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# TYPE 2 DIABETES MELLITUS;
## ANTIOXIDANT STATUS IN PATIENTS WITH VARIATION IN DURATION

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**ABSTRACT… Objectives**: To access the Antioxidant Status in Patient with Variation in Duration of Type 2 Diabetes Mellitus. **Data source**: 90 selected patients suffering from Type 2 Diabetes Mellitus (DM) and 30 subjects as control group. **Design of study**: Case Control Study. **Setting**: Rawal Institute of Health Sciences, Islamabad. **Period**: July 2013 – March 2014. **Materials & methods**: Out of 120 selected subjects, 90 were of DM type 2and 30 were assigned as control group (group 1). Based on duration, patients of DM type 2 were divided into; group 2, 3 and 4; group 2 – patients with DM (type 2) duration less than 5 years, group 3 - with DM duration between 5-10 years and group 4 - with duration of DM more than 10 years. Smokers, renal failure, coronary artery disease, thyroid disease and previous antioxidant treatment patients were excluded from the study. Diabetes mellitus type 2 was diagnosed according to the standards set by American Diabetes Association. The fasting plasma glucose levels were measured by glucose oxidase method; HbA1c by automated kit on Cobas Integra of Roche. The TAC was measured by calorimetric TAC Assay Kit (BioVision) while Vitamin C and E were measured by using ELISA Kit (HUMAN). Cut off values for HbA1c was taken as ≤6%; FBS ≤110 mg/dl; TAC ≥1.16 mmol/L; Vitamin C ≥2 mg/dl; Vitamin E ≥ 9.5nmol/ml. **Results**: As the duration of type 2 diabetes increases, it was seen that vitamin C levels and TAC levels in all groups except between groups 1 & 2 decreased significantly; however, anti-oxidant vitamin E, was found to be significantly decreased in all the groups as the duration increases. A significantly increased level of HbA1c were noticed in groups 2, 3 and 4 as compared to group 1 as the duration of diabetes increases; however, the levels were found to be non-significant when group 4 was compared with that of group 2 and group 3. When fasting blood sugar was compared between the diabetic groups a significant increased levels were noticed in all the groups with the exception between group 3 and 4. A significant differences between each group and within the groups was observed when Hb1Ac, vitamin E, vitamin C, &TAC were compared using ANOVA. A statistical significant correlation was observed when HbA1c was correlated with FBS; however, it shows an inverse relationship with TAC, vitamin C and vitamin E. A significant inverse correlation of FBS was noticed with TAC, vitamin C, and vitamin E. A significant positive correlation was seen when TAC was correlated with vitamin C and vitamin E. A similar trend of significant positive correlation was seen when vitamin C was correlated with vitamin E. **Conclusions**: The levels of total anti-oxidant capacity, vitamin C, and vitamin E gradually decrease with duration of diabetes and are associated with oxidative stress. These antioxidant vitamins (vitamin C and vitamin E) should be supplemented in diabetics to increase their quality of life. TAC status may be taken as early marker to detect complications in diabetic type 2 patients especially of longer duration.

**Key words:** Diabetes Mellitus type-2, Anti-oxidants

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## INTRODUCTION

Diabetes mellitus is a chronic disease in which there is an increased production of reactive oxygen species (ROS).The oxidative stress, through ROS, has been proposed as the root cause of insulin resistance, β-cell dysfunction, impaired glucose tolerance, type 2 diabetes mellitus and diabetic complications[1].Euglycemia and inhibition of ROS restores metabolic and vascular imbalances and blocks both the initiation and progression of diabetic complications[2].

The ROS consist of free radicals such as $O_2$•, OH• ions, non-radical oxygen derivatives such

as hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl) and singlet Oxygen ($1O_2$); produced through utilization of oxygen by peroxisomes, mitochondria, phagocytic cells, and cytochrome P450 enzymes. The ROS are detoxified by glutathione, ubiquinol, urate, bilirubin and enzymes (Super Oxide Dismutase, catalase, glutathione peroxidase) and by some dietary components like polyphenols, vitamin C, vitamin E and carotenoids[3]. Antioxidants are frequently used to preserve good health as this delayed oxidative stress[4].

Prolonged hyperglycemia results in over-production of ROS by mitochondria, insulin insensitivity and diabetic complications[5].Mitochondrial dysfunction and DNA mutations have been detected in diabetic patients. Metabolic disorders, increased levels of advanced glycation end products. Glycated and oxidized lipoproteins impair the activities of mitochondrial respiratory chain complex enzymes in the vascular endothelial cells[6].

Vitamin Eacts as antioxidant by blocking lipid peroxidation in the cell membrane. Hepatocytes transfer vitamin E (α-tocopherol) to very-low-density lipoproteins (VLDLs)that maintain blood α-tocopherol levels, through special cytosolic protein called α-Tocopherol transfer protein (α-TTP).Mutations to the α-TTP gene are associated with vitamin E deficiency characterized by peripheral nerve degeneration[7].

Vitamin C works as a cofactor in various enzymatic reactions (in collagen synthesis) and if dysfunctional,results symptoms of scurvy. Ascorbate –an anion of ascorbic acid- is required for different metabolic reactionsacting as reducing agent and capable of scavenging a number of reactive oxygen species[8]. Moderately higher blood levels of vitamin C has been found to be prospectively correlated with decreased risk of cardiovascular disease and showed an inverse relation with cancer risk in men. Ascorbic acid reduces transition metals, such as cupric ions (Cu2+) to cuprous (Cu1+), and ferric ions (Fe3+) to ferrous (Fe2+)[9]. In patients with type 2 diabetes, renal dysfunction and low-grade inflammation were closely related to low levels of vitamin C in serum[10].

Studies have shown a relation between low plasma total antioxidant capacity with presence of diabetes mellitus and arterial hypertension[11]. The total antioxidant capacity (TAC) considers all the antioxidants present in plasma and body fluids, and gives a picture of the delicate balance in vivo between oxidants and antioxidants. It may also help in the evaluation of, environmental, nutritional and physiological factors of the redox status in humans[12].

## MATERIAL AND METHODS

The study was conducted at Rawal Institute of Health Sciences Islamabad from July 2013 to March 2014. Out of 120 selected subjects (mean age = 49.11±5.98 years**)**,90 were of DM type 2 and 30 were assigned as control group (group 1). Based on duration, patients of DM type 2 were divided into 3 groups; group2, 3 and 4; group 2– patients with DM duration less than 5 years, group 3with duration between 5-10 years and group 4 with duration of DM more than 10 years.

Smokers, patients on previous antioxidant treatment, of renal failure, coronary artery disease and thyroid disease were excluded from the study. Diabetes mellitus type 2 was diagnosed according to the standards set by American Diabetes Association. Included diabetics were not getting aspirin, statins, or antihypertensive medications. Informed consent was obtained from all participants before participation.

The overnight fasting blood samples were collected from anti-cubital vein. A soft rubber tourniquet was applied above the elbow. The punctured site was cleaned with spirit swab and was air dried. Five ml of blood was collected using aseptic techniques, tourniquet was removed and the punctured site was sealed. Blood for glucose was collected in grey top (acetoacetate) tube, for HbA1c in purple(EDTA) tube and for vitamin C, vitamin E and total antioxidant capacity in red top tube.

The fasting plasma glucose levels were measured

by glucose oxidase method; HbA1c by automated kit on Cobas Integra of Roche; Ascorbic acid measured by calorimetric Assay Kit Biovision (FRASC). The TAC was measured by calorimetric TAC Assay Kit (BioVision) while Vitamin C and E were measured by using ELISA Kit(HUMAN).

Cut off values for HbA1c was taken as <6%; FBS <110 mg/dl; TAC>1.16 mmol/L; Vitamin C >2 mg/dl; Vitamin E >9.5nmol/ml.

## Ethical Consideration

The study protocol was approved by ethical committee of the institution. Previous permission regarding enrolment in the study protocol was taken and participant identify was kept secret by the use of a unique ID number.

## Statistical Analysis

Data was entered and analyzed in SPSS (Statistical package for social sciences) version 20.

Mean ± SD was used to present quantitative variables i.e. HBA1c, FBS, Vitamin E, Vitamin C and TAC. The sensitivity and specificity of individual assay and combinations of various assays was determined at 95% confidence interval (CI).

To determine the association between quantitative variables of four groups, ANOVA was applied. Pearson Correlation was computed with level of significance <0.05. The data was represented as tables. The p -value <0.05 was considered statistically significant.

## RESULTS

Table I shows levels of TAC decrease significantly (p <0.05) in all groups except between groups 1 & 2 (P = 0.563) as the duration of diabetes increases. A similar trend of significant difference(p <0.05) in the levels of vitamin C was seen in all groups of patients with type 2 diabetes mellitus as the duration of diabetes increases; while the difference was found to be non-significant (p = 0.613) when the levels were compared between group 1 & 2. On the other hand anti-oxidant vitamin E, was found to be significantly decreased (p <0.05) in all the groups as the duration of type 2 diabetes increases.

| Groups | HbA1c (%) | FBS (mg/dl) | TAC (mmol/L) | Vit. C (mg/dl) | Vit. E (nmol/ml) |
|---|---|---|---|---|---|
| Group 1 (Control ) | 4.93±0.99 | 86.62 ± 13.89 | 0.98±0.10 | 1.32±0.37 | 6.47±1.21 |
| Group 2 | 8.08±1.79 | 160.47 ± 28.00 | 0.96±0.15 | 1.28±0.35 | 3.25±0.40 |
| Group 3 | 8.41±1.87 | 182.83± 44.75 | 0.76±0.10 | 0.80±0.13 | 1.31±0.34 |
| Group 4 | 8.77±1.44 | 188.0±49.80 | 0.43±0.14 | 0.24±0.10 | 1.94±.40 |
| 95%CI group 1 & 2 (p value) | -3.957 -- -2.339 (0.000) | -92.974 – -54.717 (0.000) | -0.047 – 0.862 (0.563) | -0.103 – 0.175 (0.613) | 2.864- 3.569 (0.000) |
| 95% CI group 1 & 3 (p value) | -4.284 - -2.666 (0.000) | -115.341 – -77.083 (0.000) | 0.156– 0.289 (0.000) | 0.375– 0.654 (0.000) | 4.805 – 5.510 (0.000) |
| 95% CI group 1 & 4 (p value) | -4.644 - -3.0.265 (0.000) | -120.501 – -82.250 (0.000) | 0.482 – 0.616 (0.000) | 0.939 – 1.217 (0.000) | 4.176 – 4.881 (0.000) |
| 95%CI group 2 &3 (p value) | -1.128 – 0.475 (0.422) | -41.332 – -3.400 (0.021) | 0.137 – 0.269 (0.000) | 0.341- 617 (0.000) | 1.591 - 2.290 (0.000) |
| 95% CI group 2 & 4 (p value) | -1.488 – 0.1155 (0.407) | -46.499 – -8.567 (0.005) | 0.463– 0.596 (0.000) | .904 – 1.180 (0.000) | 0.962 – 1.661 (0.000) |
| 95% CI group 3 &4 (p value) | -1.162 - 0.442 (0.376) | -24.132 – 13.799 (0.591) | 0.260 - .392 (0.000) | 0.425– 0.701 (0.000) | -0.978 – -.0.279 (0.000) |

**Table-I. Comparison of different variables between different groups at different durations of Diabetes mellitus**

*Hb1Ac: Hemoglobin 1 Ac, FBS: Fasting blood sugar, TAC: Total Anti-oxidant Capacity*
*Vit. C: Vitamin C Vit. E: Vitamin E*

A significantly increased (p ,0.05) levels of HbA1c were noticed in groups 2, 3 and 4 as compared to group 1 as the duration of diabetes increases; however, the levels were found to be non-significant when group 4 was compared with that of group 2 (p = 0.407) and group 3 (p = 0.376).

When fasting blood sugar was compared between the diabetic groups a significant increased (p <0.05) levels were noticed in all the groups with the exception between group 3 and 4 (p =0.591).

Table II shows significant differences between each group and within the groups when Hb1Ac, vitamin E, vitamin C, & TAC were compared using ANOVA.

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| **Variable** | | **Sum of Squares** | **df** | **Mean Square** | **F** | **Significance** |
| Hb1Ac | Between Groups | 284.456 | 3 | 94.819 | 38.811 | .000 |
| | Within Groups | 283.399 | 116 | 2.443 | | |
| FBS | Between Groups | 195100.467 | 3 | 65033.489 | 47.642 | .000 |
| | Within Groups | 158344.333 | 116 | 1365.037 | | |
| TAC | Between Groups | 5.903 | 3 | 1.968 | 118.079 | .000 |
| | Within Groups | 1.933 | 116 | .017 | | |
| Vit.C | Between Groups | 22.839 | 3 | 7.613 | 104.759 | .000 |
| | Within Groups | 8.430 | 116 | .073 | | |
| Vit.E | Between Groups | 465.633 | 3 | 155.211 | 322.693 | .000 |
| | Within Groups | 55.795 | 116 | .481 | | |

**Table-II. Significance of HbA1c, FBS, TAC, Vit C, and Vit E between the groups and within groups**

*Hb1Ac: Hemoglobin 1 Ac, FBS: Fasting blood sugar, TAC: Total Anti-oxidant Capacity Vit. C: Vitamin C, Vit. E: Vitamin E*

Table III shows a statistical significant correlation when HbA1c was correlated with FBS (r=0.598; p =0.000), however, it shows an inverse relationship with TAC (r= -0.453, p = 0.000), vitamin C (r= -0.458, p = 0.000) and vitamin E (r=-0.658, p = 0.000). A significant inverse correlation of FBS was noticed with TAC (r=-0.444, p = 0.000), with vitamin C (r= -0.414, p = 0.000), and vitamin E (r=-0.687, p=0.000). A significant positive correlation was seen when TAC was correlated with vitamin C (r=0.786, p=0.000) and vitamin E (r= 0.641, p=0.000). A similar trend of significant positive correlation was seen when vitamin C was correlated with vitamin E (r=0.682, p=0.000).

| Correlation | | | | | | |
|---|---|---|---|---|---|---|
| | | **HbA1c** | **FBS** | **TAC** | **Vit.C** | **Vit.E** |
| Hb1Ac | Pearson Correlation | 1 | .598** | -.453** | -.458** | -.658** |
| | p Value | | .000 | .000 | .000 | .000 |
| FBS | Pearson Correlation | .598** | 1 | -.444** | -.414** | -.687** |
| | p Value | .000 | | .000 | .000 | .000 |
| TAC | Pearson Correlation | -.453** | -.444** | 1 | .786** | .641** |
| | p Value | .000 | .000 | | .000 | .000 |
| Vit. C | Pearson Correlation | -.458** | -.414** | .786** | 1 | .682** |
| | p Value | .000 | .000 | .000 | | .000 |
| Vit. E | Pearson Correlation | -.658** | -.687** | .641** | .682** | 1 |
| | p Value | .000 | .000 | .000 | .000 | |

**Table-III.** *Hb1Ac: Hemoglobin 1 Ac, FBS: Fasting blood sugar, TAC: Total Anti-oxidant Capacity Vit. C: Vitamin C, Vit. E: Vitamin E*

**. Correlation is significant at the 0.01 level (2-tailed).

## DISCUSSION

Our study showed an overall reduction in the levels of TAC, vitamin C and vitamin E in type 2 diabetics with time duration as compared to control group. These results are in consistent with Odum et al[13], who showed a significant reduction in the levels of vitamin C, vitamin E and TAC in type 2 diabetic patients compared to control group**.** Peerapatdit et al[14] also showed a significant reduction in the levels of vitamin E in type 2 diabetic patients and in those diabetics which were complicated with coronary heart disease. However, Savu et al[6] showed that TAC of plasma was increased in patients with uncomplicated type 2 diabetes despite high levels of oxidative stress depending upon mitochondrial functions.

An elevated oxidative stress was detected in diabetic patients as well as in animal models as a result of abnormality in mitochondrial function as well as mutation in the DNA. The mitochondria are one of major sources of reactive oxygen species (ROS) in the cell. The metabolic disorders like hyperglycemia, hypertriglyceridemia, hypercholesterolemia, hypo-alpha-lipoproteinemia, and increased levels of advanced glycation end products, glycated and oxidized lipoproteins, are associated with oxidative stress. In type 2 diabetic patients, risk of oxidative stress increase with a decrease in the levels of anti-oxidants with duration of the disease[5].

There also occurs an associated rise in lipid peroxidation mediated by free radicals. Vitamin E, a lipid soluble vitamin having antioxidant action sand shows a greater protection of membranes against damage, produced by cholesterol oxidation products[15].Vitamin E also decreases the risk of cardio metabolic events, and for the same reason, it must be given in diabetic patients on long term basis[16]. The erythrocyte vitamin E levels are more important than plasma levels of Vitamin E in case of diabetic children[17]which can be restored if vitamin C is given with metformin[18].

Our results are in consistent with Kenzo et al[10]who showed reduced levels of vitamin C in type 2 diabetics.

Vitamin C, a water-soluble vitamin, has strong antioxidant property and works through regeneration of vitamin E[18,19]. It has been shown that the individuals with increased plasma levels of Vitamin C have many fold decrease risk of diabetic complications[20].

Our results showed an inverse correlation of FBS with TAC. These results are in consistent with Akinosun et al[21]and Song et al[22]in poorly controlled Type 2 diabetics associated with a defect in antioxidant defense of the body against oxidative stress. A good control of FBS could possibly help reduce free radical activity and probably minimize the chronic complications in diabetic patients. Increased blood glucose is associated with an increase in oxidative stresses and some deficiency in the antioxidant defense. These factors cause increase oxidative DNA damage, pancreatic beta-cell dysfunction, insulin resistance and more enhanced hyperglycemia. This vicious circle is responsible for making the diabetes more deleterious[22].

In type 2 diabetics, administration of vitamins (C and E) for few months have shown many beneficial effects, including reduction in levels of hypertension, reduced blood glucose levels and a rise in super oxide dismutase and glutathione peroxidase enzyme activity that decrease insulin resistance by reduction of oxidative stress[23]. Vitamin C supplementation reduces fasting and postprandial oxidative stress thus protects diabetics from many diabetic complications. Serum Vitamin C has also a negative relation with metabolic status and serum uric acid levels[24].

## CONCLUSIONS

The levels of Total anti-oxidant capacity, vitamin C and vitamin E gradually decrease with duration of diabetes and are associated with oxidative stress. These antioxidant vitamins (vitamin C and vitamin E) should be supplemented in diabetics to increase their quality of life. TAC status may be taken as early marker to detect complications in diabetic type 2 patients especially of longer

duration.



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