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A Prospective Study of Evaluation of Oxidative Stress in Type 2 Diabetes Patients

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Abstract

Introduction: *Oxidative stress, through the production of reactive oxygen species (ROS), has been proposed to be the unifying link between the various molecular disorders underlying the development of insulin resistance, β-cell dysfunction and impaired glucose tolerance leading to the development of type 2 diabetes mellitus.*

Materials and Methods: *The study was carried out at the outpatient clinic of Department of Medicine, patients were chosen & all the biochemical studies & investigations were carried out in Department of Biochemistry, Siddhartha Medical College, Vijayawada. Our study group consists of 50 Type 2 Diabetic patient Cases of age ranging from 40-60 yrs. & 50 Controls of the similar age group. The Case & Control subjects were randomly selected with age & sex matched. The Case subjects age ranging from 40-60 years, fitted the inclusion criteria of type 2 diabetes. The case subjects were on oral hypoglycaemic drugs and were not on any kind of oral antioxidants. The exact duration of type 2 diabetes according to the history given by the Case subjects themselves were around 6-7 years. The Control subjects were healthy patients aged (40 -60 years) with no history of T2DM or antioxidant supplements, and they were included in the "Healthy criteria''. With proper understanding, the subjects have accepted to undergo this procedure and have accordingly given their consent.*

Results: *We have included 100 subjects in our study, where 50 were type 2 DM subjects & 50 were healthy individuals. The patients were from the Department of Biochemistry, Siddhartha Medical College, and Vijayawada. Data analysis was done using SPSS 20 version software. The mean HbA1c level is higher in Cases {7.09 ± 0.38} % than in Controls {4.55 ± 0.41} % [p value- < 0.001] which is given in Table 1. The mean TAC level is decreased in Cases {1.28 ± 0.19} mM than in Controls {2.3 ± 0.18} mM [p value-<0.001] which is given in Table 2.The mean MDA level is increased in Cases {2.11 ± 0.34} nmol/mL than in Controls {0.74 ± 0.13} nmol/mL [p value- < 0.001] which is shown in Table 3. A negative correlation of TAC with HbA1c of both Cases {-0.959, p value- <0.001} & Controls {- 0.991, p value- <0.001} and is shown in Table 4 respectively. There is a positive correlation of MDA with HbA1c of both Cases {0.624, p value -<0.001} & Controls {0.936, p value- 0.001} and it is shown in Table 5.*

Conclusion: *Type 2 DM is strongly associated with various risk factors, that have the ability to proceed to a complicated state, which eventually cause the patients to lead a burdened compromised life. In our study, we focused on ruling out the panel of test for screening of type 2 diabetic subjects who are at early risk of developing complication.*

Key Words: *Oxidative stress, T2DM, MDA, HbA1c*

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I. Introduction

Oxidative stress, through the production of reactive oxygen species (ROS), has been proposed to be the unifying link between the various molecular disorders underlying the development of insulin resistance, β-cell dysfunction and impaired glucose tolerance leading to the development of type 2 diabetes mellitus.[1] Oxidative stress, secondary to persistent hyperglycaemia and dyslipidemia plays a key role in the pathogenesis of T2DM and its complications by excess ROS generation, auto-oxidation of glucose, non enzymatic protein glycosylation, lipid peroxides formation, impaired glutathione metabolism, impaired activities of antioxidant defence enzymes and decreased concentrations of low molecular weight antioxidants such ascerulo plasmin and uric acid.[2]

Malondialdehyde, as TBARS (ThioBarbituric Acid Reacting Substances), is frequently used to determine the prooxidant/antioxidant balance in type 2 diabetic patients as they are stable and easily measurable lipid peroxidation products. Ceruloplasmin acting as ferroxidase decreases the availability of the iron in free

radical generating reactions.[3] Considering the pro-oxidant status of patients with T2DM, an increase in the level of CP probably favours its protective action against free radical injury. Alternatively, an increase in serum CP in type 2 diabetes could generate excess oxidized LDL, which causes atherosclerosis. It could also cause vascular injury by generating free radicals, such as hydrogen peroxide, in the course of oxidization of serum homocysteine.[4]

Uric acid is the main plasma antioxidant in humans, followed by vitamin C. Uric acid stabilizes vitamin C in plasma and protects it from oxidation. Urate, the soluble form of uric acid in the blood, can scavenge superoxide radicals, hydroxyl radicals, and singlet oxygen and can chelate transition metals. Uric acid can also block the reaction of superoxide anion with nitric oxide forming peroxynitrite which is a particularly toxic product that can injure cells by nitrosylating the tyrosine residues of proteins.[5]

II. Materials And Methods

The study was carried out at the outpatient clinic of Department of Medicine, patients were chosen & all the biochemical studies & investigations were carried out in Department of Biochemistry, Siddhartha Medical College, and Vijayawada. Our study group consists of 50 Type 2 Diabetic patient Cases of age ranging from 40-60 yrs. & 50 Controls of the similar age group.

The Case & Control subjects were randomly selected with age & sex matched. The Case subjects age ranging from 40-60 years, fitted the inclusion criteria of type 2 diabetes. The case subjects were on oral hypoglycaemic drugs and were not on any kind of oral antioxidants. The exact duration of type 2 diabetes according to the history given by the Case subjects themselves were around 6-7 years. The Control subjects were healthy patients aged (40 -60 years) with no history of T2DM or antioxidant supplements, and they were included in the "Healthy criteria''. With proper understanding, the subjects have accepted to undergo this procedure and have accordingly given their consent.

Inclusion criteria of Case subjects:

HbA1c or Glycated haemoglobin ≥ 6.5%.ORFasting Plasma Glucose >126 mg/dL. or Postprandial Plasma glucose >200 mg/dL after oral glucose intake. The test was to be performed as described by the World Health Organisation after a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water OR Random plasma glucose > 200 mg/dL.

Inclusion Criteria for Control Group

- Fasting Plasma glucose < 100 mg/dL.
- 2-hour Plasma glucose level <140 g/dL.
- HbA1c ≤ 5.7%.

Exclusion Criteria for Case & Control

- History of alcohol consumption.
- History of severe renal, hepatic & neurological disease.
- Pregnant women.
- Individuals refusing to provide informed consent.
- History of hypertensive crisis.
- History of known cardiovascular disorders.
- Not on any kind of Antioxidant supplementations.

Venous blood samples from all the participants were drawn after an overnight fast from an antecubital vein into clotted and EDTA tubes. Serum was separated and retained for analysis of TAC, MDA and HbA1c respectively. Methodology Total Antioxidant Capacity (TAC) By Colorimetric assay (BioVision Assay kit, total antioxidant capacity assay Kit, K274-100).11Malondialdehyde (MDA) It was evaluated by the Thiobarbituric Acid test.12Glycated Haemoglobin (HbA1c) It was evaluated by HPLC (Bio-Rad–D10).

III. Results

We have included 100 subjects in our study, where 50 were type 2 DM subjects & 50 were healthy individuals. The patients were from the Department of Biochemistry, Siddhartha Medical College, and Vijayawada. Data analysis was done using SPSS 20 version software.

The mean HbA1c level is higher in Cases {7.09 ± 0.38} % than in Controls {4.55 ± 0.41} % [p value- < 0.001] which is given in Table 1. The mean TAC level is decreased in Cases {1.28 ± 0.19} mM than in Controls {2.3 ± 0.18} mM [p value-<0.001] which is given in Table 2.The mean MDA level is increased in Cases {2.11 ± 0.34} nmol/mL than in Controls {0.74 ± 0.13} nmol/mL [p value- < 0.001] which is shown in Table 3. A negative correlation of TAC with HbA1c of both Cases {-0.959, p value- <0.001} & Controls {-

0.991, p value- <0.001} and is shown in Table 4 respectively. There is a positive correlation of MDA with HbA1c of both Cases {0.624, p value -<0.001} & Controls {0.936, p value- 0.001} and it is shown in Table 5.

	Group			
	Case	**Control**		
	Mean ± SD	**Mean ± SD**	**P Value**	**Significance**
HbA1c	**7.08 ± 0.38**	**4.54 ± 0.41**	**<0.001**	**Significant**

Table 1: Comparision of HbA1c of Case & Control

	Group			
	Case	**Control**		
	Mean ± SD	**Mean ± SD**	**P Value**	**Significance**
TAC mM	**1.28 ± 0.19**	**2.3 ± 0.18**	**<0.001**	**Significant**

Table 2: Comparision of TAC of Case & Control

	Group			
	Case	**Control**		
	Mean ± SD	**Mean ± SD**	**P Value**	**Significance**
TAC mM	**2.11 ± 0.34**	**0.74 ± 0.13**	**<0.001**	**Significant**

Table 3: Comparision of MDA of Case & Control

Correlations			
Group			**HbA1C**
Case	TAC mM	Pearson Correlation	-0.951
		p Value	<0.001
Control	TAC mM	Pearson Correlation	-0.991
		p Value	<0.001

Table 4: *Correlation of TAC in relation to HbA1c of (a) Case & (b) Control*

Correlations			
Group			**HbA1C**
Case	MDA nmol/mL	Pearson Correlation	0.624
		p Value	<0.001
Control	MDA nmol/mL	Pearson Correlation	0.936
		p Value	<0.001

Table 5: Correlation of MDA in relation to HbA1c of (a) Case & (b) Control

IV. Discussion

Type 2 diabetes mellitus is a disease which has a tendency to progress into a state of various complication, oxidative stress, heightened inflammatory process and eventually leading to metabolic dysregulation. Countries with the highest number of diabetics are in India (19 million), China (16 million), and the United States (14 million).[6] In our study, parameters of oxidative stress of 50 middle-aged subjects with age ranging from 40-60 years, along with healthy subjects (Control group) with age & gender matched, were evaluated with a detailed assessment of history & is included in the study according to the inclusion criteria already discussed in materials & methods, and statistical analysis is done.[7]

The mean glycated haemoglobin (HbA1c) is increased in Case group when compared with the Control group and has been found statistically significant.

Results are consistent with the study done by Kodiatte et al, Verma et al & Masram et al where Kodiatte et al, Masram et al & Verma et al stated HbA1c was increased in type 2 diabetic subjects compared to control group.[8]

As of present scenario, hyperglycaemia appears as a significant cornerstone, providing a favourable cellular environment for increased RONS (Reactive oxygen & nitrogen species) production and successively leading to increased production of free radicals and impaired antioxidant defences resulting in increased oxidative stress (OS) contributing to the development and progression of diabetes and its complications.[9] Thereby Total Antioxidant Capacity (TAC) is being assessed as an "antioxidant marker" & Malondialdehyde (MDA) as a marker for elevated lipid damage. [10]

In our study, the mean TAC levels is decreased & MDA levels is increased in type 2 diabetic subjects when compared to the control group and this has been found statistically significant.

V. Conclusion

Type 2 DM is strongly associated with various risk factors, that have the ability to proceed to a complicated state, which eventually cause the patients to lead a burdened compromised life.

In our study, we focused on ruling out the panel of test for screening of type 2 diabetic subjects who are at early risk of developing complication.

In our study, it has been found that there is a significant decrease in evaluated Oxidative stress (TAC) in T2DM Case subjects when compared with the Control group, showing that excessive free radical generation has been initiated due to the incompetency of "antioxidant defence system".

A negative correlation is also noted of TAC in respect to HbA1c, stating that due to increase in HbA1c there is a decrease in "Total Antioxidant Capacity".

In our study, there is an increase in MDA, in type 2 diabetic subjects when compared to Control group, and has been positively correlated with HbA1c, stating an increased lipid peroxidation in diabetic subjects which is due to associated dyslipidaemia.

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