International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 04(A); April 2019; Page No.18078-18080
DOI: http://dx.doi.org/10.24327/ijcar.2019.18080.3447

Research Article

EVALUATION OF VITAMIN D AND OXIDATIVE STRESS STATUS IN PATIENTS OF TYPE2DIABETES

Ashima Badyal[1]*, Sanjeev Kumar[2] and Kailash Chander[3]

[1]Department of Biochemistry, GMC Jammu, J&K
[2]Department of Medicine, GMC Kathua, J&K
[3]Consultant Physician, CHC, Bishnah, J&K

A R T I C L E I N F O

Article History:
Received 06th January, 2019
Received in revised form 14th February, 2019
Accepted 23rd March, 2019
Published online 28th April, 2019

Key words:
vitamin D3, FBG, TOS, TAD, diabetes.

A B S T R A C T

India faces concurrent epidemics of T2DM and hypovitaminosis D, across all age groups in urban as well as rural regions. Vitamin D played an important role in the metabolism of glucose. It directly stimulates insulin secretion from beta cells of the pancreas. Besides, increased oxidative stress and decreased antioxidant defence are established etiological factors of this multi-factorial disease. This study intended to estimate the total oxidative stress, total antioxidant defence and vitamin D3 levels in type 2 recently diagnosed diabetic patients. A comparative study was conducted in the Department of Medicine, GMC Jammu in collaboration with the department of Biochemistry, GMC Jammu, on 30 recently diagnosed type 2 diabetic patients and equal number of age and gender matched healthy controls. The mean level of vitamin D3 in patients was 49.26±16.48 ng/ml which was lower than the corresponding mean values in healthy controls, ie 74.95±8.81 ng/ml. This study has given significant higher values of TOS and significant lower values for TAD in subjects as compared to the controls. The fact that T2DM is associated with increased oxidative stress and decreased antioxidant defence, the low levels of circulating plasma vitamin D3 seems to be associated with type 2 diabetic patients.

Copyright©2019 ***Ashima Badyal, Sanjeev Kumar and Kailash Chander.*** *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

INTRODUCTION

Vitamin D deficiency is a major public health problem worldwide. With the prevalence of DM all over the world, it is expected that this disorder will remain as one of the main causes of morbidity and mortality.[1]In recent years, studies have shown that using vitamin D can help decrease the incidence of diabetes and adjustment of insulin and glucose.[2] Type 2 DM is the significant cause of premature morbidity and imposing enormous socioeconomic burden globally.[3] As per the current prevalence and trend of T2DM, international diabetes federation (IDF) predicted 592 million people will have T2DM by 2035 worldwide.[4]Prevalence of T2DM is escalating at rapid pace in India due to sedentary lifestyle. As per IDF report, the prevalence of T2DM will increase to 101.2 million by 2030 among Indians.[5]Several etiological factors including genetic, environmental, lifestyle and nutritional habits have been implicated in the causation of DM. One of the important emerging nutritional risk factors recognized for the development of insulin resistance (IR) and T2DM is the deficiency of vitamin D. Also it has been proposed to be associated with worsening of glycemic controls and progression of complications among T2DM individuals.[6]In spite of adequate sunlight exposure throughout the year, several studies documented deficiency of vitamin D as most prevalent finding among Indians.[7] With this context, rising surge of T2DM and hypovitaminosis D among Indians, Vitamin D plays important roles in the metabolism of glucose. It directly stimulates insulin secretion from beta cells of pancreas. It increases intracellular calcium, which attenuates insulin synthesis. Also it improves insulin sensitivity in peripheral muscle and fats cells. T2DM is a state of chronic inflammation because of anti-inflammatory nature. It is however a well established fact that diabetes mellitus is associated with oxidative stress.Vitamin D exerts beneficial effects on glycemic control and helps in the prevention of complications of T2DM. The imbalance between total oxidative stress and antioxidant defence attributes largely to the pathogenesis of T2DM and its complications.[8]

This study intended to estimate the total oxidative stress, total antioxidant defence and vitamin D3 levels in type 2 recently diagnosed diabetic patients.

MATERIAL AND METHODS

The study was conducted in the department of Medicine, GMC Jammu in collaboration with the department of Biochemistry, GMC Jammu, between November, 2018 and December, 2018 on as many as 30 recently diagnosed type 2 diabetic patients(12 males and 18 females),with their ages ranging from 20-50 years; and equal number of age and gender matched

Corresponding author:* **Ashima Badyal
Department of Biochemistry, GMC Jammu, J&K

healthy controls (14 males and 16 females) of the same age group were also recruited. The study was duly approved by the institutional ethical committee of GMC Jammu. Patients having other endocrine disorders, like type 1 diabetes, thyroid disorders; pregnant mothers; patients suffering from ploycystic ovarian disease, renal failure or any malignant disease; were excluded from the study. The data was collected through performa including gender, age, medical history, onset duration and complication of diabetes. Physical examination was done.

Sample Collection

Five ml of venous blood was drawn aseptically from superficial vein, in vials, centrifuged for 5 minutes at 3000 rpm, and done for the estimation of vitamin D levels in Abott architect chemiluminescencemicroparticle immunoassay.[9]All samples were analyzed in calibrators before every run. Another test of blood glucose levels was done by enzymatic method where glucose oxidase(GOD) convertsglucose into gluconic acid;hydrogen peroxide formed in this reaction in the presence of peroxide (POD) to produce red quinoneimine dye. This dye has absorbance at 505nm. The intensity of colour is directly proportional to the glucose in the specimen.[10]The tests for total oxidative stress and total antioxidant defence were carried out at Ranbaxy laboratory, Jammu.

Assay of total Oxidative stress (TOS)[11]

The test is based on iron catalysed breakdown of hydroperoxides into alkoxyl (RO) and peroxyl (ROO) radicals which interacts with the chromogen (N,N-dimethyl-p-phenylenediamine sulphate) towards formation of a coloured compounds, the absorbance of which is photo-metrically detectableat 505 nm. The method has been modified from the original FORT (Free Radical Oxygen Test), due to the instability of nonlypholized chromogen in solution procured and used in our laboratory. The intensity of the color correlates directly with the quantity of radical compounds.According to the lambert beers law, it can be related to the oxidative status of the sample. In the procedure, one hundred micro-litres of plasma,was dissolved in one ml of acetate buffer. Twenty-five micro-litres of working chromogen solution (N,N-dimethyl-p-phenylenediamine sulphate) was added and absorbance was taken at 505nm by 6minute time scan in UV-VIS spectrophotometer. The absorbance values obtained at 4 to 6 minutes for each sample were compared to the curve obtained using H_2O_2. Standardization of TOS by modified-FORT was prepared by using different solutions of hydrogen peroxide. In millimolar concentrations per litre and difference in absorbance values taken at 505nm in a six minute time-scan, different values by the same procedures performed in four different occasions and completed in 6minutes.The maximum intra-assay variation at 4 min was 4.763 and that at 6minutes was 4.414 and inter-assay variation at 4th and 6th minutes were 2.713 and 2.105 respectively for TOS. The maximum sensitivity of the assay was 1.22 millimol/l H2O2 and the linearity was up to 120millimol.

Assay for total Antioxidant Defence (TAD)[12]

In an acidic medium (pH = 5.2) and a suitable oxidant ($FeCl_3$) the chromogen (N,N- dimethyl-p-phenylenediamine sulphate) develops a stable and coloured radical cation that is photometrically detectable at 505nm at 37^0 C. Antioxidant compounds in the sample reduce the radical cation of the chromogen, quenching the colour and producing discoloration of the solution, which is proportional to the concentration. The absorbance values obtained for the samples are compared with a standard curve obtained using Trolox. In this procedure one ml of acetate buffer (pH=5.2) is taken ina test tube. Twenty-five micro-litre chromogen reagent that contains N,N-dimethyl-p-phenylenediamine sulphate and 10 micro-litre $FeCl_3$solutions were added. Ten microliter of 20 times diluted plasma was added to the mixture, the antioxidant compounds in the sample reduce the chromogen, quenching the colour and producing a discoloration of the solution, which is proportional to their concentration. Standardization of TAD was prepared by using different solution of trolox, a potent antioxidant and it quenches the colour of the chromogen maximally between 4^{th} and 6^{th} minute. With different concentrations of trolox, (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylicacid) a water-soluble form of vitamin E, the difference in absorbance values at 505nm at 4^{th} and 6^{th} minutes time scan was plotted and a calibration curve was constructed. The maximum intra-assay variations at 4^{th} and 6^{th} minutes were 7.913 and 9.009 respectively and inter-assay variations at 4minutes and 6 minutes were 2.173 and 4.717 respectively, where the linearity ranged from 2.173 and 4.717 respectively, where as the linearty ranged from 0.25 to 10.mmol/l equivalent of trolox.

RESULTS

The clinical and biochemical parameters of this study with respect to the cases and healthy controls show a significant and inverse relation. (Table 1) The mean level of vitamin D_3 in patients was 49.26±16.48 ng/ml which was lower than the corresponding mean values in healthy controls, ie 74.95±8.81 ng/ml.

Table 1 The biochemical parameters of type-2 diabetic patients and controls

Variables	Patient (N=30)	Control (N=30)	P value
Age (Years)	44.17±6.63	38.32±10.44	--
Gender (Male/Female)	12/18	14/16	--
Body mass index (BMI)	25.60±6.81	25.04±4.73	--
Fasting Blood Glucose (mg/dl)	123.41±22.85	80.66±15.16	<0.001*
TOS (mM/l equivalent of H202)	32.37±12.89	11.50±5.45	<0.001*
TAD (mM/l equivalent of trolox	120.00±68.60	329.40±128.70	<0.001*
Vitamin D3 (ng/ml)	49.26±16.48	74.95±8.81	<0.001*

*indicates significance (p<0.05)

For study subjects/ patients, the mean TOS value stood at: 32.37±12.89mM/l equivalent of H202and mean TAD value stood at:120.00±68.60mM/l equivalent of trolox; while for the healthy controls, the mean TOS value stood at:11.50±5.45mM/l equivalent of H202and the mean TAD value stood at: 329.40±128.70 mM/l equivalent of trolox. (Table 1)

The fact that T2DM is associated with increased oxidative stress and decreased antioxidant defence, the low levels of circulating plasma vitamin D_3 seems to be associated with type 2 diabetic patients. The plasma vitamin D_3 also shows a positive correlation with TAD values anda significant negative correlation with the TOS values in the study subjects. A rounded off value of 20 milimol of H_2O_2/l was taken as cut off for higher and lower values for total oxidative stress. A

rounded off value of 100 millimol/l was selected as mean cut off for anti-oxidant defence. (Figure: 1)

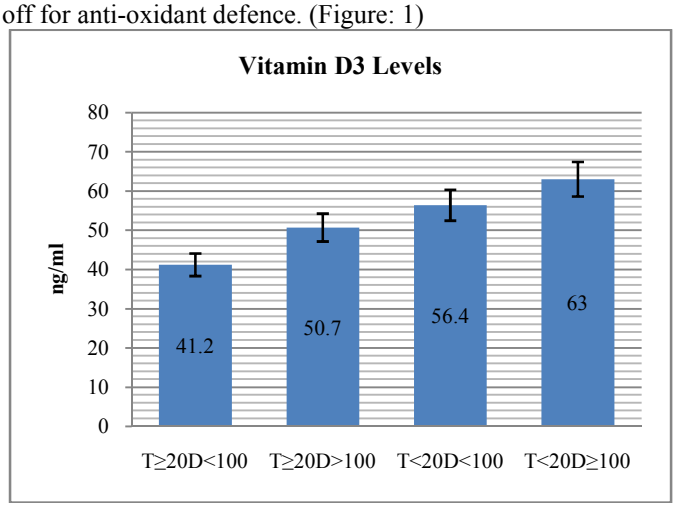

Figure 1 Vitamin D3 levels in different stress and antioxidant groups

DISCUSSION

Vitamin D receptor (VDR) has been identified in many cells, like intestinal cells, immune cells (T&B cells), kidney cells and pancreatic b-cells. The binding of $1,25(OH)_2D_3$ to the VDR/RXR (rediniod X receptor) complex and subsequent binding to its specific DNA sequence known as the vitamin D response element (VDRE) leads to and increased expression of proteins, such as calbindin-D9K fond in the intestine and calbindin-D28K fond in pancreatic β-cells, thus facilitating calcium influx into these tissues.

This study has given significant higher values of TOS and significant lower values for TAD in subjects as compared to the controls. Other researchers are supportive of this finding. In a study conducted by Dalgard *et al*,[13] more than 50% of the study population was deficient in Vitamin D. This significant association of 25-OH vitamin D_3 with antioxidant defence and significant negative correlation with oxidative stress observed in our study may lead researchers in future to investigate whether vitamin D_3 should be included in the treatment protocol of T2DM patients.

CONCLUSION

It is a new study horizon, to determine the exact role of vitamin D_3 in patients suffering from T2DM with oxidative stress as an etiological factor. Though the sample size of current study was small, it does not undermine the importance of such studies however. It is in a way indicative of the path-breaking investigations that are further in line to decode and identify our present lifestyles' stress-induced ailments.

Referrences

1. Zelaa JB, Deluca HF. Vitamin D and autoimmune diabetes. J Cell Biochem 2003; 88:216-22.
2. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Arch Dis Child 2008;93:512-17.
3. Stumvoll M, Goldstein B, Van Haeften TW.Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005; 365: 1333-46.
4. International diabetes federation. IDF diabetes atlas. 6th edition 2013.
5. IDF: One adult in ten will have diabetes by 2030. IDF press release 2011.
6. Pittas AG, Dawson-hughes B. Vitamin D and diabetes. J Steroid Biochem Mol Biol 2010;121:425-429.
7. Ritu G, Ajay G. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients 2010;6: 729-75.
8. Brijesh M,Saurav P. Prevalence of vitamin D deficiency in type 2 diabetes mellitus patients and its correlation with glycemic status. *International journal of bioassays* 2014;3: 3313-17.
9. Holick MF, Binkley NC, Bischoff-ferrari HA, Gordon CM*et al*. guidelines for preventing and treating vitamin D deficiency and insufficiency revisited.J Clin Endocrinol Metab.2012; 97: 1153-58.
10. Basak A. Development of rapid and inexpensive plasma glucose estimation by two points kineticsmethod basedon glucose-oxidase-peroxidaseenzymes. Indian J Clin Biochem 2007;22(1): 156-60.
11. Pavlatou MG, Papastamataki M, Apostalakou F. FORT and FORT:two simple and rapid assays in the evaluation of oxidative stress in patients with type2 DM. Metabolism 2009;58:1657-62.
12. Palomer X, Gonzaler-clemente JM, Blanco-vaca F. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes, Obesity and metabolism 2008;10:185-97.
13. Dalgard C, Petersen MS, Weihe P, Grandjean P. Vitamin D Status in Relation to Glucose Metabolism and Type 2 Diabetes in Septuagenarians. Diabetes Care, 2011 Jun; 34(6):1284-88.

How to cite this article:

Ashima Badyal, Sanjeev Kumar and Kailash Chander (2019) 'Evaluation of Vitamin d and Oxidative Stress Status in Patients of Type2diabetes', *International Journal of Current Advanced Research,* 08(04), pp.18078-18080.
DOI: http://dx.doi.org/10.24327/ijcar.2019.18080.3447

****