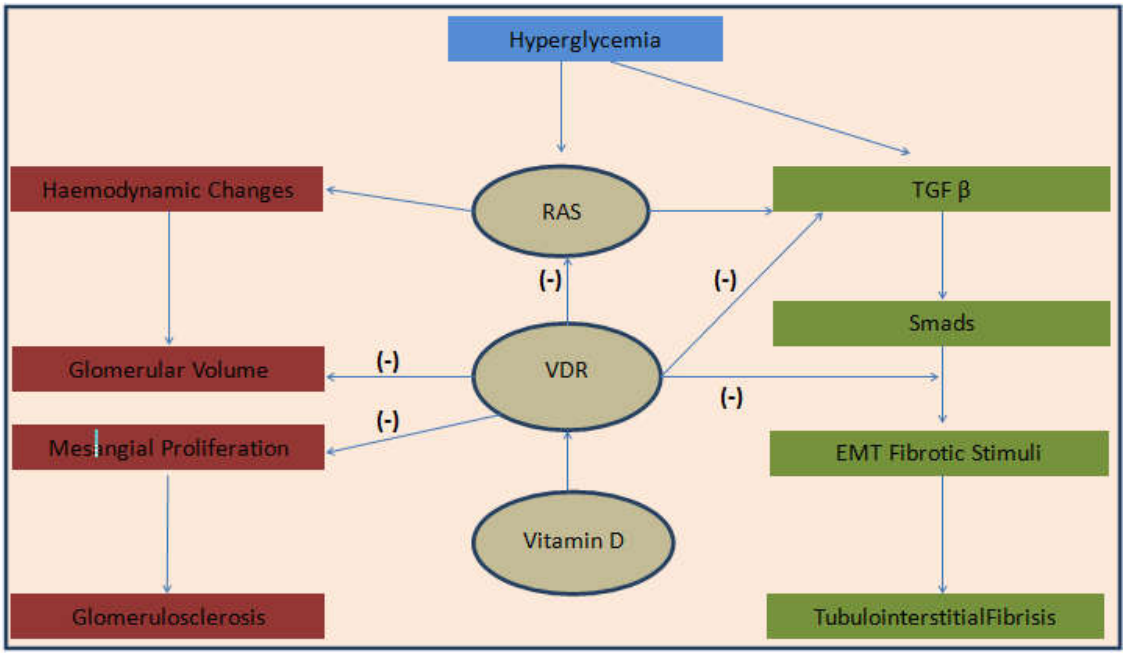

Figure 1. Flow chart states the patho-physiology of diabetic nephropathy and the inhibition of progress of diabetic nephropathy by vitamin D or its active metabolites. (-) is the inhibitory steps here

Statistical Methods

The data collected was checked for error, cleaned and double entered into MS-Excel spread sheets and checked for any entry error. Then the whole data was imported into IBM SPSS Statistics (version 20.0) and further analysis was done. Continuous variables were expressed as mean, median, ranges, and standard error (SE) or standard deviation (SD). Data was first summarized and then analyzed for test of significance e.g. chi-square test, independent sample student t-test wherever applicable using the software package. P value less than 0.05 was taken as significant.

According to stage of CKD, the cases were divided in to four groups; Stage I, Stage II, Stage III, and Stage IV, in which each stage contains 50 cases. According to age of cases, the case group was sub-divided into two groups; 18-45 yrs group and > 45 yrs group. The cases were again subdivided according to their gender and BMI (< 30 kg/m^2 and ≥ 30 kg/m^2). In the clinical history parameters; presence of hypertension and high cholesterol levels were identified and subdivided according to the stages of CKD. All these socio-demographic, anthropometric and clinical history parameters are summarized in tabular form above in Table 1. The vitamin D_3 levels were determined in the case group according to the sub-groups of ages.

And the results were compared with that of control group with same sub-groups of ages. The data were summarized and compared in Table 2.

Table 2. Comparison of levels of Vitamin D_3 according to the ages of Case and Control groups

Levels of Vitamin D_3 [Mean ± SD] (ng/ml)			P value
	Case	Control	
18-45 yrs	27.32 ± 2.43	32.41 ± 2.11	0.037
> 45 yrs	19.84 ± 3.56	28.48 ± 2.81	0.012

In the age group of 18-45 yrs, the levels of vitamin D_3 [Mean ± SD], were 27.32 ± 2.43ng/ml and 32.41 ± 2.11ng/ml in the cases and control groups respectively (p = 0.037). Again, in the age group of > 45 yrs, the levels of vitamin D_3 [Mean ± SD], were 19.84 ± 3.56ng/ml and 28.48 ± 2.81ng/ml in the cases and control groups respectively (p = 0.012). So, in the both age groups, the levels of vitamin D_3 were statistically related, but this relation is more in elder group, and it was observed that with the advancement of age of case the levels of vitamin D_3 had a great tendency to decrease. Again the vitamin D_3 levels were estimated in the case group according to the sub-groups of stages of CKD. And the results were compared with that of control group. The data were summarized and compared in Table 3.

Table 3. Comparison of levels of Vitamin D_3 according to the stages of Case and Control groups

Levels of Vitamin D_3 [Mean ± SD] (ng/ml)			
Stages of Cases	Cases	Controls	P Value
Stage I	28.41 ± 2.34	31.03 ± 2.79	0.044
Stage II	23.39 ± 2.61		0.032
Stage III	19.73 ± 2.89		0.017
Stage IV	15.52 ± 3.04		< 0.001

In cases, the estimated levels of vitamin D_3 [Mean ± SD] were 28.41 ± 2.34ng/ml, 23.39 ± 2.61 ng/ml, 19.73 ± 2.89ng/ml, and 15.52 ± 3.04ng/ml in stage I, stage II, stage III, and stage IV respectively. These values were compared with the level of vitamin D_3 [Mean ± SD] of control group (31.03 ± 2.79 ng/ml). From the above table, it was evident that all the stages were statistically related as P values were 0.044, 0.032, 0.017 and <0.001 in stage I, stage II, stage III, and stage IV respectively. And it was also revealed that in progression of the stages diabetic nephropathy, the levels of vitamin D_3 had a tendency to go downward.

DISCUSSION

Effects of active vitamin D metabolites on development of diabetic nephropathy

Increased blood sugar level (Hyperglycemia) due the state of diabetes, stimulates the intrarenal renin–angiotensin system (RAS) in kidney. Hyperglycemia is also responsible for the formation of transforming growth factor-β (TGF-β) and other cytokines. All these factors as a whole bring on proteinuria whether it may be micro-albuminuria or macro-albuminuria. The whole patho-physiological condition is diagrammed above in Figure 1. Now, this TGF-β induces tubular epithelial-to-mesenchymal transition and stimulation of profibrotic signals. Due to continuous stimulation, activity of the RAS is rapidly increased. This condition induces hemodynamic changes in the glomerulus part of the nephron. It is responsible for the increased glomerular volume, mesangial proliferation, and podocyte injury in the kidney. Vitamin D and its active metabolites attach to the vitamin D receptor (VDR) and inhibit stimulation of the RAS, changes in podocyte and as well as mesangial-cell proliferation. In addition, via the activation of hepatocyte growth factor, active vitamin D metabolites counter balance fibro genesis of renal tissue by inhibiting TGF-β indirectly. In consequence, active vitamin D metabolites resist the steps of glomerulosclerosis and progression of tubulointerstitial fibrosis. (Klaus, 2008; Rucker *et al.,* 2009)

Summary and Conclusion

In this study, it is observed that there is a strong relationship between vitamin D status and diabetic nephropathy. With the gradual progression of age and stages of CKD with diabetes, it is revealed that vitamin D insufficiency and deficiency are found in the patients of nephropathy with diabetes. Vanessa A *et al.* also got the similar type of result in their study amongst US population. (Vanessa *et al.,* 2009) Vitamin D actions have a renoprotective role in diabetic nephropathy. (Klaus, 2008; Zhang *et al.*, 2008) However, larger study population with better sample size is required for further exploration. So we should evaluate the levels of vitamin D in diabetic nephropathy patients in the very first time we meet and should start the treatment of imbalance of vitamin D as early as possible to arrest the further rapid progression of disease like diabetic nephropathy.

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