CORRELATIVE ANALYSIS OF VITAMIN D STATUS IN PEOPLE SUFFERING FROM TYPE-2 DIABETES MELLITUS IN EASTERN REGION OF NEPAL

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ABSTRACT

Introduction
Vitamin D deficiency exists as a common problem among population worldwide. The deficient vitamin D level leads to direct impact on various normal functioning of human body systems. It has been observed in few studies that decreased circulating concentration of 25-hydroxyvitamin D is associated with type-2 diabetes mellitus. The possible reason behind such occurrence is due to the direct effect of vitamin D on glucose metabolism. Vitamin D exerts influences on activity of pancreatic β-cell function from where insulin is secreted. In addition, the patients suffering from low vitamin D status show insulin resistance. These affected individuals with low vitamin D status exhibits impaired markers of glucose metabolism such as glycosylated hemoglobin. So, researchers have found vitamin D deficiency to correlate with type-2 diabetes and 80 % of obese adults suffering from vitamin D insufficiency state. In spite of such crucial significance, the correlave studies related to vitamin D status and type-2 diabetes still remains obscure in eastern region of Nepal.

Objective
We aimed to invesgate status of vitamin D among type-2 diabecs and analyze its possible correlaon

Methodology
In the present study which was hospital-based and cross-sectional one, antecubital venous blood samples were collected from patients (n=100) in plain vials with informed written consent. Blood samples were allowed to clot and centrifuged for separation of serum. The separated sera were further processed for determination of glucose (fasting as well as post-prandial) by spectrophotometry and esmaon of vitamin D with use of microwell Enzyme-linked immunosorbant assay (ELISA) technique. The data was interpreted by using SPSS soware version 16.

Results
We found that 75 % type-2 diabetics had suffered from “state of vitamin D deficiency”. Among them, females, in contrast, were predominang which was interesng in an analycal point of view.

Conclusion
Our study reviews evidence on disorders related to type-2 diabetes mellitus and vitamin D status, especially in older people. The biological processes that lead to synthesis of vitamin D in human body tissues become less efficient when an individual’s age advances with in addional occurrences of type-2 diabetes mellitus. We should therefore maintain the repleon of vitamin D in healthy older people via supplementary intakes and sensible sun-exposure.

KEY WORDS
Diabetes, Deficiency, Vitamin D
INTRODUCTION

The vitamin D is accessible in natural foods meaning that humans should consume them on regular basis. The vitamin D deficiency, however, exist as common problem in population. The deficient level of vitamin D exhibits direct impact on normal functioning of human body systems. Bones and tendons require sufficient amount of vitamin D as normal growth requirement. The reduced level of 25-hydroxyvitamin D is associated with type-2 diabetics mellitus. The already known fact behind this occurrence is due to the direct effect of vitamin D on glucose metabolism. Vitamin D exerts influences on activity of pancreatic β-cell function from where insulin is secreted. These affected individuals with low vitamin D status exhibits impaired HbA1C. The inadequacy of vitamin D, further, retards physical growth with a risk in fracture of hip bones among elderly people. Maintenance of normal levels of vitamin D, therefore, is essential for appropriate regulation of systemic processes. Thus, vitamin D deficiency is emerging as a serious health problem. Hence, estimation of its plasma levels is essential for determining the grade of an individual who may stand either under-deficiency or over-nutrition state. As it transforms metabolic processes of glucose and deficiency is worldwide being prevalent in stages that range from degree of newborn to infancy and adulthood, determination of vitamin D is crucial.

Moreover, numerous studies have reported for existence of higher incidences of vitamin D deficiency among type-2 diabetics. So, continuation of vitamin D deficiency may affect type-2 diabetes control. Vitamin D deficiency typically associates with worsening glycemic index and increased insulin requirements. As per the data put forwarded by World Health Organization, a pandemic disease called diabetes mellitus affects 170 million patients with having an effect on more than double to 370 million patients by year 2030. Researchers, in contrast, have found vitamin D deficiency to correlate with type-2 diabetes and 80 % of obese adults suffering from vitamin D insufficiency state. Scientists have shown low vitamin D status to associate with development of type-2 diabetes as well as metabolic syndrome so that these consequences reflect as incidences of vital clinical importance. In spite of suchcrucial significance, the correlave studies related to vitamin D status in collected blood samples. The informed written consent was taken from all patients and study was approved by Institutional Ethical Committee. All the participants had undergone detailed history, clinical examination and laboratory investigations using Performa designed for this study.

All patients were subjected to following investigations: determination of fasting blood sugar (FBS) and post-prandial blood sugar (PPBS) as well as estimation of vitamin D.

Sample collection and serum preparation:

Venipuncture was performed to collect blood samples under universal attentiveness as described in manufacturer’s protocol. Antecubital venous blood was collected from patients in plain vials with informed written consent, strictly as per the norms recommended by Institutional Ethical Committee. Blood samples were allowed to clot for five min and centrifuged at 3000 rpm for 10 min in order to separation of serum. Sera were stored at-20 °C until assessment. All steps were carried out under sterile conditions and precautions were taken to prevent blood samples from hemolysis, as described earlier.

Determination of glucose in serum:

The determination of glucose levels in serum was done using spectrophotometer and glucose oxidase-peroxidase assay kit by Tinder’s method. The unhemolysed serum samples and normal as well as abnormal controls were used while estimation, according to the instructions provided by manufacturers.

Estimation of vitamin D:

Competitive immunoassay was performed for estimation of serum levels of vitamin D using direct chemiluminescent technology with ADVIA Centaur vitamin D assay kit. The ADVIA Centaur XP system was utilized while performing assay. The system was prepared after loading reagent packs containing anti-vitamin D-antibody. Instrument automatically performed certain actions that include: dispensing 100 μl serum into cuvette, adding 115 μl dithiothreitol, 200 μl solid-phase reagent followed by incubation at 37 °C for 5 min and administering 200 μl light reagent with incubation at 37°C for 2.5 min. Then machine had separated, aspirated and washed the cuvettes with reagent water. Afterwards instrument had dispensed 300 μl each of acid and base reagents to initiate chemiluminescent reaction. The patients were declared deficient if they demonstrated serum levels of vitamin D < 10, insufficient when concentrations resided between 10-30, sufficient after values > 30 and toxicity if > 100 ng/ml. Results were obtained according to selection option in system. Based on setting up of the assay, system had reported serum levels of vitamin D in terms of ng/ml.

Data interpretation:

The validity and reliability of test results were determined using control sera (from Bio-RAD). Data were analyzed under Software Package for Social Science version 16 (SPSS 16).
RESULTS

Strategy to investigation of vitamin D status among type-2 diabetic patients: Strictly as per the rules prescribed by American Diabetic Association and guidelines provided by World Health Organization, all participants had undergone through detailed history and clinical examination. A total of 100 patients with age-groups ranging from 20- to 60-years-old were enrolled for investigation. All the patients were subjected to investigation for FBS and PPBS. These individuals, if having FBS ≥7.1 mmol/L (or 126 mg/dl) and PPBS ≥ 15.2 mmol/L (or 200 mg/dl), were registered as type-2 diabetics (1st & 2nd bars, respectively, in Figures 1 & 2) in present study. All of them were then subjected to further investigation for vitamin D status.

Diabetics had wide range of circulating vitamin D levels, but no toxicity: In a total 100 type-2 diabetics, we found values of FBS and PPBS ranging as 7.2-8.3 and 15.2-18.6 mmol/L (1st & 2nd bars in Figures 1 & 2), respectively, and so these had been the target population. Further, our data demonstrated that they had their circulating levels of vitamin D ranging from 8 and 59 ng/ml (3rd bar; Figure-1); suggestive of a fact that such patients had wide variation in circulating levels vitamin D. In contrast, none of them had relatively shown the values > 100 ng/ml (data not shown); indicative to absence of vitamin D toxicity among these type-2 diabetic individuals.

Majority of type-2 diabetics suffered from “the state of vitamin D deficiency”: With respect to above finding on demonstration of a wide variation in vitamin D status in type-2 diabetics, population was then grouped based on their vitamin D levels. In contrast as serum levels of vitamin D < 30 ng/ml require corresponding supplementation under therapeutic measure, we reasoned to consider both vitamin D-deficient and -insufficient groups (with D < 10 and between 10-30 ng/ml, respectively) into a single set as under “the state of vitamin D deficiency” and those having > 30 ng/ml to subsist in normal regime.

With above postulation and among 100 number of total type-2 diabetics, we found that 25 people had vitamin D levels in normal limit i.e., > 30 ng/ml (3rd bar; Figure-2). Remarkably the remaining large group (n = 75) had vitamin D< 30 (4th bar; Figure-2); suggesting that majority (means 75 %) of type-2 diabetics in locality suffered from “the state of vitamin D deficiency”.

Correlation of vitamin D status among type-2 diabetics with progression of age: we divided 75 % of type-2 diabetics (who suffered from “the state of vitamin D deficiency”) into four sets like ‘A’, ‘B’, ‘C’ & ‘D’ consisting of individuals as 21-30, 31-40, 41-50 & 51-60 years-old, respectively.

Out of total type-2 diabetics who suffered from state of vitamin D deficiency (n = 75), eight individuals stood in age-group of 21-30 years-old (1st bar; Figure-3). Further vitamin D deficiency appeared in 19 diabetics with age-group of 41-50 years-old (3rd bar; Figure-3), suggesting that generality was 25 %. Interestingly when we had look upon a residual group ‘D’ holding the type-2 diabetics with 51-60 years-old, immensely around 50 % (n = 37) were patients (4th bar; Figure-3), suggestive of predominant existence of vitamin D deficiency.
DISCUSSION

In present study, we observed that majority (75%) of type-2 diabetics who attended the hospital in eastern part of Nepal suffered from "state of vitamin D deficiency" (Figure 2). Based on few studies carried out previously in relative perspective, vitamin D deficiency was shown to be more common among type-2 diabetics. Our observation stands in accordance with these reports. By contrast, in eastern part of Nepal, frequency of vitamin D status remained unknown. Thus we screened type-2 diabetic individuals to investigate existence of associated status of vitamin D and observed preponderant significance of vitamin D deficiency in this area (Figure 2).

Vitamin D deficiency exists as a common clinical problem these days. According to the survey carried out by Martina R., more than half of the adult people in Germany had vitamin D levels below common threshold. Since then there had been a lot of attention for vitamin D in scientific community. Other study carried out by Daga et al in North India, 91.1% of type-2 diabetic patients had vitamin D deficiency. In this study, vitamin D concentrations were 7.88±1.2 and 16.64±13.6 ng/ml in diabetic and non-diabetic patients, respectively. In addition, Yousef A.S. had reported about prevalence of vitamin D deficiency in the Kingdom of Saudi Arabia in every age group. According to Colorado prevalence study, Alejandro S. had shown commonness of vitamin D inadequacy in Portuguese older adults. In such study, nationwide cluster sample of 1500 Portuguese subjects > 65 years of age suffered from vitamin D deficiency. In the present study, we observed that 75% people who had suffered from type-2 diabetes mellitus stood in the state of vitamin D deficiency. When we divided such 75% type-2 diabetics into four sets designated as 'A', 'B', 'C' and 'D' consisting of individuals as described above, our data led us to note that 50% (n=37) were patients s of vitamin D deficiency (4th bar, Figure-3). Our findings were therefore suggestive of predominant existence of vitamin D deficiency. According to study previously carried out by Barbara J.B., hypovitaminosis D exist as common problem worldwide, but it is more common and severe in older people as we observed in our study.

In age group 51-60 years females had higher prevalence of Vitamin D deficiency as compared to male. This has been the striking prevalence among old-aged type-2 diabetics. In this analytical point of view to discuss, Hanan A.H. had also obtained the similar data and so had reported the fact that vitamin D levels were significantly higher than females. These findings, in the study carried out by Hanan A.H., had been suggestive to the fact that vitamin D levels were affected by age, nationality, gender and health statues. In beneficial point of view to the patients having vitamin D deficiency, sensible sun exposure cause health benefits and maintains the strong bones.

CONCLUSION

Our study reviews evidence on disorders related to type-2 diabetes mellitus and vitamin D status, especially in older people. Vitamin D is as essential for bone health in adults and in children to prevent osteomalacia and muscle weakness. Skin provided vitamin D synthesis followed by UVB irradiation from summer sunshine and to lesser extent via absorption from food. However, these processes become less efficient when an individual's age advances with in additional occurrences of type-2 diabetes mellitus. We should therefore maintain the repletion of vitamin D in healthy older people via supplementary intakes and sensible sun exposure.

RECOMMENDATIONS

Based on our observation in the present study, we strongly recommend to supplement vitamin D in both diabetic and non-diabetic older people.

LIMITATIONS OF THE STUDY

As the present study has been carried out in an urban city Biratnagar, it would be more applicable if the rural areas would have been included.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.
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