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# ROLE OF VITAMIN D AND OXIDATIVE STRESS MARKERS IN THE DEVELOPMENT AND PROGRESSION OF TYPE 2 DIABETES MELLITUS

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## ABSTRACT

**BACKGROUND**: Vitamin D has major role in the calcium homeostasis. Recent advancements in the field of medicine and science enable the researchers to discover the whole new dimensions regarding the role of vitamin D. Deficiency of vitamin D is often associated with the non-skeletal diseases. One of these is type 2 diabetes mellitus (T2DM) and thus, with the discovery of vitamin D receptors on pancreatic beta cells have even increased their interest in the role of vitamin D as a causative factor of T2DM. Moreover, oxygen intermediates (ROI) is considered as one major factor for the onset and development of diabetes supported by recent evidences also compel the scientists to look into this domain too. **AIMS AND OBJECTIVES**: Aim and objective of the current study includes the role of vitamin D and oxidative stress in the development and progression of type II diabetes mellitus.

**METHODOLOGY**: For the current study fifty patients (n=50) with T2DM and thirty (n=50) age and sex matched controls were substituted. All of the research work was carried out after the approval from the research ethical committee of the Institute of molecular biology and biotechnology (IMBB), The University of Lahore. An informed consent was obtained from the patients before including them into the study. Five ml of blood was taken from cubital vein, centrifuged and stored at -70˚C for further analysis. The samples were processed and analyzed for the estimation of Vitamin-D, 8-OHdG and MDA with the help of their respective methods.

**RESULT**: Current study shows the significantly elevated levels of oxidative stress markers i.e., MDA (0.07±0.006, p=0.01) and 8-OHdG (0.07±0.006, p=0.01) as compared to the control group (0.06±0.01 and 0.58±0.001 respectively). Furthermore, with the increases of ROS there is a noticeable decrease in the vitamin D levels (17.26±5.23, p=0.021) as compared to the control group (32.26±4.56) in patients having type 2 diabetes mellitus.

**CONCLUSION**: From the results of this study it can be concluded that vitamin D deficiency or elevated levels of reactive oxygen species may have a contributing role in the development and progression of type 2 diabetes mellitus. Hence, therapies including the vitamin D supplementation may have significant role in the prevention and treatment of type 2 diabetes mellitus.

**Keywords: Vitamin D, Type 2 Diabetes Mellitus, MDA, 8-OHdG**

## INTRODUCTION

Diabetes mellitus type 2 is considered one of the primitive chronic metabolic diseases that are known to human being. Its prevalence has been increasing rapidly in the world. According to International diabetes federation (IDF) in 2017, 425 million people have diabetes in the world and more than 39 million people belongs to Middle East countries and North Africa (MENA). It is estimated that the number of diabetes patient will rise to 67 million in MENA region by 2045. Pakistan is included in IDF MENA region and according to the data there were 7.4 million adult cases of diabetes in Pakistan in 2017 with prevalence rate of 6.9%. Rapid increase in the number of diabetic patients in last few decades have put innumerous financial burden on health sector. In developing countries like Pakistan it is very challenging to deal with such a huge number. Thus, the pathophysiology and causative factors should be investigated in depth to prevent the onset of this disease. In this way as a researcher we can help our government

to maintain a good socio-economic status of Pakistani citizens. There are large number of contributory factors in the development of diabetes mellitus i.e. obesity, sedentary life style, food habits and family history as well. Typically, the pathogenesis of T2DM is insulin resistance which tends to reduce the ability of the insulin receptor sensitivity to the cells. However, the causes of T2DM incidences are still not well understood. In most of the cases, it begins with risk factors, co-morbidities status and autoimmune conditions, which stimulates inflammatory mediators and leads to metabolic syndromes. Previous study investigating the relationship between inflammation and T2DM incident has unified enough data on the incidence of insulin resistance, particularly in the signal molecules involved in the insulin signal mechanism. Discovery of vitamin D receptors on beta cells of pancreas open the new horizon to investigate the role of another factor in the development and progression of T2DM. Role of vitamin D is not only limited to the calcium homeostasis. During last few decades, deficiency of vitamin D has been associated with many non-skeletal diseases. One of them is Type 2 diabetes mellitus (T2DM). Deficiency of vitamin D also play functional role in aging, obesity, osteoporosis, cardiovascular disease, hormonal disorders even in different types of malignancies such as breast and prostate cancer. Recent studies provide evidence that vitamin D deficiency and T2DM are related to one another as vitamin D has important role to play in glucose tolerance and insulin sensitivity **[1]**. The relationship between type one diabetes mellitus and vitamin D deficiency has been extensively reportable. Vitamin D treatment has been shown to boost, and even stop, type one diabetes mellitus in each human and animal models **[2]**. These effects are mainly attributed to the immunomodulatory actions of vitamin D **[3]**. However, less is thought on the association between vitamin D and T2DM. Vitamin D deficiency causes reduced insulin secretion in rats and humans, and its supplementation improves β-cell perform and glucose tolerance **[4]**. Moreover, bound gene variations within the vitamin D receptor (VDR) and vitamin D–binding protein (DBP) would possibly influence aldohexose tolerance and internal secretion, therefore contributory to the genetic risk for T2DM **[5]**. Rosen **[6]** in 2001 summaries the role of reactive oxygen species in onset and progression of T2DM in UNESCO-MCBN supported symposium. There is convincing experimental and clinical proof that showed reactive oxygen species (ROS) is multiplied

in each type of diabetes as compare to non-diabetes. It was also found that the onset of diabetes is intently associated with oxidative stress. Nevertheless there is controversy about which markers of oxidative stress are most reliable and appropriate for medical practice. There are various mechanisms that make contributions to the formation of reactive oxygen intermediates (ROI). It is found in many cases that vascular cells, especially the endothelium end up by producing ROI. A very essential position of oxidative stress for the improvement of vascular and neurological issues is suggested by experimental and medical studies. The mechanisms with the aid of which oxidative stress might also speed up the development of complications in diabetes are still to investigate **[7]**. ROI interfere with insulin signaling at various points and are in a position to inhibit the translocation of GLUT4 in the plasma membrane. Aims and objectives of the current study includes the role of vitamin D and oxidative stress in the development and progression of T2DM.

## MATERIALS AND METHODS

For the current study fifty patients (n=50) with T2DM and fifty (n=50) age and sex matched controls were substituted. All of the research work was carried out after the approval from the research ethical committee of the Institute of molecular biology and biotechnology (IMBB), The University of Lahore. An informed consent was obtained from the patients before including them into the study. Five ml of blood was taken from cubital vein and stored at -70˚C for further analysis. The samples were processed and analyzed for the estimation of Vitamin-D, 8-OHdG and MDA with the help of their respective methods.

## INCLUSION CRITERIA

Diagnosed cases of T2DM were included in the following study

## EXCLUSION CRITERIA

Patients suffering from hypertension, myocardial infarction or any other hepatic or renal diseases were excluded from the present study. **Sample Collection** 5.0 ml of venous blood was drawn from healthy individuals (controls) and T2DM patients undergoing ant diabetic therapy.

## BIOCHEMICAL ANALYSIS

The samples were processed and analyzed for the estimation of Vitamin-D and, 8-OHdG, and MDA.

## DETERMINATION OF MDA

Patients were screened for the determination of increased lipid peroxidation due to stress by determining the amount of MDA with the help of spectrophotometer. Lipid peroxidation (MDA) in blood was

estimated calorimetrically by measuring Thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al* (1979). 0.2ml of sample, 0.2ml of 8.1% Sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA was added. After centrifugation at 3000 rpm for 10 min the upper organic layer was taken and its OD was read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as milimoles of Thiobarbituric acid reactive substances (TBARS)/g of blood using standard curve.

**ESTIMATION OF 8-OHdG**

8-Hydroxydeoxyguanosine was estimated in the diseased group by the help of commercially available ELISA kit by (ENZO chemicals).

**ESTIMATION OF VITAMIN D**

Patients of T2DM were determined for the level of Vitamin D in their serum samples by the help of ELISA kit by (Caymen, USA).

**STATISTICAL ANALYSIS**

Results have been expressed as mean±SD (Standard Deviation). Statistical significance was determined by one way analysis of variance and spearman correlation (Two Tailed) were used to correlate the different variables. The difference was considered significant at $p<0.05$.

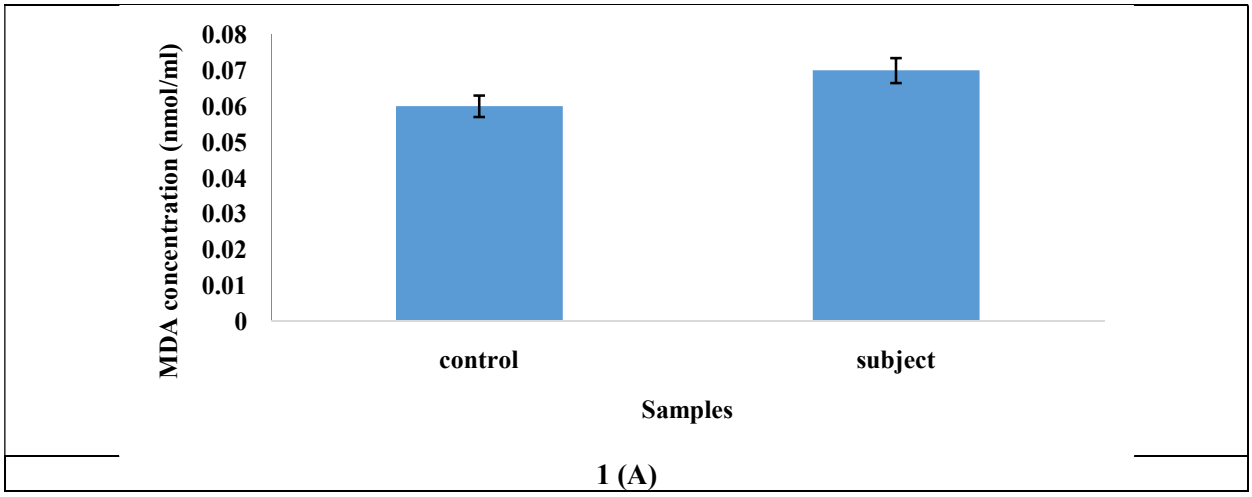
**RESULTS**

For the current study total of fifty (50) patients of T2DM and thirty (30) age and sex matched controls were added in the study and have been analyzed for their vitamin D and reactive oxygen species. All of the statistical evaluation was completed with the assist of SPSS version 16. It depicted that in the case of T2DM the patients had elevated lipid peroxidation which used to be estimated in terms of Malondialdehyde (MDA) as it is believed to be the end-product of the lipid peroxidation. Furthermore, elevated lipid peroxidation is signified by way of the increased ranges of MDA. **Figure (1A)** shows that MDA level was increased significantly in patients of T2DM (0.07±0.006, p=0.01) when compared with the healthy group of population (0.06±0.01). Increased level of MDA displays aggravation of a condition known as oxidative stress and decreased levels of antioxidants in the body. Similarly **Figure (1B)** describes elevated levels of 8-OHdG in the diseased group (2.55±0.78, p=0.001) as compared to controls (0.58±0.001). When MDA is extended notably it performs indispensable role in the formation of DNA adducts and its damage which is on the later

stages is estimated in phrases of 8-OHdG. On the other hand when the patients were estimated for the degree of vitamin D in their body they have reflected closer to new sight. As known foremost source of vitamin D is through sunlight (UV-B)-induced photo-biosynthesis in the skin and it is determined to play its principal function in skin and bones; however significant amount of vitamin D can be involved in the pathogenesis of T2DM. In case of current study a similar fashion was once seen which states decline in the level of Vitamin D in diseased group as shown in **Figure 1C** in subjects vitamin D used to be recorded as (17.26±5.23, p=0.021) while in controls it remained as (32.26±4.56). Reduction in the level of vitamin D signifies towards the new roots which can also be involved in the pathogenesis of the disease and its aggravation. There may also be various reasons involved including socio-demographic distribution etc.

**Table 1: Levels of extrapolative variables of medical importance and their interplay in the development of T2DM**

| VARIABLES | CONTROL (n=50) | SUBJECT (n=50) | *P*- VALUE (0.05) |
|---|---|---|---|
| MDA (nmol/ml) | 0.06±0.01 | 0.07±0.006 | 0.01 |
| 8-OHdG (pg/ml) | 0.58±0.001 | 2.55±0.78 | 0.001 |
| Vit-D(nmol/L) | 32.26±4.56 | 17.26±5.23 | 0.021 |




**1 (A)**

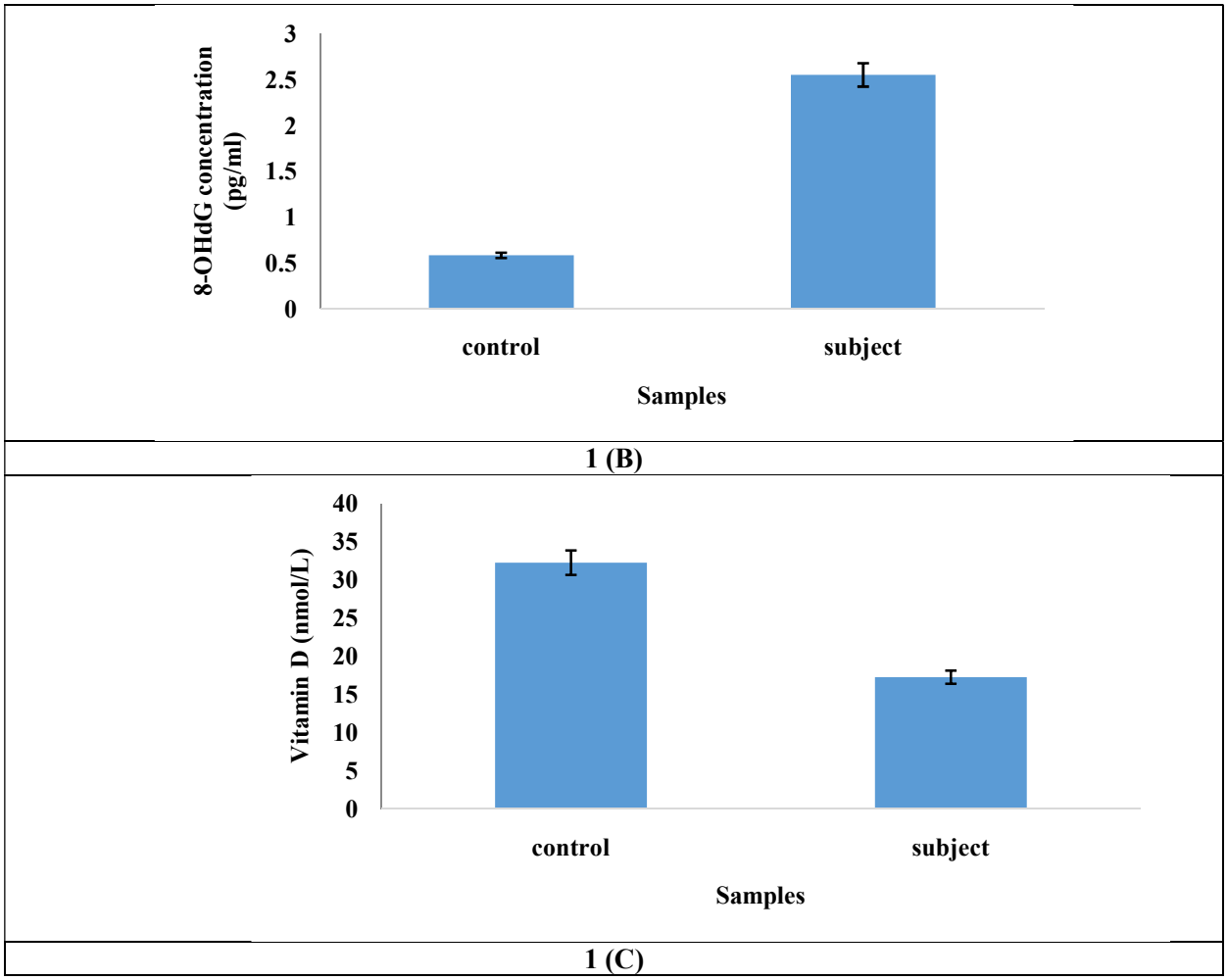



**Figure 1: (A) Showed the concentration of MDA, (B) showed the concentration of 8-OHdG and (C) Vitamin D concentration**

## DISCUSSION

Diabetes mellitus type 2 is a chronic multifactorial metabolic disease. A lot of work has been done to understand its pathophysiology. Vitamin D is being reported as a contributory factor in the development of T2DM. Talaei *et al* investigated the results of vitamin D supplementation on glucose homeostasis **[7]**. The outcomes showed that vitamin D supplementation substantially reduced fasting plasma levels of glucose (FPG), insulin resistance and Hemostatic model assessment-Insulin resistance (HOMA-IR) in patients with T2DM. It was found that there was an inverse relationship of ultimate FPG and basal 25 (OH) D concentrations. In other words, higher serum basal 25(OH) D led to decrease final FPG **[7]**. The research showed that effects of vitamin D on insulin resistance was enormous when vitamin D concentration of plasma was 40–60 ng/ml (100–150 nmol/l) whereas low vitamin D concentration didn't affect the insulin resistance. Similar

results were noticed in Inzucchi **[8]** showed that there was a 60% improvement in insulin sensitivity when we increased serum 25 (OH) D concentration from 10 to 30 ng/ml (25 to75nmol/l). Effects of vitamin D supplementation on glucose homeostasis have been shown in several other studies. Ken determined an inverse relation between 25(OH) vitamin D concentration and FPG, however a direct relation with insulin sensitivity **[9]**. Like other studies, this research also found that mean FPG was appreciably decreased after accelerated vitamin D intake **[10]**. Monthly supplementation with 120,000 units of vitamin D additionally expanded insulin sensitivity **[11]**. The results of current study also support this type of relationship between vitamin D and T2DM. There are some proposed mechanisms of vitamin D action due to which it shows anti-diabetic activity. One of them is the presences of vitamin D receptor on pancreatic β cells other are vitamin D activating 1α hydroxylase is expressed in pancreatic β cells **[12]**. Presence of vitamin D response factor in the insulin gene, presence of vitamin D receptor in skeletal muscle and the fact that 1, 25(OH) D increases transcription of insulin receptor genes, and additionally suppresses the renin gene hyperglycemic effect in pancreatic β cells. Protective effect of vitamin D on diabetes, may be due to its anti-inflammatory properties **[13, 14]**. It appears that vitamin D is responsible to increase calcium content in the cells, so it increased glucose transportation into the muscle **[15]**. Vitamin D also responsible to control the regulatory of nuclear PPAR (Peroxisome proliferative activated receptor) which plays a significant role in the insulin sensitivity. The deficiency of Vitamin D is related to increases in inflammation. Vitamin D reduces the value of the expression of pro-inflammatory cytokines that are involved in insulin resistance like interleukins, IL-1, IL-6 and TNF-α. It also down regulate NF-Kb (Nuclear factor) activity **[16]**. For this purpose, It would be more significant to approach the other studies to find more about the effect and the mechanism of vitamin D on both alpha and islet β-cell function along with the processes determining insulin resistance. The above evidence augment the results of current study in which vitamin D has an important role in the pathogenesis of T2DM. The status of vitamin D can affect insulin sensitivity, β-cell function or both. Moreover, several genes which are related to vitamin D have shown links with different pathogenetic traits of the disease. Hence, vitamin D and its related

metabolic and immune pathways might be responsible in the pathogenesis of T2DM at different levels, one at environmental level and the other at genetic level. So more knowledge on this issue can identify new targets in the treatment and prevention of the disease. Therefore, further investigations are continued in this field of research **[14]**. There are some other evidence that reveled the role of vitamin D deficiency in the pathogenesis of T2DM i.e. decreased level of vitamin D is a contributory factor of obesity that may leads to the insulin resistance. There is a study on animals which also proved that vitamin D receptors and vitamin D can play important role in T2DM and obesity. A latest research is carried out on 9649 adults that suggested that the inverse correlation between CRP's (C-reactive proteins) and serum vitamin D. Increased CRP status can be associated with complications which are related to obesity. The justification for this relationship is not known. However, the supplementation of 25-(OH) vitamin D can reduce obesity related metabolic complicated problems and inflammations **[17]**. More researches are needed to trace the processes underlying vitamin D related obesity and its complications. Finally, in current research, however the impact of vitamin D supplements on measures of glycemic control, β-cell function and insulin resistance did not follow, but it was proved that levels of vitamin D (17.26±5.23, p=0.021) in the diseased group significantly low as compared to the control groups (32.26±4.56). At present reactive oxygen species (ROS) have been taken as the unifying link of diabetes and its complications. For example serum MDA level was notably higher in T2DM patients with micro albuminuria when they were compared to patients without micro albuminuria. This result is in agreement to preceding studies. This great expansion in MDA tiers may be due to accelerated production of free radicals in diabetes mellitus patients. MDA is an end product of lipid peroxidation could be responsible for the intermolecular linking of collagen via MDA, leads to its stabilization and further glycation. This starts a vicious cycle as glycated collagen initiates similarly lipid peroxidation releasing more MDA. Superoxide dismutase (SOD), a superoxide radical scavenging enzyme, is viewed the first line of defense against the deleterious effect of oxygen radicals in the cells. The presence of superoxide dismutase (SOD) in a variety of compartments of the body allows it to dismutase $O_2^-$ radicals straight away and protects the cells from oxidative damage. Serum SOD level was extensively lowered in

patients of T2DM with micro albuminuria as compared to patients besides micro albuminuria. Literature supports the findings of the research. A widespread inhibition in SOD exercise in T2DM with micro albuminuria may effects in an elevated flux of O2 - radical and subsequently reflects the tissue injury. This observation is in accordance with the hypothesis that make bigger MDA stage and reduced GPx level would possibly play a role in tissue damage **[18]**. There is a proof in Rosen **[6]** study that the generation of reactive oxygen intermediates (ROI) is one among major factors in the onset and the progress of diabetes and related complications. Previous researches of the last 10 years signify that oxidative stress has a significant role in this pathway as a link between hyperglycemia and the typical observed pathophysiological features of diabetes. Currently, a research has also showed an oxidative stress-induced inactivation of the signal pathway which is present between the insulin receptor and the glucose transporter system. This pathway is defective in the skeletal muscle in the patients of T2DM. So it can be stated by taking in account the results of previous and current study that oxidative stress may be a significant underlying factor which leads to the onset and development of insulin resistance in patients of T2DM. So the antioxidants may be helpful for treatment of diabetic patients and their complications. A large variety of antioxidants, for example ascorbate, glutathione, acetylcysteine and others, is available. But only two α-lipoic acid and RRR-α-tocopherol have been shown in various experimental and clinical studies which have beneficial effects on diabetes and its complications. RRRα-Tocopherol decreases LDL oxidation and autoantibody titers against LDL modification proteins that are prominent and established risk factors for the manifestation of atherosclerosis **[19]**. Clinical and epidemiological researches have proposed that there is an inverse relationship between RRR-α-tocopherol and the rate of myocardial infarction. It was also proved that RRR-α-Tocopherol exerted positive effects on blood flow. α-Lipoic acid has been proved in several researches to interfere with the development of diabetic polyneuropathy. It has shown that clinically, it reduces neuropathic symptoms and it develops autonomic and peripheral functions positively in patients of diabetes. It has been presented in previous researches that α-Lipoic acid reduces the imbalance between oxidative stress measures and antioxidant capacity in diabetic patients. This antioxidant effect can give an explanation for the

inactivation of the oxidative stress sensitive transcriptional factor, NFkB, in plasma mononuclear cells, which in turn may be related with the improvement of renal and endothelial characteristic in diabetic patients. Improvement of insulin-stimulated glucose disposal in T2DM patients strengthens its therapeutic value in the presence of α-lipoic-acid. These experimental and scientific effects advise the significance of research to further evaluate the potential value of antioxidants for the treatment of diabetes and its complications. Large-scale clinical trials, such as the DCCT Study or the UKPDS Study investigate the long-term effects of these antioxidants in diabetic patients. The clinical and socio-economic burden of diabetes and its problems in the near future demand further innovation in diabetes therapy. Therefore attainable therapeutic agents such as antioxidants ought to be examined potential studies **[20]**. Reactive oxygen species can damage DNA. It has been well explain in vitro and in humans by the presence of DNA damage products in human plasma including 8-hydroxydeoxyguanosin (8-OHdG) and Malondialdehyde (MDA) by the thiobarbituric acid (TBA) test **[21, 22]**. Dandona **[22]** described an approximately four-fold higher median concentration of 8-OHdG in mononuclear cells of diabetic patients when they were compared and contrasted to corresponding controls. The difference in results was important statistically. It was demonstrated for the first time greater oxidative damage to DNA in diabetic patients when compared to controls **[23]**. Thornally **[23]** described a decline in the endogenous antioxidant, glutathione, in the erythrocytes of diabetic patients. Current study also as showed similar results. Significant elevated levels of 8-OHdG (2.55±0.78, p=0.001) and MDA (0.07±0.006, p=0.01) in diabetes group as compare to the controls (0.58±0.001 and 0.06±0.01 respectively) indicating the increased levels of ROS. Conversely, Baynes **[24]** in 1991 gave proofs that oxidative stress may not arise earlier in the disease development of diabetes. It may be an elementary pathogenic factor in the development and progression of the disease. One among the serious problems in evaluating the questions concerning how and when oxidative damage takes place in the process of disease and whether there is any chance of accumulation of free radical-derived tissue damage in the course of time of the disease is the stability of the oxidation products. But for DNA, it is recognized that alterations of DNA can be repaired through a sophisticated repair process. This process

utilizes repair enzymes which works through excision and replacement of the modified base or nucleotide **[25]**. In case of proteins, lipids and RNA, the kinetics of the turnover of the affected molecules seems to be the significant factor that limited the accumulation of oxygen radical damage. In case of long-lived unrepaired protein molecules, like collagen, products of oxygen radical reactions may accumulate with time. So they can act as a unique sensor for exposure to oxidative stress over time. Studies on glycation of proteins and Maillard reactions of glycated proteins have yielded indirect evidence for increased oxidative modifications of collagen in diabetes **[26]**. Results of some researches contradicted the result of current study i.e. Witham found out that vitamin D intake (at unique dosage) had no consequences on insulin resistance or on HbA1c as did Lind **[14]**. Nagpal **[11]** results manifested that vitamin D supplementation had no impact on imply of insulin sensitivity but two years remedy with vitamin D did enhance HOMA-IR.

## CONCLUSION

From this study it can be concluded that deficient levels of vitamin D or elevated levels of reactive oxygen species can be the contributing factors in the development as well as in the progression of type 2 diabetes mellitus. Therefore, therapies including vitamin D and anti- oxidants supplementation might helpful in treatment or even in the prevention of type 2 diabetes mellitus.

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